Systematics and evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs

Dissertation

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Abstract

Sea anemones in genera *Adamsia, Calliactis* and *Paracalliactis* (family Hormathiidae) engage in a mutualistic symbiosis with hermit crabs. The anemone gains substrate and food in exchange for defending the crab. Some of the sea anemones also expand the living space of the crab by producing a carcinoecium, a chitinous structure that overlies the initial gastropod shell in which the hermit crab lives. The symbiosis is initiated either by the crab or by the anemone. Although behavioral and physiological aspects of this symbiosis have been studied, interpretations cannot be generalized because no previous taxonomic or molecular-based studies have focused on this group of sea anemone or implemented an evolutionary framework.

To explore evolutionary hypotheses for the group, I reconstructed relationships among members of Hormathiidae using DNA sequences (12S, 16S, 18S, 28S and COIII). I found that the association between sea anemones and hermit crabs has evolved at least twice: *Adamsia* nests within *Calliactis* in a single clade, and *Paracalliactis* belongs to a different clade within the family. The carcinoecium and complex behavioral and anatomical features associated with the symbiosis are interpreted as having evolved at least twice within Hormathiidae and seem to be phylogenetically labile. Both parsimony and model-based analyses of these DNA sequences recover similar relationships between members of Hormathiidae and

closely related families. None of the markers separately or in combination support a monophyletic origin for Hormathiidae due to the inclusion of *Actinoscyphia plebeia* (Actinoscyphiidae), *Bathyphellia australis* (Bathyphellidae), and *Nemanthus nitidus* (Nemanthidae) within Hormathiidae. Most of the characters used in the circumscription of genera within the family, such as fertility of perfect mesenteries, number of fertile and sterile mesenteries and number of mesenteries distally and proximally, are inferred to be convergent in the hormathiids included in the analysis.

To evaluate genetic diversity and phylogenetic utility of the internal transcribed spacer (ITS) region in sea anemones, five populations of the most widespread species of sea anemones symbiotic with hermit crab, *C. polypus*, and two closely related species of *Calliactis* were analyzed. I found extensive intragenomic variation, but this does not overlap with intraspecific or inter-specific variation. Thus, variation does not obscure phylogenetic signal, making ITS a potentially useful marker for analysis of closely related species of sea anemones. In addition, no population structure was found in a parsimony analysis of sequences from 36 individuals of *C. polypus* from five populations. Thus, ITS sequences were not variable enough for studies below the species level in *C. polypus*. Finally, to understand the diversity of sea anemones symbiotic with hermit crabs in family Hormathiidae, I undertook a monograph of *Adamsia*, *Calliactis*, and *Paracalliactis*, The monograph provides a comprehensive perspective on diversity and variation within these genera and includes important taxonomic tasks such as the validation of names and circumscription of genera. Eighteen species of sea anemones symbiotic

with hermit crabs are described in detail, including two new species and one new combination.

Dedication

Dedicated to my parents, Juliana and Ribeiro, and to my grandparents Maria do Céu, Euterpe, Dolores and Ferro.

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Fields of study

Major Field: Evolution, Ecology and Organismal Biology.

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Chapter 1: Introduction

Sea anemones (Cnidaria: Actiniaria) are solitary marine invertebrates that have achieved great ecological success despite their structural simplicity. Essentially laminar organisms, their two-dimensional epithelial construction has shaped both behavioral and physiological responses and led to great diversity (Shick 1992), as evidenced by their presence in all marine habitats, from the intertidal zone to deep-sea hydrothermal vents and whale falls (Rodriguez and Daly 2010). The ecological success of sea anemones may be partly attributed to their ability to engage in associations with other organisms.

Associations of actiniarians with macrofauna such as crustaceans (Bach and Hernnkind 1980; Crawford 1992), mollusks (Smith 1971; Ross and Kikuchi 1976; Pastorino 1993; Riemann- Zürneck 1994; Ates 1997; Mercier and Hamel 2008; Rodriguez and Lopez-Gonzalez 2008), and fishes (Fautin1991; Fautin and Allen 1992) and microorganisms such as dinoflagellates (zooxathellae) (Berner et. al. 1993) are some of the most familiar marine symbioses. Although these symbioses have been known for more than a century, their taxonomic and evolutionary components are poorly understood (Dunn 1981; Geller and Walton 2001). The association of sea anemones and hermit crabs is one of the most interesting and eye catching symbiosis in the sea. This association is common, occurring at most depths

and latitudes, and involves one or more anemones living on a shell inhabited by a hermit crab (reviewed in Gusmão and Daly 2010). This symbiosis is established through a variety of mechanisms and may entail a considerable degree of species recognition and discrimination (Ross 1974a). In most cases, the crab actively engages the anemone, detaching it from the substrate and placing it on its shell (Brooks 1991). In other cases, the anemone transfers itself through complex and poorly understood mechanisms mediated by a molluscan substance in the shell used by the hermit crab (McFarlane 1969). The latter mechanism is among the most complex behaviors performed by cnidarians (Ross 1974b). Ecological and behavioral studies have shown that these associations are, predominantly, a result of choice, not chance, with isolated cases where members of the pair may live independently (Ross 1974a).

The anemone-crab partnership is a mutualism in which anemones are transported, avoiding unsatisfactory local conditions (e.g., temperature and oxygen) and receiving food, in exchange for defending the crab from predators (Ross 1971). In some instances, the anemone also provides the crab with a means of avoiding the critical necessity of replacing shells by forming a living "cloak" that effectively expands the living space of the crab (Ross 1974b; Daly et. al. 2004). Members of the genus *Adamsia* produce a chitin carcinoecium under the living cloak, which allows the hermit crab to grow larger where big shells are scarce and calcium carbonate dissolves quickly, providing an extraordinary instance of coevolution (Dunn et al. 1980).

Most of the knowledge of the symbiosis between hermit crabs and sea anemones is based on the work of D.M. Ross and his collaborators (Hand 1975). Ross divided the study of this association in four areas: behavior and physiology, ecology, taxonomy and systematics and biogeography. As the most prolific researcher in his field, he produced in the 70's most of what it's known in these areas of research.

Behavior and physiology have been the most active areas of study of this symbiosis, with many works describing the wide range of responses involved in the establishment of the associations (e.g., Faurot 1910; Davenport et al. 1961; Ross and Sutton 1961; Cutress and Ross 1969; Mainardi and Rossi 1969; Ross 1974a; McFarlane and Shelton 1975; Ross 1979; Ross and Boletzky 1979). Response to shells, activity by crabs and relaxation and detachment are the most important patterns and are known for many of the species described (Ross 1974b). Nonetheless, many adaptive and ecological aspects are still obscure, consisting, predominantly of inferences drawn from collections and laboratory experiments. Most studies have focused on documenting the associations between the members of the pairs, ranging from a temporary association, to a permanent association that yields structural protection, avoiding the critical necessity of replacing shells by the hermit crab, or protection from predators (Ross 1971; Brooks 1989).

The evolution of these associations is difficult to study due to confusion on the identities of species of both sea anemones and hermit crabs. In some cases, the literature is old and many of the descriptions are poor, with currently relevant

taxonomic characters not discussed. Single records of sea anemones are also a problem and, unfortunately, in many cases, only the anemone or the hermit crab is retained and identified depending on the interest of the collector (Ross 1974a). As a result, the taxonomy and systematics of the group consists mainly of opportunistic descriptions of new species (Hand 1975; Riemann-Zurneck 1975; Ross and Zamponi 1982; Fautin 1987). Generic revisions, such as the work of Daly et. al. (2004), where the authors address the circumscription of *Adamsia*, *Calliactis,* and *Paracalliactis* and the summary of the taxonomy and the problems associated with the symbiotic anemones by Ross (1974a) are exceptions.

Although the distribution of the symbiosis is known at a large scale, the local distributions remain unknown (Ross 1974a). Ross (1974a) attempted to summarize the distributions of the associated anemones and, although he did not have as many records as are available today, he characterized their distribution as confined to warmer seas and at depths less than a few hundred meters. Ross (1974b) also pointed out that the biogeography of this group could shed much light on its evolution, including the obligation of its members and the boundaries between hermit crab and gastropod associated species.

This study integrates taxonomy, analyses of species genetic diversity, and phylogenetic analysis of broader evolutionary relationships to understand the diversity and diversification of sea anemones symbiotic with hermit crabs. No previous taxonomic or molecular-based studies have focused on this group of sea anemone. The morphological revision will help the identification of symbiotic sea

anemones and will accurately account for the diversity of the group. Broader phylogenetic relationships within and between the hermit crab symbiotic anemones will aid in finding hypotheses of relationships for the group. Population-level studies will further contribute to a clarified and stable classification for the hermit-crab symbiotic anemones and will inform future phylogeographic studies in sea anemones.

Overview of chapters

To understand the origin, diversity and diversification of sea anemones symbiotic with hermit crabs in family Hormathiidae, I undertook a monograph of *Adamsia*, *Calliactis*, and *Paracalliactis*, analyzed species genetic diversity, and explored broad-scale phylogenetic analysis of Hormathiidae. Here I describe how I organized the chapters of this dissertation and briefly explain the major findings of each of these projects.

Chapter 2 addresses family-level diversity within Hormathiidae to resolve broader evolutionary questions about the origin and diversification of the group of sea anemones symbiotic with hermit crabs. I used DNA sequences from three mitochondrial markers (12S, 16S, COIII) and two nuclear markers (18S, 28S) to reconstruct, for the first time, relationships among members of Hormathiidae and the superfamily Acontiaria. Specific objectives of the analyses include an investigation of whether the symbiosis arose once or multiple times in the lineage that includes the three symbiotic genera (*Adamsia, Calliactis* and *Paracalliactis*), and the identification of their closest non-symbiotic relative(s), and the exploration of the morphological and biological transformations that occurred as the symbiosis evolved

were also examined. I found that association between sea anemones and hermit crabs has evolved at least twice within family Hormathiidae: *Adamsia* nests within *Calliactis* in a single clade, and *Paracalliactis* belongs to a different clade within the family. The carcinoecium and complex behavioral and anatomical features associated with the symbiosis are interpreted as having evolved at least twice within Hormathiidae, and, thus, despite their complexity, seem to be phylogenetically labile. Additionally, the hypothesis that symbiosis with gastropods is a precursor or otherwise associated with the evolution of the symbiosis with hermit crabs could not be rejected and needs further investigation.

In Chapter 3, I explore the data of the analyses described in Chapter 2 to further the discussion of the phylogenetic relationships within the superfamily Acontiaria and the family Hormathiidae. I found that none of the markers (separately or in combination) support a monophyletic origin for Acontiaria or Hormathiidae. More important to the present study, however, is the consistent finding of a non-monophyletic Hormathiidae. The inclusion of *Actinoscyphia plebeia* (Actinoscyphiidae), *Bathyphellia australis* (Bathyphelliidae) and *Nemanthus nitidus* (Nemanthidae) renders Hormathiidae paraphyletic. The inclusion of *Acy. plebeia* within Hormathiidae confirms morphological (Riemann-Zürneck 1978; Rodríguez et. al. 2008) and phylogenetic hypotheses (Daly et al. 2008) that regard members of Actinoscyphiidae as hormathiids that have lost acontia. The phylogenetic position of *B. australis* within Hormathiidae may illustrate that nematocyst type present in the acontium is a good indicator of evolutionary relationships within Acontiaria.

Although instances of loss of acontia are known (e.g., *Actinoscyphia*), when present, the types of nematocysts in the acontia seem to track evolutionary history and are a synapomorphy for the clade to which Hormathiidae belongs. *Nemanthus nitidus* is always recovered as the sister group to a clade that includes three hormathiids and one species of the family Bathyphellidae (*B. australis*). Based on these results we hypothesize that acontioids are homologous to true acontia. Relationships among genera within Hormathiidae showed that most of the characters used by Carlgren (1949) in his classification of Hormathiidae, such as fertility of perfect mesenteries, number of fertile and sterile mesenteries and the direction of mesentery growth, presence of cinclides, as well as a symbiotic habit, are convergent at broad taxonomic scales.

Chapter 4 is an assessment of genetic diversity of the most widespread species of sea anemone symbiotic with hermit crabs and an evaluation of the potential phylogenetic utility of the internal transcribed spacer region (ITS-1, 5.8S, and ITS-2) in sea anemones. I used ITS sequences from 36 specimens from five populations of *C. polypus* and two closely related species (*C. tricolor* and *C. parasitica*). I found variation at intragenomic, intraspecific and interspecific levels, but intragenomic variation does not overlap with intraspecific or interspecific variation, and does not obscure phylogenetic signal. This indicates that ITS is a potentially useful marker for analysis of closely related species. However, ITS sequences do not seem variable enough for studies below the species level. I found no geographic structure for populations of *C. polypus* in a parsimony or maximum likelihood analyses. In

addition, I found that internal transcribed spacers of more distantly related species are highly divergent and cannot be unambiguously aligned. Given the potential of ITS as a species-specific marker, the use of secondary structure to guide alignments is a solution to include more distantly related species of sea anemones in the same analysis.

Chapter 5 is the taxonomic monograph of *Adamsia*, *Calliactis,* and *Paracalliactis* and is the largest component of this project. The complexity of taxonomic issues and the overlapping definitions of taxa necessitated that the three genera be considered simultaneously. The monograph addresses important taxonomic tasks such as validation of names and provides a comprehensive perspective on diversity and variation within the group. The monograph and phylogenetic analyses emphasize characters conventionally used in the taxonomy of sea anemones: external and internal anatomy, microanatomy, and cnidae. Eighteen species of sea anemones symbiotic with hermit crabs in genera *Adamsia*, *Calliactis* and *Paracalliactis* are examined. One new species of *Calliactis* and one new species of *Paracalliactis* are described.

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Chapter 2: Evolution of sea anemones (Cnidaria: Actinaria: Hormathiidae) symbiotic with hermit crabs

Introduction

Approximately 100 species of cnidarians are reported to live on shells inhabited by hermit crabs (Williams and McDermott 2004). However, sea anemones in the lineage Acontiaria and family Hormathiidae, particularly of genera *Adamsia, Calliactis* and *Paracalliactis*, are the only cnidarian symbionts known to be actively positioned on these shells by the hermit crabs. This is in contrast to many other invertebrates, which become fixed randomly on shells as larvae transitioning from a pelagic to a benthic life (Balasch and Cuadras 1977), or which settle and survive preferentially on shells without active intervention by the crab.

In this association, one or more sea anemones live on a gastropod shell inhabited by a hermit crab. The sea anemone-crab partnership is a mutualism (Brooks and Gwaltney 1993; Mainardi and Rossi 1969) in which the sea anemone increases its dispersal capability (Balss 1924) and gains a suitable substrate (Nyblade 1966; Riemann-Zürneck 1994) in exchange for defending the crab from predators using its nematocysts (Ross 1971; Ross 1974a; Ross 1974b; Ross and Boletzky 1979; Christidis et al. 1997). Increased access to food captured by mobile, scavenging hermit crabs might be an additional benefit for the sea anemone (Ross 1960; Stachowitsch 1979, 1980; Chintiroglou and Koukoras 1991; Fautin 1992;

Chintiroglou et al.1996). Whether the hermit crab directly feeds its symbiotic sea anemone (e.g., Wortley 1863; Fox 1965) is controversial (Ross 1974a).

Defense of a hermit crab by its sea anemone is less controversial: a hermit crab is either partially or completely defended by its sea anemone (Ross 1971; Ross 1984; McLean and Mariscal 1973), with crabs bearing one or more symbiotic sea anemones more likely to survive encounters with predators (Brooks 1989). The presence of hermit crab predators, such as octopus, encourages active acquisition of sea anemones by hermit crabs in the laboratory (Balasch and Mental 1974; Bach and Herrnkind 1980; Ross and Boletzky 1979). The congruent distributions of the predator *Octopus vulgarism* and hermit crabs bearing the symbiotic sea anemone *Calliactis parasitica* in Europe further support the hypothesis that predation maintains activity of the crab in nature (Ross 1971) or, at least, reinforces the operational trigger of this association (Ross and Boletzky 1979).

Additional benefits of this symbiosis to the crab may include structural defense: in some associations, the sea anemone forms a living "cloak" around the shell inhabited by the hermit crab, reinforcing the shell and expanding the living space of the crab (Faurot 1910; Doumenc 1975; Ross 1984). Members of the genus *Adamsia* secrete a chitin replica of the shell under the living cloak, known as a carcinoecium, which allows the hermit crab to avoid changing shells as it grows (Dunn et al. 1980). A similar structure is produced by sea anemones of the genus *Stylobates* Dall, 1903 (Dunn et al. 1980; Fautin 1987); because *Stylobates* and *Adamsia* belong to distantly related lineages (e.g., Daly et al. 2008), the carcinoecium

is inferred to be the product of convergent evolution.

The association between sea anemones and hermit crabs is established through a variety of mechanisms that entail some species recognition and discrimination by the hermit crab (reviewed in Ross 1974b; Brooks and Mariscal 1986). In most cases, it is the crab that initiates the symbiosis, using tactile stimulation to detach a sea anemone from the substrate and then placing the sea anemone on its shell (Brunel 1910; Cowles 1919; Ross 1970). Predation pressure and balance affect the placement of sea anemones on shells by hermit crabs: a sea anemone is typically placed close to the aperture of the shell (Cutress and Ross 1969; Brooks 1988, 1991) and arranged in accordance with the center of gravity of the shells (Balasch et al. 1977). Less frequently, the sea anemone initiates the symbiosis, transferring itself to the shell upon perception of a molluscan substance in the shell inhabited by the hermit crab (Ross and Sutton 1961; Davenport et al. 1961; Ross 1959, 1965). This unaided settlement on shell is one of the most complex behaviors performed by a cnidarian (Ross 1974b) and known only in three species of *Calliactis* and one species of *Paracalliactis.* No species of *Adamsia* is known to exhibit such behavior. In most instances, a combination of hermit crab and sea anemone behavior is necessary for the establishment of the association, with the success of the crab partly depending on the sea anemone's cooperation (Cutress and Ross 1969; McFarlane 1969; Ross 1974b).

Sea anemones belonging to genera *Adamsia* (three valid species)*, Calliactis* (19 valid species) and *Paracalliactis* (nine valid species) form a complex whose

members are distinguished by a mosaic of features (Daly et al. 2004). Some of these features are summarized in Table 2.1. Members of *Adamsia* and *Calliactis* share morphological characters that include micro-anatomical characteristics and a smooth column with pores (cinclides) through which protrude threads of nematocyst-batteries called acontia. Acontia are important in the defense of the hermit crab. *Adamsia* and *Paracalliactis* are similar in morphological attributes associated with a symbiotic habit: both have a bi-lobed base that enwraps the shell completely and produce either a carcinoecium or a less developed, plate-like cuticle. *Adamsia* and *Paracalliactis* differ in column nematocysts and absence in cinclides: *Paracalliactis* has microbasic *p*-mastigophores in the column, has tubercles, and lacks cinclides. Mesentery arrangement alone suggests an affinity between *Paracalliactis* and *Calliactis*, which differ in column anatomy (*Calliactis* has cinclides, *Paracalliactis* lacks them), the morphology of the base (generally ovoid in *Calliactis*, bi-lobed in *Paracalliactis*), the production of a carcinoecium or a chitin plate (not reported for any species of *Calliactis*) and complement of cnidae (= condom).

The difficulty of interpreting these conflicting suites of morphological characters has resulted in problematic species identifications and descriptions and thus in frequent taxonomic reassignments (e.g., Duerden 1902; Hand 1975a; Daly et al. 2004). Because the distinction between *Adamsia*, *Calliactis,* and *Paracalliactis* is primarily based on characters related to a symbiotic habit, functional rather than phylogenetic explanations may be more relevant, and these characters may not be appropriate to delimit monophyletic groupings (Daly et al. 2004). Although some

have hypothesized that these anemones may not represent a single monophyletic group (e.g., Ross 1974a), monophyly of hermit-crab symbiotic sea anemones has been justified by the remarkable nature of the biological adaptation and complex behavior exhibited by the partners (Ates 1997a). This assumption, however, may be biased by the morphological characters related to the symbiosis. The occurrence of isolated associations involving anemones from different lineages (e.g., *Stylobates*, see Dunn et al. 1980; Fautin 1987) demonstrates that a symbiotic habit with hermit crabs has arisen at least twice. Furthermore, many hormathiids engage in interactions with gastropods or other sessile invertebrates (e.g., Ross and Kikuchi 1976; Riemann-Zürneck 1994; Ates 1997a, b; Mercier and Hamel 2008), suggesting that some elements of the behavior are shared much more widely across Hormathiidae.

The diversity of interactions across the hormathiid genera also raises critical questions about the emergence and evolution of the symbiosis. Competing hypotheses concerning the emergence of these associations include a "crab-driven" hypothesis and a "shell-response" hypothesis (Ross 1974a). The "crab-driven" hypothesis proposes that hermit crabs are the driving force in the partnership, picking up sea anemones and placing them on their shells, first as a means of camouflage, with defense evolving later (Ross 1974a). This behavior is perpetuated because it has a positive effect on fitness, providing both concealment and defense for the crab. This hypothesis is supported by studies showing that most partnerships between hermit crabs and sea anemones are initiated through activity of the crab, and by the more frequent (or exclusive) occurrence of sea anemones on shells occupied by hermit

crabs, even in areas where gastropods are also common. Ross (1974b) has also shown with laboratory experiments that only anemones symbiotic with hermit crabs respond tactile stimulation by hermit crabs. Hermit crabs also steal sea anemones from other crabs, illustrating the importance of the association to the crab (Mainardi and Rossi 1969; Ross 1979). In contrast, the shell-response hypothesis predicts that the shell mounting behavior led to evolution of the symbiosis. The association is hypothesized to have started with anemones transferring themselves to gastropod shells because the sea anemone shell-response is based on a shell factor that is stronger in a shell still associated with the gastropod that made it than in a discarded shell used by a hermit crab. Since the primary benefit for the anemone in this association is transport, this tendency was transferred to even more mobile hermit crabs. Presumably, once hermit crabs started to benefit from having anemones on their shells, they developed the behavior of picking up anemones. Some facts argue against this hypothesis, including the much smaller number of sea anemones living on gastropods or inactive crabs.

Only 12 species of sea anemones are known to associate with gastropods. From these, seven are hormathiids: three species are found exclusively on gastropods, *Allantactis parasitica*, *Hormathia digitata* and *Hormathianthus* sp. and four species, *Calliactis tricolor*, *Calliactis parasitica*, *Calliactis conchicola* and *Paracalliactis rosea*, associate with gastropods or hermit crabs (Ates 1997a). All of these gastropod symbionts exhibit shell-mounting behavior. Although he articulated both hypotheses, Ross (1974a) preferred the shell-response hypothesis, in which anemones first associated with gastropods, then with hermit crabs that later developed an active

behavior towards sea anemones.

Questions about monophyly of hormathiid sea anemones symbiotic with hermit crabs, and about the evolution of the symbiosis, are best addressed in the context of a phylogenetic tree for Hormathiidae. To address these questions, we collected and analyzed more than 5.4 kb of sequence data from three mitochondrial markers (12S, 16S and COIII) and two nuclear markers (18S and partial 28S) for a broad range of Hormathiidae and its presumed relatives, including representatives of all of the hermit crab symbiotic taxa in this lineage. This is the first phylogenetic tree of Acontiaria and the most extensive talon sampling study focusing on the family Hormathiidae.

Material and methods

Taxon sampling

Specimens were collected by hand, during SCUBA dives, or via trawls, depending on the depth and location. Thirty-seven samples corresponding to 34 in-group and three out-group species were used in this study. The in-group includes 33 aconite species, corresponding to nine out of 13 families in the superfamily (Daly et al. 2007). We included multiple representatives of Hormathiidae (15 species, 9/15 genera). Among these are six hermit crab-symbiotic species: one species of *Adamsia* (*A. palliata*), four species of *Calliactis* (*C. japonica, C. parasitica, C. polypus* – including one specimen from Hawaii and one from Japan - and *C. tricolor*), and one species of *Paracalliactis* (*Paracalliactis* sp.). We also included *Actinoscyphia plebeia*

(Actinoscyphiidae) and *Andvakia boninensis* (Andvakiidae), species belonging to the larger Acontiaria-Boloceroidaria-Mesomyaria clade of Daly et al. (2008). The outgroups *Nematostella vectensis*, *Bunodactis verrucosa* and *Isosicyonis striata* are drawn from outside this clade, and represent the other two major clades in the actiniarian suborder Nynantheae (Daly et al. 2008). One species included in this analysis is commonly found associated with gastropods: *All. parasitica*. All specimens were identified based on formalin vouchers (see Table 2.2) following standard taxonomic protocols that include morphological, microanatomical, and nematocyst studies (e.g., Daly and Gusmão 2007).

Molecular data collection and analysis

Genomic DNA was isolated from tentacles, pedal disc, or column with the DNAeasy Kit (Qiagen) using the modified rat tail protocol supplied by the manufacturer. PCR products for three mitochondrial (12S, 16S and COIII) and two nuclear markers (18S, partial 28S) were amplified on an Eppendorf Mastercycler using published primers and standard protocols (e.g., Geller and Walton 2001; Daly et al. 2008). Annealing temperatures ranged from 40-42oC. PCR products were cleaned using AmPure magnetic bead solution (AgenCourt) and rehydrated with sterile, autoclaved, double-distilled, de-ionized water. All PCR products were sequenced on an ABI 3730*xl* by staff at Genaissance (New Haven, CT) and Cogenics (Houston, TX). Contiguous sequences were assembled and edited using Sequencher v.4.7 (Gene Codes Corporation). Sequences were blasted against Embank to check

for successful amplification of target loci and taxa. All new sequences (110) were deposited in Embank; these were compared to 74 sequences drawn from Embank (Table 2.2). A total of 184 sequences were analyzed.

For each marker, assembled sequences were aligned using the default settings in Muscle 3.6 (Edgar 2004). All sequences for all markers were readily alienable and lacked alignment-ambiguous regions. Incongruence between individual data sets and between the mitochondrial and nuclear data sets was assessed using the Incongruence Length Difference Test (ILD, Farris et al. 1994; Farris et al. 1995). The appropriate model of nucleotide substitution for each gene was evaluated using Modeltest 3.7 (Posada and Crandall 1998); selection of models was based on the Akaike Information Criterion (AIC), which rewards models for good fit but penalizes them for unnecessary parameters (Posada and Buckley 2004). This combined alignment is archived in TreeBase (http://www.treebase.org/treebase/index.html), and was used for all phylogenetic inference.

TNT v.1.1 (Goloboff et al. 2008) was used for parsimony analyses of individual and combined data sets; searches implemented random and sartorial searches, tree drifting, and 10 rounds of tree fusing to find minimum length trees 10 times. Gaps were treated as missing data. To evaluate relative support for individual clades, searches were followed by 1000 jackknife replicates (36% probability removal, collapse clades with <50% support). To assess the level of homoplasy of separate and combined datasets, the consistency index (CI), retention index (RI) and rescaled consistency (RC) were calculated. To assess the information content of each

gene partition, nuclear, mitochondrial and combined datasets, the Data Decisiveness index (DD; Goloboff 1991) was calculated, using the formula: DD=(S*-S)/(S*-M), where S* is the average length of all trees, S is the length of the most parsimonious tree(s) and M is the minimum possible number of steps for the data set. The average length of all trees was estimated with PAUP version 4.0b10 (Sawford 2003) using 100,000 random trees.

Maximum likelihood analyses were performed in Ram 7.0.4 (Stamata is 2006), using 1000 replications. Model parameters were estimated by Ram. In the combined analysis, these parameters were estimated separately for each gene partition. Clade support was assessed with 1000 rounds of bootstrap re-sampling. Bayesian analyses were performed in Morays V3.1 (Huelsenbeck and Ronquist 2001) under the same model used for the maximum likelihood analyses. Two independent runs were started from random trees using one cold and seven heated chains for five million generations, each with trees sampled every 1000th generation. To increase the probability of chain convergence, we sampled trees after the standard deviation values of the two runs were < 0.01.

Hypothesis testing

The hypothesis of a single origin for the hermit crab symbiosis within Hormathiidae was tested using constrained parsimony, maximum likelihood and Bayesian analyses to examine support for suboptimal solutions. Under parsimony, monophyly of the symbiotic hormathiids was enforced in a replicate analysis, and

resulting trees containing the monophyletic group were retained for comparison. Differences in length were assessed for significance using the Wilcoxon signed-ranks tests (Templeton 1983) and winning-sites tests (Pager and Wilson 1988) as implemented in PAUP* version 4.0b10 (Sawford 2003). The Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999) was used to compare constrained and unconstrained maximum likelihood topologies. A third approach was used to evaluate this hypothesis in the Bayesian analysis: trees that included the symbiotic sea anemones as a monophyletic group were filtered from the pool of trees generated by the analyses (5000 trees from 5 million generations). The percentage of trees including the symbiotic sea anemones as monophyletic gives the posterior probability of this hypothesis.

Results

Sequencing, alignment and data set congruence

Sequences for five markers for a total of over 5.4 kb were successfully obtained for all 37 taxa. PCR products varied in length from 437 (16S) to 1935 (18S) bp. All positions were retained in the analysis. Sequence characteristics of the five gene partitions and nuclear, mitochondrial and combined analysis are summarized in Table 2.3.

The proportion of parsimony informative sites differed among data sets, with 18S being the least variable (6.5%) and 28S the most variable (26.8%). When combined, mitochondrial (14.5%) and nuclear (13.0%) markers showed roughly the

same percentage of parsimony informative sites. Of 5408 total characters, 732 were parsimony informative in the combined data set (13.5%). No significant differences in nucleotide frequencies were detected in any gene partition or between the nuclear and mitochondrial data sets. When all genes were combined, frequencies for the four nucleotides were equal (Table 2.3). The ILD test showed significant incongruence for some pair wise gene comparisons, but not for the comparison between mitochondrial and nuclear data sets (results not shown). Because combining data from different sources is more likely to accurately reflect evolutionary history (e.g., Eernisse and Kluge 1993), these two partitions were combined into a single dataset that was used for all analyses.

Parsimony analysis

Combined analysis of all five partitions resulted in a single most parsimonious tree of 5002 steps (Figure 2.1). Information for this tree and trees resulting from analysis of each individual partition (trees not shown) is given in Table 2.4. Individual gene partitions and the nuclear, mitochondrial, and combined datasets vary in amount of homoplasy, degree of resolution, and data decisiveness. Of the separate gene partitions, 16S shows the least homoplasy (CI=0.79; RI=0.86; RC=0.68) and is the most decisive (DD=0.92), even though it presents the greatest number of equally parsimonious trees (30). 28S shows the most homoplasy (CI=0.55; RI=0.68; RC=0.32) and is the least decisive (DD=0.55). When gene partitions are combined, homoplasy increases and the dataset becomes less decisive: the nuclear,

mitochondrial, and total combined datasets present much higher levels of homoplasy and are less decisive than their constituent data sets. Nonetheless, the phylogenetic signal is strong in the total combined analysis, resulting in a single most parsimonious tree with 17/35 nodes having jackknife support of more than 70%.

In this tree, the aconite species are divided into three clades: the basal-most clade corresponds to family Aiptasiidae; the second clade includes families Sagartiidae, Metridiidae, Diadumenidae, and Haliplanellidae; and the third clade corresponds to Kadosactidae, Nemanthidae, Hormathiidae, Andvakiidae, Actinoscyphiidae, Bathyphelliidae, and the sagartiid *Phellia gausapata*. Neither of the two most speciose aconite families, Sagartiidae and Hormathiidae, are monophyletic in this tree. Sagartiidae is paraphyletic with regard to Metridiidae, Haliplanellidae, and Diadumenidae in the parsimony analysis (Figure 2.1) and to these plus Aiptasiidae in the model-based analyses (Figure 2.2). Furthermore, in all analyses, the sagartiid *P. gausapata* clusters with members of Hormathiidae rather than with the other sagartiids. The placement of the nemanthid *Nemanthus nitidus,* the bathyphellid *Bathyphellia australis*, and the actinoscyphiid *Acy. plebeia* within Hormathiidae renders Hormathiidae paraphyletic. *Hormathia,* the type genus of Hormathiidae, is not monophyletic. A close affinity between Nemanthidae and Hormathiidae is also recovered.

None of the markers separately or in combination support a monophyletic origin for the hormathiid sea anemones symbiotic with hermit crabs. In all parsimony results, *Paracalliactis* shows more affinity to *Paraphelliactis* and *Chondrophellia*

than to *Calliactis* or *Adamsia*, grouping within a clade that also includes *Bathyphellia* and *Nemanthus*, but never with members of *Calliactis* or *Adamsia.* All of the analyses recover a paraphyletic *Calliactis* due to the inclusion of *Adamsia palliata* within the clade that includes the remaining species of *Calliactis*. In the combined analysis, the clade corresponding to *Calliactis* + *Adamsia* is well supported (80% jackknife support) with *C. japonica* as the sister to a clade that includes all remaining species of *Calliactis* and also *A. palliata*. *C. parasitica* and *A. palliata* are each others closest relative (100% jackknife support), and are sister to a clade including both specimens of *C. polypus* and *C. tricolor*. Individuals of *C. polypus* are not recovered as sister taxa. The larger clade that includes *Adamsia* and *Calliactis* also includes *Hormathia lacunifera* and *Hormathia armada*.

Maximum likelihood and Bayesian analyses

The preferred model for the different markers differs and is shown in Table 2.3. In general, the model-based topologies have greater support than the parsimony trees. The Bayesian tree has higher support compared to the parsimony and likelihood trees, with 26/35 nodes showing posterior probabilities of 0.70 or more.

Because the best trees from the maximum likelihood and Bayesian analyses are identical with regard to relationships within Hormathiidae and between this family and its closest relatives, only the best maximum likelihood tree is shown (Figure 2.2). For comparison, posterior probabilities are also indicated in the best maximum likelihood tree. These trees recover two major clades: one that corresponds to families Aiptasiidae, Sagartiidae, Metridiidae, Diadumenidae, Haliplanellidae and

Kadosactidae and another corresponding to Hormathiidae, Actinoscyphiidae, Andvakiidae, Nemanthidae, Bathyphelliidae, and the sagartiid *P. gausapata*. Results are broadly consistent with topologies recovered in the parsimony analysis, but the position of Aiptasiidae and of *Kadosactis antarctica* is not consistent across phylogenetic methods. As is the case for parsimony analysis, the model-based topologies do not recover a monophyletic Sagartiidae or Hormathiidae.

In the Bayesian and maximum likelihood analyses, sea anemones symbiotic with hermit crabs never form a single monophyletic group. As in the parsimony analysis, *Calliactis* and *A. palliata* form a monophyletic group. *Paracalliactis* sp., however, is never included in this clade, grouping instead with *Paraphelliactis* sp. (0.9 posterior probability) in a larger clade that also includes *Chondrophellia*, *Bathyphellia* and *Nemanthus.* The affinity between *A. palliata* and *C. parasitica* (1.0 posterior probability; 100 bootstrap) and between *C. polypus* and *C. tricolor* (0.93 posterior probability) is also apparent in these analyses.

Hypothesis Testing and Character Optimization

Three trees of 5047 steps were produced in the parsimony analysis under the constraint of monophyly for the hermit crab symbionts (*Adamsia* + *Calliactis* + *Paracalliactis*). These constraint trees were significantly different from the shortest tree found without the constraint of monophyly, based on the non parametric Templeton test (Wilcoxon signed-ranks; p=0.0001) and winning-sites tests (p=0.0001). The Shimodaira-Hasegawa test rejected the constrained maximum

likelihood topology with a p-value of 0.0253. None of the filtered trees from the Bayesian analysis included a monophyletic group of symbiotic hormathiids, conferring a posterior probability of zero on this hypothesis. Thus, alternative suboptimal topologies suggesting a monophyletic origin for the group of sea anemones symbiotic with hermit crabs are statistically rejected.

The carcinoecium optimizes unambiguously and identically on the parsimony (Figure 2.1) and likelihood trees. For ease of evaluation, it is shown only on the parsimony tree; the shorter internal nodes of the likelihood tree, and the dual support values depicted on those nodes complicate the depiction of additional information. In both cases, the carcinoecium is interpreted as a convergent feature, evolving separately in *Paracalliactis* and *Adamsia*. This structure is interpreted as evolving after the establishment of the symbiosis in the case of *Adamsia*, and is coincident with the establishment of the symbiosis in *Paracalliactis*.

Discussion

The origin and evolution of the hermit crab symbiosis in Hormathiidae

The pattern of relationships within Hormathiidae suggests two independent origins for the symbiosis between sea anemones and hermit crabs. Because *Adamsia* and *Calliactis* are each others' closest relatives but are not closely related to *Paracalliactis*, our results do not support monophyly of the hermit crab symbionts within Hormathiidae. Our confidence in these results is further bolstered by the constraint analysis, which did not recover monophyly of all three symbiotic genera. A

close evolutionary relationship among sea anemones symbiotic with hermit crabs has been hypothesized based on morphological (e.g., Duerden 1902; Cutress and Ross 1969; Hand 1975a; Daly et al. 2004) and behavioral studies (Ross 1974b; Hand 1975b; Ates 1997a). Similarities in the morphology of these sea anemones have led to considerations of some genera of symbiotic hormathiids as a single species complex (e.g., *Paracalliactis* and *Calliactis* by Hand 1975a; *Calliactis* and *Adamsia* by Duerden 1902). The capacity to respond to tactile stimulus from hermit crabs, for example, leads to relaxation and detachment only in anemones that live in association with crabs (Ross 1974b). Furthermore, the shell mounting behavior is also largely confined to sea anemones living in symbiosis with either gastropods or hermit crabs (McFarlane 1969). In light of our results, however, similarities in morphology and behavior among some genera in the group have to be interpreted as related to the ways in which a symbiotic habit arises and is maintained, and not due to shared evolutionary history.

Our results support Ross' hypothesis (1974a) that anemone-crab associations evolved independently on a number of occasions within Actiniaria. Difficulties arise when trying to translate evolutionary considerations from Ross' studies, given the largely non-phylogenetic nature of his work. For example, in his hypotheses about the evolution of this symbiosis, Ross (1974a) does not specify whether each symbiotic hormathiid genus evolved independently or if some of them are more closely related to each other. We interpret Ross' hypothesis (1974a) as stating that all three symbiotic genera evolved independently of each other. A sister group relationship

between *Adamsia* and *Calliactis* was explicitly denied by Ross (1974a). We reject Ross' hypothesis, because parsimony and model-based analyses find *Adamsia* nested within *Calliactis* (Figures 2.1, 2.2), arguing for a common origin of the symbiosis for the members of these genera.

Acontiarians, particularly *Adamsia* and *Paracalliactis*, seem more inclined to produce chitin than anemones in other lineages (Dunn and Lieberman 1983). The production of a chitinous carcinoecium is a complex anatomical and biological feature that could be interpreted as a sign of a shared evolutionary history of *Paracalliactis* and *Adamsia*. Production of large amounts of chitin was first described in endomyarian sea anemones in *Stylobates* (Dunn and Lieberman 1983). Our results support Dunn and Liberman's (1983) hypothesis that although ancient, the biochemical pathways for the production of chitin are rarely entirely lost in any single lineage, and are retained in isolated species of anemones. All the chemical cues ("shell factors") involved in the shell mounting behavior and association with hermit crab might stimulate the synthesis of chitin, activating the pathway with cues not commonly experienced by other sea anemones.

In his discussion of the possibility of multiple independent origins for the symbiosis between sea anemones and hermit crabs, Ross (1974b) indicated that *Calliactis* is particularly crucial to understanding the evolutionary history of the symbiosis: it is the most widespread and diverse genus of symbiotic sea anemones, and it includes most of the commonly found symbiotic anemones. The genus currently comprises 19 valid species (Fautin 2009) and includes species that live in

symbiosis with hermit crabs and species whose members may live independently of a hermit crab, at least in some part of their range. The behavior involved in the establishment of the symbiosis differs slightly across the genus, with some species relaying more on the activity of the crab and others on the response to shells or a mixture of these two behaviors. Our results do not support monophyly of *Calliactis*, due to the inclusion of *A. palliata* within *Calliactis* as the sister of *C. parasitica*. These two species occur in sympatry in the Northern Atlantic and live in association with different species of crabs in the genera *Pagurus* (Family Paguridae) and *Dardanus* (Family Diogenidae). Of these species, *A. palliata* is more conspicuous and obligate in its association, a fact reflected in its bi-lobed pedal disc and secretion of carcinoecium. The extreme morphology of *A. palliata* might be related to the crab symbiont and the differences in the symbiosis it engages in, compared to its sister *C. parasitica*: *Pagurus prideauxi* exhibits active behavior towards *A. palliata*, whereas crabs that associate with *C. parasitica* are not active, at least in some parts of the anemone's distribution.

Ross (1974a) hypothesized that the Caribbean and Indo-Pacific symbiotic *Calliactis* have lost elements of the ancestral shell response. Although we support Ross's hypothesis of a close relationship between the Caribbean *C. tricolor* and the Indo-Pacific *C. polypus*, the Japanese representative of *C. polypus* is more closely related to *C. parasitica* and *A. palliata* than to the Hawaiian specimen of *C. polypus* and the Caribbean *C. tricolor*. The polarity implied by Ross's hypothesis is refuted by our results. Among the sampled species, only *C. parasitica* has a strong shell

response; *C. tricolor* has a weak response, and *C. polypus* and *A. palliata* have no response. We also do not support the hypothesis of Brooks et al. (1995) that *C. polypus* is the most "advanced" species of *Calliactis* as evidenced by the lack of a shell response and to a greater dependence on active crabs. The two specimens of *C. polypus* nest within the *Calliactis* clade, but are not crown members. Furthermore, the two specimens of *C. polypus* collected in Japan and Hawaii are not each other's closest relatives: *C. polypus* from Hawaii is sister to the Caribbean *C. tricolor.* The widespread *C. polypus* contains many junior synonyms described from more restricted locales (reviewed in England 1971); an extensive revision of *C. polypus* is necessary to establish boundaries between this species and other species of *Calliactis*.

Although similarities in shell-mounting behavior have been proposed as evidence of shared ancestry between sea anemones symbiotic with hermit crabs and sea anemones symbiotic with gastropods (Ates 1997b), our results do not support a single origin for a gastropod and hermit crab symbioses within Hormathiidae. Although *All. parasitica*, a gastropod symbiont found exclusively in the Atlantic, is in the same clade as *Calliactis* and *Adamsia*, this clade also includes species of *Hormathia* (*H. lacunifera* and *H. armada*) that do not exhibit shell-mounting behavior and are never found in association with gastropods or hermit crabs. Because the position of *All. parasitica* relative to the *Calliactis* + *A. palliata* clade is only weakly supported, we conducted an additional constraint analysis to evaluate if a tree that includes *All. parasitica* as the sister group to *Calliactis* + *A. palliata* is significantly different than the most parsimonious trees. Trees grouping *All.*

parasitica with the clade of hermit crab symbionts are not significantly different from the best trees, and thus we cannot reject the hypothesis that symbiosis with gastropods is associated with the evolution of the symbiosis with hermit crabs.

A close affinity between *Adamsia* and *Calliactis* has also been proposed on morphological grounds (Duerden 1902; Daly et al. 2004), and the history of species reassignments exemplifies the difficulties involved in the identification and description of species in these two genera. Various characters have been given greater or lesser importance in the differentiation of these genera, including posture on the shell and shape of the pedal disc (Verrill 1869), and number of perfect sterile mesenteries (Carlgren 1928a, 1949). Our phylogenetic analyses provide insight into this situation through two avenues. First, the difficulty of differentiating and defining these genera is exemplified by our finding of a close relationship between the type species of *Adamsia* and *Calliactis* and the lack of monophyly of *Calliactis*. Second, our results provide a context for evaluating characters that have been traditionally used for delimiting taxa in this group. Based on our phylogenetic trees, characters related to a symbiotic habit, such as the presence of a bi-lobed (wrapping) base and the production of a carcinoecium, should not be used to differentiate genera as they do not define monophyletic groups. Similarly, the affinity of *Adamsia* and *Calliactis* demonstrates that other anatomical and micro-anatomical characters, including the occurrence of cinclides, carry phylogenetic signal and should have a greater role in taxonomy.

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Feature	***Adamsia***	***Calliactis***	***Paracalliactis***
Base	Bi-lobed	Circular /Ovoid	Bi-lobed
Column	Smooth	Smooth	Smooth or with tubercles
Cinclides	Present	Present	Absent
Cnidae of the column	Basitrichs	Basitrichs and microbasic *p*mastigophores	Basitrichs
Carcinoecium	Present	Absent	Present
Pairs of perfect sterile mesenteries	6 or 12 pairs	6 pairss	6 pairs

Table 2.1. Taxonomically important anatomical and micro-anatomical characters of *Adamsia*, *Calliactis* and *Paracalliactis*.

Table 2.2. List of species included in this study with voucher location and Genbank accession numbers. Samples are organized alphabetically within their current family. New sequences indicated in bold. Museum Acronyms: KUNHM, University of Kansas Natural History Museum; BEIM, Collection of Biodiversidad y Ecología de Invertebrados Marinos, University of Seville; AMNH, American Museum of Natural History; ZSM, Bavarian State Collection of Zoology; CMHN, Natural History Museum and Institute, Chiba.

Table 2.2

Family	Species	Locality	Voucher	12S	16S	18S	28S	COIII
Actiniidae	*Bunodactis verrucosa*	Northern Ireland	KUNHM	EU190723	EU190766	EU190854	EU190812	**FJ489484**
	Isosicyonis striata	Antarctica	BEIM	EU190736	EU190781	EU190864	**FJ489463**	**FJ489493**
Actinoscyphiidae	*Actinoscyphia plebeia*	Antarctica	BEIM	EU190712	EU190754	**FJ489437**	EU190800	**FJ489476**
Aiptasiidae	*Aiptasia mutabilis*	Ireland	KUNHM	**FJ489408**	**FJ489418**	**FJ489438**	**FJ489469**	**FJ489505**
	Aiptasia pulchella	Japan	KUNHM	EU190715	EU190757	EU190846	EU190803	**FJ489477**
	Bartholomea annulata	Aquarium trade	KUNHM	EU190721	EU190763	EU190851	EU190809	**FJ489483**
Andvakiidae	*Andvakia boninensis*	Saipan	KUNHM	EU190717	EU190759	EU190848	EU190805	**FJ489479**
Bathyphelliidae	*Bathyphellia australis*	Antarctica	KUNHM	**FJ489402**	**FJ489422**	EF589063	EF589086	**FJ489482**
Diadumenidae	*Diadumene cincta*	England	KUNHM	EU190725	EU190769	EU190856	EU190814	**FJ489490**
Edwardsiidae	*Nematostella vectensis*	Maryland, USA	AMNH	EU190750	AY16930	AF254382	EU190838	**FJ489501**
Haliplanellidae	*Haliplanella lineata*	Massachusetts, USA	KUNHM	EU190730	EU190774	EU190860	EU190819	**FJ489506**

Hormathiidae	*Actinauge richardi*	Ireland	KUNHM	EU190719	EU190761	EU190850	EU190807	**FJ489480**
	Adamsia palliata	Ireland	KUNHM	**FJ489398**	**FJ489419**	**FJ489436**	**FJ489452**	**FJ489474**
	Allantactis parasitica	North Atlantic	KUNHM	**FJ489399**	**FJ489420**	**FJ489439**	**FJ489454**	**FJ489478**
	Amphianthus sp.	Lau	KUNHM	**FJ489413**	**FJ489432**	**FJ489450**	**FJ489467**	**FJ489502**
	Calliactis japonica	Japan	KUNHM	**FJ489403**	**FJ489423**	**FJ489441**	**FJ489456**	**FJ489486**
	Calliactis parasitica	France	KUNHM	EU190711	EU190752	EU190842	EU190799	**FJ489475**
	Calliactis polypus	Hawaii	KUNHM	**FJ489407**	**FJ489427**	**FJ489445**	**FJ489459**	**FJ489485**
	Calliactis polypus	Japan	KUNHM	**FJ489404**	**FJ489424**	**FJ489442**	**FJ489457**	**FJ489487**
	Calliactis tricolor	Gulf of Mexico	KUNHM	**FJ489405**	**FJ489425**	**FJ489443**	**FJ489458**	**FJ489488**
	Chondrophellia sp.	Gulf of Mexico	KUNHM	**FJ489406**	**FJ489426**	**FJ489444**	----------	**FJ489489**
	Hormathia armata	Antarctica	BEIM	EU190731	EU190775	EU190861	**FJ489460**	**FJ489491**
	Hormathia lacunifera	Antarctica	BEIM	**FJ489409**	**FJ489428**	**FJ489446**	**FJ489461**	**FJ489492**
	Hormathia pectinata	Chile	ZSM	**FJ489415**	**FJ489430**	**FJ489448**	**FJ489465**	**FJ489497**
	Paracalliactis sp.	Japan	CMHN	**FJ489411**	**FJ489429**	**FJ489447**	**FJ489464**	**FJ489496**
	Paraphelliactis sp.	North	KUNHM	**FJ489412**	**FJ489431**	**FJ489449**	**FJ489466**	**FJ489498**

		Pacific						
Kadosactidae	*Kadosactis antarctica*	Antarctica	BEIM	**FJ489410**	EU190782	EU190865	EU190825	**FJ489504**
Metridiidae	*Metridium senile*	Washington, USA	KUNHM	EU190740	EU190786	AF052889	EU190829	**FJ489494**
Nemanthidae	*Nemanthus nitidus*	Japan	KUNHM	EU190741	EU190787	EU190868	EU190830	**FJ489495**
Sagartiidae	*Actinothoe sphyrodeta*	Ireland	ZSM	**FJ489401**	**FJ489421**	**FJ489440**	**FJ489455**	**FJ489481**
	Anthothoe chilensis	Chile	ZSM	**FJ489397**	**FJ489416**	**FJ489434**	**FJ489453**	**FJ489470**
	Cereus pedunculatus	Chile	KUNHM	EU190724	EU190767	EU190855	EU190813	**FJ489471**
	Phellia gausapata	Chile	ZSM	EU190744	EU190790	EU190870	EU190833	**FJ489473**
	Sagartia troglodytes	Ireland	KUNHM	EU190746	EU190792	EU190872	EU190834	**FJ489499**
	Sagartiogeton laceratus	Ireland	KUNHM	EU190748	EU190794	EU190874	EU190836	**FJ489500**
	Sagartiogeton undatus	France	KUNHM	**FJ489400**	**FJ489417**	**FJ489435**	**FJ489462**	**FJ489472**
	Verrillactis paguri	Hawaii	KUNHM	**FJ489414**	**FJ489433**	**FJ489440**	**FJ489468**	**FJ489503**

Summary statistics	Length of PCR products	# Chars.	# PI sites (%)	Empirical base frequencies (%)				Best-fitting model	Gamma shape parameter	Prop. invariant sites
				G	A	T	C			
12S	769 -889	805	91 (11.3)	26.0	31.5	25.5	17.0	GTR+G+I	0.6919	0.4682
16S	437-494	471	46 (9.8)	20.9	29.1	29.3	20.7	TVM+G	0.3966	0
18S	1714-1935	2223	144 (6.5)	25.0	25.0	25.0	25.0	SYM+G+I	0.2400	0.3000
28S (partial)	770-990	1172	306 (26.8)	29.6	20.7	20.3	29.4	GTR+G+I	0.4033	0.1576
COIII	468-723	737	145 (19.7)	20.2	24.8	35.9	19.1	GTR+G	0.7930	0
Mt DNA	-	1949	282 (14.5)	23.6	28.7	29.7	18.0	GTR+G+I	0.6474	0.3504
Nu DNA	-	3459	450 (13.0)	23.7	28.7	29.7	17.9	GTR+G	0.2959	0
All data	-	5408	732 (13.5)	25.0	25.0	25.0	25.0	GTR+G+I	0.4441	0.1621

Table 2.3. Summary of sequence information for separate gene partitions and combined analysis of data sets. PI = Parsimony informative, Chars = characters.

Partition	# MPT	L	CI	RI	RC	Resolved nodes in consensus	DD
12S	1	257	0.74	0.84	0.62	35/35	0.83
16S	30	114	0.79	0.86	0.68	14/35	0.92
18S	13	1192	0.83	0.60	0.50	24/35	0.61
28S (partial)	9	1610	0.55	0.68	0.32	19/34	0.55
COIII	20	1276	0.64	0.63	0.40	19/35	0.58
Mt DNA	32	1887	0.59	0.57	0.33	23/35	0.58
Nu DNA	20	2908	0.66	0.56	0.37	21/35	0.49
All data	1	5002	0.60	0.50	0.30	35/35	0.43

Table 2.4. Tree information for parsimony analyses of separate and combined partitions. MPT = most parsimonious trees; L = length of trees; CI = consistency index; RI = retention index; RC = rescaled consistency index; Resolved nodes in consensus = number of nodes in the strict consensus of all MPT / total number of nodes; DD = data decisiveness.

Figure 2.1. Single most parsimonious tree from a combined analysis of sequences from 12S, 16S, 18S, 28S (partial) ribosomal genes and Cytochrome Oxidase subunit III. Only jackknife values higher than 70% are shown above branches. Grey rectangles indicate species of Hormathiidae symbiotic with hermit crabs (HC symbionts) and gastropods (GA symbiont). Closed circles indicate the clades whose members produce a carcinoecium.

Figure 2.1

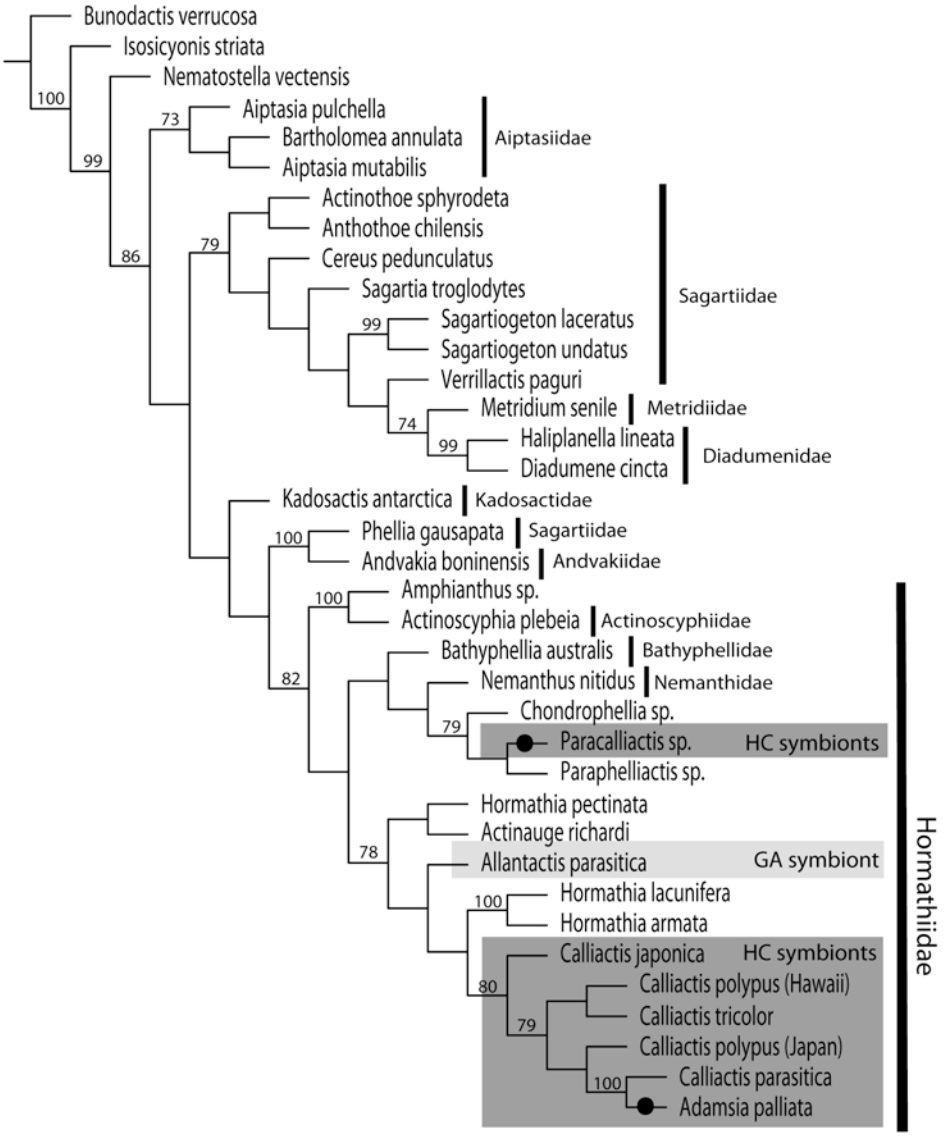
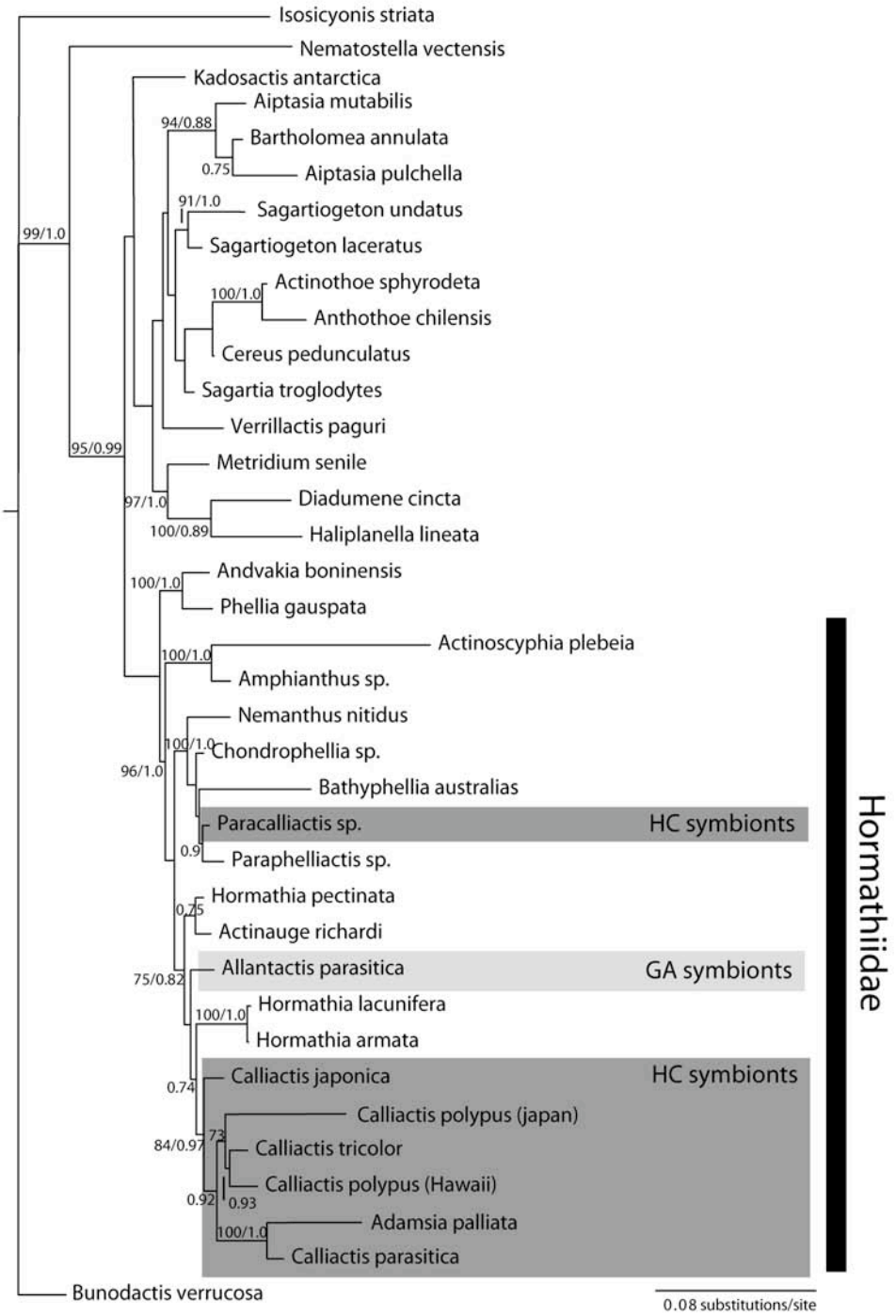

Bunodactis verrucosa
Isosicyonis striata
Nematostella vectensis
Aiptasia pulchella
Bartholomea annulata
Aiptasia mutabilis
Aiptasiidae
Actinothoe sphyrodeta
Anthothoe chilensis
Cereus pedunculatus
Sagartia troglodytes
Sagartiogeton laceratus
Sagartiogeton undatus
Verrillactis paguri
Sagartiidae
Metridium senile
Metridiidae
Haliplanella lineata
Diadumene cincta
Diadumenidae
Kadosactis antarctica
Kadosactidae
Phellia gausapata
Sagartiidae
Andvakia boninensis
Andvakiidae
Amphianthus sp.
Actinoscyphia plebeia
Actinoscyphiidae
Bathyphellia australis
Bathyphellidae
Nemanthus nitidus
Nemanthidae
Chondrophellia sp.
Paracalliactis sp.
HC symbionts
Paraphelliactis sp.
Hormathia pectinata
Actinauge richardi
Allantactis parasitica
GA symbiont
Hormathia lacunifera
Hormathia armata
Calliactis japonica
HC symbionts
Calliactis polypus (Hawaii)
Calliactis tricolor
Calliactis polypus (Japan)
Calliactis parasitica
Adamsia palliata
Hormathiidae

Figure 2.2. Maximum likelihood tree resulting from a combined analysis of Sequences from 12S, 16S, 18S, 28S (partial) ribosomal genes and Cytochrome Oxidase subunit III. Numbers on branches are bootstrapvalues from maximum likelihood analyses and posterior probabilities from Bayesian analyses, respectively. Only bootstrap values higher than 70% and posterior probabilities greater than 0.70 are shown. Grey rectangles indicate species of Hormathiidae symbiotic with hermit crabs (HC symbionts) and gastropods (GAsymbionts).

Figure 2.2.


Isosicyonis striata
Nematostella vectensis
Kadosactis antarctica
Aiptasia mutabilis
94/0.88
Bartholomea annulata
0.75
Aiptasia pulchella
91/1.0
Sagartiogeton undatus
99/1.0
Sagartiogeton laceratus
Actinothoe sphyrodeta
100/1.0
Anthothoe chilensis
Cereus pedunculatus
Sagartia troglodytes
Verrillactis paguri
95/0.99
Metridium senile
97/1.0
Diadumene cincta
100/0.89
Haliplanella lineata
Andvakia boninensis
100/1.0
Phellia gauspata
Actinoscyphia plebeia
100/1.0
Amphianthus sp.
Nemanthus nitidus
100/1.0
Chondrophellia sp.
96/1.0
Bathyphellia australias
Paracalliactis sp.
HC symbionts
0.9
Paraphelliactis sp.
Hormathia pectinata
0.75
Actinauge richardi
Allantactis parasitica
GA symbionts
75/0.82
Hormathia lacunifera
100/1.0
Hormathia armata
Calliactis japonica
HC symbionts
0.74
Calliactis polypus (japan)
73
84/0.97
Calliactis tricolor
Calliactis polypus (Hawaii)
0.92
0.93
Adamsia palliata
100/1.0
Calliactis parasitica
Bunodactis verrucosa
Hormathiidae
0.08 substitutions/site

Chapter 3: Phylogeny of Hormathiidae

Introduction

Family Hormathiidae belongs to Acontiaria, a superfamily that includes species that possess a distinct base, a mesogleal sphincter (in most cases), and thread-like extensions of the mesenterial filaments packed with nematocysts called acontia (Carlgren 1925). The type of nematocyst in the acontia is partly how families in Acontiaria are defined (Carlgren 1949). In family Hormathiidae the acontia have only basitrichs. This group is also distinguished by the morphology of the mesenteries, which are monomophic, few in number, with usually only 6 pairs of perfect mesenteries that are rarely fertile. Additionally, members of Hormathiidae are characterized by spirocysts that are large and broad.

Hormathiidae is the third largest family of sea anemones and the most diverse and species-rich family of Acontaria. With fifteen valid genera and approximatelly 110 described species (Carlgren 1949; Daly et. al, 2007; Fautin 2010), Hormathiidae is comprised of four genera that have more than 15 described species, six genera that have 2-8 species, and five genera that are monotypic (Fautin 2010). Characters that distinguish genera within Hormathiidae, include, the number of perfect mesenteries, the fertiliy, the direction of growth of the mesenteries, the general morphology of the column, the specializations of the column specializations, the distribution of

cinclides, and the musculature of the tentacles (Carlgren 1945). In addition to morphological characters, features that may be the result of interactions with the environment, such as the morphology of the pedal disc (cup-like vs. not cup-like) and the secretion of a carcinoecium also define genera within the family. Because most Hormathiidae genera are defined by a mosaic of these features, identification is often difficult.

Hormathiidae is distributed world-wide and is represented in many environments, from the poles to the tropics (Fautin 2010). Its bathymetric distribution is also broad, ranging from the intertidal to the deep sea, being especially dominant at the deep sea up to 4000 meters (Fautin and Barber 1999). As might be expected given this distribution, hormathiids exhibit a variety of morphological specializations and have diverse ecological and physiological attributes. Adult specimens in this group range in size from 1 to 20 cm, and may have column and specializations. Species that are found in the deep sea often have very thick mesoglea that may help support the body in the high pressure environments and also exhibit a tendency to attach to organic or artificial substrates that are very scarce in the deep sea (Manuel 1981). This tendency is carried to an extreme in members of the genera *Calliactis*, *Adamsia*, and *Paracalliactis*, whose members are commonly found in symbiosis with hermit crabs (e.g., Doumenc 1975; Ross 1959; Ross 1970; Ross 1984; Daly et. al. 2004; reviewed in Ross 1974) and in *Allantactis* (Mercier and Hamel 2008) and *Hormathia,* whose members can be found in symbiotic relationships with gastropods (Riemann-Zürneck 1994).

Despite its wide morphological variation and distribution, Hormathiidae is one of the best defined and most homogenous families within Acontiaria (Gusmão and Daly 2010). Due to the combination of a mesogleal sphincter and acontia with only basitrichs, a hormathiid is usually easily identified and disinguished from most other acontiarians (LCG pers. obs). Morphological homogeneity, however, may not necessarily mean that members of the family form a monophyletic group, as some of the characters that define the family may not be synapomorphic (Gusmao and Daly 2010). Members of the family Bathyphellidae, for example, also have a mesogleal sphincter and only basitrichs in the acontia. Bathyphellids, however, possess dimorphic mesenteries divisible into macrocnemes and microcnemes (Carlgren 1949). As is the case of most families of sea anemones that have never been subject of a thorough phylogenetic analysis (Daly et. al. 2007), relationships within Hormathiidae and between this family and other acontiarians have only been recently explored. In elucidating relationships among major groups, Daly et. al. (2008) found that the hormathiids were in a larger clade that included not only acontiarians, but also members of different superfamilies. More importantly, the three exemplar species, corresponding to three genera of Hormathiidae, clustered together in a monophyletic group with relatively high support. Due to the inclusion of only three species (out of 110), this study cannot be considered a strong test of monophyly for Hormathiidae and does not elucidate the relationships of genera within this family and between this family and other acontiarians.

Gusmão and Daly (2010) presented the most complete phylogeny of Acontiaria. This phylogeny included 35 acontiates and 15 species of Hormathiidae, but their focus was not the relationships within the superfamily Acontiaria or family Hormathiidae, but the placement and origin of a group of hormathiids symbiotic with hermit crabs (genera *Calliactis*, *Adamsia* and *Paracalliactis*). For this reason, although Gusmao and Daly (2010) found that neither Acontiaria nor Hormathiidae are monophyletic, due to the focus of their study, a more general discussion of these findings or the relationships among taxa in their analyses were not indicated and discussion was omitted.

Here, I use the results published by Gusmao and Daly (2010) to further the discussion of the monophyly of Hormathiidae and the relationships within it. I use these results to explore the importance of characters that are traditionally used to distinguish genera in this group.

Material and Methods

Taxon sampling

Specimens were collected by hand, during SCUBA dives, or via trawls, depending on the depth and location. Thirty-seven samples corresponding to 34 ingroup and three outgroup species were used in this study. The ingroup includes 33 acontiate species, corresponding to nine out of 13 families in the superfamily (Daly et al. 2007). We included multiple representatives of Hormathiidae (15 species, 9/15 genera). We also included *Actinoscyphia plebeia* (Actinoscyphiidae) and *Andvakia boninensis*

(Andvakiidae), species belonging to the larger Acontiaria-Boloceroidaria-Mesomyaria clade of Daly et al. (2008). The outgroups *Nematostella vectensis*, *Bunodactis verrucosa*, and *Isosicyonis striata* are drawn from outside this clade, and represent the other two major clades in the actiniarian suborder Nynantheae (Daly et al. 2008). One species included in this analysis is commonly found associated with gastropods: *All. parasitica*. All specimens were identified based on formalin vouchers (see Table 2.2) following standard taxonomic protocols that include morphological, microanatomical, and nematocyst studies (e.g., Daly and Gusmão 2007).

Molecular data collection and analysis

Genomic DNA was isolated from tentacles, pedal disc, or column with the DNAeasy Kit (Qiagen) using the modified rat tail protocol supplied by the manufacturer. PCR products for three mitochondrial (12S, 16S and COIII) and two nuclear markers (18S, partial 28S) were amplified using published primers and standard protocols (e.g., Geller and Walton 2001; Daly et al. 2008). All PCR products were sequenced on an ABI 3730xl by staff at Genaissance (New Haven, CT) and Cogenics (Houston, TX). Contiguous sequences were assembled and edited using Sequencher v.4.9 (Gene Codes Corporation). Sequences were blasted against GenBank to check for contamination and all new sequences (110) were deposited in GenBank; these were compared to 74 sequences drawn from GenBank (Table 2.1). A total of 109 sequences were analyzed.

For each marker, assembled sequences were aligned using the default settings in Muscle 3.6 (Edgar 2004). All sequences were unambiguously aligned. Incongruence between individual data sets and between the mitochondrial and nuclear data sets was assessed using the Incongruence Length Difference Test (ILD, Farris et al. 1994, 1995). Selection of models was based on the Akaike Information Criterion (AIC), which rewards models for good fit but penalizes them for unnecessary parameters (Posada and Buckley 2004), using the Modeltest 3.7 (Posada and Crandall 1998). A combined alignment can be found in Dryad (http://www.datadryad.org/), and was used for all phylogenetic inference. TNT v.1.1 (Goloboff et al. 2008) was used for parsimony analyses of individual and combined data sets; searches implemented random and sectorial searches, tree drifting, and 10 rounds of tree fusing to find minimum length trees 10 times. Gaps were treated as missing data. Trees of minimum length were found at least ten times. Support for clades was estimated using 10,000 rounds of jackknife re-sampling. To assess the level of homoplasy of separate and combined datasets, the consistency index (CI), retention index (RI) and rescaled consistency (RC) were calculated. To assess the information content of each gene partition, nuclear, mitochondrial and combined datasets, the Data Decisiveness index (DD; Goloboff 1991) was calculated, using the formula: DD = (S* S)/(S* M), where S* is the average length of all trees, S is the length of the most parsimonious tree(s) and M is the minimum possible number of steps for the data set.

One thousand rounds of likelihood bootstrapping were conducted using RAxML HPC-2 (Stamatakis 2006). Model parameters were estimated by RAxML. In

the combined analysis, these parameters were estimated separately for each gene partition. Clade support was assessed with 1000 rounds of bootstrap re-sampling. Consensus trees were visualized in Treeview X (Page 1996).

Hypothesis testing

The hypothesis of a single origin for the family Hormathiidae was tested using constrained parsimony, maximum likelihood and Bayesian analyses to examine support for suboptimal solutions. Under parsimony, monophyly of the symbiotic hormathiids was enforced in a replicate analysis, and resulting trees containing the monophyletic group were retained for comparison. Differences in length were assessed for significance using the Wilcoxon signed-ranks tests (Templeton 1983) and winning-sites tests (Prager and Wilson 1988) as implemented in PAUP* version 4.0b10 (Swofford 2003). The Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999) was used to compare constrained and unconstrained maximum likelihood topologies. A third approach was used to evaluate this hypothesis in the Bayesian analysis: trees that included the family Hormathiidae as a monophyletic group were filtered from the pool of trees generated by the analyses (5000 trees from five million generations). The percentage of trees including the symbiotic sea anemones as monophyletic gives the posterior probability of this hypothesis.

Results

Dataset properties

Sequences for five markers for a total of over 5.4 kb were successfully obtained for all 37 taxa. PCR products varied in length from 437 (16S) to 1935 (18S) bp. All positions were retained in the analysis. Sequence characteristics of the five gene partitions and nuclear, mitochondrial and combined analysis are summarized in Table 2.3.

The proportion of parsimony informative sites differed among data sets, with 18S being the least variable (6.5%) and 28S the most variable (26.8%). When combined, mitochondrial (14.5%) and nuclear (13.0%) markers showed roughly the same percentage of parsimony informative sites. Of 5408 total characters, 732 were parsimony informative in the combined data set (13.5%). No significant differences in nucleotide frequencies were detected in any gene partition or between the nuclear and mitochondrial data sets. When all genes were combined, frequencies for the four nucleotides were comparable (Table 2.3). The ILD test showed significant incongruence for some pairwise gene comparisons, but not for the comparison between mitochondrial and nuclear data sets (results not shown). Because combining data from different sources is more likely to accurately reflect evolutionary history (e.g., Eernisse and Kluge 1993), these two partitions were combined into a single dataset that was used for all analyses.

Phylogenetic relationships within Acontiaria

Combined analysis of all five partitions resulted in a single most parsimonious tree of 5002 steps (Figure 2.1). Information for this tree and trees resulting from analysis of each individual partition (trees not shown) is given in Table 4. Individual gene partitions and the nuclear, mitochondrial, and combined datasets vary in amount of homoplasy, degree of resolution, and data decisiveness. Of the separate gene partitions, 16S shows the least homoplasy (CI=0.79; RI=0.86; RC=0.68) and is the most decisive (DD=0.92), even though it presents the greatest number of equally parsimonious trees (30). 28S shows the most homoplasy (CI=0.55; RI=0.68; RC=0.32) and is the least decisive (DD=0.55). Nonetheless, the phylogenetic signal is strong in the total combined analysis, resulting in a single most parsimonious tree with 17/35 nodes having jackknife support of more than 70%.

The preferred model for the different markers differs and is shown in Table 3. In general, the model-based topologies have greater support than the parsimony trees. The Bayesian tree has higher support compared to the parsimony and likelihood trees, with 26/35 nodes showing posterior probabilities of 0.70 or more. Because the best trees from the maximum likelihood and Bayesian analyses are identical with regard to relationships within Hormathiidae and between this family and its closest relatives, only the best maximum likelihood tree is shown (Figure 2.2). For comparison, posterior probabilities are also indicated in the best maximum likelihood tree.

Results from parsimony (Figure 2.1) and model-based analyses (Figure 2.2) are broadly consistent and presented similar groupings within Acontiaria. In general,

the model-based topologies have greater support than the parsimony trees. The Bayesian tree has higher support compared to the parsimony and likelihood trees, with 26/35 nodes showing posterior probabilities of 0.70 or more. Because the best trees from the maximum likelihood and Bayesian analyses are identical with regard to relationships within Hormathiidae and between this family and its closest relatives, only the best maximum likelihood tree is shown (Figure 2.2). For comparison, posterior probabilities are also indicated in the best maximum likelihood tree.

Acontiate species are divided into three major clades: a first clade corresponds to family Aiptasiidae (clade 1), a second clade includes members of families Sagartiidae, Metridiidae, Diadumenidae, and Haliplanellidae (clade 2) and a third clade corresponds to Kadosactidae, Nemanthidae, Hormathiidae, Andvakiidae, Actinoscyphiidae, Bathyphelliidae, and the sagartiid *Phellia gausapata* (clade 3). The difference between the two analyses consists in the relationships between these three clades: in the parsimony analysis, clade 1 is basal to a group that includes clade 2 and clade 3, while in the model-based analyses, clade 1 is sister to calde 2 and these two form a group that is sister to clade 3.

Two sister group relationships are found in all analyses: *Metridium senile* is sister to the clade that includes *Diadumene cincta* and *Haliplanella lineata* and *Andavakia boninensis* is sister to Sagartiid *Phellia gausapata*. Additionally, the three members of Aiptasiidae (*Aiptasia mutabilis*, *Aiptasia pallida* and *Bartholomea annulata*) cluster in a monophyletic group in both parsimony and model-based analyses. Neither of the two most speciose acontiate families, Sagartiidae and

Hormathiidae, are monophyletic in any analyses. Sagartiidae is paraphyletic with regard to Metridiidae, Haliplanellidae, and Diadumenidae in the parsimony analysis (Figure 2.1) and to these plus Aiptasiidae in the model-based analyses (Figure 2.2). Furthermore, in all analyses, the sagartiid *P. gausapata* clusters with members of Hormathiidae rather than with the other sagartiids. The placement of the nemanthid *Nemanthus nitidus*, the bathyphellid *Bathyphellia australis*, and the actinoscyphiid *Acy. plebeia* within Hormathiidae renders Hormathiidae paraphyletic. A close affinity between Nemanthidae and Hormathiidae is also recovered.

Phylogenetic relationships within Hormathiidae

Both parsimony and model-based analyses recover similar relationships between members of Hormathiidae and closely related families. The basal-most clade includes *Amphianthus* sp. and *Acy. plebeia* (Actinoscyphiidae). In all parsimony results, *Paracalliactis* cluster with *Paraphelliactis* and *Chondrophellia*, grouping within a second clade that also includes *Bathyphellia* and *Nemanthus*. Members of genera *Hormathia*, *Actinauge*, *Allantactis*, *Calliactis* and *Adamsia* form a third clade within Hormathiidae.

None of the markers separately or in combination support a monophyletic origin for the two hormathiid genera for which multiple species were included. In both parsimony and model-based analyses, the genus *Hormathia*, the type genus of family Hormathiidae, is not monophyletic as *H. pectinata* is more closely related to *Actinauge richardi* than to *H. lacunifera* or *H. armata*. Similarly, *Calliactis* is

paraphyletic due to the inclusion of *Adamsia palliata* within the clade that includes the remaining species of *Calliactis*. In the combined analysis, the clade corresponding to *Calliactis* + *Adamsia* is well supported (80% jackknife support) with *C. japonica* as the sister to a clade that includes all remaining species of *Calliactis* and also *A. palliata*. *C. parasitica* and *A. palliata* are each others closest relative (100% jackknife support; 1.0 posterior probability), and are sister to a clade including both specimens *of C. polypus* and *C. tricolor*. Individuals of *C. polypus* are not recovered as sister taxa. The larger clade that includes *Adamsia* and *Calliactis* also includes *H. lacunifera and H. armata.*

Hypothesis testing

One tree of 5267 steps was produced in the parsimony analysis under the constraint of monophyly for the family Hormathiidae. This constrained tree was significantly different from the shortest tree found without the constraint of monophyly based on the non parametric Templeton test (Wilcoxon signed-ranks; p=0.0001) and winning-sites tests (p=0.0001). The Shimodaira-Hasegawa test rejected the constrained maximum likelihood topology with a p-value of 0.0356. None of the filtered trees from the Bayesian analysis included a monophyletic group of symbiotic hormathiids, conferring a posterior probability of zero on this hypothesis. Thus, alternative suboptimal topologies suggesting a monophyletic origin for the members of family Hormathiidae are statistically rejected.

Discussion

Phylogeny of Acontiaria

The phylogeny of acontiate sea anemones inferred by parsimony, maximum likelihood and Bayesian analyses is largely congruent and in agreement with the general conclusions of other analyses (Daly et al. 2008; Rodriguez et. al. unpublished data). A common element is the paraphyly of the group Carlgren (1949) called Acontiaria, due to the inclusion of *Acy. plebeia* and *A. boninensis* within the ingroup. The major division of Acontiaria into a group that has acontia with numerous nematocysts and members of Nemanthidae that possesses acontia like-organs (acontioids) with few nematocysts is not supported in this analysis. *Nemanthus nitidus* is nested well within the clade that includes members of Hormathiidae and its allies. That is not surprising given the uncertainty surrounding the phylogenetic importance of acontia or the poor definition of acontioids. One of the most evident problems with the group Acontiaria is the presence of acontia in members of Athenaria (see Figure 2.1, 2.2*)*, a different lineage of sea anemones (Hand 1966). Carlgren (1949) recognizes the subtribe (=superfamily) Acontiaria in his classification for practical reasons, but notes that the group was probably not genetically homogeneous.

Schmidt (1974) does not regard anemones with acontia as a distinct taxonomic group, in marked contrast with other authors (e.g., Stephenson 1922; Carlgren 1949; for a detailed discussion see Daly et al. 2008). Instead of subdividing sea anemones that possess a mesogleal sphincter into Mesomyaria (without acontia)

and Acontiaria (with acontia), Schmidt (1974) divides species with both acontia and mesogleal sphincter into Early Mesomyaria (Diadumenidae, Aiptasiidae, Hormathiidae, Nemanthidae) and Late Mesomyaria (Isophellidae, Sagartiidae, Halcampidae, Metridiidae, Actinostolidae), based on the type of cnidae present in the acontia, filament, and pharynx. No partition or any analyses recovers the groups hypothesized by Schmidt (1974). In fact, the Early Mesomyaria members of the family Diadumenidae, Aiptasiidae and Hormathiidae plus Nemanthidae correspond to the three distinct clades recovered by all analyses in this study.

Phylogeny of Hormathiidae

More important to the present study, however, is the consistent finding of a non-monophyletic Hormathiidae. The inclusion of *Acy. plebeia* (Actinoscyphiidae), *B. australis* (Bathyphellidae), and *N. nitidus* (Nemanthidae) renders Hormathiidae paraphyletic. Our confidence in these results if further supported by the constraint analyses, which never recovered a monophyletic origin for the members of Hormathiidae.

A monophyletic Hormathiidae is recovered by Daly et al. (2008), but as already mentioned, that analysis includes only three hormathiids and thus does not represent a strong test of the monophyly of the family. The inclusion of *Acy. Plebeia* within Hormathiidae confirms morphological (Riemann-Zürneck 1978; Rodríguez et. al. 2008) and phylogenetic hypotheses (Daly et al. 2008; Rodríguez and Daly 2010) that regard members of Actinoscyphiidae as hormathiids that have lost acontia. A

common element of all analysis is an assemblage of acontiate species that comprises two clades corresponding to taxa that either have basitrichs and microbasic *p*-mastigophores or only basitrichs in the acontia. Clade 3 comprises Hormathiidae and Bathyphellidae, two families that possess only basitrichs in the acontia. This may illustrate the conflicting signals in some characters used by Carlgren (1949) to differentiate families and genera within the superfamily Acontiaria, as the phylogenetic position of *B. australis* within Hormathiidae may illustrate that nematocyst type present in the acontia is a good indicator of evolutionary relationships within Acontiaria. Although instances of loss of acontia are known (e.g., *Actinoscyphia*), when present, the types of nematocysts in the acontia seem to track evolutionary history and are a synapomorphy for Hormathiidae.

The relative importance of mesentery morphology, however, may be challenged by the position of *B. australis* in the present analyses. Although only one species included in this study has dimorphic mesenteries (*B. australis*), its nested position within a clade of species having monomorphic mesenteries indicate that the morphology of mesenteries should not be used to indicate close relationships among families in Acontiaria. Inclusion of species of Isophellidae, another taxa with dimorphic mesenteries, in future studies may help to elucidate some of these questions.

Daly et al. (2008) finds a close association between *N. nitidus* and the exemplar species of Hormathiidae. Nemanthidae includes three species (Rodríguez et al. 2008; Fautin et. a.l. 2007; Fautin 2010) that lack true acontia but have acontia-like

organs called “acontioids” that lack the battery of nematocysts characteristic of an acontium. Carlgren (1940) hypothesizes that acontioids could be regarded as either transformed acontia, acontia in development or a structure not homologous to true acontia. Later, Carlgren (1943) hypothesizes that acontioids and true acontia share a common ancestry as differentiations of the lowermost part of filaments, but he was not sure whether this structure was different enough for *Nemanthus* species to be regarded as a different family. The distribution of nematocysts in acontioids is not well known and it was impossible to determine with confidence which types of nematocysts were present in these structures when the family Nemanthidae was ereceted (Carlgren 1940). When describing the third species of *Nemanthus* (*N. annamensis*), Calgren (1943) indentifies two types of nematocysts in the acontioids: basitrichs and microbasic *p*-mastigophores. In our analyses, *N. nitidus* is always recovered as the sister group to a clade that includes three hormathiids and one species of the family Bathyphellidae (*B. australis*). Based on these results we hypothesize that acontioids are homologous to true acontia. A morphological phylogenetic analyses of all genera of Hormathiidae is underway and should shed more light on the evolutionary relationships between Hormathiidae and Nemanthidae. A closer examination of acontioids from a species of *Nemathus* reveals that only basitrichs are present in these structures (LGG pers.obs.). It remains to be determined if acontioids possess microbasic p-mastigophores or if their presence was the result of contamination from filament tissue.

The relationship between *A. boninensis* and *P. gausapata* and the affinity of this clade with Hormathiidae, although difficult to interpret from a morphological standpoint, is also recovered in the analysis of Daly et al. (2008). Both species have acontia with basitrichs and microbasic *p*-mastigophores and are the sister group to the remaining hormathiids and species in families Bathyphellidae, Actinoscyphiidae and Nemanthidae.

Although no hypothesis of relationship among genera of Hormathiidae exists, the characters used by Carlgren (1949) to define he genera of hormathiids have been assumed to carry phylogenetic signal and his classification has been used as a framework to test evolutionary relationships within Actinaria (Schmidt 1974; Daly et.al. 2008; Gusmao and Daly 2010).

The consistent find of a clade that includes the two species *H. lacunifera* and *H. armata* as the sister group to *Calliactis* and *Adamsia* renders *Hormathia*, the type of Hormathiidae, paraphyletic. The close relationship between *H. lacunifera* and *H. armata* and the *Calliactis* + *Adamsia*, is credible on the basis of morphology. These three genera, as well as *Allantactis parasitica* and *Actinauge richardi* exhibit a tendency to attach to organic substrates and are distinguished within Hormathiidae by having six pairs of perfect and sterile mesenteries, while other genera exhibit 6 pairs of perfect but fertile mesenteries. Although *A. palliata*, the type species of *Adamsia*, has been described as having 12 pairs of perfect sterile mesenteries (Carlgren 1928a; Manuel 1981), Schmidt (1972) reports only one cycle (=6 pairs) of perfect mesenteries for *A. palliata*. Likewise, LCG (pers.obs) while revising the genus

Adamsia, finds that *A. palliata* as well as *A. sociabilis* possess only one cycle of perfect sterile mesenteries.

Similarly, although not previously hypothesized, an exclusive relationship between *Paracalliactis*, *Chondrophellia*, and *Paraphelliactis* is supported by a number of shared morphological characters: column differentiated in scapus and scapulus, absence of cinclides, column provided with corona of tubercles and a cuticle and same number of mesenteries proximally and distally. Additionally, while *Chondrophellia* possess 6-12 pairs of perfect and fertile mesenteries, *Paracalliactis* is sister to *Paraphelliactis* and both possess six perfect and sterile mesenteries. The importance of cinclides has been emphasized by several authors as a shared similarity between *Calliactis* and *Adamsia* (Carlgren, 1928b; Hand, 1975; Daly et al., 2004), and thus the association of *Paracalliactis* with groups whose members lack cinclides is not be surprising.

Most of the characters used by Carlgren (1949) in his classification of Hormathiidae, such as fertility of perfect mesenteries, number of fertile and sterile mesenteries and the direction of mesentery growth, presence of cinclides, as well as a symbiotic habit, are inferred to be convergent in the hormathiids included in this analysis. Although these characters may carry some phylogenetic signal, they are usually found scattered across the topologies found in this study and define smaller groups within the family.

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Chapter 4: Genetic diversity and phylogenetic utility of the ribosomal Internal Transcribed spacers (ITS-1, 5.8S, ITS-2) region in the sea anemone *Calliactis polypus* (Cnidaria: Actiniaria: Hormathiidae)

Introduction

Sea anemones belong to Class Anthozoa, which exhibit one of the slowest rates of molecular evolution among metazoans (Geller and Walton 2001; Worheide et. al. 2006; Hellberg 2006; Park et al. 2007; Huang et al. 2008; Shearer and Coffroth 2008). Mitochondrial markers may evolve 100 times slower than in other marine invertebrates (Shearer et.al. 2002; Hellberg 2007), while nuclear markers have been less studied but seem to be only a little more variable (Hellberg 2007; Daly et al. 2010; Gusmão and Daly 2010). When used in phylogenetic studies of sea anemones, these markers are useful at the order, family, and genus levels, but generally do not permit resolution between closely related species or populations. This lack of variation renders sequence-based studies at lower levels less effective and, consequently, most of the population and species-level studies have used techniques such as isozymes and allozyme electrophoretic analyses (e.g., Solé-Cava and Thorpe 1992; Thorpe and Solé-Cava 1994; Monteiro et al. 1997), DNA fingerprinting (Edmands and Potts 1997) and Amplified Fragment Length Polymorphisms (AFLP) (Douek et al. 2002; Ting and Weller 2000; Darling et al. 2004). These techniques have been applied to investigate reproductive plasticity, species introduction (Ting

and Geller 2000; Darling et al. 2004), and cryptic speciation (Douek et al. 2002) among sea anemones.

The use of fast evolving markers, such as the nuclear ribosomal Internal Transcribed Spacers (ITS), is an alternative to fragment analyses and can be easily incorporporated into a phylogenetic framework. The complete ITS region is found in tandem repeats and is situated between the small nuclear ribosomal unit (18S) and the large subunit (28S) and it includes the ITS-1, 5.8S and ITS-2 fragments. Polymorphism among repeat units in the genome is possible, but concerted evolution is assumed to homogenize these units and reduce intragenomic diversity (Li 1997), leaving differences between species preserved (Hillis and Dixon 1991). The the 5.8S gene shows a slower rate of evolutionary change, but both spacers show higher sequence variation and have been widely used to infer phylogenetic relationships from populations to families and even higher taxonomic levels in many organisms (Baldwin 1992; Fritz et. al. 1994; Vogler and Desalle 1994; Schlötterer et al. 1994; Gómez-Xurita et. al. 2000; Chu et. al. 2001; Alvarez and Hoy 2002; Chen et. al. 2002; Shaw et. al. 2002; Worheide et al. 2006; Addis and Peterson 2005; Drake et. al. 2007; Hedenas 2009; Miranda et. al. 2010; reviewed in Hills and Dixon 1990). The level of divergence in the spacer region is especially useful for detecting differences between conspecific individuals, providing a potential useful marker with which to study populations and closely related species of anthozoans (Beauchamp and Powers 1996; Chen and Miller 1996; Hunter et al. 1997; Lopez and Knowlton 1997; Odorico and Miller 1997; Medina et. al. 1999; Dawson and Jacobs 2001; Lam and Morton

2003; Reimer et al 2007a, b; Sanchez et. al. 2007; Sinninger et. al. 2008; Acuña et. al. 2009; Gutiérrez-Rodríguez et. al. 2009;Aguilar and Reimer 2010).

Many studies have found polymorphism in the ITS region of anthozoans and used this markers with sucess at various taxonomic levels (e.g, Beauchamp and Powers 1996; Chen et al. 1996; Chen and Miller 1996; Odorico and Miller 1997; McFadden et al. 2001; Van Oppen et al. 2002; Acuña et. al. 2007; Sanchez and Dorado 2009). A few studies, however, did not find enough variation in the spacers (Lee and Song 2000; Calderón et. al.2008), could not unambigously align divergent sequences (Reimer et. al. 2007a) or found high intragenomic variation that can obscure phylogenetic signal (Vollmer and Palumbi 2004; Sánchez and Dorado 2008; McFadden et. al. 2010). Only recently, the genetic variability of this marker in sea anemones was explored (Ting and Geller 2000; Stoletzki and Schierwater 2005; Acuña et al. 2007). Stoletzki and Schierwater (2005) sequenced and cloned the 5.8S and ITS-2 to investigate population structure in *Condylactis gigantea* from Jamaica, while Acuña et. al. (2007) direct sequenced the complete ITS region to infer phylogenetic relationships within the genus *Aulactinia*. The results of these two studies are somewhat at odds. While Stoletzki and Schierwater (2005) found diversity at all levels (intragenomic, intra-specific and inter-specific), with intra-specific divergence higher than inter-specific divergence, Acuña et. al. (2007) found high inter-specific divergence among three species in the genus *Aulactinia* and little intraspecific variation within each species. Acuña et al. (2007) direct sequenced the ITS region and did not test for intragenomic variation. Both studies were carried out

at a local scale with populations no more than 30 Km apart.

Originally described from the Red Sea, *C. polypus* is the most widespread species of hermit crab symbiotic anemone. Its range spans the Red Sea (Djibouti, Egypt, Israel, Saudi Arabia), the Pacific Ocean (Australia, French Polynesia, Galapagos Islands, Guam, Hawaii, Kiribati, Japan, Marshall Islands, Micronesia, Palau, Philippines, among other localities) and the Indian Ocean (Maldives; Seychelles, South Africa, Tanzania) (Fautin 2010; LCG pers.obs.). This wide distribution is the result of synonymies and reassignments of geographically distant populations based on morphological characters (e.g., England 1975). Morphological differentiation, however, is not straightforward and populations of *C. polypus* show a degree of differentiation that is hard to interpret. While taxonomic decisions should not be taken based on genetic data alone, this source of information is useful in cases where species boundaries are unclear. Here we will use five population of *C. polypus* to study population structure and cryptic speciation and delimit diversity between closely related species in *Calliactis*.

In the present study the feasibility of using the internal transcribed spacer (ITS) region of ribosomal DNA as a molecular marker in sea anemones was explored. We present the results from cloning and sequencing of the ITS region of 36 individuals of *C. polypus* taken from 5 different populations across its distribution range. Two closely related species (*C. tricolor* and *C. parasitica*), as well as species of Hormathiidae, the family to which *C. polypus* belong, were examined for comparison. We assess levels of variation and the phylogenetic utility of this genomic region in

resolving taxonomic questions at and below the species level. Specific goals were to examine (I) levels of intragenomic; (II) intraspecific and (III) interspecific within and between *C. polypus* and two closely related species.

Material and Methods

Taxon sampling and DNA extraction

Thirty-six individuals of *C. polypus* were collected by hand on intertidal or using SCUBA gear from 5 localities: Hawaii, Japan, Maldives, Saipan and South Africa. Whole animals or pieces of tissue from each specimen were preserved in 96% ethanol. Genomic DNA was isolated from tentacles, pedal disc, or column with the DNAeasy Kit (Qiagen) using the modified rat tail protocol supplied by the manufacturer. We also amplified and sequenced clones from *C. tricolor* and *C. parasitica*, two closely related species (Gusmão and Daly 2010) for inter-specific comparison and use as potential outgroups in the phylogenetic analysis. We also attempted to include sequences from other species of family Hormathiidae in the analysis. Too much variation among distantly related species prevented a reliable alignment of these sequences.

ITS rDNA amplification, cloning and sequencing

For molecular analyses we amplified the complete ITS rDNA fragment spanning ITS-1, 5.8S and ITS-2 using the primers 18SUniv.fw. 5'-GGTTTCCGTAGGTGAACCTGCGGAAGGATC-3' and 28SAct.rev. 5'-

GTTCCCGCTTCATTCGCCATTAC-3' (from Stoletzki and Schierwater 2005). PCRs products were cloned into pCR II-TOPO vectors using a TOPO TA Cloning Kit (Invitrogen, La Jolla, CA). Transformations followed the manufacturer's supplied protocol. We re-amplified 10-12 positive colonies per individual. PCR products were sequenced using the same primers as in the amplification reaction on an ABI 3730xl by staff at the sequencing facilities of Genaissance (Houston, TX) and Agencourt (Beverly, MA). Forward and reverse sequences were assembled using Sequencher v.4.9 (Gene Codes Corporation, Ann Harbor, MI) and blasted against the nucleotide database of Genbank to check for contaminants. Unique haplotypes were deposited in Genbank (table 3.1).

Sequence alignment and phylogenetic analysis

The boundaries between ITS-1 5,8S and ITS-2 regions were determined using the flanking conserved sequences of 18S, 5.8S and ITS-2 of published sequences of the sea anemones *Heteractis magnifica* (AF050211) and *Aulactinia marplatensis* (EF026595) and the zoanthid *Abyssoanthus nankaiensis* (AB247643). The comparison indicated that the amplified region containing the sequences of 18S, 5.8S and 28S is sufficiently conserved, thus permitting unambiguous alignments within and among species. The ITS-1, 5.8S and ITS-2 regions were assembled and analysed as a single sequence (=complete ITS sequence) or as separate data partitions. Sequences were aligned in MUSCLE (Edgar 2004) using default parameters. Alignment of sequences of all three species was not problematic and implied only

short insertions/deletions. Base composition, pairwise distance (Kimura 2-parameter), substitution patterns, and transition /transversion ratios were generated using MEGA 4.0 (Tamura et. al. 2007). Number of polymorphic sites and haplotype frequencies were calculated using DNAsp (Librado and Rozas 2009). In the phylogenetic analysis, we considered four different data matrices: ITS-1 only, ITS-2 only, 5.8S only and all three fragments combined (combined matrix). Results based on the combined dataset are shown when these reflect the results found with separate data partitions. We used TNT (Goloboff et al. 2008) to generate parsimony trees for all data sets and to calculate tree statistics. RaxML (Stamatakis 2006) was used on the Cipres Portal (Miller et.al. 2010) to generate maximum likelihood trees.

Results

Length of sequences and G+C content

Three hundred and eighty four complete ITS sequences (ITS-1, 5.8S and ITS-2) were analyzed. All three species showed little length variation in the complete ITS sequences, varying from 521-522 bp in *C. tricolor* to 525-530bp in *C. polypus* and 567-568 in *C. parasitica* (Table 3.2). Length variation was due entirely to variation in the ITS-1 and ITS-2 fragments, as 5.8S sequences were constant at 148 bp. ITS-1 and ITS-2 were of comparable sizes in *C. polypus* and *C. tricolor*, but ITS-2 was longer than ITS-1 in *C. parasitica*. The final data set of complete sequences exhibited similar single-base frequencies for all three species and the average percentage of nucleotide composition was: A=21.38%, C=28.64%, G= 27.44% and T= 22.54%.

The G+C content of the complete ITS region do not vary significantly between *Calliactis* species and range from 53.94% in *C. tricolor* to 56.62% in *C. parasitica* (Table 3.2). ITS-2 exhibited consistent higher G+C content, ranging from 61.13% in *C. tricolor* to 66.12% in *C. polypus*. Sample sizes, length of sequences and G+C content are summarized in Table 3.2.

Sequence variation

Intragenomic/ intraindividual variation

By sequencing multiple clones from the same individual, we detected intragenomic variation in all three species of *Calliactis*. The single individual of *C. parasitica* exhibited five distinct ITS copies, while the single individual of *C. tricolor* had four copies (Table 3.2). All individuals of *C. polypus* had at least two distinct complete ITS sequences (=haplotypes), with an average of 6.28 sequences per individual in a total of 165 distinct haplotypes (Table 3.2). Most of the individuals of *C. polypus* (32/36) exhibited intra-individual variation in the ITS-1, while all individuals of *C. polypus* showed intra-individual variation in the ITS-2. 5.8S was less variable, with only 29/36 individuals presenting intra-individual variation in ITS sequences. Table 3.2 summarizes the number of haplotypes per species, individuals and populations for each ITS partition and the complete ITS region.

The average intragenomic divergence (Kimura 2 parameter) in *C. polypus* was 0.007 (ranging from 0-0.020). Intragenomic differences, however, were frequently only discrete base substitutions or single insertions or deletions. Additionally, most of

the differences between copies were confined to certain regions of the ITS-1, 5.8S and ITS-2. Thus, it seems that the intragenomic variation observed is not an artifact of cloning or polymerase mis-incorporation since it's unlikely that the polymerase errors would occur repeatedly and independently at the same positions.

Intra-specific variation

Intraspecific variation was highest in the ITS-2 region for all three species, while the 5.8S region was the least variable (Table 3.2). Because only one individual of *C. parasitica* and *C. tricolor* were analyzed, intraspecific and intragenomic variation coincide in these species and results are mostly based on *C. polypus*. As expected given the number of individuals examined, the number of haplotypes found in *C. polypus* was higher than the other two species (362 clones; 165 haplotypes). *Calliactis polypus* exhibited 74 distinct haplotypes of ITS-2, with the most widespread haplotype found in 161/361 sequences (33/36 individuals). ITS-1, on the other hand, exhibited 63 distinct haplotypes, with the most widespread haplotype found in 223/361 sequences and all individuals. Forty-nine haplotypes of 5.8S was found, with the most widespread haplotype found in 297/361 sequences and all individuals (seven individuals possessed only this haplotype).

Calliactis polypus complete ITS sequences were defined by 78 polymorphic sites arising from base substitutions alone. Among these base substitutions, there was a preponderance of transitions over transversions. The average intra-specific genetic divergence was less than 1% for all three species, ranging from 0.7% in *C. polypus* to

0. 9% in both *C. parasitica* and *C. tricolor* (Table 3.2). Two distinct variants (variant I and variant II) were found in the complete ITS sequences. These variants could be detected by specific regions of the ITS-2 that contained nine point mutations and five indels. Most ITS sequences belonged to variant I (85.45%), while variant II exhibited 24 distinct haplotypes (14.55%). Both variant I and variant II presented one haplotype that was very widespread with the remaining haplotypes being unique and found mostly in single individuals. The most common haplotype of variant I represented 97/361 (26.87%) sequences and it was found in 25/36 individuals and the most widespread haplotype of variant II represented 19/46 (41.30%) sequences in 9/36 individuals. All individuals possessed variant I, 23/36 individuals possessed only variant I and only 13/36 individuals possessed both variants I and II. Pairwise divergence between variants I and II varied from 0 to 1.7%, which could be higher than the difference between individuals. The variation within variants between the two variants was 0-0.015.

Polymorphism within populations of *C. polypus* was low, with the average ranging from 0.007-0.010 (Table 3.2). Variation among populations ranged from 0.004 in the Maldives to 0.010 in South Africa (Table 3.2), with the greatest inter-populational variation found between Saipan and South Africa. The japanese population exhibited the highest number of haplotypes, while South Africa was the least variable (Table 3.2). However, most of the south african specimens exhibited sequences belonging to variant II, which made the average distance within this population highest. Individuals from the Maldives, on the other hand, did not present

any ITS belonging to Variant II. The most common ITS haplotype was the only one shared between all populations. Most haplotypes were unique to individuals and not shared between individuals or across populations. No clear pattern in the sequences that could differentiate species were observed. Specific information on population differentiation for ITS-1, ITS-2 and 5.8S are shown in table 2.

Inter-specific variation

Comparisons of sequences among *calliactis* species suggest that substantial differences have accumulated in the ITS region in the time since these species diverged. Inter-specific divergence based on complete ITS sequences was higher than intraspecific and inter-individual divergence and exhibited maximum values between *C. parasitica* and *C. polypus* (0.232) and *C. parasitica* and *C. tricolor* (0.225) and it was lower between *C. polypus* and *C. tricolor* (0.060)(Table 3.3). Despite high inter-specific divergence between species, sequences of all three species of *Calliactis* were unambiguously aligned. In addition, no haplotypes were shared across species. In average, inter-specific divergence was highest in the ITS-1 and lowest in the 5.8S. Table 3.3 shows ITS average pairwise nucleotide divergence between *Calliactis* species for all three ITS partitions. At this time, only a comparison between these three closely related species can be made. Extensive interspecific variation in the ITS-1 and ITS-2 made the alignment of more distantly related taxa unreliable. We consequently restricted our analysis to the three closely related species of *Calliactis* that could be aligned with relatively little ambiguity.

Phylogenetic analysis

Of the 571 positions in the alignment of the combined dataset, 247 were variable between taxa and 164 of these sites were informative for parsimony analysis. The analysis of the combined dataset including the complete ITS region yielded 49 most parsimonious trees of 377 steps (CI=0.41; RI=0.48). A summary of all parsimony analyses is given in Table 3.4. Tree topologies for all parsimony analysis were identical irrespective of the set of sequences analyzed (ITS-1, 5.8S, ITS-2 partitions). The topology presented in figure 1 is the result of the parsimony analysis using the complete ITS region and illustrates the relationships recovered for all analyses and data sets.

Both parsimony and maximum likelihood topologies consistently recovered three clades corresponding to the three species of *Calliactis* (Figure 3.1). Strong reciprocal monophyly of all three species of *Calliactis* is supported by jackniffe and bootstrap values higher than 98% for all three species. The twelve sequences of *C. parasitica* clustered together with high jackknife (100%) and bootstrap support (100%). Similarly, the 12 sequences of *C. tricolor* formed a monophyletic group with high support (98% jackknife and 99% bootstrap). *C. polypus* sequences formed a single cluster with the both jackknife and bootstrap support of 100%.Within *C. polypus*, however, topologies did not show any clear geographical pattern. Instead, sequences were separated into two groups, representing variant I and variant II sequences. This dichotomy was strongly supported by jackniffe and bootstrap values of 99% and 89%, respectively.

Discussion

Molecular characteristics of Calliactis ITS

The conserved 18S and 28S flanking regions were nearly identical among the three species of *Calliactis* and even among species with highly divergent ITS-1 and ITS-2 that could not be unambiguously aligned. No significant intragenomic length variation of individual spacers were detected in the ITS region of *Calliactis*. ITS-1 and ITS-2 exhibited similar length in *C. tricolor* and *C. polypus*, but *C. parasitica* exhibited a slightly longer ITS-2. The length of sequences examined here fell within the values found by Acuña et.al. (2007) for sea anemones in the genus *Aulactinia*. While our 5.8S sequences showed similar lengths, ITS-2 sequences are slightly longer than those of *Condylactis* (Stoletski and Schierwater 2005). Values for G+C content for the complete ITS region in *Calliactis* are comparable to those found in other anthozoans (Chen and Miller 1996; Odorico and Miller 1997; Chen et. al. 2004; Forsman et. al. 2005; Moothien-Pillay et. al. 2006; Reimer et. al. 2007; Dorado and Sanchez 2009). A higher G+C content in the ITS-2 has also been described in other scleractinian corals (Chen et.al. 2004; Forsman et al. 2005).

A considerable number of bases in some regions of the ITS were difficult to determine with precision in species of sea anemones that were direct sequenced (LCG pers.obs.). This could be due to the presence of variation in heterozygous alleles or between multiple copies in the genome. This variation was later confirmed when multiple clones from a single individual were sequenced. The nucleotide variation between distinct copies in individuals of *C. polypus* was low, but, in most cases,

individuals possessed at least two distinct sequences: a common ITS haplotype and some unique ones. Despite the use of a single individual of *C. parasitica* and *C. tricolor* in this study, the degree of intra-individual sequence variation in these two species was very similar to that observed in the 36 individuals of *C. polypus* examined. Similar intragenomic variation was found in *Condylactis gigantea* where many individuals possessed multiple ITS copies belonging to two major variants (Stoletski and Schierwater 2005). Intragenomic variation is not rare and it has been found in other anthozoans (McFadden et. al. 2001; Fujiwara et. al. 2003; Vollmer and Palumbi 2004; Calderon et. al. 2006; Moothien-Pillay 2006; Reimer et. al. 2007; Dorado and Sánchez, 2009; Gutiérrez-Rodríguez 2009), but the the intragenomic variation in *Calliactis* is very low compared to the levels of intragenomic variation found in the scleractinian species of *Acropora* (Odorico and Miller 1997; Hatta et. al 1999; Medinal et.al. 1999; Diekmann et al. 2001; Fukami et.al. 2001; Van Oppen et.al. 2000, 2002; Marquez et.al. 2003).

The "typical" intraspecific sequence divergence of the ITS region (ITS-1, 5.8S, ITS-2) in sea anemones is largely unexplored. The low intra-specific variation found in the three species of *Calliactis* (0.7-0.9% divergence) falls within the range of variation found in other species of sea anemones (Stoletski and Schierwater 2005) and is considered acceptable (<5% following Worheide et.al. 2006). The analysis of ITS-1 and 5.8S sequences in *Condylactis gigantea* showed that the levels of intra-specific divergence in this species are comparable to the degree of variation found by others for these markers (Stoletski and Schierwater 2005). This variation consisted of

a few base subsititutions between two ITS variants and it was relatively high when compared to the inter-specific comparison between this species and its mediterranean relative *C. aurantiaca*. Acuña et al. (2007) did not examine intra-specific variation within *Aulactinia* spp, but LCG (pers.obs) examined their published sequences and found that the variation within species was similar to the one found in this study.

Comparable low levels of intra-specific variation can be found in other anthozoans, particularly in corallimorpharians that, in come cases, may exhibit no intraspecific variation (Chen et al. 1996; Chen and Miller, 1996). Zoanthids in genera *Zoanthus* and *Palythoa* can also have low levels of variation within species (Reimer et. al. 2007a,b) or present extremely high intraspecific sequence variation, ranging 14.9-32.1% in *P. tuberculosa* and *P. mutuki* (Reimer et. al. 2007b). Most scleractinians present low levels of variation, including those in genera *Madractis* (Diekmann et al. 2005), *Balanophyllia* (Beauchamp and Powers 1996), *Siderastrea* (Forsman et. al. 2005), *Montastrea* (Lopez and Knowlton 1997), *Heliofungia* (Takabayashi et al. 1998). Although extreme ITS heterogeneity has also been found in corals, particularly those in genera *Acropora* (Odorico and Miller 1997; Van Oppen et. al. 2001; Vollmer and Palumbi 2004; Wei et. al. 2006) showed that this high sequence variation is probably a unique characteristic of this genus and not a common feature of coral ITS.

Although intraspecific variation is low, interspecific variation presented intermediate levels, being lowest in the 5.8S (0.3%-3.0%) and highest in the ITS1 (7.6-27.4%). The complete ITS region presented levels of variation that depicts the

sister taxa relationships between these two species, with variation between *C. parasitica* and both *C. polypus* (avg. 19.9%) and *C. tricolor* (avg. 19.3%) higher than that between *C. polypus* and *C. tricolor* (avg. 5.7%), which are more closely related (Gusmão and Daly 2010). The interspecific variation found in *Calliactis* corresponds to the range of variation found in other anthozoans, including some scleractinian corals (e.g., Vollmer and Palumbi 2001; Forsman et. al. 2005; Moothien-Pillay et. al. 2006; Wei et. al. 2006), but are only in the lower end of the range found in species of corallimorpharians in the genus *Rhodactis* (range 30.97-73.19%) (Chen and Miller 1996) and in intergeneric comparisons between genera *Rhodactis*, *Amplexidiscus* and *Actinodiscus* (Chen and Miller 1996). ITS1 and ITS2 are very divergent in zoanthids of the genus *Zoanthus* and cannot be readily aligned, however, estimates of sequence divergence reach ~45% in *Zoanthus* spp. (70% in ITS1) (Reimer et. al. 2007a). Sequence divergence in the aligned sequences of 5.8S in *Zoanthus* ranges from 2.5-7.0% (Reimer et al. 2007a) and is higher when compared to the divergence observed in *Calliactis* (0.3-3.0%). Similarly, octocorals exhibit low to intermediate interspecific variation, ranging from 1.2% in the ITS1+ITS2 (Rodriguez-Lannety and Hoegh-Guldberg, 2002) to a maximum of 10.86% in the ITS2 in *Eunicella cavolinii* (Calderón et. al. 2006).

All analyses and data partitions supported the hypothesis that *C. polypus*, *C. parasitica* and *C. tricolor* should be considered separate species. Individual and combined analyses of the ITS region supported reciprocal monophyly of the three species with very high support. Additionally, no haplotypes were shared between

these species. This result was expected, as these three species are among the most well defined and characterized species of sea symbiotic sea anemones in the genus *Calliactis*. They are also geographically isolated, with *C. parasitica* confined to the Mediterranean Sea and North Atlantic and *C. tricolor* found from the Caribbean to southern Brazil and *C. polypus* found in the Indian and Pacific Ocean (Fautin 2010). All sequences of *C. polypus* clustered together as a single clade, with low intraspecific diversity, which indicates that this species may be a truly cosmopolitan species. A morphological revision of *C. polypus* (LCG unplished data) including populations throught its geographic range has also shown that no clear anatomical differences can be found among these populations and this may be a truly widespread species. Most sea anemones present plantotrophic larvae, but little is known regarding its settlement or recruitment (Shick 1992) and Siebert (1974) has shown that larvae of *Anthopleura elegantissima* can swim for at least two weeks before settlement. During this time, the distances between the populations studied may be further achieved with the help of their symbiotic partners (hermit crabs) that may serve as stepping stones between populations that are more distantly located.

The ITS sequences, however, did not yielded any pattern of population structure by geography within *C. polypus*, as the five populations examined did not form distinct clades in the parsimony or the maximum likelihood analyses. This may be indicative of the low evolutionary rate of the ITS region in *C. polypus* that does not capture a signal of population differentiation in this species. Sequences of *C. polypus* are divided in two clades that corresponds to the two variants of ITS, which

further supports the existence of two ITS variants. The finding of distinct ITS variants is not rare in anthozoans (e.g., Odorico and Miller 1997; Stoletski and Schierwater 2005; Reimer et. al. 2007a; Aguilar and Reimer 2010). It's possible that one of the ITS variants represent orphons or repeats organized in small clusters that can only be detected by cloning. Another possibility is that the occurrence of chromosomal regions homogenized for different variants by intrachromosonal recombination (Polanco et. al. 1998; Wang et. al. 2007; Freire et. al. 2010) or the divergence between ITS copies are different due to unequal crossing-between copies in the center or middle of the chromosome (Ruiz Linares et. al. 1994; Trontin et. al. 1999).

Phylogenetic utility of ITS in sea anemones

Potential problems of using a fast evolving marker (or any marker) in a phylogenetic analysis at low taxonomic levels may include high intragenomic diversity that obscures phylogenetic signal at higher levels and difficulties with sequence alignment due to high sequence divergence. Substantial amounts of intragenomic variation might negatively impact phylogeny estimation and population level studies based on ribosomal spacers, especially at shallow phylogenetic levels (Onyave and Conn 1999; Vollmer and Palumbi 2004). It is necessary to first determine the extent of homogenization of a multigene family at several hierarchical levels (within individual, population and species) before employing them in phylogenetic studies or as diagnostic markers because high levels of intragenomic variation may obscure phylogenetic signal (Onyave and Conn 1999).

Although intragenomic polymorphism was detected, this variation was not high and did not obscure the phylogenetic signal in the analysis. The relatively high level of variation between species with no overlap between intraspecific and interspecific levels of variation indicate that ITS may be a valuable marker to infer relationships between closely related species that do not possess highly divergent ITS sequences and that can be readily aligned. The population study using the ITS region in *C. polypus*, however, does not seem promissing given the very low level of intraspecific variation and the lack of population structure in the phylogenetic trees and lead us to recommend locating more variable regions to infer population differentiation in sea anemones.

It has been shown that phylogenetic signal can be improved by using secondary structure to guide alignments in cases where sequence divergence is too high or when distantly related species are compared (McFadden et. al. 2001). This has been shown to work for other anthozoans (Aguilar and Sanchez 2007, Grajales et al. 2007; Gutierrez-Rodriguez 2009). In addition to making homology statements between highly divergent sequences possible, secondary structure prediction can incoporate important characters in the analysis (Aguilar and Reimer 2010; McFadden et. al. 2010). Although ITS secondary structure could be used to guide the alignment and make interpretation about the ITS region more general if other species could be included in this study, ITS structural does not exist for sea anemones and the development of one was beyond the scope of this study. Thus, future studies should incorporate the exploration of secondary structure to guide alignments to include

distantly related species of sea anemones and m Takabayashi ake generalizations of ITS diversity and phylogenetic analysis possible.

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Species	Genbank Acc. No.	No. of ind.	No. of clones / ind.	ITS-1		5.8S		ITS-2		ITS region	
				Length (bp)	%GC	Length (bp)	%GC	Length (bp)	%GC	Length (bp)	%GC
Calliactis polypus	HQ156276-HQ156440	36	10	189-191	49.90	148	52.04	186-191	66.12	525-530	56.02
Calliactis tricolor	HQ156441-HQ156452	1	12	186-187	48.68	148	52.02	186-188	61.13	521-522	53.94
Calliactis parasitica	HQ156453-HQ156456	1	12	197-198	50.50	148	52.02	222	65.13	567-568	56.62

Table 4.1. Genbank accession numbers, length and G+C content (%) in ITS partitions and complete ITS region in the three species of *Calliactis* examined.

Taxa	# haplotypes				# haplotypes/ individual				Intra-specific pairwise distance (K2P)			
	ITS-1	5.8S	ITS-2	ITS region	ITS-1	5.8S	ITS-2	ITS region	ITS-1	5.8S	ITS-2	ITS region
C. parasitica	3	3	3	4	3	3	3	4	0.012	0.002	0.016	0.009
C. tricolor	4	6	4	12	4	6	4	12	0.005	0.004	0.011	0.009
C. polypus	63	49	74	165	3.58	2.61	4.71	6.28	0.006	0.003	0.013	0.007
Hawaii	36	20	45	50	3.60	2.00	4.50	5.00	0.006	0.002	0.015	0.008
Japan	50	43	66	72	3.33	2.87	4.40	4.80	0.005	0.003	0.011	0.007
Maldives	14	12	14	23	2.80	2.40	2.80	4.60	0.003	0.003	0.006	0.004
Saipan	11	10	12	19	5.50	5.00	6.00	9.50	0.007	0.006	0.016	0.010
South Africa	18	9	13	12	4.50	2.25	3.25	2.40	0.009	0.001	0.016	0.009

Table 4.2. Number of haplotypes per species, individual and population for each internal transcribed spacer (ITS) partition. Intra-specific distanes (%) calculated using the Kimura 2-parameter. K2P= Kimura 2-parameter.

Species	*Calliactis polypus*				*Calliactis tricolor*			
	ITS-1	5.8S	ITS-2	ITS region	ITS-1	5.8S	ITS-2	ITS region
Calliactis tricolor	0.080	0.003	0.087	0.060				
Calliactis parasitica	0.353	0.030	0.306	0.232	0.344	0.031	0.295	0.225

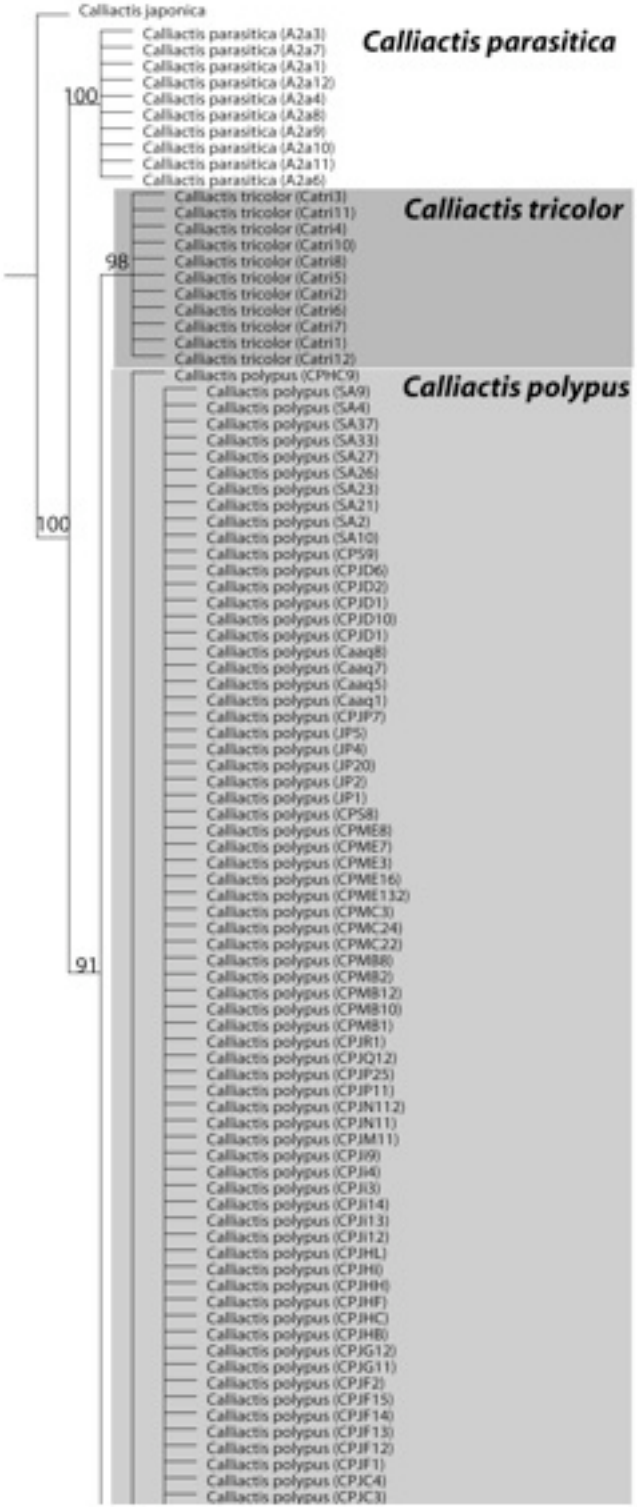
Table 4.3. Average interspecific divergence (Kimura 2 parameter) between the three *Calliactis* species.

Partition	# Chars.	# PI sites (%)	# MPT	L	Best-fitting model
ITS-1	200	75 (37.5)	31	137	SYM+I
ITS-2	223	68 (30.49)	43	148	TVM+I
5.8S	148	21 (14.19)	2	60	GTR
ITS region	571	164 (28.72)	49	377	TVM+G

Table 4.4. Summary of sequence information for separate gene partitions and combined analysis and tree information for parsimony and maximum likelihood analyses. Chars = characters, PI = Parsimony informative, MPT = most parsimonious trees, L = length of trees.

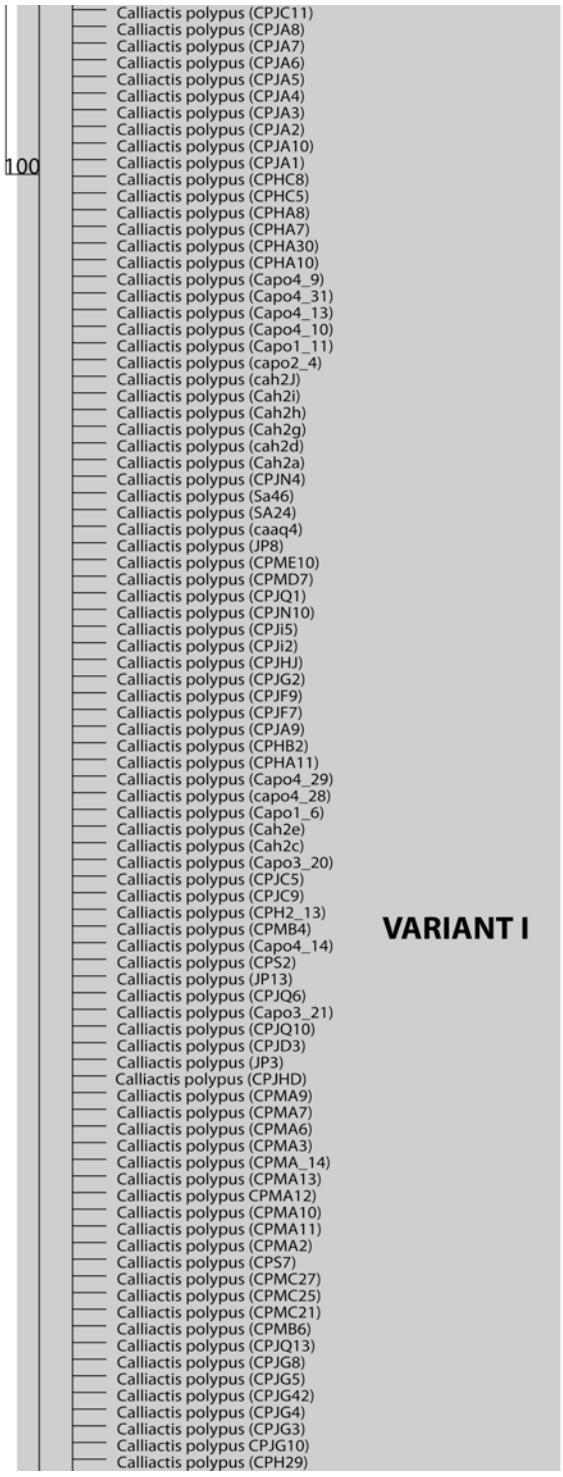
4.1. Strict consensus of the most parsimonious trees resulting from an analysis of all internal transcribed spacers (ITS) sequences. Numbers on branches are jackknife values. Only jackknife values higher than 50% are shown above branches. Grey rectangles indicate the different species of *Calliactis* and internal transcribed spacers (ITS) variants I and II.

Figure 4.1



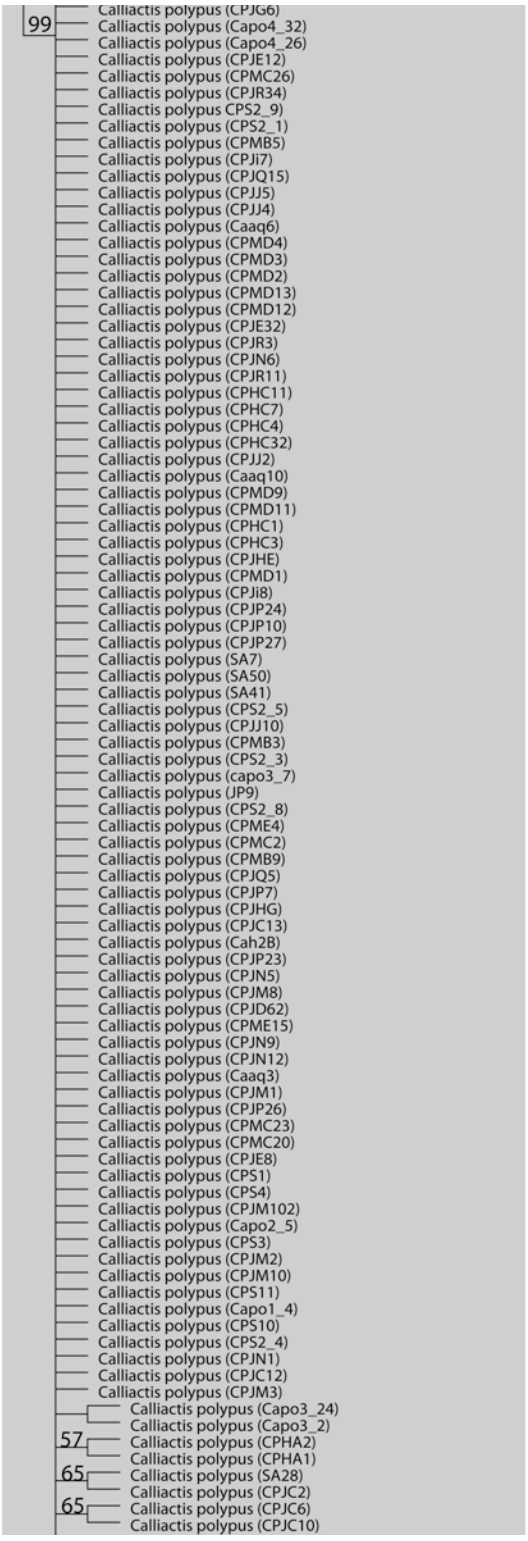
Continued

Figure 4.1 Continued



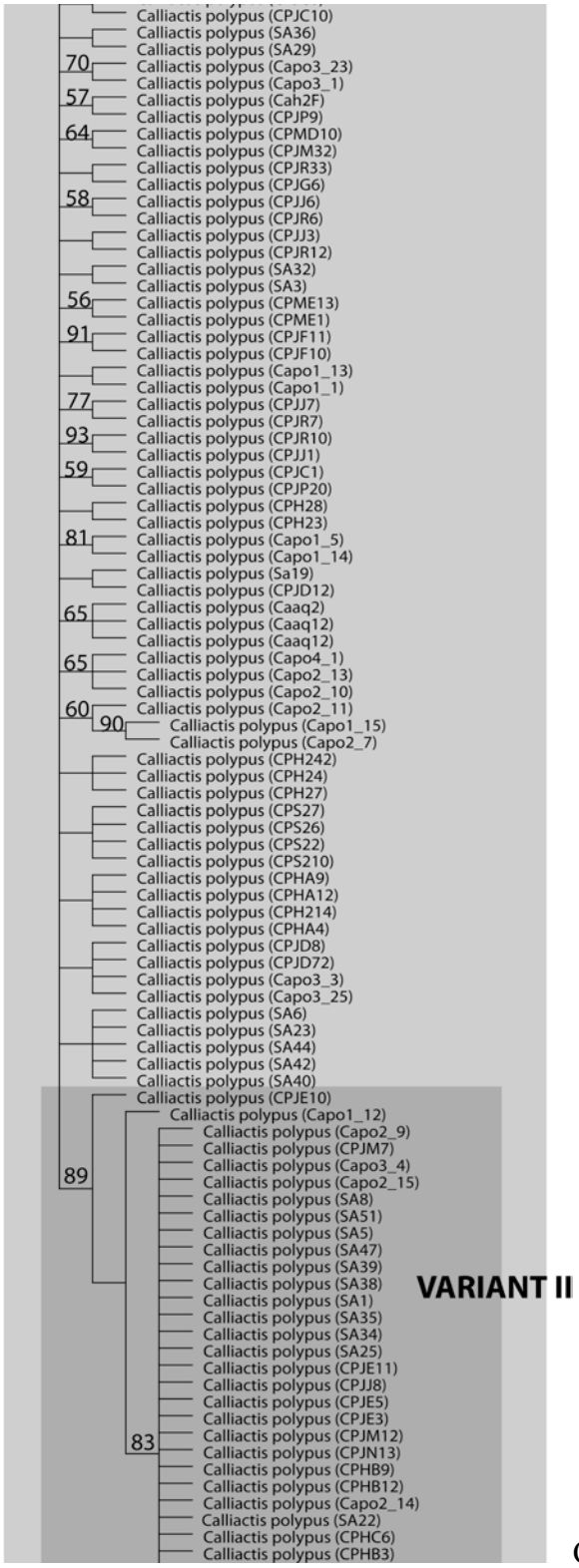
Continued

Figure 4.1 continued



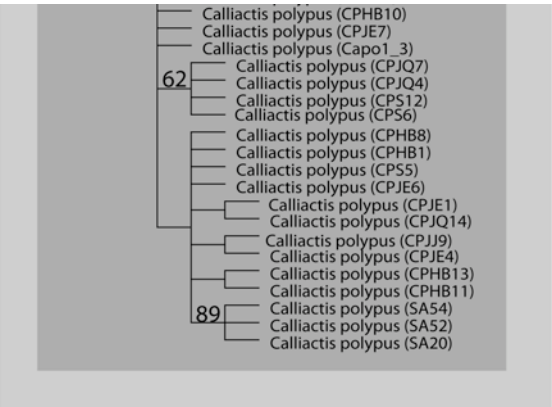
Continued

Figure 4.1. Continued



Continued

Figure 4.1. Continued


Calliactis polypus (CPHB10)
Calliactis polypus (CPJE7)
Calliactis polypus (Capo1_3)
62
Calliactis polypus (CPJQ7)
Calliactis polypus (CPJQ4)
Calliactis polypus (CPS12)
Calliactis polypus (CPS6)
Calliactis polypus (CPHB8)
Calliactis polypus (CPHB1)
Calliactis polypus (CPS5)
Calliactis polypus (CPJE6)
Calliactis polypus (CPJE1)
Calliactis polypus (CPJQ14)
Calliactis polypus (CPJJ9)
Calliactis polypus (CPJE4)
Calliactis polypus (CPHB13)
Calliactis polypus (CPHB11)
89
Calliactis polypus (SA54)
Calliactis polypus (SA52)
Calliactis polypus (SA20)

Chapter 5: Morphological revision of *Adamsia, Calliactis* and *Paracalliactis*

Introduction

Sea anemones symbiotic with hermit crabs belong to six genera in three families: Actiniidae (*Stylobates*), Hormathiidae *(Adamsia, Calliactis, Paracalliactis*) and Sagartiidae (*Carcinactis*, *Verrillactis*). The vast majority of anemones that engage in symbiosis with hermit crabs belong to family Hormathiidae (26/32 species). This partnership consists of one or more anemones living on a shell inhabited by a hermit crab, and often entails extreme modification of the sea anemone's body to life on a shell (Ross 1974a; Daly et.al. 2004; Gusmao and Daly 2010). In most cases, the association with a hermit crab profoundly affects the anemone's anatomy, with animals usually exhibiting the pedal disc much greater than the oral disc to enwrap the shell, producing a carcinoecium and having some mesenteries displaced as they grow to follow the curve of the shell (Daly et. al. 2004). Additionally, crab-symbiotic anemones may exhibit a peculiar position on the shell: the oral disc of the anemone may be positioned right below the aperture of the shell, just beneath the legs of the hermit crab, or may be located away from the aperture of the shell. Differences in the position of the anemone on the shell have consequences for the way the pedal disc wraps the shell and produces the carcinoecium (Hand 1975a; LCG pers.obs.).

The symbiosis can be established by either partner: the sea anemone may transfer itself unaided to a shell in one of the most complex behavior exhibited by any cnidarian, or the hermit crab may discriminate among different species of anemones, using tactile stimuli to detach the desired anemone from the substrate and put it on its shell (Ross 1974b). The sophistication of the responses and coordination between the partners has no parallel among other marine symbioses (Ross 1974a). Three possible types of benefit may occur in this symbiosis: defensive, phoretic, and trophic (Ross, 1974a; Dunn et. al. 1981; Daly et. al. 2004). The crab may be defended by sea anemones, more specifically by the stinging capsules (nematocysts) made by them (Ross and Boletzky 1979) or, in some instances, the anemone may provides the crab with a means of avoiding the critical necessity of replacing shells as it grows by forming a living "cloak" that expands the living space of the crab (Dunn et. al. 1981; Ross, 1974a; Doumenc 1975). Especially in the deep sea where calcium carbonate (the major constituent of shells) is easily dissolved and large shells are scarce, the association with a sea anemone is extremely beneficial to the crab (Fautin, 1992). Additionally, the sea anemone increases its dispersal capability and gains a consolidated substrate and additional access to food by the scavenger hermit crabs (Ross 1974b; Williams and McDermott 2004). Although these benefits explain the maintenance of the symbiosis, they cannot explain its origin or evolution.

Prior to this taxonomic revision, the group of sea anemones symbiotic with hermit crabs in family Hormathiidae included 26 nominal species (Fautin, 2010). The hormathiid genera *Adamsia, Calliactis and Paracalliactis* form a complex whose

members differ morphologically in degree rather than kind (Daly et. al. 2004; Gusmao and Daly 2010), making generic and specific identification difficult. The 3 species of *Adamsia* present the highest degree of obligation and are distinguished from *Calliactis* by attributes related to their obligate symbiosis, such as secretion of carcinoecium and asymmetry of the pedal disc, and reproductive characters (Daly et al. 2004). Currently comprised of 18 valid species, *Calliactis* is the most diverse genus and includes anemones that may live independently of hermit crabs. *Paracalliactis* comprises eight species of anemones that resemble *Adamsia* in that they secrete a cuticle over the surface of the host shell and present extreme adapation of the pedal disc to a symbiotic habit. The same internal characteristics that distinguish *Calliactis* from *Adamsia* unite the former and *Paracalliactis*; both *Calliactis* and *Adamsia* differ from *Paracalliactis* in having perforations called cinclides in the column. Because genera are distinguished by a mosaic of features, the boundaries between taxa are not clear and the taxonomic history of the group is problematic (Duerden 1902; Carlgren 1928b; Doumenc 1975; Hand 1975a; Daly et. al. 2004; Gusmao and Daly 2010).

The taxonomy of these animals is further obscured because most descriptions of these animals are old, and many are poor, lacking discussions of currently relevant taxonomic characters (Hand 1975a; Daly et al.. 2004; Gusmao and Daly 2010). For example, not all descriptions of species include mention of whether the column bears cinclides, although this attribute is the most critical feature distinguishing *Paracalliactis* from *Adamsia* or *Calliactis* (Daly et al. 2004). Many species are

known from a single collection of one or few specimens, and so variability in anatomy or in the size ranges of nematocysts cannot be evaluated. Furthermore, many of the taxonomically relevant features, such as the secretion of a carcinoecium, or the asymmetry of the pedal disc, arise as a consequence of symbiosis, and thus convergence and ecophenotypic plasticity may present problems (Daly et al. 2004).

Phylogenetics

Monophyly of the group of sea anemones symbiotic with hermit crabs

In his revision of symbiotic sea anemones, Ross (1974a) was the first to propose evolutionary hypotheses for the symbiotic sea anemones in family Hormathiidae. Difficulties arise when trying to translate evolutionary considerations from Ross' studies into trees, given the largely non-phylogenetic nature of his work. For example, in his hypotheses about the evolution of this symbiosis, Ross (1974a) does not specify whether each symbiotic hormathiid genus evolved independently or if some of them are more closely related to each other (Gusmao and Daly 2010). We interpret Ross' hypothesis (1974a) as stating that all three symbiotic genera evolved independently of each other. A sister group relationship between *Adamsia* and *Calliactis* was explicitly denied by Ross (1974a). However, close evolutionary relationship among sea anemones symbiotic with hermit crabs in genera *Calliactis*, *Adamsia* and *Paracallicatis* has been hypothesized based on morphology (e.g., Duerden 1902; Cutress and Ross 1969; Hand 1975a; Daly et al. 2004) and behavior (Ross 1974b; Hand 1975b; Ates 1997). Monophyly of hermit-crab symbiotic

anemones has been justified by the remarkable nature of the biological adaptation and complex behavior exhibited by the partners (Ates 1997). The production of a chitinous carcinoecium, for example, is a complex anatomical and biological feature that could be interpreted as a sign of a shared evolutionary history of *Paracalliactis* and *Adamsia*. Similarities in the morphology of sea anemones in this group have led to considerations of some genera of symbiotic hormathiids as a single species complex (e.g., *Paracalliactis* and *Calliactis* by Hand, 1975a; *Calliactis* and *Adamsia* by Duerden 1902). A close affinity between *Adamsia* and *Calliactis* has been proposed on morphological grounds (Duerden 1902; Daly et al. 2004): they are similar in morphological attributes associated with a symbiotic habit (both have a bi-lobed base that enwraps the shell completely and produce either a carcinoecium or a less developed, plate-like cuticle).

The assumption of monophyly for the group, however, may be biased by the morphological characters related to the symbiosis (see Table 2.1). Because the distinction between *Adamsia*, *Calliactis,* and *Paracalliactis* is primarily based on characters related to a symbiotic habit, functional rather than phylogenetic explanations may be more relevant, and these characters may not be appropriate to delimit monophyletic groupings (Daly et al. 2004). Furthermore, various characters have been given greater or lesser importance in the differentiation of these genera, including posture on the shell and shape of the pedal disc (Verrill 1869), and number of perfect sterile mesenteries (Carlgren 1928a, 1949), complicating the identification and description of species.

Behavioral studies have shown that the capacity to respond to tactile stimulus from hermit crabs leads to relaxation and detachment only in anemones that live in association with crabs (Ross 1974b). The shell mounting behavior is also largely confined to sea anemones living in symbiosis with either gastropods or hermit crabs (McFarlane 1969). On the other hand, many hormathiids engage in interactions with gastropods or other sessile invertebrates (e.g., Ross and Kikuchi 1976; Riemann-Zürneck 1994; Ates 1997a, b; Mercier and Hamel 2008), suggesting that some elements of the behavior are shared much more widely across Hormathiidae.

The first modern comprehensive treatment of the evolution of sea anemones symbiotic with hermit crabs using phylogenetic methods was that of Gusmão and Daly (2010). This multi-locus molecular phylogeny provides a context for evaluating characters that have been traditionally used for delimiting taxa in this group. For example, Gusmão and Daly (2010) found no support for the monophyly of the hermit crab symbionts: *Adamsia* and *Calliactis* are each others' closest relatives but they are not closely related to *Paracalliactis*. This demonstrates that similarities in morphology and behavior among some genera in the group have to be interpreted as related to the ways in which a symbiotic habit arises and is maintained, and not due to shared evolutionary history.

Characters related to a symbiotic habit, such as the presence of a bi-lobed (wrapping) base and the production of a carcinoecium, should not be used to differentiate genera as they do not define monophyletic groups. The carcinoecium is interpreted as a convergent feature, evolving separately in *Paracalliactis* and

Adamsia. Similarly, the affinity of *Adamsia* and *Calliactis* demonstrates that other anatomical and micro-anatomical characters, including the occurrence of cinclides, carry phylogenetic signal and should have a greater role in taxonomy. Additionally, *Calliactis* is not monophyletic due to the inclusion of *Adamsia palliata* within *Calliactis* as the sister of *C. parasitica* (Gusmão and Daly 2010). The hypothesis of Brooks et al. (1995) that *C. polypus* is the most "advanced" species of *Calliactis* as evidenced by the lack of a shell response and a greater dependence on active crabs is also rejected by Gusmão and Daly (2010). The widespread *C. polypus* contains many junior synonyms described from more restricted locales (reviewed in England 1971); an extensive revision of *C. polypus* is necessary to establish boundaries between this species and other species of *Calliactis*. Gusmão & Daly (2010) only included one species of *Adamsia* and *Paracalliactis*, leaving the monophyly of these two genera untested.

Objectives

The primary goal of this taxonomic revision of genera *Calliactis, Paracalliactis* and *Adamsia* is to document the diversity of the group and limit and circumscribe species and genera. It provides a comprehensive perspective on diversity and variation within the group because it includes most nominal species and addresses important taxonomic tasks such as validation of names and designation of neotypes. The complexity of taxonomic issues and the overlapping definitions of taxa necessitate that the three genera be considered simultaneously. This strategy will

alleviate difficulties associated with species description and circumscription of genera and make the identification of species easier.

Material and methods

Material

The results of this study are based on examination of more than 500 specimens. Most of the material examined for this monograph was loaned from museum collections worldwide. Complete information (museum collection, catalog number, number of specimens examined, locality, depth) about specimens used is given in each species description and summarized in Appendix A.

In this study three genera in the family Hormathiidae were examined. The classification follows Carlgren (1949):

Order Actiniaria

Suborder Nynantheae Carlgren 1899

Tribe Acontiaria Carlgren in Stephenson 1935

Family Hormathiidae Carlgren 1925

Genus *Adamsia* Forbes 1840

Adamsia palliata (Muller 1776)

Adamsia sociabilis Verrill 1882

Genus *Calliactis* Verrill 1869

Calliactis algoaensis Carlgren 1938

Calliactis androgyna Riemann-Zürneck 1975

Calliactis annulata Carlgren 1922

Calliactis brevicornis (Studer 1879)

Calliactis conchiola Parry 1952

Calliactis japonica Carlgren 1928

Calliactis parasitica (Couch 1842)

Calliactis polypus (Forsskål 1775)

Calliactis tricolor (Le Sueur 1817)

Calliactis tigre sp. nov.

Calliactis valdiviae Carlgren 1938

Genus *Paracalliactis* Carlgren 1928

Paracalliactis consors (Verrill 1882)

Paracalliactis michaelsarsi Carlgren 1928

Paracalliactis rosea Hand 1976

Paracalliactis valdiviae Carlgren 1928

Paracalliactis obvolva (Daly, Cha and Fautin 2004)

Paracalliactis niwa sp. nov.

Collection, anesthesia and fixation

Live animals were collected either by hand in the intertidal zone or by SCUBA in deeper waters (up to 35m). After collection, animals were taken to the laboratory, where live observations were made based on specimens kept in aquaria. Live observations are important, as many characters, such as coloration, number and cycles

of tentacles, morphology and specializations of the column and behavior are more easily or exclusively observed. In these occasions, live preparations of cnidae were also made to visualize and identify exploded capsules. All live specimens were photo documented both in the field and in the laboratory.

After live observations were made, the material was anesthetized using menthol. Few crystals were added to the water where animals were kept to avoid violent contractions of the body and tentacles. This could take hours or days, depending on the size and state of the animal. When completely anesthetized (no response to poking using a forceps), the animals were fixed, preferentially in 10% formalin, but most of the time in 75% ethanol. When fixed in formalin, animals were later transferred to 75% ethanol for storage (2-3 months later). During fixation of live animals, little pieces of the base, column or tentacle were also preserved in absolute ethanol (~96%) for molecular studies.

Morphology and anatomy

Most of the material used in this revision is preserved museum specimens (see Appendix A). Specimens were examined whole and in dissection for external and internal anatomical characters used in anemone taxonomy using a stereomicroscope. Morphological characters used in this monograph include: number and cycles of tentacles, specializations of the column, number and cycles of mesenteries, fertility of mesenteries, number of directive mesenteries, number of syphonoglyphs, direction of mesentery growth, etc. Measurements include: oral disc width, column height and

width, base width and, when possible, size of the longest and shortest tentacles. To examine the internal anatomy, a series of transversal cuts along the column were made to see the number and cycle of mesenteries and direction of growth of the mesenteries. These cuts were examined under a stereomicroscope and the number and cycles of mesenteries, number of directive mesenteries, number of siphonoglyphs and distribution of gonads (fertility) were analyzed. Furthermore, drawings of mesentery distribution were made to facilitate the visualization of the anatomical irregularities in the specimens studied.

Histology

For microanatomical study, especially of the musculature and reproductive anatomy, well-fixed and relaxed individuals were chosen for histology. Depending on the size of the individual, whole specimens (for small individuals) or pieces of tissue (for large individuals) previously fixed in formalin (preferentially) or in 75% ethanol were used. The whole specimen or piece of tissue was first dehydrated and embedded in Paraplast X-tra using an Automatic Tissue Processor LEICA TP1020. Blocks using the tissue were prepared using paraplast and serial sections of 10 μm were made using a manual microtome. Histological sections were stained using Masson's trichrome (Presnell and Schreibman 1997).

Cnidae

The identification of cnidae is more realiable and easily made when discharged

capsules are observed because the visualization of the nematocyst and its filament and shaft are clearer. For this reason, the identification of cnidae was based, when possible, on squash preparations using live tissue from animals kept in aquaria. When using live animals, only types of cnidae were identified following the nomenclature of Weil (1934) modified by Carlgren (1940), with measurements made using undischarged capsules. Forceps were used, during anesthesia, to take small fragments of tentacles, column, pharynx, filament and acontia. These fragments were macerated under a stereomicroscope with the help of needles until the tissue was completely dissociated. Slides were examined under 1000x magnification with differential interference optics.

After identification, nematocysts length and width were measured using undischarged capsules in preserved specimens. Nematocysts change slightly in shape and size after explosion, so undischarged capsules were measured using slides made from material fixed in 75% ethanol. Different cnidae types of each tissue were photographed for reference. For each species, at least five specimens were used to measure at least 15 cnidae per tissue (when capsules were abundant). Results are presented with average values maximum and minimum length and width for each type of cnida per tissue. When distinct size peaks were found they were classified as distinct classes even when there was continuity in the length/width of a particular cnidae type.

How many species are there?

The monographic revision was based mostly on preserved specimens from museum collections (Appendix A). For this reason, species will be differentiated based on morphological characters using the Morphological Species Concept (sensu de Queiroz 1999).

Character description and phylogenetic analysis

The data set containing characters relevant to the relationships among *Adamsia*, *Calliactis* and *Paracalliactis* and the outgroups used in this study comprised 25 characters (see Appendix B). Character definitions include written description and, if necessary, figures. The data matrix includes characters related to external morphology, internal anatomy, microanatomy and cnidae. Character descriptions and definitions are given in Appendix 1. Most characters used in the present study were coded using reductive (binary) coding with inapplicables treated as '-', combined with multistate characters (see Strong and Lipscomb 1999). All multistate characters are treated as unordered. Missing information was represented by a '?' in the data matrix. Morphological characters were coded according to direct observations made based on preserved specimens. In the few cases where polymorphism among specimens was found, the characters were scored after the condition in the type material was observed.

Parsimony analyses were run on TNT (Goloboff et. al. 2008) and node support was estimated 1000 rounds of parsimony jackknifing. Winclada (Nixon 2002) and

McClade 4.01 (Maddison and Maddison 2002) were used to optimize and trace character state changes on the preferred tree and to calculate the number of characters that support each node.

Results

Phylogenetic analysis

The morphological analysis of the species of sea anemones symbiotic with hermit crabs in genera *Adamsia, Calliactis* and *Paracalliactis* plus two outgroups resulted in a single most parsimonious tree of 76 steps (CI=0.59; RI=0.78) (Figure 5.1). The monophyly of each genus is supported at jackknife values of 90% or greater.

In the single most parsimonious tree, the species of symbiotic sea anemones are divided into two larger clades. The first clade comprise of all nine species of *Paracalliactis,* and this is sister to a clade comprising the two species of *Adamsia* and all ten species of *Calliactis*. The sister group relationship between *Adamsia* and *Calliactis* in this second clade is supported by a jackknife value of 90%. In addition, a few pairs of sister taxa are recovered: *P. sinica* is sister to *P. azorica*, *P. consors* is sister to *P. obvolva*, *C. polypus* is sister to *C. parasitica*, *C. tigre* is sister to *C. tricolor*, and *C. conchicola* is sister to *C. androgyna*. All of these sister-taxa relationships are supported by at least two morphological characters.

Species description

Family Hormathiidae Carlgren 1932

Diagnoses (modified from Carlgren 1949 and Daly et. al. 2004)

Acontiaria with distinct base and strong mesogleal sphincter. Mesenteries not divisible into macro- and micro-cnemes. Usually six pairs of perfect mesenteries, sometimes more, but never numerous. Perfect mesenteries usually sterile, rarely fertile. Nematocysts of the acontia basitrichs only. Spirocysts usually dimorphic: gracile spirocysts have narrower tubule and smaller capsule than robust spirocysts. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores.

Genus *Adamsia* Forbes 1840

Diagnoses (modified from Carlgren 1949 and Daly et. al. 2004).

Hormathiidae with very wide base divisible into two lobes, usually of greater diameter than oral disc. Column smooth, not differentiated into scapus, scapulus and capitulum. Cinclides are present close to the base and sometimes situated on slight elevations. Column nematocysts basitrichs only. Tentacles in several cycles, never long. Twelve pairs of perfect, sterile mesenteries, two pairs of directives associated with one siphonoglyph each. Mesenteries more numerous at the margin. Retractors and parietobasilar muscles weak. Species of this genus live attached to shells inhabited by hermit crabs and secrete a carcinoecium. Due to the symbiotic habit, the

oral disc of the anemone is always situated downwards, beneath of the mouth of the crab. Cnidom: spirocysts, basitrichs and microbasic *p*-mastigophores.

Type species: *Adamsia palliata* (Muller 1776)

Valid species: *Adamsia palliata* (Muller 1776)

Adamsia sociabilis Verrill 1882

Adamsia fusca (Quoy and Gaimard 1833)

Adamsia palliata **(Muller 1776)**

Medusa palliata Fabricius 1779: 328

Actinia maculata Adams 1800: 8-9

Actinia carciniopados Otto 1823: 288-292; no *Actinia carciniopados* Delle Chiaje 1822: 242-243;

Actinia cancincopados Forbes 1840: 183

Actinia picta Risso 1826: 286

Cribrina palliata Ehrenberg 1834: 41; Brandt 1835: 15

Adamsia palliata Andres 1881: 306, 324-325, 338; Gosse and Johnston 1847: 201-210; Fischer 1889: 254, 272; Haddon 1890: 373; Carlgren 1942: 43; Carlgren 1945: 130, 157; Pax 1952: 13-16; Schmidt 1972: 35-38; Chintiroglou Doumenc and Koukouras 1985: 517, 525; Zamponi 1985: 71; Loukimidou Doumenc and Chintiroglou 1996: 98; Ates 1997: 15, 27; Vafidis and Chintiroglou 2002: 90-91

Sagartia palliata Duerden 1905: 507

Adamsia carciniopados Manuel 1981: 176-177; Ocaña and Den Hartog 2002: 39, 48; Wirtz Ocaña and Molodtsova 2003: 116.

Material examined: See Appendix A.

External anatomy (Figure 5.2)

Pedal disc and carcinoecium: Pedal disc very adherent and composed of two lobes (bi-lobed). These lobes grow around the shell inhabited by the hermit crab and unite beneath it. In larger specimens, the pedal disc completely wraps the shell, but smaller specimens may not completely wrap the shell. The pedal disc secretes a thin but conspicuous, golden or brown-colored carcinoecium that may extend beyond the hermit crab shell. In fact, most specimens do not present any sign of a shell, which has probably been completely dissolved. Pedal disc diameter of the specimens examined from 1.8 to 7.0 cm.

Column: Column thin, smooth, not divisible into a scapus and scapulus, with cinclides in one row (sometimes two rows) located proximally, close to region where the two lobes of the base meet. These cinclides may be located in small elevations, which may give the column an appearance of not being smooth. Abundant acontia usually protrude through cinclides in preserved specimens. In live animals, the column is yellow, becoming dark bege distally, with pink or purple dark spots.

Preserved specimens are yellow, bege or light pink. In preserved specimens, column height 0.5 – 3.8 cm, width 0.4 – 3.0 cm.

Oral disc and tentacles: Due to morphology of the base and the position of the anemone on the shell, the oral disc is situated beneath the hermit crab's mouth. Oral disc light yellow in color in live animals and light bege in preserved specimens, width up to 2.4 cm. Small, central mouth that can be pink or yellow in color. Tentacles very numerous, up to 200, short, distributed in 4 or 5 cycles, inner tentacles of first and second cycle slightly longer (up to 1 cm). Tentacles light yellow, uniform in color.

Internal anatomy and microanatomy

Mesenteries regularly arranged in 5 cycles proximally (6+6+12+24+48). Same number of mesenteries proximally and distally. First and second cycles of mesenteries perfect and sterile and third and fourth cycles imperfect and fertile. A fifth cycle is regularly arranged, but very reduced without filaments or gonads. Two pairs of directives associated with one siphonoglyph each. Retractor and parietobasilar muscles weak and diffuse. Sphincter mesogleal and strong. Acontia very abundant, coiled, and present in all pairs of mesenteries, except for those of the fifth cycles. Acontia are purple or pink in live specimens and light yellow or bege in preserved specimens.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.3). For measurement of capsules see Table 5.1.

Distribution: *Adamsia palliata* has the widest geographic distribution among *Adamsia* species. Most of the records for this species are from the North Atlantic, including Norway, Scotland, Wales, Northern Ireland, Ireland, England, and France. *A. palliata* has also been collected from the Madeira Archipelago and the Canary Islands (Ocanã and den Hartog 2002). Additional records include Italy in the Mediterranean Sea (Loukmidou et. al. 1996) and Croatia in the Adriatic Sea (Kruzic, 2002). The bathymetric range of *A. palliata* is wide and this species can be found between 1 and 613 meters. Abundant between the depths of 15 and 60 meters.

Discussion

Due to its wide distribution in the North Atlantic and in the Mediterranean sea, *A. palliata* has been included extensively in European inventories of cnidarians and marine invertebrates (e.g., Jourdan 1880; Pax and Muller 1962; Doumenc et. al. 1985; Chintiroglou and Den Hartog 1995; Loukmidou et. al. 1996; Kruzic 2002). The original description and most of the subsequent studies, however, do not include information on the characters used in modern taxonomy and rarely go beyond the description of the anemone's external morphology **(**e.g., Muller 1776; Forbes 1840; Dalyell 1848; Johnston 1847; Gosse 1858; Thompson 1858; Andres 1883; Haddon 1890; Pax 1914**)**. This might be due to the fact that the species can be identified

without taking into consideration its internal anatomy and microanatomy characteristics or to difficulties in observing these characters in anemones with such peculiar and extreme morphology. In addition, *A. palliata* is easily distinguished from *Calliactis parasitica*, the only other sea anemone symbiotic with hermit crabs found in the same geographic and bathymetric range. For this reason, the internal anatomy and nematocyst distribution of *A. palliata* have only been described in a few studies (Stephenson 1935; Schmidt 1972) and issues regarding its internal anatomy, including the number of pairs of perfect and sterile mesenteries, a character that has been used to diagnose *Adamsia*, for example, is still in unclear.

The specimens of *A. palliata* examined in the present study agree with descriptions of external morphology and coloration of *A. palliata* made by several authors (e.g., Stephenson 1935; Schmidt 1972; Doumenc et. al. 1985). The distribution and dimensions for the nematocysts of *A. palliata* found in the specimens examined here largely agree with those described by others (Stephenson 1935; Carlgren 1945; Schmidt 1972; Doumenc et. al. 1985). A few differences were found, however, with Zamponi (1985). For example, spirocysts is only found in the tentacles of *A. palliata*, not in the column, pharynx, filament or acontia as described by Zamponi (1985). Similarly, the presence of atrichs in the pharynx and filament of *A. palliata* found by Zamponi (1985) is not confirmed in this study. The presence of spirocysts in the structures given by Zamponi (1985) is probably due to contamination as spirocysts were never observed in the present study despite measuring a very large number of capsules (Table 5.X). Although Zamponi (1985)

reports atrichs in the pharynx and filament, these were never observed in the many *A. palliata* examined. In addition, the presence of spirocysts in the acontia of *A. palliata* is largely unlikely given that the family Hormathiidae is characterized by having acontia with basitrichs only. Another difference between the cnidae described here and previous reports is that a second size of basitrichs in the column was relatively common in the specimens examined. This difference has never been described before for the species..

Daly et. al. (2004) raises the question of whether all species of *Adamsia* are characterized by a second cycle of perfect and sterile mesenteries or if this feature is unique to *A. palliata*. Although Stephenson (1935), Carlgren (1928, 1949) and Manuel (1981) all report the second cycle of perfect and sterile mesenteries, Schmidt (1972) did not. After an extensive study of the internal anatomy of *A. palliata*, the present study confirms a second cycle of perfect and sterile mesenteries in this species. These can be visualized especially in those mesenteries that are located proximally, where mesenteries are more regularly spaced.

Adamsia sociabilis Verrill 1882

Adamsia sociabilis Verrill 1883: 45; Verrill 1885: 534; Parker 1900: 755; Carlgren 1949: 98; Carlgren 1950: 26-27; Sebens 1998: 12, 15, 28, 53-54; Daly 2002: 212, 223.

Material examined: See Appendix A.

External anatomy (Figure 5.4)

Pedal disc and carcinoecium: Pedal disc very adherent and expanded, forming two lobes that surround the aperture of the shell and unite close to it. Pedal disc much wider than the column diameter. The pedal disc secretes a thin, brown or golden-colored carcinoecium that usually extends beyond the shell, increasing the living space of the crab. Sometimes, parts of the shell can be seen in smaller specimens but, in most cases, only the carcinoecium, not the shell, is observed. The shell has been probably completely dissolved. Pedal disc diameter in preserved specimens range from 0.5 to 1.8 cm.

Column: Column very thin, smooth, not divisible into a scapus and scapulus, with one row of cinclides close to the base. Cinclides are conspicuous in some specimens, but in others they can only be assumed by the presence of acontia coming though the pore. Clear mesenterial insertions can be seen in most specimens examined. The color of the column in preserved specimens is pinkish, bege, or light brown. Live specimens have a translucent column with pink and white longitudinal stripes

(Verrill, 1883). In preserved specimens, column height 0.2 – 0.5 cm, width 0.2 – 0.4 cm.

Oral disc and tentacles: The oral disc was only observed in a few specimens as most specimens examined had their oral disc fully retracted into the gastrovascular cavity. In these specimens, mesenterial insertions and a small central mouth were observed. The oral disc is positioned downwards, beneath and behind the crab legs (close to the aperture of the shell). Oral disc bege in preserved specimens, diameter 0.1 – 0.3 cm. Given the size of the animal, it was very difficult to count the number of tentacles, but it was clear that they were never more numerous than 96, and possibly distributed in 4 cycles. Inner tentacles longer than outer ones, same color as the oral disc in preserved specimens (pinkish in live specimens: Verrill 1883).

Internal anatomy and microanatomy

Forty-eight pairs of mesenteries are found proximally, distributed in four cycles (6+6+12+24). Mesenteries are more numerous distally (below the oral disc), where up to 200 pairs were counted. These extra pairs of mesenteries located distally are small, without developed retractor muscles and without filaments, forming a fifth cycle that is irregularly arranged. Mesenteries of the first and second cycles perfect and sterile, those of the third and forth cycle imperfect and fertile. Retractor and parietobasilar muscles weak and diffuse. Two pairs of directives associated with two siphonoglyphs. Acontia bege, not very abundant in preserved specimens.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.3). For measurement of capsules see Table 5.2.

Distribution: *Adamsia sociabilis* is has a very narrow geographic distribution compared to *A. palliata* being known only from western Atlantic Ocean. This species has been mostly collected from Martha'sVineyard, Massachusetts, USA (Sebens 1998). Two additional records includes five specimens from Southern New England, USA and three specimens from Long Island, NY, USA (Sebens 1998). The bathymetric distribution of this species is 138 – 900 meters; the majority of specimens are from 140 – 235 meters.

Discussion

The original description of *A. sociabilis* (Verrill 1882a) is not very complete, including only information about coloration and size of live specimens collected off the coast of Massachusetts. Verrill (1882b; 1883), however, later adds more information to the species description, including characteristics of external anatomy, such as column and base morphology, presence of a carcinoecium, morphology and number of tentacles and position of the anemone on the shell. *Adamsia sociabilis* external morphology is characteristic of species of *Adamsia*: smooth column with cinclides close to the base, the base becomes bi-lobed and wraps the shell uniting when they become in contact, secreting a carcinoecium that dissolves the shell as it

expands. Carlgren (1950) is the first to describe the cnidae of *A. sociabilis*, but does not find acontia. The internal anatomy and microanatomy, as well as the external anatomy and cnidae, of the species is only described much later in Sebens (1998) faunal guide of hexacorallians from the eastern United States.

The syntypes of *A. sociabilis* examined in this study agree with accounts of external and internal anatomy made by others (Verrill 1882a,b; 1883; Carlgren 1950; Sebens 1998). A second cycle of perfect and sterile mesenteries is confirmed in the present study after examination of syntypes of *A. sociabilis* in agreement with Sebens (1998). Acontia are less abundant in this species compared to *A. palliata* and/or other hormathiids symbiotic with hermit crabs, it is still present. The distribution and size of nematocysts largely correspond with those described by Carlgren (1950) and Sebens (1998), with a few notably differences: Sebens (1998) found spirocysts in the column and microbasic *p*-mastigophores in the tentacles, whereas the present study and Carlgren (1950) does not; the basitrichs of the pharynx described by Sebens (1998) are considered a different category (much smaller) than those reported by Carlgren (1950) or the in present study. The differences found between the cnidae of *A. sociabilis* by Sebens (1998) in the tentacles and column nematocysts might be explained as contamination from different tissues. This is particularly plausible as these nematocysts are described as rare, possibly contamination, by the author himself. The cnidae of the acontia of *A. sociabilis* is described in the present study for the first time.

Genus *Calliactis* Verrill 1869

Diagnoses (modified from Verrill, 1869, Carlgren 1949, Hand, 1975a, and Daly et. al. 2004).

Hormathiidae with circular or ovoid pedal disc, never bi-lobed as in *Adamsia*. The pedal disc does not secretes a cuticle or a carcinoecium and is only slightly wider than the column and oral disc. Column may be divisible into scapulus and scapulus, smooth or, rarely, with scattered tubercles. Column may be have a thin, decidous cuticle. Cinclides *always* present. Basitrichs are always found in the column. Microbasic *p*-mastigophores may be present in the column. Tentacles usually in several cycles. Mesenteries hexamerously arranged, six pairs of perfect and sterile mesenteries and two pairs of directives, each attached to a siphonoglyph. Retractor and parietobasilar muscles weak. Sphincter strong. Species belonging to this genus live attached to gastropod shells inhabited by hermit crabs with its oral disc always dorsally (directed away from the aperture of the shell).

Type species: *Calliactis polypus* **(**Forsskål 1775)

Valid species: *Calliactis polypus* **(**Forsskål 1775)

Calliactis tricolor (Le Sueur 1817)

Calliactis parasitica (Couch, 1842)

Calliactis brevicornis (Studer 1879)

Calliactis annulata Carlgren 1922

Calliactis japonica Carlgren 1928

Calliactis algoaensis Carlgren 1938

Calliactis conchicola Parry 1952

Calliactis androgyna Riemann-Zurneck 1975

Calliactis argentacorolata Pei 1996

Calliactis polypores Pei 1996

Calliactis xishaensis Pei 1996

Calliactis tigre nov. Sp.

***Calliactis polypus* (Forsskål 1775)**

Priapus polypus Forsskål 1775:102

Actinia polypus de Blainville 1830: 293

Cribrina polypus Ehrenberg 1834:264 – 265; Carlgren 1899: 16

Actinia decorata Dana 1848: 139 – 140

Adamsia decorata Milne Edwards 1857: 281

Adamsia priapus Milne Edwards 1857: 280

Calliactis decorata Verrill 1869: 481 – 482; Hertwig 1882: 74

Calliactis polypus Hertwig 1882: 65 – 67; Carlgren 1949: 97; England 1987: 206, 214, 279-280, 282; England 1971: 23 – 29; den Hartog 1997: 360; Fautin Crowther and Wallace 2008: 39, 40.

No *Calliactis polypus* Dawson 1966:176.

Adamsia decorata Andres 1883: 162 – 163

No *Adamsia rondeletti* Andres 1883: 159 – 162

Adamsia miriam Haddon and Shackleton 1893: 117, 130 – 131; Carlgren 1896: 174; *Calliactis miriam* Haddon 1898: 398, 457; Stephenson 1920: 529; Carlgren 1950: 427, 444-445.

Pro parte *Calliactis armillatas* Verrill 1928: 20 – 21

Calliactis sinensis Verrill 1869: 54; Carlgren 1949: 98.

Calliactis marmorata Studer 1879: 543

Calliactis valdiviae Carlgren 1938: 77; Carlgren 1949: 97

Calliactis variegata Verrill 1869: 481 – 482; Carlgren 1949: 97; Carlgren 1951: 429 – 430.

Material examined: see Appendix A.

External anatomy (Figure 5.5)

Pedal disc: Pedal disc adherent, thin, irregularly shaped, may be twice the diameter of the column, but never enwraps the shell completely. The fine pedal disc is bege or slightly transparent with marked white mesenterial insertions. No cuticle is present in the specimens examined. The diameter of the pedal disc is usually twice the diameter of the oral disc. Pedal disc diameter in preserved specimens range from 1.8 – 4.5 cm.

Column: Column smooth, vertically ridged in contraction, divided into scapus and scapulus. The scapus tubercles scattered proximally, but becomes smooth towards the

pedal disc. Above the pedal disc one or sometimes two rows of very conspicuous cinclides occur on slight elevations of the column. These elevations are white and contrast with the color of the column, making the cinclides very easy to observe. A thin, deciduous cuticle may be present in the scapus. The scapulus is marked with numerous longitudinal ridges and is narrow and delicate. The scapulus is not covered by a cuticle. The color of the column is variable, but generally exhibits are bege or brown background against which red, white or brown spots are scattered. These spots may form a variety of patterns in the column, including patterns where these colors alternate in more or less obvious longitudinal stripes. Most of the individuals of *C. polypus* also present a pink ring around the column very close to the pedal disc in which the column becomes very thin and white mesenterial insertions are seen. Within this pink ring, the white elevations of the column that bear cinclides are located. In preserved specimens, column height 1.1– 5.9 cm, width 1.0 – 5.1 cm.

Oral disc and tentacles: The oral disc is circular, opaque white or bege, with a diameter that may generally the diameter of the column, especially in live individuals. Given the position of the anemone on the shell, the oral disc is always located upwards. The mouth is central and small, white or bege, with two conspicuous siphonoglyphs. The pharynx is short and can be seen through the mouth of live specimens. The pharynx may possess 12 lobes that get inflated when the animal is disturbed. Tentacles are light bege or transparent and some present brown horizontal bands that alternate with white spots along the second half of each tentacle. Tentacles

are restricted to two thirds of the area of the oral disc. Tentacles are short, conical, with inner tentacles longer than outer ones. Up 240 tentacles distributed in 7 cycles (6+6+12+24+48+96+192). Tentacles are not strictly hexamerously arranged in the last two cycles. Oral disc diameter: 0.3 – 5.3 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, transversally stratified. It occupies the entire mesoglea close to the margin but tapers away from the ectoderm at its lower end. Mesenteries are more numerous close to the margin than at the center of the column. Up to 96 pairs proximally, hexamerously distributed in 5 cycles (6+6+12+24+48). At the center of the column, up to 48 pairs were distributed in 4 cycles (6+6+12+24). The first cycle of mesenteries is perfect and sterile, while those from the second, third and fourth cycle are imperfect and fertile. The mesenteries of the fifth cycle are very reduced, without musculature, gonads, filament and acontia. All mesenteries, except for those of the fifth, cycle with filaments and acontia. Retractors of the mesenteries of the first to the fourth cycles are weak and diffuse. Parietobasilar and basilar muscles weak.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.6). For measurement of capsules see Table 5.3.

Distribution: *C. polypus* is the most widespread species of *Calliactis* and has been recorded from Saudi Arabia, Djibouti and Egypt in the Red Sea (Forsskal 1775; Kluzinger 1877; Krenmpf 1905); Tanzania, Seychelles, Maldives, South Africa and Madagascar in the Indian Ocean (Carlgren 1900; Carlgren 1938; England 1987; den Hartog 1994); Philippines, Marshall Islands, Australia, Hawaii, French Polynesia, Galápagos Islands in the Pacific Ocean, China (Dana 1846; Verrill 1869; Studer 1879; Haddon and Shackleton 1893; Verrill 1928; Carlgren 1950; England 1971; Cutress 1977; Cutress and Arneson 1987; Fautin et. al. 2007; Fautin et. al. 2008). After the present study, the distribution of this species is extended to the east coast of Africa, off the coast of Somaliland and from Nias Island, Indonesia. *Calliactis polypus* is found between 0 – 81 meters.

Discussion

Calliactis polypus was first recorded by Forsskal (1775) for the Red Sea. Klunzinger (1877) added additional information after examining specimens from the type locality. Later, Hertwig (1882) gave a very detailed description of the species based on individuals collected by the Challenger. *Calliactis polypus* is the most widespread species of sea anemone symbiotic with hermit crabs, with records in the Indian Ocean, Red Sea and Pacific Ocean. This species is among the *Calliactis* found at shallow depths, generally at SCUBA dive depths (0 – 40 meters), which may contribute to the large number of museum specimens available for the species. At least 2 individuals are found in each shell inhabited by the hermit crab, but usually

many more are attached to a single shell. Up to 11 specimens have been observed living attached to a shell collected from Hawaii.

Despite its wide geographic range, and after examination of several populations of *C. polypus* spanning its entire geographic distribution, it was not possible to differentiate specimens collected from populations isolated by hundreds or thousands of kilometers. The finding of very low levels of intra-specific diversity in internal transcribed spacer (ITS) sequences present in Chapter 4 with no geographic differentiation between five geographically isolated populations of *C. polypus* supports the morphological analysis of *C. polypus* presented here. Populations from Hawaii, for example, in the Pacific Ocean, did not show consistent differences in external or internal anatomy, microanatomy or cnidae from populations collected in the Maldives in the Indian Ocean. In fact, specimens of this species are extremely constant in number and cycle of tentacles and mesenteries, musculature and cnidae. A difference that is important to discuss is the extreme coloration diversity in *C. polypus*. Although most individuals observed in the field and in aquaria had more or less the same colors, these colors formed very diverse patterns in the column. The diversity range in coloration pattern, however, is high even among individuals found in the same shell in a single population. Coloration pattern, however, may be related to environmental factors and are not traditionally used to distinguish species of sea anemones. Some individuals collected in Japan, for example, showed a pattern that matched the coloration of the crab and the shell, which is an indication that coloration

may be used as camouflage. For this reason, although coloration is not acknowledge in the present study, it was not used to distinguish populations of *C. polypus*.

In addition, the present study synonymized a few species originally described from specimens collected in the Indian and Pacific Ocean with *C. polypus*. In most cases, these species are known from single records and only from its type material. After examining material for the species *C. armillatas*, *C. valdiviae* and *C. variegata,* it became evident that they were individuals similar and could not be distinguished from specimens of *C. polypus*.

Adamsia miriam was described by Haddon and Shackleton (1893) from specimens the Great Barrier Reef. The species was transferred to *Calliactis* by Haddon (1898). Later, Carlgren (1900) hypothesizes that the species is a synonym of *C. polypus* but the material was lost and no other details of were given to the species. England (1971) re-examines the cnidae of specimens of *C. miriam* and *C. polypus* and found great similarity. Three specimens identified as *C. miriam* from Australia are examined in the present study and similar results are found. For this reason, these two species are considered synonyms here.

Calliactis valdiviae originally described from specimens collected off the coast of Somalia is differentiated by Carlgren (1938) from *C. polypus* based on the larger size of basitrichs in the acontia. Here, after examining specimens of *C. valdiviae*, this difference was not found. Both *C. polypus* and *C. valdiviae* exhibited a very similar range for basitrichs in the acontia, with all other morphological

characters agreeing with those described for *C. polypus*. Here these two specimens are synonymized.

Calliactis armillatas is described by Verrill (1928) from Hawaii. England (1971) later examines the type material of *C. armillatas* and finds that two distinct species are present. Some specimens are, in fact, what Verrill (1928) describes as *C. armillatas*, but other specimens are re-described as the new species *Verrillactis paguri*. This new species is placed in the family Sagartiidae and is differentiated from *C. armillatas* based on the presence of basitrichs and microbasic *p*-mastigophores in the acontia, its position on the shell (always close to the shell aperture), and the fact that this species reproduces by pedal laceration. Although the remaining specimens of the type material identified as *C. armillatas* by Verrill (1928) shows some variance in the range of nematocysts, England (1971) notes that all the other characters of internal and external anatomy and microanatomy agree with those described for *C. polypus*. Thus, these two species are synonymized by England (1971). The present revision agrees with England (1971) in that no consistent differences are found between *C. armillaas* and *C. polypus*. Furthermore, they are found at the same locality and at the same depth. Although slight variance in the range of nematocysts is described here, these differences are not considered sufficient to recognize two separate species.

Verrill (1869) describes the species *C. sinensis* from the North Pacific. Later, in the original description of *C. armillatas* (Verrill 1928), *C. armillatas* is differentiated from *C. sinensis* based on the coloration pattern of the column. As

already mentioned, although coloration is often included in the description of sea anemones, this character should not be used as diagnostic given the variation of coloration that is observed in sea anemones. Similar variation has been observed in individuals of *C. polypus* and *C. parasitica*, for example. Furthermore, after examining the type material, no differences are found between specimens of *C. sinensis* and *C. polypus*. Both species possess the same general morphology, with a circular pedal disc, one row of cinclides proximally, same number of tentacles, same number and arrangement of mesenteries and very similar musculature. In addition, the range of nematocysts is also very similar (see Table 5.3) and the slight differences can be attributed to populational or individual variation. For this reason, *C. sinensis* is considered a junior synonym of *C. polypus*.

Calliactis tricolor **(Le Sueur 1817)**

Actinia tricolor Le Sueur 1817: 171.

Actinia bicolor Le Sueur 1817: 171.

Adamsia tricolor Milne-Edwards 1857: 281; Duchassaing and Michelotti 1866:134; McMurrich 1898: 234.

Adamsia egletes Duchassaing and Michelotti 1866: 134.

Calliactis bicolor Verrill 1869: 481.

Adamsia sol McMurrich 1893: 183.

Adamsia bicolor Andres 1883: 179.

Adamsia tricolor Andres 1883: 180.

Calliactis tricolor Duerden 1902: 329, 259; Stephenson 1920: Pax 1924: 119-120; 528; Carlgren 1949: 97; Hedgpeth 1954: 287-289; Corrêa 1964:50, 113-117, 129, 130; Cutress and Ross 1969: 225-241; Corrêa 1973: 458; Dube 1974:52-54, 66, 68; Dube, 1978: 31; Zamponi Belem Schlenz and Acuna 1998:34, 37-40.

Material examined: see Appendix A.

External anatomy (Figure 5.7)

Pedal disc: Pedal disc very adherent to shell. Well developed, circular shape in smaller specimens, with irregular shape in larger specimens. Pedal disc generally slightly wider than diameter of the column. Pedal disc wall fine marked by numerous mesenterial insertions. In live specimens pedal disc is dark green with white mesenterial insertions. Pedal disc bege in preserved material. Bright orange acontia visible through the pedal disc in live specimens. Pedal disc diameter in preserved specimens from 1.3 – 3.7 cm.

Column: Column short in retracted individuals but relatively long in live specimens. Column is narrow close to the base and becomes progressively wider distally, closer to the oral disc. The column has a very firm consistency, with a thick mesoglea, and is divided into a scapus and narrow, delicate and transparent capitulum. The latter is much more evident in live animals. The scapus presents longitudinal and transversal ridges that give the column a wrinkled texture. A fine cuticle is sometimes present in

the scapus, but never in the capitulum. Two rows of cinclides are found close to the pedal disc. Most specimens examined exhibited an irregular number of cinclides, although some individuals were regular and presented 24 cinclides in each row. The first row is found more proximally and is a lot smaller than the second row of cinclides that are usually found in slight elevations of the column. These cinclides contrast with the color of the column due to their darker red or brown color, with purple margins. Through these cinclides, spirilized acontia can be seen. These acontia are bright orange in live animals. The scapus exhibits a dark green coloration pattern with orange or white spots scattered throughout the column in live individuals. In preserved specimens this coloration pattern is still visible but not with the bright colors of live individuals. In preserved specimens, column height 1.5– 3.8 cm, width 1.7 – 4.5 cm.

Oral disc and tentacles: The oral disc is circular, dark green as the column, with white mesenterial insertions. Given the position of the anemone on the shell, the oral disc is always located opposite to the gastropod shell aperture. The mouth is small, central, with clear lips through which the pharynx is visible. Two conspicuous siphonoglyphs are present. In live animals, the mouth is white and the tentacles bege or transparent with numerous brown horizontal bands throughout its entire length. Tentacles of first and second cycle present an additional dark brown band proximally. Tentacles are short, smooth, highly retractile and densely packed in the oral disc. Inner tentacles longer than outer ones. Up to 190 tentacles arranged in 5 cycles (6+12+24+48+96).

Sometimes, tentacles are not strictly hexamerously arranged. Coloration: yellow, grey. Oral disc diameter: 0.3 – 3.0 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, transversally stratified. The sphincter occupies the entire length of the capitulum. Mesenteries up to 48 pairs, distributed in four cycles (6+6+12+24). Same number of mesenteries proximally and distally. Mesenteries of first cycle perfect and sterile, those of the second and third imperfect and fertile. Two pairs of directives associated with two siphonoglyphs. Mesenteries of the second and third cycles with retractors, filaments and acontia, while the fourth cycle has very reduced mesenteries without filaments, gonads or acontia. Retractor of the mesenteries and parietal muscle weak and diffuse. Basilar muscle well developed. Acontia are abundant in the first, second and third cycles, but absent in the fourth cycle. Acontia can be seen through the cinclides of preserved specimens.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.6). For measurement of capsules see Table 5.4.

Distribution: *Calliactis tricolor* is originally described from Barbados (Le Sueur, 1817) but this species has a very widespread distribution from North Carolina, USA (Field 1949) to the South of Brazil (Correa 1964; Zamponi et. al. 1998). *Calliactis tricolor* is known from the Gulf of Mexico (Bach and Herrnkind, 1980; Carlgren and

Hedgpeth 1952) through the Caribbean, including Cuba (McMurrich 1898), Puerto Rico (Duerden 1902), the Virgin Islands (Duchassaing and Michelotti 1864), Curacao (Pax 1924) and Jamaica (Duerden 1898). Most specimens collected from 0 – 45 meters.

Discussion

Verrill (1869) erects *Calliactis* and includes in the genus *Actinia tricolor* of Le Sueur (1817). Different names have been used to described *C. tricolor*, including *Actinia bicolor* from St. Vincent (Le Sueur 1817), *Adamsia egletes* from St. Thomas (Duchassaing and Michelotti 1864) and *Actinia sol* from the eastern coast of the USA (Verrill 1874). These species are synonymized with *C. tricolor* by McMurrich (1898). As a result, *C. tricolor* is the only species of the genus distributed in the western Atlantic Ocean, from the eastern coast of the USA to the southern coast of Brazil (Fautin 2010).

Le Sueur (1817) and Duchassaing and Michelotti (1864) gives early descriptions of the external morphology, including coloration and information on number and cycle of tentacles, and size of individuals for the species *C. tricolor*. Duerden (1902) and Pax (1924) describes specimens of *C. tricolor* collected from Puerto Rico and Curacao, respectively, in detail and gave information not only about their external anatomy, but also of internal anatomy and nematocysts. Carlgren and Hedgpeth (1952) and Hedgpeth (1954) further contribute to the description of specimens of *C. tricolor* from the Gulf of Mexico. Material of *C. tricolor* have also

been examined from the southernmost part of its range in Brazil, and described in extensive detail by Correa (1964, 1973) and Dube (1978). As a result of these studies, the external and internal anatomy of *C. tricolor*, as well as the cnidae and microanatomy are well known and *C. tricolor* is one of the best described species of the genus *Calliactis*.

The material of *C. tricolor* examined in the present study corresponds closely to descriptions of external morphology given by other studies (Le Sueur 1817; Duchassaing and Michelotti 1864; Duerden, 1902; Pax 1924; Carlgren and Hedgpeth, 1952; Correa 1964; Dube 1978). The coloration of live animals, however, seems to be highly variable and may differ depending on the population examined (pers.obs). A few differences should be noted between the specimens examined in this study and those examined by Duerden (1902). In addition, some earlier studies described up to three rows of cinclides (Correa 1964; Duerden 1902), while only one or two rows of cinclides were observed in the specimens examined here. While Duerden (1902) observes a cuticle covering the column of specimens from Puerto Rico, I observe this structure in most well-preserved and live animals used in the present study. In addition, I do not confirm the presence of a cuticle secreted by the pedal disc of *C. tricolor*, as found by Duerden (1902). It should be noted that, sometimes, when specimens of *C. tricolor* were detached from the shell in which it was collected on, part of the surface of the shell was detached with the pedal disc of the anemone. This gave the impression that there was a cuticle secreted by the pedal disc, but the examination of the shell confirmed that it was not the case for this species. In regards

to internal anatomy and microanatomy, specimens of *C. tricolor* are very regular and the present study is in agreement with the results of other studies (Duerden 1902; Pax 1924; Carlgren and Hedgpeth 1952; Correa 1964; Dube 1978).

Although the distribution and measurements of cnidae for *C. tricolor* examined here agree with previous studies (Carlgren and Hedgpeth 1952; Correa 1964; Dube 1978; Riemann-Zurneck 1975), some differences should be noted. These differences include: presence of microbasic *p*-mastigophores in the tentacles, presence of a second class of basitrichs in the column, presence of a second class of microbasic *p*-mastigophores in the filament. The differences in cnidae for *C. tricolor* presented here and previous accounts are probably the result of a more extensive examination of the nematocysts for the species, not a real difference between populations examined here and by other authors.

Calliactis reticulata is originally described from the southern coast of Brazil at 72 meters (Stephenson 1920) but it's never recorded again for the region. *Calliactis reticulata* is sympatric with *C. tricolor*, a species widely distributed from the Caribbean to the southern coast of Brazil. *Calliactis reticulata* is distinguished from *C. tricolor* based on differences of cnidae, including: the presence of microbasic *p*-mastigophores in the tentacles and basitrichs of two sizes in the acontia. In addition, *C. reticulata* had only 160 tentacles compared to up to 184 in *C. tricolor*.

After examining the type material of *C. reticulata*, the differences reported by Stephenson (1920) regarding the number of tentacles and cnidae were confirmed in part, but they do not distinguish this species from *C. tricolor*. The reported

nematocyst differences between *C. reticulata* and *C. tricolor* are not confirmed. Regarding the number of tentacles, the variability found in specimens of *C. tricolor* encompasses the number of tentacles found in *C. reticulata*. No morphological difference is found between the species *C. tricolor* and *C. reticulata*. For this reason, the synonymy between *C. reticulata* and *C. tricolor* is proposed here.

Calliactis parasitica **(Couch 1842)**

Priapus polypus Forsskal 1775: 102.

Actinia effeta Risso 1826: 285.

Actinia effoeta de Blainville 1830: 291-292.

Cibrina polypus Ehrenberg 1834: 264; Brandt 1835: 15.

Actinia rondeleti Delle Chiaje 1841: 137.

Actinia parasitica Couch 1842: 60; Couch 1844: 80-81; Johnston 1847: 228;

Sagartia parasitica Gosse 1858: 416.

Calliactis parasitica Hertwig 1882: 8, 44, 74-76, 82; Carlgren 1928: 172-173; Stephenson 1928: 111; Carlgren 1949: 97; Pax 1952: 12-13; Davenport 1955: 39; Schmidt 1969: 290; Manuel 1981: 174-176; Chintiroglou Doumenc and Kaikoras 1985: 516, 525; Song and Lee 1998: 229, 234-237, 240; Cha and Song 2001: 104, 109; Uchida and Soyama 2001: 150, 153;

Adamsia rondeletii Andres 1883: 159-162.

Actinia parasitica Haddon 1889: 324.

Calliactis rondeleti Stephenson 1918: 135.

Material examined: see Appendix A.

External anatomy (Figure 5.8)

Pedal disc: The pedal disc is circular and, in live specimens, is very adherent to the shell to which is attached. The pedal disc never covers the shell entirely and is only slightly wider than the column. In both live and preserved specimens, the pedal disc is white. Pedal disc diameter in preserved specimens range from 3.5 – 8.1 cm.

Column: Smooth, divisible into scapus and capitulum. The scapus is partly covered by a thin deciduous cuticle. The column is narrow close to the base and becomes progressively wider distally, closer to the oral disc. The column has a very firm consistency, with a thick mesoglea. The scapus presents longitudinal and transversal ridges that give the column a wrinkled texture. One or two rows of cinclides are distributed close to the pedal disc. These cinclides are not very conspicuous as they do not contrast in color to the column or are situated in slight elevations of the column. Acontia are either bege or yellow. The scapus exhibits a coloration pattern that is variable, but includes yellow or light brown backgrounds with densely red, pink or brown spots scattered throughout the column. These spots sometimes tend to form alternating longitudinal stripes that run from the pedal disc to the limit of the scapus and capitulum. In preserved specimens this coloration pattern is still visible but is not as obvious as in live specimens. In preserved specimens, column height 2.6– 7.2 cm, width 2.7 – 4.3 cm.

Oral disc and tentacles: The oral disc is circular, white or bege, with white mesenterial insertions. The mouth is a small, central, through which two conspicuous siphonoglyphs and the pharynx are visible. The tentacles are conical, short, smooth and very numerous. The inner tentacles are longer than the outer ones. Up to 720 tentacles were counted in larger individuals. Most tentacles are hexamerously arranged in approximately 7 cycles (6+12+24+48+96+192+384), except for tentacles of the sixth and seventh cycle that may be irregularly arranged. Tentacles are transparent, but with numerous horizontal darker bands that may alternate with white spots. Furthermore, the base of the tentacles of the last cycle are alternatively colored in white and dark brown to create a pattern that is very characteristics for this species.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, showing a tendency to transversal stratification. The sphincter occupies the entire length of the capitulum, but does not extend far into the scapus. Mesenteries up to 96 pairs proximally, distributed in five cycles (6+6+12+24+48). Same number of mesenteries proximally and distally. Mesenteries of the first cycle perfect and sterile, including two pairs of directives associated with two siphonoglyphs. Mesenteries of the second, third and fourth cycles imperfect, fertile, with retractors, filament and acontia. The fifth cycle is composed of very reduced mesenteries that may not be strictly hexamerously distributed and do not

possess filament or acontia. Retractor muscle of the mesenteries is weak and diffuse. The parietal muscle is very weak and, often, hard to observe.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores. (Figure 5.6). For measurement of capsules see Table 5.5.

Distribution: *Calliactis parasitica* is widely distributed in the North Atlantic Ocean, including, England (Couch 1844; Cocks 1851; Gosse 1860; Stephenson 1935; Robins 1969), France (Teissier 1950, 1965; Ates 1997), Madeira Archipelago (Johnson 1861; Ocaña and den Hartog 2002), Canary Islands – Gran Canaria, Fuerteventura and Lanzarote - (Ocanã and Hartog 2002). *Calliactis parasitica* is also distributed in the Mediterranean Sea (Enhrenberg, 1834), including Italy (Delle Chiaje 1841; Schmidt and Béress 1971) and Spain (Rioja and Martín 1906; Ates, 1997), in the Aegen Sea (Chintiroglou and Koukoras 1991; Christidis et. al. 1997) and in the Adriatic Sea (Riedel et. al. 2008). *Calliactis parasitica* bathymetric range is found between 0 – 80 meters.

Discussion

Calliactis parasitica is very widespread in the North Atlantic and Mediterranean and, for this reason, it is listed in many faunistic inventories for that region (e.g., Hertwig 1882; Haddon, 1889; Pax and Muller 1954; Pax and Muller, 1962; Schmidt 1972; Manuel 1981; Doumenc et. al. 1985; Loukmidou et. al. 1996;

Christidis et. al. 1997; Ocana and den Hartog 2002; Wirtz et. al. 2003). Most of these studies, however, do not describe the species in detail, including only external characteristics, coloration and size. Only a few studies have included characters of internal anatomy, microanatomy or cnidae (Stephenson 1935; Schmidt 1972; Manuel 1981).

Calliactis parasitica is fairly homogenous in external and internal anatomy and is characterized by the very high number of tentacles (up to 720) in approximately 7 cycles and cinclides that are not very easily observed. In addition, there are proximally (close to the pedal disc) about twice as many mesenteries (96 pairs) as there are in the middle of the column (48 pairs). The specimens of *C. parasitica* examined in the present study belong to different populations spanning the wide range of the species. They agree with previous accounts of anatomy and cnidae (Stephenson 1935; Schmidt 1972; Manuel 1981) Variability in the number of tentacles and the number of mesenteries is detected, but is scattered and occurs in specimens from many populations. Thus, it is considered part of the natural variation found in a species rather than justification for different species.

The cnidae of *C. parasitica* described here is in agreement in general lines with the account given by Schmidt (1972). No differences in the types of nematocyst in each tissue were found, but the range of capsules is extended for a few, in particular the spirocysts in the tentacles and the basitrichs in the column and filament.

Even after examining specimens from many populations, *C. parasitica* is very well defined and easily distinguished from other species such as *C. polypus* and *C.*

tricolor based on the number and cycle of tentacles, the number of mesenteries and size and distribution of cnidae.

Calliactis brevicornis **(Studer 1879)**

Cereus brevicornis Studer 1879: 542-543, 677; Pax 1908: 476-477.

Heliactis brevicornis Andres 1883: 182.

Calliactis brevicornis Carlgren 1928: 199- 201; Carlgren 1949: 97.

Material examined: see Appendix A.

External anatomy (Figure 5.9)

Pedal disc: Pedal disc is very adherent to the shell to which is attached, circular in shape, wider than the column and approximately twice as wide as the diameter of the oral disc. The pedal disc does not secrete a cuticle, but when the individual is detached from the shell, part of the surface of the shell comes off attached to the pedal disc. Thus, if the shell is not present for comparison, it would appear as the pedal disc secreted a fine cuticle. Pedal disc white, same color as the column. Pedal disc diameter in preserved specimens range from 1.8 to 3.3 cm.

Column: Column short in preserved individuals, very wide close to the base and becoming progressively narrower distally, closer to the oral disc. The column has a very firm consistency, with a thick mesoglea, and cannot be differentiated into scapus

and scapulus/capitulum. The column is smooth and may present a fine, deciduous cuticle, especially proximally, closer to the pedal disc. One row of cinclides is present close to the pedal disc. These cinclides are not distributed regularly, 28 in number. These are not very conspicuous and do not contrast in color or texture to the rest of the column. Through these cinclides, spirilized acontia were visible in a few specimens Acontia are usually yellow . In preserved specimens, column height 1.7–2.6 cm, width 2.5 – 3.9 cm.

Oral disc and tentacles: The oral disc is circular, white, without any clear coloration pattern. The mouth is small, central, with clear lips and two siphonoglyphs. The pharynx is clearly visible and everted in some specimens. Tentacles are short, smooth, conical and distributed only in half of the oral disc area. Inner tentacles longer than outer ones. Up to 194 tentacles arranged in 5 cycles (6+12+24+48+96). Sometimes, tentacles are not strictly hexamerously arranged, especially in the last cycle. Coloration: yellow or bege. Oral disc diameter in preserved specimens: 0.2 – 1.5 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, transversally stratified. Mesenteries up to 48 pairs, distributed in four cycles (6+6+12+24) in the center of the column. A fifth cycle of mesenteries may be present proximally. Mesenteries of the first cycle perfect and sterile, those of the second and third imperfect and fertile. Two pairs of directives

associated with two siphonoglyphs. Mesenteries of the second and third cycles with retractors, filaments and acontia, while the fourth and fifth cycles have very reduced mesenteries without filaments, gonads or acontia. Retractor of the mesenteries and parietal muscle weak and diffuse. Basilar muscle weak. Acontia are abundant in the first, second and third cycles, but absent in the fourth and fifth cycles. Acontia can be seen through the cinclides of preserved specimens.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.6). For measurement of capsules see Table 5.6.

Distribution: *Calliactis brevicornis* was described from specimens collected in the eastern Atlantic Ocean, Bijoga Island, Guinea Bissau, west Africa between 214 – 274 meters (Studer 1879). Additional specimens were collected from the eastern Atlantic Ocean, off the coast of Congo at 214 meters (Carlgren 1928a).

Discussion

The original description of *Cereus brevicornis* (Studer 1879) from specimens collected off the coast of Guinea Bissau is very short and includes only the external morphology, coloration and size of individuals. Carlgren (1928a) re-describes *C. brevicornis* from specimens collected off the coast of Congo and transfer the species to *Calliactis*, and provides a very detailed description for this species.

The results presented here are based on the examination of the type material collected by Studer (1879). This account agrees with the descriptions given by Studer (1879) and Carlgren (1928a) for *C. brevicornis*. One point needs to be further discussed: the presence of cinclides in the species. Although Carlgren (1928a) says that no cinclides were visible in the material of *C. brevicornis* collected off the coast of Congo, in the discussion of the species, he noted that they probably existed and were not conspicuous. In addition, Carlgren (1928a) also affirms that when he examined the type material of *C. brevicornis*, cinclides were visible. The present study confirms that the type material of *C. brevicornis* possesses one row of cinclides and that the specimens examined by Carlgren (1928a) probably also possess them. As noted for other species of *Calliactis* (e.g., *C. japonica*, *C. parasitica*, *C. annulata*), cinclides are not always situated on slight elevations of the column, which makes them harder to see.

The cnidae of *C. brevicornis* is partly described by Carlgren (1928a) who presented measuraments for all tissues except filaments. The present study differs from the cnidae account given by Carlgren (1928a) in the measurements of classes of basitrichs in the column and microbasic *p*-mastigophores in the pharynx. Additionally, the distribution and measurements for nematocysts of the filament are described for the first time.

Calliactis annulata Carlgren 1922

Calliactis annulata Carlgren 1922: 146 – 148; England 1971: 27, 29.

Material examined: See Appendix A.

External anatomy (Figure 5.10)

Pedal disc: Pedal disc of irregular shape. The diameter of the pedal disc is smaller than the column and oral disc. The holotype examined is not attached to a shell and does not present the wide and irregular pedal disc characteristic of species of *Calliactis*. Pedal disc diameter in preserved specimens is 3.5 cm.

Column: Column long, smooth, narrow close to the pedal disc and becoming wider close to the oral disc. The column is not divisible into a scapus and scapulus. The column has a very firm consistency, with a thick mesoglea. No cuticle is found covering the column of preserved specimen. The column presents longitudinal and transversal ridges that give the column a wrinkled texture. One row of cinclides is found close to the pedal, but these cinclides are not very conspicuous and not easily observed. Preserved specimens are predominatly white with brown and yellow spots scattered through the column that does not form any clear stripes or patterns. In most specimens the coloration pattern is still visible but not with the bright colors of live individuals. In preserved specimens, column height 6.5 cm, width 4.2 cm.

Oral disc and tentacles: The oral disc is circular and provided with radial grooves. The pharynx possesses longitudinal ridges. The mouth is small, central and possesses two clear siphonoglyphs. Tentacles are short, conical and hexamerously arranged up to 96 in 5 cycles (6+6+12+24+48). Inner tentacles are more than twice as long as the outer. Half of the oral disc without tentacles, with weak radial furrows. Oral disc diameter: 4.2 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, transversally stratified. Mesenteries up to 48 pairs, distributed in four cycles (6+6+12+24) in the middle of the column. Close to the margin an extra cycle may exist in a total of 96 pairs of mesenteries. Mesenteries of the first cycle perfect and sterile, those of the second and third imperfect and fertile. Two pairs of directives associated with two siphonoglyphs. Mesenteries of the second ,third and fourth cycles with retractors, filaments and acontia, while the fifth cycle has very reduced mesenteries without filaments, gonads or acontia. Retractor of the mesenteries and parietal muscle weak and diffuse. Acontia are abundant in the first, second and third cycles, but absent in the fourth cycle.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.6). For measurement of capsules see Table 5.7.

Distribution: *Calliactis annulata* is only known from its type locality in the Juan Fernandez Islands in the eastern Pacific Ocean (Carlgren 1922). The holotype was collected at 50 meters (Carlgren 1922).

Discussion

Calliactis annulata is described in detail by Carlgren (1922) from one specimen collected from the Juan Fernandez Islands. Details of external morphology, internal anatomy, musculature, and cnidae are given by Carlgren (1922). The only difference between the original description of the species and the present study is the confirmation of cinclides in the specimen. Carlgren (1922) does not find any cinclides in the species examined, but noted that they probably existed and were not easily observed at the time of his study.

Although distribution and measurements of the nematocysts of the tentacles, column, and acontia largely agree with those of Carlgren (1922), the range of most of these nematocysts is extended in both extremes. Furthermore, microbasic *p*-mastigophores are seen in the pharynx. The cnidae of the filament is described for the first time; they include basitrichs and microbasic *p*-mastigophores.

Unfortunately, the only specimen of *C. annulata* examined in the present study is the holotype (Göteborg 927): because no other material for this species was found in any museum visited. For this reason, it is hard to give a more detailed account of the characteristics of the species or describe populational variation. However, *C. annulata* is easily distinguished from the other species of *Calliactis*

found in the eastern Pacific, *C. polypus*, based on the number and cycle of tentacles and the distribution and size of cnidae.

***Calliactis japonica* Carlgren 1928**

Calliactis japonica Carlgren 1928: 172-173; Carlgren 1949: 98; Song and Lee 1998: 229, 234-237, 240; Cha and Song 2001: 104, 109; Uchida and Soyama 2001: 150, 153.

Material examined: see Appendix A.

External anatomy (Figure 5.11)

Pedal disc: The pedal disc in live specimens is very adherent to the shell to which is attached and, most of the times, completely covers the shell. The pedal disc is irregular and usually covers the entire shell, but never extends beyond the aperture of the shell or secretes any cuticle. Usually only one individual is found in each examined shell, but in two rare cases, two individuals were found in the same shell. When this was the case, one of the individuals was much larger than the other. In live specimens, the pedal disc is of the same width of the rest of the column and may be slightly narrower than the oral disc diameter. In preserved specimens, the diameter of the pedal disc is only slightly larger than that of the column and the oral disc. In both live and preserved specimens, the pedal disc is white. Pedal disc diameter in preserved specimens range from 3.1 to 6.6 cm.

Column: Column generally smooth, but few tubercles are irregularly scattered throughout its length. *C. japonica* is among the larger species in the genus, but the column is short compared to its diameter. The column has a very firm consistency, with a thick mesoglea, and is not divisible into scapus and scapulus. The column is white with numerous red spots similar to a leopard pattern and the capitulum is white and exhibits a bright red ring distally. The column does not present a cuticle. Cinclides are distributed in a single ring proximally, vey close to the pedal disc. These cinclides are not easily observed in preserved specimens as they are not positioned in elevations of the scapus or present a distinct color that contrasts to the column as in *C. tricolor*, *C. parasitica* and *C. polypus*. Acontia are opaque white in live animals and bege in preserved specimens. In preserved specimens, column height 3.1– 7.7 cm, width 3.2 – 7.5 cm.

Oral disc and tentacles: The oral disc is circular, bege, with a clear white ring between the mouth and the first cycle of tentacles. White mesenterial insertions are visible as well as two clear siphonophyphs. Given the position of the anemone on the shell, the oral disc is always located opposite to the gastropod shell aperture. The mouth is a slit in the center of the oral disc through which the pharynx is visible. Tentacles are numerous, short, smooth, and conical. Inner tentacles always longer than outer ones. Tentacles are bege as the rest of the oral disc and possess no band or coloration pattern. Up to 200 tentacles arranged in 7 cycles (6+6+12+12+24+48+96).

Tentacles are not strictly hexamerously in the fifth cycle. Coloration: yellow, grey. Oral disc diameter: 0.6 – 6.9 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, transversally stratified, occupying the entire length of the capitulum. Mesenteries up to 48 pairs and distributed hexamerously in four cycles (6+6+12+24). Same number of mesenteries proximally and distally. The mesenteries of the first cycle are perfect and sterile, including two pairs of directives associated with two siphonoglyphs. Mesenteries of the second, third and fourth cycles are imperfect and fertile, with retractors, filaments and acontia. Retractors of the mesenteries and parietobasilar muscle very weak and diffuse. Acontia are abundant in all cycles.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.6). For measurement of capsules see Table 5.8.

Distribution: *Calliactis japonica* is originally described from Sagami Bay, Japan, between 0 – 210 meters (Carlgren 1928b). Additionally, *C. japonica* has been collected in the Tanabe Bay and off Enoshima in Japan (Yusa et. al. 2001). *C. japonica* is also known from South Korea (Cheju Island, Geojedo Island and Cheonsgsando) where it was collected from 7 – 100 meters (Song and Lee 1998).

Discussion

The original description of *C. japonica* (Carlgren 1928b) is very short and does not include many details about the morphology of the species, citing only the number of mesenteries and tentacles, the morphology of the sphincter and nematocyst types and size measurements for the tentacles, pharynx and acontia. *Calliactis japonica* is differentiated from *C. parasitica* based on the smaller number of mesenteries proximally (48 pairs vs. 96 pairs in *C. parasitica*) and the significantly larger basitrichs in the acontia of *C. japonica*.

Material examined in the present study includes the holotype of *C. japonica* (UUZM 688), new collections from Japan and museum material (see Table 5.1). This material corresponds in general lines to the information given by Carlgren (1928b). *Calliactis japonica* is among the very largest species of *Calliactis* and is easily distinguished by a coloration pattern that is maintained even after preservation and storage in ethanol. In the original description, Carlgren (1928b) includes a question mark close to the word "cinclides", but also says that these cinclides are very numerous. There are many cinclides forming one ring very close to the pedal disc. These cinclides are not very easily observed, especially in preserved specimens, but are evident in live animals especially when acontia are emitted. These cinclides are not situated on slight elevations of the column as they are in *C. tricolor* or *C. polypus*, and are not distinguished from the column by a different color. This might explain Carlgren (1928b) uncertain ability to identify such structures as cinclides in *C. japonica*.

In the present study, details are described for the first time for the external anatomy, including the number and cycles of tentacles, the morphology of the pedal disc and oral disc as well as the internal anatomy and microanatomy, including number and cycle of mesenteries, fertility and musculature. Nematocyst distribution and measurements are given, for the first time, for column and filaments of *C. japonica*. \The results agree with the distribution and measurements given by Carlgren (1928b) for the tentacles, pharynx and acontia, although the range of a few nematocysts is extended. These differences, however, should be regarded as populational variation.

***Calliactis conchicola* Parry 1952**

Calliactis conchicola Parry 1952: 127 – 129; Hand 1975: 494 – 500; Dawson 1992: 41.

Material examined: see Appendix A.

External anatomy (Figure 5.13)

Pedal disc: Pedal disc adherent, thin, circular or irregularly shaped. In some specimens, the pedal disc tends to be circular and not completely enwrap the shell, while in other specimens the pedal disc covers the shell entirely and may extend beyond the aperture of the shell. The fine pedal disc is bege or slightly transparent with marked white mesenterial insertions. No cuticle is present in the specimens

examined. The diameter of the pedal disc is usually twice the diameter of the oral disc. Pedal disc diameter in preserved specimens range from 3.6 – 7.7 cm.

Column: Column with a very firm consistency, with a thick mesoglea, and divided into a scapus and a narrow and delicate scapulus. The scapulus is marked with numerous longitudinal ridges. The scapus presents longitudinal and transversal ridges that leave the column corrugated in preserved specimens. A fine deciduous cuticle is present in the scapus, but not in the capitulum. The cuticle is hardly noticeable in some individuals. One row of more or less conspicuous cinclides are located close to the pedal disc. These cinclides may have a darker coloration in preserved specimens. Cinclides are not regularly arranged and may be hardly visible in some individuals unless acontia have been emitted through them. The color of the scapus is white or bege and the cuticle is brown when present. The scapulus is white as the rest of the column or may be slightly pink. In preserved specimens, column height 1.6– 4.7 cm, width 1.8 – 6.6 cm.

Oral disc and tentacles: The oral disc is circular, opaque white or bege, with white mesenterial insertions and two clear siphonophyphs visible. Given the position of the anemone on the shell, the oral disc is always located opposite to the gastropod shell aperture. The oral disc is bege always small than diameter of the pedal disc. The mouth is central and small. Tentacles are pale pink or white and some have a longitudinal white line along its length. Tentacles are restricted to a small portion of

the oral disc. They are short, conical, with inner tentacles longer than outer ones. Up to 112 tentacles hexamerously arranged in 5 cycles (6+12+12+24+48). Sometimes, tentacles are not strictly hexamerously arranged. Oral disc diameter: 0.5 – 2.1 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, well-developed, transversally stratified. It occupies the majority of the scapulus and runs a considerable length into the scapus. Mesenteries up to 48 pairs, distributed in four cycles (6+6+12+24). In two larger individuals, a fifth cycle was present, but the mesenteries of this fifth cycle were scattered and not regularly arranged. These mesenteries are scattered and do not follow any clear pattern. The first cycle of mesenteries is perfect and sterile and include two pairs of directives associated with two siphonoglyphs. Imperfect mesenteries of the second and third cycles fertile. All mesenteries, except those of the fourth cycle, have filaments and acontia. Retractors of the mesenteries of the first, second and third cycles are weak and diffuse. The fourth cycle of mesenteries has no retractors or gonads.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.6). For measurement of capsules see Table 5.10.

Distribution: *C. conchicola* is originally described from specimens collected in Lyttelton Harbour, New Zealand in the Southern Pacific Ocean at 72 meters (Parry

1952). *C. conchicola* is also known from Kaikoura and Otago Bay, New Zealand, between 15 – 120 meters (Hand 1975a).

Discussion

Calliactis conchicola is described by Parry (1952) and later re-described by Hand (1975a) based on specimens collected from the eastern coast of New Zealand. No type material for this species was deposited by Parry and no specimens from her study are known. The specimens examined here include loans from the National Institute of Water and Atmospheric Research (NIWA) and two lots of *C. conchicola* deposited by Hand (1975a) in the National Museum of New Zealand (NMNZ).

The specimens of *C. conchicola* examined here correspond to the previous descriptions for the species (Parry 1952; Hand 1975a). Two differences concern the morphology and number of tentacles and the number and cycles of mesenteries. Hand (1975a) noted that the tentacles of live specimens of *C. conchicola* did not possess the morphology that is characteristic of *Calliactis*: short and conical. Instead, Hand (1975a) describes the tentacles of *C. conchicola* as long and slender, but noted that this may be the result of the plastic nature of tentacles in sea anemones. Here, I confirm that after preservation these tentacles are, in fact, short and conical, which can be easily explained by the contraction that usually happens when live specimens of sea anemones are fixed without anesthesia. As mentioned by Hand (1975a) this characteristic should not be taken as significant.

Another difference noted by Hand (1975a) in comparison with the description of Parry (1952) is the number of tentacles compared to the number of mesenteries proximally. Hand (1975a) finds that the number of tentacles was equal to the number of mesenteries close to the pedal disc (both 96). After examination of the lots deposited by Hand and additional specimens from its type locality, I found that the number of tentacles is actually slightly higher than the number of mesenteries proximally. For this reason, I do not agree with the modification of the diagnosis of *Calliactis* made Hand (1975a) based on the number of tentacles in *C. conchicola*. A higher number of tentacles, compared to the number of mesenteries proximally, is still a diagnostic character for members of *Calliactis*. Based on the specimens of *C. conchicola* examined here, a fifth cycle of mesenteries is never complete and mesenteries of a fifth cycle that are present only close to the margin are very reduced, scattered and not regularly arranged. These results are not in agreement with Parry (1952), who described specimens that possessed a complete fifth cycle of mesenteries.

The cnidae of *C. conchicola* is only partially described in its original description, as Parry (1952) includes only nematocysts present in the tentacles and acontia. Hand (1975a) gives a more detailed account of the cnidae for this species. The present study largely agrees with the distribution and measurements given by Hand (1975a). The only difference found between this study and that of Hand (1975a) is the absence of small basitrichs in the tentacles of *C. conchicola*. The variation found in the remaining nematocysts can be attributed to population or individual

variations and are not significant. I also confirm here the presence of microbasic *p*-mastigophores in the column of *C. conchicola*. Most species of *Calliactis* do not possess microbasic *p*-mastigophores in the column, except for *C. conchicola* and *C. androgyna*. Instead this is a characteristic that is found in all species of *Paracalliactis*.

***Calliactis androgyna* Riemann-Zurneck 1975**

Calliactis androgyna Riemann-Zurneck 1975: 388-394.

Material examined: see Appendix A.

External anatomy (Figure 5.10 A, B)

Pedal disc: Pedal disc very irregularly shaped, but only slightly larger than the diameter of the column. The pedal disc does not secrete a cuticle. In the preserved material the pedal disc is dark brown and no mesenterial insertions are present. Pedal disc diameter in the preserved specimen is 2.3 cm.

Column: Column short, narrow close to the base and becomes progressively wider distally, closer to the oral disc. Divided into scapus and scapulus, with a deciduous cuticle present in the scapus, but not scapulus. The column has a very firm consistency, with a thick mesoglea The scapus presents longitudinal and transversal furrows that give the column a leathery texture. Two rows of conspicuous cinclides

are found close to the pedal disc. These cinclides have a darker color compared to the rest of the column. Through these cinclides, spirilized acontia are emitted. In preserved specimens, column height 2.4 cm, width 2.2 cm.

Oral disc and tentacles: The oral disc is very irregularly shaped and wider than the diameter of the column. The mouth is large, central, with a broad pharynx and two very clear siphonoglyphs are visible. Tentacles are short, conical, smooth, densely packed in an area of about half of the oral disc. The inner tentacles longer than the outer ones. Up to 382 tentacles arranged in 7 cycles (6+6+12+54+48+96+192). Sometimes, tentacles are not strictly hexamerously arranged, especially in the last cycles. The coloration of the oral disc is yellow or bege and the oral disc diameter is 2.4 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, transversally stratified, becoming reticular towards its proximal end. The sphincter occupies the entire length of the scapulus and runs down into the scapus. Mesenteries up to 82 pairs, distributed in 5 cycles. The first cycle consists of 6 pairs of perfect and sterile mesenteries that are hexamerously arranged perfect and sterile. The second to fourth cycles present a variable number of mesenteries that are not hexamerously arranged. All of these mesenteries are imperfect and fertile and possess filaments and acontia. A fifth cycle of very reduced mesenteries that are scattered and not hexamerously arranged is present. Some of

these mesenteries possess gonads and filaments, others do not possess them. Acontia very abundant. Retractor of the mesenteries of the first to the fourth cycle are weak and diffuse. Parietobasilar muscle very weak.

Cnidom: Spirocysts, basitrichs, microbasic *p*-mastigophores, microbasic *b*-mastigophores (Figure 5.6). For measurement of capsules see Table 5.11.

Distribution: *Calliactis androgyna* has been collected once from its type locality in the southern Atlantic Ocean off the coast of Brazil at 40 meters (Riemann-Zurneck, 1975).

Discussion

Calliactis androgyna is described based on one specimen collected off the southern coast of Brazil (Riemann-Zurneck 1975). It is sympatric with *C. tricolor* and is differentiated from this species by the irregular arrangement of the mesenteries of the second to fourth cycles, microbasic *p*-mastigophores in the column and basitrichs in the column that do not belong to two size classes.

The holotype (ZMH 7629) examined here corresponds very closely to the description given by Riemann-Zurneck (1975). The irregularity of the second to fourth cycles and scattered and irregularly arranged mesenteries of a fifth cycle that are characteristic of *C. androgyna* were also found here. The differences in the cnidae of *C. androgyna* and *C. tricolor* described here correspond to those described by

Riemann-Zurneck (1975). Another difference is that *C. tricolor* presents one class of basitrichs, whereas *C. androgyna* presents two classes of basitrichs.

The irregularities found in the mesenteries of *C. androgyna* are unique among any species of *Calliactis*. Members of the genus *Calliactis* present mesenteries that are strictly hexamerous and no members of the genus are known to reproduce asexually. Thus, these irregularities in *C. androgyna* set this species apart from other species in the genus. It is difficult, however, to appreciate whether this difference is the result of regeneration or any other abnormality, as only one individual of the species is known. Specimens of *C. tricolor* from the same type locality of *C. androgyna* were examined in this study and none of them presented these anatomical characteristics. Because other differences in the distribution of nematocysts are present in *C. androgyna,* I consider *C. androgyna* distinct from *C. tricolor*. More specimens, especially those from the type locality, are required to evaluate the status of *C. androgyna*.

***Calliactis tigre* nov. sp.**

Material examined: see Apendix A.

External anatomy (Figure 5.14)

Pedal disc: Pedal disc, circular, not very adherent. It usually covers a limited area of the hermit crab shell and it does not enwrap the shell completely. In cases where two

specimens coexist in the same shell, individuals may occupy most of the area of the shell. No cuticle is present in the specimens examined. The diameter of the pedal disc is usually the same diameter or slightly wider than the column diameter. Pedal disc diameter in preserved specimens range from 3.3 – 8.8 cm.

Column: Column with a very firm consistency, with a thick mesoglea, not divisible into scapus and scapulus. The capitulum is marked with numerous ectodermal pits. Cuticle never present in the column. One row of unconspicuous cinclides are located close to the pedal disc. As in *C. japonica*, cinclides are not regularly arranged and may be hardly visible in some individuals unless acontia have been emitted through them. The coloration of the column is very characteristic even in preserved specimens. Few preserved specimens do not present this coloration pattern The background color of the column is white with numerous dark red marks that do not form longitudinal stripes as commonly found in other *Calliactis* species. In preserved specimens, column height 3.3 – 8.6 cm, width 3.5 – 8.2 cm.

Oral disc and tentacles: The oral disc is circular and wide in relaxed specimens. Opaque white or bege, with white mesenterial insertions and two clear siphonophyphs visible. The oral disc is always erect and located opposite to the gastropod shell aperture as in other *Calliactis*. Oral disc diameter slightly wider than the pedal disc. The mouth is central and small. Tentacles are white or bege with transveral darker line along its length. Tentacles are restricted to approximately half of the oral disc.

They are short, conical, with inner tentacles longer than outer ones. Up to 96 tentacles hexamerously arranged in 5 cycles (6+6+12+24+48). Oral disc diameter: 1.0– 4.1 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, well-developed, transversally stratified. Mesenteries up to 48 pairs, hexamerously distributed in four cycles (6+6+12+24). Same number of mesenteries proximally and distally. Mesenteries of the first cycle are perfect and sterile and include two pais of directives associated with one siphonoglyph each. Second, third and fourth cycles with imperfect and fertile mesenteries. Filament and acontia restricted to the first, second and third cycles. Retractor of the mesenteries weak in the mesenteries of the first, second and third cycles. Mesenteries of the fourth cycle very reduced with very weak retractors. Parietobasilar and basilar muscles very weak.

Cnidom: Spirocysts, basitrichs and microbasic *p*-mastigophores (Figure 5.6). For measurement of capsules see Table 5.12.

Distribution: *Calliactis tigre* is described from specimens collected from the western Pacific Ocean, off the coast of Queensland and New South Wales in Australia. The new species is found between 166 – 508 meters.

Discussion

The presence of acontia with basitrichs as the only nematocyst, six pairs of perfect and sterile mesenteries, presence of cinclides in the column near the base, strong mesogleal sphincter, and weak parietobasilar and basilar muscles. *Calliactis tigre* is the second valid species of *Calliactis* recorded from the eastern coast of Australia. The new species is easily distinguished from *C. polypus*, also recorded from Australia, based on external and internal anatomy and nematocysts. Externally, *C. tigre* is much bigger than *C. polypus* and doesn't present cinclides that are conspicuously located on slight elevations of the column. Internally, *Calliactis tigre* differs from *C. polypus* by the number of mesenteries: *C. tigre* has 46 pairs of mesenteries, whereas *C. polypus* presents twice as many pairs of mesenteries (96 pairs). *Calliactis tigre* is further distinguished from *C. polypus* based on the number of mesenteries found proximally and distally: the same in *C. tigre* and higher distally in *C. polypus.* In addition, *C polypus* has up to 240 tentacles divided in seven cycles, whereas *C. tigre* has only 96 tentacles divided into 4 cycles. The size of basitrichs in the acontia also differs between the two species with no overlap. Although both species are found in the same locality, they are not in sympatry: while *C. tigre* is found between 160 – 508 meters, *C. polypus* is found, predominantly at shallower depths (0 – 45 meters).

Similarly, *C. tigre* is distinguished from *C. japonica*, a species that is recorded from Japan and is similar in size and external anatomy, based on the presence of tubercles in the column in the latter, the number and cycle of tentacles, and

differences in the development of mesenteries, and presence of filaments and acontia in the fourth cycle of mesenteries. In addition, details of nematocyst distinguish *C. tigre* from *C. japonica*: the presence of microbasic *p*-mastigophores in the pharynx in *C. japonica*, whereas *C. tigre* has only basitrichs in the pharynx; the size of basitrichs and microbasic *p*-mastigophores in the filament that does not overlap in these two species. Additionally, the bathymetric range of both species overlap only slightly: while *C. japonica* is collected between 0 – 200 meters, *C. tigre* is found between 160 – 508, predominantly at depths superior to 300 meters.

Calliactis tigre is distinguished from the remaining species of *Calliactis* based on number and cycles of mesenteries: while *C. tigre* has 48 pairs of mesenteries, *C. androgyna* has 82 pairs of mesenteries and *C. parasitica* has 96 pairs of mesenteries. In addition, the number and cycles of tentacles differ between *C. tigre* and other species of *Calliactis*: while *C. tigre* has up to 96 tentacles, *C. brevicornis* has up to 194 tentacles, *C. conchicola* has up to 112 tentacles and *C. tricolor* has up to 190 tentacles. *C. algoaensis* and *C. annulata* are distinguished from *C. tigre* based on their size, aspects of external morphology (division of the column in regions, location and development of cinclides, etc) and cnidae.

Genus *Paracalliactis* Carlgren 1928

Diagnoses (modified from Carlgren 1949, Doumenc 1975, Hand 1975a and Daly et. al. 2004).

Hormathiidae with pedal disc of greater diameter than oral disc, asymmetric but not bi-lobed as in *Adamsia*. The pedal disc secretes a cuticle that may or may not project beyond the shell aperture forming a carcinoecium. Column divisible into scapus and scapulus, without cinclides; may have a thin, easily deciduous cuticle. Column smooth or with distal tubercles that may form a complete corona. Column diameter greater than column height. Column nematocysts microbasic *p*-mastigophores and basitrichs. Same number of mesenteries proximally and distally. Mesenteries hexamerously arranged, six pairs of perfect and sterile mesenteries and two pairs of directives, each attached to a siphonoglyph. Retractor and parietobasilar muscles weak. Sphincter strong. Species belonging to this genus live attached to gastropod shells inhabited by hermit crabs with its oral disc positioned either dorsally (directed away from the aperture of the shell) or laterally, never ventrally as in *Adamsia*.

Type species: *Paracalliactis valdiviae* Carlgren 1928

Valid species: *Paracalliactis valdiviae* Carlgren 1928

Paracalliactis michaelsarsi Carlgren 1928

Paracalliactis consors (Verrill 1882)

Paracalliactis lacazei Dechancé and Dufaure 1959

Paracalliactis azorica Doumenc 1975

Paracalliactis rosea Hand 1976

Paracalliactis sinica Pei 1982

Paracalliactis obvolva Daly Ardelean Cha and Fautin 2004

Paracalliactis niwa sp. nov.

***Paracalliactis valdiviae* Carlgren 1928**

Paracalliactis valdiviae Carlgren 1928a: 193-196; Carlgren 1945:18; Carlgren 1949: 95.

Paracalliactis stephensoni Carlgren 1928b: 170-172; Carlgren 1945: 18; Carlgren 1949: 95; Doumenc 1975: 161-163.

Material examined: See Appendix A.

External anatomy (Figure 5.15)

Pedal disc and cuticle: Pedal disc wide and irregularly shaped. The degree of pedal disc development varies, with some specimens exhibiting a pedal disc that completely enwraps the shell and others where parts of the shell are not covered. The pedal disc, however, is always wider than the diameter of the oral disc or the diameter of the column. The cuticle secreted by the pedal disc is very thin and, in some specimens, can only be visualized because the column is very transparent proximally. The cuticle does not enlarge the living space of the crab. The pedal disc varies in color, from pink

to transparent. The cuticle is usually brown, but may take a darker golden color, in some cases. Pedal disc diameter: 1.2 – 3.3 cm.

Column: The column is divisible into scapus and scapulus. In some specimens, the scapus has a brown, thin cuticle, but, in most cases, specimens do not present a clear cuticle. The fragile nature of the cuticle could probably explain the absence of a cuticle in some specimens due to handling during collection and preservation. The scapus is smooth until distal part, close to the scapulus, where it exhibits one row of 12 very well developed tubercles that form a complete corona. Some of the tubercles are still covered by a thin, brown layer of cuticle. The scapulus is short and free of cuticle, with markedly longitudinal ridges. No cinclides. The color of the column in preserved specimens is white, but a few specimens are a dark yellow. In preserved individuals, column height 1.0 –2.7 cm, width 0.8 – 2.2 cm.

Oral disc and tentacles: The oral disc is located directed away from the aperture of the shell (dorsally) or lateral to the aperture of the shell. Most preserved specimens had their oral disc fully retracted, but when expanded, mesenterial insertions are generally visible, as well as two well- developed siphonoglyphs. In some cases, a short pharynx can be seen through the oral disc and acontia may be extruded through the mouth. Up to 94 tentacles were counted, which were divided into 4 cycles. Tentacles are usually short and do not present any coloration pattern, being white or light pink in its entire

length, with the inner longer than the outer ones. Oral disc diameter ranged from 0.3 – 1.9 cm.

Internal anatomy and microanatomy

Sphincter mesogleal and strong with occasional major lamellae of mesoglea crossing from side to side and with a tendency to transversal stratification. Mesenteries are regularly arranged in a total of 48 pairs distributed in four cycles (6+6+12+24). Same number of mesenteries proximally and distally. Six pairs of mesenteries form the first cycle and are perfect and sterile, including two pairs of directives associated with one siphonoglyph each. The rest of the mesenteries are imperfect and fertile, distributed in three cycles. All pairs of mesenteries present acontia and filaments and run from the margin to the base. Acontia are abundant when present and many acontia can be seen close to the mouth of preserved specimens. Retractor of the mesenteries and parietobasilar muscles weak and diffuse. The parietobasilar muscles are weak in the perfect mesenteries than in the imperfect mesenteries of the second, third, and fourth cycles.

Cnidom: Spirocysts, basitrichs, microbasic *p*-mastigophores, (Figure 5.16). For measurement of capsules see Table 5.13.

Distribution: *Paracalliactis valdiviae* is known from off the coast of Somaliland in east Africa at depths between 628 and 823 meters (Carlgren, 1928a). *Paracalliactis*

stephensoni is known from the North Atlantic Ocean between 1590 and 2700 meters (Carlgren 1928b; Doumenc 1975).

Discussion

The anatomy and microanatomy of the type material of *P. valdiviae*, the type species of *Paracalliactis*, examined in this study corresponds closely to the original description of Carlgren (1928a). In his work, Carlgren (1928a) makes a very detailed description of the species, including information not only about the external morphology of the anemone, but also information on the internal anatomy and microanatomy. Although Carlgren (1928a) notes that while some of specimens he designated as syntypes present tubercles, other specimens do not clearly present such structures. In the re-examination of the syntypes, I confirm that only some of them have clear tubercles forming a more or less complete corona. The specimens that do not present clear tubercles, however, do not present a smooth appearance either, maybe indicating that the presence or absence of tubercles in preserved specimens may be related to either biological reasons or to preservation reasons (or to both).

The distribution and measurements of nematocysts in the original description of *P. valdiviae* (Carlgren, 1928a) is not complete; it includes values for tentacles, pharynx and acontia, but not column or filaments. Later, Carlgren (1945) re-examines the type material and although his results does not contradict his earlier work, it makes a more complete account of the cnidae of *P. valdiviae,* including structures not included in the original description of the species (Carlgren 1928a). The cnidae

described in the present study differs from that of Carlgren (1928a) and Carlgren (1945) in a few respects: although rare, a second category of small basitrichs is found in the column of most syntypes re-examined (3/4); microbasic *p*-mastigophores were found in high abundance in the pharynx of the specimens examined (4/4); a new category of small basitrichs were found in the acontia of all specimens examined. The range of the remaining categories of nematocysts found in the present study and in Carlgren (1928a) and Carlgren (1945) are very similar and indicate that the differences found may be attributed to the variability of the species itself. The absence of a type or size of a nematocyst in any specimen does not implicate its complete absence, especially if the capsule is rare when present. Thus, the finding of new categories of nematocysts in the syntypes of *P. valdiviae* is probably due to a more comprehensive account of the cnidae of the species and perhaps more precision in the measurements of these nematocysts.

The specimens of *P. stephensoni* in this study correspond in general terms to the originally described by Carlgren (1928b). Carlgren (1928b) makes a short description, including only basic information on external and internal anatomy, microanatomy and cnidae. Although fairly abundant in the North Atlantic, type material for this species is unknown (see Fautin 2010). In his description of *P. stephensoni*, Carlgren (1928b) notes that most individuals possess a clear row of tubercles forming a complete corona in the scapus, whereas others exhibit a degree of tubercle development that varies from unclear to completely lacking, especially in younger individuals. Doumenc (1975) proposes that both *P. valdiviae* and *P.*

stephensoni present a wide degree of variability regarding tubercle development. All specimens examined by him present tubercles. The specimens examined in the present study also show the varying degree of tubercle variation, including specimens with and without tubercles in a single lot.

Similarly, the variability of pedal disc and cuticle development present in *P. stephensoni* described by Carlgren (1928b) and Doumenc (1975) is confirmed by the present study. Specimens of *P. stephensoni* exhibit a pedal disc that may completely enwrap the shell to which is attached, enlarging the living space of the hermit crab in, sometimes more than 1 cm or only a few millimeters. In some cases, similarly to *P. valdiviae* the cuticle can only be visualized through the column due to its transparency.

Although the nematocysts data included in the original description of *P. stephensoni* (Carlgren 1928b) is not complete, Carlgren (1945) expands the information based on examination of the type material that largely agrees to his previous work. The cnidae of *P. stephensoni* of Doumenc (1975) differs from that published by Carlgren (1928b, 1945) in the column, filament and in the acontia; Doumenc (1975) finds an extra category of smaller basitrichs in both column and acontia and does not find basitrichs in the filaments. The cnidae reported in the present study agrees in part to Carlgren (1928b, 1945) and to Doumenc (1975): although rare, a second category of small basitrichs in the column is confirmed here and in the acontia (similarly to Doumenc (1975), and basitrichs are also found in the filament (as in Carlgren 1928b, 1945). The discrepancies found in this study and

previous works are not sufficient to differentiate the specimens examined here from those of Carlgren (1928b, 1945) and Doumenc (1975).

Based on examination of specimens of *P. valdiviae* (including the type material) and *P. stephensoni* and considering the external and internal anatomical characters, as well as microanatomy and nematocyst information, the species *P. valdiviae* and *P. stephensoni* cannot be distinguished at this point and should be considered the same species: *P. valdiviae*. The synonymy of these two species is questioned by Carlgren (1928b), when he noted that the newly described species, *P. stephensonsi* is very similar to *P. valdiviae*, also described by him (Carlgren, 1928a). In his discussion of *Paracalliactis*, Doumenc (1975) only differentiates these two species morphologically on the basis of the slightly larger cnidae measurements of *P. stephensoni.* Although a tendency for slightly longer measurements of some cnidae types in *P. stephensoni* is confirmed in the present study (e.g., spirocysts in the tentacle and microbasic *p*-mastigophores in the column), this small discrepancy can be attributed to variability of the species and is not higher than the variability found in individuals from a single population or from distinct populations (pers. obs.).

A second difference between *P. valdiviae* and *P. stephensoni* is the hermit crab species on which they are found. While *P. valdiviae* is always found on a shell inhabited by *Parapagurus bicristatus* or *Pp. andersoni*, *P. stephensoni* is symbiotic to *Pp. pilosimanus* or *Pp. nudus* (Doumenc 1975). The two anemones species are also found in geographically disjunct localities: *P. valdiviae* has been only collected from its type locality off the east coast of Africa (Somaliland), whereas *P. stephensoni* has

been originally collected from the west coast of Ireland, but is widely distributed in the north Atlantic.

***Paracalliactis michaelsarsi* Carlgren 1928**

Paracalliactis michaelsarsi Carlgren 1928b: 172; Carlgren 1934: 13; Carlgren 1949: 95; Doumenc 1975: 163-166; Pei, 1982: 68.

Material examined: See Appendix A.

External anatomy (Figure 5.17)

Pedal disc and carcinoecium: In all specimens examined, the pedal disc is wide and asymmetric and completely enwraps the shell. The pedal disc is much wider than the column diameter, getting especially thick proximally and secretes a very well-developed carcinoecium. In most specimens, no shell is found associated with the anemone, but in the few cases where it is still present, the carcinoecium extends well beyond the aperture of the shell and greatly greatly enlarges the living space of the crab. 1.4 cm. The carcinoecium has a bronze coloration and may enlarge the shell at approximately 1.4 cm in larger specimens. Pedal disc diameter in preserved specimens range from 1.8 to 3.5 cm.

Column: Column divisible into scapus and scapulus. The scapus is completely smooth, with no tubercles. A very thin, slightly disappearing cuticle is observed in the

scapus of some specimens, especially in the larger ones. The scapulus does not exhibit a cuticle, but is marked by shallow longitudinal ridges. No cinclides. The color of the column in preserved specimens is white, almost transparent proximally. In preserved individuals, column height 2.0 – 4.0 cm, width 1.4 – 3. cm.

Oral disc and tentacles: The oral disc is located either dorsally (opposite to the aperture of the shell) or laterally (lateral to the aperture of the shell). When expanded, the oral disc has clear mesenterial insertions and short tentacles that do not exhibit any coloration pattern, with inner tentacles longer than outer ones. Ninety-six tentacles are present, divided into 5 cycles. Tentacles white, bege or pink. Oral disc diameter ranges from 0.2 – 2.2 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, with its fibers showing a transversal stratification. Same number of mesenteries proximally and distally distributed in four cycles in a total of 48 pairs (6+6+12+24). The first cycle with six pairs of perfect and sterile mesenteries, including two pairs of directives. Each pair of directives associated with one siphonoglyph. The remaining cycles are formed by imperfect and fertile mesenteries. The second and third cycles both exhibit filaments and acontia, but the fourth cycle is reduced and lack filaments or acontia. The retractor muscles of the mesenteries are very small and weak and have a diffuse nature. Parietobasilar muscles also weak and diffuse.

Cnidom: Spirocysts, basitrichs, microbasic *p*-mastigophores, (Figure 5.16). For measurement of capsules see Table 5.14.

Distribution: *Paracalliactis michaelsarsi* is found in the North Atlantic Ocean (Carlgren 1928b; Doumenc 1975) with parts of its range overlapping with the distribution of *P. stephensoni*. The bathymetric range of *P. michaelsarsi* is 4166 – 4700 meters vs. 1340 – 2456 meters of *P. stephensoni*.

Discussion

Described by Carlgren (1928b) from the North Atlantic Ocean, *P. michaelsarsi* is only known from depths of more than 4000 meters and is distinguished from other species by its relative larger size and cnidae. Carlgren, 1928b) differentiates it from *P. stephensoni*, also known from the North Atlantic Ocean, by its symbiotic habit, including a carcinoecium that greatly enlarges the living space of the crab and by the absence of tubercles in the column of *P. michaelsarsi*. Additionally, both species are differentiated by the position of the anemone on the shell with individuals of *P. michaelsarsi* having the oral disc always oriented close to the shell aperture and by small differences in the cnidae. Doumenc (1975) further distinguishes these species based on the space between the striations of the carcinoecium, with *P. michaelsarsi* exhibiting striations 1mm apart and *P. stephensoni* with striations of 0.1mm or 1mm apart. In addition, small differences in

the size and distribution of cnidae further distinguish these two species, with those from *P. michaelsarsi* slightly longer than those reported for *P. stephensoni*.

The specimens of *P. michaelsarsi* examined in the present study largely agree with previous descriptions of its external morphology, internal anatomy, and microanatomy published by Carlgren (1928b, 1934) and Doumenc (1975). After examining the type material of *P. michaelsarsi* and additional specimens collected from different localities of the North Atlantic Ocean, the smooth nature of column is confirmed, as well as the orientation on the shell. Confidence that the lack of tubercles in the column of *P. michaelsarsi* is not a preservation artifact is raised by the fact that none of the specimens examined showed any sign of tubercles. Additionally, the position of the anemone on the shell in this species is very constant and only in two cases specimens have been oriented in a different position on the shell which contradicts the findings of Doumenc (1975) who finds specimens located both close to the aperture of the shell or directed away from the aperture of the shell.

After examining more than 30 specimens of each species, the striation on the carcinoecium secreted by the pedal disc of both *P. michaelsarsi* and *P. stephensoni* did not show any regularity. This character does not seem to be a constant characteristic among individuals of the same species, This shows that it might be related to biological or ecological interactions with the environment. For this reason, the utility of this character is compromised and it will not be used to distinguish species in the genus *Paracalliactis*.

The distribution and size of cnidae in *P. michaelsarsi* reported in the present study are largely in agreement with those reported by Carlgren (1928b) in the original description of the species and later by Doumenc (1975). The small range discrepancy found in different tissues of the species are not very important and are probably related to the differences found in any species. These differences are not enough to distinguish the specimens studied in the present study from those described by others (Carlgren 1928b; Doumenc 1975). A few differences, however, are found, including: the present study detected microbasic *p*-mastigophores in the column of *P. michaelsarsi*, while Carlgren (1928b) does not (Doumenc, 1975 did not report cnidae from the column); the size of *p*-mastigophores in the pharynx of the species were only detected in the present study, not by others. Carlgren (1928b) notes that the ectoderm of *P. michaelsarsi* was almost ripped off in the specimens he used to describe the species. This might explain the absence of microbasic *p*-mastigophores in the column of *P. michaelsarsi*, since this is an important and very constant character that defines the genus *Paracalliactis*.

In addition to the morphological distinction found between *P. michaelsarsi* and *P. stephensoni*, nematocyst differences were also detected in the present study. We confirm that the cnidae of *P. michaelsarsi* is invariably larger than those of *P. stephensoni*. Differences in the classes of the cnidae found in different tissues are also important. For example, a small class of basitrichs is found in *P. stephensoni;* the microbasic *p*-mastigophores found in the *P. stephensoni* is much smaller than found in *P. michaelsarsi;* basitrichs of the filament of *P. stephensoni* are of a different and

smaller class; the microbasic *p*-mastigophores in *P. stephensoni* belongs to a smaller class compared to *P. michaelsarsi* which have two different classes of much larger microbasic *p*-mastigophores. Additionally, *P. stephensoni* exhibits two sizes of basitrichs in the acontia, while *P. michaelsarsi* present only one size (a larger size) of basitrichs.

***Paracalliactis consors* (Verrill 1882)**

Urticina consors Verrill 1882: 225; Verrill 1883: 49-50.

Actinauge consors Verrill 1885: 534

Adamsia involvens McMurrich 1893: 182-183; Carlgren 1896: 174; Carlgren 1947: 15.

Paracalliactis involvens Carlgren 1949: 95; Dechancé and Dufaure 1959: 1567; Sebens 1998: 28.

Paracalliactis consors Daly et. al. 2004: 392-395.

Material examined: See Appendix A.

External anatomy (Figure 5.18)

Pedal disc and carcinoecium: Pedal disc circular and irregularly shaped, but never bi-lobed. Pedal disc much wider than column diameter. The pedal disc wraps the shell entirely, at least in larger individuals. Smaller specimens may not cover the shell entirely. When the pedal disc completely enwraps the shell a carcinoecium is present.

In individuals that do not completely enwrap the shell, a less developed cuticle is present. Various degrees of cuticle development is found, especially when smaller and larger specimens are compared, but the shell is always present, even when a more developed carcinoecium is found. When a cuticle is present it does not enlarge the shell of the hermit crab, but the pedal disc is thickened close to the limbus, close to the edge of the shell. Pedal disc is white and the disc diameter in preserved specimens range from 0.8 to 2.5 cm.

Column: Column divisible into scapus and scapulus. A thin cuticle is found in the scapus of most specimens, but not in the scapulus. The scapulus is longitudinally furrowed in preserved specimens. In the distal part of the column, tubercles are more or less developed and may form a complete corona of tubercles. In some specimens, the tubercles are not easily identified and the column appears to be smooth. This is probably an artifact of preservation, because the same lot contains specimens with and without clearly identified tubercles. No cinclides. The color of the column in preserved specimens is light brown or light bege, with mesenterial insertions very clear, especially close to the base. In preserved specimens, column height 1.0 –3.0 cm, width 0.8 – 3.2 cm.

Oral disc and tentacles: The oral disc is located either in the opposite side of the gastropod shell aperture or beneath the shell aperture. Most preserved specimens had their oral disc fully retracted, but when the oral disc was expanded, up to 96 tentacles were present, distributed in five cycles. Inner tentacles longer than outer ones,

ranging from 0.5 – 1.0 cm. Tentacles and oral disc light bege. Oral disc diameter is about half of the diameter of the base, ranging from 0.5 – 1.5 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, strong, with transversal stratification. Mesenteries up to 48 pairs, distributed in four cycles (6+6+12+24). Same number of mesenteries proximally and distally. Mesenteries of the first cycle perfect and sterile, those of the second, third and fourth cycle imperfect and fertile. Mesenteries of the fourth cycle are very reduced, with no filaments and acontia. Retractor of the mesenteries and parietobasilar muscles weak and diffuse. Two pairs of directives associated with two large siphonoglyphs. Acontia are abundant when present and many acontia can be seen close to the mouth of preserved specimens.

Cnidom: Spirocysts, basitrichs, microbasic *p*-mastigophores, microbasic *b*-mastigophores (Figure 5.16). For measurement of capsules see Table 5.15.

Distribution: *Paracalliactis consors* is distributed in the western Atlantic Ocean (eastern United States)(Verrill 1882; Sebens 1998; Daly et. al. 2004) and the eastern Pacific Ocean (Galápagos Islands, Ecuador)(McMurrich 1893; Daly et. al. 2004). Its bathymetric range is between 600 –660 meters.

Discussion

McMurrich (1893) describes the species *Adamsia involvens* from material collected at 1344 meters from the Galapagos Islands (Ecuador). In the original description of *A. involvens*, however, McMurrich (1893) notes that the new species secreted a carcinoecium but no cinclides were present and the acontia were emitted from the mouth of the individuals, not from the column wall as is the case when cinclides are present. McMurrich (1893) decides to place the species in the genus *Adamsia* due to the secretion of a carcinoecium, but notes that the absence of cinclides may indicate that this is not the correct placement for this new species. In fact, after examining the type material of *A. involvens*, Carlgren (1947) transfer the species to the genus *Paracalliactis*.

In his work of hexacorallians of the eastern coast of the United States, Sebens (1998) refers to the material collected off the coast of Massachusetts and New England as *P. involvens*. This is an indication that Sebens (1998) considers *Urticina consors*, a species that was originally described by Verrill (1882) for the same locality, a synonym of *P. involvens*. Daly et. al. (2004) agrees with the synonym of these two species of *Paracalliactis* given by Sebens (1998), but notes that *U. consors* is the older name and since no other names have been used recently in publications, except Sebens (1998), the species should be called *P. consors* (ICZN 1999, Art. 23.9.1).

The original description of *P. consors* (Verrill 1882) and a subsequent re-description by Verrill (1883) are very incomplete and include only information about

external anatomy, such as column, oral disc and tentacle morphology, as well as size and coloration of specimens. Although Verrill (1882) places this new species in the genus *Urticina*, he notes that this placement may not be correct and the species may not be a true *Urticina.* Verrill (1882) finds similarities between the *U. consors* a certain species of the family Sagartiidae, but was prevented from placing the new species in that family because no acontia was found in the material used to describe *U. consors*. Sebens (1998) is the first to examine and describe the internal anatomy and microanatomy of *P. consors*, while Daly et. al. (2004) re-describes *P. involvens* as *P. consors* to reflect the synonym of those two species.

After examining the type material of *A. involvens*, the placement of the species in the genus *Paracalliactis* made by Carlgren (1947) is confirmed in the present study. The type material of *A. involvens* exhibits characters that are clearly present in species of *Paracalliactis*, including: although the pedal disc enwraps the shell, it is not bi-lobed as in *Adamsia*; the oral disc is always directed away from the aperture of the shell; secretion of a carcinoecium; absence of cinclides in the column; same number of mesenteries proximally and distally; same number of tentacles and mesenteries; presence of microbasic *p*-mastigophores in the column. The re-examination of the type material of *A. involvens* and additional specimens collected from in the type locality however, differ from the original description of *A. involvens* in that tentacles are not distributed in three cycles, instead tentacles are arranged in four cycles. This difference is probably due to the degree of contraction of the type

specimens, making the correct distribution of tentacles very difficult, something noted by McMurrich (1893) in the original description of the species.

The syntypes of *U. consors* and *A. involvens* examined in the present study corresponds closely to previous descriptions of each of these species (Verrill 1882; 1883; Sebens 1998; Daly et. al. 2004). The differences between *P. consors* and *P. involvens* includes the number and cycles of tentacles, the presence or absence of tubercles, the size of spirocysts in the tentacles and their geographic distribution. While Verrill (1882, 1883) estimates that *P. consors* had four cycles of tentacles, McMurrich finds only three cycles of tentacles in *P. involvens*. As mentioned, the present study agrees with Daly et. al. (2004) and Sebens (1998) in that both species present ~96 tentacles and confirms that they are distributed in 4 cycles. The presence of tubercles in some syntypes of *U. consors* and absence of these structures in *P. involvens* noted by Daly et. al. (2004) and in the present study should be regarded as individual or preservation artifact. In fact, this type of variation in tubercle development has been found in other species of *Paracalliactis*, including the type species of the genus, *P. valdiviae*, *P. stephensoni* and *P. michaelsarsi* (pers.obs). The reported size difference of the spirocysts in the tentacles of *P. consors* and *P. involvens* of Daly et. al. (2004) is not confirmed after the examination of syntypes of both species. While the range of cnidae reported here for both species are very similar, they differ from previous accounts in that although rare, microbasic *b*-mastigophores were found in the tentacles of *P. involvens* and *P. consors*. Additionally, small differences in the size of basitrichs and microbasic *p*-

mastigophores in the filament and basitrichs in the acontia were between the two species. These small differences can be attributed to the variability found in individuals of these two species and should not be regarded a reason to distinguish them. Although *P. involvens* and *P. consors* were described from widely different localities, no morphological differences that would distinguish these two species were found. For this reason, the present study confirms the synonym of *P. involvens* and *P. consors* proposed by Sebens (1998) and agrees with Daly et. al. (2004) that the valid name for the species is *P. consors*.

***Paracalliactis rosea* Hand 1975**

Paracalliactis rosea Hand 1975a: 501-506; Hand 1975b: 514-519; Hicks et. al. 1975: 7; Pei, 1982: 68; Ates 1989: 71; Ates 1997: 12; Dawson 1992: 41.

Material examined: See Appendix A.

External anatomy (Figure 5.19)

Pedal disc and cuticle: Pedal disc irregularly shaped, if may be circular or elongated and oval, always asymmetric. The degree of pedal disc development varies, but in most cases it completely wrapping the gastropod shell. In all cases, the pedal disc is much wider than the column diameter. The pedal disc secretes a cuticle that may be very well developed in some specimens, especially in the larger ones, or may be less developed. It never extends beyond the shell aperture. The pedal disc is fragile and

easily destroyed as one tries to remove the anemone from the shell. In most cases, the shell is still present, but in a few specimens. Pedal disc diameter in preserved specimens range from 1.2 to 3.5 cm. The pedal disc varies in color, from white or light bege, to transparent. The carcinoecium is usually bright golden in color, but may take a dark golden color, in some cases. Pedal disc diameter 0.9 – 3.2 cm.

Column: Column clearly divisible into scapus and scapulus. In some specimens, the scapus has a brown, thin cuticle, but, in most cases, specimens do not present a clear cuticle. The fragile nature of the cuticle could probably explain the absence of a cuticle in some specimens due to handling during collection and preservation. The scapus is smooth until its distal part, close to the scapulus, where it exhibits one row of 12 very well developed tubercles that form a complete corona. Some of the tubercles are still covered by a thin, brown layer of cuticle. The scapulus is short and free of cuticle, with markedly longitudinal ridges. No cinclides. The color of the column in preserved specimens is white, but a few specimens may be dark yellow or pink. In preserved individuals, column height 1.0 –2.7 cm, width 0.8 – 2.2 cm.

Oral disc and tentacles: The oral disc is always located in the opposite side of the gastropod shell aperture. Most preserved specimens had their oral disc fully retracted, but when expanded, mesenterial insertions are generally visible, as well as two well-developed siphonoglyphs. In some cases, a short pharynx can be seen through the oral disc and acontia may be extruded through the mouth. Up to 96 tentacles were

counted, which are divided into 4 cycles. Tentacles are usually short and do not present any pattern, being white or light pink in its entire length, with the inner longer than the outer ones. Oral disc diameter ranges from 0.3 – 1.9 cm.

Internal anatomy and microanatomy

Sphincter mesogleal and strong with a tendency to transversal stratification. Mesenteries are regularly arranged in a total of four cycles (6+6+12+24). Same number of mesenteries proximally and distally. Six pairs of mesenteries form the first cycle and are perfect and sterile, including two pairs of directives associated with one siphonoglyph each. The rest of the mesenteries are imperfect and fertile, distributed in three cycles. All pairs of mesenteries present acontia and filaments and run from the margin to the base. Acontia are abundant when present and many acontia can be seen close to the mouth of preserved specimens. Retractor of the mesenteries and parietobasilar muscles weak and diffuse. The parietobasilar muscle is weaker in the perfect mesenteries and becomes more obvious in the imperfect mesenteries of the second, third and fourth cycles.

Cnidom: Spirocysts, basitrichs, microbasic *p*-mastigophores, (Figure 5.16). For measurement of capsules see Table 5.16.

Distribution: *Paracalliactis rosea* is distributed in the western Pacific Ocean, off the east coast of New Zealand (Hand 1975a; pers. obs.). Specimens of *P. rosea* have been

collected at depths between 50 – 140 meters and between 600 – 660 meters (Hand 1975a; pers. obs.).

Discussion

The species *P. rosea* described from New Zealand by Hand (1975a) is the best well described species in the genus *Paracalliactis*. The absence of cinclides, the position of the anemone on the shell, the presence of a cuticle, and the presence of microbasic *p*-mastigophores in the column are some characters that confirm the identity of this species as a *Paracalliactis*. Additionally, *P. rosea* exhibit the same number of tentacles and mesenteries, a strong mesogleal sphincter and retractor and parietobasilar muscles weak and diffuse. Contrary to other species of the genus *Paracalliactis*, however, *P. rosea* is found from much shallower depths, being collected from 100 – 1000 meters continuously, while most specimens have their bathymetric range starting at 1000 meters and going as deep as 4750 meters.

The specimens studied here correspond very closely to the original description of *P. rosea*. A few differences from the study of Hand (1975a), however, should be discussed. Hand (1975a) notes that specimens of *P. rosea* may vary in the appearance of tubercles present in the distal part of the scapus. All specimens examined in the present study, however, including the holotype and paratype of these species possess clear and well-developed tubercles. The variation in the development of tubercles, however, seem to be widespread in the genus *Paracalliactis* and is found in the other species, including *P. valdiviae* (type species of the genus *Paracalliactis*), *P.*

stephensoni and *P. michaelsarsi*. The absence of tubercles in preserved specimens seems to be a preservation artifact and do not represent the morphology present in the anemones while they are alive.

The re-examination of the cnidae of *P. rosea* exhibited a few differences to the distribution and range of nematocysts given by Hand (1975a) in the original description. These differences include: rare small basitrichs in the column; no large basitrichs in the in the material of *P. rosea* examined; expanded ranges for basitrichs in the column, microbasic *p*-mastigophores in the pharynx and large basitrichs in the tentacles. The variability in the nematocyst measurements showed here are based in the type material and vouchers and so probably reflect real variation and some differences in methods of sampling or measurement equipment.

The name *Paracalliactis rosea* is used by Ross (1974b: 287-288) in his review of behavior patterns in associations of cnidarians and other animals. At the time of publication, however, no formal description had been made for the species *P. rosea*. For this reason, Hand (1975a) considers the name a *nomen nudum*.

Paracalliactis obvolva **new. comb.**

Adamsia obvolva Daly et al. 2004: 385-392, 394, 397.

Material examined: See Appendix A.

External anatomy (Figure 5.20)

Pedal disc and carcinoecium: Pedal disc very irregular, completely wrapping the gastropod shell inhabited by the hermit crab, but not bi-lobed. Pedal disc much wider than the column diameter. The pedal disc secretes a bronze-colored carcinoecium that does not extend beyond the pedal disc of the anemone. Only the carcinoecium, not the shell, is usually observed in the specimens examined. The shell has been probably completely dissolved. Pedal disc diameter in preserved specimens range from 1.7 to 4.2 cm.

Column: Column smooth, with a short scapulus. Cinclides were not observed in any of the specimens examined, although sometimes, close to the oral disc, some specimens may have parts of the column sunken and that may appear like a cinclide. Mesenterial insertions can be seen in most specimens examined, especially proximally. No cinclides. The color of the column in preserved specimens is pinkish or light bege. Live specimens have a bright pink column with slightly darker longitudinal stripes. In preserved specimens, column height 2.3– 4.8 cm, width 1.9 – 3.6 cm.

Oral disc and tentacles: The oral disc is located in the opposite side of the gastropod shell aperture. Most preserved specimens had their oral disc fully retracted. Those that had the oral disc fully expanded presented up to 190 short tentacles divided in 5 cycles (probably 6+12+24+48+96), inner tentacles longer than outer ones. Inner

tentacles range from 0.2 – 1.2 cm. Tentacles and oral disc present the same color: pink or light bege.

Internal anatomy and microanatomy

Sphincter mesogleal, transversally striated, strong. Mesenteries up to 48 pairs in number, distributed in four cycles (6+6+12+24). Mesenteries of the first cycle perfect and sterile, those of the second, third and fourth cycle imperfect and fertile. A fifth cycle of very reduced mesenteries without filaments, gonads or acontia close to the margin. Retractor of the mesenteries and parietobasilar muscles weak and diffuse. Two pairs of directives associated with two siphonoglyphs. Acontia are not abundant and pink in preserved specimens. Many acontia can be seen close to the mouth of preserved specimens, something common in specimens that do not possess cinclides.

Cnidom: Spirocysts, basitrichs, microbasic *p*-mastigophores, microbasic *b*-mastigophores (Figure 5.16). For measurement of capsules see Table 5.17.

Distribution: *Adamsia obvolva* is known only from the Gulf of Mexico and its bathymetric range is 379 –731 meters (Daly et. al. 2004). Most specimens of *A. obvolva* were collected between 379 – 538 meters (pers. obs.).

Discussion

The species *A. obvolva* is described by Daly et. al. (2004) to differentiate specimens that were misidentified as *P. involvens* collected from the Gulf of Mexico but that possessed cinclides and about half the number of tentacles as *P. involvens*. The reason to consider *A. obvolva* a member of the genus *Paracalliactis*, including the absence of cinclides and presence of microbasic *p*-mastigophores in the column of those specimens, has already been discussed. Here, the focus will be the distinction between *A. obvolva* and *P. consors*, species that were collected from the same locality (Gulf of Mexico), that live at similar depths and live in symbiosis with the same species of hermit crab (*Parapagurus pictus*). Additionally, *P. obvolva* and *P. consors* both secrete a carcinoecium, have similar size, mesentery arrangement and musculature morphology. *Paracalliactis obvolva* and *P. consors* are distinguished morphologically by the number of tentacles and by differences in the range of nematocysts in the tentacles, filament and acontia and the absence of microbasic *p*-mastigophores in the column of *A. obvolva*.

After an extensive examination of the type material of *A. obvolva* as well as additional material collected from the type locality, most differences between this species and *P. consors* were not confirmed. Reported differences in the size of basitrichs in the tentacles and in the acontia and in the microbasic *p*-mastigophores in the filament were not found and are in agreement with the range reported for the species *P. consors* by the present study. Additionally, the presence of microbasic *p*-mastigophores in the column of *A. obvolva* given by Daly et. al. (2004) should not be

used in the differentiation of this species from *P. consors*, since these nematocysts although rare, were present in the specimens examined in that study. The re-examination of specimens of *A. obvolva* further confirmed the presence of microbasic *p*-mastigophores.

Although a discrepancy between the numbers of tentacles in *P. obvolva* has been found between those reported by Daly et. al. (2004) and the present study, the difference in tentacles number is the only remaining character that distinguish the species *P. obvolva* and *P. consors*. Until further examination of additional specimens of *P. obvolva* from the Gulf of Mexico are carried out and although the similarities between these two species are present, because the characters that distinguishes species within *Paracalliactis* can be sometimes very sutil, the species *P. obvolva* will remain separated from the species *P. consors*.

***Paracalliactis niwa* nov. sp.**

Material examined: See Appendix A.

External anatomy (Figure 5.21)

Pedal disc and cuticle: In most specimens examined, the pedal disc has an irregular outline, but does not completely enwrap the shell. Pedal disc is wider than the column diameter and it secrets a thin, fragile, bronze cuticle that does not extend beyond the aperture of the shell and does not enlarge the living space of the crab. The pedal disc

is fragile and easily destroyed as one tries to remove the anemone from the shell. The pedal disc has very clear mesenterial insertions represented by dark lines that can be seen through the pedal disc (and the column). In all specimens, the shell is still present. The pedal disc is light brown, but becomes almost transparent proximally. Pedal disc diameter in preserved specimens range from 2.1 – 3.7 cm.

Column: Column divisible into scapus and scapulus. The scapus exhibits a very thin deciduous cuticle that in most specimens has already been scrapped off from the ectoderm. The scapus is mostly brown, but it presents a very characteristic coloration pattern: longitudinal darker brown stripes constrast with the light brown color of the column. Additionally, the column is has deep longitudinal furrows that coincide with the coloration pattern. All specimens present scattered ectodermal pits that can be seen when the column is examined in low magnification of a dissecting scope, as well as in cross sections of the animal in histological sections of the column. Otherwise, the scapus is smooth and does not present tubercles or other specializations. The scapulus is free of a cuticle, short and with deep longitudinal furrows. No cinclides.. In preserved individuals, column height 1.7 – 3.0 cm, width 1.4 – 2.4 cm.

Oral disc and tentacles: The oral disc is always located in the opposite side of the gastropod shell aperture. When expanded, the oral disc is circular, with white striations corresponding to the mesenterial insertions. The mouth is small, with prominent lips, and marked by the two broad siphonoglyphs. Up to 172 smooth

tentacles, hexamerously arranged in 5 cycles (6+6+12+24+48) are present. Inner tentacles usually longer than outer ones. Tentacles are dark white or transparent. Oral disc diameter ranges from 0.2 – 2.2 cm.

Internal anatomy and microanatomy

Sphincter mesogleal, moderately strong, with transversal stratification. Mesenteries are hexamerously arranged in a total of 96 pairs of mesenteries, distributed in 5 cycles (6+6+12+24+48). Same number of mesenteries proximally and distally. The first cycle perfect and sterile; second ,third and fourth cycles imperfect and fertile; fifth cycle imperfect and very reduced, without filaments or acontia. The retractors of the mesenteries and the parietobasilar muscles are weak. The spirilized acontia are present in all cycles, but especially abundant in the first four cycles.

Cnidom: Spirocysts, basitrichs, microbasic *p*-mastigophores, (Figure 5.16). For measurement of capsules see Table 5.18.

Distribution: *Paracalliactis* niwa nov. sp. is currently only known from the Tasman Sea off the western coast of New Zealand. Its bathymetric range is 2417 – 2421 meters.

Discussion

The new species *P. niwa* is placed in the genus *Paracalliactis* due to the absence of cinclides in the column of the species, presence of six pairs of perfect and sterile mesenteries, the secretion of a cuticle, the presence of microbasic *p*-mastigophores in the column and the same number of mesenteries distally and proximally. Additionally, the column is divisible into a scapus and scapulus, the number of tentacles and mesenteries are the same, presence of a strong mesogleal sphincter, and retractors and parietobasilar muscles weak.

The external morphology of the new species *P. niwa* is clearly different from *P. rosea*, the other species in the genus *Paracalliactis* recorded in New Zealand. It has a smooth column that lacks tubercles in the distal most part of the scapus, ectodermal pits in the entire extension of the scapus, and an extra cycle of tentacles and mesenteries. Furthermore, the cnidae of *P. niwa* and *P. rosea* are clearly different in every type of tissue examined. Differences include the size of spirocysts in the tentacles, microbasic *p*-mastigophores and a smaller class of basitrichs in the tentacles of *P. rosea*; slightly longer basitrichs and microbasic *p*-mastigophores in the column of the *P. niwa*; a smaller class of microbasic *p*-mastigophores in *P. niwa*; microbasic *p*-mastigophores in the filament belong to mutually exclusive classes in both species; and basitrichs in the acontia.

In addition, the geographic range of *P. niwa* and *P. rosea* do not overlap. *Paracalliactis niwa* is recorded from the western coast of New Zealand in the Tasman Sea, but *P. rosea* is recorded from the western Pacific Ocean off the eastern coast of

New Zealand. The bathymetric ranges of the species differs: *P. niwa* is known from depths of 2417 – 2421 meters and *P. rosea* has a continuous range from 100 to 1000 meters.

The new species will be further differentiated from other described species of the genus in the discussion of *Paracalliactis*.

Discussion

Phylogenetic analysis

The three genera of sea anemones symbiotic with hermit crabs (*Adamsia*, *Calliactis* and *Paracalliactis*) are recovered as reciprocally monophyletic in the morphological analysis. The separation of species of sea anemones symbiotic with hermit crabs into distinct genera in the present study supports the classification of Carlgren (1949) based on traditional morphological characters. In his work, Ross (1974a) hypothesizes that species of sea anemones symbiotic with hermit crabs had independent origins and does not form a monophyletic group. Given the largely non-phylogenetic nature of his work, Ross (1974a) does not specify if members of each genus forms a monophyletic group, but states that members of *Adamsia* and *Calliactis* are probably closely related, with *Adamsia* exhibiting a more 'derived' morphology. Here, the affinity between *Adamsia* and *Calliactis* is found with high support (90% jackknife value), but given the sister-group relationship, there is no evidence of a more 'derived' position for *Adamsia*.

Over time, different morphological characters have been used to justify the

affinity between the *Adamsia*, *Calliactis* or *Paracalliactis*. Duerden (1902) hypothesized that *Adamsia* and *Calliactis* formed a single group. When examining specimens of *C. tricolor* from Puerto Rico, Duerden (1902) notes that both genera have cinclides and the characters that differentiate members of *Calliactis* from members of *Adamsia* are related to the symbiotic habit (shape of the pedal disc and the secretion of a carcinoecium by *Adamsia*). Duerden (1902) considers that these differences may not be sufficient to differentiate these two genera. However, Duerden (1902) ignores that these two genera are also distinguished from each other based on the number of perfect and sterile mesenteries (two cycles in *Adamsia* and one cycle in *Calliactis*). Similarly, Hand (1975a) while working with species of *Calliactis* and *Paracalliactis* notes that they are very similar based on internal anatomy and are only differentiated by cinclides and the secretion of a cuticle or carcinoecium by *Paracalliactis*. The present analysis shows that characters related to a symbiotic habit, such as the pedal disc shape, may carry phylogenetic signal and could be used to identify members of *Adamsia* from *Calliactis*.

The close relationship between *Adamsia* and *Calliactis* is also found by Gusmão and Daly (2010), who shared with molecular data that *A. palliata* nests within a clade comprised of species of *Calliactis*, whereas *Paracalliactis* is not closely related to these two genera. The present study confirms the affinity between *Adamsia* and *Calliactis*. A clear synapomorphy for the group is the presence of cinclides. In the present study, however, *Calliactis* is monophyletic, not paraphyletic as in the study of Gusmao and Daly (2010). Because only one species of *Adamsia* and

Paracalliactis is included in the analysis of Gusmão and Daly (2010), the monophyly of *Adamsia* and *Paracalliactis* is not tested.

A close affinity between *Adamsia* and *Paracalliactis* due to their symbiotic habit is illustrated by the description of *A. involvens*. McMurrich (1983) places the new species in the genus *Adamsia* due to the extreme modification of the pedal disc and the secretion of a carcinoecium despite the total absence of cinclides that characterize *Adamsia*. The species is later transferred to *Paracalliactis* by Carlgren (1947) who also adds that the species contained microbasic *p*-mastigophores in the column, a characters present in all members of *Paracalliactis*.

The morphological similarities of members of *C. conchicola* and *P. rosea* examined by Hand (1975a), led to the hypothesis of both genera forming a single group. Hand (1975a) considers that the single cycle of perfect and sterile mesenteries in both species was an indication of their close relationship. This affinity, however, is not supported in the present analysis or by the study of Gusmão and Daly (2010).

The monophyly of *Adamsia* is unambiguously supported by the bi-lobed base, annular body shape, the second cycle of mesenteries that are perfect and sterile and by its position on the shell (always located close to the aperture of the shell and with its oral disc downwards and below the mouth of the hermit crab). Monophyly of *Calliactis* is unambiguously supported by the circular/ovoid base. The clade of *Adamsia* plus *Calliactis* is supported by the occurrance of cinclides in both genera. The monophyly of *Paracalliactis* is supported by the shape of the pedal disc which is always irregular but never bi-lobed.

Many of the characters that are traditionally used in the taxonomy of sea anemones to distinguish genera are largely invariant in the genera of sea anemones symbiotic with hermit crabs. For example, the musculature of species in these genera does not vary in nature, the number of directive mesenteries, the number of siphonoglyphs, and the cnidae in species of *Adamsia, Calliactis* and *Paracalliactis* are largely constant. Characters that may distinguish species in other genera, such as the number and cycles of mesenteries, musculature and external anatomy are diagnostic characters at the generic level in genus *Calliactis,* for example. Other characters, such as the number and cycles of tentacles, proportion of tentacles to mesenteries, fertility of mesenteries, presence of column specializations and division of the body in different regions do not carry phylogenetic signal in the three genera of sea anemones symbiotic with hermit crabs.

The secretion of a carcinoecium, a highly distinctive structure in sea anemones, has three independent origins in the phylogeny presented here: three species of *Paracalliactis* (*P. consors*, *P. michaelsarsi* and *P. obvolva*) and two species of *Adamsia* (*A. palliata* and *A. sociabilis*) secrete a carcinoecium. Thus, the carcinoecium, a complex behavioral and anatomical feature associated with the symbiosis, is interpreted as having evolved at least three times within Hormathiidae and seem to be phylogenetically labile. This is in agreement with the results of Gusmão and Daly (2010).

The sister-taxa relationship between *P. sinica* and *P. azorica* is supported by two characters: the flat morphology of the body, with the diameter of the pedal disc

exceeding the height of the column, and the absence of regions in the body. The species *P. consors* and *P. obvolva* form a group that is supported by microbasic *b*-mastigophores in the tentacles and the secretion of a carcinoecium. *Calliactis tigre*, *C. tricolor*, *C. conchicola* and *C. androgyna* form a group supported by microbasic *p*-mastigophores in the tentacles. Futhermore, *C. conchicola* and *C. androgyna* have two size of basitrichs in the column. A close relationship between *C. polypus* and *C. parasitica* is supported by the number of cycles of mesenteries, the number of cycles and total number of mesenteries and by the number of cycles of tentacles.

Taxonomy

Discussion of *Adamsia*

The genus *Adamsia* is created by Forbes (1840) to include the species *Actinia maculata* collected from Wales and described by Adams (1800). Forbes (1840) emphasizes the morphological adaptation of an anemone that lives in symbiosis with a hermit crab to define the genus *Adamsia*, including in his diagnosis those sea anemones that have a bi-lobed body adhering by a broad base to a shell. Additionally, *Adamsia* also includes sea anemones with simple, sub-retractile tentacles. Although Forbes (1840) notes that species of *Adamsia* produced a cuticle that expanded the shell inhabited by a hermit crab, he does not include that characteristic in his diagnosis. Forbes (1840) also notes that the species *Actinia carciniopados* described by Otto (1823) and *Actinia picta* of Risso (1826) are probably synonyms of *Actinia maculata*.

In the original description of *Calliactis*, a new genus of hermit crab symbiotic anemones, Verrill (1869) notes that members of both genera have cinclides close to the base, but emphasizes the shape of the base to distinguish *Adamsia* from the new genus. Verrill (1869) characterizes members of *Adamsia* as having a low spreading pedal disc with two lobes that unite and give the body of the anemone an annular shape. Verrill (1889) also adds that the pedal disc of these sea anemones secrete a tough carcinoecium that extends the aperture of the shell.

The morphological adaptation of the base to a symbiotic life was subsequently emphasized by many to define members of *Adamsia*. McMurrich (1893) includes in the definition of *Adamsia* characteristics of the pedal disc (adherent base that secrets a membrane), but also the presence of a series of cinclides close to the base. Similarly, Haddon (1898) emphasizes the bi-lobed shape of the pedal disc and the presence of a cuticle secreted by the pedal disc to characterize the genus. In addition, the presence of cinclides in the proximal part of the column was also used by Haddon (1898) to define *Adamsia*.

Carlgren (1945) maintains characters related to the shape of the pedal disc and symbiotic habit in his generic definition of *Adamsia*, but adds, for the first time in the generic definition of *Adamsia,* characters of internal anatomy and microanatomy: 2 pairs of directive mesenteries attached to one siphonoglyph each, 12 pairs of perfect and sterile mesenteries, mesenteries more numerous distally. In his classification, Carlgren (1949) summarizes the characters used by him in Carlgren (1945) to

characterize the genus *Adamsia*, maintaining characters related to the symbiotic habit as well as information on internal anatomy and microanatomy.

More recently, Daly et. al. (2004) modifies the diagnosis of *Adamsia* given by Carlgren (1949) to emphasize the shape of the pedal disc and the secretion of a carcinoecium, which is a complex feature highly distinctive within Actiniaria, and to help the distinction between *Adamsia* and other genera of hermit crab symbionts. Additionally, Daly et. al. (2004) includes in the generic definition characters pertaining to the position of the cinclides (in mid-column), nematocysts (basitrichs only in the column), and microanatomy (retractor and parietobasilar muscles weak). To include the newly described species *A. obvolva* in the genus, Daly et. al. (2004) modifies the diagnosis of *Adamsia* to include species that present six pairs of perfect and sterile mesenteries (not twelve as in *A. palliata* and *A. sociabilis*) and cinclides in mid-column (not close to the base as in *A. palliata* and *A. sociabilis*).

The present study confirms the presence of a second pair of perfect and sterile mesenteries in the type species of the genus, *A. palliata*, and in the species *A. sociabilis*. A second pair of perfect and sterile mesenteries is also reported by Stephenson (1935) for *A. palliata* and this character was used by Carlgren (1928a, 1949) and Manuel (1981) to define members of *Adamsia* and differentiate it from *Paracalliactis* and *Calliactis*. Although the internal anatomy of *A. fusca* (Quoy and Gaimard 1833) has never been described and cannot be examined because type specimens are unknown, the re-examination of the type species of *Adamsia*, *A. palliata,* and the examination of the internal anatomy of *A. sociabilis* can be used to

infer that species of *Adamsia* possess a second cycle of mesenteries that are perfect and sterile and that this character should used to differentiate it from the *Calliactis* and *Paracalliactis,* the other genera of sea anemones symbiotic with hermit crabs.

The position of specimens of *A. palliata* and *A. sociabilis* on the shell of the hermit crab, with its oral disc downwards and close to the aperture of the shell has been proven by the present study to be extremely regular and of great importance to define the genus. After many specimens of *A. palliata* and *A. sociabilis* were examined, not a single one was not located with its oral disc close to the aperture of the shell, beneath the mouth of the hermit crab. Although the species *Paracalliactis stephensoni*, in a different genus seem to occupy the same position on the hermit crab shell, this species in most of the cases is located in the dorsal part of the shell, while *Adamsia* species are less flexible and can only be found close to the aperture of the shell.

In the diagnosis of *Adamsia* present in this study, I reconcile the intentions of Forbes (1840) and Verrill (1869) that emphasize the posture of the anemone and pedal disc shape with that of Carlgren (1945, 1949) and Daly et. al. (2004) that also add features of the internal anatomy and musculature to the diagnosis. Two characteristics included by Daly et. al. (2004) in the diagnosis of *Adamsia*, however, need further exploration and the diagnosis present here partly reflects the new findings and reexamination. For example, cinclides in the two valid species of *Adamsia* examined in the present study are located proximally, very close to the base of the animals, not in mid-column as indicated by Daly et. al. (2004). In fact, the

cinclides illustrated by Daly et. al. (2004) for *A. obvolva* that would be located in the mid-column, were not found after an extensive examination of the holotype and paratypes. Instead, parts of the upper column and mid-column of *A. obvolva* present longitudinal ridges, which is characteristic of species of *Paracalliactis*, and may become indented both longitudinally and transversally (Doumenc 1975; Hand 1975a; pers. obs.). This characteristic may give the impression of small perforations in the column, similar to cinclides, but under close inspection they are not true cinclides as found in other species of *Adamsia.* Because *A. obvolva* lacks cinclides and a bi-lobed base, characters that define *Adamsia*, and presents features that are characteristic of the genus *Paracalliactis*, including the presence of microbasic *p*-mastigophores in the column and six perfect and sterile mesenteries, this species will be dealt with other species of *Paracalliactis*.

After the examination of two valid species of *Adamsia* a few conclusions can be made about the genus. The presence of a carcinoecium, a row of cinclides close to the base, a bi-lobed base that wraps the hermit crab shell and unites behind it when they become in contact, as well as the position of both specie s on the shell with the oral disc downwards close to the aperture of the shell, giving the body an annular shape are the external characters that should be used to differentiate this genus from the other two genera of crab-symbiotic anemones (*Calliactis* and *Paracalliactis*). Additionally, the column of *Adamsia* is not divisible into a scapus and scapulus. Two cycles of perfect and sterile mesenteries differentiate these two species of *Adamsia* from those of *Calliactis* and *Paracalliactis* that possess only the first cycle of

mesenteries perfect and sterile. In terms of its microanatomical features, the genus *Adamsia* possesses a strong mesogleal sphincter and retractor and parietobasilar muscles weak and diffuse.

Adamsia palliata and *A. sociabilis* can be distinguished based on characters of external morphology, internal anatomy and cnidae. In *A. palliata,* tentacles are much more numerous (around 200), than *A. sociabilis* (up to 96 tentacles). The color patterns exhibited by both species are very characteristic: *A. palliata* is known as the spotted anemone, presenting pink spots in its column, while *A. sociabilis* presents longitudinal stripes. The cnidae can also differentiate both species: *A. palliata* presents much larger spirocysts in the tentacles than *A. sociabilis*; *A. palliata* presents two size of basitrichs in the column, while *A. sociabilis* presents only a smaller class of basitrichs; the length of basitrichs in the acontia are mutually exclusive, ranging from 25.40 – 33.68 μ in *A. palliata* and 15.77 – 21.48 μ in *A. sociabilis*. In addition, these two species have disjunct distributions: *A. palliata* is known from the North Atlantic, from Norway to the Madeira Archipelago and Canary Islands, and from the Mediterranean Sea; *A. sociabilis* has a much more restrict distribution, being found in the western Atlantic Ocean, in the east coast of the United States, from Massachusetts to New England.

A nomenclatural issue raised by Manuel (1981) pertains to the name of the type species of the genus *Adamsia*. As Manuel (1981) notes, the type species of the genus, *Medusa palliata*, was described in the work of Bohadsch (1761) whose names were ruled invalid for the purpose of nomenclature by the International Code of

Zoological Nomenclature (ICZN, 1999) because his work was not consistently binomial. For this reason, and to promote stability, Manuel (1981) proposed that the next available name would be *Adamsia carciniopados* from *Actinia carciniopados* Otto 1823, because *Actinia maculata*, a, earlier name given by Adams (1800) was preoccupied. Daly et. al. (2004) argued that a re-description of *M. palliata* by Fabricius (1779) before the work of Otto (1823) made the name *A. palliata* available for nomenclatural purposes.

Uncited by Manuel (1981) or Daly et al. (2004), however, was the earlier work of Muller (1776) in which he uses the name *M. palliata* and objectively cites Bohadsch's plate and figure by number and text, and, thus, makes the name *M. palliata* available. As explained by Cornelius and Ates (2003), a name included in a rejected work is made available if used by a subsequent author and if no alternative name has been introduce in the meantime (ICZN 1999: Article 12.2.2). Although Ates (1985) pointed out the earlier work of Muller (1776) in a dutch periodical, this information was not readily available for more recent workers, and some did not apply the name *A. palliata* (e.g., Manuel 1981, 1995; Williams 1997). I agree with Ates (1985), Cornelius and Ates (2003) and Sherborn (1902), who correctly listed the combination *M. palliata* as used by Muller (1776), in that the type species of *Adamsia* is *A. palliata* (Muller, 1776).

Discussion of *Calliactis*

Diagnosis of *Calliactis*

Verrill (1869) erects the genus *Calliactis* and describes the new species *C. variegata* from Panama Bay. As defined by Verrill (1869), members of *Calliactis* possess a column changeable in form with a broadly expanded base that in contraction forms a broad, low and flattened cone; have a smooth column with one or more rows of cinclides with thickened and permanently raised borders close to the base; have numerous tentacles; highly developed acontia emitted through cinclides. Verrill (1869) notes that *Calliactis* is allied to *Adamsia* by having cinclides close to the base; he differentiates these genera based on the morphology of the pedal disc, the position of the anemone on the shell, the general morphology of body and the secretion of a carcinoecium.

Adamsia is characterized by (Verrill 1869) by a low, spreading body, whereas *Calliactis* is changeable in form, having a subcylindrical body and a broadly expanded base. In *Adamsia*, the pedal disc extends around the aperture of the shell in two lobes that unite where they come in contact, the body of the anemone has an annular form. Species of *Calliactis*, on the other hand, present a pedal disc that is roughly circular or ovoid and have a relatively tall column. In addition, whereas *Adamsia* always situates its oral disc downwards beneath the legs of the crab, *Calliactis* always presents an up-right position with its oral disc always directed away from the aperture of the shell. In addition, members of *Calliactis* never secrete a carcinoecium, a characteristic of found in species of *Adamsia.*

While describing the new species *C. miriam* from Australia, Haddon (1898), in agreement with Verrill (1869), emphasizes the morphology of the column and pedal disc, the cinclides in the column, the high number of tentacles and the development of acontia that are emitted freely from the cinclides as generic features of *Calliactis*. Haddon (1898) differentiates members of *Calliactis* from *Adamsia* based on the morphology of the column (higher in *Calliactis*) and the morphology of the pedal disc (less expanded in *Calliactis*) and by the morphology of the tentacles (longer in *Calliactis*). Haddon (1898), however, did not include the absence of a carcinoecium as a characteristic that would differentiate *Calliactis* from *Adamsia*.

Duerden (1902) re-describes *C. tricolor* from Puerto Rico in great detail and modifies the diagnosis given by Haddon (1898) to include the secretion of a cuticle by the pedal disc of *Calliactis*. Duerden (1902) also includes in the diagnosis of *Calliactis* that the column is usually divided into scapus and capitulum, with the scapus secreting a deciduous cuticle. Duerden (1902) notes that the characters of *Calliactis* and *Adamsia* are very similar and casts doubt on whether the growth of the pedal disc is sufficient to for generic distinction. As an example of this similarity, Duerden (1902) cites *C. polypus*, cited by Hertwig (1882) in the Challenger report in as *Calliactis*, but transferred in the supplement of that work (Hertwig, 1888) to *Adamsia*. To reinforce his position, Duerden (1902) also notes that while Haddon (1989) and Carlgren (1900) retains the distinction between *Calliactis* and *Adamsia*, McMurrich (1893) places the species *A. involvens* in *Adamsia*. However, *A. involvens* was later transferred to *Paracalliactis* by Daly et. al. (2004), not to *Calliactis*. This

case exemplifies that the confusion is not restricted to the species of *Adamsia* and *Calliactis*, but also involves those of *Paracalliactis*.

Carlgren (1928a) re-describes *C. polypus* and *C. brevicornis,* modifying Verrill (1869) diagnosis to include characteristics of the sphincter and mesenteries. In addition, Carlgren (1928a) distinguishes members of *Calliactis* from members of *Adamsia* by the number of perfect and sterile mesenteries: species of *Calliactis* have one cycle of perfect and sterile mesenteries (6 pairs), those of *Adamsia* have two cycles of perfect and sterile mesenteries (12 pairs). Carlgren (1928a) questions whether all species of *Calliactis* possesses cinclides in the column, but noted that if this is true, it might be one of the most valuable characters to distinguish members of *Calliactis* from *Paracalliactis*. The distinction between *Calliactis* and *Paracalliactis* also includes the higher number of mesenteries close to the margin (Carlgren 1928a).

England (1971) characterizes the species *C. polypus* as having acontia with basitrichs only, more mesenteries at the margin than at the base, six pairs of perfect and sterile mesenteries and a row of cinclides near the pedal disc. While examining populations of *C. polypus* and *C.miriam* from the Pacific Ocean, England (1971) also confirms that cinclides are a generic characteristic of all species of *Calliactis,* adding this to the diagnosis of the genus. In agreement with Carlgren (1928a), England (1971) also distinguishes members of *Calliactis* from *Adamsia* on the basis of the fertility of the mesenteries, with members of *Calliatis* always exhibiting one cycle (6 pairs) of perfect and sterile mesenteries.

Hand (1975a) describes the new species *C. conchicola* from New Zealand. To accommodate the new species in the genus *Calliactis*, Hand (1975a) modifies the diagnosis of the genus to include species that, like *C. conchicola,* presented long and slender tentacles and the same number of tentacles as the number of mesenteries close to the pedal disc. The shape of the tentacles observed by Hand (1975a), however, should not be given much significance due the plastic nature of the shape of tentacles in sea anemones. Hand (1975a) notes that in the preserved state, the tentacles of *C. conchicola* are short and conical as described for other species of *Calliactis*. Another difference between *C. conchicola* and other species given by Hand (1975a) is the microbasic *p*-mastigophores in the column. *Calliactis conchicola,* however, is not the only species of *Calliactis* that has microbasic *p*-mastigophores in the column: *C. androgyna* described by Riemann-Zurneck (1975) also presents microbasic *p*-mastigophores in the column.

Daly et. al. (2004) revises the characters included by Verrill (1869) and Carlgren (1928a, 1949) in the diagnosis of *Calliactis*, and modifies the definition of the genus to include the following characteristics: a circular or ovoid pedal disc that is equal or slightly larger than the oral disc, the absence of a carcinoecium, the column is typically less than the column height and the column has only basitrichs. Daly et. al., (2004) maintains in the diagnosis of *Calliactis* characters included by others (Verrill 1828; Carlgren 1928a, 1949; Hand 1975a),such as a column that possesses cinclides and can be differentiated into scapus and scapulus, scapus may present a

deciduous cuticle, tentacles in several cycles as numerous as mesenteries proximally, two pairs of siphonoglyphs and retractor and parietobasilar muscles weak.

After examining 15 of the 18 valid species of *Calliactis,* a few conclusions can be made. Some similarities that are shared by all species of *Calliactis* and define the genus include: a circular or ovoid pedal disc, pedal disc equal to or only slightly larger in diameter than oral disc, column smooth that may or may not be differentiated into scapus and scapulus, column diameter less than column height, numerous tentacles divided in several cycles, diameter less than column height, only one cycle of perfect and sterile mesenteries (6 pairs), two pairs of directives associated with one siphonoglyph each, mesogleal sphincter strong, retractor and parietobasilar muscles weak. Other characters that define the genus *Calliactis* but that will be further discussed here: the presence of cinclides in the column, the absence of a carcinoecium or cuticle, the presence of microbasic *p*-mastigophores in the column and the position of the anemone on the shell.

Although part of the original description (Verrill, 1869) and in subsequent studies (e.g., Duerden 1902; Haddon 1898; England 1971; England 1987), cinclides in the column of species of was put in doubt by Carlgren (1928a, 1949) and later by others (e.g., Hand 1975a; Correa 1964). Here I confirm the presence of cinclides in all examined species of *Calliactis*, but note that these cinclides are not always conspicuous. In some species, these cinclides are only perforations on the column that may be visualized more easily when acontia are emitted through the pores. The proximity of cinclides to the pedal disc as in *C. japonica*, for example, may also make

the visualization of such structures even harder. In other species, however, cinclides are more obvious, due to their location in slight elevations of the column (e.g., *C. tricolor, C. polypus*). After preservation, these cinclides are also easily visualized, although the elevations of the column are not as obvious as in live individuals. Even though not all specimens have acontia emitted through their cinclides, at least one individual of every lot exhibits these structures, which makes identification of cinclides easier.

In all species of *Calliactis,* animals examined are positioned on the shell with the body erect and oral disc directed away from the shell. This represents a major difference from species of *Adamsia,* in which the oral disc is always positioned downwards, beneath the legs of the hermit crab, and close to the aperture of the shell. In addition, the pedal disc of *Calliactis* is never bi-lobed as in *Adamsia* and does not, consequently, present the annular form of those anemones. The pedal disc that is circular and ovoid equal to or slightly larger than the diameter of the column also differentiates *Calliactis* from *Paracalliactis*, since members of the latter have a very wide base that is always wider than the column diameter.

The secretion of a cuticle that is observed by Duerden (1902) for specimens of *C. tricolor* from Puerto Rico is not observed in the material examined here. None of the specimens of any species of *Calliactis* possesses a cuticle as in *Paracalliactis* or a carcinoecium as in *Adamsia* and some *Paracalliactis*. This is in agreement with the majority of studies where *Calliactis* was examined (e.g., Verrill 1869; Haddon 1898; Carlgren 1928a, 1949; Correa 1964; England 1971, 1987; Hand 1975; Dube 1978)

and is a characteristic that can be used to differentiate *Calliactis* from both *Adamsia* and *Paracalliactis*.

Most of the species of *Calliactis* present only basitrichs on the column (one or two classes of size), but two species, *C. androgyna* and *C. conchicola*, exhibit in addition to basitrichs, microbasic *p*-mastigophores. Although this characteristic is not common for the *Calliactis*, I do not agree with Daly et. al. (2004) and, thus, do not include the presence of only basitrichs in the column as a diagnostic characters for the genus *Calliactis*.

Riemann-Zurneck (1975) while describing the species *C. androgyna* from the southern coast of Brazil, notes that species of *Calliactis* are not well diffentiated. Carlgren (1949) lists 14 valid species for the genus *Calliactis*. Later, England (1971) reduces this number to 12 valid species, from which Riemann-Zurneck (1975) considers only five well-characterized. These species are: *C. algoaensis*, *C. parasitica*, *C. tricolor*, *C. reticulata*, and *C. polypus*. Riemann-Zurneck (1975) questioned the validity of the remaining seven species, including: *C. decorata*, *C. valdiviae*, *C. brevicornis*, *C. fusca*, *C. sinensis*, *C. japonica* and *C. variegata*.

Previous to this revision, 18 valid species of *Calliactis* existed. After the synonyms and the description of one new species, a total of 13 species are considered valid, not including the three species that could not examined in the present study. The reduction in the number of valid species is mostly due to the species that were synonimized with the species *C. polypus*. This species is very widespread in the

Indian Ocean, the Red Sea and the Pacific Ocean and the morphology and cnidae of the different populations examined here did not show any significant differences.

Species of *Calliactis* not included in the study

Three species of *Calliactis* described by Pei (1996) for the east and south China Sea could not be included in the present study. The loan of the type material of *C. polypores*, *C. xishaensis* and *C. argentacoloratus* from the Institute of Oceanology of the Chinese Academy of Sciences was not possible. In addition, no material of sea anemones symbiotic with hermit crabs was found for the locality in which these specimens were collected.

Discussion of the genus *Paracalliactis*

Diagnosis of *Paracalliactis*

In the original description of *Paracalliactis*, Carlgren (1928a) includes in the new genus hormathiids that live in symbiosis with hermit crabs and that, like *Adamsia*, may secrete a carcinoecium that enlarges the living space of the crab. In addition, members of *Paracalliactis* are positioned on the shell in a way that the oral disc of the anemone may be directed away from the shell or may be lateral or ventral in relation to the aperture of the shell; the column is divisible into scapus and scapulus; the scapus does not possess cinclides and may or may not present a row of tubercles in its distal most part; the row of tubercles may form a complete corona; the scapulus present deep longitudinal furrows; the sphincter distal with a tendency to

transversal stratification; tentacles thin and hexamerously arranged, same or nearly same number of tentacles as mesenteries at the margin; two siphonoglyphs present; pairs or mesenteries hexamerously arranged, six pairs of perfect and sterile mesenteries; retractors and parietobasilar muscles of the mesenteries weak; acontia thin, long and possessing only one type of nematocysts. Carlgren (1928a) describes the type species *P. valdiviae*.

Carlgren (1928b) describes two new species of *Paracalliactis* (*P. stephensoni* and *P. michaelsarsi*) from the North Atlantic Ocean and noted that members of *Paracalliactis* are biologically intermediates between *Adamsia* and *Calliactis*. Carlgren (1928b) illustrates this idea by emphasizing the secretion of a cuticle or a carcinoecium as a characteristic that is shared between *Adamsia* and *Paracalliactis* and that distinguishes these genera from *Calliactis*. The position of the anemone on the shell, however, is a character that unites members of *Paracalliactis* and *Calliactis* and distinguish them from *Adamsia*.

Carlgren (1949), in his classification of sea anemones, maintains most of the characters in the diagnoses of *Paracalliactis* unchanged compared to his the original description (Carlgren 1928a), but included an additional characteristic to members of that genus: the presence of microbasic *p*-mastigophores in the column. Carlgren (1949) also noted that species of *Paracalliactis* are usually positioned on the shell either ventrally (close to the aperture of the shell) or dorsally (directed away from the aperture of the shell). The latter point represents a difference from the original

description of *Paracalliactis* (Carlgren 1928a) when members of the genus were positioned dorsally, ventrally or laterally to the aperture of the shell.

While working with deep sea anemones from the North Atlantic Ocean, Doumenc (1975) describes a new species of *Paracalliactis*, *P. azorica* from Azores, and re-describes *P. stephensoni* and *P. michaelsarsi*. Doumenc (1975) differentiates members of *Paracalliactis* from genus *Adamsia* based on the absence of cinclides close to the base, the same number of mesenteries proximally and distally and the presence of 6 pairs of mesenteries perfect and sterile in *Paracalliactis*. Species of *Paracalliactis*, on the other hand, are differentiated by Doumenc (1975) based on the secretion of a carcinoecium and absence of cinclides. In addition, Doumenc (1975) noted that species of *Paracalliactis* are generally found at depths of more than 2000 meters, predominantly between 2000 and 4700 meters.

Hand (1975) describes a new species of *Paracalliactis*, *P. rosea*, from New Zealand and modified the diagnosis of the genera given by Carlgren (1949) to include that species of *Paracalliactis* never have cinclides in their column. This has already been noted by other workers, but it had never been formally included in the diagnosis of *Paracalliactis*. Additionally, Hand (1975) notes that *P. rosea* is distributed continuously from shallow (50-100 meters) to rather great depths (up to 1000 meters), while four out of five species of *Paracalliactis* known at the time were found at waters deeper than 1000 meters.

Daly et. al. (2004) while re-describing the species *P. involvens* as *P. consors* modifies the diagnosis of *Paracalliactis* to differentiate these species from the newly

described species *A. obvolva*. The diagnosis of *Paracalliactis* given by Daly et. al. (2004) differs from that of Carlgren (1949) in five major points: it adds that members of *Paracalliactis* are characterized by having a pedal disc divisible in two lobes, a pedal disc of greater diameter than oral disc diameter, a column without cinclides (as in Hand, 1975a), the column diameter greater than column height and column nematocysts basitrichs and microbasic *p*-mastigophores.

The present study of six species of *Paracalliactis* largely agrees with the diagnoses of the genus given by others (Carlgren 1928a, 1949; Doumenc 1975; Hand, 1975a; Daly et. al. 2004), A few characters included in these studies to characterize the genus *Paracalliactis* will be further discussed to help differentiate it from other genera of sea anemones symbiotic with hermit crabs. These characters are: the shape of the pedal disc, the secretion of a cuticle or carcinoecium, the presence or absence of tubercles in the column of *Paracalliactis* and the position of the anemone on the shell and its consequence to the general appearance of the body of the anemone.

Daly et. al. (2004) characterizes the pedal disc of both *Paracalliactis* and *Adamsia* as divisible in two lobes to differentiate it from the pedal disc of *Calliactis* that is circular or oval, but never bi-lobed. After examining species of *Adamsia* and *Paracalliactis*, the present study confirms the bi-lobed nature of the pedal disc of *A. sociabilis* and *A. palliata*, however, the pedal disc of *Paracalliactis* although irregular and asymmetric is never bi-lobed as in *Adamsia*. The nature of the pedal disc of *Paracalliactis* varies from completely wrapping the shell, in which case the pedal disc is characterized as irregular and asymmetric (found in *P. valdiviae*, *P. rosea*, *P.*

new species) to truly enwrapping the shell and secreting a true carcinoecium (*P. michaelsarsi*, *P. consors*, *P. rosea, P. obvolva*). The nature of the pedal disc found in species of *Adamsia* is different from that found in species of *Paracalliactis* in that it is truly bi-lobed and unites at the back of the shell when they become in contact, while the pedal disc of *Paracalliactis* enwraps the shell as a single structure but doesn't present the pedal disc in two lobes that united in the back of the shell.

While the pedal disc of *Adamsia* always produces a carcinoecium that enlarges the shell inhabited by the hermit crab, the secretion of some species of *Paracalliactis* (*P. valdiviae P. rosea*, P. *new species*) do not form a carcinoecium, only a cuticle that does not extend beyond the shell aperture. Thus, the cuticle does not enlarge the living space of the crab. Other species, including *P. michaelsarsi*, *P. consors*, *P. obvolva*, produce a carcinoecium that enlarges the living space of the crab. The variability of the secretion of the pedal disc of the anemone is not related to the depth in which these anemones are found, but may be related to other unknown environmental factors. Although species of *Paracalliactis* vary in the secretion by the pedal disc*,* all the species of the genus secrete either a cuticle or a carcinoecium. Furthermore, *Paracalliactis* is clearly differentiated from *Adamsia* or *Calliactis* based on other characters, including: the absence of cinclides, the presence of basitrichs and microbasic *p*-mastigophores in the column, the same number of mesenteries distally and proximally, six pairs of perfect and sterile mesenteries, the pedal disc shape and position of the anemone on the shell.

Species of *Paracalliactis* may present variability in the presence of tubercles in their column, with some specimens exhibiting tubercles that may form a complete corona and others have less clear tubercles or completely lack them. While, specimens of *P. rosea* examined always presented tubercles, specimens of *P. michaelsarsi, P. obvolva* and *P. new species* never presented such structures. *Paracalliactis valdiviae* and *P. consors*, on the other hand, may or may not present them. These differences are found even in the same lot where specimens were collected from the same locality and preserved in the same way. For this reason, although the presence or absence of tubercles may be still used as a taxonomic character, especially when a high number of individuals are examined, it should not be used to distinguish species that are closely allied until the reason for such variability is identified. It is unclear whether this variation is biological and caused by environmental factors or if it's a contraction artifact as assumed by Daly et. al. (2004).

The position of the anemone on the shell is a character that clearly differentiates species of *Paracalliactis* from *Adamsia* and *Calliactis*. As in *Calliactis*, most species in the genus *Paracalliactis* are positioned on the shell in a way that the oral disc of the anemone is directed away from the aperture of the shell (dorsally), but it's clearly distinguished from it by much wider shape of the pedal disc in *Paracalliactis* that enwraps the shell. In some cases, however, *Paracalliactis* may present species with their oral disc close to the aperture of the shell, a position that resembles the species *A. sociabilis* and *A. palliata*. It's important to note, however,

that this position always leaves the oral disc of the anemone lateral to the aperture of the shell in *Paracalliactis* and never beneath the mouth and legs of the hermit crab as is the case in *Adamsia*. In addition, Sebens (1998) distinguishes *A. sociabilis* from *P. consors* based on the shape of the anemone's body. Members of *A. sociabilis* have a column folded around the hermit crabs abdomen to form a conical base and an annular body, with the base uniting on the back of the shell with tentacles oriented downwards close to the aperture of the shell. *Paracalliactis consors*, on the other hand, has a column not folded to form a cone, with a deeply concave base forming the cavity for the hermit crabs abdomen. The oral disc of *P. consors* is oriented laterally on the shell of the hermit crab.

Distinguishing species of *Paracalliactis*

Species of *Paracalliactis* are very constant in their internal anatomy and microanatomy. Most species, except for the new species *P. niwa*, possesses 48 pairs of mesenteries distributed in 4 cycles, with the first cycle perfect and sterile and the remaining cycles imperfect and fertile (Carlgren 1928a,b; Doumenc 1975; Hand 1975; Daly et. al. 2004). The sphincter is mesogleal, strong and the retractor and parietobasilar muscles are weak (Carlgren 1949; Daly et. al. 2004). Species of *Paracalliactis* can be distinguished into two groups: those that possess a carcinoecium that enlarges the living species of the crab (*P. michaelsarsi*, *P. consors* and *P. obvolva*) and those that produce a less developed cuticle that doesn't extend beyond the aperture of the shell (*P. valdiviae*, *P. rosea* and *P. niwa*). Among those

that produce carninoecium, *P. michaelsarsi* and *P. obvolva* have a smooth column without tubercles, while *P. consors* possesses a complete corona of tubercles. *Paracalliactis michaelsarsi* and *P. obvolva*, on the other hand, can be distinguished based on the number of categories of basitrichs in the acontia: *P. michaelsarsi* presents one size of basitrichs and *P. obvolva* presents two sizes of basitrichs in the acontia. In addition, *P. obvolva* is distinguished from *P. michaelsarsi* and *P. consors* by having up to 96 tentacles, whereas *P. obvolva* has up to 190 tentacles (or up to 250 tentacles, see Daly et. al. 2004).

Among the remaining species that produce a less developed cuticle, *P. valdiviae* and *P. rosea* present tubercles in their column that form a complete corona, whereas *P. niwa* has a smooth column. As mentioned, *P. niwa* is easily distinguished from all other *Paracalliactis* in that it possesses an extra cycle of mesenteries (96 pairs of mesenteries total) and tentacles (up to 172 tentacles). *Paracalliactis valdiviae* and *P. rosea* are distinguished by extensive cnidae differences not only in measurements but also categories of nematocysts present in every tissue.

Species of *Paracalliactis* not included in the present study

Three species of *Paracalliactis* could not be included in the present revision of the genus: *P. lacazei*, *P. azorica* and *P. sinica*. No type material or vouchers are known for the species *P. lacazei*. The type material of *P. azorica* has been displaced and no additional specimens are known (pers. obs.) Arrangements for the loan of the type material of *P. sinica* could not be made and no additional specimens were found

in sea anemone collections worldwide. A short discussion of these three species is given below.

Dechancé and Dufaure (1959) describes a new species of *Paracalliactis*, *P. lacazei,* from Banyuls-su-Mer in the Mediterranean Sea. This new species possesses many of the generic features of *Paracalliactis*, including an irregular base that secretes a cuticle, column divisible into scapus and scapulus, same number of mesenteries proximally and distally, only one cycle of mesenteries perfect and sterile and lives in symbiosis with a hermit crab (*Anapagurus laevis*). Dechancé and Dufaure (1959), however, reports in this new species a ring of cinclides close to the base through which acontia are protruded, a character that is not present in any of the described species of *Paracalliactis*. Due to the presence of a ring of cinclides near the base, the placement of *P. lacazei* in the genus *Paracalliactis* must be considered in doubt. Although, *P. lacazei* possesses a ring of cinclides close to the base, secretes a cuticle, occurs in the same position on the shell and presents the same geographic distribution and bathymetric range of *A. palliata*, this species is probably not correctly placed in the genus *Adamsia*. *Paracalliactis lacazei* differs from *A. palliata* in that it presents the column divisible into scapus and scapulus, the same number of mesenteries proximally and distally and only one cycle of perfect and sterile mesenteries. *Paracalliactis lacazei*, however, has all of the generic characters of a *Cataphellia*, but its generic placement cannot be confirmed because no type material is known for this species and no specimens exhibiting such characteristics were found in any of the collections of sea anemones world-wide (pers.obs.).

The species *P. azorica* is described by Doumenc (1975) from specimens collected in the North Atlantic Ocean off the coast of the Azores at 2900 – 3506 meters. This species is found in sympatry with *P. stephensoni*, but differs from it in the number and cycles of mesenteries, the presence of a pedal disc cone and details of its cnidae. Particularly interesting is the presence of the pedal disc cone, which is a region of the pedal disc of the anemone that gets fused and forms a special structure that resembles a cone. This structure has never been found in any other *Paracalliactis* species and is highly distinctive. Because the location of the type material of *P. azorica* is currently unknown and no specimens from Azores examined in the present study exhibited such characteristics, this species could not be included in the present revision. Future investigations are necessary to confirm the status of the species and the nature of its characteristic pedal disc cone.

The species *P. sinica* described by Pei (1982) from the East China Sea could not be examined in the present revision due to difficulties in the arrangement of a loan of the type material from the Institute of Oceanology of the Chinese Academy of Sciences. In addition, no *Paracalliactis* specimens from China were found in any of catalogued or uncatalogued material in sea anemone collections visited worldwide. Based on its original description (Pei 1982), however, *P. sinica* resembles the species *P. valdiviae,* especially in terms of external morphology but it's distinguished from it based on the surface of the body wall that is reticulate in *P. sinica*, the higher number of tentacles (between 192 – 394 tentacles), the size of the basitrichs in the acontia and other details of the cnidae. The species *P. sinica*, however, exhibits characters that

may put in question the identity of the species, including: *P. sinica* possesses five cycles of mesenteries that presents retractors of the mesenteries and the parietobasilar muscle rather strong, lack microbasic *p*-mastigophores in the column, and present a very shallow bathymetric range, between 39-47 meters, that is unusual among other species of *Paracalliactis*. Although *P. sinica* is described as lacking cinclides, no cuticle or carcinoecium is present. It's important to note that cinclides can be very hard to observe in preserved individuals (pers. obs.). In the future, it will be important to examine the type material of *P. sinica* as many of its characters lead to the conclusion that the species would be better placed in the genus *Calliactis*.

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Tissue/cnidae type	Capsule length x width	N	P	F	Doumenc et. al. (1985)
TENTACLES					
Spirocysts	17.13 – 32.69 x 1.83 – 3.50	403	10/10	+++	14.0 – 28.9 x 1.9 – 4.2
Basitrichs	16.50 – 26.73 x 1.70 – 2.96	252	10/10	+++	14.0 – 21.7 x 1.8 – 2.1
COLUMN					
Small basitrichs	08.10 – 11.52 x 1.62 – 2.06	97	10/10	++	----------
Large basitrichs	15.65 – 19.50 x 1.72 – 2.50	121	10/10	++	12.0 – 19.1 x 2.1 – 3.4
PHARYNX					
Basitrichs	19.52 – 25.35 x 1.64 – 2.54	142	10/10	++	17.0 – 24.0 x 2.0 – 3.0
Mc. *p*-mastigophore	17.20 – 23.18 x 2.42 – 3.56	139	10/10	++	17.0 – 24.0 x 2.4 – 3.8
FILAMENT					
Basitrichs	12.54 – 14.59 x 1.48 – 1.82	66	07/10	+	12.0 – 17.0 x 1.9 – 2.8
Mc. *p*-mastigophore	15.32 – 18.11 x 2.31 – 3.40	123	10/10	+++	13.0 – 18.4 x 2.8 – 4.7
ACONTIA					
Basitrichs	25.40 – 33.68 x 2.31 – 3.40	367	10/10	+++	24.0 – 33.2 x 2.9 – 4.7

Table 5.1. Size and distribution of cnidae of *Adamsia palliata* (Muller 1776). Measurements are in micrometers (μm). N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare; Exceptional sizes in parenthesis. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length x width	N	P	F	Carlgren (1950)	Sebens (1998)
TENTACLES						
Spirocysts	15.78 – 20.92 x 2.56 – 3.01		6/6	+	----------	08.0 – 19.0
Basitrichs	11.22 – 18.43 x 2.29 – 3.10	98	6/6	+	09.2– 15.5 x 2.5 – 2.8	09.0 – 15.0
Mc. *p*-mastigophore	----------				----------	12.0 – 13.0*
COLUMN						
Spirocysts	----------				----------	12.0 – 18.0*
Basitrichs	10.03 – 15.99 x 1.54 – 2.66	80	5/6	+	19.7 – 25.4 x 3.5 – 4.2	12.0 – 22.0
Mc. *p*-mastigophore	----------	109	6/6	+	----------	13.0 *
PHARYNX						
Basitrichs	21.14 – 27.66 x 2.78 – 3.49	79	6/6	+	19.7 – 25.4 x 3.0	10.0 – 15.0
Mc. *p*-mastigophore	15.52 – 18.91 x 3.47 – 4.12	101	6/6	+	14.0 x 4.0	09.0 – 22.0
FILAMENT						
Basitrichs	13.21 – 16.20 x 2.31 – 2.71	93	6/6	+	11.3 – 14.8 x 2.2 – 2.5	08.0 – 14.0
Mc. *p*-mastigophore	10.03 – 17.29 x 3.16 – 3.85	105	6/6	++	11.3 – 13.4 x 3.5 – 4.2	10.0 – 14.0
ACONTIA						
Basitrichs	15.77 – 21.48 x 2.45 – 3.28	112	6/6	++	----------	----------

Table 5.2. Size and distribution of cnidae of *Adamsia sociabilis* Verrill 1882. Measurements are in micrometers (µm). N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare; Exceptional sizes in parenthesis. Abbreviation, Mc=microbasic.*=rare nematocyst, may or may not be a contamination.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	*Calliactis armillatas*
TENTACLES					
Spirocysts	14.21 – 36.09 (26.87) x 1.89 – 3.98 (2.83)	256	15/15	+++	14.40 – 37.25 (25.99) x 2.11 – 3.58 (2.91)
Basitrichs	19.25 – 30.45 (26.92) x 1.65 – 2.54 (2.09)	281	15/15	++	18.11 – 29.62 (26.23) x 1.59 – 2.71 (2.12)
COLUMN					
Basitrichs	06.88 – 11.55 (09.77) x 1.51 – 2.35 (1.92)	236	15/15	+++	06.96 – 12.44 (10.25) x 1.63 – 2.74 (1.95)
Mc. *p*-mastigophores	11.66 – 17.74 (14.72) x 2.32 – 3.19 (2.86)	225	15/15	++	11.66 – 17.74 (14.72) x 2.32 – 3.19 (2.86)
PHARYNX					
Small basitrichs	11.23 – 14.74 (12.86) x 1.52 – 2.48 (2.09)	239	15/15	++	11.78 – 15.24 (12.90) x 1.61 – 2.82 (2.21)
Large basitrichs	17.48 – 26.53 (22.35) x 2.27 – 3.18 (2.72)	268	15/15	+++	17.22 – 27.15 (22.95) x 2.21 – 3.06 (2.65)
FILAMENT					
Basitrichs	10.22 – 14.84 (12.11) x 1.58 – 2.13 (1.76)	223	15/15	++	10.88 – 15.35 (12.32) x 1.54 – 2.55 (1.89)
Mc. *p*-mastigophores	16.25 – 26.08 (21.93) x 2.27 – 4.07 (3.41)	258	15/15	+++	16.85 – 27.15 (20.86) x 2.36 – 9.97 (3.35)
ACONTIA					
Basitrichs	16.42 – 26.78 (22.88) x 2.34 – 3.20 (2.85)	312	15/15	+++	15.98 – 25.76 (22.43) x 2.41 – 3.18 (2.76)

Table 5.3. Size and distribution of cnidae of *Calliactis polypus* (Forsskål 1775). Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic. *= rare nematocyst.

Continued

Table 5.3. continued

Tissue/cnidae type	*Calliactis marmorata*	*Calliactis miriam*	*Calliactis sinensis*
TENTACLES			
Spirocysts	14.21 – 36.09 (26.87) x 1.89 – 3.98 (2.83)	13.58 – 33.41 (25.05) x 1.93 – 3.59 (2.70)	15.45 – 32.82 (26.23) x 2.10 – 3.68 (2.81)
Basitrichs	19.25 – 30.45 (26.92) x 1.65 – 2.54 (2.09)	21.08 – 29.64 (25.02) x 1.52 – 2.39 (2.13)	22.14 – 28.65 (24.68) x 1.47 – 2.55 (2.20)
COLUMN			
Basitrichs	06.88 – 11.55 (09.77) x 1.51 – 2.35 (1.92)	08.23 – 12.47 (10.87) x 1.64 – 3.04 (2.65)	07.84 – 13.47 (11.04) x 1.77 – 2.86 (2.42)
Mc. *p*-mastigophores	11.66 – 17.74 (14.72) x 2.32 – 3.19 (2.86)	12.38 – 18.36 (15.39) x 2.58 – 3.07 (2.62)	12.05 – 18.55 (15.83) x 2.24 – 3.07 (2.70)
PHARYNX			
Small basitrichs	11.23 – 14.74 (12.86) x 1.52 – 2.48 (2.09)	12.69 – 15.98 (13.58) x 1.35 – 2.35 (2.01)	11.21 – 17.04 (14.52) x 1.58 – 2.87 (2.38)
Large basitrichs	17.48 – 26.53 (22.35) x 2.27 – 3.18 (2.72)	18.77 – 27.32 (23.28) x 2.14 – 3.25 (2.86)	17.59 – 25.89 (22.41) x 2.08 – 3.12 (2.68)
FILAMENT			
Basitrichs	10.22 – 14.84 (12.11) x 1.58 – 2.13 (1.76)	09.35 – 14.83 (12.18) x 1.69 – 2.57 (1.92)	11.27 – 15.86 (12.96) x 1.48 – 2.32 (1.89)
Mc. *p*-mastigophores	16.25 – 26.08 (21.93) x 2.27 – 4.07 (3.41)	17.64 – 27.92 (22.88) x 2.55 – 3.97 (3.28)	16.58 – 28.07 (22.11) x 2.39 – 3.85 (3.18)
ACONTIA			
Basitrichs	16.42 – 26.78 (22.88) x 2.34 – 3.20 (2.85)	17.02 – 28.25 (23.56) x 2.52 – 3.39 (2.96)	16.77 – 28.04 (23.71) x 2.21 – 3.44 (2.90)

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Continued

Table 5.3. continued

Tissue/cnidae type	*Calliactis valdiviae*	Capsule length (x) x width (x)
TENTACLES		
Spirocysts	15.21 – 35.14 (25.47) x 1.92 – 3.85 (2.69)	14.21 – 36.09 (26.87) x 1.89 – 3.98 (2.83)
Basitrichs	18.20 – 31.08 (25.92) x 1.52 – 2.68 (2.18)	19.25 – 30.45 (26.92) x 1.65 – 2.54 (2.09)
COLUMN		
Basitrichs	07.82 – 12.20 (10.08) x 1.48 – 2.46 (1.87)	06.88 – 11.55 (09.77) x 1.51 – 2.35 (1.92)
Mc. *p*-mastigophores	12.15 – 18.08 (15.10) x 2.42 – 3.24 (2.91)	11.66 – 17.74 (14.72) x 2.32 – 3.19 (2.86)
PHARYNX		
Small basitrichs	12.40 – 15.17 (13.14) x 1.48 – 2.71 (2.15)	11.23 – 14.74 (12.86) x 1.52 – 2.48 (2.09)
Large basitrichs	18.23 – 25.87 (21.68) x 2.40 – 3.09 (2.81)	17.48 – 26.53 (22.35) x 2.27 – 3.18 (2.72)
FILAMENT		
Basitrichs	10.88 – 15.91 (13.14) x 1.65 – 2.39 (1.90)	10.22 – 14.84 (12.11) x 1.58 – 2.13 (1.76)
Mc. *p*-mastigophores	17.05 – 27.20 (22.13) x 2.42 – 3.85 (3.24)	16.25 – 26.08 (21.93) x 2.27 – 4.07 (3.41)
ACONTIA		
Basitrichs	17.82 – 27.33 (23.10) x 2.47 – 3.31 (2.91)	16.42 – 26.78 (22.88) x 2.34 – 3.20 (2.85)

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	Carlgren and Hedgpeth (1952)	Correa (1964)
TENTACLES					
Spirocysts	14.12 – 25.56 (18.45) x 1.51 – 3.52 (2.23)	97	6/6	----------	14.4 – 25.2 x 1.8 – 3.6
Basitrichs	24.31 – 34.56 (27.29) x 4.52 – 5.23 (4.78)	95	6/6	21.0 – 24.0 x 2.5 – 3.0	18.0 – 25.2 x 1.8 – 3.6
Mc. *p*-mastigophores	18.21 – 25.43 (19.45) x 2.52 – 3.94 (3.24)	59	5/6	----------	----------
COLUMN					
Small basitrichs	10.43 – 14.55 (12.59) x 0.92 – 1.82 (1.24)	75	6/6	12.0 -14.0 x 2.2	10.8 – 14.4 x 1.8
Large basitrichs	19.66 – 27.67 (21.83) x 3.41 – 4.12 (3.70)	89	6/6	----------	----------
PHARYNX					
Basitrichs	15.52 – 25.67 (18.42) x 1.81 – 3.54 (2.76)	99	6/6	21.0 – 26.8 x 3.0	18.0 – 25.2 x 1.8 – 3.6
FILAMENT					
Basitrichs	15.26 – 27.10 (19.34) x 1.91 – 3.10 (2.29)	96	6/6	11.3 – 12.7 x 1.5	18.0 – 25.2 x 1.8 – 3.6
Mc. *p*-mastigophores 1	10.35 – 15.12 (12.29) x 2.66 – 4.52 (3.85)	105	6/6	19.0 – 24.0 x 4.2 – 5.0	18.0 – 25.2 x 1.8 – 3.6
Mc. *p*-mastigophores 2	19.77 – 22.52 (20.83) x 4.50 – 5.89 (5.31)	88	6/6	----------	----------
ACONTIA					
Basitrichs	17.74 – 32.52 (27.26) x 1.82 – 3.17 (2.28)	112	6/6	21.0 – 26.8 x 3.0	18.0 – 25.2 x 1.8 – 3.6

Table 5.4. Size and distribution of cnidae of *Calliactis tricolor* (Le Sueur 1817)). Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	Schmidt (1972)
TENTACLES					
Spirocysts	20.74 – 42.65 (34.68) x 2.63 – 4.28 (3.07)	112	8/8	+++	19.5 – 36.0 (32.8) x 2.4 – 4.5 (3.1)
Basitrichs	21.06 – 33.58 (28.55) x 2.20 – 3.14 (2.69)	142	8/8	+++	22.5 – 30.0 (26.0) x 2.0 – 2.8 (2.5)
COLUMN					
Basitrichs	11.85 – 18.52 (15.33) x 1.74 – 2.90 (2.45)	98	8/8	++	13.0 – 16.9 (14.5) x 1.9 – 2.6 (2.3)
PHARYNX					
Basitrichs	23.45 – 31.69 (27.04) x 2.21 – 2.97 (2.58)	105	8/8	++	25.0 – 30.0 (28.5) x 1.9 – 2.6 (2.3)
FILAMENT					
Basitrichs	10.58 – 16.81 (14.22) x 1.36 – 2.89 (2.18)	125	8/8	++	11.7 – 14.3 (13.3) x 1.6 – 2.3 (2.0)
Mc. *p*-mastigophores	20.39 – 31.07 (26.88) x 2.85 – 4.96 (3.97)	120	8/8	++	22.6 – 28.8 (25.9) x 3.1 – 5.2 (4.1)
ACONTIA					
Basitrichs	25.72 – 33.05 (28.91) x 2.37 – 4.53 (3.16)	136	8/8	+++	24.7 – 32.5 (29.3) x 2.5 – 4.0 (3.3)

Table 5.5. Size and distribution of cnidae of *Calliactis parasitica* (Couch 1842). Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P= proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic. *= rare nematocyst.

Tissue/cnidae type	Capsule length (x) x width (y)	N	P	F	Carlgren (1928a)
TENTACLES					
Spirocysts	15.69 – 29.41 (22.68) x 2.0 – 3.28 (2.88)	87	4/4	+++	17.0 – 29.0 x 1.5 – 5.0
Basitrichs	16.08 – 26.39 (21.55) x 1.87 – 2.39 (2.13)	69	4/4	++	17.0 – 25.0 x 2.0 – 2.5
COLUMN					
Small basitrichs	09.23 – 14.12 (12.07) x 1.13 – 2.08 (1.75)	71	3/4	+	----------
Large basitrichs	17.54 – 24.04 (23.25) x 2.27 – 2.89 (2.41)	63	4/4	++	17.0 – 22.0 x 2.0
PHARYNX					
Basitrichs	24.10 – 29.67 (23.65) x 2.27 – 3.02 (2.68)	68	4/4	++	25.0 – 31.0 x 2.5 – 3.0
Mc. *p*-mastigophores 1	15.23 – 27.89 (21.88) x 2.36 – 3.24 (2.81)	65	4/4	++	----------
FILAMENT					
Basitrichs	10.12 – 15.69 (12.92) x 1.13 – 1.78 (1.42)	72	4/4	++	----------
Mc. *p*-mastigophores 1	15.77 – 20.19 (18.06) x 2.52 – 3.09 (2.74)	67	4/4	+++	----------
ACONTIA					
Basitrichs	25.58 – 32.67 (29.06) x 2.21 – 2.86 (2.42)	82	4/4	+++	24.0 – 31.0 x 3.0 – 3.5

Table 5.6. Size and distribution of cnidae *Calliactis brevicornis* (Studer 1879). Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	Carlgren (1922)
TENTACLES					
Spirocysts	12.32 – 38.91 (30.01) x 1.66 – 5.54 (4.15)	82	1/1	++	14.0 – 36.0 x 1.5 – 5.0
Basitrichs	15.50 – 24.06 (20.77) x 1.25 – 2.58 (2.04)	57	1/1	++	17.0 – 22.0 x 1.5
COLUMN					
Small basitrichs	08.36 – 15.24 (12.04) x 0.94 – 1.91 (1.40)	61	1/1	+	10.0 – 13.0 x ~1.0
Large basitrichs	15.88 – 21.61 (18.96) x 1.19 – 2.67 (2.19)	83	1/1	++	14.0 – 19.0 x 1.5
PHARYNX					
Basitrichs	23.75 – 30.06 (27.63) x 1.78 – 2.89 (2.21)	75	1/1	++	25.0 – 31.0 x 2.0 – 2.5
Mc. *p*-mastigophores	18.20 – 25.84 (21.69) x 2.38 – 3.61 (2.99)	62	1/1	+	----------
FILAMENT					
Basitrichs	14.06 – 26.98 (20.11) x 1.80 – 3.18 (2.44)	88	1/1	++	----------
Mc. *p*-mastigophores	11.66 – 17.02 (13.32) x 2.47 – 4.26 (3.84)	76	1/1	++	----------
ACONTIA					
Basitrichs	32.18 – 38.47 (35.41) x 2.68 – 3.40 (3.18)	80	1/1	+++	34.0 – 36.0 x 2.5 – 3.0

Table 5.7. Size and distribution of cnidae of *Calliactis annulata* Carlgren 1922. Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++= very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	Carlgren (1928b)
TENTACLES					
Spirocysts	16.86 – 32.65 (27.54) x 1.94 – 3.89 (2.78)	102	5/5	+++	17.0 – 34.0 x 2.0 – 4.0
Basitrichs	18.34 – 24.98 (20.92) x 1.79 – 2.38 (2.11)	98	5/5	+++	17.0 – 22.0 x ~2.0
COLUMN					
Bastrichs	10.16 – 17.67 (15.03) x 1.10 – 1.79 (1.44)	87	5/5	+++	---------- ----------
PHARYNX					
Basitrichs	22.67 – 33.21 (28.78) x 2.24 – 3.87 (3.29)	95	5/5	+++	25.0 – 31.0 x 2.5 – 3.5
Mc. *p*-mastigophores	17.65 – 23.77 (20.81) x 2.32 – 3.56 (3.02)	26	3/5	-	----------
FILAMENT					
Basitrichs	18.23 – 26.80 (23.67) x 2.34 – 3.49 (3.15)	106	5/5	++	----------
Mc. *p*-mastigophores	24.89 – 35.77 (29.32) x 2.59 – 3.89 (3.21)	89	5/5	++	---------- ----------
ACONTIA					
Basitrichs	35.66 – 42.31 (38.90) x 2.29 – 3.19 (2.76)	145	5/5	+++	37.0 – 43.0 x 2.5 – 3.0

Table 5.8. Size and distribution of cnidae of *Calliactis japonica* Carlgren 1928. Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	Hand (1975a)
TENTACLES					
Spirocysts	15.69 – 32.54 (23.51) x 1.88 – 3 (3.22)	108	6/6	+++	15.0 – 30.0 x 2.0 – 3.5
Small basitrichs	----------				10.0 – 13.0 x 1.0 – 1.5*
Large basitrichs	18.78 – 27.03 (22.05) x 2.14 – 3.26 (2.89)	111	6/6	+++	20.0 – 25.0 x 2.0 – 3.0
COLUMN					
Small basitrichs	09.12 – 15.20 (12.56) x 0.92 – 1.86 (1.33)	98	6/6	+++	10.0 – 13.0 x 1.0 – 1.5
Large basitrichs	15.36 – 23.14 (19.68) x 1.93 – 3.34 (2.68)	89	6/6	++	14.0 – 20.0 x 2.0 – 3.0
Mc. *p*-mastigophores	16.91 – 22.51 (20.06) x 2.31 – 3.22 (2.72)	31	6/6	-	15.0 – 20.0 x 2.5 – 3.0*
PHARYNX					
Small basitrichs	09.78 – 15.97 (12.77) x 1.20 – 1.85 (1.45)	101	6/6	++	11.0 – 14.0 x 1.0 – 1.5
Large basitrichs	20.17 – 29.04 (25.89) x 2.41 – 3.82 (3.19)	105	6/6	++	22.0 – 31.0 x 2.5 – 3.5
Mc. *p*-mastigophores	15.66 – 21.98 (18.44) x 3.54 – 5.23 (4.63)	56	4/6	+	17.0 – 20.0 x 4.0 – 5.0*
FILAMENT					
Basitrichs	12.84 – 18.71 (13.58) x 1.22 – 1.91 (1.62)	92	6/6	++	11.0 – 14.0 x 1.0 – 1.5
Mc. *p*-mastigophores	19.65 – 28.14 (23.04) x 2.20 – 3.61 (2.97)	89	6/6	++	18.0 – 25.0 x 2.5 – 3.5
ACONTIA					
Small basitrichs	08.56 – 16.02 (12.71) x 1.20 – 2.03 (1.47)	103	6/6	+++	10.0 – 14.0 x 1.0 – 1.5
Large basitrichs	20.72 – 33.45 (27.89) x 2.82 – 4.24 (3.56)	91	6/6	++	22.0 – 34.0 x 3.0 – 4.0

Table 5.10. Size and distribution of cnidae of *Calliactis conchicola* Parry 1952. Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare; * = rare nematocyst. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	Riemann-Zurneck (1975)
TENTACLES					
Basitrichs	23.55 – 28.91 (25.11) x 2.29 – 3.05 (2.78)	59	1/1	++	22.0 – 25.0 x 2.5
Spirocysts	20.36 – 34.20 (26.74) x 3.15 – 3.74 (3.43)	61	1/1	++	up to 31.0 x 3.0 – 3.5
COLUMN					
Basitrichs	10.04 – 25.55 (20.06) x 2.32 – 3.68 (3.09)	69	1/1	++	11.5 – 23.0 x 2.5 – 3.0
Mc. *p*-mastigophores	17.16 – 20.05 (18.29) x 2.85 – 3.79 (3.17)	35	1/1	-	16.0 – 17.5 x 3.0 – 3.5
PHARYNX					
Basitrichs	20.35 – 28.69 (23.48) x 3.18 – 4.36 (3.81)	67	1/1	++	22.0 – 26.0 x 3.0 – 4.0
FILAMENT					
Basitrichs	21.72 – 24.98 (22.23) x 2.59 – 3.18 (2.83)	62	1/1	++	22.0 – 23.0 x 3.0
Mc. *p*-mastigophores	18.63 – 25.64 (21.99) x 3.27 – 5.02 (4.15)	58	1/1	++	19.5 – 23.5 x 3.5 – 4.5
ACONTIA					
Small basitrichs	11.62 – 16.09 (13.23) x 1.77 – 2.35 (2.06)	70	1/1	++	14.5 x 2.0
Large basitrichs	19.54 – 26.87 (22.98) x 2.39 – 3.24 (2.89)	59	1/1	++	21.0 – 27.0 x 2.5 – 3.0

Table 5.11. Size and distribution of cnidae of *Calliactis androgyna* Riemann-Zurneck 1975. Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	*Calliactis polypus* (England 1975)
TENTACLES					
Spirocysts	15.97 – 22.40 (18.78) x 1.83 – 2.86 (2.34)	152	10/10	+++	16.86 – 32.65 (27.54) x 1.94 – 3.89 (2.78)
Basitrichs	16.09 – 22.56 (17.97) x 1.48 – 2.74 (2.21)	149	10/10	++	18.34 – 24.98 (20.92) x 1.79 – 2.38 (2.11)
	25.46 – 33.27 (29.04) x 2.98 – 4.49 (3.85)	122	10/10	++	
COLUMN					
Small basitrichs	07.14 – 12.00 (10.23) x 1.15 – 1.87 (1.56)	137	10/10	++	10.16 – 17.67 (15.03) x 1.10 – 1.79 (1.44)
Large basitrichs	14.71 – 16.85 (15.34) x 1.47 – 2.56 (2.17)	115	10/10	++	----------
PHARYNX					
Basitrichs	22.02 –33.75 (27.89) x .1.89 –3.62 (2.95)	157	10/10	++	22.67 – 33.21 (28.78) x 2.24 – 3.87 (3.29)
Mc. *p*-mastigophores	----------				17.65 – 23.77 (20.81) x 2.32 – 3.56 (3.02)
FILAMENT					
Basitrichs	09.24 – 14.37 (12.29) x 1.20 – 2.31 (2.05)	189	10/10	++	18.23 – 26.80 (23.67) x 2.34 – 3.49 (3.15)
Mc. *p*-mastigophores	15.98 – 23.43 (18.77) x 2.47 – 3.99 (3.12)	237	10/10	+++	24.89 – 35.77 (29.32) x 2.59 – 3.89 (3.21)
ACONTIA					
Basitrichs	31.28 – 38.49 (34.78) x 2.31 – 3.64 (3.10)	215	10/10	+++	35.66 – 42.31 (38.90) x 2.29 – 3.19 (2.76)

Table 5.12. Size and distribution of cnidae of *Calliactis tigre* sp. nov. Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	*Paracalliactis stephensoni*
TENTACLES					
Spirocysts	30.31 – 51.29 (43.70) x 4.88 – 6.27 (5.62)	115	4/4	+++	30.31 – 51.29 (43.70) x 4.88 – 6.27 (5.62)
Basitrichs	18.02 – 26.69 (23.88) x 2.11 – 3.08 (2.53)	122	4/4	+++	18.02 – 26.69 (23.88) x 2.11 – 3.08 (2.53)
COLUMN					
Small basitrichs	10.08 – 14.21 (13.05) x 1.10 – 1.72 (1.51)	57	3/4	-	10.08 – 14.21 (13.05) x 1.10 – 1.72 (1.51)
Large basitrichs	14.71 – 20.10 (17.82) x 1.93 – 2.34 (2.14)	89	4/4	++	14.71 – 20.10 (17.82) x 1.93 – 2.34 (2.14)
Mc. *p*-mastigophores	13.24 – 18.79 (16.75) x 2.42 – 3.35 (2.91)	92	4/4	+++	13.24 – 18.79 (16.75) x 2.42 – 3.35 (2.91)
PHARYNX					
Basitrichs	17.23 – 30.72 (27.33) x 2.56 – 4.18 (3.59)	49	3/4	-	----------
Mc. *p*-mastigophores	24.27 – 29.86 (26.82) x 3.29 – 4.30 (3.91)	93	4/4	++	17.23 – 30.72 (27.33) x 2.56 – 4.18 (3.59)
		80	4/4	++	24.27 – 29.86 (26.82) x 3.29 – 4.30 (3.91)
FILAMENT					
Basitrichs	11.51 – 14.93 (13.65) x 1.43 – 1.82 (1.70)	65	4/4	+	11.51 – 14.93 (13.65) x 1.43 – 1.82 (1.70)
Mc. *p*-mastigophores	18.18 – 26.80 (21.77) x 3.14 – 3.85 (3.42)	87	4/4	++	18.18 – 26.80 (21.77) x 3.14 – 3.85 (3.42)
ACONTIA					
Small basitrichs	13.78 – 17.67 (15.56) x 1.79 – 2.11 (1.91)	80	4/4	+	13.78 – 17.67 (15.56) x 1.79 – 2.11 (1.91)
Large basitrichs	21.74 – 29.24 (25.28) x 2.40 – 3.12 (2.79)	96	4/4	++	21.74 – 29.24 (25.28) x 2.40 – 3.12 (2.79)

Table 5.13. Size and distribution of cnidae of *Paracalliactis valdiviae* Carlgren 1928. Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	Doumenc, 1975
TENTACLES					
Spirocysts	20.78 – 35.88 (31.90) x 2.86 – 4.78 (3.82)	108	6/6	+++	----------
Small basitrichs	18.29 – 23.80 (21.55) x 1.77 – 2.48 (2.20)	56	4/6	++	----------
Large basitrichs	25.68 – 30.34 (28.20) x 3.32 – 4.38 (4.01)	89	6/6	++	25.0 – 35.0 x 1.7 – 3.5
COLUMN					
Basitrichs	18.55 – 23.20 (20.97) x 2.03 – 2.64 (2.28)	57	5/6	+	----------
Mc. *p*-mastigophores	18.20 – 22.83 (20.85) x 2.24 – 3.21 (2.82)	79	5/6	++	----------
PHARYNX					
Basitrichs	23.90 – 32.64 (28.91) x 2.87 – 3.95 (3.34)	85	6/6	++	25.0 – 35.0 x 3.5 – 4.0
Mc. *p*-mastigophores	15.65 – 18.72 (16.89) x 2.02 – 3.19 (2.88)	29	4/6	-	----------
FILAMENT					
Basitrichs	28.70 – 39.65 (34.82) x 3.89 – 4.42 (4.19)	96	6/6	+++	30.0 – 40.0 x 4.0 – 6.0
Mc. *p*-mastigophores 1	32.86 – 38.91 (36.29) x 4.45 – 5.28 (4.94)	89	6/6	++	35.0 – 37.0 x 5.1
Mc. *p*-mastigophores 2	70.68 – 83.76 (78.34) x 8.67 – 10.10 (9.65)	40	5/6	+	78.0 – 98.0 x 9.0 – 11.0
ACONTIA					
Basitrichs	28.81 – 34.23 (30.49) x 3.28 – 4.76 (4.27)	102	6/6	+++	31.0 – 35.0 x 4.0 – 5.0

Table 5.14. Size and distribution of cnidae of *Paracalliactis michaelsarsi* Carlgren 1928. Measurements are in micrometers (μm). N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare; Exceptional sizes in parenthesis. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	*Paracalliactis involvens*
TENTACLES					
Spirocysts	18.22 – 45.31 (30.76) x 2.14 – 4.68 (3.36)	136	6/6	+++	18.25 – 48.53 (28.02) x 2.12 – 4.65 (3.22)
Small basitrichs	14.20 - 24.58 (21.55) x 2.14 – 4.20 (3.23)	120	6/6	++	10.10 – 24.83 (19.69) x 1.38 – 2.99 (2.74)
Large basitrichs	----------				15.08 – 27.68 (21.56) x 2.24 – 4.87 (3.91)
Mc. *b*-mastigophores	15.57 – 27.92 (21.88) x 2.34 – 4.86 (3.29)	23	4/6	-	----------
COLUMN					
Small basitrichs	08.57 – 16.32 (12.45) x 1.12 – 2.98 (2.21)	118	6/6	++	08.64 – 28.74 (19.02) x 1.25 – 2.98 (2.29)
Large basitrichs	18.88 – 32.70 (26.36) x 2.57 – 4.19 (3.45)	125	6/6	+++	20.83 – 31.32 (26.87) x 2.21 – 5.18 (3.58)
Mc. *p*-mastigophores	19.69 – 32.53 (25.74) x 1.90 – 4.87 (3.29)	82	5/6	++	20.25 – 30.58 (26.04) x 1.98 – 4.54 (3.15)
PHARYNX					
Basitrichs	19.87 – 33.65 (25.83) x 2.65 – 4.11 (3.20)	110	6/6	+++	22.45 – 29.89 (26.05) x 3.02 – 4.35 (3.86)
Mc. *p*-mastigophores	17.43 – 41.82 (28.99) x 2.85 – 5.81 (3.93)	159	6/6	+++	16.90 – 37.45 (29.65) x 2.88 – 5.81 (4.56)
FILAMENT					
Small basitrichs	09.68 – 17.80 (15.59) x 1.37 – 2.74 (2.29)	102	6/6	++	12.88 – 19.36 (16.74) x 1.69 – 3.10 (2.63)
Large basitrichs	19.88 – 33.46 (26.31) x 2.52 – 4.69 (3.28)	115	6/6	++	26.65 – 31.08 (28.03) x 3.11 – 4.39 (3.82)
Mc. *p*-mastigophores	18.01 – 30.44 (27.23) x 2.97 – 4.09 (3.45)	162	6/6	+++	18.39 – 33.87 (26.98) x 3.66 – 5.34 (4.63)
ACONTIA					
Small basitrichs	08.93 – 20.12 (16.23) x 1.25 – 2.86 (2.27)	148	6/6	++	08.61 – 18.56 (13.75) x 1.35 – 2.45 (1.99)
Large basitrichs	20.85 – 42.83 (26.20) x 2.82 – 4.88 (3.89)	156	6/6	+++	18.52 – 45.84 (32.14) x 3.07 – 4.32 (3.69)

Table 5.15. Size and distribution of cnidae of *Paracalliactis consors* (Verrill 1882). Measurements are in micrometers (μm), average size in parenthesis. N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare; Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	Hand, 1975a	N
TENTACLES						
Spirocysts	25.00 – 37.22 (32.56) x 2.90 – 5.33 (4.68)	86	7/7	+++	20.0 – 34.0 x 3.0 – 5.5	30
Small basitrichs	10.12 – 15.68 (12.87) x 1.40 – 2.34 (1.89)	25	2/7	-	09.0 – 13.0 x 1.5 – 2.0	30
Large basitrichs	20.10 – 23.79 (21.65) x 2.02 – 2.94 (2.58)	91	7/7	++	19.0 – 23.0 x 2.0 – 3.0	30
Mc. *p*-mastigophores	18.85 – 25.90 (23.25) x 3.82 – 5.37 (4.75)	95	7/7	++	21.0 – 27.0 x 4.0 – 5.5	30
COLUMN						
Small basitrichs	06.55 – 09.40 (08.76) x 0.95 – 1.55 (1.32)	82	7/7	++	07.0 x 08.5 x 1.0 – 1.5	30
Large basitrichs	14.59 – 18.24 (16.99) x 2.25 – 2.84 (2.56)	85	7/7	++	14.0 – 17.0 x 2.5 – 3.0	30
Mc. *p*-mastigophores	13.88 – 17.83 (16.83) x 3.56 – 4.78 (4.25)	90	7/7	+++	12.0 – 17.0 x 4.0 – 5.0	30
PHARYNX						
Basitrichs	25.68 – 32.76 (30.45) x 2.62 – 3.78 (3.24)	93	7/7	++	28.0 – 34.0 x 2.5 – 3.5	30
Mc. *p*-mastigophores	19.92 – 28.54 (25.68) x 4.23 – 6.03 (5.14)	91	7/7	++	21.0 – 27.0 x 4.0 – 5.5	30
FILAMENT						
Small basitrichs	10.20 – 13.45 (12.34) x 1.25 – 2.32 (1.82)	68	7/7	+	09.0 – 11.0 x 1.0 – 2.0	30
Large basitrichs	----------	0	0/7		28.0 – 34.0 x 3.0 – 3.5	30
Mc. *p*-mastigophores	17.46 – 23.36 (20.98) x 2.29 – 3.26 (2.78)	102	7/7	+++	19.0 – 24.0 x 2.5 – 3.0	30
ACONTIA						
Small basitrichs	10.88 – 16.42 (14.50) x 1.39 – 2.11 (1.75)	64	6/7	+	11.0 – 15.0 x 1.5 – 2.0	30
Large basitrichs	30.17 – 39.63 (36.81) x 3.29 – 4.32 (3.90)	120	7/7	+++	28.0 – 37.0 x 3.0 – 4.5	30

Table 5.16. Size and distribution of cnidae of *Paracalliactis rosea* Hand 1975. Measurements are in micrometers (μm). N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare; Exceptional sizes in parenthesis. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F	Daly et. al. (2004)	N	P
TENTACLES							
Small spirocysts	14.87 – 45.99 (29.87) x 2.58 – 8.92 (5.88)	108	6/6	+++	15.8 – 30.3 x 2.2 – 4.4	106	8/8
Large spirocysts	----------				18.1 – 48.1 x 3.2 – 10.3	79	8/8
Basitrichs	08.56 – 21.58 (18.78) x 1.52 – 3.08 (2.69)	90	6/6	++	07.2 – 14.5 x 1.2 – 3.3	69	8/8
Mc. *b*-mastigophores	16.68 – 25.77 (22.64) x 2.18 – 4.80 (3.25)	46	4/6	-	14.9 – 29.7 x 2.0 – 5.1	204	8/8
COLUMN							
Small basitrichs	09.82 – 30.36 (19.23) x 1.67 – 3.88 (2.99)	92	6/6	++	06.5 – 14.1 x 0.9 – 3.1	97	5/5
Basitrichs	----------				15.8 – 34.3 x 2.3 – 4.2	148	5/5
Mc. *p*-mastigophores	25.87 – 33.74 (29.02) x 1.59 – 4.88 (3.39)	96	6/6	++	22.5 – 30.2 x 1.7 – 5.4	10	3/5
PHARYNX							
Basitrichs	18.05 – 33.56 (25.22) x 2.30 – 4.15 (2.98)	112	6/6	++	15.4 – 38.8 x 2.0 – 4.5	66	7/7
Mc. *p*-mastigophores	18.29 – 35.68 (29.20) x 3.10 – 6.35 (4.44)	109	6/6	++	20.1 – 40.0 x 3.0 – 6.7	54	7/7
FILAMENT							
Basitrichs	10.64 – 28.25 (22.01) x 1.42 – 4.65 (3.18)	88	6/6	++	8.25 – 16.5 x 1.1 – 3.4	47	6/6
Large basitrichs	----------				16.3 – 30.1 x 2.3 – 5.0	60	6/6
Mc. *p*-mastigophores	19.47 – 31.58 (25.66) x 3.38 – 4.90 (4.28)	95	6/6	+++	17.9 – 28.3 x 3.2 – 5.1	130	6/6
ACONTIA							
Small basitrichs	10.61 – 20.40 (15.04) x 1.25 – 3.24 (2.29)	123	6/6	+++	10.3 – 21.2 x 1.4 – 3.5	74	8/8
Large basitrichs	24.53 – 41.35 (28.87) x 2.97 – 4.10 (3.45)	135	6/6	+++	22.3 – 51.3 x 3.2 – 5.7	173	8/8

Table 5.17. Size and distribution of cnidae of *Paracalliactis obvolva* Daly, Ardelean, Cha, Campbell, Fautin 2004. Measurements are in micrometers (µm). N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare; Exceptional sizes in parenthesis. Abbreviation, Mc=microbasic.

Tissue/cnidae type	Capsule length (x) x width (x)	N	P	F
TENTACLES				
Small spirocysts	15.78 – 23.79 (20.72) x 2.26 – 3.29 (2.86)	118	4/4	+++
Large spirocysts	29.85 – 37.91 (34.70) x 3.56 – 4.15 (3.91)	102	4/4	++
Basitrichs	22.18 – 28.02 (26.88) x 2.69 – 3.42 (3.09)	113	4/4	+++
COLUMN				
Small basitrichs	09.55 – 15.89 (12.80) x 1.26 – 2.18 (1.84)	88	4/4	++
Large basitrichs	18.76 – 23.84 (20.12) x 2.68 – 3.45 (2.94)	70	4/4	++
Mc. *p*-mastigophores	19.63 – 25.67 (22.85) x 2.55 – 3.72 (3.14)	98	4/4	+++
PHARYNX				
Basitrichs	15.78 – 21.34 (18.94) x 2.36 – 3.41 (2.92)	101	4/4	++
Small mc. *p*-mastigophores	10.14 – 15.86 (12.85) x 1.97 – 2.39 (2.18)	85	4/4	++
Large mc. *p*-mastigophores	18.25 – 28.38 (24.05) x 2.78 – 3.65 (3.20)	80	4/4	+++
FILAMENT				
Basitrichs	10.22 – 15.65 (12.92) x 2.06 – 3.16 (2.89)	98	4/4	++
Mc. *p*-mastigophores	12.81 – 16.88 (14.68) x 1.86 – 2.24 (2.10)	103	4/4	+++
ACONTIA				
Basitrichs	18.93 – 29.02 (25.69) x 2.32 – 4.01 (3.12)	134	4/4	+++

Table 5.18. Size and distribution of cnidae of *Paracalliactis* niwa nov. sp. Measurements are in micrometers (μm). N= number of capsules measured; P = proportion of specimens that showed a particular kind of cnidae; F=frequency of each type of cnidae: +++ = very common, ++ = common, + =not common, - = rare; Exceptional sizes in parenthesis. Abbreviation, Mc=microbasic.

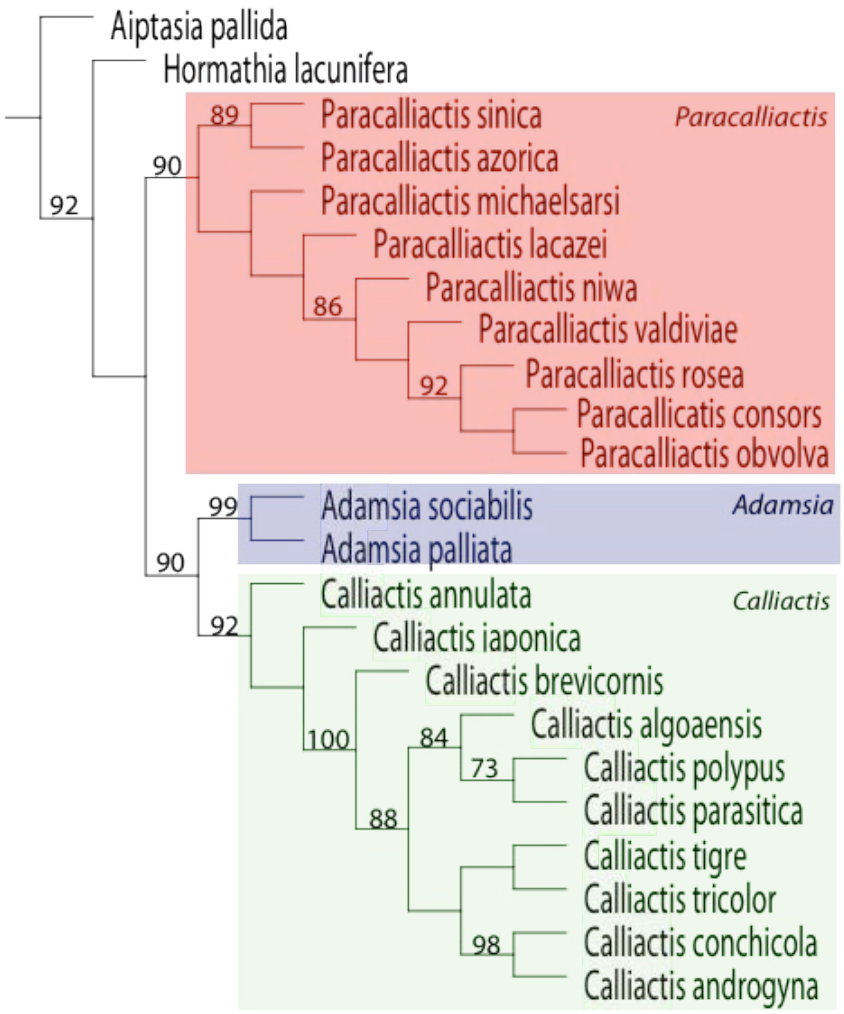

Figure 5.1. Single most parsimonious tree from a morphological analysis of genera *Adamsia*, *Calliactis* and *Paracalliactis*. Numbers above nodes are jackknife values Only jackknife values *higher* than 70% are shown above branches.

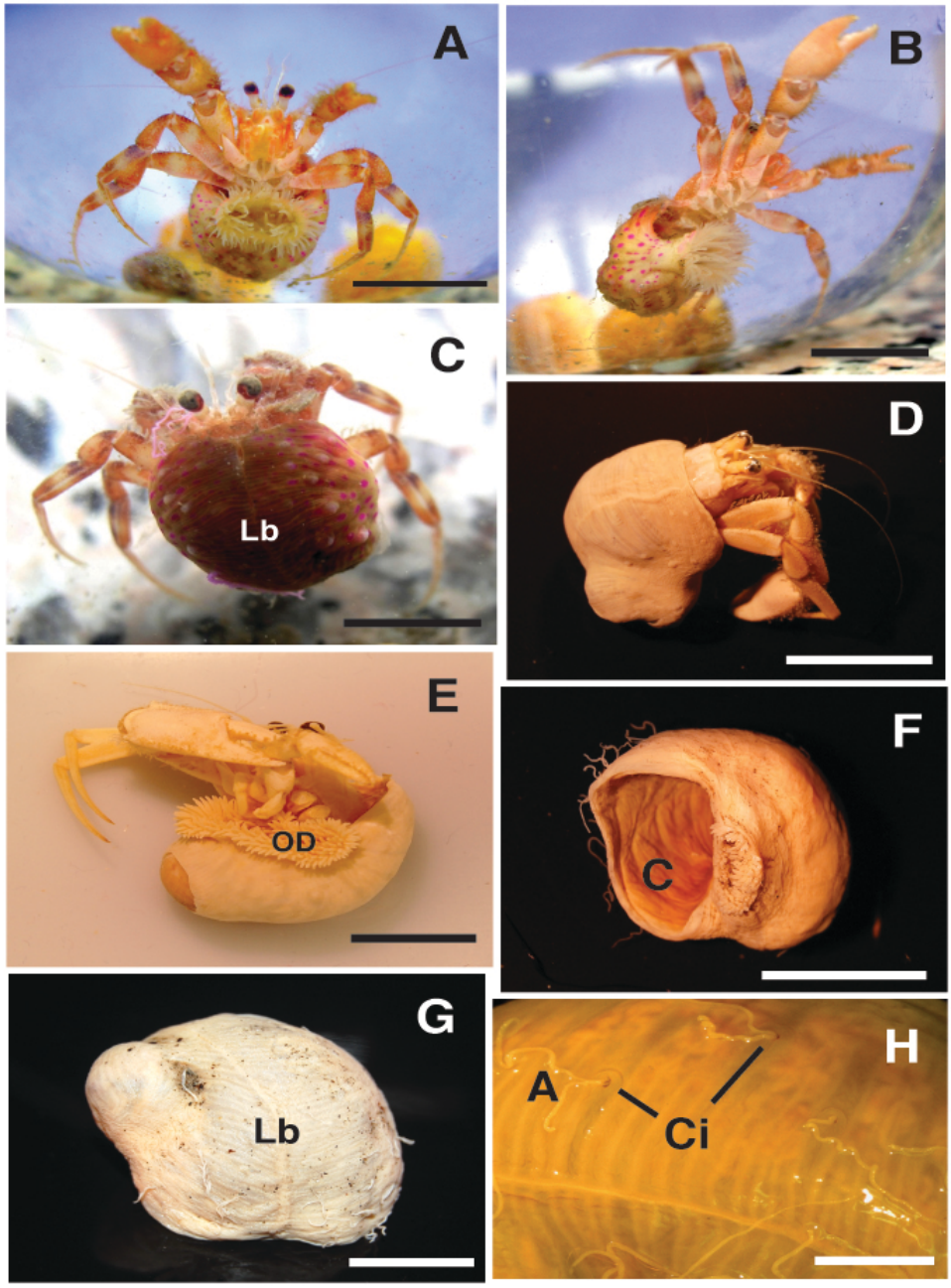

Figure 5.2 *Adamsia palliata* (Muller 1776). External anatomy: A-B) Live specimens showing its natural position on the shell; C) Live specimen showing the coloration of the column and the bi-lobed (Lb) pedal disc; D) Preserved specimens showing cinclides located on small elevations of the column; E) Preserved specimen showing the position of the oral disc (OD); F) Preserved specimens showing the carcinoecium (C); G) Preserved specimens showing the bi-lobed pedal disc (Lb); H) Detail of the column showing cinclides (Ci) and acontia (A). Scales: A, 2.0 cm; B, 1.8 cm; C, 2.3 cm; D, 1.9 cm; E, 2.1 cm; F,1.7 cm; G, 2.4 cm; H, 0.2 cm.

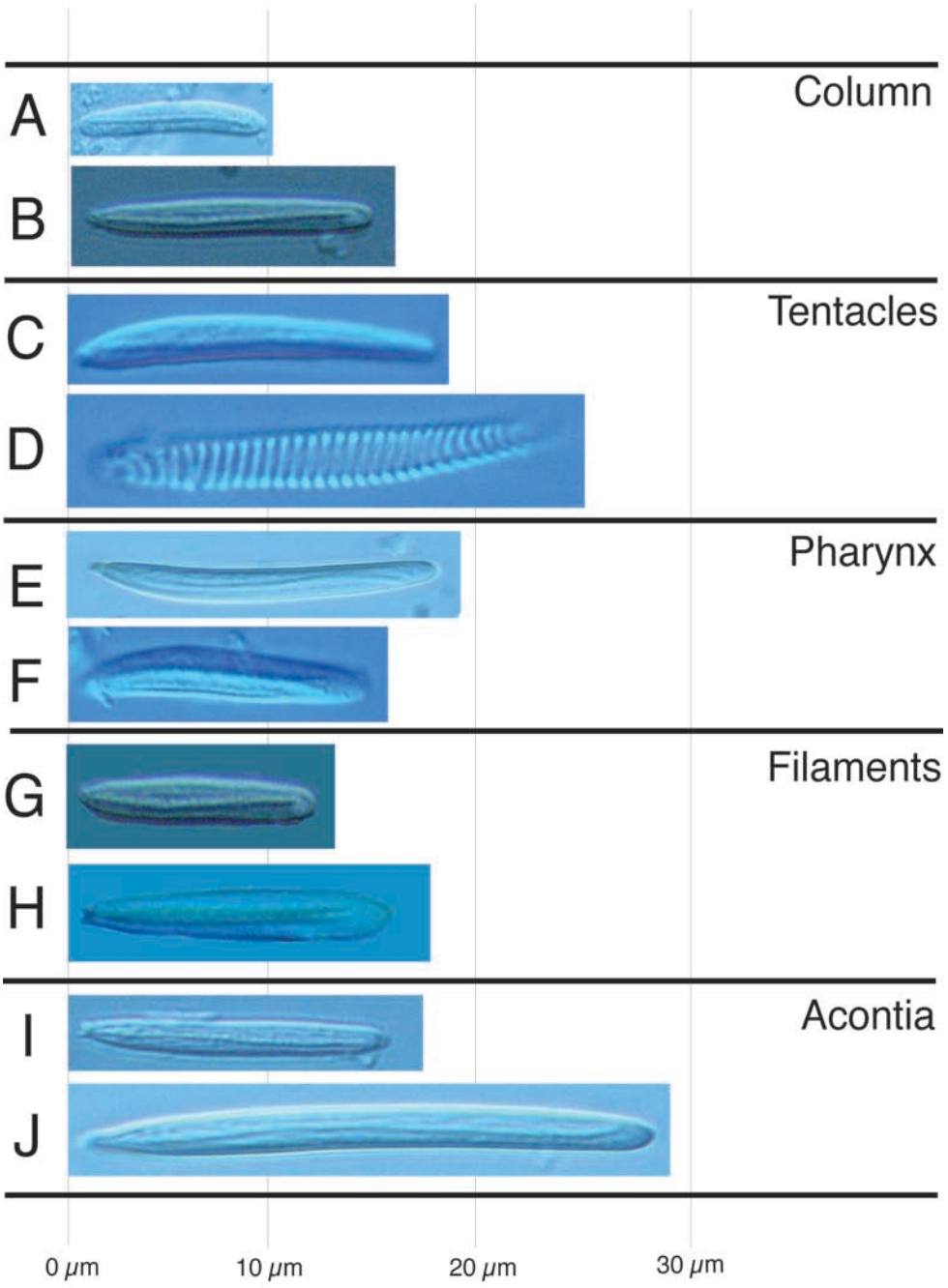

Figure 5.3. *Adamsia* Forbes 1840. Cnidae. A) Basitrich; B) Basitrich; C) Basitrich; D) Spirocyst; E) Basitrich; F) Microbasic *p*-mastigophore; G) Basitrich; H) Microbasic *p*-mastigophore; I) Basitrich; J) Basitrich.

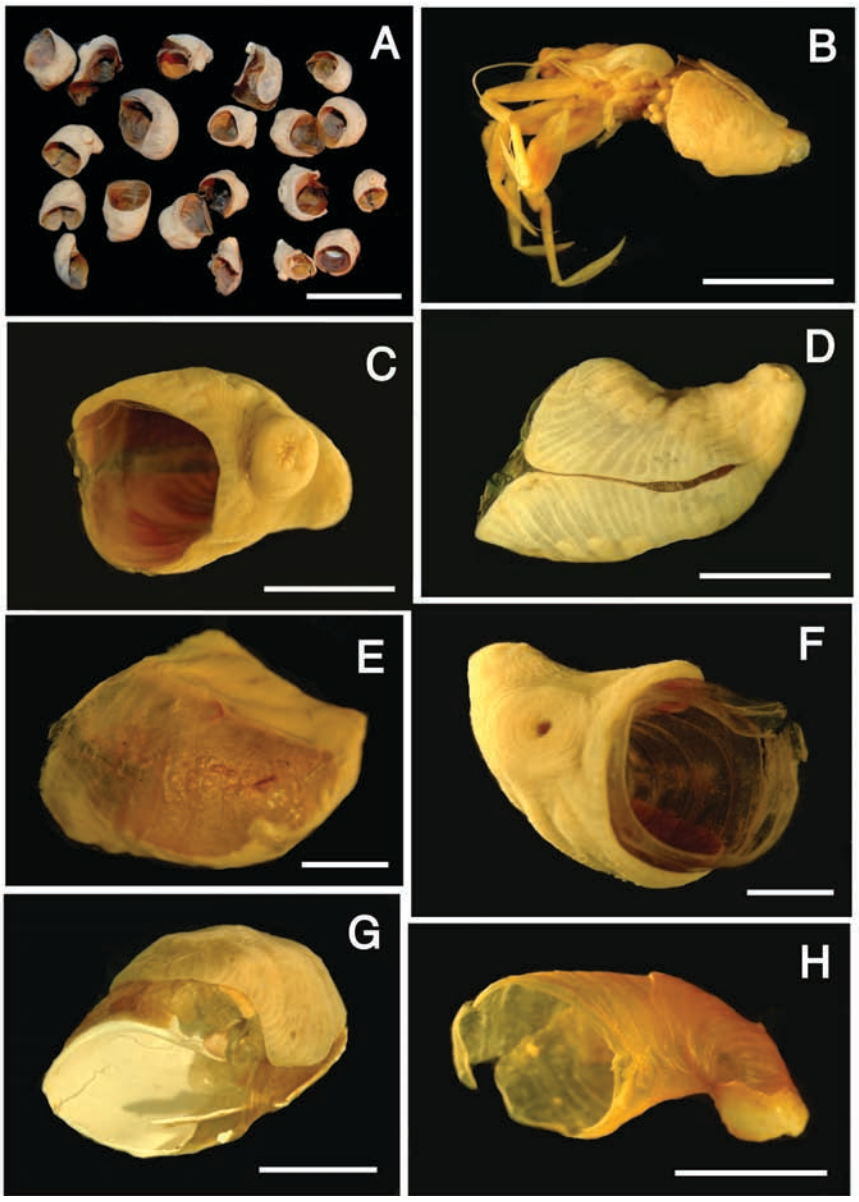

Figure 5.4. *Adamsia sociabilis* (Verrill 1882). External anatomy: A) Syntypes (YPM 9421); B) Preserved specimen on enwrapping the abdomen of a hermit crab; C) Preserved specimen showing the oral disc and the carcinoecium; D) Dorsal view of a preserved specimen showing the bi-lobed pedal disc; E) Ornamentation of a carcinoecium; F) Preserved specimen with a visible carcinoecium; G) Preserved specimen on a shell that has not been dissolved; Carcinoecium. Scales: A, 1.8 cm; B, 0.9 cm; C, 0.6 cm; D, 0.4 cm; E, 0.2 cm; F, 0.2 cm; G, 0.4 cm; H, 0.6 cm.

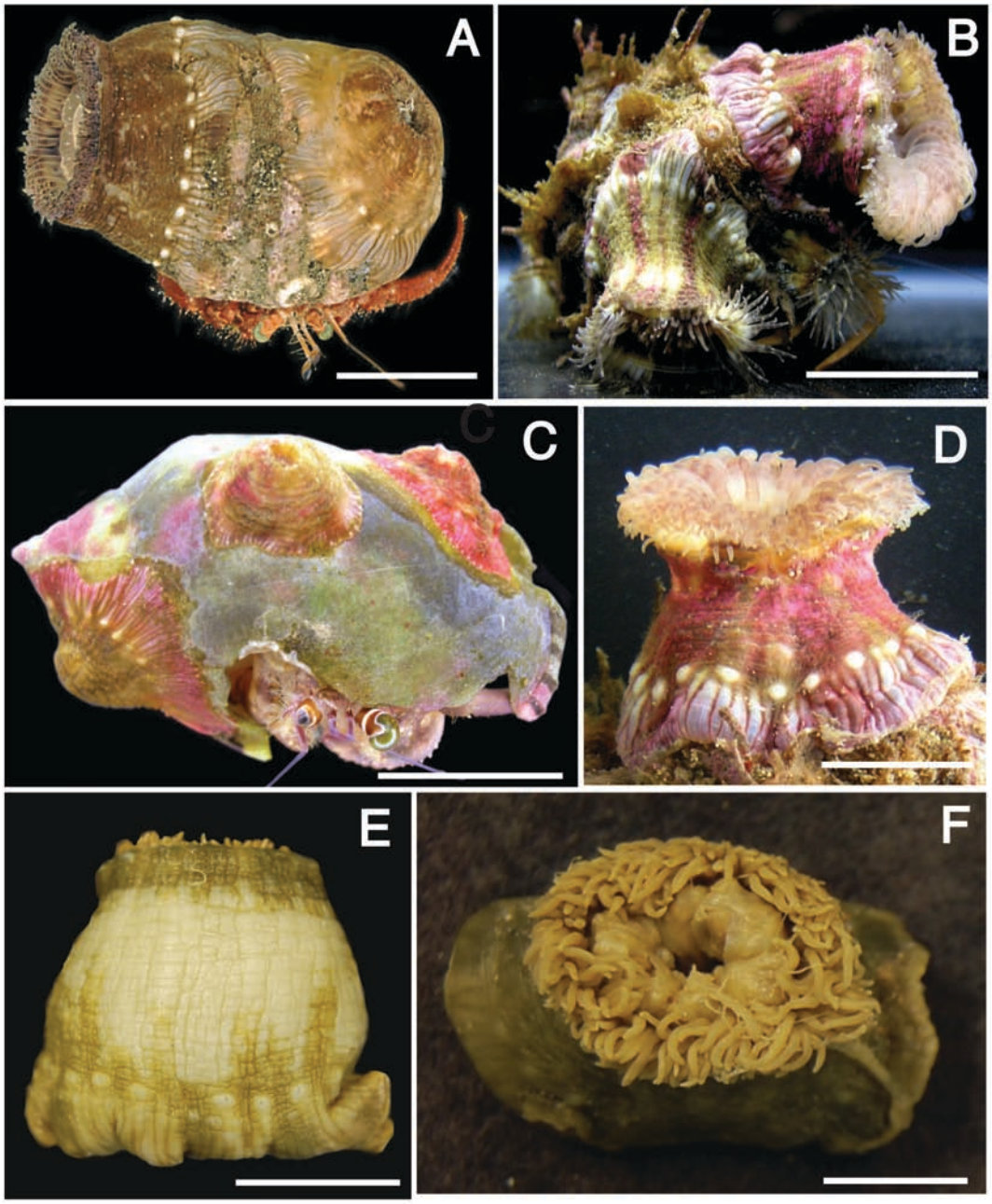

Figure 5.5 *Calliactis polypus* (Forsskål 1775). External anatomy: A) Coloration of two live specimens from the Galapagos Islands, Ecuador; B) Coloration of two live specimens from Japan; C) Coloration of three live specimens from Hawaii; D) Live specimen showing the morphology of the column and a row of white cinclides close to the pedal disc; E) Lateral view of a preserved specimen with cuticle in the proximal part of the column and one row of cinclides; F) Oral view of a preserved specimen showing short tentacles and a central mouth. Scales: A, 2.5 cm; B, 4.0 cm; C, 3.5 cm; D, 2.5 cm; E, 2.0 cm; F, 1.5 cm.

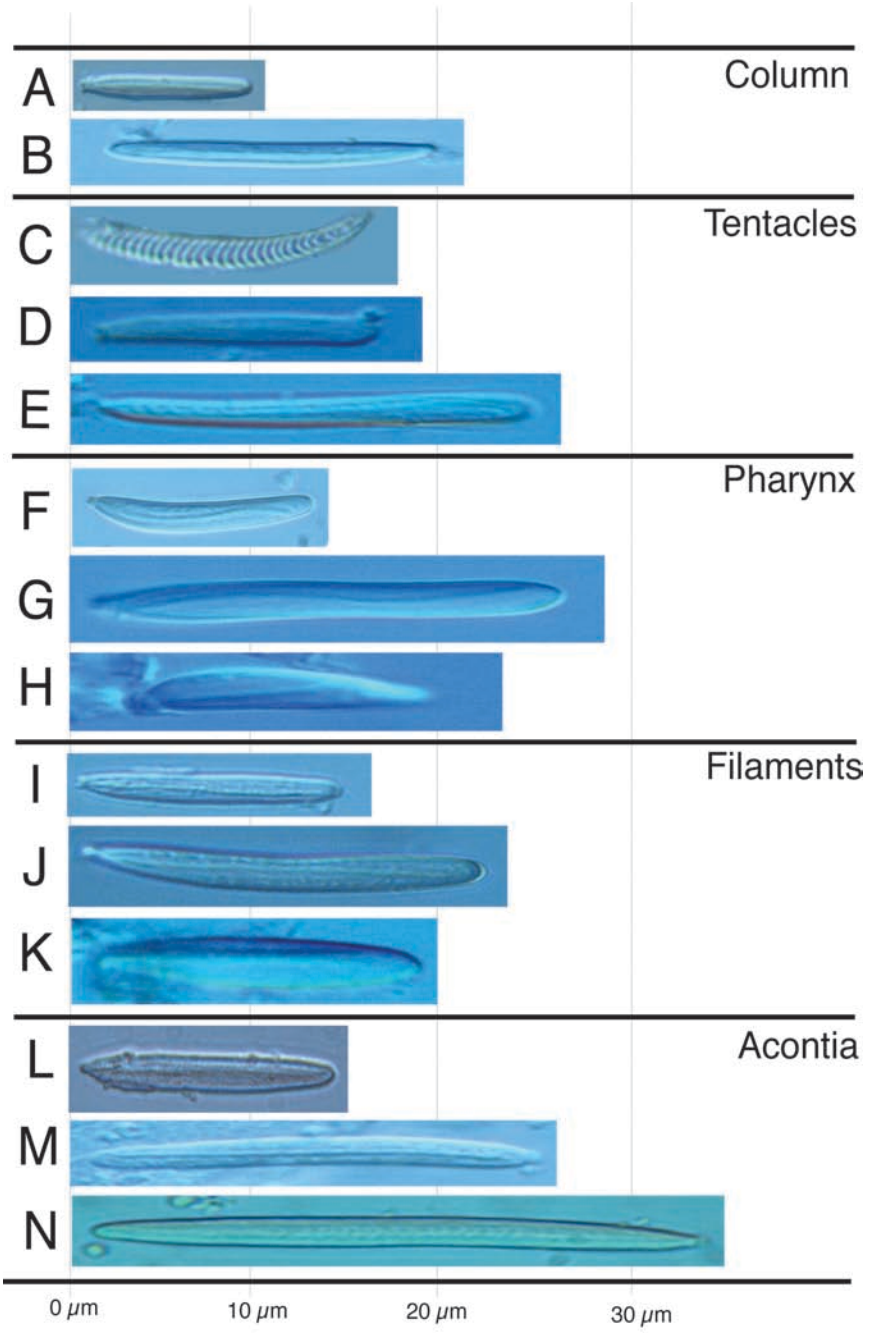

Figure 5.6 *Calliactis* Verrill 1869. Cnidae: A) Basitrich; B) Basitrich; C) Spirocyst; D) Basitrich; E) Basitrich; F) Basitrich; G) Basitrich; H) Microbasic *p*-mastigophore; I) Basitrich; J) Basitrich; K) Microbasic *p*-mastigophore; L) Basitrich; M) Basitrich; N) Basitrich.

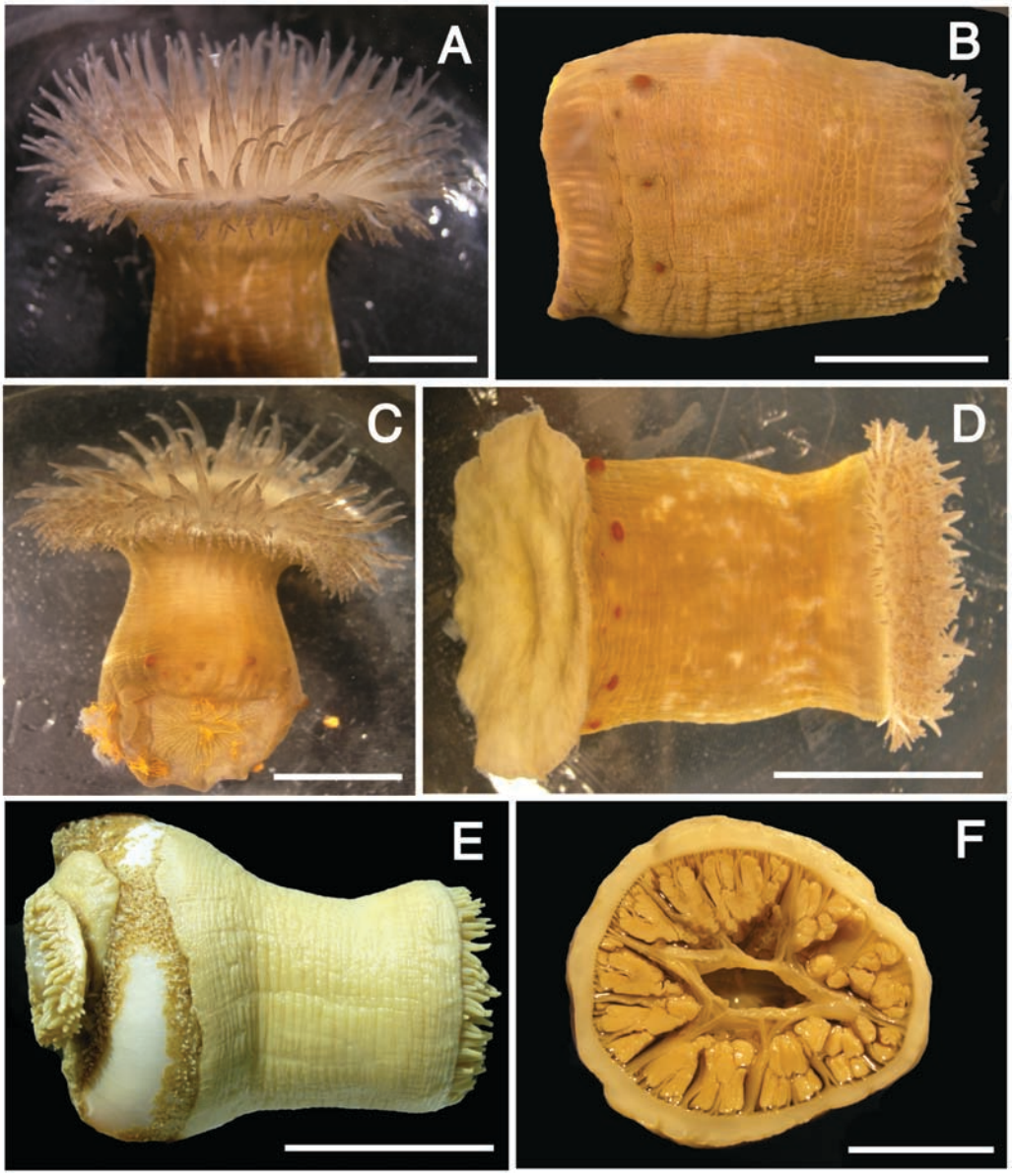

Figure 5.7 *Calliactis tricolor* (Le Sueur 1817). External anatomy: A) Live specimen showing numerous cycles of tentacles; B) Lateral view of a live specimen showing the texture of the column and the red cinclices close to the pedal disc; C) Lateral view of an expanded specimen with orange acontia protruded from cinclides; D) Lateral view of a live specimen showing the shape of the column and the oral disc; E) Lateral view of a preserved specimen; F) Transversal section at the pharynx level from a preserved specimen showing the thick mesoglea and the six cycles of perfect mesenteries attached to the pharynx. Scales: A, 2.0 cm; B, 1.8 cm; C, 2.5 cm; D, 3.0 cm; E, 3.0 cm; F, 1.4 cm.

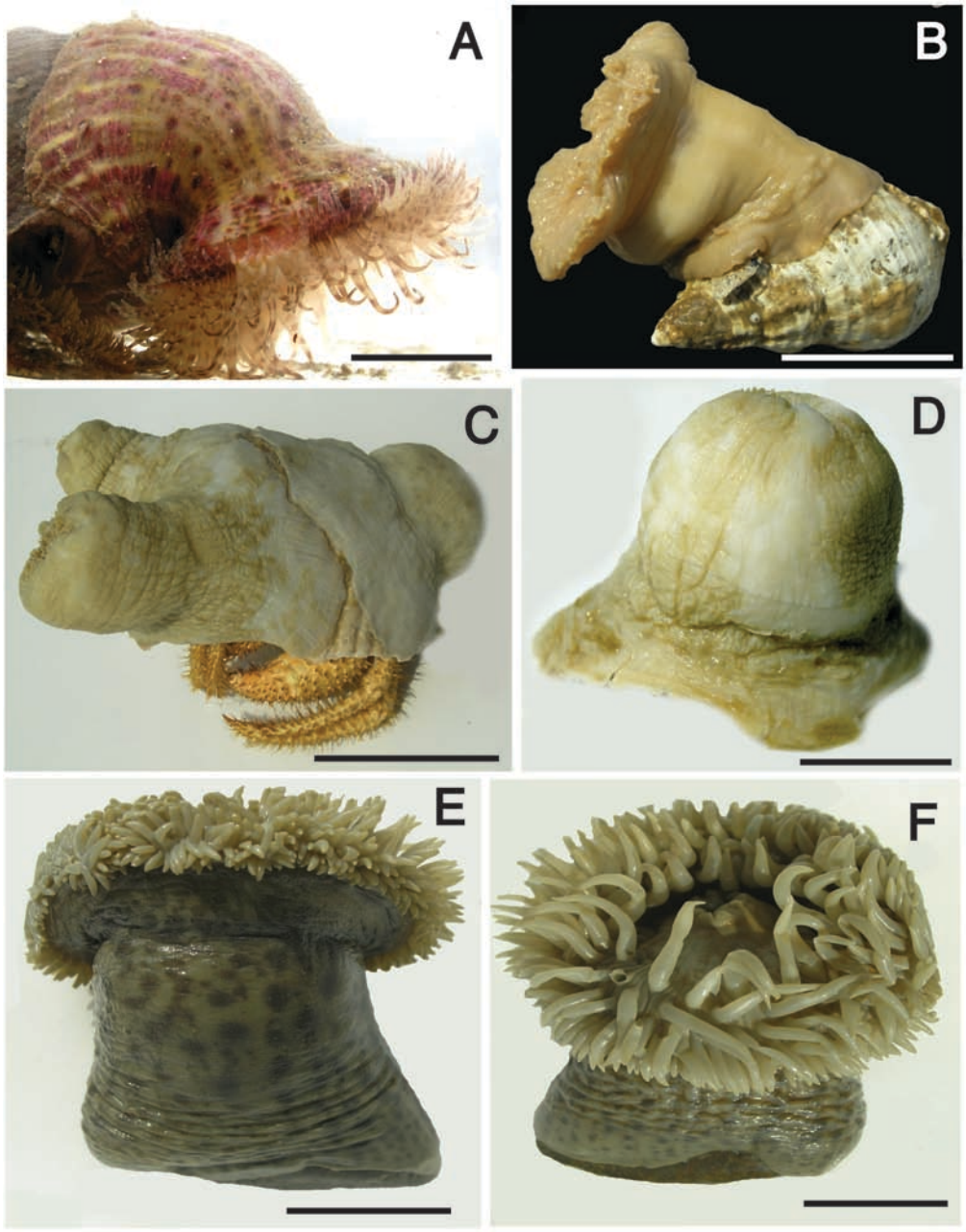

Figure 5.8 *Calliactis parasitica* (Couch 1842). External anatomy: A) Lateral view of a live specimen; B) Lateral view of a preserved specimen showing cinclides close to the pedal disc; C) Three preserved specimens on a shell showing details of the column; D) Lateral view of a contracted preserved specimen showing the cuticle of the column; E) Lateral view of an expanded and preserved specimen showing the shape of the body and the coloration pattern of the column; F) Oral disc of a preserved specimen showing numerous short tentacles arranged in several cycles. Scales: A, 2.0 cm; B, 4.0 cm; C, 3.0 cm; D, 2.8 cm; E, 2.4 cm; F, 2.2 cm

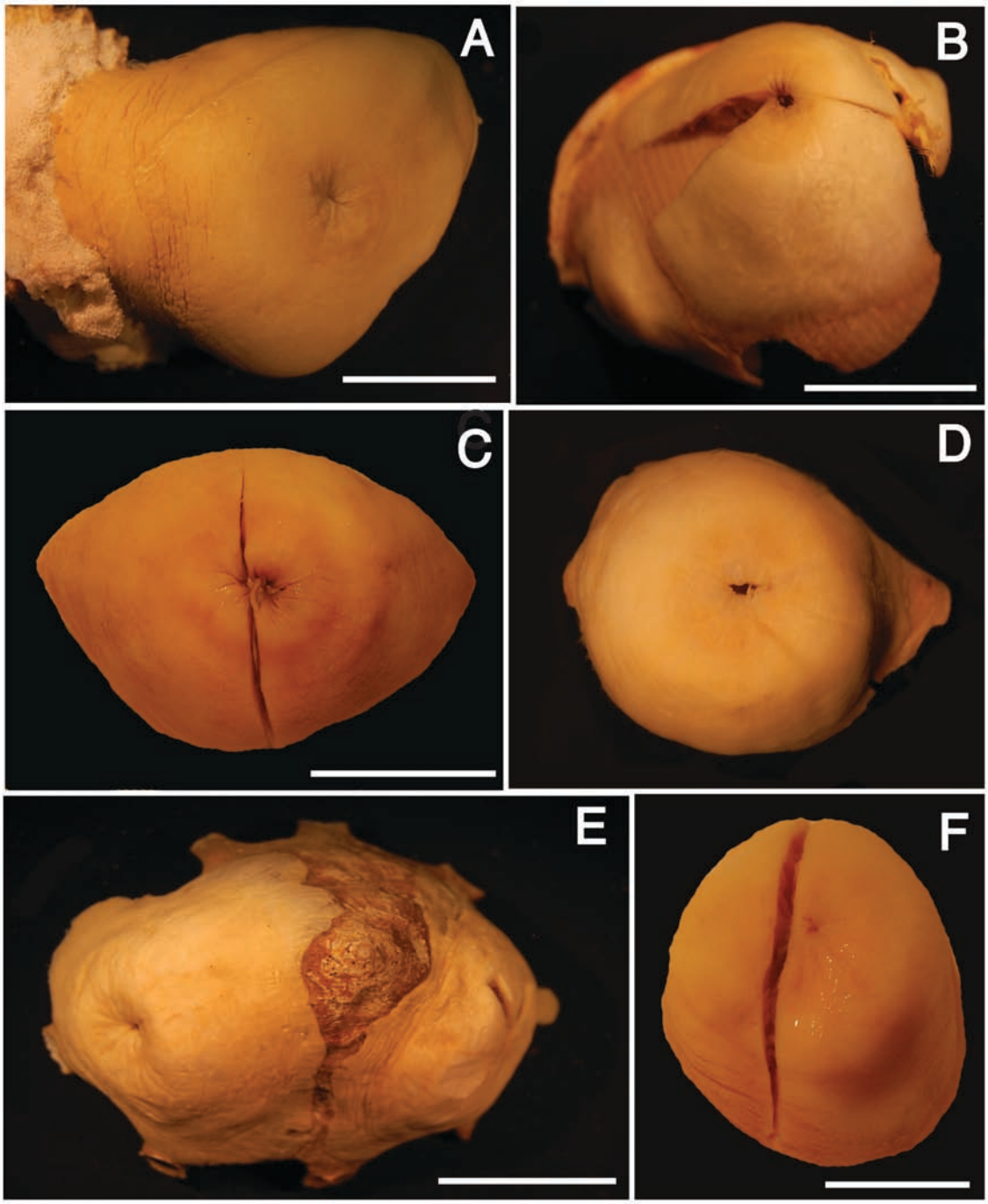

Figure 5.9. *Calliactis brevicornis* (Studer 1879). External antomy: A) Lateral view of a preserved specimen; B, C, D, F) Oral view of preserved specimens; E) Two preserved specimens on a shell. Scales: A, 1.3 cm; B, 1.7 cm; C, 1.5 cm; E, 2.0 cm, F, 1.2 cm.

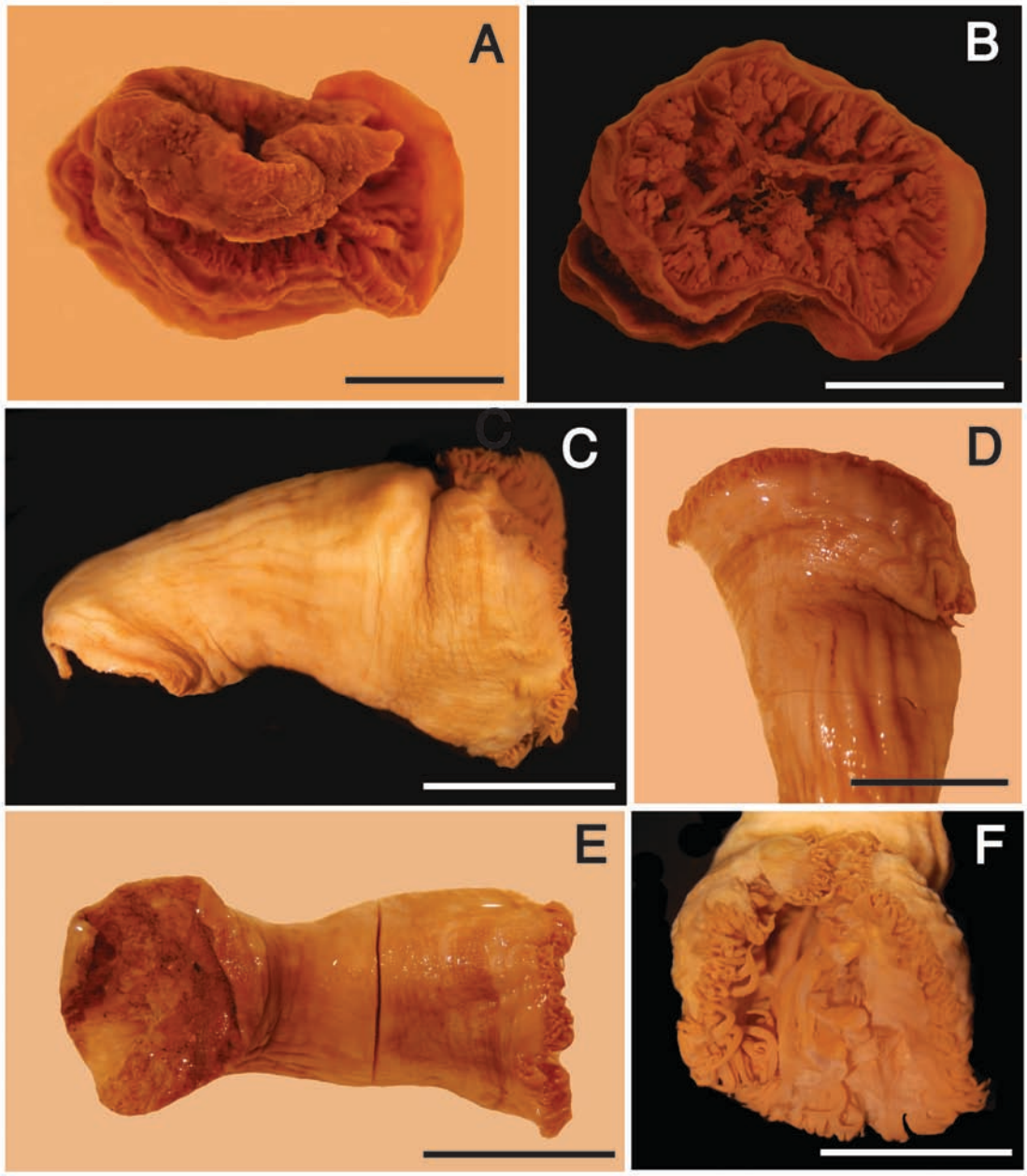

Figure 5.10 *Calliactis androgyna* Riemann-Zurneck 1975. External anatomy: A) Oral view of a preserved specimen; B) Transversal ection through the column of a preserved specimen; *Calliactis annulata* Carlgren 1922. External anatomy: C) Lateral view of a preserved specimen; D) Lateral view of a preserved specimen showing the morphology of the column; E) Lateral view of a preserved specimen showing the scapus and scapulus and the shape of the pedal disc; F) Oral view of a preserved specimen showing the oral disc and tentacles. Scales: A, 0.9 cm; B, 1.0 cm; C,1.9 cm; D, 2.1 cm; E, 2.4 cm; F, 2.8 cm.

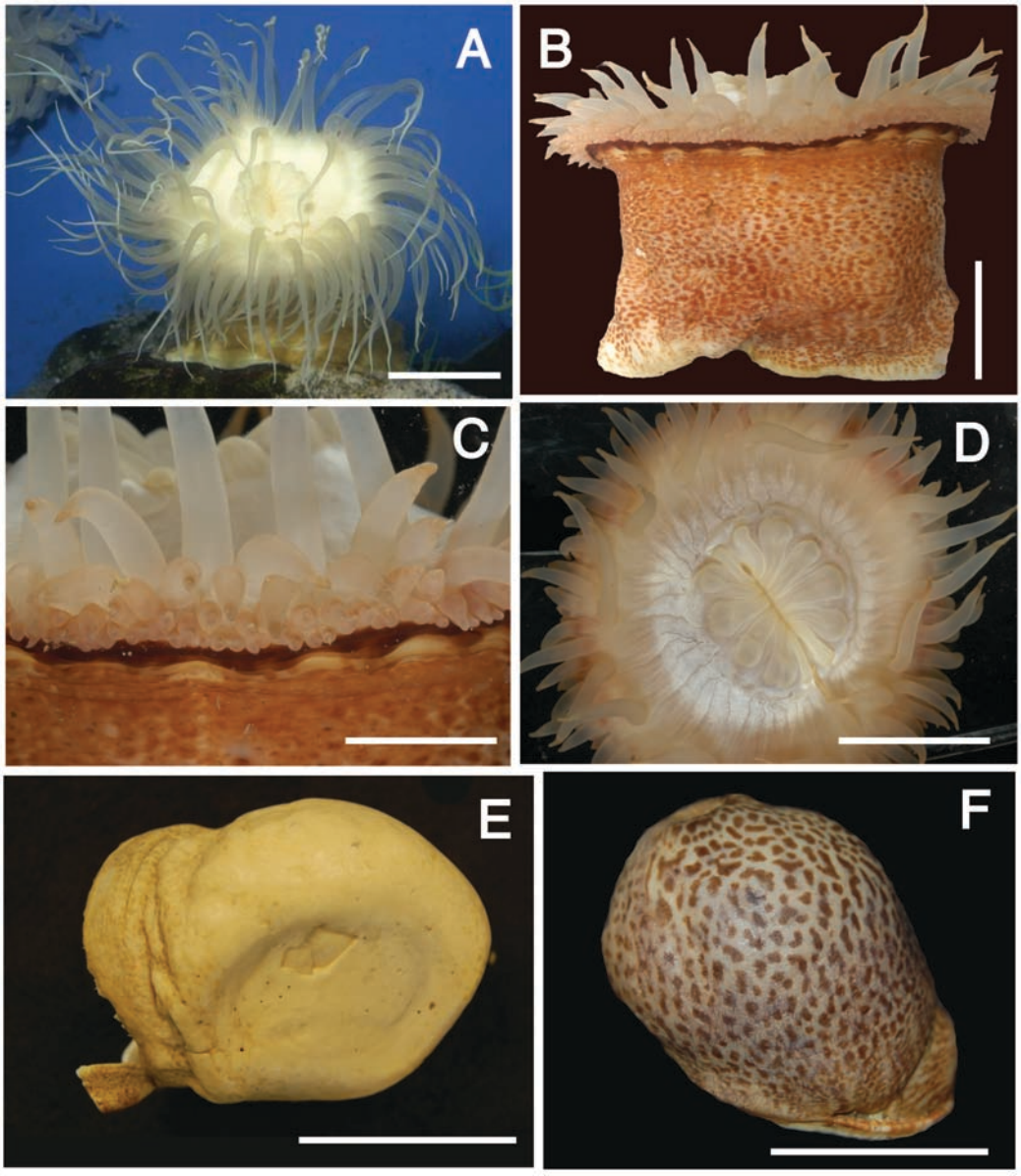

Figure 5.11. *Calliactis japonica* Carlgren 1928. External anatomy: A) Oral view of a live specimen; B) Oral view of a live specimen showing the coloration pattern and the column shape; C) Detail of the distal part of the column and the tentacles; D) Oral disc of a live specimen showing the central mouth, two clear siphonoglyphs and cycles of tentacles; E) Lateral view of a preserved specimen; F) Lateral view of a preserved a specimen with the characteristic coloration pattern of the species. Scales: A, 2.5 cm; B, 1.6 cm; C, 1.0 cm; D, 2.2 cm; E, 4.2 cm; F, 3.1 cm.

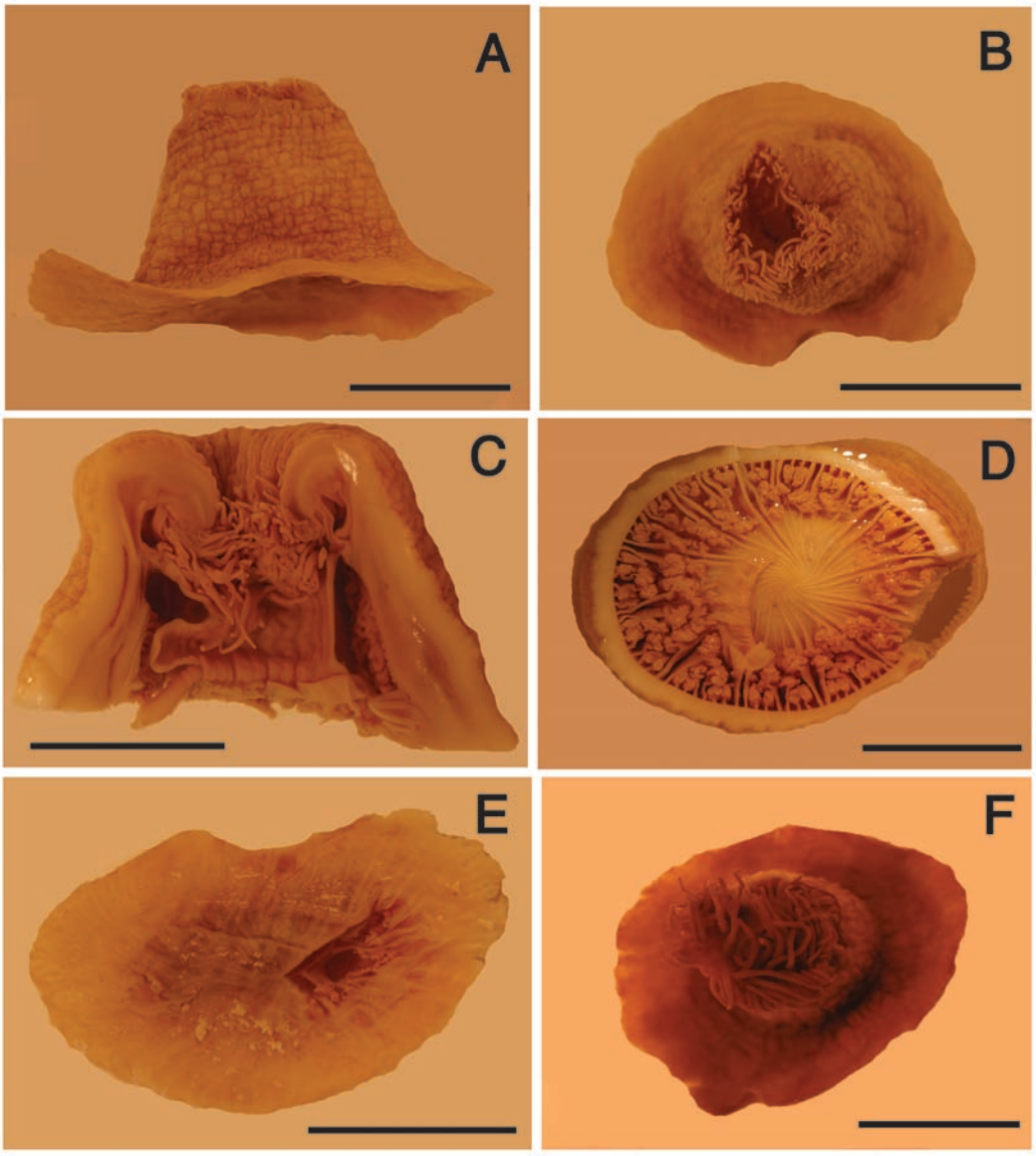

Figure 5.12 *Calliactis algoaensis* Carlgren 1938. External anatomy: A) Lateral view of a preserved specimen showing the shape of the column; B) Oral view of a preserved specimen showing the oral disc with short tentacles; C) Longitudinal section of the upper part of the column showing the thick mesoglea and the lobed pharynx; D) Transversal section through the column of a preserved specimen showing the pairs of mesenteries; E) Pedal disc of a preserved specimen; F) Oral view of a preserved specimen showing the tentacles. Scales: A, 0.5 cm; B, 0.7 cm; C, 1.0 cm; D, 1.6 cm; E, 0.5 cm; F, 0.6 cm.

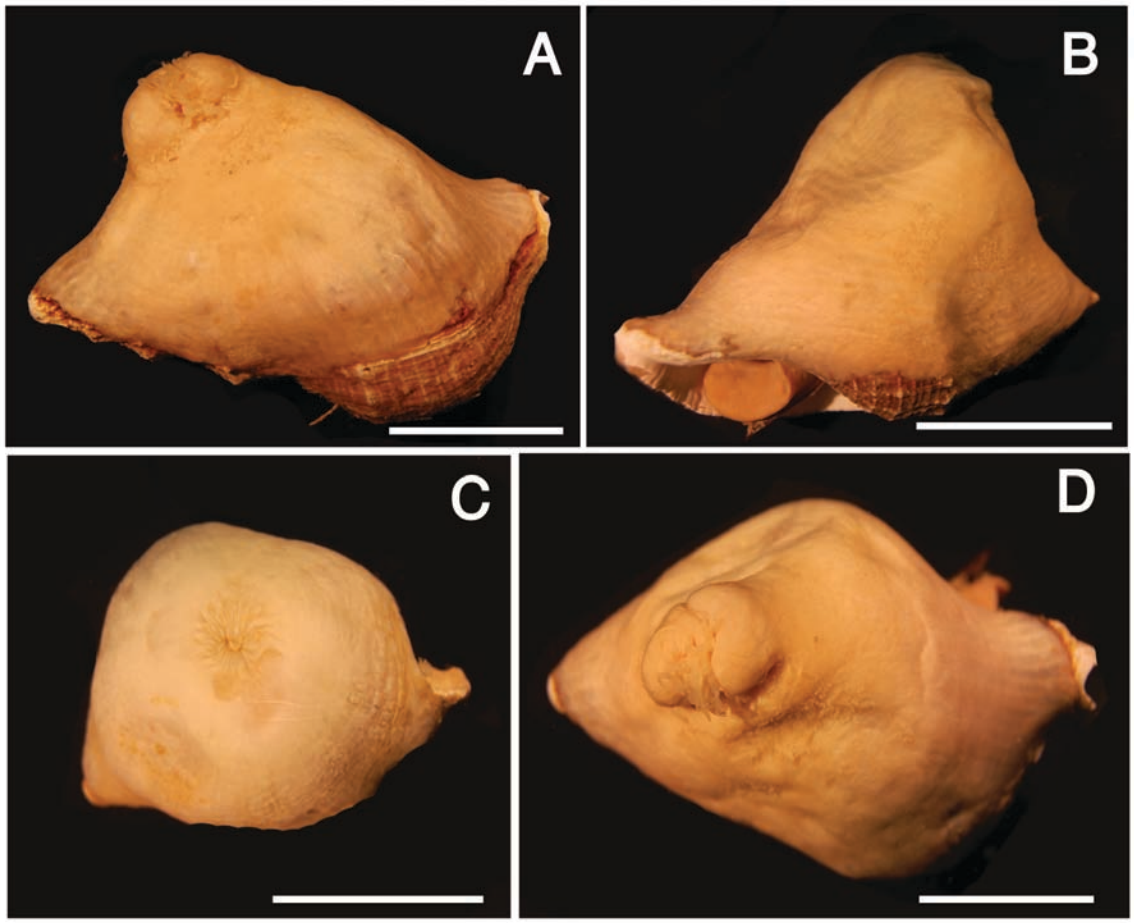

Figure 5.13 *Calliactis conchicola* Parry, 1952. External anatomy: A) Lateral view of a specimen with mesenterial insertions visible close to the pedal disc; B) Lateral view of a preserved specimen showing the smooth column; C) and D) Oral view of a preserved specimen. Scales: A, 2.0 cm; B, 2.2 cm; C, 1.4 cm; D, 1.9 cm.

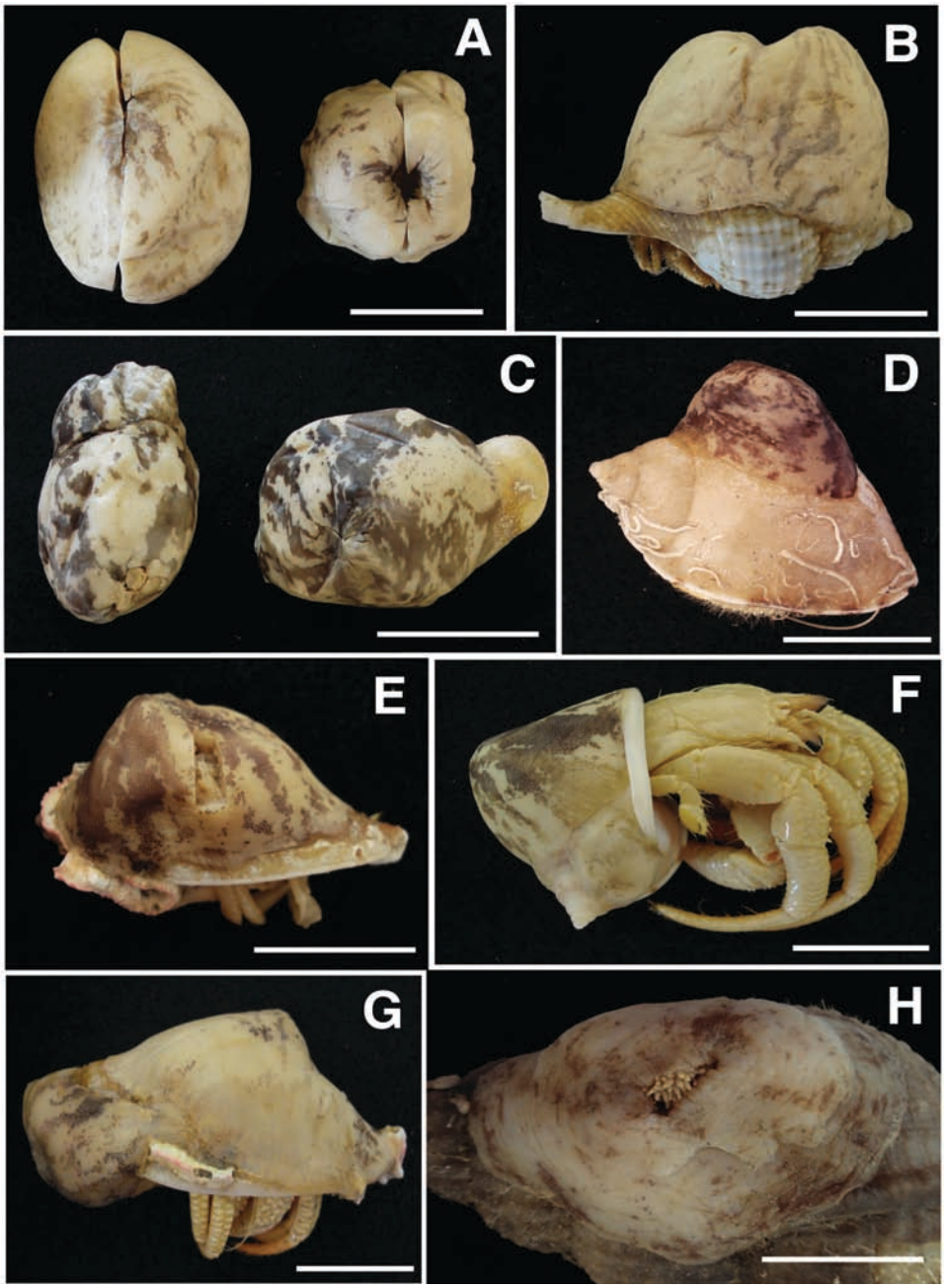

Figure 5.14. *Calliactis tigre* nov. sp. External antomy: A) Oral view of preserved specimens; B) Lateral view of a preserved specimen in its natural position on the shell; C) Preserved specimens showing the coloration pattern of the species; D-F) Small specimens on a shell; G) Two specimens on a single shell; H) Detail of the column of a preserved specimen. Scales: A, 6.0 cm; B, 4.5 cm; C, 5.0 cm; D, 4.8 cm; E, 4.6 cm; F, 3.2 cm; G, 2.1 cm; H, 2.0 cm.

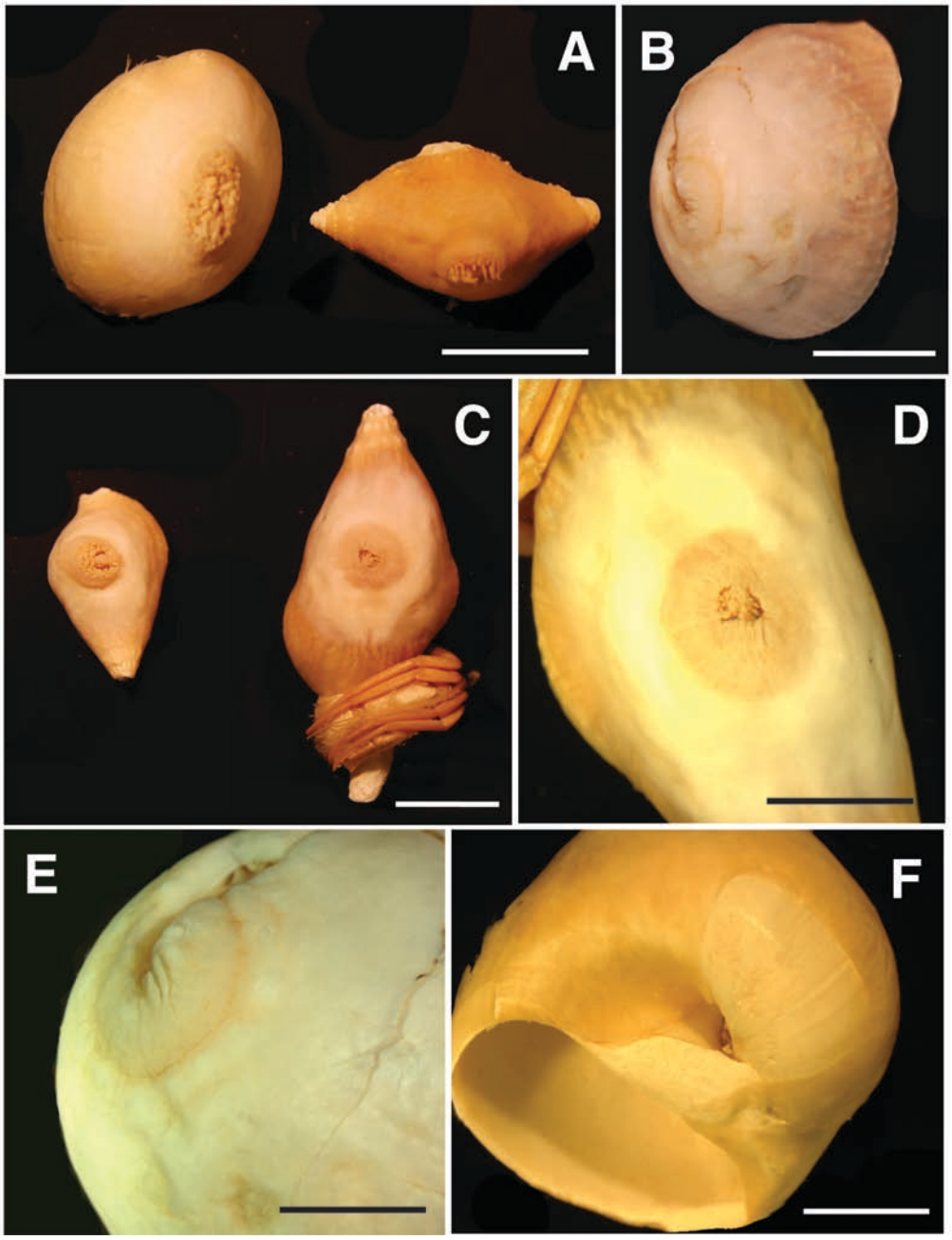

Figure 5.15 *Paracalliactis valdiviae* Carlgren 1928. External anatomy: A) Oral view of two preserved specimens; B) Lateral view of a specimens showing the irregular shape of the pedal disc; C) Preserved specimens showing the mesenterial insertions close to the pedal disc; D) Detail of the scapus and scapulus of a preserved specimen; E) Detail of the distal part of the column of a preserved specimen; F) Detail of the irregular shape of the pedal disc of a preserved specimens. Scales: A, 1.2 cm; B, 1.1 cm; C, 1.3 cm; D, 1.4 cm; E, 0.3 cm; 0.8 cm.

Figure 5.16. *Paracalliactis* Carlgren 1928. Cnidae: A) Basitrich; B) Basitrich; C) Microbasic *p*-mastigophore; D) Microbasic *p*-mastigophore; E) Basitrich; F) Basitrich; G) Basitrich; H) Spirocyst; I) Spirocyst; J)Basitrich; K) Basitrich; L) Basitrich; M) Microbasic *p*-mastigophore; N) Microbasic *p*-mastigophore; O) Basitrich; P) Basitrich; Q) Basitrich; R) Microbasic *p*-mastigophore; S) Basitrich; T) Basitrich; U) Basitrich.

Figure 5.16

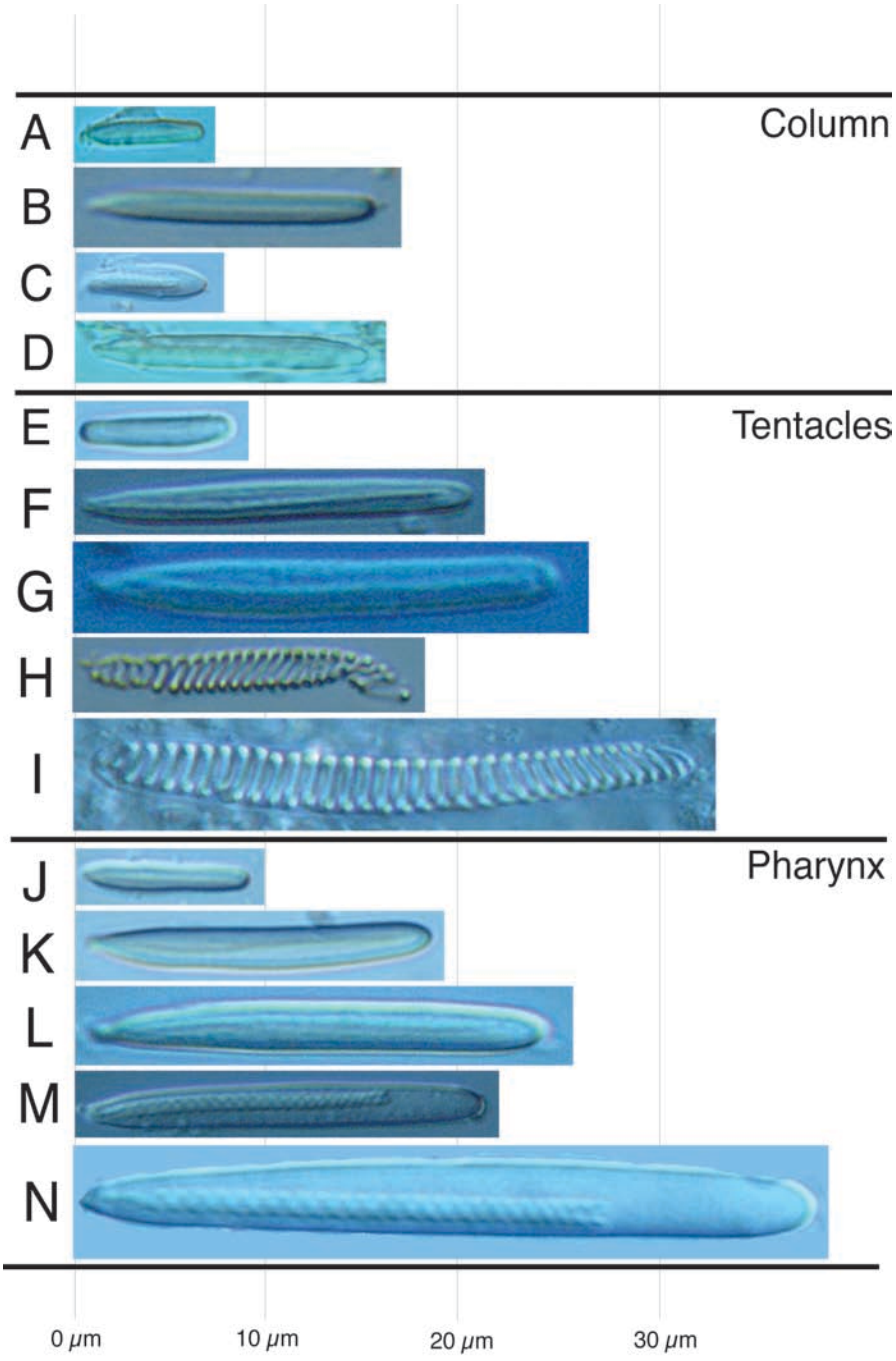

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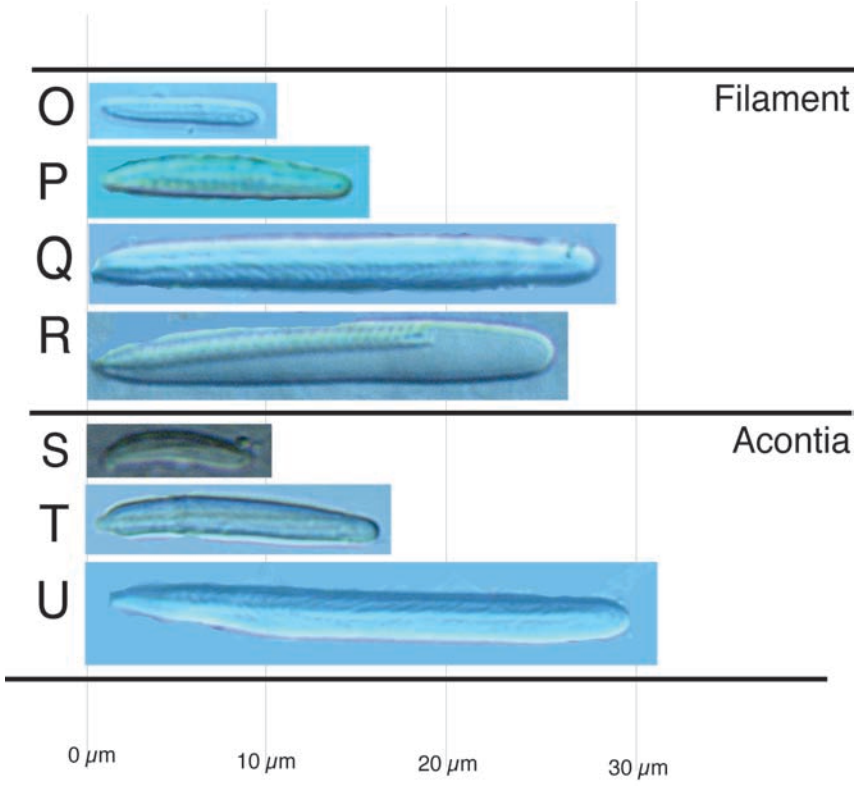
Figure 5.16 continued



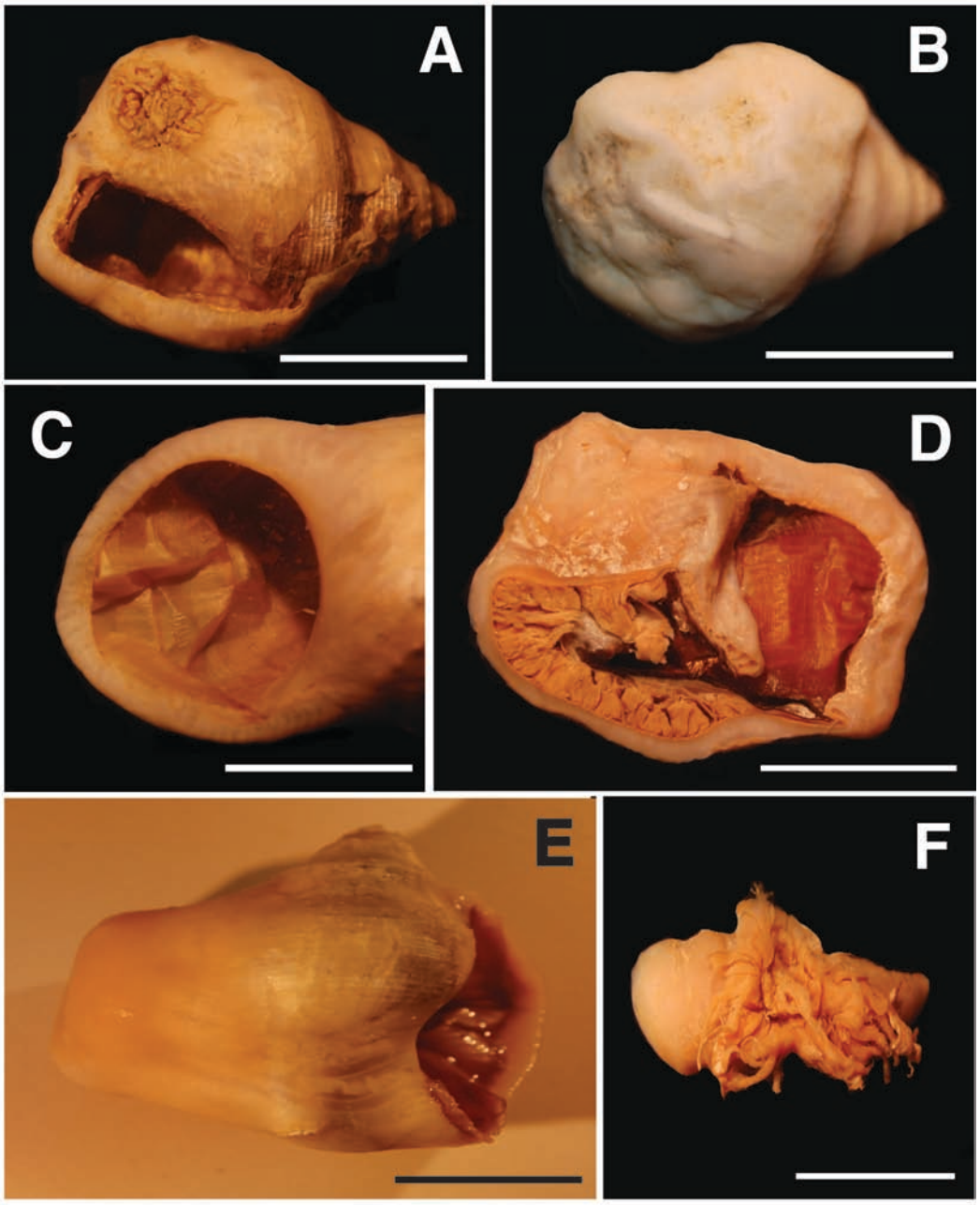


Figure 5.17 *Paracalliactis michaelsarsi* Carlgren 1928. External anatomy: A) Lateral view of a preserved specimen showing the natural position of the anemone on the shell; B) Dorsal view of a specimen on a shell; C) Detail of the carcinoecium; D) Transversal section through the column of a preserved specimen; E) Lateral view of a preserved specimen showing the carcinoecium; F) Oral disc and tentacles. Scales: A, 1.8 cm; B, 1.5 cm; D, 1.2 cm; E, 1.0 cm; F, 1.2 cm.

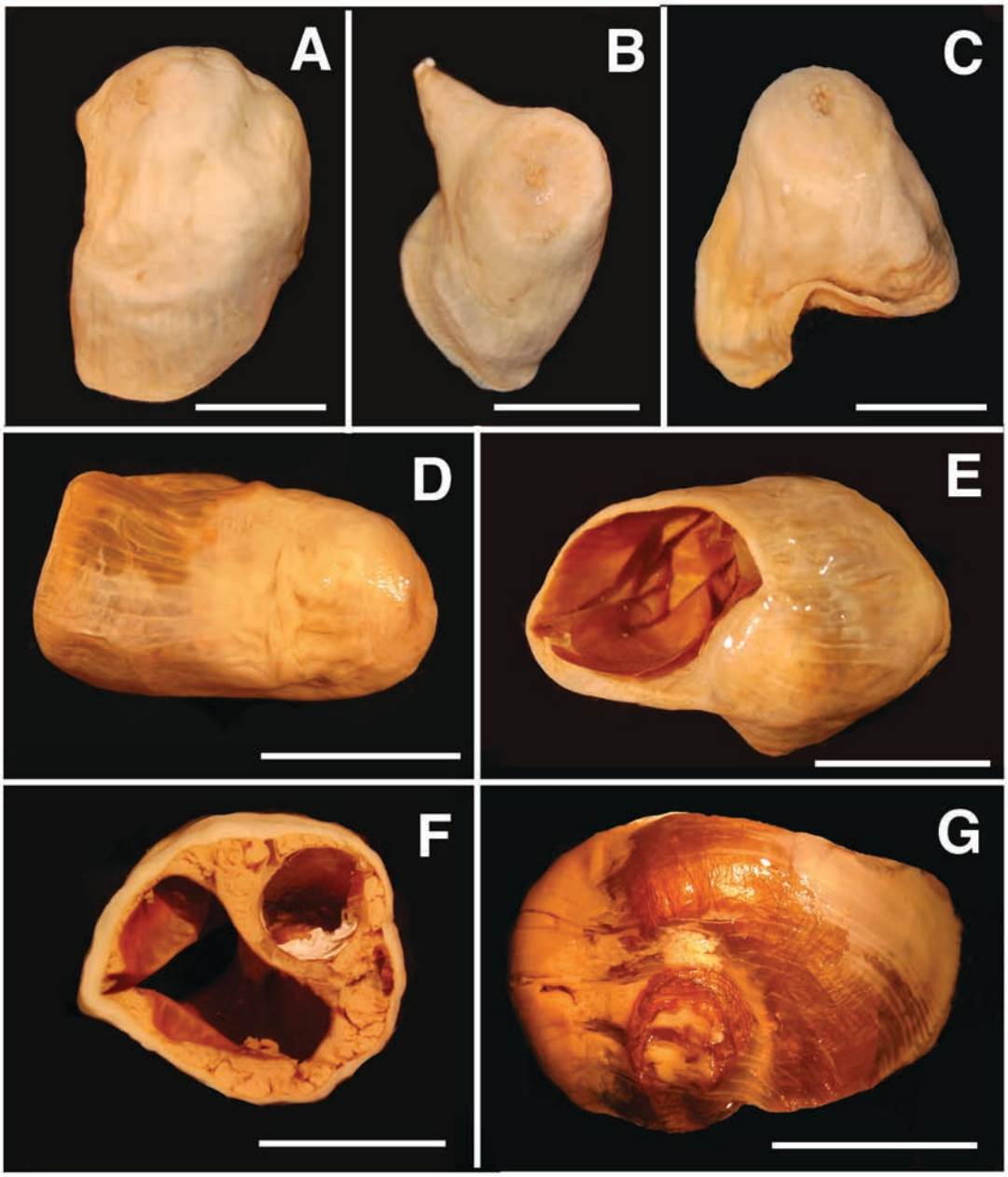


Figure 5.18 *Paracalliactis consors* (Verrill 1882). External anatomy: A-C)Lateral view of preserved specimens; D) Lateral view of a preserved specimen showing the expanded pedal disc with visible mesenterial insertions; E) Lateral view of a preserved specimen showing the bronze carcinoecium; F) Transversal section through the column of a preserved specimen showing how the irregular growth affects the anemone's body; G) Carcinoecium. Scales: A, 1.6 cm; B, 1.0 cm; C, 1.3 cm; D, 2.1 cm; E, 2.5 cm; F; 3.0 cm; G, 3.2 cm.

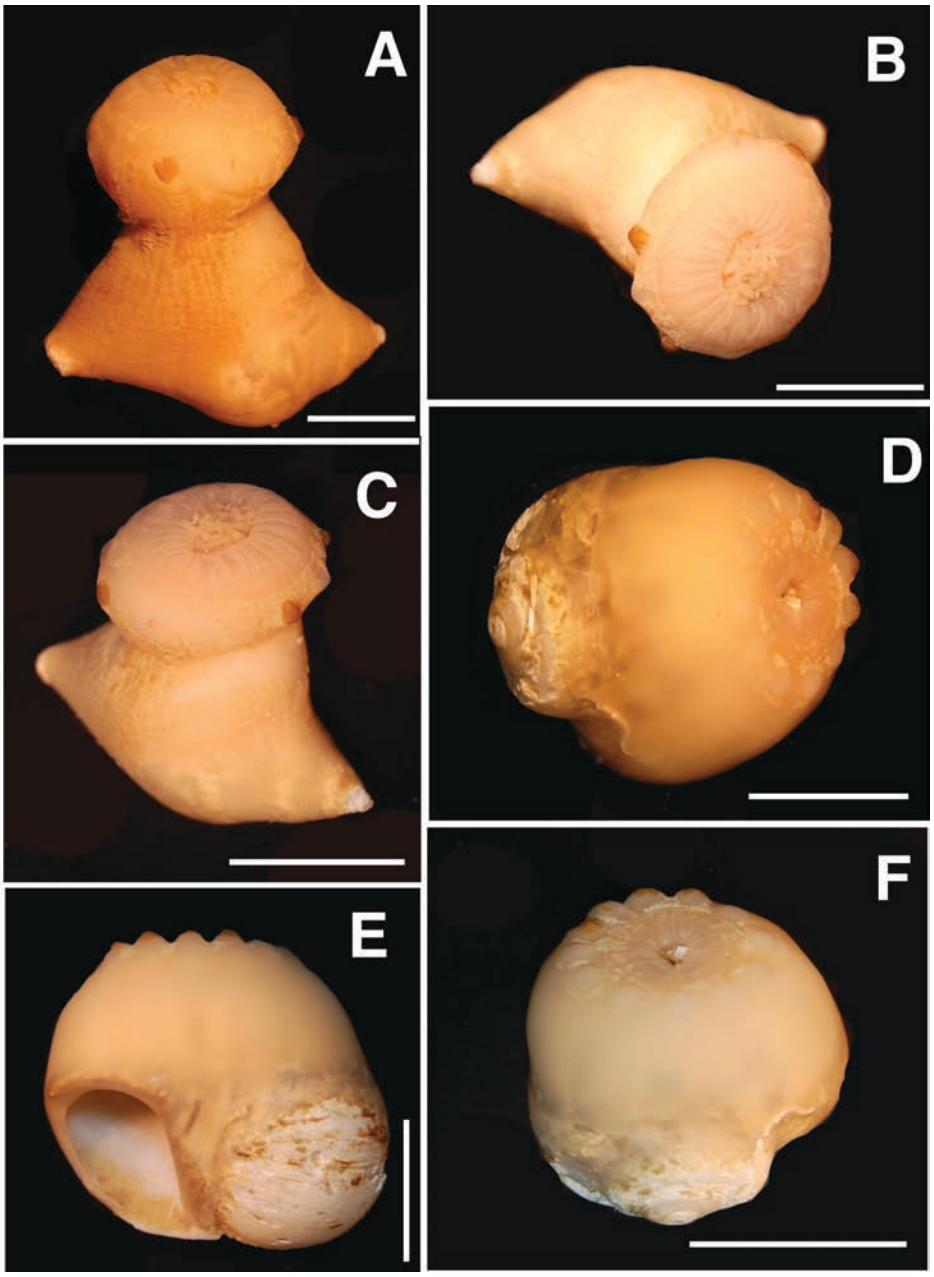


Figure 5.19. *Paracalliactis rosea* Hand 1975. External anatomy: A) Lateral view of a preserved specimen showing the column partially covered by cuticle; B) Oral view of a preserved specimen showing the tubercles; C) Lateral view of a preserved specimen showing tubercles covered by cuticle; D) Lateral view of a preserved specimen showing the transparent pedal disc; E) Lateral view of a preserved specimen showing the irregular shape of the pedal disc; F) Lateral view of a preserved specimen showing the complete corona of tubercles. Scales: A, 0.6 cm; B, 1.1 cm; C, 1.0 cm; D, 1.5 cm; E, 1.3 cm; F, 2.1 cm.

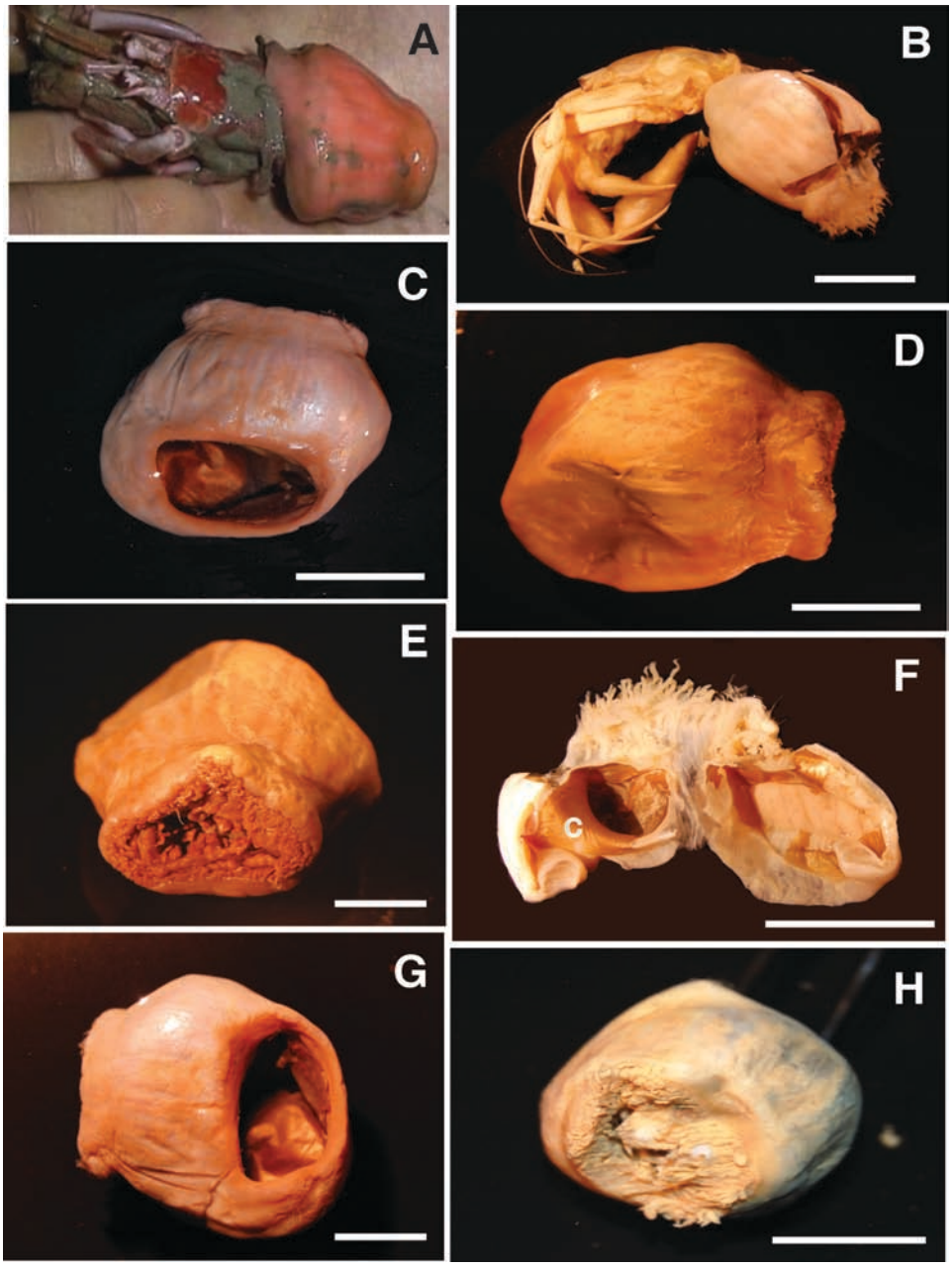


Figure 5.20. *Paracalliactis obvolva* Daly, Ardelean, Cha, Campbel, Fautin 2004. External anatomy: A) Live specimen; B) Preserved specimen in its original position with oral disc erect and away from the aperture of the shell; C) Lateral view of a preserved specimen showing the carcinoecium; D) Lateral view of a preserved specimen; E) Preserved specimen showing oral disc and tentacles; F) Longitudinal cut of a preserved specimen showing the carcinoecium (C); G) Lateral view of the holotype (KUNHM 01595); H) Oral view of the holotype (KUNHM 01595). Scales: B, 3.1 cm; C, 2.3 cm; D, 2.0 cm; E, 1.7 cm; F, 3.9 cm; G, 1.5 cm; H, 3.5 cm.

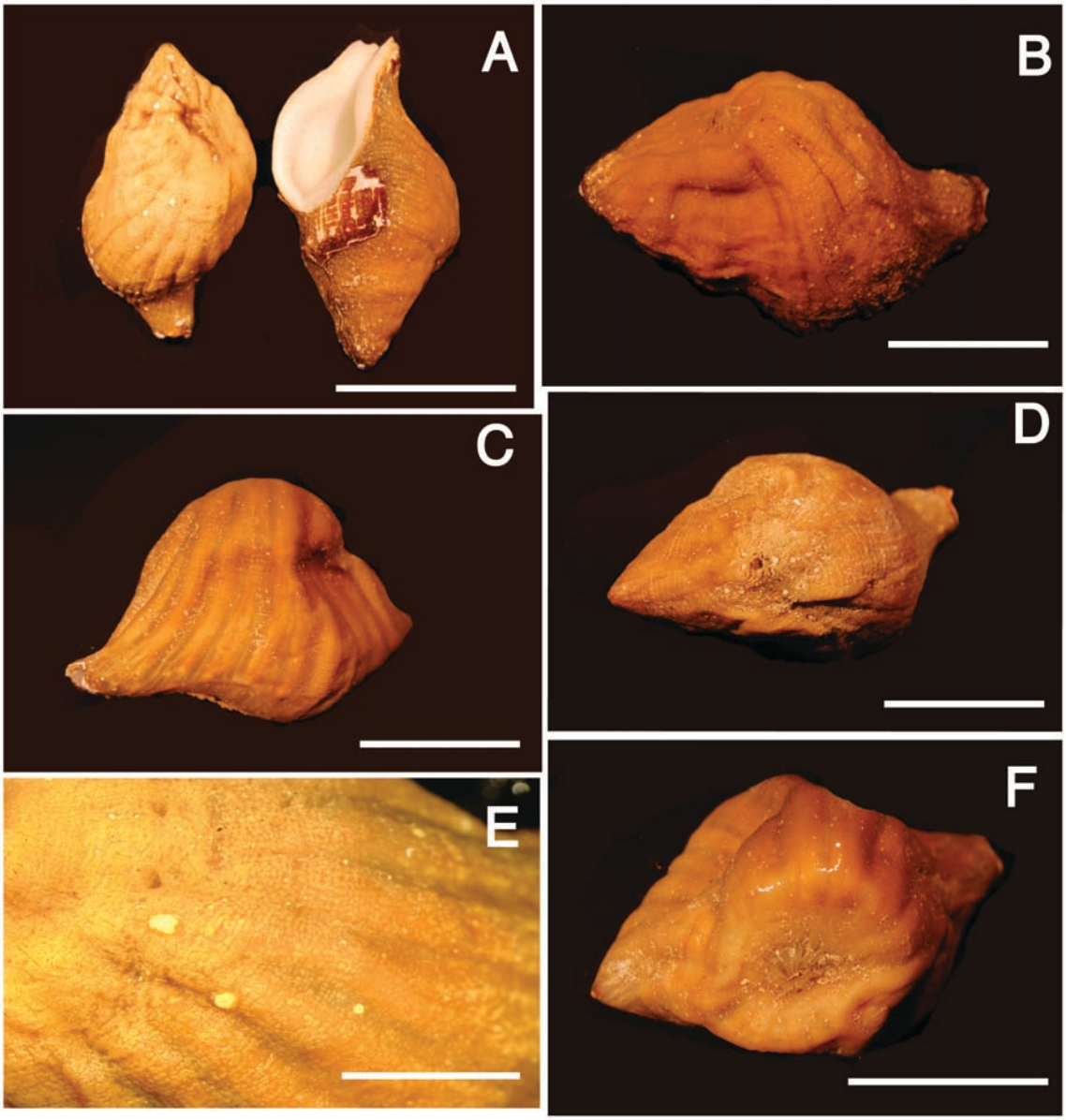


Figure 5.21 *Paracalliactis niwa* sp. nov. External anatomy: A) Preserved specimens showing the position of the anemone on the shell; B) Lateral view of a preserved specimen showing the morphology of the column; C) Lateral view of a specimen showing the longitudinal bands in the column; D) Oral view of a preserved specimen; e) Detail of the column ectodermal pits; F) Oral view of a preserved specimen showing the scapulus. Scales: A, 2.1; B, 1.7 cm; C, 2.0 cm; D, 1.9 cm; E, 0.3 cm; F, 1.7 cm.

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Appendix A: List of material examined

List of material used in this study, including complete information on museum collection and catalog number, number of specimens examined, locality and depth. Museum Acronyms : AM, Australian Museum; BPBM, Bernice P. Bishop Museum, Hawaii; BMNH, British Museum of Natural History; CAS, California Academy of Sciences; GOT, Natural History Museum, Göteborg; KUNHM Kansas University Natural History Museum; LO, Museum of Zoology, Lund University; MNB, Museum für Naturkunde der Humboldt Universität; MNHN, Muséum National d'Histoire Naturelle; NIWA, National Institute of Water `SMNH, Swedish Museum of Natural History; SBMNH, Santa Barbara Museum of Natural History; UF, University of Florida, Gainesville; USNM, United States Natural History Museum; UUZM, Uppsala University Zoological Museum; ZMBN**,** Zoological Museum of Bergen; ZMH, Zoologisches Museum Hamburg; YPM, Yale Peabody Museum.

Species	Museum	Catalog #	# spec	Locality	Depth
Adamsia sociabilis	USNM	8285	9	Off Martha's Vineyard;	157 – 749 m
	USNM	223665	5	Off Martha's Vineyard	226 m
	USNM	23684	1	Off Martha's Vineyard	226 m
	USNM	23685	2	Off Martha's Vineyard	210 m
	USNM	22422	12	Off Martha's Vineyard	210 m
	USNM	23686;	2	Martha's Vineyard	177 m
	USNM;	23688;	3	Martha's Vineyard	369 m

	YPM	9418	8 SYNTYPES	S. of Martha's Vineyard (39^{0}56'00"N \| 70^{0}54'18"W);	230 m
	USNM	22493	50	North Atlantic Ocean, United States, Massachusetts, Martha's Vineyard (39^{0}57'N \| 62^{0}28'W)	219 m
	YPM	9420	2	S. of Martha's Vineyard (39^{0}58'N \| 69^{0}42'W)	369 m
	YPM	9421	24	S. of Martha's Vineyard (39^{0}56'00"N \| 70^{0}54'18"W);	230 m
	YPM	9419	2	S. of Martha's Vineyard (40^{0}01'15"N \| 70^{0}22'00"W)	179 m
	YPM	9424	3	S. of Martha's Vineyard (39^{0}58'N \| 70^{0}35'W);	210 m
	USNM	51057	3	100 miles SE Long Island, NY, USA	900 m
	YPM	9427.	2 SYNTYPES	S. of Martha's Vineyard (40^{0}02'54"N \| 70^{0}23'40"W	210 m
	YPM	9422	15	S. of Martha's Vineyard (39^{0}57'00"N \| 70^{0}56'00"W);	219 m
	USNM	52257	1	Barbados, W.I.;	**200 fms**
	SMNH	4264	2	Pacific Ocean; 01^{0}03'N \| 80^{0}15'W	1349 m
Adamsia palliata					
	Goteborg	27	1	Bergen, Norway.	-
	Bergen	65635	1	Husnesfjorden, Norway	30 m
	Bergen	10135	4	Norway	-
	Bergen	Uncatalogued	6	Hardanger Fjord Undersokelsene, Norway	29 – 42 m
	Bergen	5497	1	Nordsjoen, Norway.	-

`	Bergen	5846	1	Bergen, Norway	-
	YPM	7147	8	Off Port Vandres, France	60 – 100 m
	Bergen	36845	?	Norway	-
	Bergen	19073	1	Mostrafjord, Ryfyke, Norway;	100 m
	Bergen	17452	2	Gordusuml, Norway	-
	Bergen	65610	3	Bildosundet, Norway	1-15 m
	Bergen	65707	2	Guleskjaer, Norway	13-20 m
	Stockholm	19008	1	Naples, Italy.	-
	Kansas		3	Rathlin Island, Northern Ireland	15 m
	Bergen	65614	1	Ramsholmene, Moster, Norway	11-26m.
	Bergen	65729	1	Samlafj. Norway	7-11m.
	Bergen	5845	1	Raftebik, Jondal, Nordfj. Norway.	-
	MHNH	Uncatalogued	3	Cap Béar (42^{0}28$'$40$''$N \| 03^{0}10$'$50$''$E)	62 m
	YPM	7146	8	; Off Port Vandres, France	60 – 100 m
	YPM	7145	5	Off Port Vandres, France	60 –100 m
	MNHN	Uncatalogued	3	Cap Béar (42^{0}29$'$01$''$N \| 03^{0}22$'$02$''$E);	110 – 112 m
	MNHN	uncatalogued	17	Jean Charcot (48^{0}01$'$N \| 07^{0}03$'$W)	110 – 112m.
	Bergen	10242	6	Jondal, Handanger, Norway	10 – 50 m
	Bergen	10134	5	Miffagosund (?), Norway	20 – 45 m
Paracalliactis obvolva	CAS	052750	1	Gulf of Mexico, 80 miles off New Orleans, Louisiana, USA	685 – 731 m

	USNM	1004627	1 PARATYPE	Gulf of Mexico, Louisiana, USA (27°45'32"N | 91°13'37"W)	457 – 472 m
	Texas A&M	uncatologued	3	Gulf of Mexico (19°37.5'N | 92°39.2'W);	511 m
	KUNHM	01595	1 HOLOTYPE	N. Gulf of Mexico (27° 35'8" N | 93° 01' W)	538 m
	USNM	1004629	1	Gulf of Mexico, Louisiana, USA (27°45'32"N | 91°13'37"W	457 – 472 m
	YPM	28174	1 PARATYPE	NW Gulf of Mexico	-
	KUNHM	01591	1 PARATYPE	NW Gulf of Mexico	-
	USNM	1004628	1	Gulf of Mexico, Louisiana, USA (27°45'32"N | 91°13'37"W);	457 – 472 m.
	USNM	1004630	1 PARATYPE	Gulf of Mexico, USA ,Louisiana (27°33'47"N | 93°01'00"W);	538 m
	SBMNH	347282	1 VOUCHER	NW Gulf of Mexico, Veracruz, Mexico (29°21'30"N | 88°28'48"W);	379 – 430 m
	SBMNH	347281	1 VOUCHER	NW Gulf of Mexico, Veracruz, Mexico (27°35'48"N | 93°01'00"W)	538 m
Paracalliactis niwa	NIWA	43817	4	-43.92; -176.67 D0885	59m
	NIWA	43816	4	-43.67; -176.33 Station D0880	78m
Paracalliactis rosea	NIWA	34558	3	-38.58; 167.15 Station TAN0707/56	974 m
	NIWA	43804	30	-38.62; 165.60 Station U0226	2417 – 2421 m
	NIWA	34485	1	-42.91°, 185.53°	1190 – 1210

					m
	NIWA	34540	1	-42.54^0, 175.14^0	1812 – 1813 m
	NIWA	43812	2	-38.62; 165.60 Station U0226	2417-2421 m
Paracalliactis consors	USNM	22502	1 SYNTYPE	Martha's Vineyard, Massachusetts, USA (39^{0}58'N, 70.38'W)	313 m
	USNM	22363	2 SYNTYPE	Massachusetts, USA (39^{0}53"00'N, 69^{0}50"30'W)	483 m
	USNM	22440	1	Massachusetts, USA (39^{0}57"06'N, 69^{0}16"00'W)	838 m
	USNM	22445	3 SYNTYPES	Massachusetts, USA (40^{0}04"00'N, 68^{0}49"00'W)	
Paracalliactis valdiviae	SMNH	5687	2	Somalia, Indian Ocean	838 m
	MNB	7198	2	Somalia, Indian Ocean	625 m
	MNB	7199	1	Somalia, Indian Ocean	836 m
	MNB	7178	2	Somalia, Indian Ocean	758 m
Paracalliactis involvens	USNM	50281	2	Juan Fernandez Island, Chile, South Pacific Ocean	2515 m
	USNM	50282	3	North Atlantic Ocean (47.10N, 36.08W)	4206 m
	USNM	50283	1	Virginia, USA (37^{0}56"20'N, 07^{0}57"30'W)	3506 m
	USNM	50284	4	Cocos Island, Costa Rica (05^{0}43"00'N, 85^{0}50"00'W)	1789 m
	USNM	50285	7	Galapagos Island, Ecuador (00^{0}24"00'S, 89^{0}06"00'W)	1485 m
	USNM	50286	2	Texas, Gulf of Mexico (27.15N, 96.0W)	430 m
	USNM	50287	9	Louisiana, USA (29^{0}03"15'N, 88^{0}16"00'W)	593 m

	USNM	50782	3	Mississipi, Gulf of Mexico (29.10N, 88.08W)	439 m
	USNM	87465	1	New Jersey, USA ($38^0$44”25’N, $72^0$41”17’W)	2100 m
Paracalliactis michaelsarsi	NOCS	Discovery Collections, uncatalogued	7	North Atlantic Ocean; St. 9640#1	3749 – 3757 m
	NOCS	Discovery Collections, uncatalogued	5	North Atlantic Ocean; St. 50811	4350 – 4400 m
	NOCS	Discovery Collections, uncatalogued	3	North Atlantic Ocean; St. 15062#1	3072 – 3184 m
	NOCS	Discovery Collections, uncatalogued	2	North Atlantic Ocean; St. 9132#5	3089 – 3109 m
	NOCS	Discovery Collections, uncatalogued	5	North Atlantic Ocean; St. 13941	3975 m
	MNHN	Uncatalogued	1	North Atlantic Ocean, Biacores St.149 ($45^0$50’N, $17^0$32’W)	4620 – 4690 m
	MNHN	Uncatalogued	1	North Atlantic Ocean, Biacores St.ChG251 ($47^0$38’N, $08^0$56’W)	
Paracalliactis stephensoni	MNHN	Uncatalogued	2	North Atlantic Ocean, Bay of Biscay. BIOGAS IX, St. 9 ($47^0$32’N, $08^0$25’W)	1970 m
	MNHN	Uncatalogued	2	North Atlantic Ocean, Bay of Biscay. BIOGAS IX, St. 9 ($47^0$32’N, $08^0$25’W)	1331 m
	MNHN	uncatalogued	4	North Atlantic Ocean, Bay of Biscay. Polygas St.CVII ($47^0$29.1’N, $08^0$16.1’W)	2141 m
	MNHN	Uncatalogued	5	North Atlantic Ocean, Bay of Biscay. Polygas, St. 124 ($47^0$28’N, $08^0$25’W)	2149 m

	MNHN	Uncatalogued	4	North Atlantic Ocean, Bay of Biscay. Polygas, CV09 St. 1 ($47^0$31'N, $08^0$44'W)	2119 m
	MNHN	Uncatalogued	3	North Atlantic Ocean, Bay of Biscay. Polygas, St. CV24 ($47^0$34'N, $08^0$34'W)	2025 m
	MNHN	Uncatalogued	11	North Atlantic Ocean, BIOGASIII, St. CB22#2 ($47^0$41.8'N, $08^0$18.7'W)	1896 m
	MNHN	Uncatalogued	3	North Atlantic Ocean, Bay of Biscay. BIOGASII, St. CV20 ($47^0$37'N, $08^0$34'W)	2282 m
	MNHN	Uncatalogued	14	North Atlantic Ocean, Azores. BIACORES Sta. ChG+F252 ($47^0$35'N, $08^0$47'W)	2120 m
	MNHN	Uncatalogued	5	North Atlantic Ocean. BIACORES Sta. ChG196 ($37^0$50'N, $24^0$55'W)	1146 – 1191 m
	MNHN	Uncatalogued	10	North Atlantic Ocean. BIOGAS Sta. CP24 ($44^0$08.1'N, $04^0$15.2'W)	1995 m
	MNHN	Uncatalogued	12	North Atlantic Ocean. Cryos Sta. CP90 ($34^0$21'N, $07^0$24)	890 m
Calliactis japonica	UUZM	688a	1 SYNTYPE	Japan, Honshu, Sagami Bay, Kanagawa-ken, Misaki, Gote [Goto] Island ; 34.00, 132.60.	165 m
	UUZM	688b	1 SYNTYPE	Japan, Honshu, Sagami Bay, Kanagawa-ken, Misaki, Gote [Goto] Island 34.00, 132.60.	110 m
	UUZM	116c	2 SYNTYPES	Japan, Honshu, Sagami Bay, Kanagawa-ken, Misaki, Gote	210 m

				[Goto] Island ; 34.00, 132.60.	
	OMNH	5267	1	Off Matsuzaki, Suruga Bay, Japan	-
	OMNH	5257	1	Off Matsuzaki, Suruga Bay, Japan	-
	OMNH	5258	1	Off Matsuzaki, Suruga Bay, Japan	-
	OMNH	7423	1	Off Toda, Suruga Bay, Japan	-
	OMNH	Uncatalogued	2	Off Wase, Mieprefecture, Japan	170 – 340 m
	USNM	20290	4	Suruga Bay, Honshu island, Shizuoka, Japan; 35.033, 138.767.	110 – 128 m
	USNM	50289	1	Japan; 36.000, 138.000	-
	USNM	50291	2	Hong Kong, China; 21.500, 116.533	256 m
Calliactis conchicola	NIWA	43808	3	-49.50^0, 166.22^0	616 m
	NIWA	43805	10	-47.76^0, -179.28^0	648 m
	NIWA	43807	3	-43.06^0, 173.20^0	42 – 44 m
	NIWA	1112372	4	South Pacific Ocean, New Zealand, Chatham Islands	302 m
	NIWA	43814	2	-53.23^0, 171.80^0	435 m
	NIWA	43813	1	-42.58^0, 173.55^0	165 m
	NIWA	43819	3	-36.96^0, 176.32-36.96^0	535 m
	NIWA	43815	2	-49.46^0, 174.00^0	501 m
	NIWA	43810	2	-41.54^0, 174.43^0	91 m
Calliactis brevicornis	MNB	1844	1 SYNTYPE	West Africa, Bijoga Island; 10.12^0, -17.28^0	150 m
	ZMH	7164	4 SYNTYPES		-
	ZMH	C11608	6		-

	ZMH	C11609	1		-
	ZMH	C11630	1		-
Calliactis androgyna	ZMH	C7629	1 HOLOTYPE	-31.10^0, -50.60^0	40 m
Calliactis annulata	GOT	927	1 HOLOTYPE	Pacific Ocean, Juan Fernandez Islands, Masatierra; -33.63^0, -78.88^0	50 m
Calliactis algoaensis	SMNH	1175	3 SYNTYPES	South Africa, Eastern Cape, East London; -32.96^0, 27.93^0	-
	SMNH	1176	2	South Africa, Eastern Cape Province, Algoa Bay; -33.80^0, 25.70^0	91 m
Calliactis marmorata	MNB	1668	3	Australia, Western Australia, Mermaid Strait; -20.53^0, 116.74^0	-
Calliactis armillatas	BPBM	D114	9 SYNTYPES	USA, Hawaiian Islands, Laysan Island – Tanger; 25.77^0, -171.73^0	0 m
	BPBM	D295	1 SYNTYPE	USA, Hawaiian Islands, Laysan Island – Tanger; 25.77^0, -171.73^0	0 m
Calliactis parasitica	USNM	19331	2	Mediterranean Sea, Italy	0 m
	USNM	50474	4	Mediterranean Sea, Algeria	10 m
Calliactis polypus	CAS	071082	3	Philippines, Little Santa Cruz Island	1 – 3 m
	NIWA	43806	7	-23.65^0, -178.92^0	5m
	MNHN	Uncatalogued	5	Polinaise Francaise, Archipel des Australes; $23^0$22.8'S, $150^0$43.3' W	90 – 95 m
	UF	4983	8	Hawaii; 23.81^0, -166.38^0	72 – 79 m
	BMNH	1985.2.13.2-6	6	Indian Ocean, Maldives, Addu Atoll, Gan Island; -	-

				0.69^0, 73.15^0	
	OMNH	4432	3	Sabiura, Kushimoto-town, wakayama Prefecture, Japan	-
	AM	G.17480	4	Australia, New South Wales, south of Smoky Cape, (30°56'27"S, 153°05'58"E)	20 m
	AM	G.17481	5	Tasman Sea, Middleton Reef, reef near Fuku Maru wreck, (29°28'48"S, 159°07'30"E)	0 m
	AM	G.17487	4	French Polynesia, Austral Isles, Rurutu Island, Avera Bay, (29°22'S, 151°21'03"E)	6 m
Calliactis reticulata	BMNH	1918.5.12.23-24	7	(-22.93^0, -41.57^0)	73 m
Calliactis valdiviae	MNB	7229	2 SYNTYPES	Somalia; -0.46^0, 42.79^0	638 m
Calliactis variegata	LO	432	1 SYNTYPE	Panama Gulf, Panama; (8.90^0, -79.30^0)	3.5 – 11 m
	USNM	50301	6	San Francisco, California, USA; (37.46^0, -123.00^0)	505 m
	USNM	49404	2	Concepcion Bay, Gulf of California, Baja California, Mexico	-
Calliactis tricolor	USNM	50006	2	Texas, Gulf of Mexico, USA; (24.83^0, 97.05^0)	-
	USNM	54164	16	Florida, Gulf of Mexico, USA	-
	USNM	51330	3	Suriname, North Atlantic Ocean; (6.50^0, -55.86^0)	31 m
	USNM	54121	2	E. of Beaufort Bay, North Carolina, USA	12 m
	USNM	50479	3	Florida, Gulf of Mexico, USA	-

	USNM	51056	3	Grand Island, Louisiana, USA	9 m
	USNM	17143	77	Fort Macon, North Carolina, USA	-
	USNM	22130	7	Mayaguez, Puerto Rico, Caribbean Ocean, North Atlantic Ocean	-
	USNM	53274	1	Turrumote Island, Puerto Rico.	20 m
	USNM	44042	3	Florida, USA; 28.13^0, -79.90^0	183 m
	USNM	59298	3	Off North Carolina, USA; 33.33^0, -77.76^0	25 m
	USNM	50979	10	Sapelo Island, Georgia, USA	-
	USNM	51667	3	St. John, Virgin Island	15 m
	USNM	1106024	2	Cape Orange, Amapa, Brazil; 4.70^0, -51.46^0	37 m
Calliactis polypus	USNM	50460	20	Oahu Island, Waikiki, Hawaii	9 – 37 m
	USNM	50453	2	Gilbert Islands, Onotoa Atoll, Kiribati, S. Pacific Ocean	-
	USNM	50462	4	Comiguin Island, Mindanao Sea, Philippines; 9.11^0, 124.78^0	-
	USNM	1121785	6	Elat, Israel, gulf of Aqaba, Red Sea	2 m
	USNM	50481	3	Guam Agat Bay, North Pacific Ocean	10 m
	USNM	50276131	3	Society Islandsm Tahiti, French Polynesia, South Pacific Ocean	5 m
	USNM	50279	2	Kagoshima, Japan, North Pacific Ocean	2 m

	USNM	50475	3	Carolina Islands, Micronesia, North Pacific Ocean	-
	USNM	50459	6	Ratak Chain, Kamajuro Atoll, Marshall Islands	1 m
	USNM	50280	2	Ras Tannurah, Tarut Bay, Saudi Arabia, Indian Ocean	12 m
	USNM	50279	5	Jurayd Island, Saudi Arabia, Arabian Sea, Persian Gulf, Indian Ocean	16 m
	USNM	52386	13	Palau, North Pacific Ocean	5 m
	USNM	1112501	2	Addu Atoll, Gan Island, Maldives, Indian Ocean	3 m
	USNM	1110239	15	Aldabra Atoll, Seychelles, Indian Ocean	10 m
	USNM	1110375	4	Ha'apai Group, Tonga, South Pacific Ocean	2 m
	USNM	50277	6	Ras Tannurah, Saudi Arabia, Persian Gulf, Indian Ocean	1 m
	USNM	50461	3	Mindanao Island, Butuan Bay, Philippines, North Pacific Ocean	3 m
	USNM	50483	3	Oahu Island, Honolulu, Hawaii, North Pacific Ocean; 21.30^0, -157.85^0	10 m
	USNM	525410	3	Palau, Urukthapel Island; (7.26^0, 134.45^0)	1 m
	AM	G.17480	6	Between Sydney & Port Stephens, New South Wales Australia, (33°50'S, 151°13'E)	10 m
	AM	G.16899	3	Minnie Waters south of Clarence Riber Mouth, New	5 m

				South Wales (19 47'S, 153 18E)	
	AM	G.15831	3	Northeast of Long Reef, New South Wales, Australia (33°42'S, 151°54'E)	0 m
	AM	G.16894	4	Northeast of Long Reef, New South Wales, Australia (33°42'S, 151°54'E)	0 m
	AM	G.15827	3	Palau, North Pacific Ocean	12 m
	AM	G. 17481	3	Off Moreton Bay, Queensland, Australia (QLD) 27 27'22"S, 153 39'E)	77 m
	AM	G.16895	4	Pago Pago, Samoa (14 16'S, 170 43'W)	10 m
	AM	G. 16894	3	Sandon Beach, Queensland	0 m
	AM	G.16900	2	Between Sydney & Port Stephens, New South Wales Australia, (33°50'S, 151°13'E)	5 m
Calliactis tigre	UF	5018	2	French Frigate Shoald, Hawaiian Islands (23.772^0, 166.3915^0)	279 – 373 m
	UF	4964	1	French Frigate Shoald, Hawaiian Islands (23.62^0, 166.06^0)	255 – 287 m
	AM	G.17483	2	East of Mooloolaba, Queensland, Australia (26°52'44"S, 153°35'20"E)	60 m
	AM	G.17484	2	East of Mooloolaba, Queensland, Australia, (26°52'44"S, 153°35'20"E),	160 m
	AM	G.17485	3	East of Mooloolaba,	160 m

				Queensland, Australia, (26°52'44"S, 153°35'20"E)	
	AM	G.17486	5	Northeast of Long Reef, New South Wales, Australia (33°42'S, 151°54'E)	466 m
	AM	G.17482	2	East of Bulli, New South Wales, Australia (34°15'S, 151°25'E)	275 m
	AM	G.17489	4	Between Sydney & Port Stephens, New South Wales Australia, (33°50'S, 151°13'E)	365 m
	AM	G.15586	2	East of Mooloolaba, Queensland, Australia (26°52'44"S, 153°35'20"E)	350 m
	AM	G.16897	2	East of Mooloolaba, Queensland, Australia, (26°52'44"S, 153°35'20"E)	300 m
	AM	G.16907	1	Northeast of Long Reef, New South Wales, Australia (33°42'S, 151°54'E)	450 m
	AM	G.17491	2	East of Mooloolaba, Queensland, Australia, (26°52'44"S, 153°35'20"E),	312 m

Appendix B: Character description and comments

1. <u>Morphology of the pedal disc</u>: (0) bi-lobed; (1) irregular; (2) circular/ovoid

The body of an anemone is a hollow cylinder closed in by one disc at the upper end (oral disc) and the pedal disc at the lower end. The pedal disc may have different morphologies, the most common of which is a thin plate of tissue by means of which the animal adheres to the substrate. The pedal disc of anemones that live in symbiosis with hermit crabs can be circular or ovoid, with the diameter of the pedal disc equal or slightly wider than the diameter of the column (Figure A1) or may be modified to completely enwrap the shell to which is attached, forming two lobes that united when they come in contact (Figure A2). In some cases, the pedal disc is irregularly shaped, which means it is always wider than the diameter of the column, but never forms two lobes (Figure A3).

2. <u>Type of secretion produced by the pedal disc</u>: (0) none; (1) cuticle; (2) carcinoecium

The pedal disc of most species of sea anemones do not secrete any special material, however, some species of sea anemones symbiotic with hermit crabs may form a living "cloak" around the shell inhabited by the crab, reinforcing the shell and expanding the living space of the crab. Some species secrete a chitinous material under the living cloak, known as a carcinoecium (Figure A4), which allows the hermit crab to avoid changing

shells as it grows. In most cases, the shell is completely dissolved in species where the carcinoecium is present. A carcinoecium is especially beneficial at great depths where the calcium carbonate of shells dissolve quickly and larger shells are scarce. Other species crab-symbiotic anemones secrete a cuticular material (=cuticle, Figure A5) that covers the shell in which it's attached but does not enlarge the living space of the crab. In this case, the shell is always present.

3. <u>Column divided into scapus and scapulus/capitulum</u>: (0) absent; (1) present

The cylindrical body wall of an anemone is known as the column and in its simplest state it forms a smooth cylindrical wall identical in structure from the pedal disc to the oral disc (Stephenson, 1928). In some species, the column is externally divisible into regions: scapus, scapulus and capitulum (Figure A6). The scapus is the longest and most conspicuous zone of the column that is often provided with column specializations, such as tubercles or a cuticle. The capitulum is located at the distal most part of the column, in the region below the tentacles, and is differentiated from the scapus by the lack of ectodermal specializations that may be present in the scapus. In addition, the scapulus is thin-walled and translucent in live animals. The scapulus lies proximal to the capitulum and like the capitulum, is without periderm and ectodermal specializations, but has slightly thicker mesoglea and ectoderm (Stephenson, 1935). The distinction between scapulus and capitulum is not made by all authors (e.g., Carlgren, 1921, 1950; Stephenson, 1928) and will not be made here.

4. Morphology of the body: (0) annular; (1) cylindrical; (2) flat

Sea anemones symbiotic with hermit crabs may possess an annular body shape that covers the abdomen of the hermit crab completely (Figure A7) or may be erect and cylindrical, with the diameter of the pedal disc smaller than the column height (Figure A8). In other species, the pedal disc diameter is much wider than the column height, giving the anemone a flat shape (Figure A9).

5. Cinlides in the column: (0) absent; (1) present

Cinclides (Figure A10) are small perforations of the column wall found in some species of sea anemones that function as safety-valves that allow fine jets of water to escape from the gastrovascular cavity during rapid contraction, supplementing the mouth as a means of exit and helping to prevent rupture of the body wall. In addition, when acontia are present, they are emitted through these cinclides.

6. Cuticle: (0) absent; (1) present

In some species, a deciduous cuticle is present in the scapus (not scapulus/capitulum). The cuticle is held on the scapus by the action of the numerous longitudinal and horizontal ridges that give the scapus a wrinked texture.

7. Tubercles: (0) absent; (1) present

Tubercles are specializations of the column that are not adherent and possibly ornamental. They consist of thickenings of the mesoglea, solid, rounded or conical

covered by ectoderm and cuticle (Figure A11). They occur on the scapus of species with though body wall and thick mesoglea (Stephenson, 1928).

8. Position of the oral disc: (0) downwards close to the aperture of the shell; (1) lateral to the aperture of the shell; (2) erect and directed away from the aperture of the shell.

The position of the anemone on the shell inhabited by the hermit crab is variable. In some cases, the oral disc of the anemone is always located close to the aperture of the shell, either laterally (Figure A12) or downwards, immediately behind the legs of the hermit (Figure A13). In other cases, the oral disc of the anemone is directed away from the aperture of the shell (Figure A14)

9. Number of cycles of tentacles: (0) four cycles; (1) five cycles; (2) six cycles; (3) seven cycles.

The outer part of the oral disc gives rise to the tentacles that are disposed in alternating circles that are placed progressively further away from the mouth located on its center. Each circle of tentacles is known as a cycle. The number of cycles present varies from one species to another.

10. Same number of tentacles as mesenteries proximally: (0) absent; (1) present

11. Number of siphonoglyphs: (0) none; (1) one; (2) two.

Siphonoglyphs are anatomically differentiated grooves that run down the actinopharynx from the mouth to its inner end (Figure x). Siphonoglyphs have modified walls with an ectoderm that differs in structure from the rest of the actinopharynx. The number of siphonoglyphs present in each species is often constant, but may vary due to asexual reproduction.

12. <u>Marginal sphincter:</u> (0) endodermal; (1) mesogleal

Below the margin, the circular muscle sheet is concentrated into a special circular band called the marginal sphincter (Stephenson, 1935). The primary function of this muscle is to draw the margin inwards over the tentacles during retraction. When the sphincter lies entirely outside of the mesoglea of the column and their processes project into the endoderm, they are called endodermal sphincters. If the sphincter is embedded in the mesoglea, it's called mesogleal sphincter.

13 <u>Number of mesenteries</u>: (0) 48 pairs; (1) 82 pairs; (2) 96 pairs

A mesentery is a membranous lamella consisting of a middle layer of mesogloea, covered by a sheet of endoderm on either face that divides the gastrovascular cavity (Stephenson, 1928). Mesenteries are arranged in pairs, each consisting of two mesenteries adjacent to one another. The mesenteries are attached to the underside of the oral disc at their top edge, the column wall at their outer edge, and the pedal disc at their bottom edge. The inner edge of the mesenteries attach to the actinopharynx in the upper column, and is free in the lower column.

14. Number of cycles of mesenteries: (0) four cycles; (1) five cycles; (2) six cycles

Pairs of mesenteries are arranged in cycles that are distinguished by the size and development of the mesenteries. In most species of sea anemones, the arrangement of mesenteries is hexamerous, but irregularities may be present, especially when asexual reproduction or regeneration processes exist.

15. Distal vs. proximal number of mesenteries: (0) same; (1) fewer; (2) more

In some species, an extra cycle of mesenteries may be present proximally (close to the pedal disc) or distally (close to the oral disc). In other species, the number of mesenteries is constant throughout the column of the individual.

16. First cycle of mesenteries perfect and sterile: (0) absent; (1) present.

Mesenteries that reach the actinopharynx in the upper region of the column are called perfect mesenteries, while those that are narrower so that the inner edge of the mesentery does not reach the actinopharynx are called imperfect mesenteries (Figure x). The number of perfect and imperfect mesenteries may vary among species. Gonads develop parallel to the edge of the mesenteries as a thickened band that involves the endoderm and the mesoglea of the mesentery. These bands are constructed in a way that the gonad appear as series of independent packets of cells, when in fact, they are one continuous band.

17. Second cycle of mesenteries perfect and sterile: (0) absent; (1) present

18. <u>Retractor muscles of the mesenteries:</u> (0) weak; (1) strong

One side of each mesentery is occupied y longitudinal muscles called retractors. When these muscles can are concentrated, they form a strong retractor. A rectrator that is not strongly concentrated is called diffuse. The main function of the retractor muscle is to draw the oral disc and the upper part of the body wall downwards and inwards.

19. <u>Parietalbasilar muscle</u>: (0) weak; (1) strong

The parietobasilar muscle forms a triangular area in the angle between the base and the column wall stretching across from one to another (Stephenson, 1928). The fibers run diagonally across this angle, and are often supported by well developed processes. Sometimes, the edge of the muscle that is not attached to the base or the column forms a distinct free edge that is visible as a ridge or flap on the surface of the mesentery. The parietobasilar muscle may be well developed in which case is called strong (Figure x), or may be underdeveloped and weak.

The nematocysts present in different tissues of a species are considered diagnostic at various levels. The presence or absence of a nematocyst type is of particular importance in ordinal, familia and generic designations (Carlgren, 1949; Cutresss, 1955; Fautin, 1988). The presence of certain types of nematocysts in different tissue has been used to differentiate different genera of sea anemones symbiotic with hermit crabs (e.g., Daly et. al. 2004). The distribution, size and shape of nematocysts can be important at less lower taxonomic levels (Carlgren, 1943; Williams, 1981). In the present study nematocyst size will not be used as a character for the phylogenetic analysis given the problematic nature

of using this kind of data. Characters related to the cnidae of the examined species are listed below.

20. Microbasic *b*-mastigophores in the tentacles: (0) absent; (1) present

21. Microbasic *p*-mastigophores in the column: (0) absent; (1) present

22. Basitrichs only in the pharynx: (0) absent; (1) present

23. Two sizes of basitrichs in the acontia: (0) absent; (1) present