MORPHOLOGICAL AND MOLECULAR EVOLUTION OF SEA ANEMONES AS REVEALED BY AN EMERGING MODEL ORGANISM: *AIPTASIA* (CNIDARIA: ACTINIARIA: AIPTASIIDAE)

A Dissertation
submitted to the Faculty of
The Richard Gilder Graduate School
at the
American Museum of Natural History
in partial fulfillment of the requirements for the
degree of
Doctor of Philosophy

By

Alejandro Grajales, M.Sc.

Richard Gilder Graduate School
at the
American Museum of Natural History
New York, NY
January 31, 2014

MORPHOLOGICAL AND MOLECULAR EVOLUTION OF SEA ANEMONES AS REVEALED BY AN EMERGING MODEL ORGANISM: *AIPTASIA* (CNIDARIA:ACTINIARIA:AIPTASIIDAE)

Alejandro Grajales M.Sc.

Chair: Estefanía Rodriguez, Ph.D.

ABSTRACT

Sea anemones (Cnidaria: Actiniaria) of the family Aiptasiidae Carlgren, 1924 are conspicuous members of shallow-water environments and several species within the family are widely used as model systems for studies of cnidarian-dinoflagellate symbiosis and coral bleaching. Although previously published phylogenetic studies of sea anemones recovered the family Aiptasiidae as monophyletic, they only included partial sampling of its diversity. This study explores the diversity within this group of organisms in an integrative way, from the family to population levels. In this study, I explored the morphological and molecular diversity of the group using newly collected material covering the distribution of most of the described genera and species. The family Aiptasiidae was found to be a monophyletic lineage. Similarly, most of the genera within the family represent monophyletic lineages, with the exception of the genus *Aiptasia*, now divided into *Aiptasia* and *Exaiptasia*. *Bellactis* Dube, 1983 and *Ragactis* Andres, 1883 are now genera included within Aiptasiidae, in agreement with previous morphological studies and the morphological homogeneity observed here by the members of this family. In addition, I discovered new diagnostic morphological characters supporting the relationships among the major clades within the family. The molecular phylogenetic results provided evidence to diagnose two species within *Exaiptasia: E. pallida*—a single widespread species—and a new cryptic species, *E. brasilensis* sp. nov.—restricted geographically to the southwestern Caribbean Sea and the southwestern Atlantic Ocean. Finally, although the algal diversity within the group showed no evidence of coevolution at the family level, an interesting pattern of adaptation and cladogenesis of the endosymbiotic algae (*Symbiodinium* spp.) was found within host species.

ACKNOWLEDGEMENTS

I am very grateful to colleagues who assisted with fieldwork, particularly to C. Arvanitidis (Hellenic Centre for Marine Research, MRC, Greece), A. Baird (James Cook University, Townsville, Australia), J. Campbell (Smithsonian Marine Station at Fort Pierce, USA) R. Collins (Smithsonian Tropical Research Institute, STRI, Panama), V. Cumbo (James Cook University, Townsville, Australia), J. Frommlet (Centro de Estudios do Ambiente e do Mar, CESAM, Portugal), R. González (Universidad Nacional Autónoma de Mexico, UNAM) L. Gusmão (Universidade de São Paulo, Brazil), K. Hiscock (Marine Biological Association Plymouth, U.K.), P.J. López González (Universidad de Sevilla, Spain), P. Manent (Instituto Canario de Ciencias Marinas, Spain), O. Nir (Haifa University, Israel), J. Reimer (Ryukyus University, Okinawa, Japan) M. Takabayashi (U. Hawaii, Hilo) and P. Wirtz. Comments from M. Brugler (AMNH) M. Daly (Ohio State University) and D. Thornhill (Defenders of Wildlife, Washington) improved this manuscript. An RGGS Graduate Fellowship and student research funds and the Lerner-Gray Fund for Marine Research (AMNH) provided support for this work, as did a Doctoral Dissertation Improvement Grant (NSF DEB 1110754) to AG and ER, and the Bermuda Institute of Ocean Sciences (BIOS, Bermuda).

TABLE OF CONTENTS

FIGURES AND TABLES

Figures

Tables

Appendices

CHAPTER I

BACKGROUND

Sea anemones (Cnidaria: Actiniaria) are ubiquitous components of marine environments. These organisms are adapted to a wider range of environmental conditions compared to other members of the subclass Hexacorallia (e.g. hard corals), with representatives in shallow and abyssal waters, tropical, temperate and polar waters, or chemosynthetic environments. Their ecological success has been attributed to their morphological diversity, the plasticity of their reproductive strategies as well as the associations they form with other organisms. Although these adaptations have been recognized for a long time, efforts to examine these traits within an evolutionary context for sea anemones are very recent. A broad phylogenetic framework is now available for the order Actiniaria (Daly et al. 2008) but it only accounts for one of four traditionally recognized suborders (Fautin 2010). Although recently some phylogenetic work also has been done at the family level (Gusmão and Daly 2010; Rodríguez et al. 2008; Rodríguez and Daly 2010), those workers also emphasize the necessity of increasing efforts in taxonomy and systematics within families and genera. As a consequence, the potential for the formulation of hypotheses concerning the evolution of morphological characters and reproductive strategies is still limited. One of the main difficulties working with sea anemones is associated with the challenges of obtaining material. Due to their long evolutionary history, monophyletic groups within anemones often have extensive and discontinuous distributions, and specimen collection usually requires extensive fieldwork, combined with revision of material scattered around the world. Although these characteristics also make them an interesting group from a biogeographic point of view, comprehensive studies of this topic are scarce for sea anemones (Riemann-Zürneck, 1986; Rodríguez et al. 2007). Because the diversity of the group remains poorly understood, this type of research is often limited to a summary of occurrence records from taxonomic descriptions (e.g. Fautin 1999, 2005). Another important limiting factor for the research at lower taxonomic levels is the uncertainty about species boundaries. As a consequence of this, population-level studies are often limited to very reduced geographical scales (e.g. Edmands and Potts, 1997 Douek et al. 2002).

Species within the genus *Aiptasia* present a suitable opportunity to address hypothesis within an evolutionary framework, both at the population and above-species levels. *Aiptasia* species are conspicuous components of shallow water environments worldwide. A rapid growth rate, allowed by a symbiotic relationship with a dinoflagellate (*Symbiodinium* spp.) combined with asexual reproduction by pedal laceration (Clayton et al. 1985; Lin et al. 1992) make them excellent competitors that usually overgrow other organisms. These features, that are undesirable in some contexts (they are considered aquarium pests), make them excellent model organisms. Research on *Aiptasia* spp. (a group usually making reference to specimens within the distribution of *A.pallida*, *A.californica* or *A. pulchella*) over more than 30 years has significantly improved our understanding of dinoflagellate-cnidarian symbiosis (e.g. Rodriguez-Lanetty et al. 2006; Sunagawa et al. 2008, 2009). They have also been selected as models to understand the processes responsible for coral bleaching (e.g., Sawyer and Muscatine, 2001). As opposed to corals, which usually die, these anemones are able to survive long time without their symbionts and can be subsequently re-infected (Weis et al. 2008). Despite its importance and accessibility, to date there have been no comprehensive analyses of the systematics of the group. The latest compilation accounted 16 species records (Fautin, 2006). This scenario, however, is far from being settled. Species within *Aiptasia* show very disparate distributions (See figure 1) and most of them have descriptions that are incomplete by modern standards. Additionally recent phylogenetic studies of sea anemones showed that the genus is paraphyletic (Daly et al. 2008; Gusmão and Daly 2010; Rodríguez and Daly, 2010) although only two species of the genus were included in those analyses. Without an adequate phylogenetic framework in hand, hypotheses concerning the diversification and evolution of this group cannot be formulated. Population-level studies have also being hindered because of the lack of clearly defined taxonomic units within *Aiptasia*.

Given the lack of a phylogenetic hypothesis, controversy arises about whether this “model organism” is actually composed of one or a few cosmopolitan species, or by a more diverse group of cryptic or insufficiently described ones. To test both hypotheses, this thesis project studied this group of sea anemones from the population to the family level, integrating a comprehensive taxonomic revision, molecular phylogenetics analysis as well as a study of the endosymbiotic algal diversity across the family. These three elements comprise the three following chapters of this dissertation.

References

Amaral F. D., Hudson M. M., da Silveira F. L., Migotto Á. E., Pinto S. M., and Longo L., 2002. Cnidarians of Saint peter and St. Paul Archipelago, northeastern Brazil. Proc. 9th Intl. Coral Reef Symp.**1**: 567-571

Andres. A. 1881 Prodromus neapolitanae actiniarum faunae addito generalis actiniarum bibliographiae catalogo Mitt. Zool. Stn. Neapel **2**: 305-371

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. USA **98**:4563–4568.

Carlgren, O.1943**.** East-Asiatic Corallimorpharia and Actiniaria. K. Svenska Vetenskapsakad. Handl. **20**:1-43

Carlgren, O. 1949. A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria.K. Svenska Vetenskaps-Akad. Handl. **1**:1–121.

Carlgren O. and Hedgpeth J. W. 1952. Actiniaria, Zoantharia and Ceriantharia from Shallow Water in the northwestern Gulf of Mexico. Publ. Inst. Mar. Sci. (Univ. Texas) **2**:143-172

Chen C, Soong K, and Chaloum C.A. 2008. The Smallest Oocytes among Broadcast-Spawning Actiniarians and a Unique Lunar Reproductive Cycle in a Unisexual Population of the Sea Anemone, Aiptasia pulchella (Anthozoa: Actiniaria). Zool Stud. **47**: 37-45

Clayton PD, JD Collins.1992. Reproduction and feeding ethology of a tropical, intertidal sand-swelling anemone (Actinoporus elongatus, Carlgren 1900). Hydrobiol. **237**: 31-38.

Crawford N.G. 2009. Smogd: Software for the measurement of genetic diversity. Mol. Ecol. Res. **10**: 556–557.

Daly, M., Brugler, M., Cartwright, P., Collins, A.G., Dawson, M.N., France S.C., Fautin, D.G., McFadden, C.S., Opresko, D.M., Rodriguez, E., Romano, S.L., and J.L. Stake. 2007. The phylum Cnidaria: A review of phylogenetic patterns and diversity three hundred years after Linnaeus. Zootaxa **1668**: 127-186.

Daly, M., A. Chaudhuri, L. Gusmão, and E. Rodriguez. 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). Mol. Phyl. Evol. **48**:292–301.

Darling J.A., Reitzel A.M., Finnerty J.R. 2004. Regional population structure of a widely introduced estuarine invertebrate: Nematostella vectensis Stephenson in New England. Mol. Ecol:**13** 2969–2981

Delle-Chiaje, S. 1822. Memorie sulla storia e notomia degli animali senza vertebre del regno di Napoli. Napoli

Douek, J., Barki, Y., Gateño, D. & Rinkevich, B. 2002 Possible cryptic speciationwithin the sea anemone Actinia equina complex detected by AFLP markers. Zool. J. Linn. Soc.**136**: 315–320

Duchassaing de Fombressin P. and Michelotti G.1866. Supplément au mémoire sur les Coralliaires des Antilles. Memorie Reale Accademia delle Scienze di Torino. **8**:97-

206.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nuc. Ac. Res. 32: 1792–1797.

Edmands, S. 1995 Mating systems in the sea anemone genus Epiactis. Mar Biol 123:723-733.

Edmands S, Potts D.C.1997. Population genetic structure in brooding sea anemones (Epiactis spp.) with contrasting reproductive modes. Marine Biol. 127: 485-98.

Excoffier L., Laval G., Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics dataanalysis. Evol. Bioinfs Online. 1: 47–50.

Fautin, D. G., Barber, B. R. 1999. Maractis rimicarivora, a new genus and species of sea anemone (Cnidaria: Anthozoa:Actiniaria: Actinostolidae) from an Atlantic hydrothermal vent. Proceedings of the Biological Society of Washington,112: 624–631.

Fautin, D. G. 2005 Three species of intertidal sea anemones (Anthozoa: Actiniidae) from the tropical Pacific: description of Anthopleura buddemeieri n. sp., with remarks on Anthopleura asiatica and Gyractis sesere. Pacific Science 59, 379–391.

Fautin DG. 2006. Hexacorallians of the World. Available at http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm.

Fautin. D.G., Goodwill, R.H. 2009 Neoaiptasia morbilla new species (Cnidaria: Actiniaria), a sea anemone symbiont of sand-dwelling gastropods on Saipan, Mariana Islands, with comments on some other associations. Micronesica. 41: 101-105.

Fautin D, Dalton P, Incze L.S., Leong J.C., Pautzke C., Rosenbeg A., Sandifer P., Sedberry G., Tunnel Jr. J.W., Abbot A., Brainard R.E., Brodeur M., Eldrege L.G., Feldman M., Meretzohn F., Vroom P.S., Wainstein M., Wolff N. 2010. An overview of marine biodiversity in United States waters. PLoS ONE. 5. e11914 p.

Field. L.R. 1949. Sea anemones and corals of Beafurot, North Carolina. p. 20. Duke University Press: Durham

Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. Cladistics, 24: 5.

Gravenhorst, I. L. C. 1831. Tergestina, oder Beobachtungen und Unteruchungen üver einige bei Triest im Meere lebende Arten der Gattungen Octopus, Doris, Pinna, Ascidia, Serpula, Echinus, Asterias, Ophiura, Holothuria, Actinia, Caryophyllia, Actinotus Wilhelm Gottlieb Korn: Breslau

Gunter, G. Geyer, R.A. 1955 Studies on fouling organisms of the northwest Gulf of Mexico. Publ. Inst. Mar. Sci. (Univ. Texas) 4: 38-67

Gusmão L, Daly M. 2010. Evolution of sea anemones (Cnidaria: Actiniaria:Hormathiidae) symbiotic with hermit crabs. Mol Phyl Evol. 56: 868-877.

Hellberg ME. Footprints on water: the genetic wake of dispersal among reefs. Coral Reefs 2007;26:463-73.

Herrera, A. 1981. Nuevos registros de anémonas (Coelenterata: Actiniaria y Corallimorpharia) para aguas cubanas. Poeyana: Instituto de Zoologia, Academia de Ciencias de Cuba 214: 1-3

Holard, M.H. 1848. Etudes sur l’organisation des Acinies. Imprimerie de Marc Ducloux et Ce. Paris

Huang, X. and Madan, A. 1999 CAP3: A DNA Sequence Assembly Program, Genom Res. 9: 868-877.

Huelsenbeck JP, Ronquist F. 2001 MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.

Hunter T. 1984. The energetics of asexual reproduction: pedal laceration in the symbioticsea anemone Aiptasia pulchella (Carlgren, 1943). J. Exp. Mar. Biol. Ecol. 83: 127-147.

Lin J, C-C Tsai, W-K Lai, C-P Chen. 1992. Pedal laceration in the sea anemone Aiptasia sp. (Anthozoa: Actiniaria). Chin. Biosci. 35: 33-41.

Martins WS, Lucas DCS, Neves KFS, Bertioli D.J. 2009. WebSat - A Web Software for MicroSatellite Marker Development, Bioinformation. 3:282-283

Mueller UG, Wolfenbarger LL.1999 AFLP genotyping and fingerprinting. Trends in Ecology and Evolution, 14, 389–394.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proc. of the Nat.Acad. of Sci, USA, 70: 3321–3323.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583–590.

Nylander J.A.A. 2004. MrModeltest v2. Program distributed by the author. In Evolutionary Biology Center Uppsala University, Sweden:Evolutionary Biology Center.

Nylander J.A.A., Wilgenbusch J.C., Warren D.L., Swofford D.L. 2008. AWTY (Are We

There Yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics **4**: 581-583

Parulekar. A. Neoaiptasia commensali, gen. et. sp. nov.: an actiniarian commensal of hermit crabs. J. Bombay Nat. Hist. Soc. **66**: 57-62

Pax. F. 1912. Les actinies de la cote de Perou. Mission du Service Géographique de l'Armée pour la Mesure d'un Arc de Méridien Equatorial en Amérique du Sud Sous le Contrôle Scientifique de l'Académie des Sciences. Gauthier-Villars, Imprimeur-Libraire: Paris

Peakall R, Smouse P.E., Huff D.R. 1995. Evolutionaryimplications of allozyme and RAPD variation in diploidpopulations of dioecious buffalograss Buchloe dactyloides. Molecular Ecology, **4**: 135–147.

Pritchard J.K., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus genotype data.Genetics, **155**: 945–959.

Rapp. W. 1829 Ueber die Polypen im Allgemeinen und die Actinien Grolsherzogl. Sdch Weimar.

Riemann-Zurneck, K. 1986. On some abyssal sea anemones of the North Atlantic (Actiniaria: Hormathiidae). Mitteilungenaus dem Hamburgischen Zoologischen Museum und Institut, **83**: 7–29.

Rodriguez-Lanetty M, WS Phillips, VM Weis. 2006. Transcriptome analysis of a cnidarian-dinoflagellate mutualism reveals complex modulation of host gene expression. BMC Genom. **7**: Art. no. 23

Rodriguez, E., Lopez-Gonzalez, P. J. & Gili, J. M. 2007. Biogeography of Antarctic sea anemones (Anthozoa, Actiniaria): What do they tell us about the origin of the Antarctic benthic fauna? Deep-SeaResearch II. **54**: 1876-1904.

Rodríguez E., Castorani C.N. and Daly M. 2008. Morphological phylogeny of the family Actinostolidae (Anthozoa: Actiniaria) with description of a new genus and species of hydrothermal vent sea anemone, Inv. Syst. **22**:439–452.

Rodríguez E, Daly, M. 2010. Phylogenetic relationships among deep-sea and chemosynthetic sea anemones: Actinoscyphiidae and Actinostolidae (Actiniaria: Mesomyaria). PLoS ONE. **5**:e10958.

Rozen S. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol **132**: 365-86

Sawyer S.J., Muscatine L. 2001. Cellular mechanisms underlying temperature-induced bleaching in the tropical sea anemone Aiptasia pulchella. J. Exp. Biol. **204**: 3443-3456.

Schmidt, H .1972. Die Nesselkapseln der Anthozoen und ihre Bedeutung fur die phylogenetische Systematik. Helgol. Wiss. Meeres. **23**: 422–458.

Shearer, T. L., M. J. Van Oppen, S. L. Romano, and G. Worheide. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol. Ecol. **11**:2475–2487.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Stimpson, W. 1856. Descriptions of some of the new marine invertebrata from the Chinese and Japanese seas. Proc. Acad. Nat. Sci. Phila. 7: 375-384

Sunagawa S, Choi J, Forman HJ, Medina, M. 2008. Hyperthermic stress- induced increase in the expression of glutamate-cysteine ligase and glutathione levels in the symbiotic sea anemone Aiptasia pallida. Comp Biochem Physiol B: Biochem Mol Biol, 151:133-138.

Sunagawa S, Wilson E.C., Thaler M., Smith M.L., Caruso C., Pringle J.R., Weis V.M., Medina M., Schwarz J.A. 2009. Generation and analysis of transcriptomic resources for a model system on the rise: the sea anemone Aiptasia pallida and its dinoflagellate endosymbiont. BMC Genomics 10:258.

Ting JH, Geller J.B. 2000. Clonal diversity in introduced populations of an Asian sea anemone in North America. Biol. Invasions 2:23–32.

Toth G, Gaspari Z, Jurka J. 2000. Microsatellites in different eukaryotic genomes: survey and analysis. Genome Res. 10:967–981.

Verrill, E.E. 1864.Revision of the Polypi of the eastern coast of the United States. Mem. Boston Soc. Nat. Hist. 1:1-45

Wang S, Zhang L, Matz M. 2009. Microsatellite characterization and marker development from public EST and WGS databases in the reef-building coral Acropora millepora (Cnidaria, Anthozoa, Scleractinia). J. Hered. 100:329–37.

Watzle, O. 1922 Die Aciniarien der Bahamainsein Ark. Zool. 14: 1-89.

Weis V.M., Davy S.K., Hoegh-Guldberg O., Rodriguez-Lanetty M., Pringle J.R. 2008 Cell biology in model systems as the key to understanding corals. Trends Ecol Evol. 23:369-376.

Zamponi M. O., Belém M. J. d., Schlenz E., and Acuña F. H.1998. Distribution and some ecological aspects of Corallimorpharia and Actiniaria from shallow waters of the South American Atlantic coasts. Physis 55: 31-45

CHAPTER II

MORPHOLOGICAL REVISION OF THE GENUS *AIPTASIA* AND THE FAMILY AIPTASIIDAE (CNIDARIA, ANTHOZOA, ACTINIARIA, METRIDIOIDEA)

(Adapted from Grajales, A. & Rodriguez, E. Submitted. Morphological revision of the genus *Aiptasia* and the family Aiptasiidae (Cnidaria, Actiniaria, Metridioidea))

ABSTRACT

Sea anemones of the genus *Aiptasia* Gosse, 1858 are conspicuous members of shallow-water environments worldwide and serve as a model system for studies of cnidarian-dinoflagellate symbiosis. However, to date there have been no comprehensive analyses investigating the systematics of the group. In addition, previously published phylogenetic studies of sea anemones have shown that the genus is not monophyletic. Herein I revise the genus *Aiptasia* and the family Aiptasiidae Carlgren, 1924 using newly-collected material. I find that the formerly-named *A. pallida* (Agassiz in Verrill, 1864) (now *Exaiptasia pallida* comb. nov.) encompasses a single, widespread species from the tropics and subtropics; I erect a new genus, *Exaiptasia* gen. nov., for this species primarily based on cnidae, mode of asexual reproduction and symbionts. I also find morphological evidence that supports splitting *A. mutabilis* (Gravenhorst, 1831) into two species: *A. couchii* Cocks, 1851 and *A. mutabilis*. In addition, I find that *Bellactis* Dube, 1983 (formerly placed within Sagartiidae Gosse, 1858) and *Ragactis* Andres, 1883 (whose familial placement was previously uncertain) within Aiptasiidae. Aiptasiidae is a morphological homogeneous family whose members are characterized by ectodermal longitudinal muscles in the distal column, rows of cinclides in mid-column, microbasic *b*-

mastigophores in the column, and acontia with basitrichs and microbasic *p*-amastigophores.

KEY WORDS. *Aiptasia pallida*; Anthozoa; cnidom; microbasic *p*-amastigophores, nomenclature, synonym.

INTRODUCTION

Sea anemones (Cnidaria: Actiniaria) of the genus *Aiptasia* Gosse, 1858 are conspicuous members of tropical and subtropical shallow-water marine environments worldwide. More than 30 years of research on *Aiptasia* has vastly improved our understanding of dinoflagellate-cnidarian symbiosis (e.g. Rodriguez-Lanetty *et al.* 2006; Sunagawa *et al.* 2008, 2009). Aiptasiids have also been selected as model systems to understand the processes responsible for coral bleaching (e.g. Sawyer & Muscatine 2001). However, despite their importance, accessibility and the fact that publications using *Aiptasia* spp. as focal taxa are common, to date there has not been a comprehensive systematic analysis of the group (e.g. Dunn *et al.* 2002; Muller Parker & Davy 2001; Weis *et al.* 2008; LaJeunesse *et al.* 2010).

The latest compilation of the genus included 14 species distributed worldwide (Fautin 2013); however, most of these descriptions are incomplete by modern standards. Additionally, molecular phylogenetic studies of sea anemones have shown that the genus *Aiptasia*, the type genus of the family is not monophyletic (e.g. Daly *et al.* 2008 *Bartholomea annulata*, appears as the sister group to *A. mutabilis*. only two species within the genus were included. Similarly, molecular and morphological evidence show that membership and diagnostic features of the family Aiptasiiidae Carlgren, 1924 need to be revised (Rodríguez *et al.* 2012; González-Muñoz *et al.* 2012). Without an adequate phylogenetic framework, hypotheses concerning the diversification and evolution of this group cannot be formulated. Population-level studies have also been hindered due to the lack of clearly-defined taxonomic units within *Aiptasia*.

Herein, I revise the genus *Aiptasia* and the family Aiptasiidae based on newly-collected specimens. I erect a new genus for the former species *A. pallida* (Agassiz in Verrill, 1864) (now *Exaiptasia pallida* comb. nov.) and synonymize several species under this name. This species is distributed worldwide in the tropics and subtropics. I consider the formerly-named *A. mutabilis* (Gravenhorst, 1831) to comprise two different species, *A. mutabilis* and *A. couchii* Cocks, 1851. I revise Aiptasiidae and amend the diagnosis to reflect our new findings: i.e., members of the family have microbasic *p*-amastigophores in the acontia (~ *p*-rhabdoibs B2 *sensu* Schmidt 1969) and microbasic *b*-mastigophores in the column (~ *b*-rhabdoibs *sensu* Schmidt 1969). In addition, I move the genus *Bellactis* Dube, 1983 within Aiptasiidae, based on the combination of two diagnostic morphological characters, the presence of ectodermal musculature at the distal end of the column and the presence of *b*-mastigophores in the scapus. Finally, I confirm that *Ragactis* Andres, 1883 also belongs in Aiptasiidae.

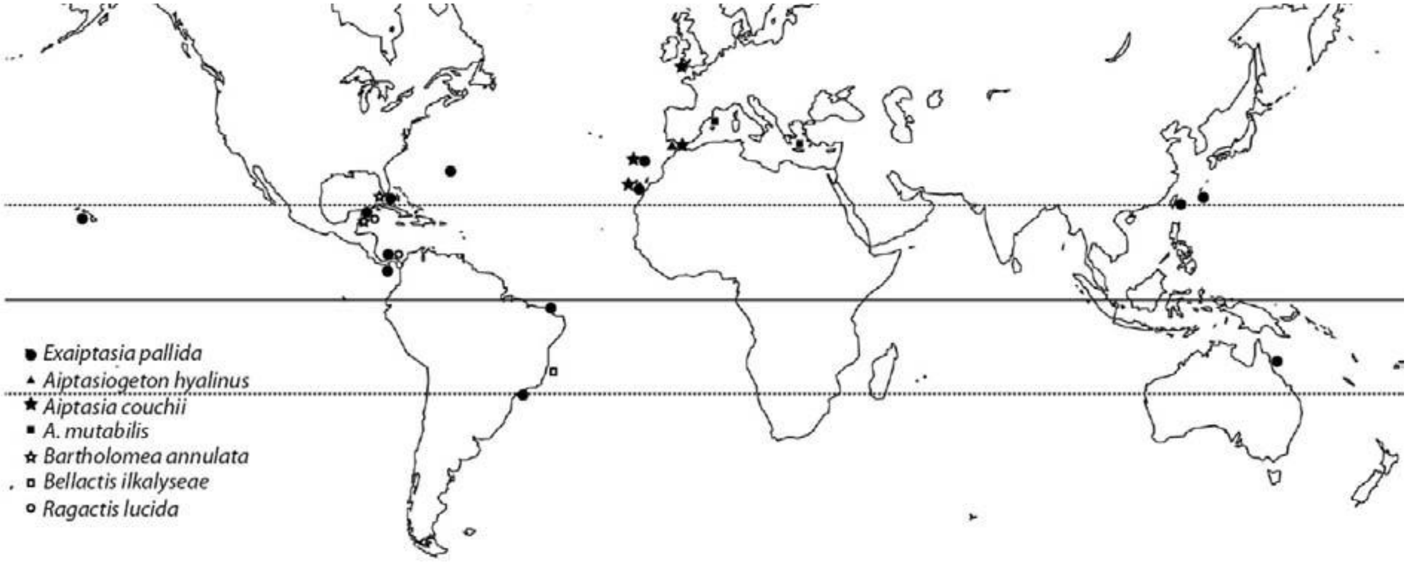
Materials and Methods

The material studied was collected between 2009–2012 from 18 different localities spanning the globe (Fig. 2.1, Appendix 2.1).

Sea anemones were relaxed using menthol crystals and photographed alive. Small pieces of tissue from selected specimens were preserved in absolute ethanol for DNA analysis, and subsequently fixed in 10% seawater-buffered formalin. All preserved specimens were examined whole, while a subset were dissected. Histological sections 7–8 µm thick from parts of several specimens were made (Johansen 1940) and stained with Ramón y Cajal's Triple Stain (Gabe 1968). Measurements of cnidae were made from preserved material; small pieces of tissue were smeared on slides and examined using DIC microscopy at 1000X magnification. I scanned through the slides and randomly chose 15 capsules of each type (when possible) to measure to generate a range: frequencies given are subjective impressions based on all the cnidae seen on the slides. For each type, a mean and standard deviation has been provided to give an idea of the distribution of sizes; these are not statistically significant (see Williams 1998, 2000 for minimal requirements for statistical significance in cnida sizes) but provide some qualitative information about variability in capsule size for each type of nematocyst. Cnida terminology follows Mariscal (1974); however, I allude to Schmidt’s (1969) terminology and correspondences between classifications because the latter is more detailed.

The studied material has been deposited in the American Museum of Natural History in New York (AMNH) and in the Museu de Zoologia da Universidade de São Paulo (MZUSP) in Brazil (Appendix 2.1).

FIGURE 2.1. Map showing localities where studied species were collected.


● Exaiptasia pallida
▲ Aiptasiogeton hyalinus
★ Aiptasia couchii
■ A. mutabilis
☆ Bartholomea annulata
□ Bellactis ilkalyseae
○ Ragactis lucida

I have followed the taxonomic classification implemented in Fautin (2013) but incorporated modifications from Rodríguez *et al.* (2012). Taxa are presented in alphabetical order except in the case of *Exaiptasia* gen. nov.; I described *Exaiptasia* following *Aiptasia* because members in the former genus were previously included within the latter and it makes the comparison between genera clearer. I modified the order and wording of the genus-level diagnoses to homogenise them, but did not mark these changes; other more significant modifications from prior diagnoses are marked in italics. The lists of citations for described species are not exhaustive; I provide only references to the first citation of a species by a particular name. With regards to taxonomic remarks of the species, in some cases I have spelled out the names of the genera to avoid confusion.

RESULTS AND DISCUSSION

Order Actiniaria Hertwig, 1882

Suborder Nynantheae Carlgren, 1899

Superfamily Metridioidea Carlgren, 1893

Family Aiptasiidae

Diagnosis (after Carlgren 1949 and Rodríguez *et al.* 2012). *Metridioidea with well developed pedal disc and basilar muscles*. *Column sometimes distinctly divided into regions*. Tentacles and distal column with ectodermal longitudinal muscles. Cinclides in mid-column, margin tentaculate. Mesogleal marginal sphincter muscle. Mesenteries not divisible into macro- and micro-cnemes. Six to eight pairs of mesenteries perfect and fertile. Two siphonoglyphs. Acontia with basitrichs and *microbasic* p-*amastigophores*. Cnidom: Spirocyst, basitrichs, and microbasic *b-mastigophores* and *p*-amastigophores.

Type genus. *Aiptasia*.

Included genera. *Aiptasia*; *Aiptasiogeton* Schmidt, 1972; *Bartholomea* Duchassaing de Fombressin & Michelotti, 1864; *Bellactis*; *Carlgreniella* Watzl, 1922 (?); *Exaiptasia* gen. nov., *Paranthea* Verrill, 1868 (?); *Ragactis*.

Taxonomic remarks. I modified the familial diagnosis to reflect the recent changes in the higher-level classification of the order (see Rodríguez *et al.* 2012). I also noted that the column is not always clearly divided into regions, the mesogleal marginal sphincter muscle is not always weak, and that the cnidom of the family includes microbasic *b*-mastigophores (in the column) and microbasic *p*-amastigophores but not microbasic *p*-mastigophores (*p*-rhabdoids B2 but not *p*-rhabdoids A *sensu* Schmidt (1969), respectively).

England (1992) erected the genus *Paraiptasia* for *P. radiata* (Stimpson, 1856) and modified the diagnosis of Aiptasiidae to accommodate it within the family (England 1992). *Paraiptasia* has catch tentacles and lacks cinclides (England 1992); these features are not common to the rest of the genera within Aiptasiidae. The distribution and size ranges of cnidae among tissues within members of Aiptasiidae are relatively uniform (see Tables 2.1–2.7). However, the cnidom, cnidae and distribution and sizes of *Paraipatasia* are quite different from the other genera within the family (see England 1992: fig. 2.18 and table 2.11). For example, *Paraiptasia* does not have microbasic *b*-mastigophores in the column or the smaller microbasic *p*-amastigophores (1) in the acontia, and the large microbasic *p*-amastigophores (2) in the acontia are considerably shorter than those of the other aiptasiid genera with non-overlapping length ranges (to ~20–36 μm *vs*. 41–80 μm). Furthermore, *Paraipatasia* seems to have *p*-rhabdoid B1a (specific category of microbasic *p*-amastigophores) in the filaments (see England 1992: fig. 18), according to the size and shape of the capsule, although it was not possible to fully differentiate the type due to poor image quality, a nematocyst category not present in any other member of Aiptasiidae (Reft pers. com.). Additionally, England (1992) does not mention the longitudinal muscles in the distal column in his redescription of *P. radiata*, a distinctive character for the family. *Paraipatasia* seems unlikely to belong within Aiptasiidae and thus I do not consider it within the family. The appropriate familial placement of this genus among the remaining families remains uncertain until further revision.

The genus *Neoaiptasia* Parulekar, 1969 currently includes two species (Fautin 2013): *N. comensali* Parulekar, 1969 and *N. morbilla* Fautin & Goodwill, 2009. The original assignment of both species of *Neoaiptasia* to the family Aiptasiidae was suspect (Parulekar 1969; Fautin & Goodwill 2009). Similarly to *Paraiptasia*, *Neoaiptasia* lacks cinclides and the cnidom of both species does not correspond to other members of Aiptasiidae (see Parulekar 1969: 60–61; Fautin & Goodwill 2009: fig. 5). Furthermore, molecular evidence clearly shows that *N. morbilla* does not belong within Aiptasiidae (Rodríguez *et al.* 2012). I do not consider *Neoaiptasia* to belong within Aiptasiidae; however, the familial position of this genus remains uncertain.

Paranthea originally included three species (Fautin 2013); however, currently it only includes one species *P. armata* Verrill, 1868 (the other two species have been synonymized as species of *Aiptasia*, see below). The description of *P. armata* is incomplete to modern standards and the type material of the species is not available (Fautin 2013). Based on existing data (only color of the specimen and the approximately length and number of tentacles are provided, see Verrill 1868) it is not possible to determine the identity of *P. armata* or the familial placement of the genus *Paranthea*.

Watzl (1922) erected *Carlgreniella* for *C. robusta* Watzl, 1922 and distinguished it from *Bartholomea* based on the presence of 24 longitudinal rows of papillae in the column and a fairly strong mesogleal marginal sphincter muscle in *Carlgreniella*. Although Watzl (1922) illustrated and described in detail the papillae of *C. robusta*, Carlgren (1949) synonymized this species with *B. annulata*; he added the putative presence of papillae in the column (marking it with a question mark) in the diagnosis of *Bartholomea*. Current data are insufficient to determine whether *C. robusta* represents a different species from *B. annulata* and if the presence of rows of papillae in the column warrants a genus-level distinction within these taxa. Pending further revision I consider the synonymy of *C. robusta* and *B. annulata* and the generic status of *Carlgreniella* unresolved.

Finally, *Aiptasiodes* Stephenson, 1918 was previously placed within Aiptasiidae (Fautin 2013). Stephenson (1918) erected the genus for forms related to *Aiptasia* but having a mesogleal sphincter muscle because at the time the type species of *Aiptasia* was described without having a marginal sphincter muscle. After a mesogleal marginal sphincter muscle was noticed in *A. mutabilis*, *Aiptasioides* became a junior synonymy of *Aiptasia* and *Aiptasiodes* is no longer considered a valid genus (Fautin 2007).

Genus *Aiptasia*

Diagnosis (after Carlgren 1949). Aiptasiidae with adherent pedal disc. Column *usually* elongated, smooth, with cinclides in 2–3 longitudinal rows in mid-column*; sometimes scattered cinclides proximally*. *Column not distinctly divisible into scapus and capitulum.* Mesogleal marginal sphincter moderately strong. Strong longitudinal ectodermal muscles in distal column. Tentacles long, simple, to 192, always smooth, without projections. Same number of mesenteries distally and proximally *or more distally*. Six pairs of perfect mesenteries. First and second cycles of mesenteries fertile. Retractor muscles *diffuse to* restricted. Acontia well developed. Symbiotic with *Symbiodinium* spp. *or Amphidinium* spp. *Asexual reproduction by longitudinal or transversal fission*. Cnidom: spirocysts, basitrichs, microbasic *b*-mastigophores and *p*-amastigophores.

Type species. *Aiptasia amacha* Gosse, 1858 by monotypy (Fautin *et al.* 2007a).

Included species. *Aiptasia couchii*; *A. erythrochila* (Fisher, 1874) (?); *A. mutabilis*; *A. prima* (?).

Taxonomic remarks. The genus *Aiptasia* includes 14 species (Fautin 2013). However, molecular evidence shows that the genus is not monophyletic (Daly *et al.* 2008; Rodríguez *et al.* 2012). Similarly, I could distinguish two different genera morphologically, one corresponding to forms similar to the well-known European species

A. mutabilis and the second corresponding to those forms similar to the more widely-distributed species known up to now as *A. pallida* — for which I erect a new genus, *Exaiptasia* gen. nov. (see below). Both genera are distinguishable by gross external morphology when specimens are alive, but are difficult to distinguish once specimens are preserved. They differ in the number of mesenteries proximally and distally (more numerous distally or equal numbers proximally and distally in *Aiptasia* whereas this number is equal in *Exaiptasia* gen. nov.), the mesogleal marginal sphincter muscle (reticulated and relatively stronger in *Exaiptasia* gen. nov. than in *Aiptasia*), cnidae (particularly in the size and shape of the microbasic *b*-mastigophores in the column, those of *Aiptasia* longer and slimmer – similar to the ones found in other aiptasiid genera — than the ones in *Exaiptasia* gen. nov., shorter and thicker, similar to those of *Aiptasiogeton*; however, microbasic *p*-amastigophores in the acontia of *Exaiptasia* gen. nov. are also slightly shorter than those of *Aiptasia*: see Tables 2.1–2.3 and Schmidt 1972), mode of asexual reproduction (transverse or longitudinal fission in *Aiptasia* but pedal laceration in *Exaiptasia* gen. nov.) and in habitat (*Exaptasia* gen. nov. is restricted to tropical and subtropical waters whereas species of *Aiptasia* are restricted to subtropical and temperate waters of the Eastern Atlantic and the Mediterranean Sea (except for *A. prima* but see below)). Furthermore, both genera are associated with different species of symbiont; *Symbiodinium* sp. “A1_med” clade and *Amphidinium* sp. in *Aiptasia* whereas *Symbiodinium minutum* “B1” and *Symbiodinium* sp. “A4” are found in *Exaiptasia* gen. nov.) (Thornhill *et al.* 2013; Grajales & Rodríguez unpub.). Most of the morphological differences I find are of degree more than of kind (e.g. marginal sphincter muscle).

However, I think that the combination of morphological, cnidae, ecological and molecular differences warrant the distinction of *Aiptasia* and *Exaiptasia* gen. nov. Thus, after this revision I restrict the use of the genus *Aiptasia* to four species (see taxonomic remarks below). Nevertheless, the identity of *A. erythrochila* and *A. prima* remains unclear because available data are not conclusive (see taxonomic remarks in pages 15–16 for a discussion on these).

Aiptasia couchii Cocks, 1851

(Figs. 2.2–2.4, Table 2.1)

Synonymy

Anthea Couchii [*sic*] Cocks, 1850 (*nomen nudum*)

Anthea Couchii [*sic*] Cocks, 1851

? Actinea biserialis Forbes, 1840

? Entacmaea biserialis Milne Edwards & Haime, 1851

? Dysactis biseralis Milne Edwards, 1857

Aiptasia amacha Gosse, 1858

Aiptasia Couchii [*sic*]: Gosse 1860

Aiptasia Couchii [*sic*]: Johnson 1861

Aiptasia Couchi [*sic*] Gosse: Andres 1883 (1884)

Non Aiptasia couchii Cocks: Pax 1909

Aiptasia couchi [*sic*]: Stephenson 1920

Aiptasia mutabilis Form I: Schmidt 1972

Aiptasia mutabilis Form II: den Hartog & Ates 2011

External anatomy (Fig. 2.2): Pedal disc to 15 mm diameter, wider than column. Column smooth, to 15 mm height and 5 mm diameter in preserved specimens. Cinclides in mid-column, relatively conspicuous, in three rows, corresponding with endocoels of first two cycles of mesenteries; sometimes scattered cinclides in proximal column. Mesenterial insertions visible. Oral disc to 15 mm diameter. Tentacles to 96, smooth, tapering towards tips; inner tentacles longer than outer ones, to 15 mm and 5 mm length, respectively.

Internal anatomy and microanatomy (Fig. 2.3): Mesogleal marginal sphincter muscle diffuse, relatively strong, restricted to column margin. Same number of mesenteries proximally and distally. Mesenteries hexamerously arranged in four cycles. Only first cycle perfect; first three cycles fertile, including directives. Two pairs of directives each associated with a well-developed siphonoglyph. Gonochoric. Retractor muscles diffuse to restricted. Parietobasilar muscles differentiated, weak. Longitudinal muscles of tentacles ectodermal. Strong longitudinal ectodermal muscles in distal end of column. Basilar muscles well differentiated, relatively weak, with fibers on short mesogleal pennon. Acontia numerous, well developed.

FIGURE 2.2: External anatomy of *Aiptasia couchii*. A, B) Oral view of living specimens. C) Lateral view of living specimen; notice rows of cinclides (arrows). D) Detail of proximal column of preserved specimen; notice scattered cinclides (arrows). E) Lateral view of preserved specimen; notice cinclides and acontia (arrows). Scale bars: A–E, 10 mm.

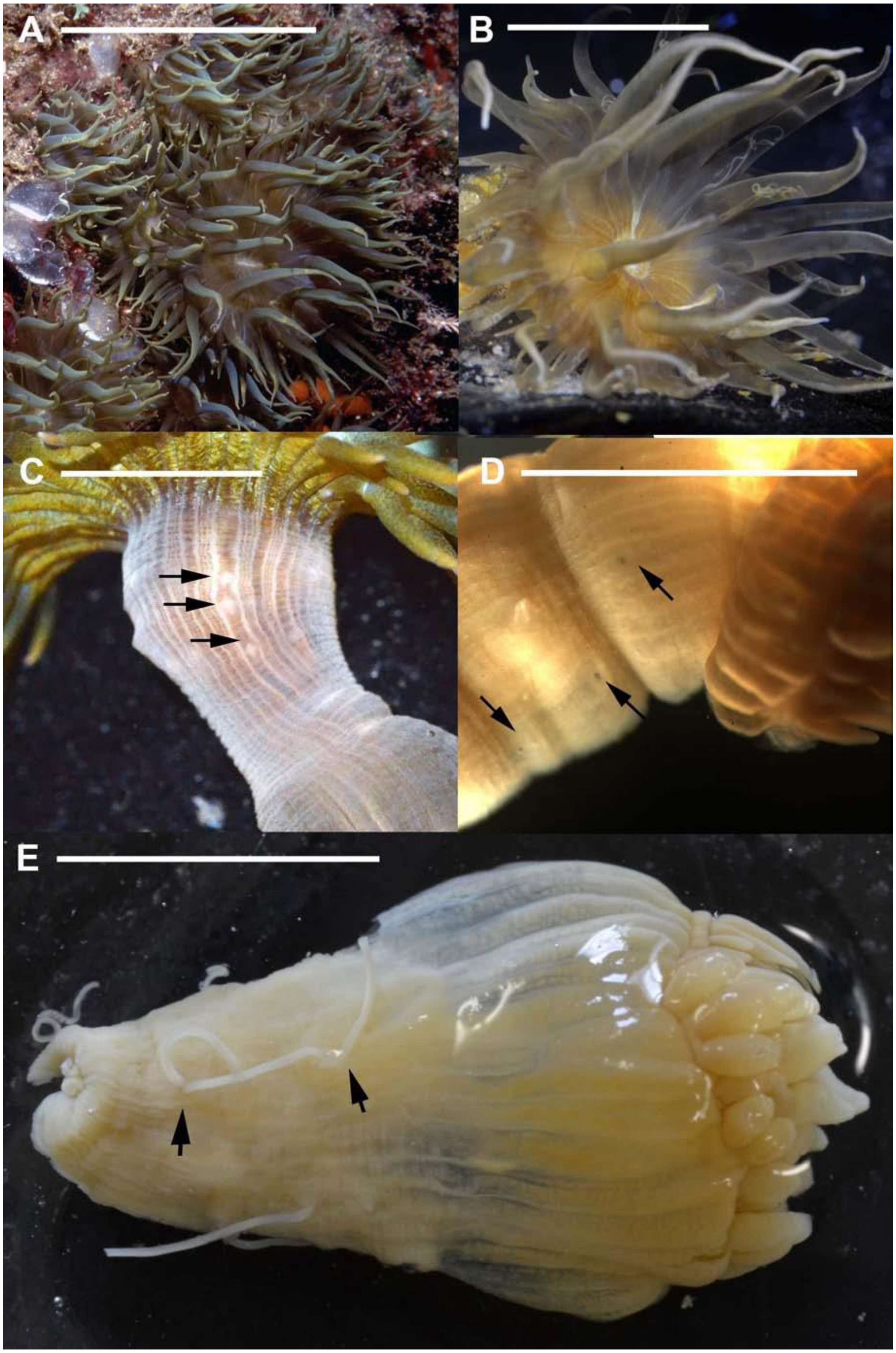

A
B
C
D
E

FIGURE 2.3. Internal anatomy of *Aiptasia couchii*. A) Cross section of the column at the level of the actinopharynx showing the cycles of mesenteries (indicated by roman numbers between pairs). B) Detail of fourth cycle of mesenteries. C) Cross section through a tentacle showing the ectodermal longitudinal musculature. D) Cross section through distal column showing the strong ectodermal longitudinal musculature. E) Detail of a longitudinal section of the proximal end showing the basilar muscles (arrows). F) Longitudinal section of distal column showing mesogleal marginal sphincter muscle. Abbreviations: ep, epidermis: ga, gastrodermis; me, mesoglea. Scale bars: A–F, 0.1 mm.

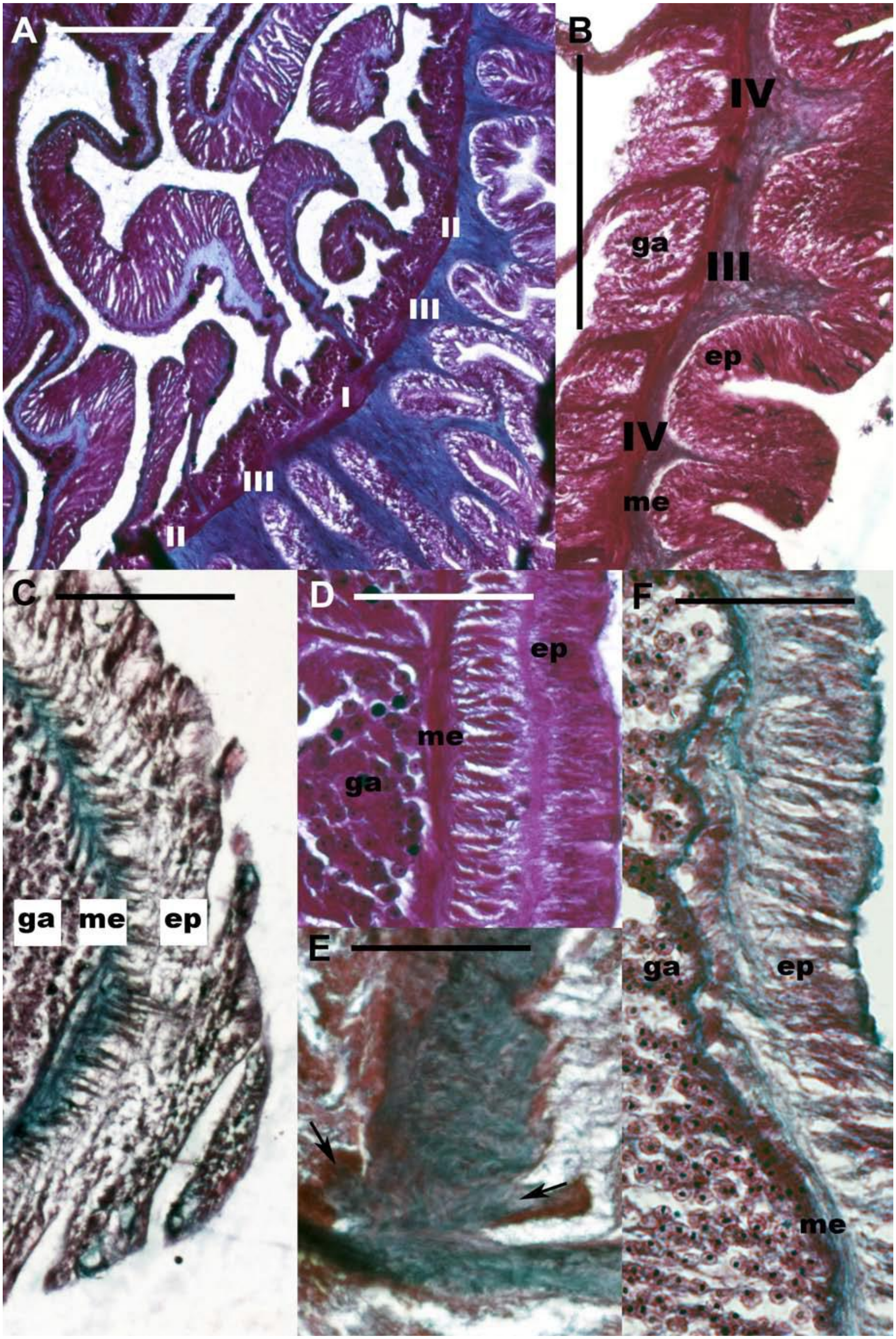

A
II
III
I
III
II
B
IV
ga
III
ep
IV
me
C
ga
me
ep
D
ep
me
ga
E
F
ga
ep
me

FIGURE 2.4. Cnidae of *Aiptasia couchii*. A, C, F, I, K, L, N, O) Microbasic *p*-amastigophores. B, D, G, J, M, P) Basitrichs. E) Microbasic *b*-mastigophore. H) Spirocyst.

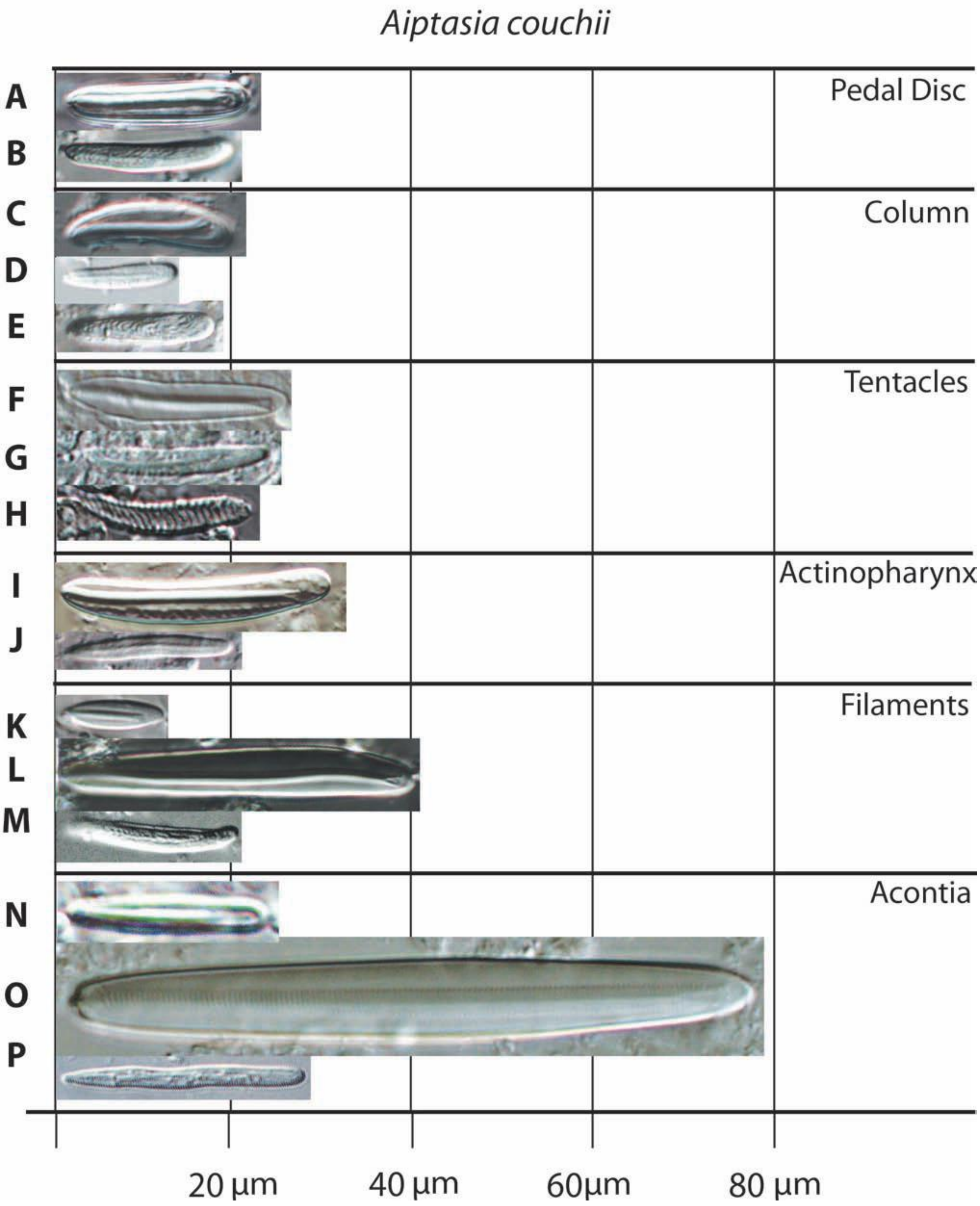

Aiptasia couchii
A
B
Pedal Disc
C
D
E
Column
F
G
H
Tentacles
I
J
Actinopharynx
K
L
M
Filaments
N
O
P
Acontia
20 µm
40 µm
60µm
80 µm

Table 2.1. Size ranges of the cnidae of *Aiptasia couchii*. $\overline{X}$, mean; SD, standard deviation; S, ratio of number of specimens in which each cnidae was found to number of specimens examined; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; Abbreviations: M, Microbasic.

Categories	Range of length and width of capsules (µm)	$\overline{X}$ ± SD	S	N	F
PEDAL DISC					
M *p*-amastigophores	(16.8–38.1) x (3.6–6.1)	22.1± 3.3 x 4.7 ±0.5	10/10	207	+++
Basitrichs	(11.1–24.5) x (1.6–4.9)	16.5±0.4 x 3.2 ±0.5	10/10	193	+++
COLUMN					
M *p*-amastigophores	(10.6–36.2) x (2.5–8.7)	23.2±5.4 x 4.6±1.2	10/10	241	+++
Basitrichs	(11.4–21.2) x (1.9–3.4)	17.1±3.9 x 2.3±0.9	10/10	157	+++
M *b*-mastigophores	(18.1–30.4) x (2.2–5.8)	23.9± 2.8 x 3.8±0.7	10/10	172	++
TENTACLES					+++
M *p*-amastigophores	(15.5–41.6) x (2.7–7.8)	29.5±5.6 x 4.7±0.9	10/10	194	+++
Basitrichs	(13.4–38.2) x (1.9–6.6)	24.1±4.9 x 3.6±0.9	10/10	199	+++
Spirocysts	(11.3–31.2) x (2.7–5.8)	19.9±4.3 x 3.9±0.7	10/10	229	+++
ACTINOPHARYNX					
M *p*-amastigophores	(10.6–38.1) x (2.4–6.1)	21.6±3.1 x 4.3±0.7	10/10	227	+++
Basitrichs	(11.1–24.5) x (1.6–4.9)	7.9±2.3 x 9.9±0.5	9/10	190	++
FILAMENTS					
M *p*-amastigophores 1	(8.7–21.4) x (1.7–7.1)	12.5±2.4 x 3.4±1.7	10/10	220	+++
M *p*-amastigophores 2	(27.6–44.5) x (3.6–6.3)	35.1±3.4 x 4.9±0.6	10/10	276	+++
Basitrichs	(9.6–21.6) x (1.3–3.2)	12.7±4.1 x 2.1±0.6	10/10	217	++
ACONTIA					
M *p*-amastigophores 1	(14.2–33.5) x (2.5–6.3)	20.1±5.8 x 3.3±1.1	8/10	215	+
M *p*-amastigophores 2	(53.4–93.6) x (5.8–10.9)	74.5±10.5 x 7.9±1.1	10/10	278	+++
Basitrichs	(17.9–35.9) x (1.4–4.6)	29.1±3.9 x 2.7±0.5	10/10	303	+++

Color (Fig. 2.2): In living specimens column translucent proximally and greyish-brownish distally; tentacles and oral disc translucent greyish, the latter often with radial whitish stripes corresponding with mesenterial insertions. Mouth and actinopharynx whitish. Preserved specimens tan to yellowish in color.

Cnidom: Spirocysts, basitrichs, microbasic *b*-mastigophores and *p*-amastigophores (Fig. 2.4). See Table 1 for size and distribution.

Geographic and bathymetric distribution. *Aiptasia couchii* is distributed in the eastern Atlantic coast of Europe, Canary Islands and Madeira, and in the Mediterranean Sea (Schmidt 1972; Manuel 1981; Ocaña *et al.* 1994; den Hartog & Ates 2011). *Aiptasia couchii* is a shallow water species preferring protected subtidal waters and usually found in crevices, between 1–5 m.

Taxonomic remarks. Schmidt (1972) revised the European species of Aiptasiidae*;* he synonymized *A. couchii* as *A. mutabilis* but stated that they were different ecotypes of the same species (*A. mutabilis* Form I = *A. couchii* and *A. mutabilis* Form II = *A. mutabilis*, see Schmidt 1972). However, *A. couchii* and *A. mutabilis* can be differentiated by size (to 15 mm *vs.* to 30 mm length), color pattern (particularly that of the tentacles and oral disc, see Figs. 2, 5), number of mesenteries distally and proximally (equal numbers *vs.* more cycles distally) and tentacles (48–96 *vs.* 96–192), cnidae (e.g. microbasic *p*-amastigophores 2 in acontia: although sizes ranges overlap, in *A. couchii*, this category is shorter than in *A. mutabilis*, which has a more restricted size range; a similar situation in found in the column and the tentacles; see Tables 2.1, 2.2), ecology (clonal *vs.* solitary), geographical distribution (*A. mutabilis* is restricted to the

Mediterranean Sea), depth (intertidal *vs.* up to 30 m), kind of symbionts (*Symbiodinium vs*. *Amphidinium*, Grajales & Rodríguez unpub.), and mode of asexual reproduction (transversal *vs.* longitudinal fission). Schmidt (1972) mainly based the synonymy of both species on his observation that one specimen of *A. couchii* kept in an aquarium could grow more than 96 tentacles representing an intermediate form between both species. In my opinion, morphological and ecological evidence clearly distinguishes Schmidt's (1972) ecotypes as different species. Furthermore, molecular evidence supports this differentiation (Grajales & Rodríguez in prep.).

Our cnidae size range data differ from those of Schmidt (1972), particularly in the microbasic *b*-mastigophores of the column and the shorter microbasic *p*-amastigophores (2) of the acontia (Tables 2.1, 2.2). Schmidt (1972) did not report these two categories in his specimens. Both categories are easily overlooked or confused with other types present in the tissue because of similarities in length and width of the capsules. The category Schmidt (1972) named as anisorhize haplonemes in the column probably corresponds partly with what I named as basitrichs. Den Hartog and Ates (2011) identified one small specimen from the northern Atlantic coast of Spain as *Aiptasia mutabilis* type II *sensu* Schmidt (1972) stating that this corresponds with the previously known as *A. couchii*. However, *A. couchii* corresponds with *A. mutabilis* Form I *sensu* Schmidt (Schmidt 1972; Manuel 1981). Den Hartog and Ates (2011) provided only cnida data from the sole examined specimen. Similarly to those of Schmidt (1972), their cnida data differ from ours in the absence of the microbasic *b*-mastigophores of the column and the shorter microbasic *p*-amastigophores (1) of the acontia. The sizes ranges provided for cnida categories in all examined tissues overlap but are more restricted than ours (den Hartog & Ates 2011).

Aiptasia mutabilis (Gravenhorst, 1831)

(Figs. 2.5–2.7, Table 2.2)

Synonymy

Actinia mutabilis Gravenhorst, 1831

Cribina punctata Schmarda, 1852

Sagartia Penoti [*sic*] Jourdan, 1880

Aiptasia turgida Andres, 1881

Aiptasia carnea Andres, 1881

Aiptasia mutabilis: Andres 1883 (1884)

Aiptasiomorpha mutabilis: Carlgren 1949

Aiptasia mutabilis bicolor Andres: Pax & Müller 1953

Aiptasia mutabilis maculata Andres: Pax & Müller 1953

Aiptasia mutabilis Form II: Schmidt 1972

External anatomy (Fig. 2.5): Pedal disc to 15 mm diameter, wider than column. Column smooth, more or less cylindrical, to 30 mm height and 28 mm diameter in preserved specimens. Cinclides in mid-column, inconspicuous, in three rows,

FIGURE 2.5: External anatomy of *Aiptasia mutabilis.* A) Oral view of living specimen. B) Detail of the color pattern of the tentacle. C) Lateral view of preserved specimen. D) Detail of pedal disc of preserved specimen. Scale bars: A–D, 10 mm.

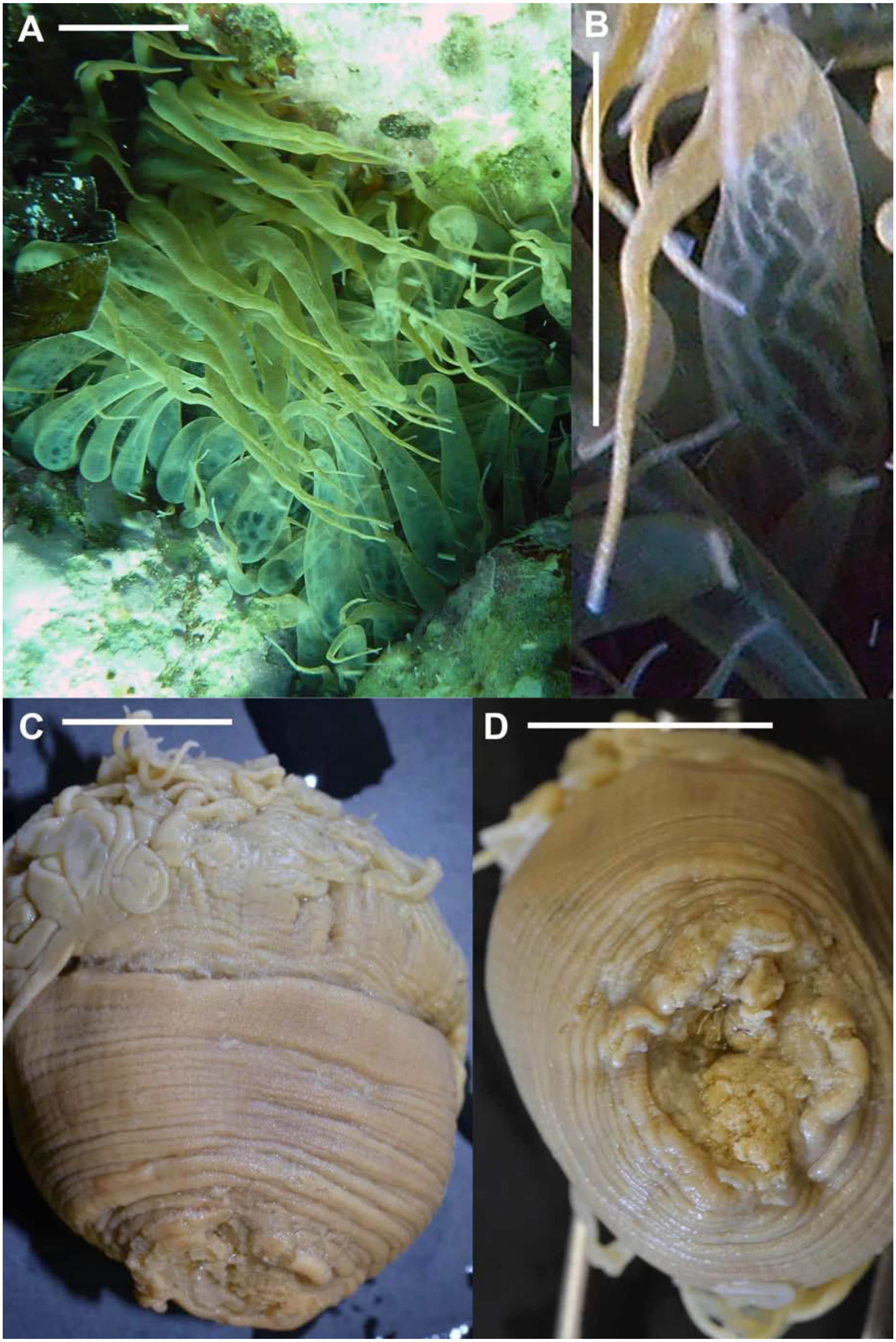

A
B
C
D

FIGURE 2.6. Internal anatomy of *Aiptasia mutabilis*. A) Cross section of the column at the level of the actinopharynx showing the cycles of mesenteries (indicated by roman numbers between pairs). B) Detail of fifth cycle of mesenteries (arrows). C) Detail of parietobasilar muscles (arrows). D) Detail of a longitudinal section of the proximal end showing the basilar muscles (arrows). E) Cross section through a tentacle showing the ectodermal longitudinal musculature. F) Cross section through distal column showing the ectodermal longitudinal musculature. G) Longitudinal section of the distal column showing the mesogleal marginal sphincter muscle. Abbreviations: ep, epidermis; ga, gastrodermis; me, mesoglea. Scale bars: A–G, 0.1 mm.

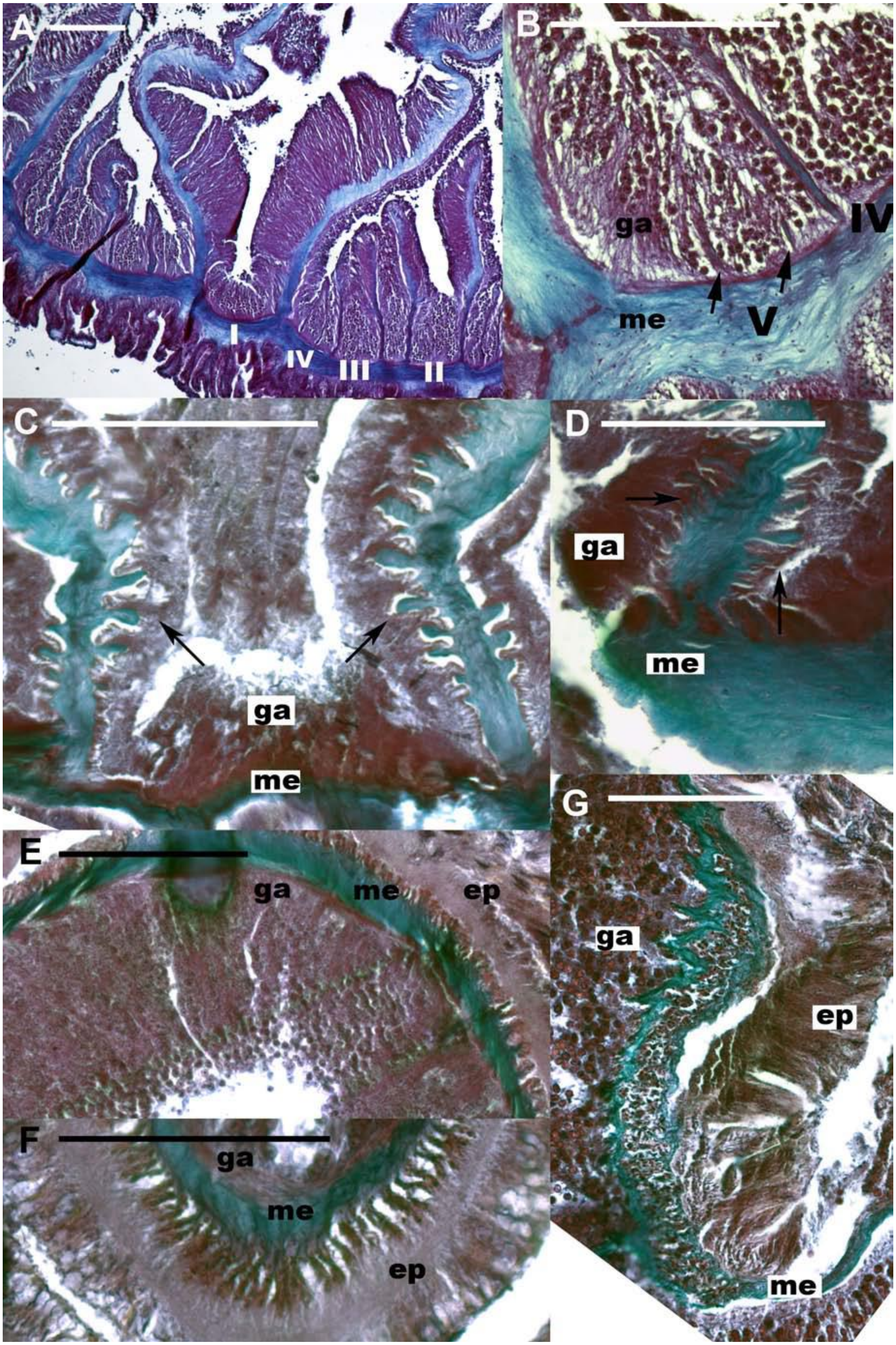

A
I
IV
III
II
B
ga
IV
me
V
C
ga
me
D
ga
me
E
ga
me
ep
F
ga
me
ep
G
ga
ep
me

FIGURE 2.7. Cnidae of *Aiptasia mutabilis*. A, C, F, I, K, L, N, O) Microbasic *p*-amastigophores. B, D, G, J, M, P) Basitrichs. E) Microbasic *b*-mastigophore. H) Spirocyst.

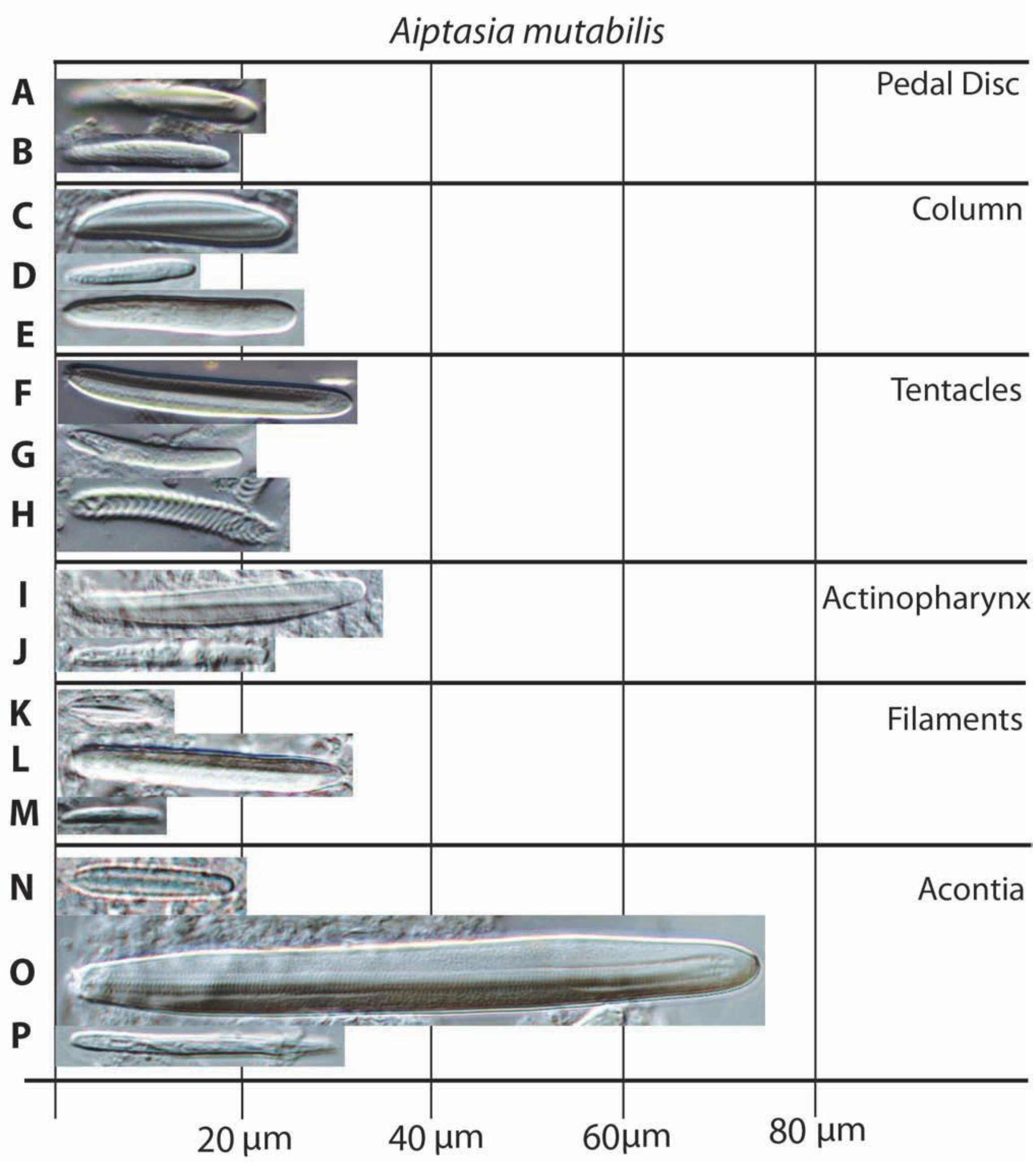

Aiptasia mutabilis
A
B
Pedal Disc
C
D
E
Column
F
G
H
Tentacles
I
J
Actinopharynx
K
L
M
Filaments
N
O
P
Acontia
20 µm
40 µm
60µm
80 µm

Table 2.2. Size ranges of the cnidae of *Aiptasia mutabilis*. $\overline{X}$, mean; SD, standard deviation; S, ratio of number of specimens in which each cnidae was found to number of specimens examined; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; Abbreviations: M, Microbasic.

Categories	Range of length and width of capsules (µm)	$\overline{X}$ ± SD	S	N	F
PEDAL DISC					
M *p*-amastigophores	(15.6–24.9) x (2.3–5.5)	20.2±1.9 x 3.6±0.9	2/2	32	+++
Basitrichs	(14.4–26.7) x (2.1–4.8)	21.1±0.6 x 3.5±4.7	2/2	25	+++
COLUMN					
M *p*-amastigophores	(18.2–21.4) x (3.5–5.4)	20.1±2.1 x 4.5±0.5	2/2	19	+
Basitrichs	(14.3–19.9) x (2.4–3.9)	17.3±1.3 x 2.9±0.4	2/2	36	+++
M *b*-mastigophores	(18.8–30.4) x (3.2–4.9)	27.4±3.1 x 4.1±0.4	2/2	26	+
TENTACLES					
M *p*-amastigophores	(28.3–38.1) x (4.2–6.3)	35.2±2.6 x 5.1±0.6	2/2	29	+++
Basitrichs	(19.4–32.3) x (1.8–6.6)	23.4±2.5 x 3.1±1.3	2/2	39	+++
Spirocysts	(12.8–28.7) x (2.2–5.5)	21.1±4.6 x 3.9±0.9	2/2	52	+++
ACTINOPHARYNX					
M *p*-amastigophores	(32.1–46.1) x (4.1–6.9)	35.2±2.4 x 5.1±0.7	2/2	39	+++
Basitrichs	(14.7–31.1) x (2.3–3.8)	25.2±3.4 x 3.0±0.3	2/2	38	+++
FILAMENTS					
M *p*-amastigophores 1	(10.7–15.9) x (1.4–3.6)	12.4±1.1 x 2.6±0.5	2/2	39	+++
M *p*-amastigophores 2	(20.9–36.9) x (3.7–5.9)	33.1±4.4 x 4.7±0.7	2/2	27	++
Basitrichs	(9.9–20.1) x (1.9–3.2)	13.3±2.7 x 2.8±0.4	2/2	27	+
ACONTIA					
M *p*-amastigophores 1	(16.4–31.7) x (2.1–4.5)	21.6±6.3 x 3.5±0.6	2/2	20	+
M *p*-amastigophores 2	(76.1–92.7) x (7.2–11.3)	84.1±4.6 x 9.1±0.8	2/2	38	+++
Basitrichs	(24.4–35.5) x (1.7–3.5)	30.3±2.3 x 2.6±0.4	2/2	59	+++

corresponding with endocoels of first two cycles of mesenteries. Oral disc to 10 mm diameter. Tentacles to 192, smooth, tapering towards tips; inner tentacles longer than outer ones, to 30 mm and 20 mm length, respectively.

Internal anatomy and microanatomy (Fig. 2.6): Mesogleal marginal sphincter muscle diffuse, relatively strong, restricted to column margin. More mesenteries distally than proximally. Mesenteries hexamerously arranged in five cycles (fifth cycle only distally). Only first cycle perfect; first forth cycles fertile, including directives. Two pairs of directives each associated with a well-developed siphonoglyph. Gonochoric. Retractor muscles diffuse to restricted. Parietobasilar muscles weak. Longitudinal muscles of tentacles ectodermal. Strong longitudinal ectodermal muscles in distal end of column. Basilar muscles weak, poorly differentiated. Acontia numerous, well developed.

Color (Fig. 2.5): In living specimens column translucent brownish; tentacles translucent brownish with distinct pattern with whitish longitudinal marks. Mouth and actinopharynx whitish. Preserved specimens tan in color.

Cnidom: Spirocysts, basitrichs, microbasic *b*-mastigophores and *p*-amastigophores (Fig. 2.7). See Table 2.2 for size and distribution.

Geographic and bathymetric distribution. Restricted to the Mediterranean Sea (Schmidt 1972), in shallow waters to 30 m.

Taxonomic remarks. Andres (1884) shorty re-described *Aiptasia erythrochila* within *Aiptasia*. Based on the available information on this species (mostly color and external anatomy) and because it is described as having 150 tentacles, it likely corresponds to *A. mutabilis*. However, the type material is not available and not enough data are available to be certain about the identity of this species.

Stephenson (1918) thoroughly described *Aiptasia prima*; however, he did not provide cnidae data for the species because it was not a common practice at the time. Thus, the generic position and identity of this species remains unclear pending an examination of the cnidae. However, based on the geographic distribution of the species (South Trinidad Islands, temperate climate) I think it probably corresponds better with *Aiptasia* rather than with the widely-distributed *Exaiptasia pallida* comb. nov.

Genus *Exaiptasia* gen. nov.

Diagnosis. Aiptasiidae with adherent pedal disc. Column elongated, smooth, with cinclides in 2–3 longitudinal rows in mid-column; *column not distinctly divisible into scapus and capitulum*. Mesogleal marginal sphincter moderately strong. Strong longitudinal ectodermal muscles in distal column. Tentacles long, simple, to 96, always smooth, without projections. Same number of mesenteries distally and proximally. Six pairs of perfect mesenteries. First and second cycles fertile. Retractor muscles restricted. Acontia well developed. Symbiotic with *Symbiodinium* spp. *Asexual reproduction by pedal laceration.* Cnidom: spirocysts, basitrichs, microbasic *b*-mastigophores and *p*-amastigophores.

Type species. *Dysactis pallida* Agassiz in Verrill, 1864 by original designation.

Included species. *Exaiptasia pallida* comb. nov., *E. insignis* (Carlgren, 1941) comb. nov. (?), *E. parva* (Carlgren, 1938) comb. nov. (?).

Taxonomic remarks. I chose to erect a new generic name for *Exaiptasia pallida* comb. nov. because all the previously used generic names in which *E. pallida* comb. nov. was included referred also to species included within the genus *Aiptasia* as currently defined.

Exaiptasia pallida comb. nov.

(Figs. 2.8–2.10, Table 2.3)

Synonymy

Actinia diaphana Rapp, 1829

Cribina diaphana: Deshayes & Milne Edwards 1840

Actinia elongata Delle Chiaje, 1841

Adamsia diaphana: Milne Edwards 1857

Dysactis pallida Agassiz in Verrill, 1864

Bartholomea Tagetes [*sic*] Duchassaing de Fombressin & Michelotti, 1864

Bartholomea Inula [*sic*] Duchassaing de Fombressin & Michelotti, 1864

Dysactis mimosa Duchassaing de Fombressin and Michelotti, 1864

Bartholomea inula: Duchassaing de Fombressin & Michelotti, 1866

Dysactis minuta Verrill, 1867 (1866)

Paranthea minuta: Verrill 1868

Paranthea pallida: Verrill 1868

Disactis Mimosa [*sic*]: Duchassaing 1870

Aiptasia saxicola Andres, 1881

Aiptasia diaphana: Andres 1883 (1884)

Aiptasia [*sic*] *Agassizii:* Andres 1883 (1884)

Aiptasia inula: Andres 1883 (1884)

Aiptasia minuta: Andres 1883 (1884)

Aiptasia mimosa: Andres 1883 (1884)

Aiptasia tagetes: Andres 1883 (1884)

Aiptasia pallida: McMurrich 1887

Aiptasia leiodactyla Pax, 1910

Aiptasioides pallida: Stephenson 1918

Aiptasiomorpha diaphana: Stephenson 1920

Aiptasiomorpha leiodactyla: Stephenson 1920

Bartholomea tagetes: Stephenson 1920

Aiptasia pulchella Carlgren, 1943

Aiptasia californica Carlgren, 1952

Aiptasia tagetes: Atoda 1954

Aiptasiomorpha minuta: Uchida & Soyama 2001

Aipstasia [*sic*] *pulchella:* Reimer *et al.* 2007

FIGURE 2.8. External anatomy of *Exaiptasia pallida* comb. nov. A) Oral view of living specimen. B) Lateral view of living specimen. C) Lateral view of preserved specimen; notice cinclides and acontia (arrows). Scale bars: A–C, 10 mm.

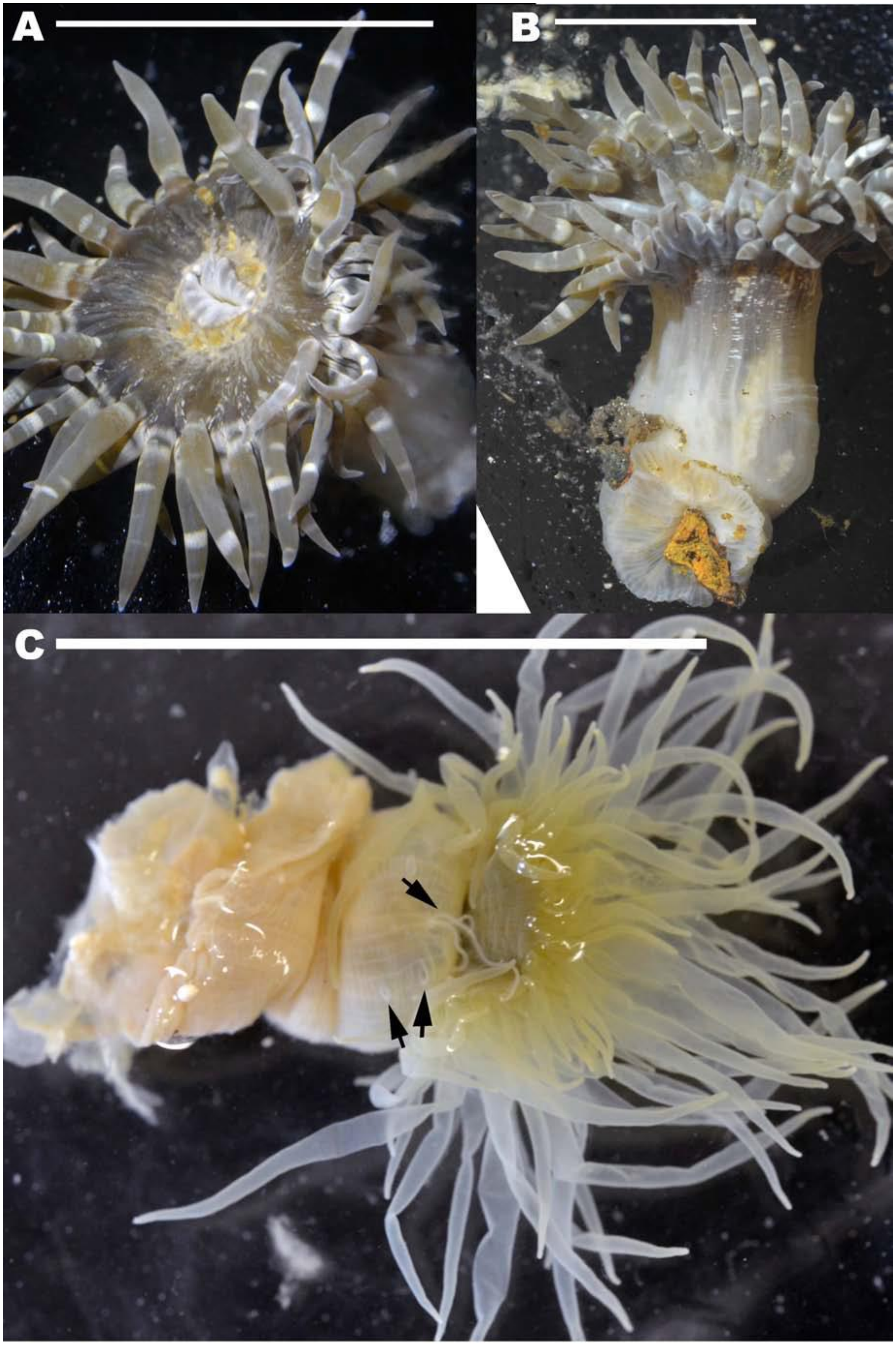

A
B
C

FIGURE 2.9. Internal anatomy of *Exaiptasia pallida* comb. nov. A) Cross section of the column at the level of the actinopharynx showing the cycles of mesenteries(indicated by roman numbers between pairs). B) Detail of fourth cycle of mesenteries (arrows). C) Cross section through a tentacle showing the ectodermal longitudinal musculature; notice zooxanthellae in gastrodermis. D) Cross section through distal column showing strong ectodermal longitudinal musculature. E) Detail of weak parietobasilar muscles. F) Detail of a longitudinal section of the proximal end showing the basilar muscles (arrows). G) Longitudinal section of the distal column showing reticulate mesogleal marginal sphincter muscle. Abbreviations: ep, epidermis; ga, gastrodermis; me, mesoglea; s, siphonoglyph. Scale bars: A–G, 0.1 mm.

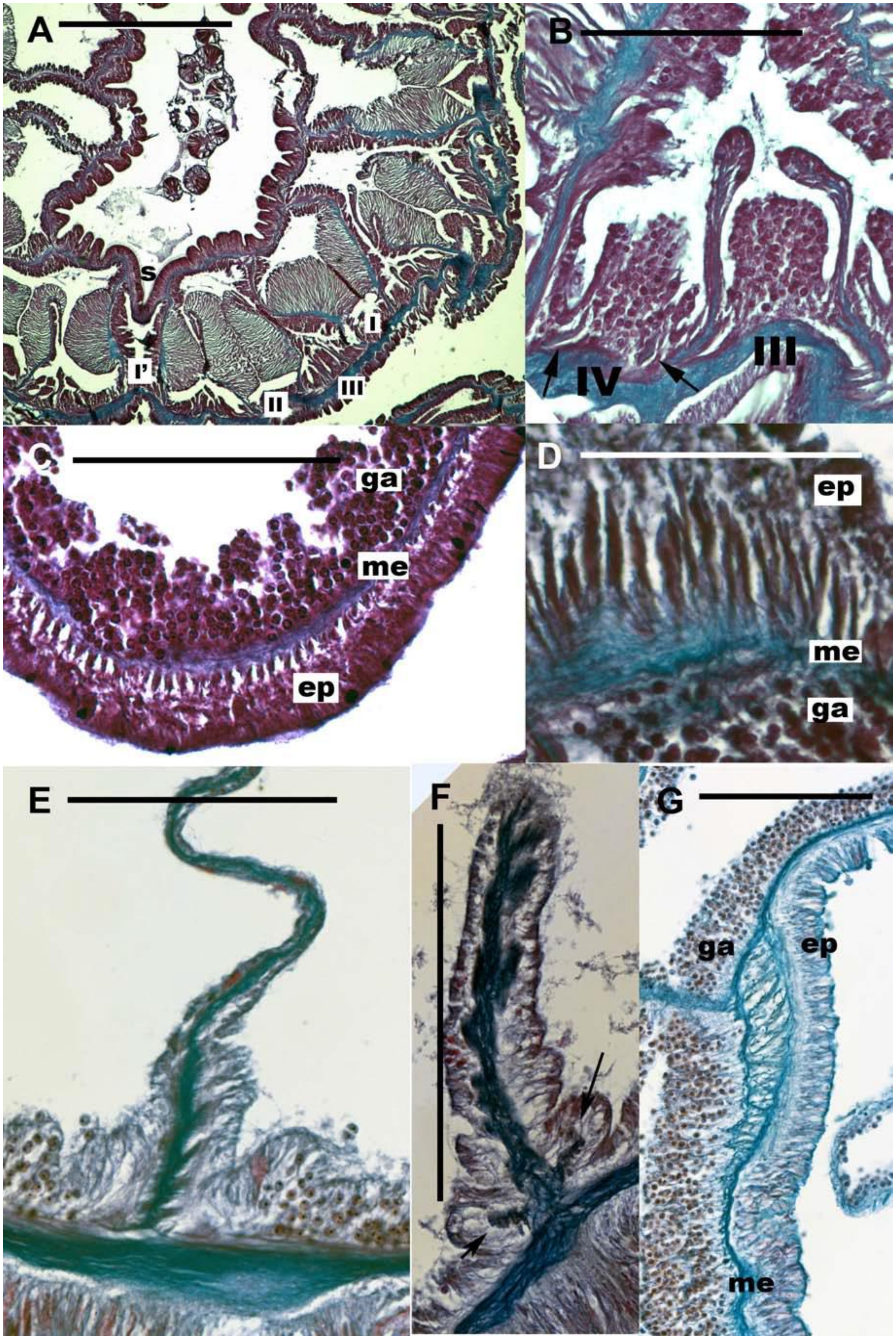

A
s
I
I'
II
III
B
IV
III
C
ga
me
ep
D
ep
me
ga
E
F
G
ga
ep
me

FIGURE 2.10. Cnidae of *Exaiptasia pallida* comb. nov. A, C, F, I, K, L, N, O) Microbasic *p*-amastigophores. B, D, G, J, M, P) Basitrichs. E) Microbasic *b*-mastigophore. H) Spirocyst.

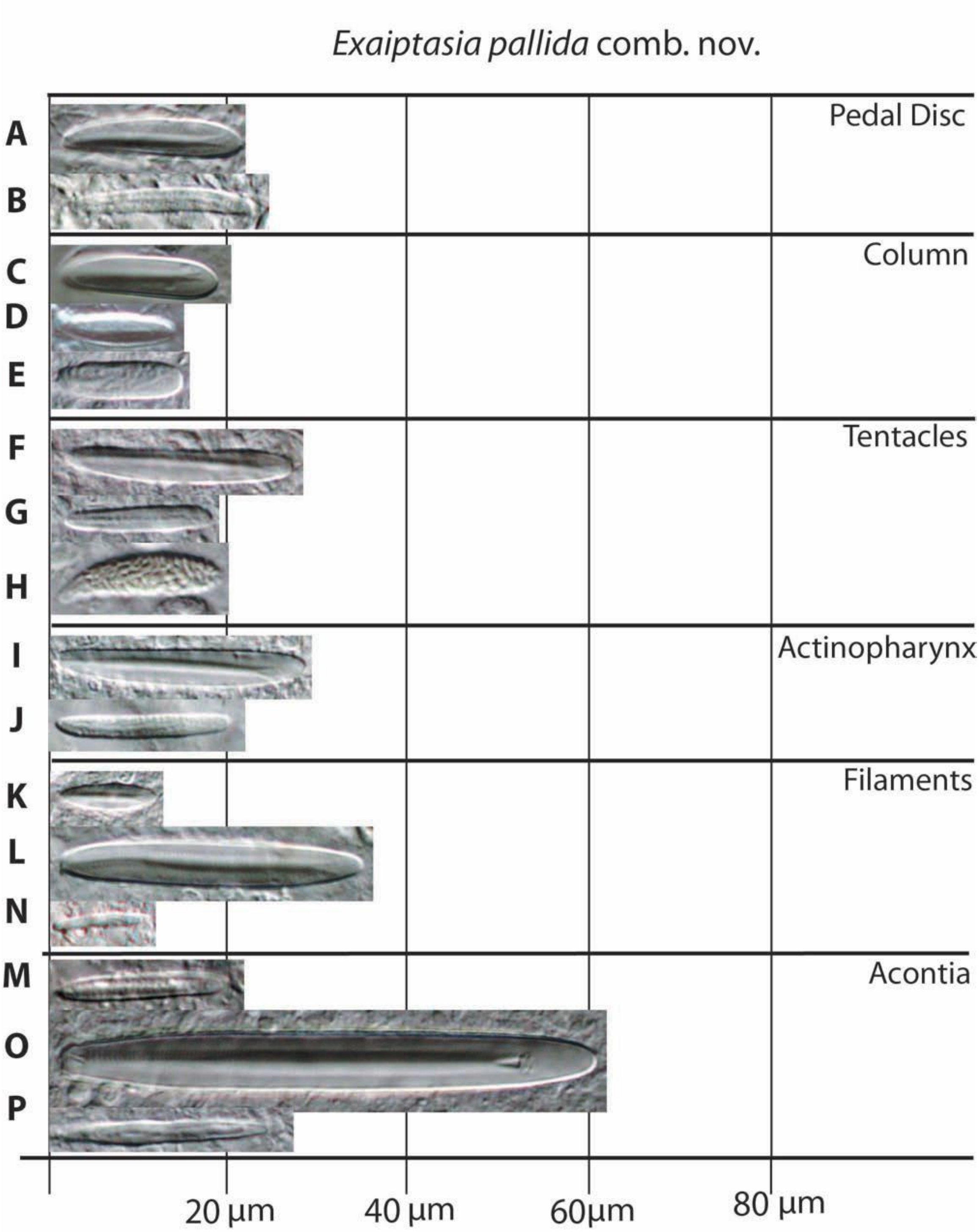

Exaiptasia pallida comb. nov.
A
B
Pedal Disc
C
D
E
Column
F
G
H
Tentacles
I
J
Actinopharynx
K
L
N
Filaments
M
O
P
Acontia
20 µm
40 µm
60µm
80 µm

Table 2.3. Size ranges of the cnidae of *Exaiptasia pallida* comb. nov. $\overline{X}$, mean; SD, standard deviation; S, ratio of number of specimens in which each cnidae was found to number of specimens examined; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; Abbreviations: M, Microbasic.

Categories	Range of length and width of capsules (µm)	$\overline{X}$ ± SD	S	N	F
PEDAL DISC					
M *p*-amastigophores	(9.7–27.5) x (1.9–6.4)	18.97±2.8 x 4.3±0.7	30/30	610	+++
Basitrichs	(9.1–19.1) x (1.2–4.4)	13.1±2.2 x 3.1±0.7	30/30	615	+++
COLUMN					
M *p*-amastigophores	(9.7–40.4) x (1.8–6.4)	18.2±4.9 x 4.2±0.7	30/30	483	+++
Basitrichs	(8.4–28.4) x (1.6–5.7)	14.1±3.1 x 3.2±0.6	30/30	493	+++
M *b*-mastigophores	(9.5–19.1) x (2.3–6.7)	14.2±2.5 x 4.3±0.7	30/30	346	++
TENTACLES					
M *p*-amastigophores	(19.5–47.8) x (2.6–7.1)	28.8±2.7 x 4.8±0.5	30/30	519	+++
Basitrichs	(9.3–25.1) x (1.4–4.9)	16.9±2.7 x 2.9±0.5	30/30	466	+++
Spirocysts	(9.2–30.6) x (2.1–6.6)	17.2±4.1 x 3.7±0.9	30/30	585	+++
ACTINOPHARYNX					
M *p*-amastigophores	(12.1–35.9) x (2.4–6.7)	24.8±4.5 x 4.1±0.5	30/30	563	+++
Basitrichs	(8.6–30.1) x (1.6–4.7)	17.3±4.5 x 2.9±0.5	30/30	511	++
FILAMENTS					
M *p*-amastigophores 1	(7.4–17.2) x (1.8–5.4)	11.6±1.7 x 3.3±0.5	30/30	578	+++
M *p*-amastigophores 2	(20.1–44.7) x (1.9–7.1)	28.6±4.1 x 4.6±1.1	30/30	435	+++
Basitrichs	(7.7–29.1) x (1.1–6.2)	18.1±6.1 x 2.8±0.6	24/30	261	+
ACONTIA					
M *p*-amastigophores 1	(10.4–31.2) x (1.9–6.2)	18.9±4.9 x 3.5±0.8	26/30	282	+
M *p*-amastigophores 2	(44.9–85.2) x (5.1–12.1)	62.1±6.1 x 7.7±1.3	30/30	439	+++
Basitrichs	(13.4–34.4) x (2.1–4.4)	24.1±2.5 x 2.9±0.4	30/30	498	+++

Material examined

See Appendix 1

Description

External anatomy (Fig. 2.8): Pedal disc to 10 mm diameter, wider than column. Column smooth, to 60 mm height and to 30 mm diameter in preserved specimens. Cinclides often conspicuous in mid-column, in 2–3 rows, with ~12 cinclides per row, alternated, corresponding with endocoels of first two cycles of mesenteries. Mesenterial insertions visible. Oral disc to 10 mm diameter in preserved specimens. Tentacles to 96, smooth, long, tapering toward tips, all of same length, to 20 mm.

Internal anatomy and microanatomy (Fig. 2.9): Mesogleal marginal sphincter muscle diffuse, strong, reticulate, relatively short, restricted to column margin; fibers occupying entire mesoglea. Mesenteries hexamerously arranged in four cycles. Only first cycle perfect; first two cycles fertile, including directives (?); third and fourth cycles poorly developed. Two pairs of directives each associated with a well-developed siphonoglyph. Gonochoric. Asexual reproduction by pedal laceration. Retractor muscles restricted, strong. Parietobasilar muscles differentiated, weak. Longitudinal muscles of tentacles ectodermal. Strong longitudinal ectodermal muscles in distal end of column. Basilar muscles well differentiated, with fibers on thin mesogleal pennon. Acontia numerous, well developed.

Color (Fig. 2.8): In living specimens column translucent proximally and greyish-brownish with scattered spots distally; oral disc and tentacles greyish, the latter with scattered white transversal stripes. Whitish mouth and actinopharynx with yellowish circle around. Preserved specimens tan in color.

Cnidom: Spirocysts, basitrichs, microbasic *b*-mastigophores and *p*-amastigophores (Fig. 2.10). See Table 2.3 and Appendix 2.2 for size and distribution.

Geographic and bathymetric distribution. *Exaiptasia pallida* comb. nov. is a widespread species recorded worldwide along the northwestern Atlantic coast (Fautin 2013), the Gulf of Mexico (e.g. Cary 1906; Gunter & Geyer 1955) and the Caribbean Sea (e.g. Silbiger & Childress 2008; González-Muñoz *et al*. 2012), the coast of Brazil in the southwestern Atlantic Ocean (e.g. Corrêa 1964, 1973; Dube 1983; Pires *et al.* 1992; Castro *et al.* 1995; Echeverria *et al.* 1997; Zamponi 1998; Farrapeira *et al.* 2007), Galapagos Islands (Fautin *et al.* 2007b), and Australia. In this study I extend the distribution of the species to the Mediterranean Sea and western Africa (geographic distribution of the former *Aiptasia diaphana*), the east and west Pacific coasts (e.g. California [geographic distribution of former *A. californica*], Japan and Hawaii [geographic distribution of former *A. pulchella*]). In this study I provide new records for the coast of Brazil, Australia, and Panama (Appendix 2.1). *Exaiptasia pallida* comb. nov. is a tropical and subtropical, shallow-water subtidal species, preferring calm and protected waters, found between 0–5 m.

Taxonomic remarks. The genus *Aiptasia* presently includes 14 nominal species (Fautin 2013). Three of these species (*A. mutabilis, A. erythrochila* and *A. prima*) I consider to belong within *Aiptasia* (see Taxonomic remarks in pag. 9). Descriptions of most of the other 11 species are rather old and incomplete for modern standards —

particularly those of species within the Caribbean Sea (González-Muñoz *et al*. 2012) — and type material is not available in most cases. Differences among these species are usually the lack of several characters – such as rows of cinclides or the marginal sphincter muscle — which are sometimes variable but also difficult to detect in some preserved specimens (e.g. differences between *A. tagetes* and *A. leiodactyla vs. E. pallida* comb. nov., see González-Muñoz *et al*. 2012). After detailed morphological and cnidae examination of newly-collected material from almost all localities reported for the 11 putative species (except South Africa, South Trinidad Islands and Saint Helena, see Appendix 2.1) I did not find any constant morphological character distinguishing among the former *Aiptasia pallida*, *A. californica*, *A. diaphana*, *A. inula*, *A. leiodactyla, A. mimosa*, *A. pulchella*, and *A. tagetes*. As mentioned, the symbiotic dinoflagellates of all collected individuals correspond to *Symbiodinium minutum* (type B1), with the exception of *A. pallida* from specimens in Florida harboring Symbiodinium sp. "A4" in addition to *S. minutum*. Appendix 2.2 shows a breakdown of cnida data of examined populations pooled by geographical regions (i.e. east Atlantic and Caribbean Sea, west Atlantic, and Pacific Ocean). Although I found slight differences in sizes ranges of the cnidae among a few localities — the most remarkable difference is longer microbasic *p*-amastigophores (2) in the filaments of specimens from Hawaii (to 35 µm *vs*. to 45 µm) — ranges always overlap and thus I do not consider this difference alone to distinguish species. Furthermore, molecular evidence supports a unique widely distributed species (Grajales & Rodríguez in prep.). Thus, I synonymized the former seven species as *E. pallida* comb. nov. *Aiptasia minuta* (Verrill, 1867) is described from Japan relatively close to the former

A. pulchella. Verrill (1866) did not detect cinclides in this species and Uchida and Soyama (2001) placed it within *Aiptasiomorpha* (Stephenson, 1920), which implies that they did not detect a marginal sphincter muscle (Carlgren 1949). However, the latter authors did not describe the species or provided cnidae data and it is not clear from their publication if they studied the species following taxonomic standards. In addition, cinclides are easily overlooked and thus I consider that this species corresponds with *E. pallida* comb. nov. (as a synonymy of the former *A. pulchella*).

According to the Principle of Priority (Art. 23 of the International Code of Zoological Nomenclature, the Code hereafter, ICZN 1999), the former *Aiptasia diaphana* is the senior subjective synonym and thus must be used over the junior synonym, the former *A. pallida.* Although the name *pallida* has currently a broader use than *diaphana* (particularly in non-taxonomic works) this name does not fulfill the requirements for a reversal of precedence of a junior synonym – i.e. Art. 23.9.1 of the Code — (ICZN 1999). However, I consider that the use of the senior synonym (*diaphana*) will cause confusion threatening stability and wish to maintain the use of the junior synonym (*pallida*). Following Art. 23.9.3 of the Code (ICZN 1999), the matter has been referred to the ICZN (Grajales & Rodríguez in press: Case 3633) and is awaiting resolution. While the case is under consideration the junior name is to be maintained (ICZN 1999); thus, here I use *Exaiptasia pallida* comb. nov.

Carlgren (1938) described *Aiptasia parva* from South Africa, including an account of cnidae sizes ranges but it is incomplete. This species seems to have accumulations of nematocysts (nematocyst batteries?) in the column, up to 70 tentacles, cinclides in mid-column but not distinctly arranged, lower number of mesenteries in mid-column and only eight mesenteries were perfect proximally in two of the examined specimens, suggesting pedal laceration (Carlgren 1938). In addition, Carlgren (1938) did not mention the presence of zooxanthellae. Thus, his description suggested that this is a different species than *E. pallida* comb. nov. Based on the size range of the longer microbasic *p*-amastigophores in the acontia and in the occurrence of pedal laceration, I think that this species is more closely related to *Exaiptasia pallida* comb. nov. rather than to species within *Aiptasia*. However, the identity of this species remains unclear until further revision.

Carlgren (1941) described shortly *Aiptasia insignis* from Santa Helena Island; he noted that it resembled *A. couchii*. However, his description fits that of *Exaiptasia* gen. nov. better than that of *Aiptasia*, especially in the size range of the longer microbasic *p*-amastigophores of the acontia (55–70.5 µm in length) and the geographic distribution (tropical and subtropical). The marginal sphincter muscle is weaker than in *E. pallida* comb. nov. and there are several differences in cnida data (mainly narrower sizes ranges than those of *E. pallida* comb. nov. and absence of small categories of cnidae – e.g., smaller category of microbasic *p*-amastigophores in acontia — these differences might be due to lower numbers of capsules and specimens measured). Thus, until further examination, I would rather consider *E. insignis* comb. nov. as a separate species, probably closely related to *E. pallida* comb. nov.

Genus *Aiptasiogeton*

Diagnosis (after Schmidt 1972). Aiptasiidae with well developed pedal disc. Column elongate, smooth, with cinclides in mid-column. *Column not distinctly divisible into scapus and capitulum.* Mesogleal marginal sphincter weak. Strong longitudinal ectodermal muscles in distal column. Tentacles rather or very long, to 96, simple, always smooth, without projections. More mesenteries distally than proximally. Eight perfect mesenteries. All cycles of mesenteries fertile. Retractor muscles restricted. Acontia well developed. Zooxanthellae absent. *Asexual reproduction by pedal laceration.* Cnidom: spirocysts, basitrichs, and microbasic *b*-mastigophores and *p*-amastigophores.

Type species. *Paractis comata* Andres, 1881 by monotypy.

Included species. *Aiptasiogeton eruptaurantia* (Field, 1949)*; Aiptasiogeton hyalinus* (Delle Chiaje, 1822).

Aiptasiogeton hyalinus

(Figs. 2.11–2.13, Table 2.4)

Synonymy

Actinia hyalina Delle Chiaje, 1822

Actinia pellucida Hollard, 1848

Non *Actinia lacerata* Dalyell, 1848

Paractis comata Andres, 1881

Aiptasia lacerata: Andres 1884 (part.)

Solecnactinia erythrochila Fisher, 1874

Sagartia erythrochila Fisher, 1889

Aiptasiogeton comatus Schmidt, 1972

Aiptasiogeton pellucidus Manuel 1981

Non Haliplanella lineata: Ramil 1987

Aiptasiogeton hyalinus: Ocaña & den Hartog 2002

FIGURE 2.11. External anatomy of *Aiptasiogeton hyalinus.* A) Oral view of living specimen; notice the distinct tentacles corresponding with directives. B) Lateral view of living specimen; notice spots. C) Lateral view of preserved specimen. D) Lateral view of living specimen. E) Oral view of living specimen undergoing pedal laceration. Scale bars: A–E, 10 mm.

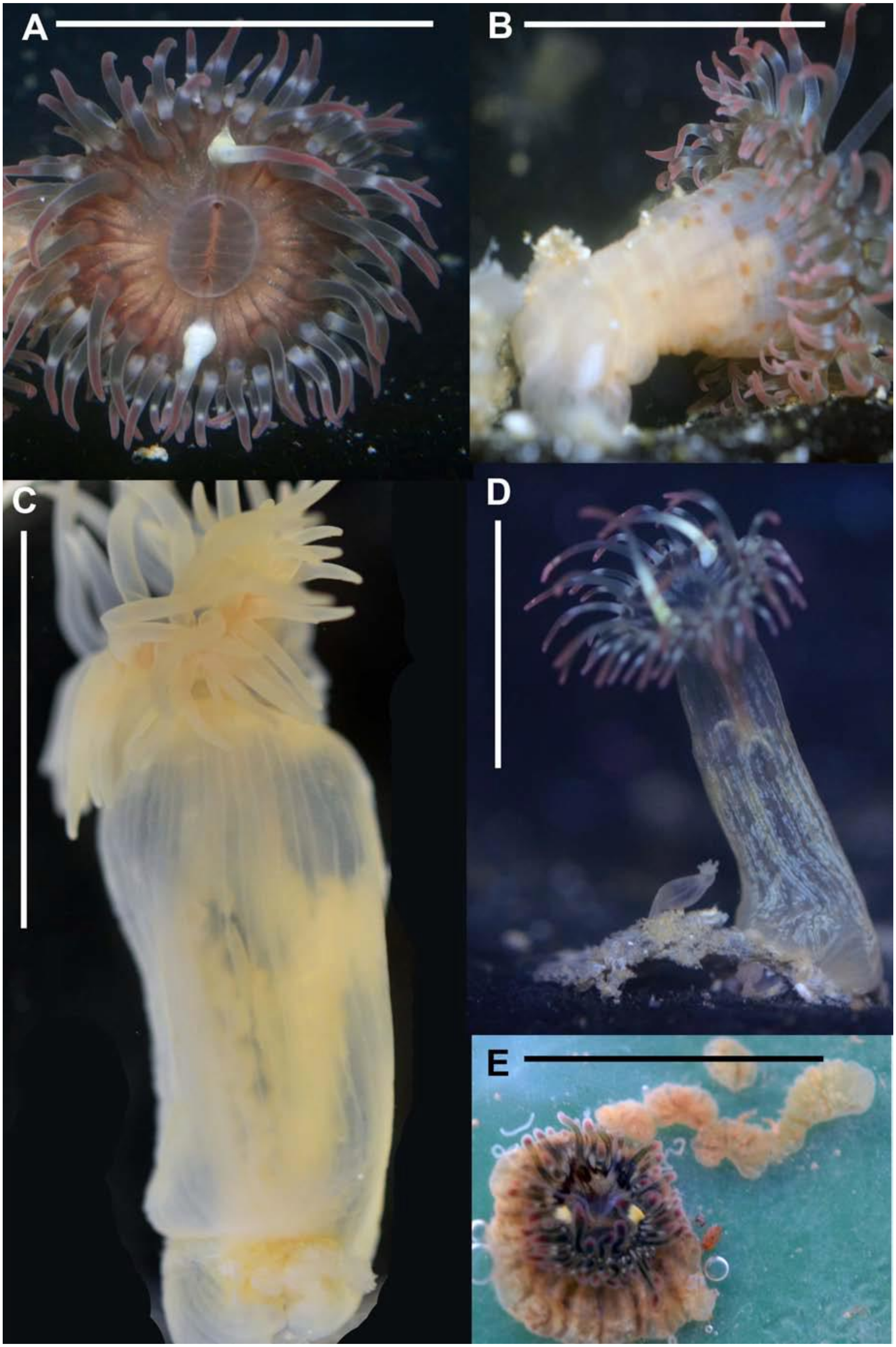

A
B
C
D
E

FIGURE 2.12. Internal anatomy of *Aiptasiogeton hyalinus.* A) Cross section of the column at the level of the actinopharynx showing the cycles of mesenteries(indicated by roman numbers between pairs). B) Detail of fourth cycle of mesenteries (arrows). C) Detail of restricted retractor muscles. D) Detail of a longitudinal section of the proximal end showing the well-developed basilar muscles (arrows). E) Cross section through a tentacle showing the ectodermal longitudinal musculature; notice the absence of zooxanthellae in the gastrodermis. F) Cross section through distal column showing strong ectodermal longitudinal musculature. G) Longitudinal section of the distal column showing strong mesogleal marginal sphincter muscle. Abbreviations: ep, epidermis; ga, gastrodermis; me, mesoglea; s, siphonoglyph. Scale bars: A–G, 0.1 mm.

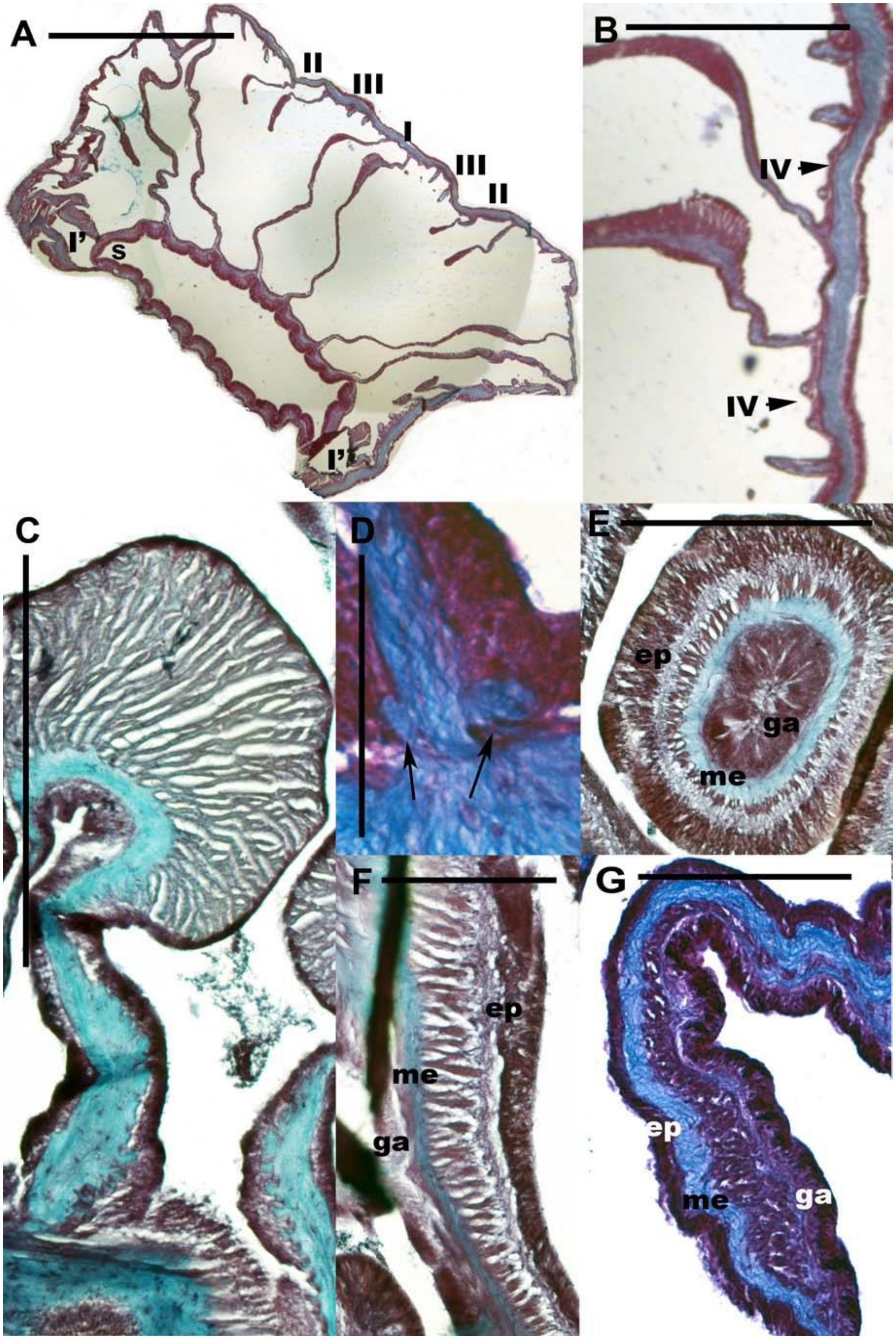

A
II
III
I
III
II
I'
s
I''
B
IV
IV
C
D
E
ep
ga
me
F
ep
me
ga
G
ep
me
ga

FIGURE 2.13. Cnidae of *Aiptasiogeton hyalinus*. A, C, F, I, K, L, N, O) Microbasic *p*-amastigophores. B, D, G, J, M, P) Basitrichs. E) Microbasic *b*-mastigophore. H) Spirocyst

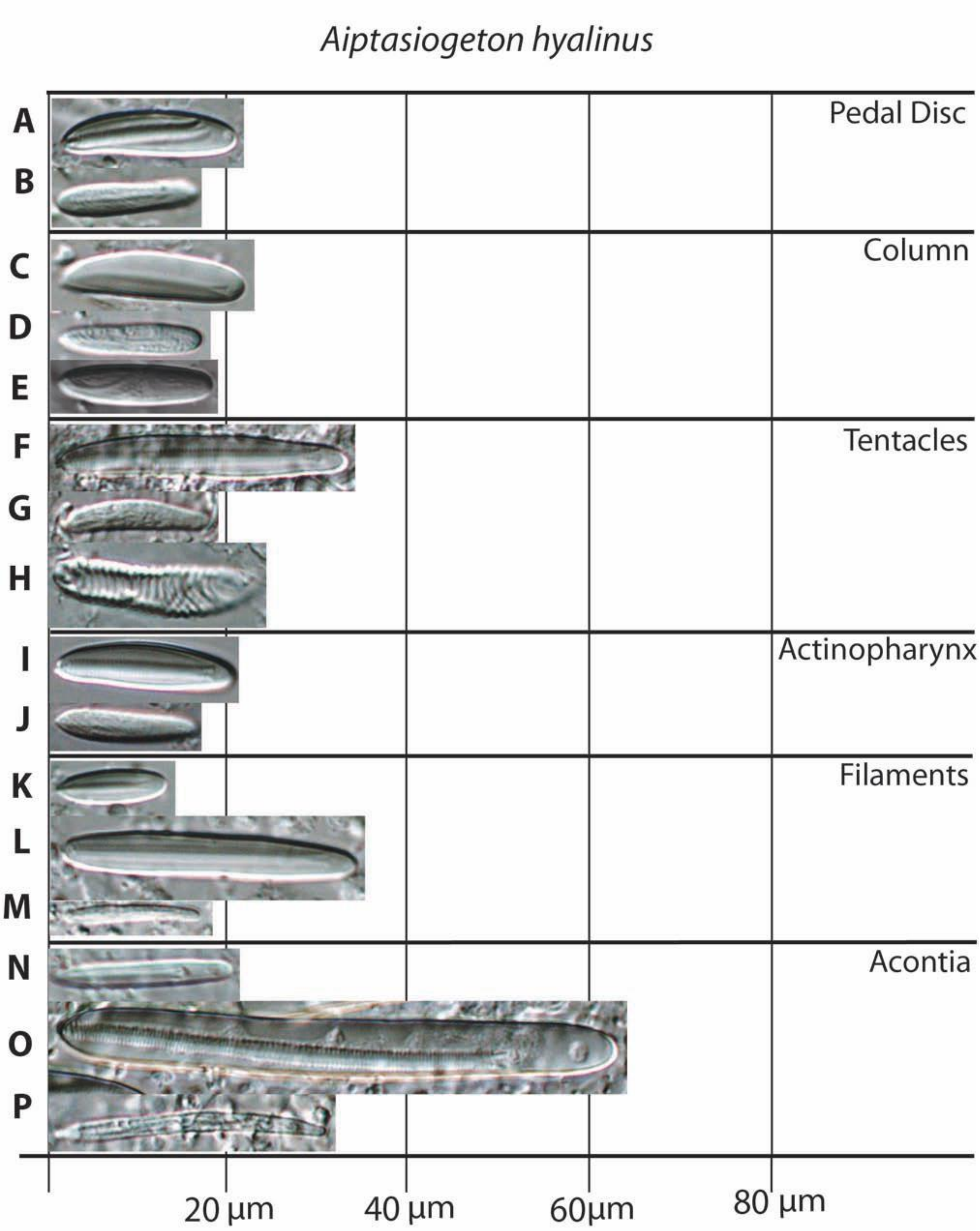

Aiptasiogeton hyalinus
Pedal Disc
Column
Tentacles
Actinopharynx
Filaments
Acontia
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
20 µm
40 µm
60µm
80 µm

Table 2.4. Size ranges of the cnidae of *Aiptasiogeton hyalinus*. $\overline{X}$, mean; SD, standard deviation; S, ratio of number of specimens in which each cnidae was found to number of specimens examined; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; Abbreviations: M, Microbasic.

Categories	Range of length and width of capsules (μm)	$\overline{X}$ ± SD	S	N	F
PEDAL DISC					
M *p*-amastigophores	(11.8–20.3) x (3.3–5.8)	16.2±2.1 x 4.2±0.6	2/2	58	+++
Basitrichs	(10.1–17.6) x (2.1–4.5)	14.3±1.7 x 3.1±0.6	2/2	38	+++
COLUMN					
M *p*-amastigophores	(15.1–22.4) x (2.9–6.1)	19.1±1.9 x 4.5±0.6	2/2	31	+++
Basitrichs	(8.9–19.1) x (2.6–4.9)	14.8±2.8 x 3.8±2.6	2/2	39	+++
M *b*-mastigophores	(11.5–19.1) x (2.1–7.8)	15.3±1.9 x 4.0±1.7	2/2	32	++
TENTACLES					
M *p*-amastigophores	(21.6–30.5) x (3.1–5.9)	26.5±2.4 x 4.4±0.7	2/2	40	+++
Basitrichs	(15.9–16.7) x (2.9–3.2)	16.2±0.5 x 3.1±0.2	2/2	32	++
Spirocysts	(10.3–21.8) x (2.4–5.7)	17.2±3.2 x 3.9±0.8	2/2	39	+++
ACTINOPHARYNX					
M *p*-amastigophores	(10.8–29.9) x (2.9-6.1)	19.4±2.8 x 4.1±0.7	2/2	34	+++
Basitrichs	(12.1–20.5) x (2.1-4.3)	15.6±2.6 x 3.2±0.6	2/2	32	+
FILAMENTS					
M *p*-amastigophores 1	(10.3–20.7) x (2.4–4.9)	13.3±2.9 x 3.4±0.5	2/2	39	+++
M *p*–amastigophores 2	(27.6–40.2) x (4.6–6.4)	33.1±3.7 x 5.4±0.5	2/2	33	++
Basitrichs	(11.6–16.7) x (1.4–3.5)	13.6±1.9 x 2.2±0.6	2/2	32	+
ACONTIA					
M *p*-amastigophores 1	(16.7–27.5) x (1.9–4.5)	22.3±3.1 x 3.2±0.6	2/2	36	++
M *p*-amastigophores 2	(45.4–69.3) x (4.6–7.5)	57.4±5.7 x 6.1±0.6	2/2	58	+++
Basitrichs	(26.8–37.1) x (1.5–3.6)	30.5±2.3 x 2.3±0.5	2/2	32	+++

Material examined

See Appendix 1

Description

External anatomy (Fig. 2.11): Pedal disc to 5 mm diameter. Column elongated, not divisible into scapus and capitulum, 7–20 mm height and 10–15 mm diameter in preserved specimens. Cinclides in mid-column, few, scattered. Mesenterial insertions visible. Oral disc to 10 mm diameter in preserved specimens. Tentacles to 96, smooth, tapering toward tips; inner tentacles longer than outer ones, 3–5 mm and 1–3 mm length in preserved specimens, respectively.

Internal anatomy and microanatomy (Fig. 2.12): Mesogleal marginal sphincter muscle diffuse, strong; fibers closer to gastrodermis. Mesenteries hexamerously arranged in five cycles (to 64 pairs). More mesenteries distally than proximally. First cycle and one pair of second cycle perfect; rest imperfect. Fifth cycle only present distally. All perfect mesenteries fertile, including directives. Two pairs of directives each associated with a siphonoglyph. Gonochoric. Retractor muscles restricted to reniform. Parietobasilar muscles differentiated, weak; fibers on thick mesogleal processes. Longitudinal muscles of tentacles ectodermal. Strong longitudinal ectodermal muscles in distal column. Basilar muscles well differentiated, with fibers on short but thick mesogleal pennon. Acontia few, well developed.

Color (Fig. 2.11): In living specimens column translucent with orange spots distally. Oral disc reddish. Tentacles translucent greyish with tips reddish to pink; those two corresponding with endocoels of directives with white base. Preserved specimens yellowish.

Cnidom: Spirocysts, basitrichs, microbasic *b*-mastigophores and *p*-amastigophores (Fig. 2.13). See Table 4 for size and distribution.

Geographic and bathymetric distribution. *Aiptasiogeton hyalinus* has been recorded in the western Mediterranean Sea, Atlantic coast of France and north of Spain, British Islands, Canary Islands, Atlantic coast of Morocco, Madeira, Azores, and Israel (reviewed in den Hartog & Ates 2011). In this study I provide a new record from the south Atlantic coast of Spain (see Appendix 1). *Aiptasiogeton hyalinus* is a shallow water species, found between 0–7 m.

Taxonomic remarks. The name of this species has a relatively complex history. Delle Chiaje (1822) provided a drawing and then in 1823 describes vaguely *Actinia hyalina*. Hollard (1848) concisely described *Aiptasiogeton hyalinus* in his thesis as *Actinia pellucida* on the French Atlantic coast. Fisher (1889) provides an adequate description of the species but named it as *Sagartia erythrochila* (Fisher, 1874) because he thought it was different than *A. pellucida*. Andres (1881) describes *Paractis comata* but then (Andres 1884) described it and *A. pellucida* under the name *Aiptasia lacerata* Dalyell, 1848 with three varieties. Two of those varieties, *A. lacerata planifrons* and *A. lacerata crucifrons* corresponded to *Aiptasiogeton hyalinus* but the third one, *A. lacerata ungicolata* corresponded to *Paractis comata* (synonym with *Sagartiogeton laceratus* (Dalyell, 1848) [Tur 1989; Fautin 2013]). In addition, Andres (1884) synonymized *Actinia hyalina* with *Aiptasia lacerata* although with some reservations.

Schmidt (1972) described the genus *Aiptasiogeton* for this species with material from the Mediterranean Sea as *A. comatus*, the same name that Manuel (1979) uses for specimens in the British Islands. However, Manuel (1981) corrected the name of the species to *A. pellucidus* because *Paractis comata* was introduced by Andres (1881) to designate a species that today is considered as *Sagartiogeton laceratus*.

According to den Hartog and Ates (2011) *Aiptasiogeton hyalinus* is the correct name for this species. I agree with those authors in considering the description of Delle Chiaje (1825) diagnostic — although the date of publication of the description provided by den Hartog and Ates (2011) is incorrect and I correct it here to 1822 (Fautin 2013). Thus, the name *A. hyalinus* precepts *A. pellucidus*. Furthermore, the name *pellucidus* is probably not available because it was published in a thesis (Tubbs 2001).

There are slight differences among the cnida data provided by Schmidt (1972), those of den Hartog and Ates (2011) and data in this study. However, I consider that most of the differences are attributable to different interpretation of cnidae categories (e.g. I consider one category of microbasic *p*-amastigophores in the filaments whereas den Hartog and Ates (2011) consider two, but the ranges of these nematocysts coincide), to low numbers of studied specimens (den Hartog and Ates (2011) only examined one specimen, and Schmidt (1972) and this work only examined two specimens) and the intraspecific variability among localities (Schmidt material was from the Mediterranean whereas den Hartog and Ates (2011) and ours are from the Atlantic coast of Spain). The studied specimens, similarly to that of den Hartog and Ates (2011), show an additional smaller category of microbasic *p*-amastigophores (1) in the acontia rather than only one as described by Schmidt (1972).

The other species of the genus, *Aiptasiogeton eruptaurantia* might be a synonymy of *A. hyalinus* (Schmidt 1972; den Hartog & Ates 2011); however, further studies are necessary to address this.

Genus *Bartholomea*

Diagnosis (after Carlgren 1949). Aiptasiidae with well developed pedal disc. Column *rather long,* smooth (or with minute papillae ?), with *up to five* rows of cinclides in mid-column; *column distinctly divisible into scapus and capitulum*. Mesogleal marginal sphincter weak. Tentacles to 192, rather long, not fully retractile, with spiral or incomplete annular raised bands with nematocyst batteries; bands throughout entire tentacle or near tip only. More mesenteries distally than proximally. Perfect mesenteries 6–8 pairs, fertile. *Oral stoma*. Retractors diffuse, sometimes fairly restricted. Symbiotic with *Symbiodinium* spp. *No asexual reproduction* (?). Cnidom: spirocysts, basitrichs, and microbasic *b*-mastigophores and *p*-amastigophores.

Type species. Currently no type species; the designation of *Actinia annulata* by Stephenson (1920) and agreed to by Carlgren (1949) is invalid because this species is not among species originally in genus (Fautin 2013).

Included species. *Bartholomea annulata*; *Bartholomea pseudotagetes* Pax, 1924 (?); *B. werneri* Watzl, 1922 (?); *B. peruviana* Pax, 1924.

Taxonomic remarks. I specified in the diagnosis of the genus that cinclides in mid-column might be arranged in up to five rows as I observed in *Bartholomea annulata*. I also added the presence of oral stoma because I detected them in *B. annulata*.

Bartholomea annulata

(Figs. 2.14–2.16, Table 2.5)

Synonymy

Actinia annulata Le Sueur, 1817

Actinia solifera Le Sueur, 1817

Non *Actinia annulata* Gay, 1854

Paractis solifera: Milne Edwards 1857

Dysactis annulata: Milne Edwards 1857

Bartholomea solifera Duchassaing de Fombressin & Michelotti, 1864.

Bartholomea [*sic*] *Solifera:* Duchassaing 1870

Aiptasia solifera: Andres 1883 (1884)

Aiptasia annulata: Andres 1883 (1884)

Aiptasia annulata solifera: Verrill 1907

Bartholomea annulata: Stephenson 1920

? *Carlgreniella robusta* Watzl, 1922

Aiptasia arrulata Atoda, 1954

Batholomea [*sic*] *annulata*: Chen *et al.* 2008

Material examined

See Appendix 1

Description

External anatomy (Fig. 2.14): Pedal disc to 40 mm diameter in preserved specimens. Column elongated, divisible into scapus and capitulum, to 40 mm height and to 20 mm diameter in preserved specimens (to 100 mm height and 40 mm diameter in living specimens). Cinclides in mid-column, in 4–5 rows. Small, rounded oral stoma. Mesenterial insertions visible. Oral disc to 13 mm diameter in preserved specimens. Tentacles to 192, tapering toward tips, rather long, all of same length, 5–11 mm in preserved specimens (50–70 mm length in living specimens). Tentacles not fully retractile, with spiral or incompletely annular raised bands with batteries of microbasic *p*-amastigophores and basitrichs; bands throughout entire tentacle.

Internal anatomy and microanatomy (Fig. 2.15): Mesogleal marginal sphincter muscle diffuse, short and weak. Mesenteries hexamerously arranged in five cycles. More mesenteries proximally than distally. Only first cycle perfect. All cycles fertile. including directives. Two pairs of directives each associated with a deep siphonoglyph. Retractor muscles diffuse, strong. Parietobasilar muscles differentiated, weak. Longitudinal muscles of tentacles ectodermal. Relatively weak longitudinal ectodermal muscles in distal end of column. Basilar muscles well differentiated, distinct, strong, with fibers on long mesogleal pennon. Acontia numerous, well developed.

FIGURE 2.14. External anatomy of *Bartholomea annulata*. A) Oral view of living specimen. B) Detail of tentacular bands (arrow). C) Lateral view of preserved and contracted specimen; notice up to five rows of cinclides. Scale bars: A–C, 10 mm.

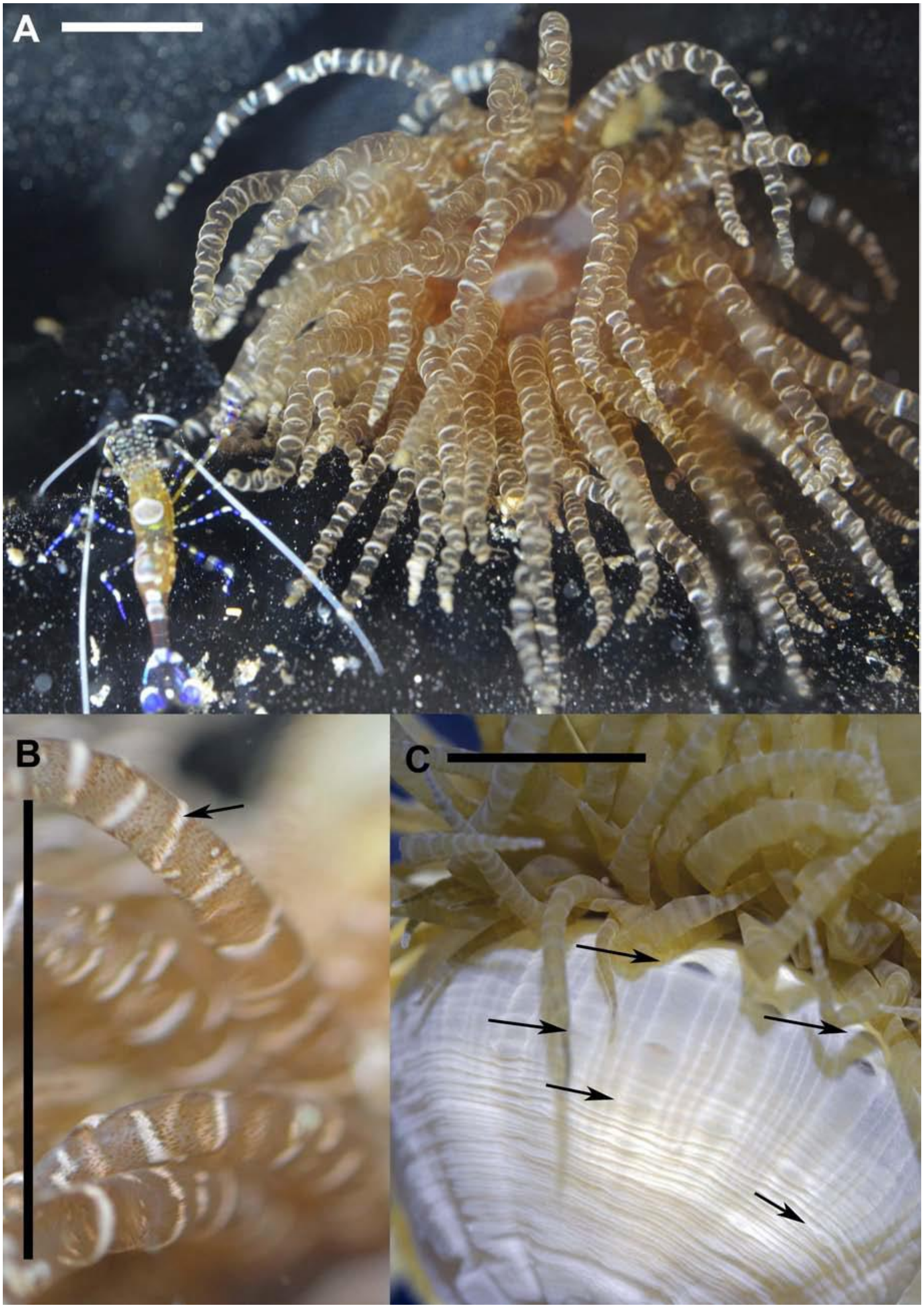

A
B
C

FIGURE 2.15. Internal anatomy of *Bartholomea annulata*. A) Cross section of the column at the level of the actinopharynx showing the cycles of mesenteries (indicated by roman numbers between pairs). B) Detail of a longitudinal section of the proximal end showing the distinct basilar muscles (arrows). C) Cross section through distal column showing the weak ectodermal longitudinal musculature. D) Longitudinal section of the distal column showing weak mesogleal marginal sphincter muscle. E) Cross section through a tentacle showing the ectodermal longitudinal musculature. F) Longitudinal section of the tentacle; notice zooxanthellae in gastrodermis and nematocyst batteries in the epidermis (arrows). Abbreviations: ep, epidermis; ga, gastrodermis; me, mesoglea; s, siphonoglyph. Scale bars: A, C–F, 0.1 mm; B, 0.05 mm.

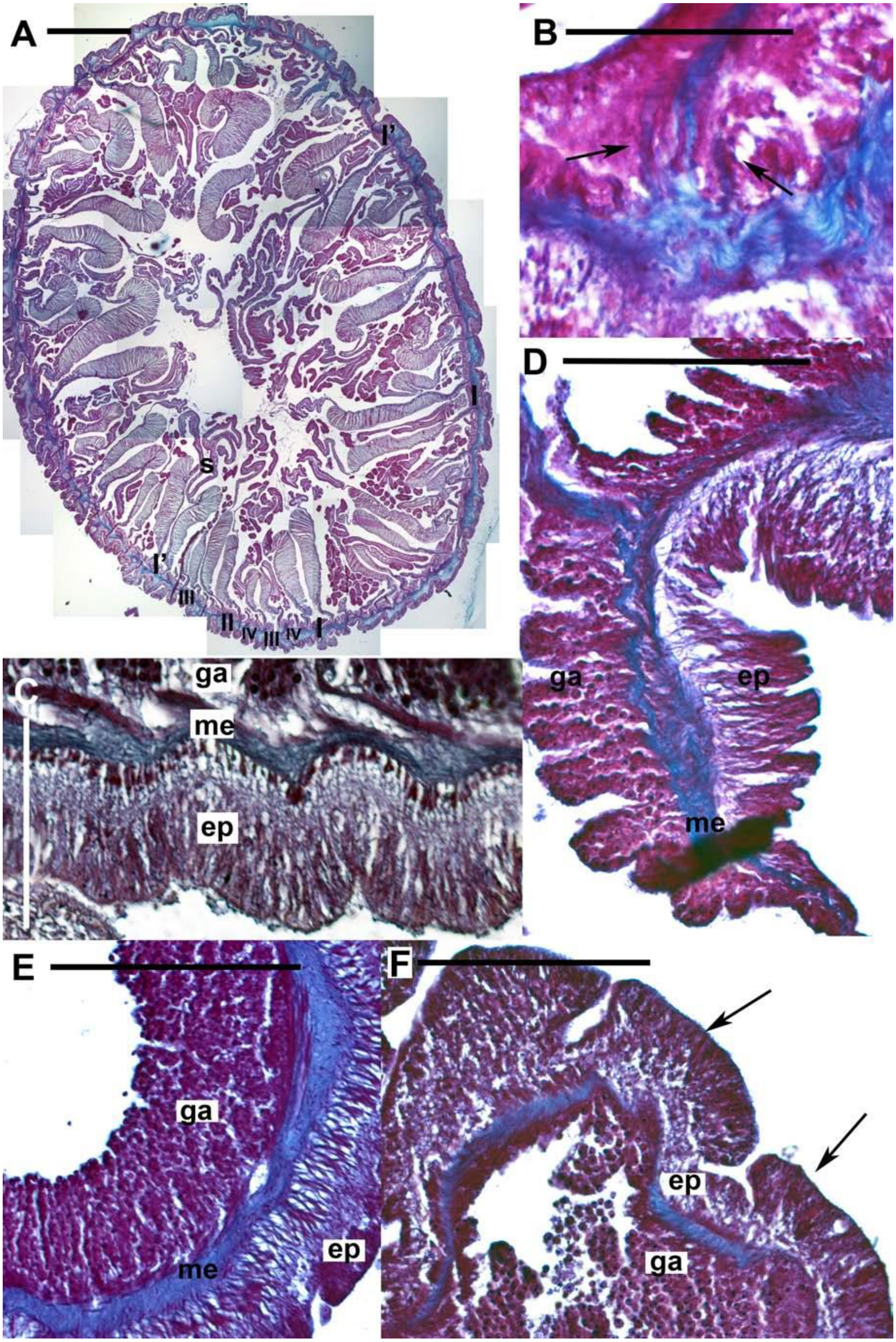

A
I'
I
S
I'
III
II
IV
III
IV
I
B
C
ga
me
ep
D
ga
ep
me
E
ga
me
ep
F
ep
ga

FIGURE 2.16. Cnidae of *Bartholomea annulata*. A, C, F, I, K, L, N, O) Microbasic *p*-amastigophores. B, D, G, J, M, P) Basitrichs. E) Microbasic *b*-mastigophore. H) Spirocyst.

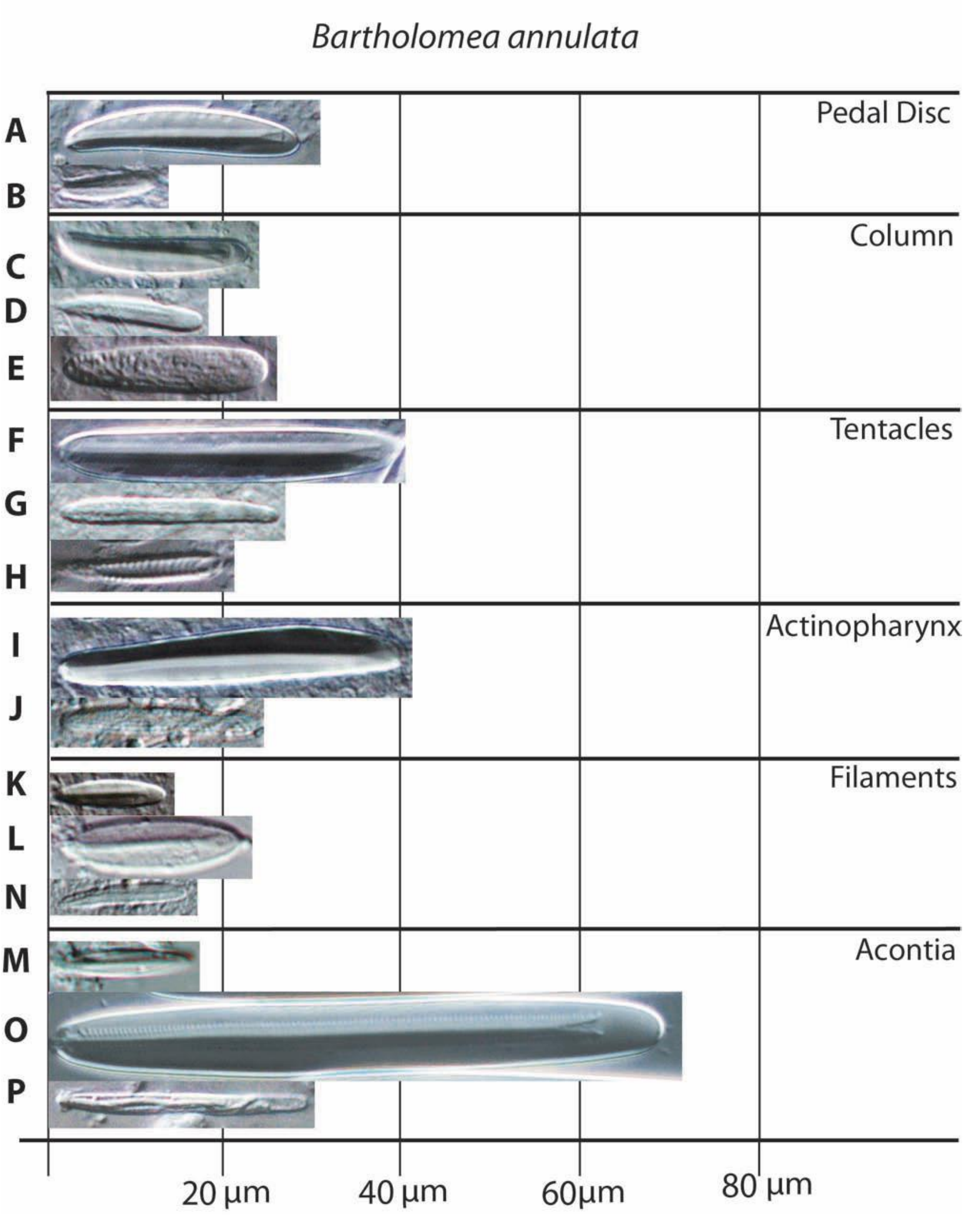

Bartholomea annulata
A
B
C
D
E
F
G
H
I
J
K
L
N
M
O
P
Pedal Disc
Column
Tentacles
Actinopharynx
Filaments
Acontia
20 µm
40 µm
60µm
80 µm

Table 2.5. Size ranges of the cnidae of *Bartholomea annulata*. $\overline{X}$, mean; SD, standard deviation; S, ratio of number of specimens in which each cnidae was found to number of specimens examined; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; Abbreviations: M, Microbasic.

Categories	Range of length and width of capsules (µm)	$\overline{X}$ ± SD	S	N	F
PEDAL DISC					
M *p*-amastigophores	(18.2–25.9) x (3.6–5.2)	22.1±2.2 x 4.5±0.4	2/2	29	+++
Basitrichs	(10.3–15.7) x (1.8–3.2)	13.1±0.4 x 2.4±0.4	2/2	26	+++
COLUMN					
M *p*-amastigophores	(20.1–25.7) x (3.8–5.7)	23.2±1.4 x 4.8±0.5	2/2	39	+++
Basitrichs	(10.7–20.2) x (2.6–3.9)	15.9±3.1 x 3.2±0.4	2/2	29	+++
M *b*-mastigophores	(18.7–26.8) x (3.4–5.7)	21.5±2.7 x 4.4±0.6	2/2	23	++
TENTACLES					
M *p*-amastigophores	(18.7–43.1) x (2.6–7.7)	37.4±5.2 x 5.5±1.1	2/2	44	+++
Basitrichs	(17.4–31.2) x (2.2–4.7)	23.1±4.1 x 3.2±0.6	2/2	43	+++
Spirocysts	(12.8–33.3) x (1.7–5.9)	19.8±6.2 x 3.5±1.1	2/2	26	+++
ACTINOPHARYNX					
M *p*-amastigophores	(31.4–40.7) x (3.9–6.6)	36.2±2.3 x 5.1±0.7	2/2	38	+++
Basitrichs	(9.9–21.5) x (2.3–4.4)	15.7±4.4 x 3.4±0.7	2/2	34	+++
FILAMENTS					
M *p*-amastigophores 1	(10.2–16.7) x (2.1–5.1)	12.9±1.3 x 3.2±0.8	2/2	33	+++
M *p*-amastigophores 2	(19.4–37.4) x (4.3–6.3)	26.4±8.3 x 5.3±0.8	2/2	34	+++
Basitrichs	(10.9–14.2) x (2.1–4.9)	12.6±1.1 x 3.4±0.8	2/2	31	+
ACONTIA					
M *p*-amastigophores 1	(14.3–19.2) x (2.2–3.5)	16.2±1.6 x 2.8±0.3	2/2	38	+++
M *p*-amastigophores 2	(60.3–71.6) x (7.7–9.9)	65.6±3.3 x 8.5±0.5	2/2	37	+++
Basitrichs	(24.4–29.3) x (1.9–3.4)	27.2±1.2 x 2.6±0.4	2/2	31	+++

Color (Fig. 2.14): Living specimens with proximal column translucent whitish, brownish with white dots distally; tentacles brown with distinct white annular bands. Preserved specimens with whitish column; tentacles dark brown when preserved.

Cnidom: Spirocysts, basitrichs microbasic *b*-mastigophores and *p*-amastigophores (Fig. 2.16). See Table 2.5 for size and distribution.

Geographic and bathymetric distribution. *Bartholomea annulata* is reported along the entire Caribbean Sea, from Bermuda to Barbados (González-Muñoz *et al.* 2012). It is a shallow-water species found between 1–20 m.

Taxonomic remarks. Currently, *Bartholomea* includes four valid species (Fautin 2013); all but *B. peruviana* (described from the coast of Peru) are distributed in the Caribbean Sea. Waltz (1922) provides a relatively detailed description of *B. werneri* and differentiates it from *B. annulata* based on the number of tentacles (to 96 *B. werneri vs.* to 192) and shape of tentacular bands (wide but flatter in *B. werneri*), and because the cinclides are not conspicuous externally in *B. werneri*. He also provides an account of cnidae sizes and ranges of both species but it is incomplete, some types of nematocysts are missing for several body regions, furthermore the provided ranges do not differ between both species. The cited differences may be due to less developed examined specimens and intraspecific variation. In addition, Waltz (1922) erroneously describes the genus *Bartholomea* with the first cycle of mesenteries sterile. I agree with Gonzalez-Muñoz *et al.* (2012) in that species descriptions for this genus (except for *B. annulata*) are not complete to modern taxonomic standards and no clear differences distinguish among the four putative species. Pending examination of the available type material (for

B. annulata and *B. werneri*) and further revision I currently consider two valid species: *B. annulata* distributed along the entire Caribbean Sea and *B. peruviana* distributed along the coast of Peru.

Genus *Bellactis*

Diagnosis (after Dube 1983). Aiptasiidae with broad base. Column elongate, smooth, thin, with 3–4 rows of cinclides in mid-column; column not distinctly divide into scapus and capitulum. Mesogleal marginal sphincter strong. Oral disc lobed. Tentacles very numerous, rather short. More mesenteries proximally than distally. Mesenteries irregularly arranged; seven pairs of perfect mesenteries; first and second cycles fertile. Retractor muscles diffuse. Symbiotic with *Symbiodinium* spp. *Asexual reproduction (?).* Cnidom: spirocysts, basitrichs, and microbasic *b*-mastigophores and *p*-amastigophores.

Type species. *Bellactis ikalyseae* Dube, 1983 by monotypy.

Included species. *Bellactis ikalyseae.*

Bellactis ikalyseae

(Figs. 2.17–2.19, Table 2.6)

Synonymy

Bellactis ikalyseae Dube, 1974 (*nomen nudum*)

Bellactis ikalyseae Dube, 1983

Bellactis ikalysae [*sic*]: Gasparini *et al.* 2005

Material examined

See Appendix 1

Description

External anatomy (Fig. 2.17): Pedal disc to 10 mm diameter in preserved specimens. Column elongated, not divisible into regions, to 22 mm height and to 10 mm diameter in preserved specimens. Cinclides not numerous, in mid-column, in 3–4 rows. Mesenterial insertions visible. Oral disc to 20 mm diameter in preserved specimens. Tentacles to ~204, smooth, tapering toward tips, 2–3 mm length in preserved specimens; irregularly arranged.

Internal anatomy and microanatomy (Fig. 2.18): Marginal sphincter muscle mesogleal, diffuse, very weak. Mesenteries irregularly arranged in four cycles. First cycle perfect, with 7 pairs of mesenteries; second (6 pairs) and third (~28 pairs) cycles imperfect; fourth cycle only proximally. First and second cycles fertile, including directives. Two pairs of directives each associated with a siphonoglyph. Retractor muscles diffuse to restricted. Parietobasilar muscles poorly developed. Longitudinal muscles of tentacles ectodermal. Relatively strong longitudinal ectodermal muscles in distal end of column. Basilar muscles well differentiated, with fibers on short and thick mesogleal pennon. Acontia very numerous, well developed.

FIGURE 2.17. External anatomy of *Bellactis ikalyseae*. A, B) Oral and lateral view of living specimen. C) Oral view of preserved specimen; notice irregular arrangement of tentacles. D) Lateral view of preserved specimen; notice cinclides and acontia (arrows). Scale bars: A–D, 10 mm.

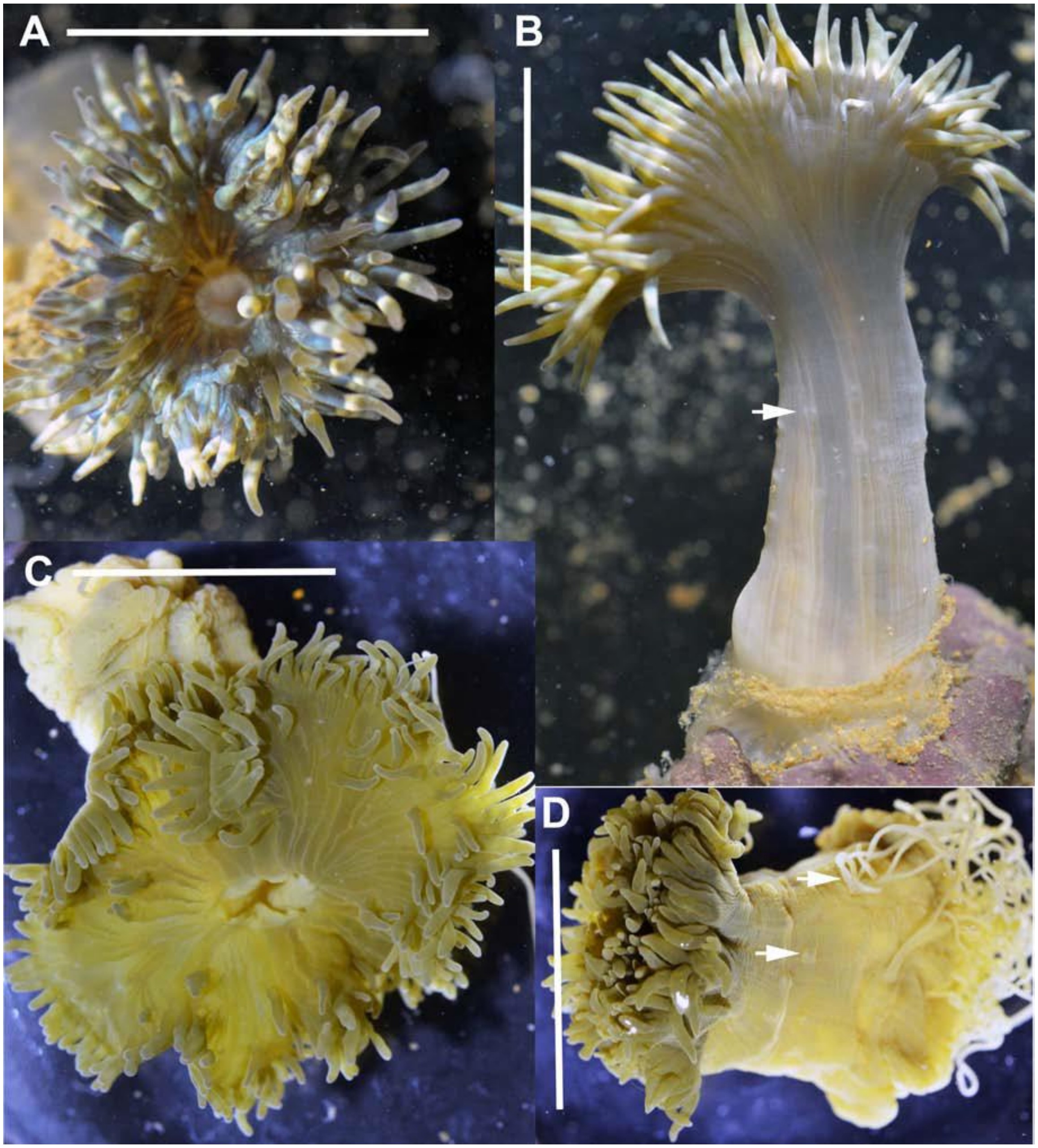

A
B
C
D

FIGURE 2.18. Internal anatomy of *Bellactis ikalyseae*. A) Cross section of the column at the level of the actinopharynx showing the cycles of mesenteries(indicated by roman numbers between pairs). B) Cross section through a tentacle showing the ectodermal longitudinal musculature; notice zooxanthellae in gastrodermis. C) Cross section through distal column showing the ectodermal longitudinal musculature. D) Detail of a longitudinal section of the proximal end showing the well-developed basilar muscles. E) Longitudinal section of the distal column showing weak mesogleal marginal sphincter muscle. Abbreviations: ep, epidermis; ga, gastrodermis; me, mesoglea. Scale bars: A–F, 0.1 mm.

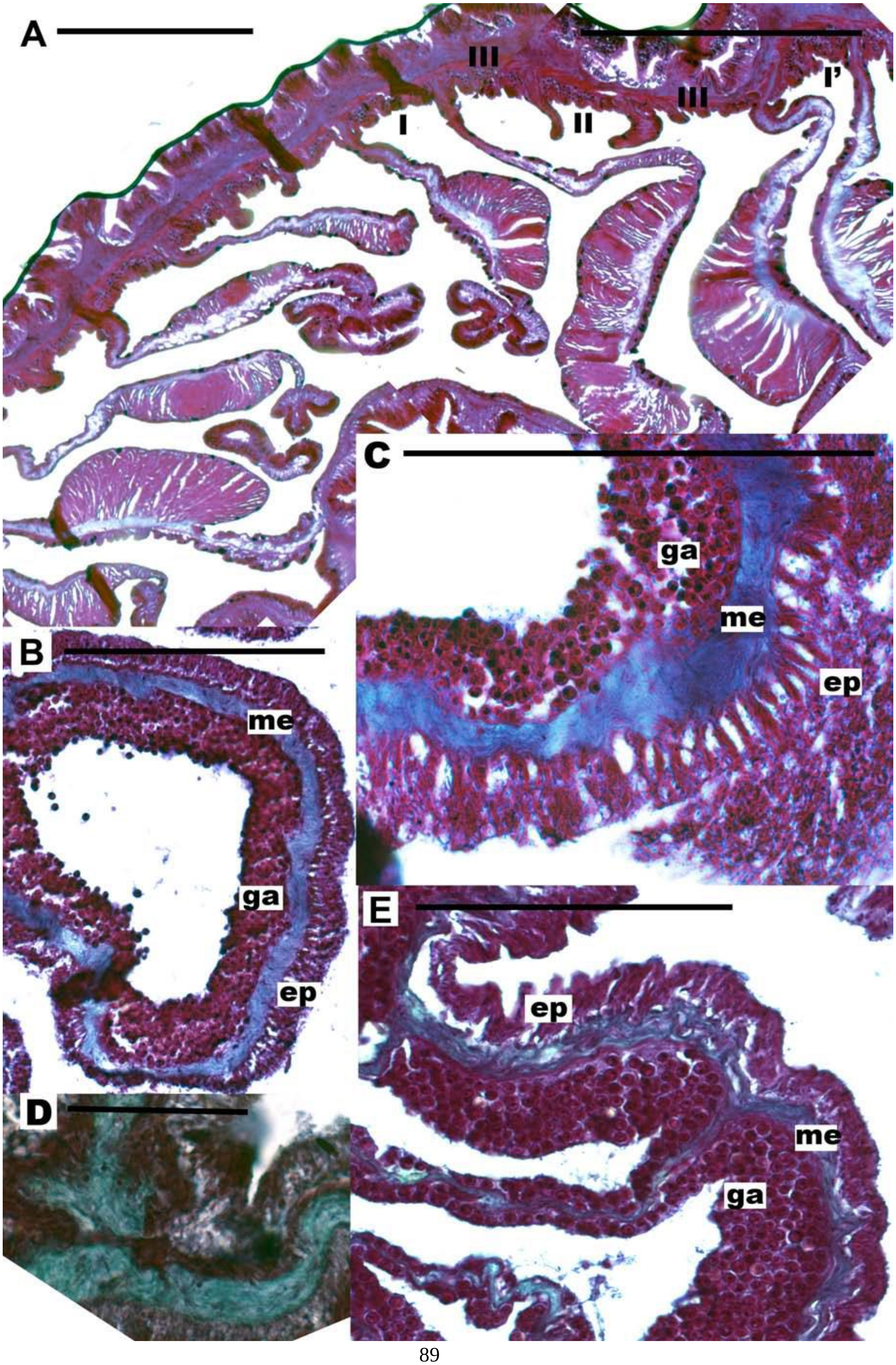

A
III
III
I
II
I'
C
ga
me
ep
B
me
ga
ep
D
E
ep
me
ga

FIGURE 2.19. Cnidae of *Bellactis ikalyseae*. A, C, F, I, K, L, N, O) Microbasic *p*-amastigophores. B, D, G, J, M, P) Basitrichs. E) Microbasic *b*-mastigophore. H) Spirocyst.

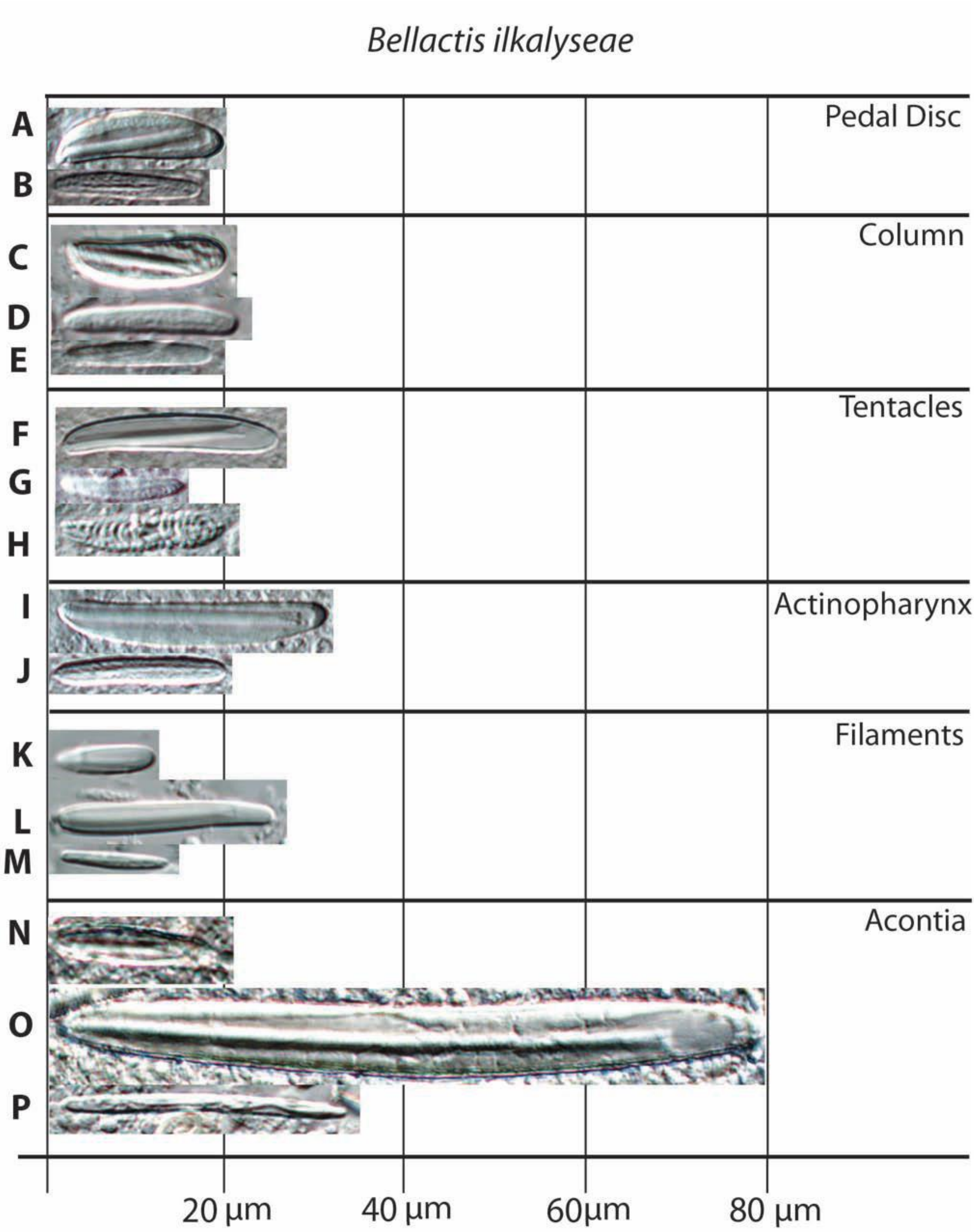

Bellactis ilkalyseae
A
B
Pedal Disc
C
D
E
Column
F
G
H
Tentacles
I
J
Actinopharynx
K
L
M
Filaments
N
O
P
Acontia
20 µm
40 µm
60µm
80 µm

Table 2.6. Size ranges of the cnidae of *Bellactis ilkalyseae*. $\overline{X}$, mean; SD, standard deviation; S, ratio of number of specimens in which each cnidae was found to number of specimens examined; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; Abbreviations: M, Microbasic.

Categories	Range of length and width of capsules (µm)	$\overline{X}$ ± SD	S	N	F
PEDAL DISC					
M *p*–amastigophores 1	(21.8-25.6) x (3.4-5.5)	19.4 ±2.3 x 4.3 ±0.6	2/2	44	+++
Basitrichs	(10.7-23.7) x (2.3-3.4)	16.2 ±0.3 x 2.8 ±0.3	2/2	34	+++
COLUMN					
M *p*–amastigophores 1	(11.7-25.4) x (3.1-5.2)	16.5 ±2.9 x 4.1 ±0.5	2/2	35	+++
Basitrichs	(9.7-22.4) x (2.3-4.3)	15.9 ±2.9 x 3.1 ±0.5	2/2	44	+++
M *b*-mastigophores	(22.2-30.6) x (3.2-5.6)	26.8 ±0.4 x 4.3 ±0.5	2/2	42	++
TENTACLES					
M *p*–amastigophores 1	(15.4-36.4) x (3.4-5.3)	24.6 ±4.5 x 4.4 ±0.4	2/2	78	+++
Basitrichs	(12.4-19.7) x (1.9-3.3)	15.9 ±1.9 x 2.7 ±0.4	2/2	34	+++
Spirocysts	(11.5-27.5) x (2.2-5.1)	17.1 ±3.7 x 3.5 ±0.6	2/2	37	+++
ACTINOPHARYNX					
M *p*–amastigophores 1	(25.2-30.1) x (3.5-4.5)	28.5 ±1.6 x 4.2 ±0.3	2/2	32	+++
Basitrichs	(15.8-22.8) x (2.3-4.1)	19.4 ±1.9 x 3.1 ±0.5	2/2	38	++
FILAMENTS					
M *p*–amastigophores 1	(9.5-12.6) x (1.9-4.1)	11.4 ±0.7 x 3.2 ±0.4	2/2	33	++
M *p*–amastigophores 2	(22.3-36.3) x (3.1-6.4)	32.1 ±3.5 x 5.2 ±0.2	2/2	37	+++
Basitrichs	(9.7-16.7) x (1.6-2.8)	11.7 ±1.7 x 2.4 ±0.4	2/2	33	+
ACONTIA					
M *p*–amastigophores 1	(13.8-18.7) x (2.1-3.4)	16.1 ±1.9 x 2.8 ±0.4	2/2	29	++
M *p*–amastigophores 2	(66.4-88.6) x (5.7-8.2)	74.7 ±5.5 x 7.1 ±0.5	2/2	43	+++
Basitrichs	(24.6-35.7) x (1.7-3.2)	30.9 ±2.5 x 2.2 ±0.3	2/2	40	+++

Color (Fig. 2.17): Living specimens with column whitish-translucent; tentacles with yellow tips, grey-bluish with irregular spots proximally. Preserved specimens with yellow column and slightly darker tentacles.

Cnidom: Spirocysts, basitrichs, microbasic *b*-mastigophores and *p*-amastigophores (Fig. 2.19). See Table 2.6 for size and distribution.

Geographic and bathymetric distribution. *Bellactis ikalyseae* is distributed in the coast of Brazil (Fautin 2013). This is an intertidal species found between 0–2 m.

Taxonomic remarks. *Bellactis* was originally placed within the family Sagartiidae (Dube 1983). However, general external and internal anatomy (particularly the presence of ectodermal longitudinal muscle along distal column) and the cnidom correspond with those of Aiptasiidae.

Our specimens agree well with the original description of the species (Dube 1983). Dube (1983) describes the retractor muscles as diffuse and relatively weak whereas in our specimens these muscles are relatively strong and restricted.

Genus *Ragactis*

Diagnosis (after Carlgren 1949). Aiptasiidae with well developed pedal disc. Column smooth, with to five rows of cinclides in mid-column; *column distinctly divisible into scapus and capitulum*. Mesogleal marginal sphincter weak. Tentacles to 192, not fully retractile with scattered, spherical hollow vesicles with nematocyst batteries and longitudinal muscles. More mesenteries distally than proximally. Six pairs of perfect and fertile mesenteries. *Oral stoma.* Retractors diffuse. Symbiotic with *Symbiodinium* spp. *No* *asexual reproduction (?).* Cnidom: spirocysts, basitrichs and *microbasic b-mastigophores* and *p*-amastigophores.

Type species. *Capnea lucida* Duchassaing de Fombressin & Michelotti, 1860 by original designation.

Included species. *Ragactis lucida* (Duchassaing de Fombressin & Michelotti, 1860); *R. hyalina* (Le Sueur, 1817) (?).

Ragactis lucida

(Figs. 2.20–2.21, Table 7)

Synonymy

Capnea lucida Duchassaing de Fombressin & Michelotti, 1860

Heteractis lucida: Duchassaing de Fombressin & Michelotti 1864

Heteractis Lucida [*sic*]: Duchassaing de Fombressin 1870

Ragactis lucida: Andres 1883 (1884)

Aiptasia lucida: Duerden 1897

Bartholomea pseudoheteractis Watzl, 1922

Bartholomea lucida: Carlgren 1949

Material examined

See Appendix 1

External anatomy (Fig. 2.20): Pedal disc to 50 mm diameter in preserved specimens. Column elongated, divisible in scapus and capitulum, to 50 mm height and to 30 mm diameter in preserved specimens (to 120 mm height and 50 mm diameter in living specimens). Cinclides in mid-column, very numerous, in to five rows, corresponding with endocoels of first two cycles of mesenteries. Rounded, relatively big oral stoma. Mesenterial insertions visible. Oral disc to 30 mm diameter in preserved specimens. Tentacles to 192, tapering toward tips, all of same length, 10–30 mm in preserved

FIGURE 2.20. External anatomy of *Ragactis lucida.* A) Lateral view of living specimen. B) Lateral view of preserved and contracted specimen. C) Detail of tentacular vesicles. Scale bars: A–C, 10 mm.

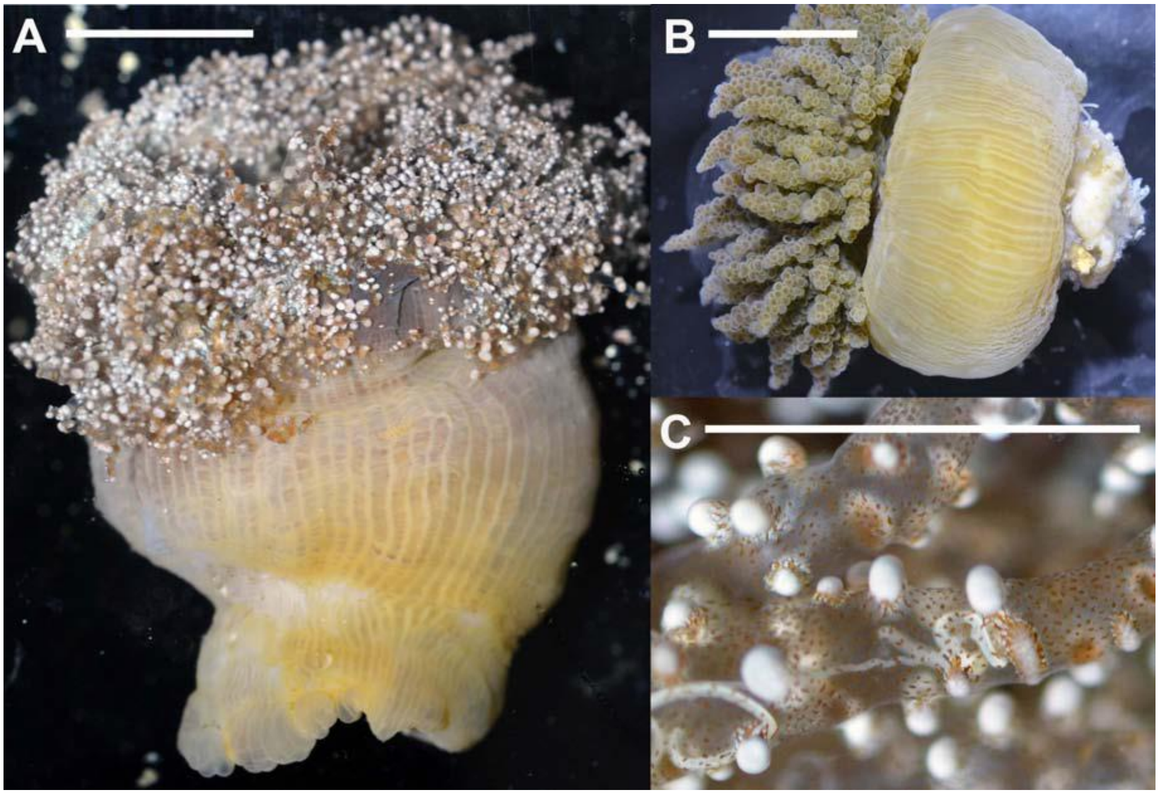

A
B
C

FIGURE 2.21. Internal anatomy of *Ragactis lucida*. A) Cross section of the column at the level of the actinopharynx showing the cycles of mesenteries(indicated by roman numbers between pairs). B) Detail of a longitudinal section of the proximal end showing the distinct basilar muscles (arrows). C) Longitudinal section of the distal column showing the weak mesogleal marginal sphincter muscle. D) Cross section through distal column showing the ectodermal longitudinal musculature. E) Cross section through a tentacle showing the ectodermal longitudinal musculature. F) Detail of longitudinal section of the tentacle showing a tentacular vesicle; notice zooxanthellae in gastrodermis and nematocyst batteries in epidermis (arrows). Abbreviations: ep, epidermis; ga, gastrodermis; me, mesoglea; s, siphonoglyph. Scale bars: A, C–F, 0.1 mm; B, 0.05 mm.

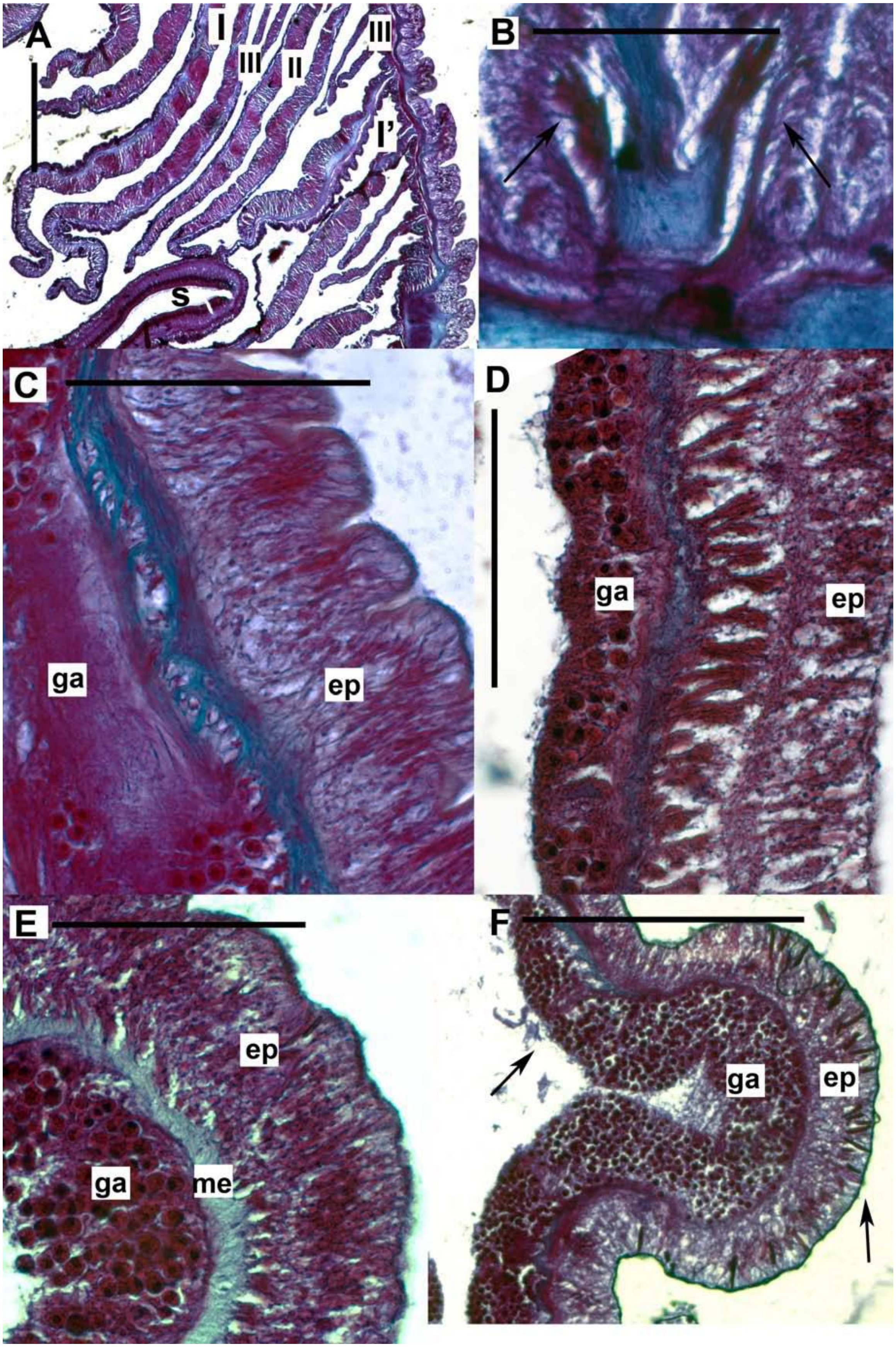
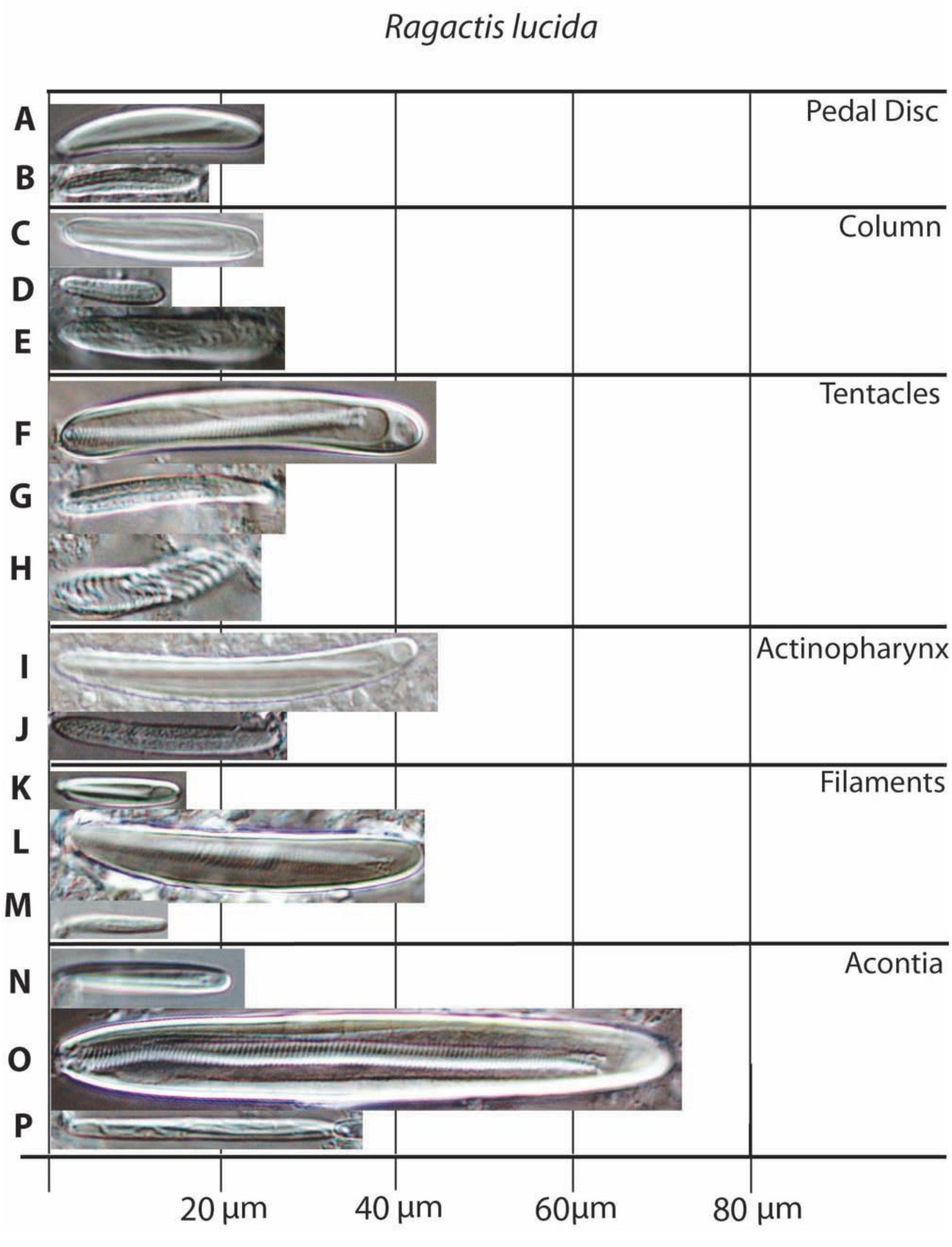

A
I
III
II
III
I'
s
B
C
ga
ep
D
ga
ep
E
ep
ga
me
F
ga
ep

FIGURE 2.22. Cnidae of *Ragactis lucida*. A, C, F, I, K, L, N, O) Microbasic *p*-amastigophores. B, D, G, J, M, P) Basitrichs. E) Microbasic *b*-mastigophore. H) Spirocyst.



Ragactis lucida
A
B
Pedal Disc
C
D
E
Column
F
G
H
Tentacles
I
J
Actinopharynx
K
L
M
Filaments
N
O
P
Acontia
20 µm
40 µm
60µm
80 µm

Table 2.7. Size ranges of the cnidae of *Ragactis lucida*. $\overline{X}$, mean; SD, standard deviation; S, ratio of number of specimens in which each cnidae was found to number of specimens examined; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; Abbreviations: M, Microbasic.

Categories	Range of length and width of capsules (μm)	$\overline{X}$ ± SD	S	N	F
PEDAL DISC					
M *p*-amastigophores	(20.4–34.7) x (3.5–6.3)	23.8±3.2 x 4.9±0.7	2/2	38	+++
Basitrichs	(11.9–27.7) x (1.9–5.7)	16.6±1.1 x 5.7±1.1	2/2	32	+++
COLUMN					
M *p*-amastigophores	(13.1–22.3) x (3.2–5.6)	17.1±2.7 x 4.2±0.5	2/2	35	+++
Basitrichs	(11.1–17.1) x (1.4–2.9)	13.6±1.8 x 2.3±0.4	2/2	34	+++
M *b*-mastigophores	(16.6–24.8) x (3.2–5.1)	21.9±1.8 x 4.1±0.5	2/2	34	+++
TENTACLES					
M *p*-amastigophores	(36.1–54.7) x (18.1–25.2)	41.8±3.8 x 6.5±0.9	2/2	30	+++
Basitrichs	(18.1–25.2) x (1.5–3.1)	21.2±2.3 x 2.5±0.5	2/2	28	++
Spirocysts	(15.1–30.3) x (2.3–6.7)	22.4±4.4 x 3.9±0.9	2/2	37	+++
ACTINOPHARYNX					
M *p*-amastigophores	(34.1–40.1) x (4.7–5.8)	37.4±1.8 x 5.1±0.4	2/2	41	+++
Basitrichs	(18.2–26.5) x (2.2–3.5)	22.3±2.4 x 2.9±0.4	2/2	38	++
FILAMENTS					
M *p*-amastigophores 1	(12.2–14.2) x (2.3–3.1)	13.2±0.6 x 2.8±0.2	2/2	31	+++
M *p*-amastigophores 2	(38.6–44.5) x (5.3–7.2)	40.7±1.8 x 6.2±0.7	2/2	30	+++
Basitrichs	(11.6–12.9) x (1.9–3.6)	12.2±0.4 x 2.4±0.6	2/2	27	+
ACONTIA					
M *p*-amastigophores 1	(61.8–71.1) x (8.4-10.3)	67.1±2.8 x 9.5±0.5	2/2	33	++
M *p*-amastigophores 2	(15.6–16.5) x (3.2–3.3)	16.1±0.6 x 3.3±0.1	2/2	32	+++
Basitrichs	(22.7–35.1) x (1.5–3.2)	30.6±2.4 x 2.3±0.4	2/2	35	+++

specimens (50–70 mm length in living specimens). Tentacles not fully retractile, with hollow vesicles with batteries of microbasic *p*-amastigophores and basitrichs.

Internal anatomy and microanatomy (Fig. 21): Marginal sphincter muscle mesogleal, diffuse, weak. More mesenteries distally than proximally. Mesenteries hexamerously arranged in five cycles. All cycles fertile, including directives. Two pairs of directives each associated with a deep siphonoglyph. Retractor muscles diffuse, long, occupying entire mesentery. Parietobasilar muscles well differentiated with strong processes. Longitudinal muscles of tentacles ectodermal. Strong longitudinal ectodermal muscles in distal end of column. Basilar muscles well differentiated, distinct, strong, with fibers on long mesogleal pennon. Acontia very numerous, well developed.

Color (Fig. 2.20): Living specimens with yellowish-translucent column proximally, darker distally; tentacles translucent with brown small spots and distinct white vesicles. Preserved specimens with yellowish column proximally and slightly darker distal column and tentacles.

Cnidom: Spirocysts, basitrichs, microbasic *b*-mastigophores and *p*-amastigophores (Fig. 2.22). See Table 2.7 for size and distribution.

Geographic and bathymetric distribution. From Bahamas to Barbados, along the entire Caribbean Sea (González-Muñoz *et al*. 2012). This is a shallow water species found between 1–15 m.

Taxonomic remarks. *Ragactis lucida* has a complicated taxonomic history (reviewed in González-Muñoz *et al*. 2012). I agree with González-Muñoz *et al*. (2012) in that based on morphology *R. lucida* clearly belongs within Aiptasiidae. Available information on the other species of the genus, *R. hyalina,* is too scarce to determine the identity of the species (Le Sueur 1817; Milne Edwards 1857); thus, the status of *R. hyalina* and its relationship with *R. lucida* remains unclear.

As previously suggested (e.g. Dunn 1981; González-Muñoz *et al*. 2012), the present results show that *R. lucida* and *B. annulata* basically differ in the morphological structure of the tentacles, and thus both genera may be synonyms. However, I prefer to keep both genera separate until the status of the additional species within both genera is studied further. Furthermore, molecular data do not support a sister relationship between *Bartholomea* and *Ragactis* (Rodríguez & Grajales in prep.).

ACKNOWLEDGEMENTS

I am very grateful to colleagues that assisted us with fieldwork, particularly to P.J. López González (Universidad de Sevilla, Spain), L. Gusmão (Universidade de São Paulo, Brazil), R. González (Universidad Nacional Autónoma de Mexico, UNAM), P. Manent (Instituto Canario de Ciencias Marinas, Spain), P. Wirtz, C. Arvanitidis (Hellinic Centre for Marine Research, MRC, Greece), R. Collins (Smithsonian Tropical Research Institute, STRI, Panama), K. Hiscock (Marine Biological Association Plymouth, U.K.), and Joerg Frommlet (Centro de Estudios do Ambiente e do Mar, CESAM, Portugal). Daphne Fautin (Kansas University, USA) and D. Lauretta (Museo Argentino de Ciencias Naturales Bernardino Rivadavia) provided helpful comments on nomenclature. Comments from A. Reft and M. Brugler (AMNH) improved this manuscript. Support for this work was provided by the RGGS Graduate Fellowship, the Lerner-Gray Fund for

Marine Research (AMNH), a Doctoral Dissertation Improvement Grant (NSF DEB 1110754) to AG and ER, and the Bermuda Institute of Ocean Sciences (BIOS, Bermuda).

References

Andres, A. (1881) Prodromus neapolitanae actiniarum faunae addito generalis actiniarium bibliographiae catalogo. *Mitteilungen aus der Zoologischen Station zu Neapel*, 2, 305–309.

Andres, A. (1883) *Le Attinie (Monografia). Bibliografia, introduzione e specigrafia*. Coi Tipi der Salviucci, Roma, 460 pp.

Andres, A. (1884) *Le Attinie (Monografia*. Verlag von Wilhelm Engelmann, Leipzig, 459 pp.

Atoda, K. (1954) The development of the sea anemone, *Diadumene luciae*, reproduced by the pedal laceration. *Science Reports of the Tohoku University*, 20(2), 123–129.

Carlgren, O. (1893) Studien über Nordische Actinien. *Kungliga Svenska Vetenskaps – Akademiens Handlingar*, 25, 1–148.

Carlgren, O. (1899) Zoantharien. *Hamburger Magalhaensische Sammelreise,* 4(1), 1–48.

Carlgren, O. (1924) Actiniaria from New Zealand and its Subantarctic Islands (Papers from Dr. Th. Mortensen's Pacific Expedition 1914–16. XXI). *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening (Copenhagen),* 77, 179–261.

Carlgren, O. (1938) South African Actiniaria and Zoantharia. *Kungliga Svenska Vetenskaps – Akademiens Handlingar,* series 3, 17(3), 1–148.

Carlgren, O. (1941) Papers from Dr. Th. Mortensen's Pacific Expedition 1914-16. LXX. The Actiniaria and Zoantharia of St. Helena. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening (Copenhagen),* 105, 1–20.

Carlgren, O. (1943) East-Asiatic Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskaps – Akademiens Handlingar,* series 3, 20(6), 1–43.

Carlgren, O. (1949) A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskaps – Akademiens Handlingar*, series 4, 1(1), 1–121.

Carlgren, O. (1952) Actiniaria from North America. *Arkiv für Zoologi*, 3(30), 373–390.

Cary L.R. (1906) A contribution to the fauna of the coast of Louisiana. *Gulf Biologic Station Bulletin*, 6, 50–59.

Castro, C.B., Echeverria, C.A., Pires, D.O., Mascarenhas, B.J.A., & Freitas, S.G. (1995) Infralittoral hard bottom distribution of Cnidaria and Echinodermata in Arraial do Cabo, Rio de Janeiro, Brazil. *Revista Brasileira de Biologia*, 55(3), 471–480.

Chen, C., Soong, K., & Chen, C.A. (2008) The smallest oocytes among broadcast-spawning actiniarians and a unique lunar reproductive cycle in a unisexual population of the sea anemone, *Aiptasia pulchella* (Anthozoa: Actiniaria). *Zoological Studies*, 47(1), 37–45.

Cocks, W.P. (1850) Contributions to the fauna of Falmouth. *Annual Report of the Royal Cornwall Polytechnic Society*, 17, 38–101.

Cocks, W.P. (1851) Actiniæ (or sea-anemones), procured in Falmouth and its neighbourhood, by W. P. Cocks, Esq., from 1843-1849. *Annual Report of the Royal Cornwall Polytechnic Society*, 19, 3–11.

Corrêa, D.D. (1964) *Corallimorpharia e Actiniaria do Atlantico Oeste Tropical*. Universidade de São Paulo, 39 pp.

Corrêa, D.D. (1973) Sobre anémonas-do-mar (Actiniaria) do Brasil. *Boletím de Zoologia e Biologia Marinha*, 30, 457–468.

Daly, M., Chaudhuri, A., Gusmão, L., & Rodriguez, E. (2008). Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular phylogenetics and evolution*, 48(1), 292–301.

Dalyell, J. G. (1848) *Rare and Remarkable Animals of Scotland*. John Van Voorst, Paternoster Row, London, 322.

Delle Chiaje, S. (1822) *Memorie sulla storia e notomia degli animali senza vertebre del regno di Napoli.* Napoli, CXI.

Delle Chiaje, S. (1823). *Memorie sulla storia e notomia degli animali senza vertebre del regno di Napoli.* Corredate di vignette e di figure incise in rame. Stamperia Della Societa Tipografica, Napoli, CXI.

Delle Chiaje, S. (1841) *Descrizione e notomia degli animali invertebrati della Sicilia Citeriore osservati vivi negli anni 1822-1830*. 5. C. Batelli e Comp., Napoli.

den Hartog, J.C., & Ates, R. (2011) Actiniaria from Ria de Arosa, Galicia, northwestern Spain, in the Netherlands Centre for Biodiversity Naturalis, Leiden. *Zoolgische Mededelingen, Leiden*, 85(2), 11-53.

Deshayes, G.P., & Milne Edwards, H. (1840) Revue et augmentée de notes présentant les faits nouveaux dont la science s'est enrichie jusqu'a ce jour. *In: Histoire Naturelle des Animaux sans Vertébres, Présentant les caractères généraux et particuliers de ces animaux, leur distribution, leurs classes, leurs familles, leurs genres, et la citation des principales espèces qui s'y rapportent*. J.B. Bailliere, Paris, 770.

Dube, V.M.C. (1974) Anêmonas-do-mar (ordem Actiniaria) do Estado da Bahia. Universidade Federal da Bahia, Salvador, Bahia. Unpublished Dissertation, 112 pp.

Dube, V.M. (1983) Contribuição ao estudo de anêmonas-do-mar do esta do da bahia. *Natura*, 83, 82–93.

Duchassaing de Fombressin, P. (1870) *Revue des Zoophytes et des Spongiaires des Antilles*. Chez Victor Masson et Fils, Paris, 52 pp.

Duchassaing de Fombressin, P. & Michelotti, G. (1860) *Mémoire sur les Coralliaires des Antilles*. Imprimerie Royale, Turin, 89 pp.

Duchassaing de Fombressin, P., & Michelotti, G. (1864) *Supplément au mémoire sur les Coralliaires des Antilles*. Imprimerie Royale, Turin, 112 pp.

Duchassaing de Fombressin, P., & Michelotti, G. (1866) Supplément au Mémoire sur les Coralliaires des Antilles. *Memorie Reale Accademia delle Scienze di Torino*, 8(2), 97–206.

Duerden, J.E. (1897) The actiniarian family Aliciidae. *Annals and Magazine of Natural History*, 20, 1–15.

Dunn, D.F. (1981) The clownfish sea anemones: Stichodactylidae (Coelenterata: Actiniaria) and other sea anemones symbiotic with pomacentrid fishes. *Transactions of the American Philosophical Society*, 71, 1–115.

Dunn, S.R., Bythell, J.C., Le Tissier, M.D., Burnett, W.J., & Thomason, J.C. (2002) Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia* sp. *Journal of Experimental Marine Biology and Ecology*, 272(1), 29–53.

Echeverria, C.A., Pires, D.O., Mederios, M.S., & Castro, C.B. (1997) Cnidarians of the Atol das rocas, *Brazil. Proceedings of the Eight International Coral Reef Symposium*, 1, 443–446.

England, K.W. (1992) Actiniaria (Cnidaria: Anthozoa) from Hong Kong with additional data on similar species from Aden, Bahrain and Singapore. *In*: B. Morton, *The Marine Flora and Fauna of Hong Kong and Southern China III*, Hong Kong University Press, Hong Kong, 49–95.

Farrapeira, C.M.R., Melo, A.V.D.O.M., Barbosa, D.F., & Silva, K.M.E.D. (2007) Ship hull fouling in the Port of Recife, Pernambuco. *Brazilian Journal of Oceanography*, 55(3), 207–221.

Fautin, D.G. (2013) Hexacorallians of the World. Available from: http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm. (June 2013).

Goodwill, R.H., Fautin, D.G., Furey, J., & Daly, M. (2009) A sea anemone symbiotic with gastropods of eight species in the Mariana Islands. *Micronesica*, 41(1), 117–130.

Fautin, D.G., Zelenchuk, T., & Raveendran, D. (2007) a Genera of orders Actiniaria and Corallimorpharia (Cnidaria, Anthozoa, Hexacorallia), and their type species. *Zootaxa*, 1668, 183–244.

Fautin D.G., Cleveland, P., Hickman, J.R., Daly, M., & Molodtsova, T. (2007) b Shallow-Water Sea Anemones (Cnidaria: Anthozoa: Actiniaria) and Tube Anemones (Cnidaria: Anthozoa: Ceriantharia) of the Galápagos Islands. *Pacific Science*, 61(4), 549–573.

Field L.R. (1949) *Sea Anemones and Corals of Beaufort, North Carolina*. Duke University Press, Durham, 39 pp.

Fischer, P. (1874) Recherches sur les Actinies des cotes océaniques de France. *Nouvelles Archives du Muséum d'Historie de Paris*, 10, 193–244.

Fischer, P. (1889) Nouvelle Contribution à l'Actinologie Française. Première Partie. *Actes de la Société Linnéenne de Bordeaux*, 43, 252–309.

Forbes, E. (1840) On the British *Actiniadae*. *Annals and Magazine of Natural History*, 5, 180–184.

Gabe, M. (1968) *Technique Histologique*. Massou et Cie, Paris, 1113 pp.

Gasparini, J.L., Floeter, S.R., Ferreira, C.E.L., & Sazima, I. (2005) Marine ornamental trade in Brazil. *Biodiversity & Conservation*, 14(12), 2883–2899.

Gay, C. (1854) *Historia Fisica y Politica de Chile*. Museo de Historia Natural de Santiago, Santiago, Chile, 499 pp.

González-Muñoz, R., Simões, N., Sanchez-Rodriguez, J., Rodríguez, E., & Segura-Puertas, L. (2012) First Inventory of Sea Anemones (Cnidaria: Actiniaria) of the Mexican Caribbean. *Zootaxa*, 3556, 1–38.

Gosse, P.H. (1858) Synopsis of the families, genera, and species of the British Actiniae. *Annals and Magazine of Natural History*, 1, 414–419.

Gosse, P.H. (1860) *A History of the British Sea-Anemones and Corals*. Van Voorst, London, 362 pp.

Grajales, A., & Rodríguez, E. Molecular phylogenetics of the family Aiptasiidae. *Molecular Phylogenetics and Evolution*, (in prep).

Grajales, A., & Rodríguez, E. Case XXXX: *Aiptasia diaphana* (Rapp, 1829) and *Aiptasia pallida* (Aggasiz in Verrill, 1864): maintaining the use of the junior synonym *A. pallida* by stability (Cnidaria: Anthozoa: Hexacorallia: Actiniaria). *Bulletin of Zoological Nomenclature* (in press).

Gravenhorst, I.L.C. (1831) *Tergestina, oder Beobachtungen und Unteruchungen über einige bei Triest im Meere lebende Arten der Gattungen Octopus, Doris, Pinna, Ascidia, Serpula, Echinus, Asterias, Ophiura, Holothuria, Actinia, Caryophyllia, Actinotus*. Wilhelm Gottlieb Korn, Breslau, 166 pp.

Gunter, G., & Geyer, R.A. (1955) Studies on fouling organisms of the northwest Gulf of Mexico. *Publications of the Institute of Marine Science (University of Texas)*, 4(1), 38–67.

Hertwig, R. (1882) *Die Actinien der Challenger Expedition*. Gustav Fischer, Jena, 119 pp.

Hollard, M.H. (1848) Études sur l'organisation des Actinies. Imprimerie de Marc Ducloux et Ce, Paris.

International Commission on Zoological Nomenclature (1999) *International Code of Zoological Nomenclature* (Fourth Edition). International Trust for Zoological Nomenclature, London.

Johansen, D.A. (1940) *Plant microtechniques.* McGraw-Hill, New York & London.

Johnson, J.Y. (1861) Notes on the sea-anemones of Madeira, with descriptions of new species. *Proceedings of the Zoological Society of London*, 1861, 298–306.

Jourdan, É. (1880) Recherches zoologiques et histologiques sur les Zoanthaires du Golfe de Marseille. *Annales des Sciences Naturelles*, 10, 1–154.

LaJeunesse, T.C., Smith, R., Walther, M., Pinzón, J., Pettay, D.T., McGinley, M., Achaffenburg, M., Medina-Rosas, P., Cupul-Magaña, A.L., Lopez Perez, A., Reytes-Bonilla, H., & Warner, M.E. (2010) Host–symbiont recombination versus natural selection in the response of coral–dinoflagellate symbioses to environmental disturbance. *Proceedings of the Royal Society B: Biological Sciences*, 277(1696), 2925–2934.

Le Sueur, C.A. (1817) Observations on several species of the genus Actinia; illustrated by figures. *Journal of the Academic of Sciences of Philadelphia*, 1, 149–154, 169–189.

Manuel, R.L. (1979). Some new records of Anthozoa from British waters. *Journal of the Marine Biological Association of the United Kingdom.* 59, 393–397.

Manuel, R.L. (1981) *British Anthozoa keys and notes for the identification of the species*. Academic Press, London, New York, Toronto, Sydney, San Fransisco, 241.

Mariscal, R.N. (1974) Nematocysts. *In:* Muscatine, L. & Lenhoff, H.M. (Eds), *Coelenterate Biology*. Academic Press, New York, pp. 129–178.

McMurrich, J.P. (1887) Notes on the fauna of Beaufort, North Carolina. *Studies at the Biological Laboratory of the John Hopkins University*, 4, 55–63.

Milne Edwards, H. (1857) *Historie Naturelle des Coralliaires ou Polypes Proprement Dits, vol 1.* Librairie Encyclopédique de Roret, Paris, 326 pp.

Milne Edwards, H., & Haime, J. (1851) *Archives du Muséum d'Historie Naturelle. 5: Monographie des polypiers fossils des terrains palfozooques, pricidie d'un tableau general de la classification des polypes*. Gide et J. Baudry, Paris, 502 pp.

Muller-Parker, G., & Davy, S.K. (2001) Temperate and tropical algal- sea anemone symbioses. *Invertebrate Biology*, 120(2), 104–123.

Ocaña, O., Nuñez, J., & Bacallado, J.J. (1994) Descriptive study of *Aiptasia mutabilis* (Gravenhorst, 1831) (Anthozoa: Actiniaria) in the Canary Islands. *Boletim do Museu Municipal do Funchal (História Natural)*, 46(255), 145–157.

Ocaña, O., & den Hartog, J.C. (2002) A catalogue of actiniaria and corallimorpharia from the Canary Islands and from Madeira. *Arquipélago. Boletim da Universidade dos Açores. Ciências Biológicas e Marinhas [Life and Marine Sciences]*, 19A, 33–54.

Parulekar, A. (1969) *Neoaiptasia commensali*, gen. et. sp. nov.: an actiniarian commensal of hermit crabs. *Journal of the Bombay Natural History Society*, 66, 57–62.

Pax, F. (1909) Aktinienstudien. I. Polyparium ambulans. II. Aktinien von Gomera. III. *Bolocera norvegica*, eine neue Aktinie von der Westküste Norwegens. *Jenaische Zeitschrift für Naturwissenschaft*, 45, 325–344.

Pax, F. (1910) Studien an westindischen Actinien. *Zoologische Jahrbücher*, 2, 157–330.

Pax, F. (1924) Actiniarien, Zoantharien und Ceriantharien von Curaçao. *Kungliga Zoologisch Genootschap Natura Artis Magistra (Amsterdam)*, 23, 93–122.

Pax, F., & Müller, I. (1953) Die Anthozoenfauna der bucht von Kaštela bei Split. *Acta Adriatica*, 5(1), 3–35.

Pires, D.D.O., Migotto, A.E., & Marques, A.C. (1992) Cnidários bentônicos do Arquipélago de Fernando de Noronha, Brasil. *Boletim do Museu Nacional (Rio de Janeiro), NS Zoologia*, 354, 1–21.

Ramil, F. (1987) Antozoos nuevos para el litoral ibérico, recolectados en Galicia. *Boletin de la Real Sociedad Española de Historia Natural,* 83(1-4), 197-204.

Rapp, W. (1829) *Über die Polypen im Allgemeinen und die Actinien*. Grolsherzogl. Sdch, Weimar, 62 pp.

Reimer, J.D., Sinniger, F., Fujiwara, Y., Hirano, S., & Maruyama, T. (2007) Morphological and molecular characterisation of *Abyssoanthus nankaiensis*, a new family, new genus and new species of deep-sea zoanthid (Anthozoa: Hexacorallia: Zoantharia) from a north-west Pacific methane cold seep. *Invertebrate Systematics*, 21(3), 255–262.

Rodríguez, E., Barbeitos, M., Daly, M., Gusmão, L., & Häussermann, V. (2012) Toward a natural classification: Phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). *Cladistics*, 28, 375–392.

Rodriguez-Lanetty, M., Phillips, W., & Weis, V. (2006) Transcriptome analysis of a cnidarian–dinoflagellate mutualism reveals complex modulation of host gene expression. *BMC Genomics*, 7(1), 23.

Sawyer, S.J., & Muscatine, L. (2001) Cellular mechanisms underlying temperature-induced bleaching in the tropical sea anemone *Aiptasia pulchella*. *Journal of Experimental Biology*, 204(20), 3443–3456.

Schmarda, L.K. (1852) Zur naturgeschichte der Adria. *Denkschriften der Kaiserlichen Akademie der Wissenschaften (Wien)*, 4, 117–137.

Schmidt, H. (1969) Die Nesselkapseln der Aktinien und ihre differentialdiagnostishce Bedeutung. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 19, 284–317.

Schmidt, H. (1972) Prodromus zu einer Monographie der mediterranean aktinien. *Zoologica*, 42, 1–121.

Silbiger, N.J., & Childress, M.J. (2008) Interspecific variation in anemone shrimp distribution and host selection in the Florida Keys (USA): implications for marine conservation. *Bulletin of Marine Science*, *83*(2), 329–345.

Stephenson, T.A. (1918) On certain Actiniaria collected off Ireland by the Fisheries Department, during the years 1899–1913. *Proceedings of the Royal Irish Academy,* 34B (7), 106–164.

Stephenson, T.A. (1920) On the classification of Actiniaria. I. *The Quarterly Journal of Microscopical Science*, 64, 425–574.

Stimpson, WM. (1856) Descriptions of some of the new marine invertebrata from the Chinese and Japanese seas. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 7, 375–384.

Sunagawa, S., Choi, J., Forman, H.J., & Medina, M. (2008) Hyperthermic stress-induced increase in the expression of glutamate-cysteine ligase and glutathione levels in the symbiotic sea anemone *Aiptasia pallida*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 151(1), 133–138.

Sunagawa, S., Wilson, E.C., Thaler, M., Smith, M.L., Caruso, C., Pringle, J.R., Weis, V.M., Medina, M. & Schwarz, J.A. (2009) Generation and analysis of transcriptomic resources for a model system on the rise: the sea anemone *Aiptasia pallida* and its dinoflagellate endosymbiont. *BMC genomics*, 10(1), 258.

Thornhill, D. J., Xiang, Y., Pettay, D. T., Zhong, M., & Santos, S. R. (2013). Population genetic data of a model symbiotic cnidarian system reveal remarkable symbiotic specificity and vectored introductions across ocean basins. *Molecular Ecology*, 22(17), 4499-4515.

Tubbs 2001. Tubbs, P.K. (2001) Availability of zoological names published in theses. *Bulletin of Zoological Nomenclature*, 58, 311–312.

Tur, J.M. (1989) *Contribució a la fauna d'actiniaris (Anthozoa) del litoral català: taxonomia i systemàtica*. Universidad de Barcelona, Unpublished thesis, 209 pp.

Uchida, H. & Soyama, I. (2001) *Sea Anemones in Japanese Waters*. TBS, Japan, 157 pp.

Verrill, A.E. (1864) a List of the polyps and corals sent by the Museum of Comparative Zoölogy to other institutions in exchange, with annotations. *Bulletin of the Museum of Comparative Zoology (Harvard University)*, 1, 29–60.

Verrill, A.E. (1864) b Revision of the Polypi of the eastern coast of the United States. *Memoirs of the Boston Society of Natural History*, 1, 1–45.

Verrill A.E. (1867) Madreporaria. *Communications of the Essex Institute*, 5, 33–50.

Verrill, A.E. (1868) Synopsis of the polyps and corals of the North Pacific Exploring Expedition, under Commodore C. Ringgold and Capt. John Rodgers, U. S. N., from

1853 to 1856. Collected by Dr. Wm. Stimpson, Naturalist to the Expedition. Part. IV. Actiniaria (First part). *Communications of the Essex Institute*, 5, 315–330.

Verrill, A.E. (1907) Additions to the fauna of the Bermudas from the Yale Expedition of 1901, with notes on other species. *In*: Verrill, A. E. (Eds.), *Zoology of Bermuda*, New Haven, Connecticut, pp. 15–62.

Watzl, O. (1922) Die Actiniarien der Bahamainseln. *Arkiv für Zoologi*, 14, 1–89.

Weis, V.M., Davy, S.K., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., & Pringle, J.R. (2008) Cell biology in model systems as the key to understanding corals. *Trends in Ecology & Evolution*, 23(7), 369–376.

Williams, R.B. (1998) Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), II: further studies of differences amongst sample means and their taxonomic relevance. *Scientia Marina*, 62(4), 361–372.

Williams, R.B. (2000) Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), III: ranges and other measures of statistical dispersion, their interrelations and taxonomic relevance. *Scientia Marina*, 64(1), 49–68.

Zamponi, M.O., Belém, M.J., Schlenz, E. & Acuña, F.H. (1998) Distribution and some ecological aspects of Corallimorpharia and Actiniaria from shallow waters of the South American Atlantic coasts. *Physis*, 55, 31–45.

CHAPTER III

MOLECULAR SYSTEMATICS OF THE FAMILY AIPTASIIDAE (CNIDARIA, ACTINIARIA, METRIDIOIDEA)

ABSTRACT

Sea anemones of the family Aiptasiidae (sensu Grajales & Rodriguez, submitted; Chapter 2 of this thesis) are conspicuous members of shallow-water environments, with several species widely used as a model system for studies of cnidarian-dinoflagellate symbiosis and coral bleaching. Although previously published phylogenetic studies of sea anemones recovered the family Aiptasiidae as monophyletic, they only included a very partial sampling in terms of its overall taxonomic diversity. This study explores the phylogenetic relationships of this family, including newly collected material from the distribution of most of the currently described genera and species. I found a monophyletic family Aiptasiidae. Most of the proposed genera were recovered as monophyletic units, with the exception of the genus *Aiptasia*, now divided into *Aiptasia* and *Exaiptasia,* a finding also supported by diagnostic morphological characters. I also included the genera *Bellactis* Dube, 1983 and *Ragactis* Andres, 1883 as members of the family Aiptasiidae, in agreement with previous morphological studies. These findings are congruent with the morphological homogeneity of the members of this family. The obtained results also allowed elucidating the evolution of morphological characters. I further found evidence for a new species, *Exaiptasia* sp. nov., based on the results from the phylogenetic analysis, geographical distribution, and the identity of their endosymbiotic algae.

Keywords: Phylogenetics, Metridioidea, Aiptasiidae, *Aiptasia*, *Exaiptasia pallida*, *Exaiptasia* sp. nov, nuclear rDNA.

INTRODUCTION

Despite their ubiquity in marine ecosystems, and their plasticity in terms of reproductive strategies, symbiotic relationships and ecological relevance, sea anemones (Cnidaria: Actiniaria) traditionally have been neglected from a taxonomic and evolutionary point of view. While some recent work has assembled a broad phylogenetic framework (Daly et al. 2003; Rodriguez et al., 2010; Rodriguez et al., in prep) much work is still needed in terms of efforts to solve the phylogenetic relationships within genera and species, except some notable exceptions (Daly et al., 2002; Gusmão et al 2010). As a consequence, the formulation of hypotheses concerning the evolution of morphological characters, taxonomic diversity through time and across habitats, and reproduction at lower levels has not been feasible. Population-level studies are also hindered by the lack of clearly defined taxonomic units – and taxonomist specialists in the field.

Species within the genus Aiptasiidae (Fig. 3.1) present a suitable opportunity to address hypotheses within an evolutionary framework, both at the population and above-species levels. Most of the species within this family of symbiotic sea anemones species are conspicuous components of shallow water environments worldwide. Previous studies of the group have recovered this family as a monophyletic clade (Rodriguez et al. 2010;

Rodriguez et al., in prep), however they only included three specimens representative of two genera. Members of the genus *Aiptasia*, in particular, have been extensively used as a model organism for studies of dinoflagellate-cnidarian symbiosis, reproduction and development (e.g. Dunn et al. 2002; Muller-Parker & Davy 2001; Weis et al. 2008; LaJeunesse et al. 2010). In addition, rapid growth rate, allowed by their symbiosis with a dinoflagellate (*Symbiodinium* spp.) combined with asexual reproduction by pedal laceration (Clayton et al., 1985; Lin et al., 1992) make them make them excellent laboratory specimens, while at the same time undesirable in some other contexts, like the aquarium trade business, where they are considered pests. However, without a phylogenetic framework in hand, hypotheses concerning the diversification and evolution of this group cannot be formulated, and the true diversity from both population and above-species levels is still unexplored. Controversy exists about whether this "model organism" is actually one cosmopolitan species, or a group of cryptic, or insufficiently described ones.

Grajales & Rodriguez (submitted) conducted a detailed taxonomic study of members of the genus *Aiptasia*, as well as the family Aiptasiidae (Cnidaria: Anthozoa: Actiniaria: Metridioidea). That study proposed major nomenclatural changes for the group based on a detailed the revision of morphological characters. The authors proposed that *Neoaiptasia* is not part of the group, and included two new genera *Bellactis* and *Ragactis,* all based on a combination of internal and external anatomical characters. Furthermore, they separated the genus *Aiptasia* into two different genera*, Aiptasia* and *Exaiptasia*, according to differences in the cnidae (the combination of different nematocysts in the body). Within genera, the authors proposed the separation of *Aiptasia* into two species, *A. mutabilis* and *A. couchii*, due size to differences in reproduction, mesentery cycles and their association with different genera of endosymbiotic algae (also reviewed in Grajales et al. b, in prep). Within *Exaiptasia*, the main focus of the taxonomic revision, the authors examined specimens corresponding to most of the reported localities of species within species other than *A. mutabilis* and *A. couchii* (still included in *Aiptasia*) and could not find morphological differences that separated currently described species, and thus synonymized all *Exaiptasia* species as a single widespread species, *Exaiptasia pallida*. Thornhill et al. (2013) also conducted a comprehensive study on the identity of the endosymbiont algae within members of *Exaiptasia*, and found that most of the studied individuals harbored a singe *Symbiodinium* algal subtype, a fact that also agrees with the hypothesis of a widespread species. In order to further test the results obtained by Grajales & Rodriguez (submitted; Chapter 2 of this thesis) I produced the first molecular phylogenetic hypothesis for the group, by sequencing more than ~8 Kbp and including all currently described genera, as well as representatives from most of the localities of previously described species within *Aiptasia*. Here, I found a monophyletic family Aiptasiidae, validated the genus *Aiptasia* and *Exaiptasia* as monophyletic, and discovered a new species, *Exaiptasia* sp. nov., restricted to the Southern Caribbean Sea and the Southwestern Atlantic Ocean.

Material and Methods

Taxonomic sampling

Multiple specimens were collected by snorkeling or during SCUBA dives, depending on depth. Ingroup sampling included 51 specimens (see Table 3.1) corresponding to genera currently included in the family Aiptasiidae (*Aiptasia*, *Aiptasiogeton, Exaiptasia* comb. nov., *Bartholomea*) according to the last taxonomic review (Fautin, 2013); as well as representatives of genera (*Ragactis, Bellactis*) that share some combination of diagnostic morphological characters typically found in members of the family Aiptasiidae (Figure 3.1). Outgroup sampling included representatives of previously identified phylogenetic clades Metridioidea, Endomyaria, Actinostolina and Edwardsiidea. (Rodriguez et al., 2012). Voucher specimens in formalin have been deposited at the American Museum of Natural History (AMNH), and the Museu de Zoologia da Universidade do Sao Paulo (MZUSP).

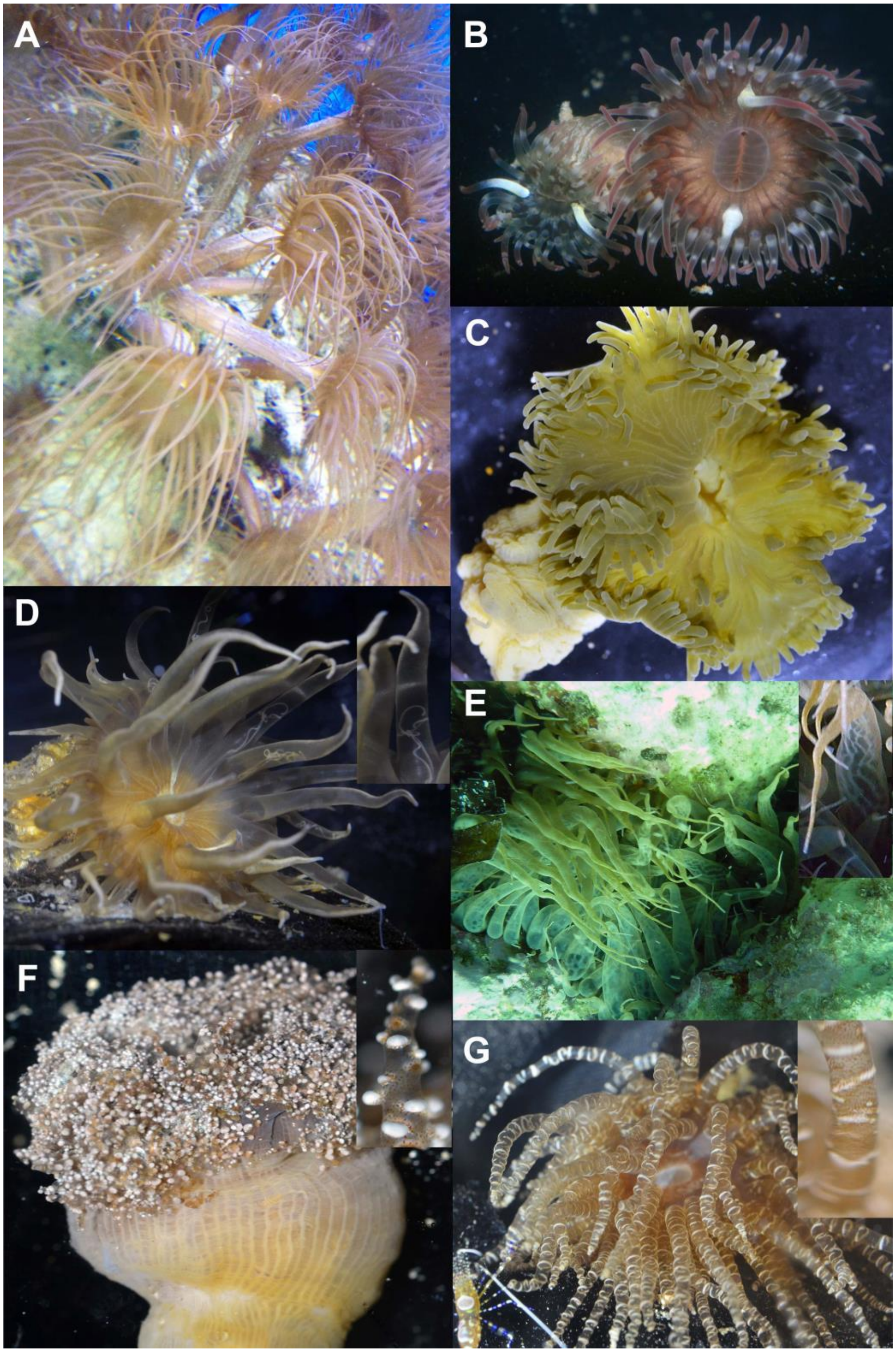
Data Collection

The material studied was collected during 2009–2012 from 40 different localities spanning the globe (Table 3.1). Sea anemones were relaxed using menthol crystals and photographed alive. Small pieces of tissue from selected specimens were preserved in absolute ethanol for DNA analysis. Preliminary identifications were made through histological sections as well as the inspection of the cnidae of each individual.

Genomic DNA was isolated from tentacle or column tissue using the Qiagen DNAasy® kit. Template DNA was amplified from genomic samples using published primers for the mitochondrial markers 12S, 16S and CO3. 18S and 28S sequences were amplified using newly developed primers specific to Actiniaria (Grajales et al., in prep a), to avoid the amplification of *Symbiodinium* nuclear ribosomal genes (See Table 3.2). PCR products were cleaned using ExosapIT. Sequencing reactions used a total of 5µL of cleaned PCR product, at a concentration of 25 ng of product for every 200 base pairs of marker length. Cleaned PCR products were sequenced using amplification primers on an ABI 3770*x* at the in-house facilities of the AMNH. Forward and reverse sequences were assembled in Geneious (version 6.16 created by Biommaters), and blasted against the nucleotide database of GenBank to confirm whether the obtained product corresponded to the target organism and not their algal symbionts. All sequences have been deposited in GenBank (Table 3.1).

Genomic DNA was isolated from tentacle or column tissue using the Qiagen DNAasy® kit. Template DNA was amplified from genomic samples using published primers for the mitochondrial markers 12S, 16S and CO3. 18S and 28S sequences were amplified using newly developed primers specific to Actiniaria (Grajales et al., in prep a), to avoid the amplification of *Symbiodinium* nuclear ribosomal genes (See Table 3.2). PCR products were cleaned using ExosapIT. Sequencing reactions used a total of 5µL of cleaned PCR product, at a concentration of 25 ng of product for every 200 base pairs of marker length. Cleaned PCR products were sequenced using amplification primers on an ABI 3770*x* at the in-house facilities of the AMNH. Forward and reverse sequences were assembled in Geneious (version 6.16 created by Biommaters), and blasted against the nucleotide database of GenBank to confirm whether the obtained product corresponded to the target organism and not their algal symbionts. All sequences have been deposited in GenBank (Table 3.1).

Figure 3.1 Representative species of the genera within the family Aiptasiidae. **A.** *Exapitasia pallida* **B.** *Aiptasiogeton hyalinus* **C**. *Bellactis ilkalyseae*. **D**. *Aiptasia couchii*. **E**. *Aiptasia mutabilis*. **F**. *Ragactis lucida*. **G**. *Bartholomea annulata*. Top right rectangles depict a detail of the tentacle, useful for field identification.


A
B
C
D
E
F
G

Data analysis

Sequences for each marker were aligned in MAFFT v 7.017 (Katoh & al. 2002) using the following settings E-INS-i option Scoring Matrix 200PAM / k=2 Gap open penalty 1.53. In order to compare the effect of using all the obtained characters (all positions in the alignments) vs. excluding ambiguously aligned positions, an increasingly common practice; two datasets were compared, the first one containing all aligned positions, and a second dataset processed with Gblocks (Castresana, 2000). The following parameters were used (Maximum number of contiguous non-conserved positions: 8, Minimum length of a block: 5, Gap positions allowed) to produce a less stringent set of conserved aligned blocks. The complete and reduced datasets were deposited in TreeBase (http://www.treebase.org/treebase/index.html).

Maximum likelihood (ML) inference. Maximum likelihood analyses were performed in RAxML v.7.6.3 (Stamatakis, 2006) implemented on the CIPRES portal (Miller et al., 2010), using the GTR + Γ (GTRGAMMA) option as model of substitution, but allowing to estimate different α shape, GTR rates and base frequencies for each marker in the combined alignment. *Cox*3 alignment data was treated as two different partitions, one corresponding to the first two-codon positions and a second one for the third position. The Majority Rule Criterion implemented in RAxML (-autoMRE) was used to assess clade support.

Maximum Parsimony.

Tree searches under maximum parsimony were conducted using random and random and constrained sectorial searches, tree drifting, and 100 rounds of tree fusing (command xmult=hits 10 rss drift css fuse 100) in TNT v1.1 (Goloboff et al., 2008). Separate analyses were run with gaps treated as missing data and as a fifth state. 1000 bootstrap replicates were run to assess clade support on the obtained strict consensus tree.

Ancestral state reconstructions:

Ancestral states reconstructions were performed under Maximum Parsimony using the Software Mesquite v.2.75 (Maddison & Maddison, 2011) counting only unequivocal states. A total of 13 characters were examined within members of Aiptasiidae.

RESULTS AND DISCUSSION

Phylogenetic position of the family Aiptasiidae.

Both ML and MP analyses recovered the family Aiptasiidae as a monophyletic clade (Figure 3.2 and supplementary figure 1), in agreement with the taxonomic hypothesis of the family Aiptasiidae being a morphologically homogeneous group. Due to the lower mutation rate in mitochondrial as well nuclear ribosomal genes mutation in Cnidaria (Shearer et al., 2002), and more specifically in Anthozoa (e.g., vanOpen, 1999), resulting in fewer segregating characters, it was relevant to test the effect of excluding characters from obtained alignments, as proposed by Castresana (2000). As expected, the number of discarded positions ranged from zero on the CO3 alignment, a protein-coding gene, to more than thousand base pairs on a nuclear ribosomal region such 28S-nrDNA (see

Table 3.1. Taxa included in this study, with voucher and location. Old species concepts, plus changes proposed in this manuscript and in Grajales et al. (2013).

Species in this study	Previous concept	Museum	Catalog #	Locality	Locality 2	Longitude	Latitude	Depth (m)
Aiptasia couchii Cocks, 1851	*A. mutabilis*, (Gravehorst 1867)	AMNH	5515.1	England	Plymouth	N 50 21 49.98	W 04 08 38.43	1
	"	AMNH	5516.2	England	Plymouth	N 50 18 57.55	W 04 04 40.02	1
	"	AMNH	5521.1	Spain	I. Medes	N 42 02 51.25	W 03 13 08.91	1
	"	AMNH	5517.1	Spain	Tarifa, Cádiz	N 36 00 35.05	W 36 04 50.22	1
	"	AMNH	5536.1	Spain	Malaga	N 37 12 27.54	W 07 02 52.78	1
	"	AMNH	5501.1	Italy	Oristano	N 39 40 34.64	E 08 26 42.66	1
	"	AMNH	5500	Italy	La Caletta	N 39 40 34.63	E 08 26 04.65	1
	"	AMNH	5542.1	France	Banyuls-sur-Mer	N 42 28 59.01	E 03 07 49.48	1
	"	AMNH	5482.1	Canary Is.	Las Palmas de Gran Canaria	N 39 21 10.34	W 09 22 45.90	1
	"	AMNH	5520.1	Canary Is.	Tenerife	N 28 33 50.35	W 16 19 55.33	1
	"	AMNH	5531.1	Portugal	Madeira – Machico	N 42 28 59.49	W 03 07 47.43	1
Aiptasia mutabilis (Gravehorst 1867)	"	AMNH	5503.1	Spain	L'Estartit, Girona	N 42 02 52.05	E 03 13 07.95	10
	"	AMNH	5517.5	Greece	Crete, Agios Nikolaos	N 35 11 40.69	E 25 43 01.87	5
Exaiptasia brasiliensis sp. nov.	-	AMNH	5504.1	Panama	Bocas del Toro	N 09 20 50.34	W 82 15 18.67	1
	-	MZUSP	002483	Brazil	Espiritu Santo – Praia dos Padres	S 19 55 23 53	W 40 06 23.98	1
	-	MZUSP	002493	Brazil	Espiritu Santo – Guarapari	S 20 40 36 64	W 40 29 59.41	1
	-	MZUSP	002512	Brazil	São Paulo, São Sebastiao	S 23 49 40.36	W 45 25 19.78	1
Exaiptasia pallida (Agassiz in Verrill, 1864)	*A. californica* Carlgren, 1952	AMNH	5366	Mexico	Baja California sur – Bahia Concepcion	N 24 15 46.46	W 110 36 52.12	1
	-	AMNH	5541.2	Panama	Venado	N 07 25 50.46	W 80 11 36.24	1
	A. diaphana (Rapp, 1829)	AMNH	5372	Portugal	Madeira – Machico	N 32 42 52.84	W 16 45 47.85	1
	"	AMNH	5528.2	Spain	Las Palmas de Gran Canaria	N 28 19 09.18	W 15 25 56.89	1

	"	AMNH		Israel	Cesarea	N 32 29 37 62	E 34 53 22.57	1
	A. pallida (Agassiz in Verrill, 1864)	AMNH	5465.10	Bermuda	Ferry reach	N 32 22 01.51	W 64 39 36.22	1
	"	AMNH	5466.1	Bermuda	Walsingham Ponds	N 32 22 12.02	W 64 61 47.33	1
	"	AMNH	5450.5	USA	Florida keys	N 25 03 61.03	W 80 25 38.02	2
	"	AMNH	5452.1	Mexico	Puerto Morelos	N 20 50 18.57	W 86 53 02.86	1
	"	AMNH	5507.2	Panama	Carenera Island	N 09 20 50.34	W 82 15 18.67	1
	"	AMNH	5509.3	Panama	Bocas del Toro	N 09 20 50.34	W 82 15 18.67	1
	"	MZUSP	002506	Brazil	Ceara, Fortaleza	S 03 43 42.93	W 38 32 35.60	1
	"	AMNH	002495	Brazil	Florianopolis	S 27 29 16.62	W 41 21 38.36	1
	A. pulchella Carlgren, 1943	AMNH	5469.4	USA	Hawaii – Hilo	N 19 43 56.37	W 155 03 06.06	1
	"	AMNH	5477.2	USA	Hawaii – Kawaii	N 22 03 38.67	E 159 19 06.61	1
	"	AMNH		Israel	Eilat	N 29 30 45.21	E 34 55 38.44	1
	"	AMNH	5464.3	Japan	Okinawa – Sesoko Island	N 26 38 10.70	E 127 51 55.03	1
	"	AMNH	5464.4	Japan	Okinawa – Sesoko Island	N 26 38 10.70	E 127 51 55.03	1
	"	AMNH		Taiwan	National Marine Aquarium, Pingtung	N 22 03 00.08	E 120 41 42.88	1
	"	AMNH		Australia	Townsville	N 19 14 46.89	E 146 49 44.02	1
Aiptasiogeton hyalinus (Delle Chiaje, 1822)	*Aiptasiogeton pellucidus* (Hollard, 1848)	AMNH	5519.8	Spain	El Portil, Huelva	N 37 12 27.54	W 07 02 52.78	1
Bartholomea annulata (LeSueur, 1817)	*Bartholomea annulata* (LeSueur, 1817)	AMNH		Bermuda	Ferry reach	N 32 22 01.51	W 64 39 36.22	1
	"	AMNH	5378	USA	Florida Keys	N 25 03 61.03	W 80 25 38.02	1
	"	AMNH	5379	Mexico	Isla Contoy	N 21 30 09.20	W 86 48 07.06	1
	"	AMNH		Panama	Bocas del Toro	N 09 20 50.34	W 82 15 18.67	1
Bellactis ilkalyseae	*Bellactis Ilkalyseae*, Dube, 1983	MZUSP	002494	Brazil	Espiritu Santo – Praia dos Padres	S 19 55 23.53	W 40 06 23.98	1

Laviactis lucida (Duchassaing de Fombressin and Michelotti, 1860)	*Ragactis lucida* (Duchassaing de Fombressin and Michelotti, 1860)	AMNH	5380	Mexico	Isla Contoy	N 21 30 09.20	W 86 48 07.06	1
	"	AMNH	5381	Panama	Carenera Island	N 09 20 50.34	W 82 15 18.67	1

monophyletic of Aiptasiidae, however the sister group of Aiptasiidae did change (Figure 3.2 and supplementary figure 2). Overall the branch lengths increased when including all the data, an expected and previously documented result (Castresana, 2000). Interestingly, the support values increased when the complete dataset was used, perhaps a counterintuitive results if the assumption was to get rid of ambiguously aligned regions. I favored the dataset including all the aligned positions, as it better resolves the internal relationships of the genera within Aiptasiidae.

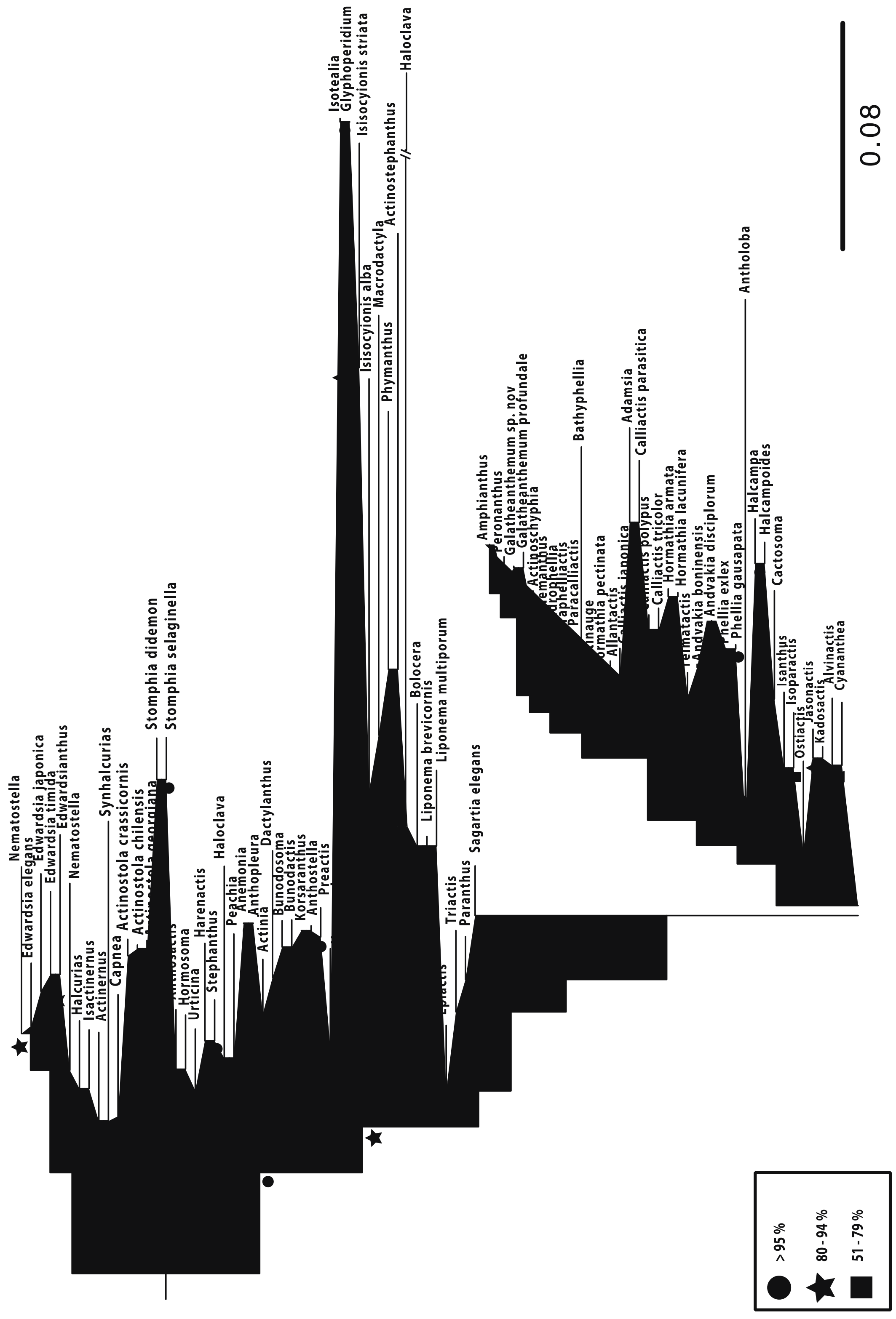
I further confirmed that the genus *Neoaiptasia* Parulekar, 1969 does not belong to the family Aiptasiidae, as shown in previous phylogenetic analyses (Rodriguez et al., 2012). This also agrees with a recent taxonomic revision (Grajales & Rodriguez, submitted; Chapter 2 of this thesis). According to the authors, the placement of this genus within the family was questionable due to the lack of morphological characters diagnostic to all other genera within the family (see Goodwill & Fautin 2009). The ML tree recovered the genus *Alicia* as a sister group of the family Aiptasiidae. The phylogenetic position of the family Aiptasiidae has been shown to vary, depending on the analyzed dataset (see Daly et al., 2008, Rodriguez et al., 2010, Rodriguez et al., submitted). However, the present study represents an significant increase in taxon sampling, which has shown to be positive affect for resolution of phylogenetic trees, both within and outside Actiniaria (e.g., Rodriguez et al., in prep.; Zwickl & Hillis, 2002; Dunn et al., 2008). Species belonging to (composed by *Gonactinia* + *Protanthea* + *Boloceroidaria*) share the presence of presence of ectodermal longitudinal muscles at the distal end of the column, a morphological character that is not prevalent among Actinaria (Rodriguez et al., in prep.). The placement of the *Sagartia* + *Sagartiogeton* and *Cereus* clades being related to Aiptasiidae is intriguing, if not strongly supported. Both *Sagartia* and *Sagartiogeton* have been shown to be polyphyletic, both in this study as well as other phylogenetic analyses (Rodriguez et al., 2012; Rodriguez et al., in prep.), confirming the necessity of further morphological revision for these genera.

Phylogenetic Relationships within genera of Aiptasiidae.

The phylogenetic relationships within the family Aiptasiidae are depicted in Figure 3.3. All genera, except *Aiptasia*, now divided into *Aiptasia* and *Exaiptasia*, were recovered as monophyletic, with strong support from both ML and MP analyses. The fact that the genus *Aiptasia* was not monophyletic was hinted at in previous studies (Daly et al., 2003; Rodriguez et al., 2012) however I more robustly confirmed this result by including representatives from all described genera and species within the family (sensu Grajales & Rodriguez, submitted; Chapter 2 of this thesis). Sampling efforts were especially exhaustive within the genus *Aiptasia*, which previously contained 16 species (Fautin et al., 2013). These efforts made possible make a significant increase in the number of included terminals; from 2 representatives included on previous studies, to a total of 41 terminals corresponding of newly collected material from localities corresponding to the distribution of currently described species within the genus, plus new records from different localities in Brazil, Panama and Australia (see Table 3.1).

Aiptasiogeton hyalinus was recovered as the sister group of all remaining members of the family Aiptasiidae. The sister clade is further subdivided in two well-supported clades, one corresponding to the genus *Exaiptasia*, and the other clade composed of all remaining genera. The newly erected genus *Exaiptasia* encompasses most of the species previously recognized as *Aiptasi*a (see Table 3.1), except for the type species of the genus, *A. mutabilis* and the species *A.couchii*, both species distributed in the Northwestern Atlantic Ocean and the Mediterranean Sea. The subdivision is further supported by two synapomorphies for the clade composed of *Aiptasia*, *Bartholomea*, *Bellactis* and *Ragactis*. These show an increase in the size of the b-mastigophores in the scapus (Figure 3.3 and 3.4l) and the lack of pedal laceration, compared to a smaller b mastigophore and the presence of pedal laceration, these features shared by both *Aiptasiogeton* and *Exaiptasia*. Despite clear morphological differences, including size, number of mesenteries, differences of endosymbiont type as well as fixed differences in the mitochondrial gene 16S, the current molecular markers did not provide enough resolution to further differentiate the two species within *Aiptasia*, *A. couchii* and *A. mutabilis*.

Figure 3.2 Phylogenetic position of the family Aiptasiidae. Maximum likelihood (ML) topology showing the hypothesized phylogenetic position of the family Aiptasiidae within the Order Actiniaria. ML bootstrap support values indicated on the bottom left box.


Nematostella
Edwardsia elegans
Edwardsia japonica
Edwardsia timida
Edwardsianthus
Nematostella
Halcurias
Isactinernus
Actinernus
Synhalcurias
Capnea
Actinostola crassicornis
Actinostola chilensis
Stomphia didemon
Stomphia selaginella
Hormosoma
Urticina
Harenactis
Stephanthus
Haloclava
Peachia
Anemonia
Anthopleura
Actinia
Dactylanthus
Bunodosoma
Bunodactis
Korsaranthus
Anthostella
Preactis
Isotealia
Glyphoperidium
Isisocyionis striata
Isisocyionis alba
Macrodactyla
Phymanthus
Actinostephanthus
Haloclava
Bolocera
Liponema brevicornis
Liponema multiporum
Epiactis
Triactis
Paranthus
Sagartia elegans
Amphianthus
Peronanthus
Galatheanthemum sp. nov
Galatheanthemum profundale
Actinoschyphia
Paracalliactis
Bathyphellia
Allantactis
Adamsia
Calliactis parasitica
Calliactis tricolor
Hormathia armata
Hormathia lacunifera
Andvakia boninensis
Andvakia disciplorum
Phellia exlex
Phellia gausapata
Antholoba
Halcampa
Halcampoides
Cactosoma
Isanthus
Isoparactis
Ostiactis
Jasonactis
Kadosactis
Alvinactis
Cyananthea
0.08
● >95 %
★ 80 - 94 %
■ 51 - 79 %

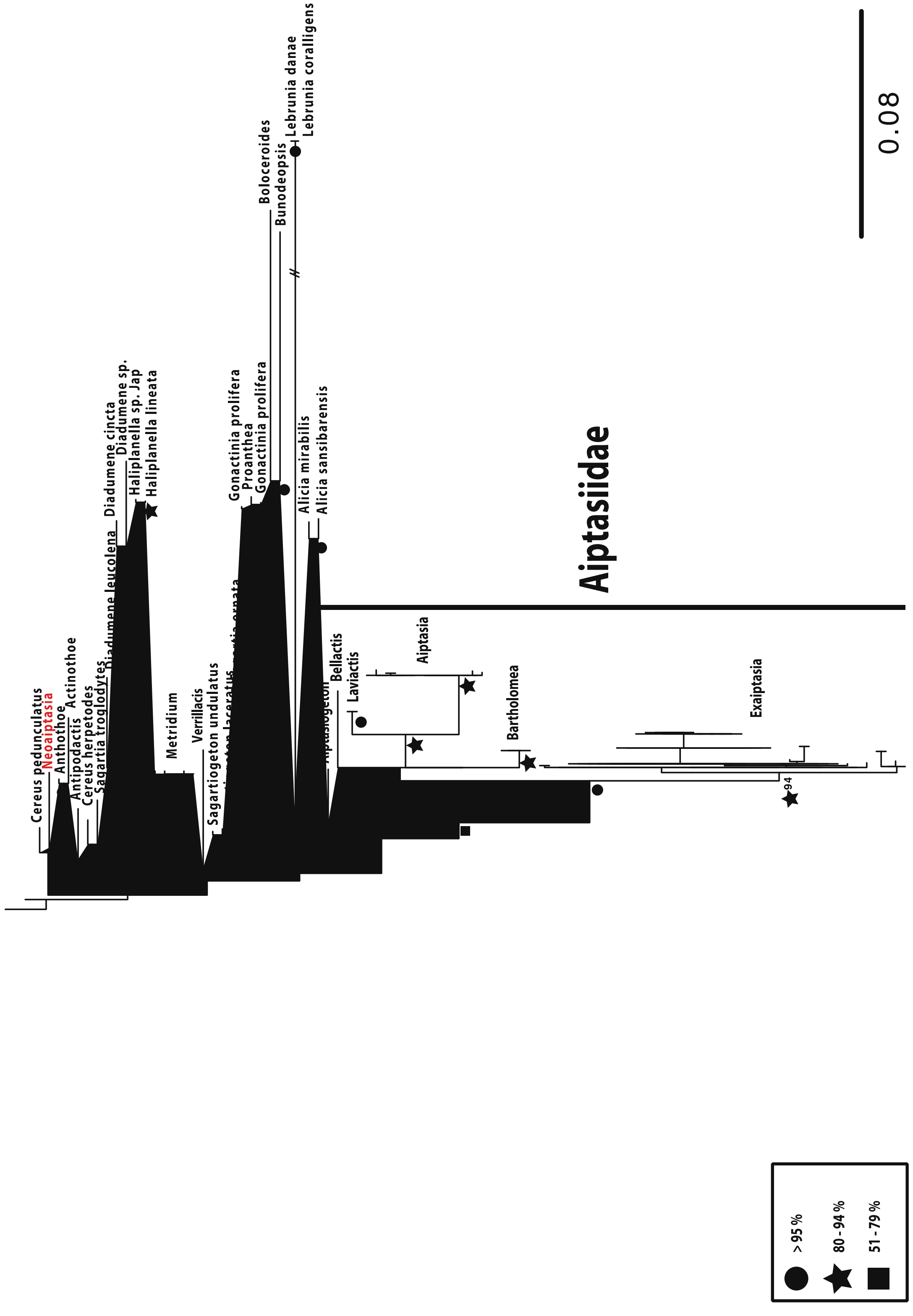
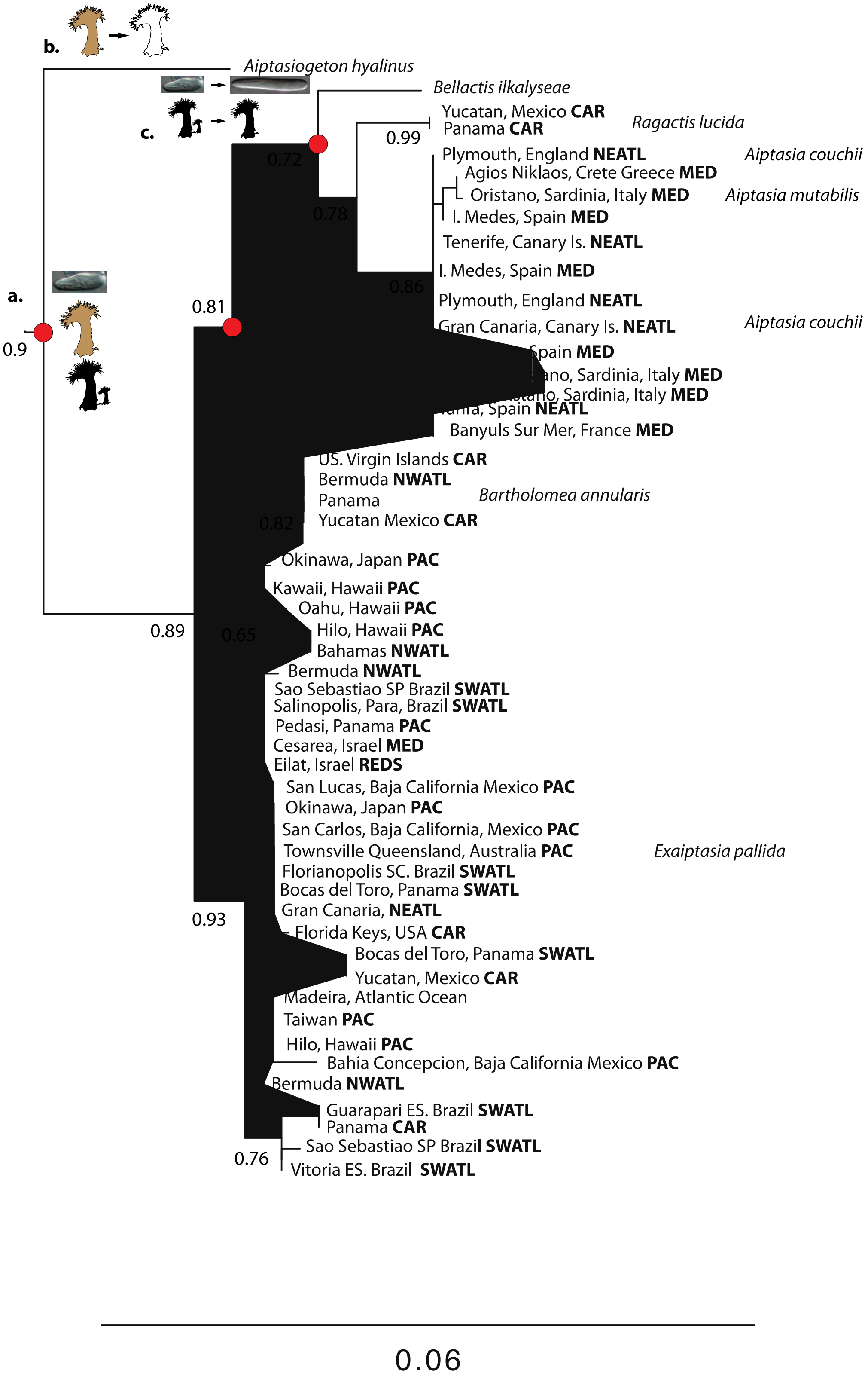

Cereus pedunculatus
Neoaiptasia
Anthothoe
Actinothoe
Antipodactis
Cereus herpetodes
Sagartia troglodytes
Diadumene leucolena
Diadumene cincta
Diadumene sp.
Haliplanella sp. Jap
Haliplanella lineata
Metridium
Verrillactis
Sagartiogeton undulatus
Gonactinia prolifera
Protanthea
Gonactinia prolifera
Boloceroides
Bunodeopsis
Lebrunia danae
Lebrunia coralligens
Alicia mirabilis
Alicia sansibarensis
Aiptasiogeton
Bellactis
Laviactis
Aiptasia
Bartholomea
Exaiptasia
94
Aiptasiidae
0.08
> 95 %
80 - 94 %
51 - 79 %

Figure 3.3. Detailed phylogenetic relationships within the family Aiptasiidae. Extract from the ML topology showing the phylogenetic relationships of genera and species within the family Aiptasiidae. Bootstrap support values (> 0.5) are shown on top of each branch. ML values left, MP values right. Detailed locality is provided for species and genera with more than one sample, followed by their general location among the different Ocean basins. **CAR**= Caribbean Sea, **MED**= Mediterranean Sea, **PAC**= Pacific Ocean, **RED**= Red Sea **SWATL**= South Western Atlantic Ocean, **NEATL**= North Eastern Atlantic Ocean, **NWATL**= North Western Atlantic Ocean.


b.
Aiptasiogeton hyalinus
Bellactis ilkalyseae
c.
0.72
0.99
Yucatan, Mexico CAR
Panama CAR
Ragactis lucida
Plymouth, England NEATL
Aiptasia couchii
Agios Niklaos, Crete Greece MED
0.78
Oristano, Sardinia, Italy MED
Aiptasia mutabilis
I. Medes, Spain MED
Tenerife, Canary Is. NEATL
I. Medes, Spain MED
0.86
a.
Plymouth, England NEATL
0.81
Gran Canaria, Canary Is. NEATL
Aiptasia couchii
0.9
Spain MED
ano, Sardinia, Italy MED
o, Sardinia, Italy MED
Spain NEATL
Banyuls Sur Mer, France MED
US. Virgin Islands CAR
Bermuda NWATL
Panama
Bartholomea annularis
0.82
Yucatan Mexico CAR
Okinawa, Japan PAC
Kawaii, Hawaii PAC
Oahu, Hawaii PAC
0.89
0.65
Hilo, Hawaii PAC
Bahamas NWATL
Bermuda NWATL
Sao Sebastiao SP Brazil SWATL
Salinopolis, Para, Brazil SWATL
Pedasi, Panama PAC
Cesarea, Israel MED
Eilat, Israel REDS
San Lucas, Baja California Mexico PAC
Okinawa, Japan PAC
San Carlos, Baja California, Mexico PAC
Townsville Queensland, Australia PAC
Exaiptasia pallida
Florianopolis SC. Brazil SWATL
Bocas del Toro, Panama SWATL
Gran Canaria, NEATL
0.93
Florida Keys, USA CAR
Bocas del Toro, Panama SWATL
Yucatan, Mexico CAR
Madeira, Atlantic Ocean
Taiwan PAC
Hilo, Hawaii PAC
Bahia Concepcion, Baja California Mexico PAC
Bermuda NWATL
Guarapari ES. Brazil SWATL
Panama CAR
0.76
Sao Sebastiao SP Brazil SWATL
Vitoria ES. Brazil SWATL
0.06

Morphological evolution within the family Aiptasiidae.

Morphologically, the family Aiptasiidae, as well as most families within the superfamily within Actiniaria, is defined by set of morphological characters, that are not exclusive for the group. Such characters have not, until recently (Rodriguez et al., in prep.) have been explicitly mapped using a phylogenetic context. As in other cases, the morphological features defining Aiptasiidae are not synapomorphies; it is rather the distinctive combination of them that makes possible to circumscribe its members. Among these, some characters like the presence of ectodermal longitudinal muscles are static within members of the family (not possible to define different states among different genera or species), while others do present some degree of variability, thus being amenable for optimization within the clade. A total of 12 morphological characters (Table 3.2) presented enough variability within the Aiptasiidae to allow further analysis. This reduced number of characters is not uncommon for morphological character-depauperate taxa such as sea anemones (e.g., Daly 2002; Gusmão & Daly, 2008; Rodriguez et al., 2008). I used the genus *Alicia* to polarize the character states. A detailed depiction of the characters in the parsimony reconstructions is shown on Figure 3.4. The obtained results allowed evaluating the degree to which some morphological characters, commonly used to define taxonomic groups, are informative of the phylogenetic relationships within the group, or instead are the product of convergence or homoplasy. The comparison between the number of mesenteries proximally and distally, the number of mesenteries distally, the number of tentacles (Figures 3.4a, 3.4b and 3.4d, respectively) show the highest degree of homoplasy, partially explaining the fact that *Aiptasia* and

Exaiptasia were previously classified together as a single genus (Carlgren, 1949). On the contrary, the number of mesenteries distally, the distribution of fertile mesenteries and the differences in the size of b-mastigophores (Figures 3.4c, 3.4f and 3.4) show no homoplasy. These results can be used as a case study from which future studies can make decisions about which characters should perhaps be considered in taxonomic determinations (i.e., merging or splitting genera and families). Usually the presence or absence of a determined nematocyst is considered a strong character to define intermediate taxonomic such as families (e.g. Carlgren 1949). At the same time, differences in the measurements of nematocysts have been traditionally used as an indication of the presence of separate species. In this example, a clear cut difference in the size of nematocysts could be used as a diagnostic character above the species level, but Grajales & Rodriguez (submitted; Chapter 2 of this thesis) showed that this is rather an exception, as all other nematocyst types did not show any indication of size differences across the entire family. It is also interesting to notice that some characters, like the tentacle shape (Figure 3.4e) and the shape of the basilar musculature (Figure 3.4j), seem to be the product of homoplasy, as opposed to the general assumption by taxonomists of these features being the product of common ancestry.

Exapitasia brasiliensis sp. nov.

Within *Exaiptasia*, two distinctive and well-supported clades could be recognized, and I considered them as two distinct cryptic species. Grajales & Rodriguez (submitted; Chapter 2 of this thesis) conducted a detailed taxonomic review of the members within Aiptasiidae, and could not find differences between specimens within *Exaiptasia*. I conclude that these two clades, namely *E. pallida* and *E. brasiliensis* can be diagnosed as different species based upon independent lines of evidence. First, they were shown to be reciprocally monophyletic. They also present different geographical patterns – *E. pallida* being a cosmopolitan species while *E. brasiliensis* is restricted to the Southwestern Caribbean Sea and the Southwestern Atlantic Ocean. It was also possible to find fixed differences in the DNA, including a nonsynonimous substitution in the CO3 gene region, a feature also observed in other taxa within Cnidaria (e.g., Medina et al., 1999; Hellberg et al, 2006), as well as some other fixed changes in 12S and 18S rDNA. Finally, *E. pallida* predominantly associates with *Symbiodinium* subtype B1 (Thornhill et al., 2013), while *E. brasiliensis* has been found to associate with at least two different *Symbiodinium* from clade A (Grajales et al., in prep b).

Thornhill et al (2013) found the first pieces of evidence for the existence of a widespread species within *Exaiptasia* by looking at the diversity of their endosymbiotic algae (*Symbiodinium* spp.) as well as some population level (SCAR) genetic markers. Despite their extensive sampling efforts, the authors did not include samples from the Southwestern Caribbean Sea or Brazil. The presence of individual specimens from both species within single localities (see Figure 3.3- Panama) was an intriguing finding, however it is not a totally unexpected observation for species within *Exaiptasia*. *E. pallida* is considered a weedy (Calado & Narciso, 2005), invasive species (Mito & Uesugi, 2004) a fact that can be enhanced by the possibility of rapid spread via pedal laceration. Thornhill et al. (2013) found very little genetic differentiation among endosymbiotic algae across different ocean basins, possibly indicative of recent introduction. The authors discussed two possible explanations for such an unusual pattern of genetic homogeneity of vectored introductions of specimens, via the aquarium trade, or ballast/fouling communities. Either possibility is likely in a highly transited area such as the Panamanian Caribbean Sea. It is worth noticing that the specimens collected in the Pacific Ocean side of Panama, from the Azuero Peninsula (>200 km away from the Panama Canal) clearly belonged to the widespread species *E. pallida.* Further clarification of this observation would be obtained by the use of population level markers that help elucidation the demographic history within each species.

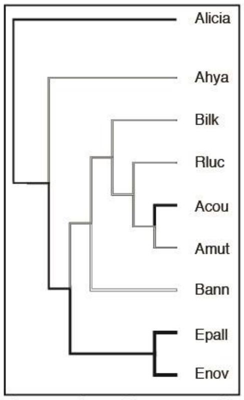
ACKNOWLEDGEMENTS

I am very grateful to colleagues who assisted us with fieldwork, particularly to C. Arvanitidis (Hellenic Centre for Marine Research, MRC, Greece), A. Baird (James Cook University, Townsville, Australia), J. Campbell (Smithsonian Marine Station at Fort Pierce, USA) R. Collins (Smithsonian Tropical Research Institute, STRI, Panama), V. Cumbo (James Cook University, Townsville, Australia), J. Frommlet (Centro de Estudios do Ambiente e do Mar, CESAM, Portugal), R. González (Universidad Nacional Autónoma de Mexico, UNAM) L. Gusmão (Universidade de São Paulo, Brazil), K. Hiscock (Marine Biological Association Plymouth, U.K.), P.J. López González (Universidad de Sevilla, Spain), P. Manent (Instituto Canario de Ciencias Marinas, Spain), O. Nir (Haifa University, Israel), J. Reimer (Ryukyus University, Okinawa, Japan) M. Takabayashi (U. Hawaii, Hilo) and P. Wirtz. Comments from M. Brugler (AMNH) M. Daly (Ohio State University) and D. Thornhill (Defenders of Wildlife, Washington) improved this manuscript. Support for this work was provided by the RGGS Graduate Fellowship, the Lerner-Gray Fund for Marine Research (AMNH), a Doctoral Dissertation Improvement Grant (NSF DEB 1110754) to AG and ER, and the Bermuda Institute of Ocean Sciences (BIOS, Bermuda).

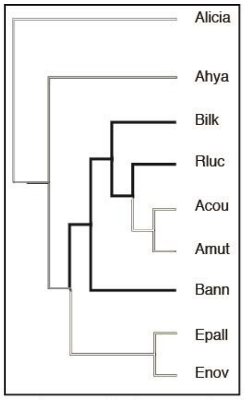
Table 3.2. Morphological characters and character states of species within the family Aiptasiidae.

Character	**States**	**Code**
Equal number of mesentery cycles	Same number of mesenterial cycles proximally and distally	0
	Different number of mesenterial cycles proximally and distally	1
Number mesenteries proximally	Four cycles	0
	More than four cycles	1
Number of mesentery cycles distally	Four cycles	0
	More than four cycles	1
Number of tentacles	96	0
	192	1
	> 192	2
Tentacle shape	Smooth	0
	Not smooth	1
Fertility	First and second cycle	0
	More than first and second cycles	1
Endosymbionts	Absent	0
	Present	1
Arrangement of cinclides	Rows	0
	Scattered	1
Pedal laceration	Present	0
	Absent	1
Basilar musculature	Lobed	0
	Flame-like	1
Scapus/Scapulus	Non differentiated	0
	Differentiated	1
Size of b-mastigophores in column (μm)	11-15	0
	16-25	1
Mesogleal sphincter	Weak	0
	Strong	1

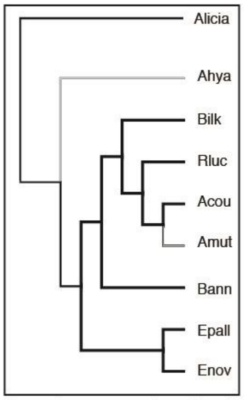
FIGURE 3.4. Parsimony character state reconstructions of morphological traits of the species within the family Aiptasiidae. Ahya = *Aiptasiogeton hyalinus*, Bilk = *Bellactis ilkalyseae*, Rluc= *Ragactis lucida*, Acou = *Aiptasia couhcii*, Amut = *Aiptasia mutabilis*, Bann = *Bartholomea annularis*, Epall = *Exaiptasia pallida*, Enov = *Exaiptasia* sp. nov.



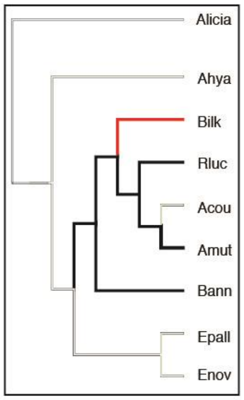
Eq. number of mesenteries



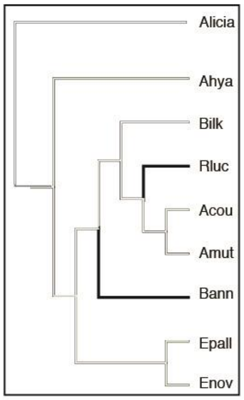
No. of mesenteries proximally



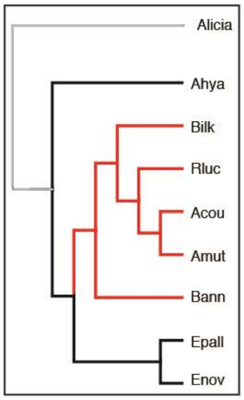
No. of mesenteries distally



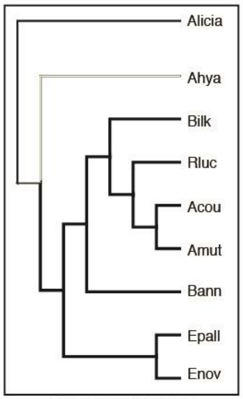
Number of tentacles



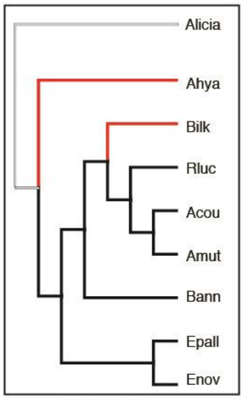
Tentacle shape



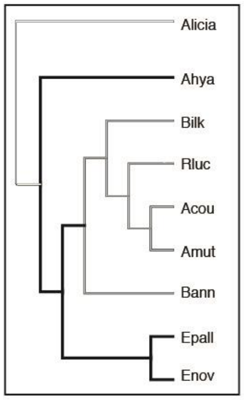
Fertility



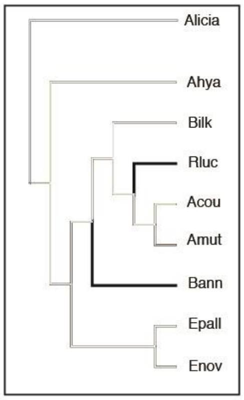
Endosymbionts



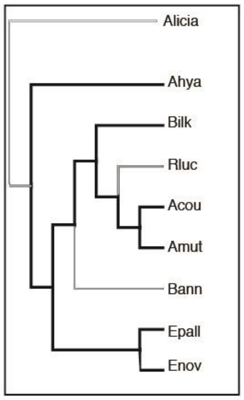
Cinclides



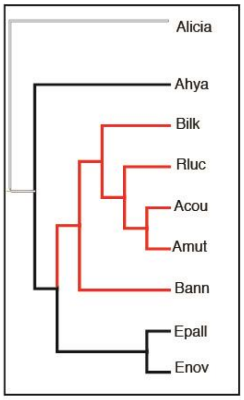
Pedal laceration



Basilar musculature



Scapus/Scapulus



B-mastigophores

References

Acuña, F. H., Excoffon, A. C., & Griffiths, C. L. (2004). First record and redescription of the introduced sea anemone Sagartia ornata (Holdsworth, 1855)(Cnidaria: Actiniaria: Sagartiidae) from South Africa. *African Zoology*, *39*(2), 314-318.

Calado, R., & Narciso, L. (2005). Ability of Monaco shrimp Lysmata seticaudata (Decapoda: Hippolytidae) to control the pest glass anemone Aiptasia pallida (Actiniaria: Aiptasidae). *Helgoland Marine Research*, *59*(2), 163-165.

Carlgren, O. (1949). A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskaps – Akademiens Handlingar*, series 4, 1(1), 1–121.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540-552.

Clayton Jr, W. S., & Lasker, H. R. (1985). Individual and population growth in the asexually reproducing anemone *Aiptasia pallida* Verrill. *Journal of experimental marine biology and ecology*, *90*(3), 249-258.

Daly, M., Lipscomb, D. L., & Allard, M. W. (2002). A simple test: Evaluating explanations for the relative simplicity of the Edwardsiidae (Cnidaria: Anthozoa). *Evolution*, *56*(3), 502-510.

Daly, M., Chaudhuri, A., Gusmao, L., & Rodriguez, E. (2008). Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular phylogenetics and evolution*, *48*(1), 292-301.

Daly, M., Gusmão, L. C., Reft, A. J., & Rodríguez, E. (2010). Phylogenetic signal in mitochondrial and nuclear markers in sea anemones (cnidaria, Actiniaria). *Integrative and comparative biology*, *50*(3), 371-388.

Dunn, S. R., Bythell, J. C., Le Tissier, M. D., Burnett, W. J., & Thomason, J. C. (2002). Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia*. sp. *Journal of Experimental Marine Biology and Ecology*, *272*(1), 29-53.

Dunn, C. W., Hejnol, A., Matus, D. Q., Pang, K., Browne, W. E., Smith, S. et al. (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature*, *452*(7188), 745-749.

Fautin, D.G. (2013). Hexacorallians of the World. Available from: http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm. (June 2013).

Goloboff, P. A., Farris, J. S., & Nixon, K. C. (2008). TNT, a free program for phylogenetic analysis. *Cladistics*, *24*(5), 774-786.

Goodwill, R.H., Fautin, D.G., Furey, J., & Daly, M. (2009) A sea anemone symbiotic with gastropods of eight species in the Mariana Islands. *Micronesica*, 41(1), 117–130.

Grajales, A., & Rodriguez, E. (Submitted). Morphological revision of the genus *Aiptasia* and the family Aiptasiidae (Cnidaria, Actiniaria, Metridioidea).

Grajales, A. Brugler, M.R. and Rodriguez, E. (In prep a). Anemone-specific primers for amplification of the nuclear ribosomal cistron in hosts that contain *Symbiodinium*, a dinoflagellate symbiont.

Grajales, A., Rodriguez, E. & Thornhill, D. (In prep b) . Patterns of *Symbiodinium* spp. specificity within the family Aiptasiidae (Cnidaria: Anthozoa: Actiniaria: Metridioidea), a monophyletic lineage of symbiotic sea anemones.

Gusmão, L. C., & Daly, M. (2010). Evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. *Molecular Phylogenetics and Evolution*, *56*(3), 868-877.

Katoh, Misawa, Kuma, Miyata (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Reseach*, 30: 3059-3066.

LaJeunesse, T. C., Smith, R., Walther, M., Pinzón, J., Pettay, D. T., McGinley, M., ... & Warner, M. E. (2010). Host–symbiont recombination versus natural selection in the response of coral–dinoflagellate symbioses to environmental disturbance. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1696), 2925-2934.

Lin, K. L., Wang, J. T., & Fang, L. S. (2000). Participation of glycoproteins on zooxanthellal cell walls in the establishment of a symbiotic relationship with the sea anemone, *Aiptasia pulchella*. *Zoological Studies*, *39*(3), 172-178.

Maddison, W. P., and D.R. Maddison. (2011). Mesquite: a modular system for evolutionary analysis. Version 2.75 http://mesquiteproject.org

Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010). "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway

Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1 - 8.

Muller-Parker, G., & Davy, S. K. (2001). Temperate and tropical algal- sea anemone symbioses. *Invertebrate Biology*, *120*(2), 104-123.

Rodríguez, E., & Daly, M. (2010). Phylogenetic relationships among deep-sea and chemosynthetic sea anemones: Actinoscyphiidae and Actinostolidae (Actiniaria: Mesomyaria). *PloS one*, *5*(6), e10958.

Rodríguez, E., Barbeitos, M., Daly, M., Gusmão, L. C., & Häussermann, V. (2012). Toward a natural classification: phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). *Cladistics*, *28*(4), 375-392.

Rodríguez, E., Barbeitos, M. S., Brugler, M.R., Crowley, L., Haussermann, V., Grajales, A., Gusmão, L., Reft, A., and Daly, M. (In prep.). Are sea anemones (Cnidaria, Anthozoa) monophyletic? The first higher-level phylogenetic classification for Actiniaria.

Shearer, T. L., Van Oppen, M. J. H., Romano, S. L., & Wörheide, G. (2002). Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria).*Molecular Ecology*, *11*(12), 2475-2487.

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models", Bioinformatics 22(21): 2688–2690.

Thornhill, D. J., Xiang, Y., Pettay, D. T., Zhong, M., & Santos, S. R. (2013). Population genetic data of a model symbiotic cnidarian system reveal remarkable symbiotic specificity and vectored introductions across ocean basins. *Molecular ecology*, *22*(17), 4499-4515.

van Oppen, M. J., Willis, B. L., & Miller, D. J. (1999). Atypically low rate of cytochrome b evolution in the scleractinian coral genus Acropora. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1415), 179-183.

Weis, V. M., Davy, S. K., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., & Pringle, J. R. (2008). Cell biology in model systems as the key to understanding corals. *Trends in Ecology & Evolution*, *23*(7), 369-376.

Zwickl, D. J., & Hillis, D. M. (2002). Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology*, *51*(4), 588-598.

CHAPTER IV

PATTERNS OF SYMBIODINIUM SPP. SPECIFICITY WITHIN THE FAMILY AIPTASIIDAE, A MONOPHYLETIC LINEAGE OF SYMBIOTIC OF SEA ANEMONE

ABSTRACT

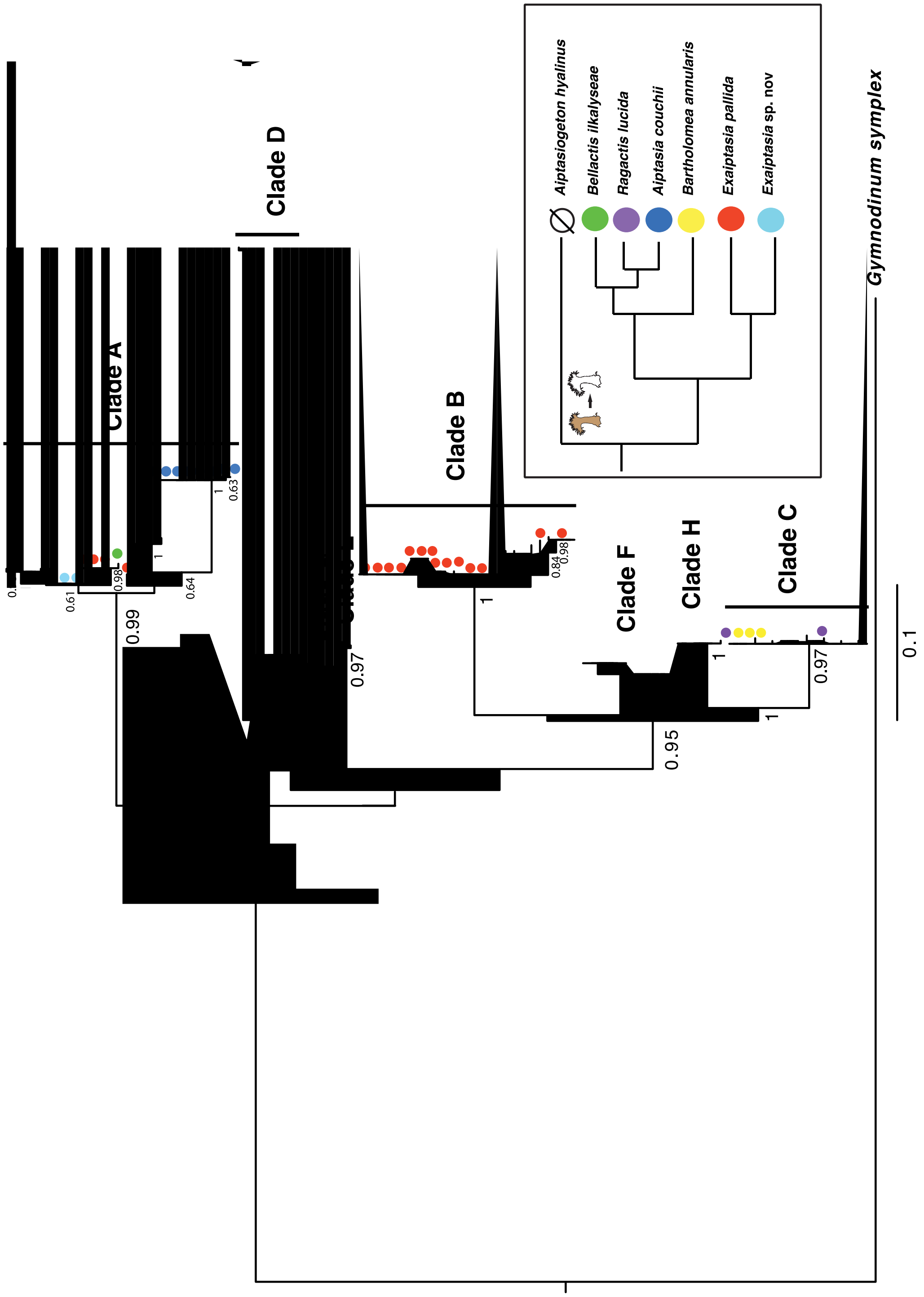
Although the symbiotic relationships between dinoflagellates and cnidarians are well recognized, few studies have examined these associations from an evolutionary perspective. This is especially true for symbiotic sea anemones, where many reports consist of an approximate species identification of the host, followed by the identification of the dinoflagellate symbiont using molecular genetic markers. To further explore the evolutionary history of sea anemone-dinoflagellate associations,I documented the diversity of *Symbiodinum* spp. in a monophyletic clade of sea anemones, the family Aiptasiidae. I combined information from several molecular genetic markers, including nuclear ITS2 and plastid cp23S-rDNA, to evaluate the patterns of evolution and diversification of *Symbiodinum* in the light of an existing phylogenetic framework for the host. At the host family level, I found no evidence for coevolution or reciprocal phylogenies between host and endosymbiont. However, within some individual host species, *Symbiodinium* spp. exhibited patterns of host specialization and cladogenesis. This pattern suggests that coevolution between host and symbiont occurred within species and genera lineages, but that this process was regularly disrupted and symbiotic partners were recombined during the longer-term evolutionary history of the Aiptasiidae. Furthermore, I observed two independent cases of *Symbiodinium* cladogenesis within a single host species, suggesting ecological speciation along an environmental gradient.

Keywords: *Aiptasia*, *Exaiptasia*, Type A4 *Symbiodinium*, Type B1 *Symbiodinium*, Adaptation.

INTRODUCTION

Symbiotic relationships between sea anemones (Cnidaria: Actiniaria) and endosymbiotic dinoflagellates (Dinophyceae: *Symbiodinium*) have been recognized for over one hundred years (Gee, 1913; Elmhirst & Sharpe, 1920), yet few studies have analyzed the evolutionary history of these associations. Only recently were the phylogenetic relationships for sea anemones explored at both broad (Daly et al., 2008; Rodríguez et al., 2010) and fine taxonomic levels (Daly et al., 2002; Gusmão et al., 2010). Most previous work on anemone-endosymbiont relationships either was conducted from the dinoflagellate perspective (e.g., LaJeunesse, 2002; LaJeunesse et al., 2004), or were species-specific, ecological studies, often occurring over latitudinal gradients (e.g., LaJeunesse & Trench, 2000; Muller-Parker & Davy, 2001; Savage et al., 2002; Venn et al., 2008). Studies such as these, combined with work in other host taxa and free-living dinoflagellates, demonstrated that *Symbiodinium* is a diverse dinoflagellate lineage comprising nine sub-generic clades (designated A through I) and many more species, phylotypes, and strains (Pochon & Gates, 2010; Finney et al., 2010; LaJeunesse and Thornhill, 2011; Pochon et al., 2012; Thornhill et al., 2013a). In contrast to the abundant data on *Symbiodinium* diversity, information about the molecular diversity and evolutionary history of the anemone host is often neglected or unavailable.

Figure 4.1 Maximum likelihood (ML) tree showing the phylogenetic position of *Symbiodinium* spp. across members of the family Aiptasiidae, based on the domain V of the cp-23S region. Only bootstrap values supporting the monophyly of each clade are shown. The diagram on the bottom right deptics the phylogenetic relationships among the host anemone species, extracted from Grajales et al., 2013b.


Clade A
Clade D
Clade B
Clade F
Clade H
Clade C
Gymnodinium symplex
Aiptasiogeton hyalinus
Bellactis ilkalyseae
Ragactis lucida
Aiptasia couchii
Bartholomea annularis
Exaiptasia pallida
Exaiptasia sp. nov
0.61
0.98
0.99
0.64
1
0.63
1
0.97
1
0.84
0.98
0.95
1
1
0.97
0.1

Most reports of *Symbiodinium* diversity within the Aiptasiidae focused on *Exaiptasia pallida* (formerly *Aiptasia pallida*, Grajales & Rodríguez, submitted [Chapter 2 of this thesis]). Notably, *E. pallida* is an important model organism for symbiosis studies, including several cultured clones lines of host and symbiont that are used in experimental manipulations (e.g., Rodriguez-Lanetty et al., 2006; Sunagawa et al., 2008, 2009; Pringle, 2012). Although few studies document the diversity and diversification of both the host anemone and their associated *Symbiodinium* spp., Thornhill et al. (2013b) recently explored this topic within *Exaiptasia* spp. This circumtropical study demonstrated that *Exaiptasia* spp. exclusively harbors *S. minutum* (ITS2 type B1*Aiptasia*) throughout most of the world, with the exception of anemones in Florida that associated with *S. minutum*, *Symbiodinium* type A4, and one lineage of *Symbiodinium* clade C. According to four SCAR genetic markers, *Exaiptasia* did not exhibit population genetic structure across most of the world, signifying a single widespread anemone species (Thornhill et al., 2013b). However, Florida *Exaiptasia* were genetically distinct compared to other localities, suggesting a differentiated population, or even an incipient species, of symbiotic anemone at that locality (Thornhill et al., 2013b). The combined host and symbiont data indicated a correlation between the diversity of each partner, with a globally distributed specific association and a Florida endemic flexible association (Thornhill et al., 2013b). Notably, Thornhill et al. (2013b) did not sample locations within the Caribbean Sea, such as Panama and Brazil and various *Symbiodinium* lineages can be induced to infect *E. pallida* in a laboratory setting (e.g., Kinzie et al., 2001; Goulet et al., 2005). Grajales & Rodríguez (in prep. [Chapter 2 of this thesis]), provided further evidence for the existence of at least two species within *Exaiptasia*, based on genetic markers. These *Exaiptasia* spp. had distinct biogeographies, including a widespread species, designated *Exaiptasia pallida*, and a second more geographically restricted species, *Exaiptasia* sp. nov. found in Brazil and Panama (Grajales & Rodríguez, in prep. [Chapter 2 of this thesis]). In contrast to Thornhill et al. (2013), Grajales & Rodríguez (in prep. [Chapter 2 of this thesis]) detected no differences between Florida *Exaiptasia* and *E. pallida* from other localities; however, the two studies used different molecular genetic markers with different taxonomic resolution (i.e., populations vs. species).

In addition to the two *Exaiptasia* species, six other species comprise the Aiptasiidae. Sister to *Exaiptasia* is a clade comprised of five species: *Bellactis ilkalyseae*, *Ragactis lucida*, *Aiptasia mutabilis*, *Aiptasia couchii*, and *Bartholomea annulata* (Fig. 1). Basal to this group and *Exaiptasia* is one final aiptasiid species: *Aipasiogeton hyalinus* (Figure 4.1). To date few studies have examined the symbiotic dinoflagellates within these hosts. Thus I elucidated dinoflagellate-anemone relationships within this group by combining molecular genetic and phylogenetic data from both symbiotic partners. I explicitly addressed whether or not anemones and symbionts follow a pattern consistent with the diversification history of the family Aiptasiidae, both between as well as within species. I also examined what environmental factors, if any, correlated with the diversification of *Symbiodinium* within this lineage.

Materials and Methods

Data Collection

The material studied was collected on 2009–2012 from 41 different localities spanning the globe (Table 4.1). All collected anemone species were collected from 0 and 5 m, except for the specimens of *Aiptasia mutabilis*, which were collected at 10 m depth. Genomic DNA was isolated from tentacle or column tissue using the Qiagen DNAasy® kit.

cp23S-rDNA:

An approximately 0.7-kb region of dinoflagellate cp23S-rDNA, corresponding to Domain V of the cp23S-rDNA molecule (Harris et al., 1994), was PCR amplified from the same isolates as those above using the primer pair 23S1 (5′-CACGACGTTGTAAAACGACGGCTGTAACTATAACGGTCC-3′) and 23S2 (5′-GGATAACAATTTCACACAGGCCATCGTATTGAACCCAGC-3′). PCRs were performed in 25-μL volumes containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 0.001% gelatin, 200 μM dNTPs, 100 pmol of each primer, 2U *Taq* polymerase, and 50–70 ng of template DNA. Reactions were carried under the following conditions: initial denaturing period of 1 min at 95 °C, 35 cycles consisting of 95 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, and a final extension period of 7 min. PCR products were cleaned using ExosapIT. Sequencing reactions used a total of 5µL of cleaned PCR product, at a concentration of 25 ng of product for every 200 base pairs of marker length. Cleaned PCR products were sequenced using amplification primers on an ABI 3770*x* at the in-house facilities of the AMNH. Forward and reverse sequences were assembled in

Geneious (version 6.16 created by Biommaters), and blasted against the nucleotide database of GenBank to confirm whether the obtained product corresponded to the target organism. All sequences have been deposited in GenBank (Table 4.2).

ITS2:

The ITS2 region was amplified from *Symbiodinium* DNA extracts for denaturing gradient gel electrophoresis (DGGE) using primers ITSintfor2 and ITS2clamp, followed by excision and DNA sequencing of all discreet, prominent bands in the DGGE profile as described by LaJeunesse (2002). Profiles and sequences were assigned an alphanumeric ITS2 type designation (*sensu* LaJeunesse 2001) representative of that fingerprint and symbiont type. This technique targets the numerically dominant sequence variants in the symbiont's genome (Thornhill et al. 2007) and is able to detect symbionts comprising approximately 10% or more of a symbiotic population (Thornhill et al. 2006). Gels were poured following manufacturer's instructions using 8% polyacrylamide (37.5:1 acrylamide/bisacrylamide ratio), approximately 20 cm long plates, 0.75 mm spacers, and a 45–80% denaturing gradient (100% denaturant contains 7 mol L^{-1} urea and 40% deionized formamide). Prior to loading samples, excess denaturant was purged from the wells using a micropipette. 20 µL of each PCR reaction was added to 10 µL of xylene cyanol loading dye (pH 7.0) and a total of 10 µl of the combined product was loaded onto each gel. Runs were performed at 60 °C. Gels were electrophoresed at 150 V for 10 h on a C.B.S. Scientific™ DGGE-1001 model apparatus (following Thornhill et al. 2010). Gels were stained with SYBR Green (Molecular Probes, 10,000× diluted in 1×TAE) for

20 min and photographed under UV light. In addition to band excision and sequencing, identifications of types were confirmed by comparison of the DGGE fingerprint for each sample to ITS2 PCR product standards from clonal *Symbiodinium* cultures representing types within Clades A, B, C, and D.

Data Analysis

23S

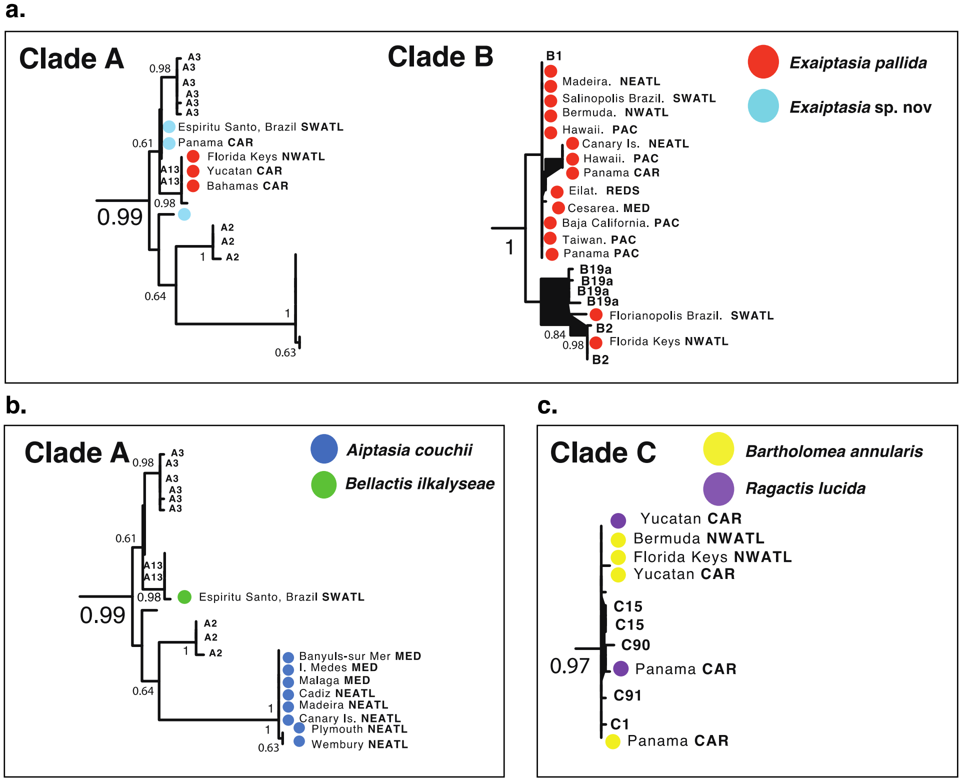
Sequences for the 23Scp-rDNA were aligned in MAFFT v 7.017 (Katoh & al. 2002) using the following settings L-INS-i option Scoring Matrix 200PAM / k=2 Gap open penalty 1.53. The produced alignment was processed with Gblocks (Castresana, 2000) to discard ambiguously aligned regions. The following parameters were used (Maximum number of contiguous non-conserved positions: 8, Minimum length of a block: 5, Gap positions allowed) to produce a less stringent set of conserved aligned blocks. The reduced alignment was deposited in TreeBase (http://www.treebase.org/treebase/index.html). A Maximum likelihood (ML) analysis was performed in RAxML v.7.6.3 (Stamatakis, 2006) implemented on the CIPRES portal (Miller et al., 2010). It included obtained sequences from this study plus voucher cp23S-rDNA sequences from cultured *Symbiodinium* types A through H, representatives of the diversity within the genus (Pochon et al., 2006). *Gymnodinium simplex* was used as an outgroup. A GTR + Γ (GTRGAMMA) was chosen as substitution model, Γ shape, GTR rates and base frequencies alignment were allowed to be estimated by the software. The

Majority Rule Criterion implemented in RAxML (-autoMRE) was used to assess clade support.

ITS2 genotyping:

Chromatograms were checked and assembled using the software Geneious (version 6.16 created by Biomaters). Sequences were aligned using MAFFT v 7.017 (Katoh & al. 2002) with reference ITS2 sequences, in order to observe whether or not they were identical to known types. New variants were characterized as potential newly discovered subtypes within each *Symbiodinium* clade.

FIGURE 4.2. Detailed view of *Symbiodinium* spp. per host genera and species **a.** *Exaiptasia pallida,* **b.** *Aiptasia couchii* and *Bellactis ilkalyseae,* **c.** *Bartholomea annularis* and *Ragactis lucida*. Detailed locality is provided for species and genera, followed by their general location among the different ocean basins. **CAR**= Caribbean Sea, **MED**= Mediterranean Sea, **PAC**= Pacific Ocean, **RED**= Red Sea **SWATL**= South Western Atlantic Ocean, **NEATL**= North Eastern Atlantic Ocean, **NWATL**= North Western Atlantic Ocean.


a.
Clade A
0.98
A3
A3
A3
A3
A3
A3
Espiritu Santo, Brazil SWATL
Panama CAR
0.61
Florida Keys NWATL
Yucatan CAR
Bahamas CAR
A13
A13
0.98
0.99
A2
A2
1
A2
0.64
1
0.63
Clade B
B1
Madeira. NEATL
Salinopolis Brazil. SWATL
Bermuda. NWATL
Hawaii. PAC
Canary Is. NEATL
Hawaii. PAC
Panama CAR
Eilat. REDS
Cesarea. MED
Baja California. PAC
Taiwan. PAC
Panama PAC
1
B19a
B19a
B19a
B19a
Florianopolis Brazil. SWATL
B2
0.84
0.98
Florida Keys NWATL
B2
Exaiptasia pallida
Exaiptasia sp. nov
b.
Clade A
0.98
A3
A3
A3
A3
A3
A3
0.61
A13
A13
0.98
Espiritu Santo, Brazil SWATL
0.99
A2
A2
1
A2
Banyuls-sur Mer MED
I. Medes MED
Malaga MED
Cadiz NEATL
Madeira NEATL
1
Canary Is. NEATL
1
Plymouth NEATL
0.63
Wembury NEATL
0.64
Aiptasia couchii
Bellactis ilkalyseae
c.
Clade C
Bartholomea annularis
Ragactis lucida
Yucatan CAR
Bermuda NWATL
Florida Keys NWATL
Yucatan CAR
C15
C15
C90
0.97
Panama CAR
C91
C1
Panama CAR

RESULTS

All but one species within the family Aiptasiidae were found to be associated with endosymbiotic dinoflagellates. The species *Aiptasiogeton hyalinus*, which is the sister clade to all other members of the family did not harbor endosymbiotic algae, a fact that was also confirmed by histological sections of the column (not shown) and the tentacles (Figure 4.1a and 4.1b). At total of three different *Symbiodinium* clades (A, B and C) were found across the different genera of Aiptasiidae (Figure 4.1).

By combining the information obtained from the phylogenetic tree of the cp23S-rDNA sequences (Figure 4.1), and the DGGE profiles and sequencing of the ITS2 (Table 4.1), it was possible to assign most of the symbiont present the studied sea anemones to known types, as well as to identify possible new types. It is now accepted that that the combination of ITS2 + DGGE profiling allows for the identification of multiple subtypes of symbionts within single individuals, as well as the utility of the markers to identify physiologically differentiated strains (see LaJeunesse, 2001, 2002), although some controversy exists (see Correa & Baker, 2009). This protocol also allows for the identification of multiple subtypes of *Symbiodinium* per individual, in the case of sea anemones, single polyps. In addition I compared the profiling of ITS2 with the information provided by the cp23S, marker, as a sort of independent marker from the chloroplast genome. In general, the information provided by the 23S sequences is consistent with the identity of the symbionts provided by ITS2, but it was not possible to confidently conclude about the specific subtypes, due to the lack of support for the depicted relationships within the clade (Figure 4.2c). All screened individuals, except for the one species of *Exapitasia pallida* from Bermuda, are associated with a single type of symbiont (Table 4.1). From a species perspective, all were found to be associated with a single symbiont clade, except for *Exaiptasia pallida*, which was found to associate with both clades A and B. *Aiptasia couchii* and *Bellactis ilkayseasae* were found to be associated with clade A *Symbiodinium,* the first species symbiont was identified as A4 subtype, according to ITS2, while all specimens from *Aiptasia couchii* were associated with a symbiont closely related to the A1 subtype (Figure 4.3a). Both *Bartholomea annularis* and *Ragactis lucida* were found to associate exclusively with type C1 according to the ITS2, while the 23S phylogenetic hypothesis recovered these specimens with the type C clade, without further resolution (Figure 4.3c). Finally, species within the genus *Exaiptasia* showed different association patterns with *Symbiodinium*. *Exaiptasia pallida* was found to be associated with clade B except from three localities in the Caribbean and one in the south of Brazil (Figure 4.3b) associated with clade A. From the specimens that harbored clade B *Symbiodinium*, most of them were associated with subtype B1, except two samples, one in the Florida Keys and in Florianopolis (south of Brazil), associated with subtype B2. The 23S information suggests that the sample from Florida belongs to subtype B2, but also suggest that the *Symbiodinium* found in the sample from the south of Brazil might be a new subtype. *Exaiptasia* sp. nov. was found to exclusively associate with clade A *Symbiodinium.* Two the samples seem to be closely related to type A13, according to the information from the 23S marker, while a third specimen, from Sao Sebastiao (Brazil), could also be a previously undiscovered subtype, given its position on the 23S tree (Figure 4.3a).

DISCUSSION

From an evolutionary point of view, the lack of endosymbionts in *Aiptasiogeton hyalinus* is likely to be a derived rather than an ancestral condition, given the phylogenetic position of the species. Grajales et al. (2013a) recovered this species as nested within a clade of symbiotic lineages, including the sister group of the family Aiptasiidae, the genus *Alicia*. The lack of symbionts in other related groups such as scleractinian corals has been found to be associated with deep-sea lineages (Barbeitos et al., 2010), while in this case this endosymbiont-free species is found between 0 and 10 m depth.

The observed phylogenetic pattern shows no evidence of coevolution between the dinoflagellates and their hosts at a broad level; the phylogenetic position of the symbionts (Figure 4.1a) does not mirror host phylogeny (Figure 4.1b). In some instances the utilized markers show lack of resolution conclude about the definite symbiont identity (Figure 4.3c), while other parts of the tree allow us to observe some interesting patterns (Figure 4.1a and 4.1b), always within a single anemone species. This fact is contrasting with what is found in other taxa, such as scleractinian corals, where the cladogenesis events seem to occurr both at the same time in both host and symbiont phylogeny (Thornhill et al., 2013), a pattern consistent with coevolution. The observed cladogenesis events in this study are more likely to be the adaptation within species towards the edges of their distribution. The first example of this pattern is observed along a latitudinal gradient along the North Eastern Atlantic Ocean. At least two symbiont subtypes were observed within individuals of *Aiptasia couchii* (Figure 4.1a). These symbionts were associated with the symbiont A1_med, a symbiont observed in other sea anemones of the North Eastern Atlantic Ocean (Savage et al., 2002), where it is described as a ‘temperate’ clade. Within this clade, two samples collected in Plymouth, classified here as A1_atl, seem to be divergent and are probably specialists only found at the northernmost distribution of the species. A parallel situation is observed within *Exaiptasia pallida*, which was found to associate with subtypes B1 and A4; it presented two exceptions, one in Florida (US) with subtype B2, and at Florianopolis (Brazil), where a divergent B2 subtype was found. Even though the latter case is less extreme in terms of latitude (correlated with seasonality changes in temperature), both cases of this pattern might reflect possible adaptations to colder environmental conditions. In the case of *A. couchii*, current evidence suggests that they subtype labeled here as A1_atl could be considered a new type, and possibly a new species. Subtype B2 was first described from an *Exaiptasia* specimen in Florida, and it has been identified as a dominant type on temperate coral populations on the Western Atlantic Ocean (Thornhill et al, 2007) while being less common in tropical latitudes. Interestingly, the divergent B2 subtype (noted here as B2*) was found in Florianopolis, Brazil, which corresponds to the southernmost report of *Exaiptasia pallida* (Grajales & Rodriguez, 2013a). Giving the geographic distance and climatic barriers between these two localities, it is striking that these two subtypes appear as strongly supported sister clades. Additional makers such as PsbA (see LaJeunesse et al., 2011, Thornhill et al., 2013) as well as an increased taxon sampling should be able to provide more resolution, in order further elucidate the mechanisms of diversification within clade B.

Our findings largely agree with the results obtained by Thornhill et al (2013b), regarding the diversity of *Symbiodinium* within the widespread species *Exaiptasia pallida*. The authors detected *Symbiodinium* B1 as the only symbiont in most localities, except Florida, a pattern consistent with that found in this study (Figure 4.3a). This subtype is a generalist and the prevalent symbiont in the Caribbean Sea, and also is present in other anthozoan taxa such as scleractinian corals and octocorals (LaJeunesse, 2001). Its presence in other localities other than the Caribbean might be associated with the possible human mediated-dispersal of its host *Exaiptasia pallida* (see Thornhill et al, 2013b for further details) rather than ancestral variation. This study increased the sampling of *Exaiptasia* within the Caribbean Sea, including localities in the Bahamas, Yucatan and Panama, as well as different localities along the coast of Brazil. Interestingly, we found an increase in the diversity of symbionts, compared to the worldwide pattern suggested by Thornhill at al. (2013b). The ITS2 identification suggested that most of the samples from the Caribbean Sea correspond to the type A4, while the 23S tree clusters the samples on a monophyletic clade, related to A13 and A3 subtypes. None of the markers used in this study seem to give enough resolution to further characterize this symbiont, in order to identify whether it is a new and endemic type, or if it is the same subtype found in other hosts within the Caribbean (Correa & Baker, 2009). On a similar note, we found that a newly described species, *Exaiptasia* sp. nov., restricted to Panama and Brazil, associates exclusively with clade A symbionts, one related to subtype A4 and a second, divergent clade, possibly representing another newly discovered subtype. Better resolution, again to be provided by molecular markers such as PsbA (LaJeunesse et al., 2011), will help further elucidate the diversification history within this clade.

ACKNOWLEDGEMENTS

We are very grateful to colleagues who assisted us with fieldwork, particularly to C. Arvanitidis (Hellenic Centre for Marine Research, MRC, Greece), A. Baird (James Cook University, Townsville, Australia), J. Campbell (Smithsonian Marine Station at Fort Pierce, USA) R. Collins (Smithsonian Tropical Research Institute, STRI, Panama), V. Cumbo (James Cook University, Townsville, Australia), J. Frommlet (Centro de Estudios do Ambiente e do Mar, CESAM, Portugal), R. González (Universidad Nacional Autónoma de Mexico, UNAM) L. Gusmão (Universidade de São Paulo, Brazil), K. Hiscock (Marine Biological Association Plymouth, U.K.), P.J. López González (Universidad de Sevilla, Spain), P. Manent (Instituto Canario de Ciencias Marinas, Spain), O. Nir (Haifa University, Israel), J. Reimer (Ryukyus University, Okinawa, Japan) M. Takabayashi (U. Hawaii, Hilo) and P. Wirtz. D. Pettay and ME Warner who greatly contributed in the identification of the ITS2 genotypes. Support for this work was provided by the RGGS Graduate Fellowship, the Lerner-Gray Fund for Marine Research (AMNH) provided support for this work, a Doctoral Dissertation Improvement Grant (NSF DEB 1110754) to AG and ER, and the Bermuda Institute of Ocean Sciences (BIOS, Bermuda).

References

Barbeitos, M. S., Romano, S. L., & Lasker, H. R. (2010). Repeated loss of coloniality and symbiosis in scleractinian corals. *Proceedings of the National Academy of Sciences*, *107*(26), 11877-11882.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular biology and evolution*, *17*(4), 540-552.

Correa, A. M. S., & Baker, A. C. (2009). Understanding diversity in coral-algal symbiosis: a cluster-based approach to interpreting fine-scale genetic variation in the genus Symbiodinium. *Coral Reefs*, *28*(1), 81-93.

Daly, M., Lipscomb, D. L., & Allard, M. W. (2002). A simple test: Evaluating explanations for the relative simplicity of the Edwardsiidae (Cnidaria: Anthozoa). *Evolution*, *56*(3), 502-510.

Daly, M., Chaudhuri, A., Gusmao, L., & Rodriguez, E. (2008). Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). *Molecular phylogenetics and evolution*, *48*(1), 292-301.

Elmhirst, R., & Sharpe, J. S. (1920). On the Colours of two Sea Anemones, *Actinia equina* and *Anemonia sulcata*: Part I. Environmental. Part II. Chemical.*Biochemical Journal*, *14*(1), 48.

Gee, W. (1913). Modifiability in the behavior of the California Shore-Anemone *Cribrina Xanthogrammica* Brandt. *Journal of Animal Behavior*, *3*(5), 305.

Grajales, A. & Rodriguez, E. (in prep). Molecular phylogenetics of the family Aiptasiidae (Cnidaria:Actiniaria:Metridioidea).

Gusmão, L. C., & Daly, M. (2010). Evolution of sea anemones (Cnidaria: Actiniaria: Hormathiidae) symbiotic with hermit crabs. *Molecular Phylogenetics and Evolution*, *56*(3), 868-877.

Harris, E. H., Boynton, J. E., & Gillham, N. W. (1994). Chloroplast ribosomes and protein synthesis. *Microbiological reviews*, *58*(4), 700.

Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, *30*(14), 3059-3066.

LaJeunesse, T. C., and R. K. Trench. (2000). "Biogeography of two species of Symbiodinium (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt)." *The Biological Bulletin* 199 (2): 126-134.

LaJeunesse, T. C. (2001). Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a “species” level marker. *Journal of Phycology*,*37*(5), 866-880.

LaJeunesse, T. (2002). Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology*, *141*(2), 387-400.

LaJeunesse, T. C., Thornhill, D. J., Cox, E. F., Stanton, F. G., Fitt, W. K., & Schmidt, G. W. (2004). High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral reefs*, *23*(4), 596-603.

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010,). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Gateway Computing Environments Workshop (GCE), 2010* (pp. 1-8). IEEE.

Muller-Parker, G., & Davy, S. K. (2001). Temperate and tropical algal- sea anemone symbioses. *Invertebrate Biology*, *120* (2), 104-123.

Pochon, X., Montoya-Burgos, J. I., Stadelmann, B., & Pawlowski, J. (2006). Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*. *Molecular phylogenetics and evolution*, *38*(1), 20-30.

Pringle, J.R. A hope for the reefs (an ode to *Aiptasia*). *Symbiosis* 57: 109-110.

Rodriguez-Lanetty, M., Phillips, W., & Weis, V. (2006) Transcriptome analysis of a cnidarian–dinoflagellate mutualism reveals complex modulation of host gene expression. *BMC Genomics*, 7(1), 23.

Rodríguez, E., Barbeitos, M., Daly, M., Gusmão, L. C., & Häussermann, V. (2012). Toward a natural classification: phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). *Cladistics*, *28*(4), 375-392.

Savage, A. M., Goodson, M. S., Visram, S., Trapido-Rosenthal, H., Wiedenmann, J., & Douglas, A. E. (2002). Molecular diversity of symbiotic algae at the latitudinal margins of their distribution: dinoflagellates of the genus *Symbiodinium* in corals and sea anemones. *Marine ecology progress series,244*, 17-26.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*(21), 2688-2690.

Sunagawa, S., Choi, J., Forman, H.J., & Medina, M. (2008) Hyperthermic stress-induced increase in the expression of glutamate-cysteine ligase and glutathione levels in the symbiotic sea anemone *Aiptasia pallida*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 151(1), 133–138.

Sunagawa, S., Wilson, E.C., Thaler, M., Smith, M.L., Caruso, C., Pringle, J.R., Weis, V.M., Medina, M. & Schwarz, J.A. (2009) Generation and analysis of transcriptomic resources for a model system on the rise: the sea anemone *Aiptasia pallida* and its dinoflagellate endosymbiont. *BMC genomics*, 10(1), 258.

Thornhill, D. J., LaJeunesse, T. C., Kemp, D. W., Fitt, W. K., & Schmidt, G. W. (2006). Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Marine Biology*, *148*(4), 711-722.

Thornhill, D. J., Lajeunesse, T. C., & Santos, S. R. (2007). Measuring rDNA diversity in eukaryotic microbial systems: how intragenomic variation, pseudogenes, and PCR artifacts confound biodiversity estimates. *Molecular ecology*, *16*(24), 5326-5340

Thornhill, D. J., Kemp, D. W., Bruns, B. U., Fitt, W. K., & Schmidt, G. W. (2008). Correspondence between cold tolerance and temperature biogrography in a western atlantic Symbiodinium (Dinophyta) lineage. *Journal of Phycology*, *44*(5), 1126-1135.

Thornhill, D. J., & Lord, J. B. (2010). Secondary structure models for the internal transcribed spacer (ITS) region 1 from symbiotic dinoflagellates. *Protist*, *161*(3), 434-451.

Thornhill, D. J., Xiang, Y., Pettay, D. T., Zhong, M., & Santos, S. R. (2013a). Population genetic data of a model symbiotic cnidarian system reveal remarkable symbiotic specificity and vectored introductions across ocean basins. *Molecular ecology*, *22*(17), 4499-4515.

Thornhill, D. J., Lewis, A. M., Wham, D. C., & LaJeunesse, T. C. (2013b). Host-specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution*.

Venn, A. A., Loram, J. E., Trapido-Rosenthal, H. G., Joyce, D. A., & Douglas, A. E. (2008). Importance of time and place: patterns in abundance of *Symbiodinium* clades A and B in the tropical sea anemone *Condylactis gigantea*. *The Biological Bulletin*, *215*(3), 243-252.

Visram, S., Wiedenmann, J., & Douglas, A. E. (2006). Molecular diversity of symbiotic algae of the genus *Symbiodinium* (Zooxanthellae) in cnidarians of the Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom*, *86*(6), 1281.

CHAPTER V

CONCLUSIONS

- By reviewing newly collected material from around the world, I was able to circumscribe the members of the family Aiptasiidae, by making a morphological revision of external and internal morphological charaters. This revision also allowed exclusión of some anemone species previously described as members of Aiptasiidae.
- The family Aiptasiidae was circumscribed morphologically by using a combination of only one synapomorphy for the group – namely the presence of b-mastigophores in the scapus, as well as a unique combination of other plesiomorphic characters prevalent in other closely related families of acontiate sea anemones. This further confirms the current lack of unique diagnostic synapomorphic morphological characters for the family, and encourages the use of current and novel molecular characters that further support the taxonomic hypothesis of monophyly.
- The family Aiptasiidae, sensu Grajales & Rodriguez (in prep) is composed of representatives that present remarkable morphological homogeneity. These findings are also corroborated by the phylogenetic hypothesis showing that this group of sea anemones forms a monophyletic clade.
- Despite their limited ability to distinguish different species within Actiniaria, the molecular makrers used in this study were able to provide enough resolution to support the phylogenetic relationships among the different genera within the

family Aiptasiidae.

- At the species levels, diagnostic DNA characters for species were mostly found on the mitochondrial markers. This finding contrasts with the fact that these markers have been found to have little to no resolution to distinguish species of other related groups, both within and outside Actiniaria.
- 16 currently valid species of the genus *Aiptasia* were separated into two different genera: *Aiptasia* and *Exapitasia* comb. nov. based on differences of their cnidom, specifically the size of the b-mastigophores in the scapus. This result confirms the importance of nematocysts for taxonomy and systematics in the Order Actiniaria, and the phylum Cnidaria, as observed in previous studies.
- The genus *Exaiptasia* was found to be composed of two species, *E. pallida* and *E. brasiliensis*. *E. pallida* is a widespread seeminlgy invasive species, present in tropical and subtropical shallow water marine environments worldwide. Most of the currently described species of *Exapitasia* (formerly part of the genus *Aiptasia*) now belong to this one species. The second species, *E. brasiliensis*, was only found in the Southwestern Caribbean Sea and some localities along the Southern Atlantic Ocean in Brazil.
- This study raises some important questions regarding the usability of certain characters that should be considered in taxonomic determinations (i.e., merging splitting genera and families). Usually the presence or absence of a particular nematocyst is considered a strong character to define intermediate taxonomic levels (i.e., families). At the same time, differences in the measurements of

nematocysts have been traditionally used as an indication of the presence of separate species. In this study, a clear cut distinction in the size of nematocysts was used as a diagnostic character, also corresponding to a cladogenesis event above the species level. This, however, proved to be rather an exception; all other nematocyst types did not show any indication of size differences across the entire family.

- It is also noteworthy that some morphological characters, like tentacle shape and the shape of the basilar musculature, seem to be the product of convergence, as opposed to the general assumption of taxonomists of these features being the product of common ancestry.
- *E. pallida* and *E.brasiliensis* could not be distinguished morphologically, but are defined by fixed nucleotides in several of the DNA sequences used in this study. Furthermore, each species show different endosymbionts (*Symbiodinium* spp.). *E. pallida* was found to be mostly associated with subtype B1, while *E. brasiliensis* is exclusively associated with subtypes of clade A, including subtype A4, and a newly underscribed subtype, also first found in this study.
- In contrast, the genus *Aiptaisa* was divided into two different species, *A. couchii* and *A. mutabilis*. These species can easily be differentiated morphologically, by their size, the number of tentacles, their mode of asexual reproduction, and their algal specificity. *A. couchii* is exclusively associated with different subtypes of clade A *Symbiodinium*, while *A. mutabilis,* also harboring algal endosymbionts, shows the presence of a different, currently unidentified alga, not belonging to the

genus *Symbiodinium*.

- The presence of *Symbiodinium minutum* in many localities and low genetic diversity within this algal endosymbiont species may be the result of human mediated-dispersal of its host *Exaiptasia pallida* rather than ancestral variation (see Thornhill et al., 2013b). Thornhill et al. (2013) reported genetic distinctions between *E. pallida* from Florida and those found at other locations. Such distinctions were not observed in the current dataset, but this may be explicable by the dissimilar rates of evolution among the different genetic markers used in the two studies.
- Thornhill et al (2013) found the first pieces of evidence for the existence of a widespread species within *Exaiptasia* by looking at the diversity of endosymbiotic algae (*Symbiodinium* spp.) as well as some population level (SCAR) genetic markers. Despite their extensive sampling efforts, those authors did not include samples from the Southwestern Caribbean Sea or Brazil, which might explain the fact that they could only distinguish a single species within this genus whereas this thesis work identified two.
- *E. pallida* and *E. brasiliensis* were both found in a single locality of the Caribbean Sea in Panama, a highly transited area that offers the opportunity for long distance transport of weedy species like *Exapitasia* via ballast water such as found in the Panamanian Caribbean Sea. It is worth noting that the specimens collected in the Pacific Ocean side of Panama, from the Azuero Peninsula (>200 km away from the Panama Canal) clearly belonged to the widespread species *E. pallida*. Further

clarification of this observation would be obtained by the use of population level markers that may help elucidate the demographic history within each species.

- This dissertation research combined information from several molecular genetic markers, including nuclear ITS2 and plastid cp23S-rDNA, to evaluate the patterns of evolution and diversification of *Symbiodinum* in the light of an existing phylogenetic framework for the host. Those different markers showed different levels of variablilty among the clades. That fact emphasizes the importance of using a multiple set of molecular markers, ideally from different genomes, in order to accurately describe the algal diversity within *Symbiodinium*, as shown in related studies.
- The observed phylogenetic pattern showed no evidence of coevolution between the dinoflagellates and their hosts at a broad level; the phylogenetic position of the symbionts does not mirror the phylogeny of their hosts. This corroborates the patterns found in other symbiotic metazoan taxa, such as scleractinian corals harboring *Symbiodinium*, in which cladogenic events do not typically coincide for both host and symbiont.
- Within some individual host species, *Symbiodinium* spp. exhibited patterns of host specialization and cladogenesis. This pattern suggests that coevolution between host and symbiont occurred within species and genera lineages, but that this process was regularly disrupted and symbiotic partners were recombined during the longer-term evolutionary history of the Aiptasiidae. Furthermore, independent cases of phylogeographic partitioning of *Symbiodinium* within a single host

species were observed.

- In addition to specificity driving the observed associations, there was evidence for sea anemone/dinoflagellate associations adapted to local environmental conditions, such as cold temperatures at the edges of the hosts' distributions. An example of this pattern occurred in *Aiptasia couchii* along a latitudinal gradient along the North Eastern Atlantic Ocean. At least two symbiont subtypes of *Symbiodinium* sp. "Mediterranean A" were observed within individuals of *Aiptasia couchii*, including a northeast Altantic haplotype and a Mediterranean haplotype. The Mediterranean haplotype has been observed in other sea anemones of the North Eastern Atlantic Ocean and is considered to be a cold-tolerant 'temperate' type. A parallel situation was observed within *Exaiptasia pallida*, which associated with B1 and subtype A4 throughout most of its range, but is associated with the cold-tolerant B2 subtype near the northern and southern limits of *E. pallida*'s distribution.
- This study corroborated the importance of thorough morphological revision and taxonomic sampling at lower ranks, in order to help resolve the interrelationships among groups of sea anemones, and possibly other groups among Anthozoa.

APPENDIX A:

SUPPLEMENTARY MATERIAL FOR CHAPTER II: MORPHOLOGICAL REVISION OF THE GENUS *AIPTASIA* AND THE FAMILY AIPTASIIDAE (SNIDARIA, ANTHOZOA, ACTINIARIA, METRIDIOIDEA)

APPENDIX A1

APPENDIX 1. Taxa included in this study, with voucher location, locality, coordinates and depth. Taxa are organized alphabetically. AMNH, American National Museum of Natural History; MZUSP, Museu de Zoologia da Universidade de São Paulo.

Species	Museum	Catalog #	# specimens	Locality	Locality 2	Longitude	Latitude	Depth (m)
Aiptasia couchii	AMNH	AMNH5360	3	Spain	Tarifa, Cádiz	N 37 12 27.54	W 07 02 52.78	1
	AMNH	AMNH5361	3	Spain	Las Palmas de Gran Canaria	N 39 21 10.34	W 09 22 45.90	1
	AMNH	AMNH5362	3	Portugal	Madeira – Machico	N 42 28 59.49	W 03 07 47.43	1
	AMNH	AMNH5363	3	UK	Plymouth	N 50 18 57.55	W 04 04 40.02	1
Aiptasia mutabilis	AMNH	AMNH5364	2	Greece	Crete, Agios Nikolaos	N 35 11 40.69	E 25 43 01.87	5
	AMNH	AMNH5365	3	Spain	L'Estartit, Girona	N 42 02 52.05	E 03 13 07.95	10
Exaiptasia pallida comb. nov.	AMNH	AMNH5366	6	Mexico	Baja California sur – Bahia Concepcion	N 24 15 46.46	W 110 36 52.12	1
	AMNH	AMNH5367	3	Panama	Venado	N 07 25 50.46	W 80 11 36.24	1
	AMNH	AMNH5368	3	Taiwan	National Marine Aquarium, Pingtung	N 22 03 00.08	E 120 41 42.88	1
	AMNH	AMNH5369	3	USA	Hawaii – Hilo	N 19 43 56.37	W 155 03 06.06	1
	AMNH	AMNH5370	3	Japan	Okinawa – Sesoko Island	N 26 38 10.70	E 127 51 55.03	1
	AMNH	AMNH5371	3	Spain	Las Palmas de Gran Canaria	N 28 19 09.18	W 15 25 56.89	1
	AMNH	AMNH5372	3	Portugal	Madeira – Machico	N 32 42 52.84	W 16 45 47.85	1
	AMNH	AMNH5373	3	USA	Florida keys	N 25 03 61.03	W 80 25 38.02	2
	AMNH	AMNH5374	3	Mexico	Puerto Morelos	N 20 50 18.57	W 86 53 02.86	1
	AMNH	AMNH5375	3	Bermuda	Ferry reach	N 32 22 01.51	W 64 39 36.22	1
	AMNH	AMNH5376	3	Panama	Carenera Island	N 09 20 50.34	W 82 15 18.67	1
	MZUSP	MZUSP002536	3	Brazil	Ceara, Fortaleza	S 03 43 42.93	W 38 32 35.60	1
	MZUSP	MZUSP002505	3	Brazil	São Paulo, São Sebastiao	S 23 49 40.36	W 45 25 19.78	1
Aiptasiogeton hyalinus	AMNH	AMNH5377	3	Spain	El Portil, Huelva	N 37 12 27.54	W 07 02 52.78	1
Bartholomea annulata	AMNH	AMNH5378	3	Mexico	Isla Contoy	N 21 30 09.20	W 86 48 07.06	1
	AMNH	AMNH5379	3	USA	Florida Keys	N 25 03 61.03	W 80 25 38.02	1
Bellactis Ilkalyseae	MZUSP	MZUSP2494	3	Brazil	Espiritu Santo – Praia dos Padres	S 19 55 23.53	W 40 06 23.98	1
Laviactis lucida comb. nov.	AMNH	AMNH5380	3	Mexico	Isla Contoy	N 21 30 09.20	W 86 48 07.06	1
	AMNH	AMNH5381	3	Panama	Carenera Island	N 09 20 50.34	W 82 15 18.67	1

APPENDIX A2

Supplementary Table S2.12. Size ranges of the cnidae of several putative *Aiptasia* species. S, ratio of number of specimens in which each cnidae was found to number of specimens examined; N, Total number of capsules measured; F, frequency; +++, very common; ++, common; +, rather common; Abbreviations: M, Microbasic.

Categories	Range of length and width of capsules (μm) ***Aiptasia pallida***	S	N	Range of length and width of capsules (μm) ***Aiptasia diaphana***	S	N	Range of length and width of capsules (μm) ***Aiptasia pulchella***	S	N
PEDAL DISC									
M *p*-amastigophores	(9.7–27.5) x (1.9–6.4)	12/12	264	(20.4–25.1) x (3.5–5.9)	6/6	120	(11.3–26.1) x (2.6–5.9)	12/12	226
Basitrichs	(9.1–19.1) x (1.2–4.4)	12/12	234	(9.1–20.6) x (1.4–4.8)	6/6	139	(8.8–20.3) x (2.2–6.8)	12/12	242
COLUMN									
M *p*-amastigophores	(9.7–29.1) x (2.4–6.4)	12/12	207	(10.1–25.1) x (1.9–5.3)	6/6	76	(9.2–40.4) X (1.8–6.4)	12/12	200
Basitrichs	(8.4–27.8) x (1.9–5.7)	12/12	214	(9.2–16.8) x (1.7 –4.4)	6/6	100	(9.8–28.4) X (2.1–5.6)	12/12	179
M *b*-mastigophores	(10.9–17.8.2) x (2.4–5.9)	12/12	112	(10.5–17.3) x (2.7–5.6)	6/6	60	(9.5–19.1) x (2.7–6.6)	12/12	174
TENTACLES									
M *p*-amastigophores	(19.5–47.8) x (2.6–6.8)	12/12	209	(24.2–36.9) x (3.3–7.1)	6/6	145	(19.8–33.4) x (3.2–5.9)	12/12	165
Basitrichs	(10.2–25.1) x (2.1–4.9)	12/12	177	(10.2–20.1) x (1.4–3.9)	6/6	92	(9.3–20.3) x (2.1–4.1)	12/12	197
Spirocysts	(9.2–29.6) x (2.2–6.6)	12/12	191	(8.4–30.6) x (2.1–6.1)	6/6	169	(10.6–29.1) x (2.2–6.1)	12/12	225
ACTINOPHARYNX									
M *p*-amastigophores	(12.1–35.9) x (2.6–6.6)	12/12	236	(15.1–28.9) x (2.6–5.1)	6/6	166	(20.1–32.7) x (2.4–6.7)	12/12	161
Basitrichs	(8.6–29.4) x (1.6–4.7)	12/12	238	(14.9–21.3) x (1.9–3.3)	6/6	75	(13.4–30.1) x (2.3–4.1)	12/12	198
FILAMENTS									
M *p*-amastigophores 1	(7.7–16.7) x (1.8–5.4)	12/12	265	(7.4–13.6) x (1.7–4.6)	6/6	118	(9.1–17.2) x (2.4–4.9)	12/12	195
M *p*-amastigophores 2	(20.1–40.1) x (1.9–7.1)	12/12	225	(20.4–36.4) x (3.1–6.7)	6/6	63	(20.2–44.7) x (3.9–7.1)	12/12	147
Basitrichs	(7.7–18.9) x (1.5–6.2)	12/12	151	(8.4–24.6) x (1.1–3.2)	2/6	25	(8.7–19.7) x (1.8–5.3)	6/12	85
ACONTIA									
M *p*-amastigophores 1	(10.4–26.5) x (2.3–4.6)	12/12	159	(15.6–31.2) x (2.9–6.2)	3/6	36	(10.8–28.7) x (2.1–5.1)	6/12	87
M *p*-amastigophores 2	(44.9–85.2) x (5.1–12.1)	12/12	168	(52.3–78.6) x (5.6–9.4)	6/6	87	(51.3–70.9) x (5.5–9.2)	12/12	184
Basitrichs	(13.4–34.4) x (2.1–4.4)	12/12	197	(13.5–29.3) x (1.3–4.2)	6/6	125	(13.5–29.1) x (2.1–3.1)	12/12	176

APPENDIX A3

Case 3633: *Dysactis pallida* Agassiz in Verrill, 1864 (currently *Aiptasia pallida*; Cnidaria, Anthozoa, Hexacorallia, Actiniaria): proposed precedence over *Aiptasia diaphana* (Rapp, 1829), *Aiptasia tagetes* (Duchassaing de Fombressin & Michelotti, 1864), *Aiptasia mimosa* (Duchassaing de Fombressin & Michelotti, 1864) and *Aiptasia inula* (Duchassaing de Fombressin & Michelotti, 1864)

Alejandro Grajales & Estefanía Rodríguez
Richard Gilder Graduate School, American Museum of Natural History,
Central Park West at 79th Street, New York, NY 10024, U.S.A.
Division of Invertebrate Zoology, American Museum of Natural History,
Central Park West at 79th Street, New York, NY 10024, U.S.A.
(e-mails: agrajales@amnh.org; erodriguez@amnh.org)

Abstract. The purpose of this application, under Article 23.9.3 of the Code, is to conserve the specific name *Aiptasia pallida* (Agassiz in Verrill, 1864) for a species of sea anemone (Cnidaria, Actiniaria) widely used as a model system for dinoflagellate cnidarian symbiosis and coral bleaching studies. The name *A. diaphana* (Rapp, 1829) is a senior subjective synonym *of A. pallida*, while *Aiptasia inula* (Duchassaing de

Fombressin & Michelotti, 1864), *Aiptasia mimosa* (Duchassaing de Fombressin & Michelotti, 1864) and *Aiptasia tagetes* (Duchassaing de Fombressin & Michelotti, 1864) are also synonyms, but published in the same year. The use of the name *A. pallida* meets the requirements for reversal of precedence of a junior synonym (Article 23.9.1) in the case of A. inula and A. mimosa, which were not used in the 20th century and are declared nomina oblita under Article 23.9.2 in this paper. The names *A. diaphana* and *A. tagetes* were used after 1899; hence the conditions of Article 23.9.1.1 are not met. However, in the interest of nomenclatural stability, we request a ruling to maintain the use of the junior synonym under the plenary power, thereby making *A. pallida* a nomen protectum, and *A. diaphana* and *A. tagetes* nomina oblita.

Keywords. Nomenclature; taxonomy; Actiniaria; *Aiptasia*; *Aiptasia pallida*; *Aiptasia diaphana*; *Aiptasia tagetes*; sea anemone.

1. Sea anemones (Cnidaria, Actiniaria) of the genus *Aiptasia* Gosse, 1858 are conspicuous members of tropical and subtropical shallow-water marine environments worldwide and serve as a model system for studies of cnidarian-dinoflagellate symbiosis. However, despite their importance, accessibility and the fact that publications using *Aiptasia spp*. as focal taxa are common (e.g. Dunn et al., 2002; Muller-Parker & Davy, 2001; Weis et al., 2008; LaJeunesse et al., 2010), to date there has not been a comprehensive systematic analysis of the group.

2. The latest inventory of the genus *Aiptasia* recorded 14 species distributed

worldwide (Fautin, 2013); however, most of the descriptions of the 14 species inventoried by Fautin (2013) are incomplete by modern standards and type material is only available in a few cases. The type series of *Aiptasia pallida* (Agassiz inVerril, 1864, p. 26) consists of two syntypes deposited in the Museum of Comparative Zoology at Harvard University (MCZ: SCOR-1004). There are no types in existence for *A. tagetes* (Duchassaing de Fombressin & Michelotti, 1864, p. 39), *A. mimosa* (Duchassaing de Fombressin & Michelotti, 1864, p. 29), A. inula (Duchassaing de Fombressin & Michelotti, 1864, p. 39) or A. diaphana (Rapp, 1829, p. 57). There were originally two syntypes of *A. tagetes* from Puerto Rico, one syntype of *A. mimosa* from the Virgin Islands, one syntype of *A. diaphana* from Naples (Italy); however, there is no information available about the museum collections were these types were deposited (Fautin, 2013), and they are thought to have been lost. After detailed morphological examination of available type and newly-collected material and cnidae from all but three of the type localities or nearby localities of the type reported for 11 of the 14 putative species within *Aiptasia,* Grajales & Rodríguez (2013 submitted) did not find any constant morphological character to distinguish between *A. diaphana, A. pallida*, *A. inula, A. mimosa, A. tagetes, A. minuta* (Verrill, 1867, p. 50), A. leiodactyla Pax, 1910, p. 178, A. pulchella Carlgren, 1943, p. 38, and A. californica Carlgren, 1952, p. 388. Thus, they proposed to synonymize these eight species. Although there is no type material in existence for *A. inula*, *A. mimosa*, *A. tagetes* or *A. diaphana*, the synonymy was possible based on available descriptions and newly-collected material from nearby localities to the type localities of these species (Grajales & Rodríguez, 2013, submitted).

3. According to the Principle of Priority, the name *Aiptasia diaphana* is the senior subjective synonym and thus must be used over the junior synonym, *A. pallida*. In addition, the names *Dysactis mimosa* (currently *A. mimosa*), *Bartholomea tagetes* (currently *A. tagetes*), and *Bartholomea inula* (currently *A. inula*) might also have priority over the name *A. pallida*. Verrill's (1864) paper was published in July 1864, while Duchassaing de Fombressin & Michelotti's (1864) supplement was published between May 1864 and January 1865 (on page 7 of the supplement Duchassaing de Fombressin & Michelotti included a footnote, which is dated 17 May 1864). Duchassaing de Fombressin & Michelotti's paper (1864) has a flyleaf note to say that it is an extract from the Memoires de l'Academie des Sciences de Turin, Serie 2, Tome 23. The supplement was indeed republished in the Memoires de l'Academie des Sciences de Turin, but only in 1866.

4. *Aiptasia pallida* has been used as a model system for research of dinoflagellatecnidarian symbiosis and the processes responsible for coral bleaching over more than 30 years (e.g. Hessinger & Lenhoff, 1973; Palinscar et al., 1989; Sawyer & Muscatine, 2001; Rodriguez-Lanetty et al., 2006; Sunagawa et al. 2008, 2009, see Appendix) and thus is currently in wider use than its senior putative synonyms: in the last 50 years the name *A. diaphana* has been used in 25 publications, *A. tagetes* in seven publications, and *A. mimosa* and *A. inula* have not been used, whereas *A. pallida* has been used in at least 50 publications. Furthermore, most of the studies using *A.*

pallida are non-taxonomic works which do not always follow formal nomenclature. In the interests of nomenclatural stability and to avoid potential confusion, it would be ideal to maintain the use of the junior synonym by reversal of precedence (Article 23.9 of the Code). The names *A. inula* and *A. mimosa* have not been used as valid names after 1899, thus meeting the conditions of Article 23.9.1.1. They are considered as nomina oblita under Article 23.9.2 of the Code. However, the names *A. diaphana* and *A. tagetes* have been used as valid after 1899 (e.g. Schmidt, 1982; den Hartog & Ocaña, 2003) and so do not meet the conditions of Article 23.9.1.1. Therefore reversal of precedence cannot be automatically granted, although the name *A. pallida* has been the most widely used in the last 50 years. We consider that the use of the senior synonyms *A. diaphana*, *A. inula*, *A. mimosa* and *A. tagetes* would cause confusion and threaten stability and, under Article 23.9.3 of the Code, we request the Commission to use its plenary power to maintain the use of the junior synonym, *A. pallida*. A list of 50 supporting references demonstrating the prevailing usage of *A. pallida* is held by the Commission Secretariat.

5. The International Commission on Zoological Nomenclature is accordingly asked:

(1) to use its plenary power to give precedence to the name pallida Agassiz in Verrill, 1864, as published in the binomen *Dysactis pallida*, over the following names, whenever they are considered to be synonyms:

(a) *diaphana* Rapp, 1829, as published in the binomen Actinia diaphana;

(b) *tagetes* Duchassaing de Fombressin & Michelotti, 1864, as published in the binomen *Bartholomea tagetes*;

(2) to place on the Official List of Specific Names in Zoology the following names:

(a) *pallida* Agassiz in Verrill, 1864, as published in the binomen *Dysactis pallida*, with the endorsement that it is to be given precedence over the names *diaphana* Rapp, 1829, as published in the binomen *Actinia diaphana*, and *tagetes* Duchassaing de Fombressin & Michelotti, 1864, as published in the binomen *Bartholomea tagetes*, whenever it and either of the other two are considered to be synonyms;

(b) *diaphana* Rapp, 1829, as published in the binomen *Actinia diaphana*, with the endorsement that it is not to be given priority over the name *pallida* Agassiz in Verrill, 1864, as published in the binomen *Dysactis pallida*, whenever the two are considered to be synonyms;

(c) *tagetes* Duchassaing de Fombressin & Michelotti, 1864, as published in the binomen *Bartholomea tagetes*, with the endorsement that is not to be given priority over the name *pallida* Agassiz in Verrill, 1864, as published in the binomen *Dysactis pallida*, whenever the two are considered to be synonyms.

Acknowledgments

We thank D. Fautin (Kansas University), M. Daly (Ohio State University) and D. Lauretta (Museo Argentino de Ciencias Naturales Bernardino Rivadavia) for helpful comments on nomenclature.

References

Carlgren, O. 1943. East-Asiatic Corallimorpharia and Actiniaria. Kungliga Svenska Vetenskaps – Akademiens Handlingar, (3)20(6): 1–43.

Carlgren, O. 1952. Actiniaria from North America. Arkiv für Zoologi, 3(30): 373–390.

den Hartog, J.C., & Ocaña, O. 2003. A new endemic Actinia species (Actiniaria: Actiniidae) from the central Macaronesian Archipelagos. Zoologische Mededelingen (Leiden), 77: 229–244.Duchassaing de Fombressin, P. & Michelotti, G. 1864. Supplément au mémoire sur les Coralliaires des Antilles. 112 pp. Imprimerie Royale, Turin.

Dunn, S.R., Bythell, J.C., Le Tissier, M.D., Burnett, W.J. & Thomason, J.C. 2002. Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone Aiptasia sp. Journal of Experimental Marine Biology and Ecology, 272(1): 29–53.

Fautin, D.G. 2013. Hexacorallians of the World. Available from: http://geoportal.kgs.ku.edu/ hexacoral/anemone2/index.cfm (accessed in July 2013).

Gosse, P.H. 1858. Synopsis of the families, genera, and species of the British Actiniae. Annals and Magazine of Natural History, 1: 414–419.

Grajales, A. & Rodríguez, E. Morphological revision of the genus Aiptasia and the family Aiptasiidae (Cnidaria, Actiniaria, Metridioidea). Zootaxa (submitted 2013).

Hessinger, D.A. & Lenhoff, H.M. 1973. Assay and properties of the hemolysis activity of pure venom from the nematocysts of the acontia of the sea anemone *Aiptasia pallida*. Archives of Biochemistry and Biophysics, 159(2): 629–638.

LaJeunesse, T.C., Smith, R., Walther, M., Pinzón, J., Pettay, D. T., McGinley, M Achaffenburg, M., Medina-Rosas, P., Cupul-Magaña, A.L., Lopez Perez, A., Reytes-Bonilla, H. & Warner, M.E. 2010. Host–symbiont recombination versus natural selection in the response of coral–dinoflagellate symbioses to environmental disturbance. Proceedings of the Royal Society B: Biological Sciences, 277(1696): 2925–2934.

Muller-Parker, G. & Davy, S.K. 2001. Temperate and tropical algal**{**sea anemone symbioses. Invertebrate Biology, 120(2): 104–123.

Palinscar, E.E., Jones, W.R., Palinscar, J.S., Glogowski, M.A. & Mastro, J.L. 1989. Bacterial aggregates within the epidermis of the sea anemone Aiptasia pallida. Biological Bulletin, 177: 130–140.

Pax, F. 1910. Studien an westindischen Actinien. Zoologische Jahrbücher, 2: 157–330.

Rapp, W. 1829. Über die Polypen im Allgemeinen und die Actinien. 62 pp. Verlag des Großherzoglich Sächsischen privileg. Landes-Industrie-Comptoirs, Weimar.

Rodriguez-Lanetty, M., Phillips, W. & Weis, V. 2006. Transcriptome analysis of a cnidarian–dinoflagellate mutualism reveals complex modulation of host gene expression. BMC Genomics, 7(1): 23.

Sawyer, S. J., & Muscatine, L. 2001. Cellular mechanisms underlying temperature-induced bleaching in the tropical sea anemone Aiptasia pulchella. Journal of Experimental Biology, 204(20): 3443–3456.

Schmidt, H. 1972. Prodromus zu einer Monographie der mediterranean aktinien. Zoologica, 42: 1–121.

Sunagawa, S., Choi, J., Forman, H. J. & Medina, M. 2008. Hyperthermic stress-induced increase in the expression of glutamate-cysteine ligase and glutathione levels in the symbiotic sea anemone Aiptasia pallida. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 151(1): 133–138.

Sunagawa, S., Wilson, E. C., Thaler, M., Smith, M. L., Caruso, C., Pringle, J. R., Weis, V.M., Medina, M. & Schwarz, J.A. 2009. Generation and analysis of transcriptomic resources for a model system on the rise: the sea anemone Aiptasia pallida and its dinoflagellate endosymbiont. BMC genomics, 10(1): 258.

Verrill, A.E. 1864. Revision of the Polypi of the eastern coast of the United States. Memoirs of the Boston Society of Natural History, 1: 1–45.

Verrill, A.E. 1867. Madreporaria. Communications of the Essex Institute, 5: 33–50.

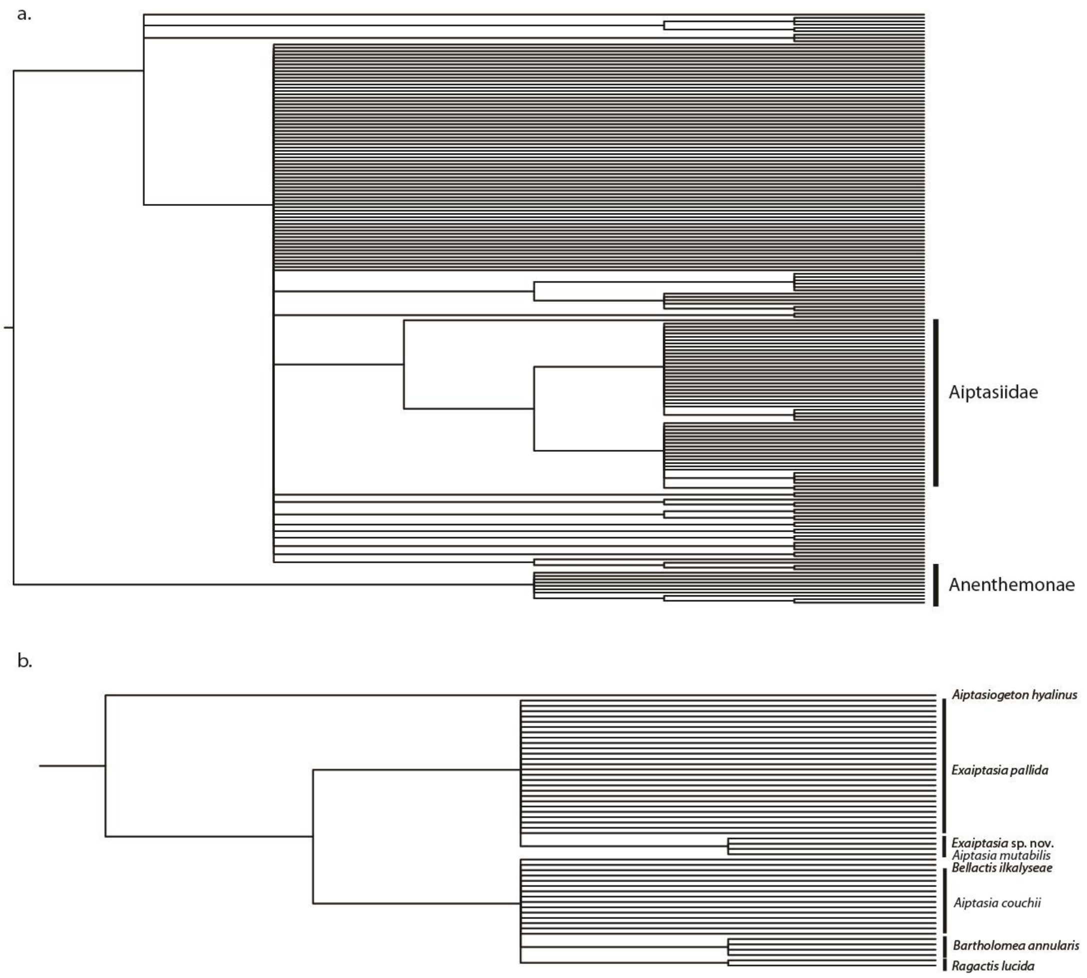
Weis, V.M., Davy, S.K., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., & Pringle, J.R. 2008. Cell biology in model systems as the key to understanding corals. Trends in Ecology & Evolution, 23(7): 369–376.

Acknowledgement of receipt of this application was published in BZN 70: 151.

APPENDIX B:

SUPPLEMENTARY MATERIAL FOR CHAPTER III: MOLECULAR SYSTEMATICS OF THE FAMILY AIPTASIIDAE (CNIDARIA, ACTINIARIA, METRIDIOIDEA)

B.1 Parsimony phylogenetic position of the family Aiptasiidae.



B. 2. Supplementary table 1. Specimens, voucher numbers and GenBank accession numbers per gene region.

Supplementary Table 1. Specimens, voucher location, and GenBank accession numbers per gene region of studied species. (-) not available; (*) clonal strains of *Exaptasia pallida*

Family	Species		Museum	12S	16S	18S	28S	COIII
Aiptasiidae	*Aiptasia*	*couchii*	AMNH	KP761199	KP761254	KP761301	-	KP761405
			AMNH	KP761200	KP761255	KP761303	-	KP761403
			AMNH	KP761201	KP761248	KP761304	-	KP761394
			AMNH	-	KP761244	-	-	KP761407
			AMNH	-	KP761245	KP761298	-	-
			AMNH	-	KP761246	KP761299	-	KP761406
			AMNH	-	KP761247	KP761300	-	-
			AMNH	KP761203	KP761258	-	-	KP761397
			AMNH	KP761201	KP761256	KP761304	-	-
			AMNH	KP761185	-	KP761295	-	KP761396
			AMNH	KP761207	KP761261	-	-	KP761261
			AMNH	KP761208	KP761262	KP761306	-	KP761399
	Aiptasia	*mutabilis*	AMNH	KP761193	KP761247	-	-	KR704269
			AMNH	KP761194	KP761248	KP761300	-	KP761404
	Exaiptasia	*brasiliensis* sp. nov.	MZUSP	-	KP761249	-	-	KP761249
			MZUSP	KP761188	KP761239	KP761312	-	KP761386
			MZUSP	KP761189	-	KP761320	-	-
			AMNH	KP761249	KP761249	KP761249	-	-
	Exaiptasia	*pallida*	AMNH	KP761184	KP761283	-	-	KP761323
			AMNH	KP761183	KP761270	KP761286	-	KP761361
			AMNH	KP761182	-	KP761279	-	-
			AMNH	KP761211	KP761266	-	-	KP761401
			AMNH	KP761209	KP761263	KP761384	-	-
			AMNH	KP761204	KP761259	-	-	KP761382
			AMNH	KP761206	KP761260	KP761284	-	-
			AMNH	KR186026	-	KR186058	-	-

			MZUSP	KP761187	KP761236	KP761311	-		KP761389 -
			MZUSP	-	KR186039	KR186056	-		-
			MZUSP	-	KP761240	-	-		KP761390
			AMNH	-	KP761228	-	KP761324		-
			AMNH	-	KP761229	KP761285	-		KP761325
			AMNH	KP761176	KP761226	KP761280	KP761327		KP761376
			AMNH	KP761197	KP761251	KP761288	-		-
			AMNH	KR186019	-	-	-		-
			AMNH	KP761175	KP761225	KP761278	KP761321		KP761375
			AMNH	KR186025	-	KR186055	-		-
			AMNH	KP761202	KP761257	KP761294	-	KP761409	P761385
	Bartholomea	*annulata*	-	KR186028	-	KR186060	-	-	P761377
			-	-	KR186042	KR186061	-	-	-
			-	KR186029	KR186043	KR186062	-	-	P761381
			-	KR186030	KR186044	KR186063	-	-	P761379
			-	KR186031	KR186045	KR186064	-	-	-
			AMNH	KP761191	KP761242	-	-	KP761392	-
			-	-	KR186046	KR186065	-	-	-
			-	KR186033	KR186047	KR186066	-	-	-
			-	KR186032	-	-	-	-	-
			-	KR186034	KR186048	KR186067	-	-	-
			-	KR186035	KR186049	KR186068	-	-	-
			-	-	KR186050	-	-	-	-
			AMNH	EU190721	EU190763	EU190851	EJ483068	FJ489483	-
			AMNH	KP761198	KP761253	KP761307	-	-	-
	Bellactis	*ilkalyseae*	MZUSP	-	KP761238	KP761316	-	KP761393	-
			MZUSP	KR186020	KR186036	KR186051	-	-	-
			MZUSP	KR186021	KR186037	KR186052	-	-	-
	Laviactis	*lucida*	-	KR186027	KR186041	KR186059	-	-	-
			AMNH	KP761192	KP761243	KP761296	-	KP761402	
			AMNH	KP761196	KP761250	KP761297	-	-	
			AMNH	KR186024	KR186038	-	-	-	
			AMNH	KR186022	-	KR186053	-	-	
			AMNH	KR186023	-	KR186054	-	-	
Aliciidae	*Alicia*	*mirabilis*	AMNH	KP761213	-	KP761310	KP761329	KP761410	
	Alicia	*sansibarensis*	AMNH	KJ482933.1	KJ482953.1	KJ483016.1	KJ483116.1	KJ483000.1	
	Lebrunia	*coralligens*	AMNH	-	KP761218	-	-	-	
	Lebrunia	*danae*	AMNH	-	KP761219	-	-	-	

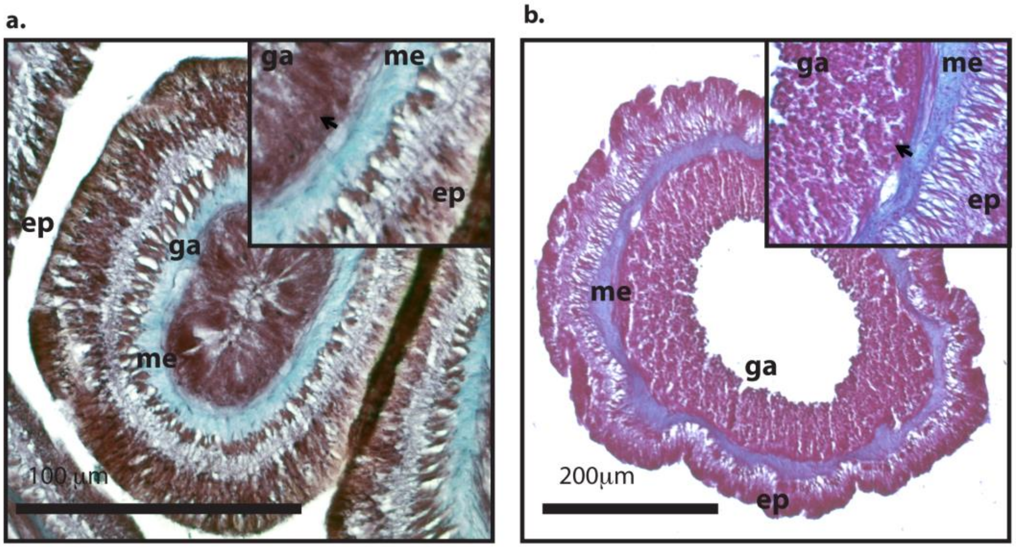
B.3. Supplementary table 2. Numbers of base pairs included in the downstream analysis, partitioned by genes.

Gene region	W/out Gblocks	Gblocks
12s	912	838
16S	421	327
18S	2157	1705
28S	4210	3121
CO3	723	723

APPENDIX C:

SUPPLEMENTARY MATERIAL FOR CHAPTER IV: MOLECULAR DIVERSITY OF *SYMBIODINIUM* SPP. (ALVEOLATA, DINOPHYCEAE) SYMBIOTIC WITH MEMBERS OF THE FAMILY AIPTASIIDAE

C.1. Detailed view of a cross section of the tentacles. **a.** *Aiptasiogeton hyalinus* and **b.** *Bartholomea annularis*. Notice the absence of algal cell in the gastrodermis of the azooxanthellate species *A. hyalinus*, in comparision with *B. annularis.* **ep** = epidermis **me** = mesoglea **ga** = gastrodermis.



C. 2. Supplementary Table 1. Genbank accession numbers from c23s-rDNA and ITS2 per specimen.

Species	**Locality**	**Accession No**	
		23S	**ITS**
Aiptasia couchii	United Kingdom	KP761369	KP761369
	United Kingdom	KP761348	-
	France	KP761350	-
	Portugal	KP761353	-
	Spain	KP761349	-
	Spain	KP761351	-
	Spain	KP761356	-
	Spain	KP761368	KP761368
Bartholomea annulata	Bermuda	-	-
	Bermuda	KR002407	KR002397
	Bermuda	KR002408	KR002401
	Bermuda	KR002409	-
	Honduras	KR002410	-
	Honduras	KR002411	-
	Honduras	KR002412	-
	Mexico	KR002405	-
	Mexico	KR002406	-
	Mexico	KP761344	-
	Panama	KR002418	-
	USA	KR002413	KR002395
	USA	KR002414	-
	USA	KT270575	KR002398
	USA	KP761339	-
	US Virgin Islands	KR002415	-
	US Virgin Islands	KR002416	-
Bellactis ilkalyseae	Brazil	KP761340	-
Exaiptasia pallida	Bahamas	KP761332	-
	Bermuda	KP761333	KP761367 KP761366
	Brazil	KP761362	KP761362
	Israel	KP761357	-
	Israel	KP761358	-
	Mexico	KP761331	-
	Mexico	KP761335	-
	Panama	KR002417	KP761364
	Panama	KP761359	-

	Portugal	KP761354	-
	Spain	KP761355	-
	Spain	KP761352	-
	St. Helena	-	KR002394
	Taiwan	KP761342	-
	USA	KP761330	-
	USA	KP761338	-
	USA	KP761334	-
	USA	KP761335	-
	USA	KP761336	-
Exaiptasia sp. nov.	Brazil	KP761374	KP761374
	Brazil	KP761373	KP761373
	Panama	KP761345	KP761370
Laviactis lucida	Honduras	KR002404	KR002400
	Mexico	KP761363	KP761363
	Panama	KR002402	KR002396
	Panama	KR002403	KR002399
	Panama	KP761346	-