María de los Angeles Mendoza Becerril

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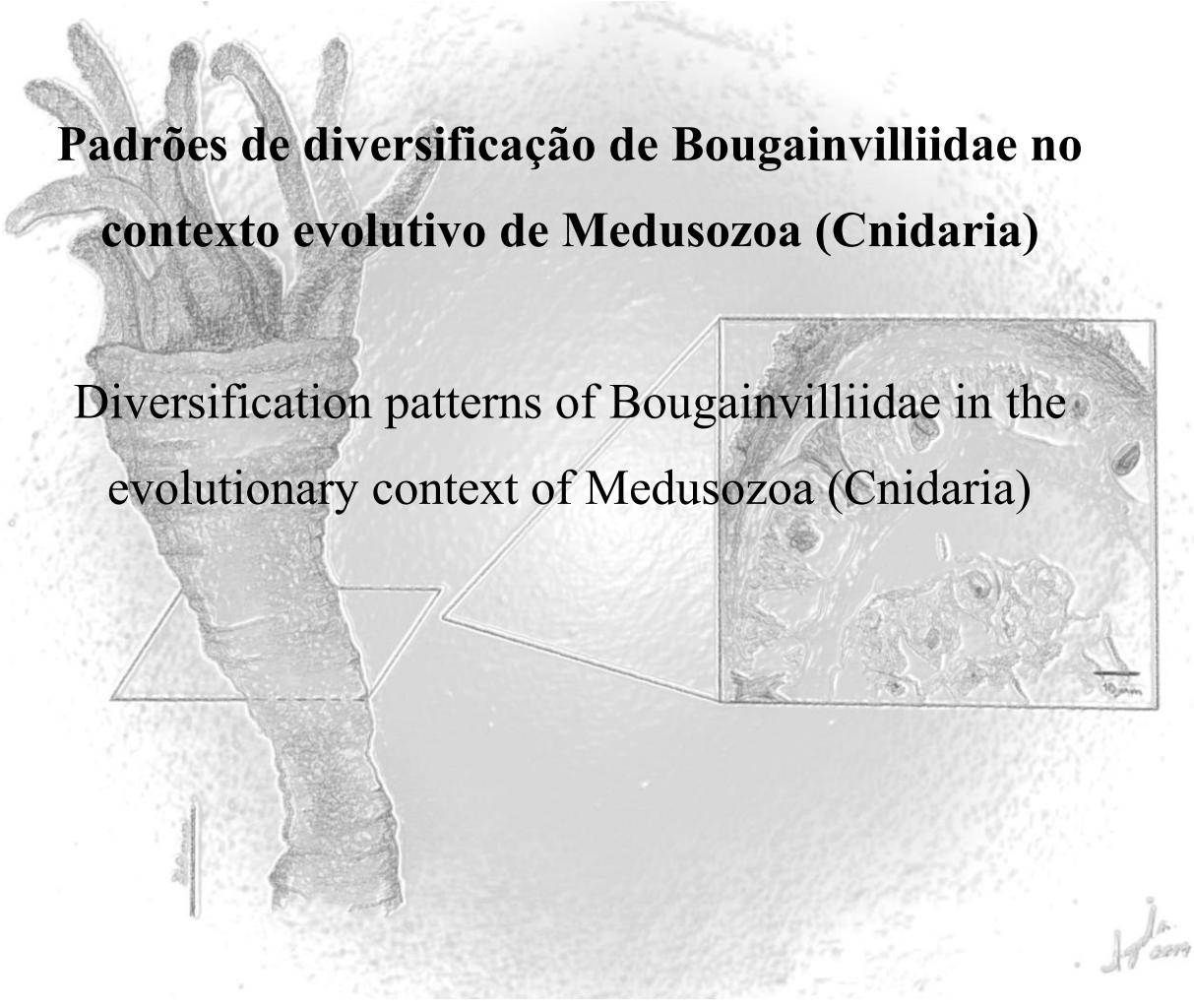

São Paulo

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências, na Área de Zoologia.

Orientador: Prof. Dr. Antonio Carlos Marques

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Prof. Dr. Antonio Carlos Marques

Aos meus grandes mestres da vida toda, meus pais.

A mis grandes maestros de toda la vida, mis padres.

Aparentemente no había nada
sólo el agua, algunas veces en protesta,
resonante e impenetrable,
otras sólo tranquila y apacible.

Aparentemente no había nada
sólo la inmensa niebla que guardas,
hasta que descubrí un mundo nuevo
y llegué a donde jamás había soñado.

Aparentemente no había nada
sólo secretos y misterios
reflejados en tus violentas
y espumosas olas.

Aparentemente no había nada
sólo tu manto azul,
acompañado de coros y mareas
que me avivaron hasta llegar aquí.

Aparentemente no había nada,
sólo arena y agua entre rocas,
resguardo de pequeños tesoros,
visibles ante sabias miradas.

Aparentemente no había nada,
sólo un infinito y majestuoso horizonte
donde se pierde el sol
y nacen nuevos sueños.

Aparentemente, não tinha nada
só água, as vezes em protesto
ressonante e impenetrável;
outras vezes somente tranquila e em paz.

Aparentemente, não tinha nada
só o imenso nevoeiro que guardas,
somente quando descobri um novo mundo
foi que cheguei onde nunca tinha sonhado.

Aparentemente, não tinha nada
só segredos e mistérios
refletidos nas suas violentas
e espumosas ondas.

Aparentemente, não tinha nada
só um manto azul,
acompanhado de coros e marés
vivificando me para chegar aqui.

Aparentemente, não tinha nada,
só areia e água entre as rochas,
resguardo de pequenos tesouros,
visíveis para um sábio olhar.

Aparentemente, não tinha nada,
só um horizonte infinito e majestoso
onde perde-se o sol
e novos sonhos nascem.

Mendoza-Becerril M.A.

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Índice

Capítulo – 1

Introdução geral

Medusozoa (Cnidaria) inclui as classes Staurozoa, Cubozoa, Scyphozoa e Hydrozoa, cujas relações filogenéticas estão baseadas em morfologia, ciclos de vida e marcadores moleculares (Marques & Collins, 2004; Collins et al., 2006; Van Iten et al., 2006, 2014). Hydroidolina é um grupo de Hydrozoa que inclui "Anthoathecata", Leptothecata e Siphonophorae (Cartwright et al., 2008), sendo "Anthoathecata" e Leptothecata os mais ricos em espécies e, conjuntamente, formando o grupo não monofilético classicamente chamado de hidroides (Daly et al., 2007; Mapstone, 2014). Hydroidolina é caracterizado pela presença de estatocistos de origem ectodérmica (Collins et al., 2006; Daly et al., 2007). Seu ciclo de vida geralmente possui dois estágios mais claros de desenvolvimento morfológica e ecologicamente distintos, nomeadamente, o pólipo e a medusa (Russell, 1953; Cornelius, 1995). Os pólipos de Hydroidolina podem ser solitários ou coloniais, e as colônias podem ser polimórficas (Daly et al., 2007).

Siphonophorae é caracterizado por uma organização colonial holopelágica (exceto a família Rhodaliidae) com alto grau de polimorfismo (cf. Mapstone, 2014). Leptothecata é caracterizado por um exoesqueleto envolvendo os hidrantes (= hidroteca) e gonóforos (= gonotheca), majoritariamente coloniais, em que os tentáculos estão dispostos em uma coroa (Cornelius, 1995; Marques & Collins, 2004). Finalmente, "Anthoathecata" é um grupo não monofilético (Cartwright et al., 2008; Cartwright & Nawrocki, 2010), caracterizado pela ausência de um exoesqueleto sobre os hidrantes (Allman, 1871; Calder, 1988) ou, em alguns casos, de qualquer estrutura exoesquelética (Daly et al., 2007). Representantes de "Anthoathecata" são coloniais ou solitários, com tentáculos dispostos em uma ou duas coroas ou dispersos no corpo do hidrante (Calder, 1988).

Estudos morfológicos tradicionais classificam representantes de "Anthoathecata" em Filifera e Capitata, cujas sinapomorfias, na fase de pólipo, são os tentáculos filiformes e capitados, respectivamente, ambos espalhados ou em coroas definidas no hidrante (Bouillon, 1985; Petersen, 1990). Essas características não encontram embasamento filogenético, a disposição de tentáculos dispersos pode ocorrer em ambos os grupos e o desenvolvimento de tentáculos capitados apresenta variação nas fases do ciclo de vida de espécies de Capitata (Millard, 1975; Petersen, 1990). Outra sinapomorfia proposta para Filifera foram os nematocistos desmonemos e euritelos microbásicos (Petersen, 1990), caracteres também

demonstrados como plesiomórficos (Marques, 2001), além da ausência de estenotelos (Schuchert, 2012). Estudos moleculares demostram que "Anthoathecata" pode ser compreendido com vários grupos não relacionados, como Aplanulata (anteriormente incluído em Capitata; Collins et al, 2006) e Capitata (Cartwright et al., 2008; Cartwright & Nawrocki, 2010; Nawrocki et al., 2010), além de diversos grupos considerados como "Filifera" (Cartwright et al., 2008; Cartwright & Nawrocki, 2010).

Conforme classicamente interpretados, "Filifera" inclui 26 famílias (Schuchert, 2015, com adição de dados de Calder et al., 2015), das quais três (Bougainvilliidae, Clathrozoellidae e Pandeidae) possuem exoesqueleto envolvendo seus hidrantes (= pseudo-hidroteca) (cf. Millard, 1975; Calder, 1988; Vervoort, 2000; Peña-Cantero et al., 2003; Schuchert, 2007). Dentre essas famílias, Bougainvilliidae destaca-se pela diversidade de sua estrutura exoesquelética e riqueza (110 espécies válidas e 15 gêneros, Mendoza-Becerril & Marques, 2013; Schuchert, 2015; Stepanjants & Chernyshev, 2015). Uma substancial parte de Bougainvilliidae é pouco conhecida, mal descrita e com registros raros. Pouco se sabe sobre o monofiletismo da família (Cartwright et al., 2008; Maronna, 2014), suas relações filogenéticas, indicações de ciclos de vida (Edwards, 1966; Calder, 1971; Schuchert, 1996, 2007), diversidade e morfologia. A "pseudo-hidroteca", por exemplo, é uma particularidade do grupo pouco investigada no que sua composição, morfologia, funcionalidade e evolução.

Nesse contexto de ausência de comparações evolutivas e de diversidade, este estudo teve como objetivos contextualizar historicamente e geograficamente o conhecimento atual sobre Bougainvilliidae, além de fazer uma predição de sua distribuição (Capítulo 2); analisar a estrutura exoesquelética em Medusozoa, comparando variações em origem, estrutura e função, e de seu papel em padrões de diversificação do grupo (Capítulo 3); analisar e comparar a estrutura e composição tecidual e exoesquelética de Bougainvilliidae e outros Hydroidolina (Capítulo 4); e esclarecer a posição filogenética de Bougainvilliidae dentre os "Anthoathecata" (Capítulo 5).

Literatura citada

Allman, G.J. 1871. A Monograph of the Gymnoblastic or Tubularian hydroids. London: The Ray Society Monograph 450 p.

Bouillon, J. 1985. Essai de classification des Hydropolypes-Hydroméduses (Hydrozoa-Cnidaria) Indo-Malayan Zoology 2: 29-243.

Calder, D.R. 1971. Hydroids and Hydromedusae of southern Chesapeake Bay. Special Papers in Marine Science 1: 1-125.

Calder, D.R. 1988. Shallow-Water Hydroids of Bermuda The Athecatae. Life Sciences Contributions 148: 1-107.

Cartwright, P. & Nawrocki, A.M. 2010. Character evolution in Hydrozoa (phylum Cnidaria). Integrative and Comparative Biology 50: 456-472.

Cartwright, P., Evans, N.M., Dunn, C.W., Marques, A.C., Miglietta, M.P., Schuchert P. & Collins, A.G. 2008. Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). Journal of the Marine Biological Association of the United Kingdom 88: 1663-1672.

Collins, A.G., Schuchert, P., Marques, A.C., Jankowski, T., Medina, M. & Schierwater, B. 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. Systematic Biology 55: 97-115.

Cornelius, P.F.S. 1995. North-west European thecate hydroids and their medusae. Part 1. Introduction, Laodiceidae to Haleciidae. Synopses of the British Fauna (New Series) 50: 1-347.

Daly, M., Brugler, M.R., Cartwright, P., Collins, A.G., Dawson, M.N., Fautin, D.G., France, S.C., McFadden, C.S., Opresko, D.M., Rodriguez, E., Romano, S.L. & Stake, J.L. 2007. The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa 1668: 127-182.

Edwards, C. 1966. The hydroid and the medusa *Bougainvillia principis*, and a review of the British species of Bougainvillia. Journal of the Marine Biological Association of the United Kingdom 46: 129-152.

Maronna, M.M. 2014. Análise filogenética de Leptothecata (Hydrozoa; Hydroidolina) e evolução da metagênese. Tese – Doutorado, Instituto de Biociências da Universidade de São Paulo, Departamento de Genética e Biologia Evolutiva 188 p.

Marques, A.C. 2001. O gênero *Eudendrium* (Hydrozoa, Anthomedusae, Eudendriidae) no Brasil. Papéis Avulsos de Zoologia 41: 329-405.

Marques, A.C. & Collins, A.G. 2004. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebrate Biology 123: 23-42.

Mapstone, G.M. 2014. Global Diversity and Review of Siphonophorae (Cnidaria: Hydrozoa). PLoS ONE 9: e87737. DOI: 10.1371/journal.pone.0087737.

Mendoza-Becerril, M.A. & Marques, A.C. 2013. Synopsis on the knowledge and distribution of the family Bougainvilliidae (Hydrozoa, Hydroidolina). Latin American Journal Aquatic Research 41: 908-924.

Millard, N.A.H. 1975. Monograph on the Hydroida of Southern Africa. Annals of the South African Museum 68: 1-513.

Nawrocki, A.M., Schuchert, P. & Cartwright, P. 2010. Phylogenetics and evolution of Capitata (Cnidaria: Hydrozoa), and the systematics of Corynidae. Zoologica Scripta 39: 290-304.

Peña-Cantero, A.L., Vervoort, W. & Watson, J.E. 2003. On Clathrozoellidae (Cnidaria, Hydrozoa, Anthoathecatae), a new family of rare deep-water leptolids, with the description of three new species. Zoologische Verhandlungen, Leiden 345: 281-296.

Petersen, K.W. 1990. Evolution and taxonomy in capitate hydroids and medusae (Cnidaria: Hydrozoa). Zoological Journal of the Linnean Society 100: 101-231.

Russell, F.S. 1953. The medusa of the British Isles. I-Anthomedusae, Leptomedusae, Limnomedusae, Trachymedusae and Narcomedusae. London: Cambridge University Press 530 p.

Schuchert, P. 1996. The marine fauna of New Zealand: Athecate hydroids and their medusae (Cnidaria: Hydrozoa). New Zealand Oceanographic Institute Memoir 106: 1-159.

Schuchert, P. 2007. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 2. Revue Suisse de Zoologie 114: 195-396.

Schuchert, P. 2012. North-West European Athecate Hydroids and their Medusae. Synopses of the British Fauna (New Series) 59. London: The Linnean Society of London 364 p.

Schuchert, P. 2015. Filifera. Accessed through: World Hydrozoa database at http://www.marinespecies.org/hydrozoa/aphia.php?p=taxdetails&id=16352 on 2015-04-11.

Stepanjants, S.D. & Chernyshev, A.V. 2015. Deep-sea epibiotic hydroids from the abyssal plain adjacent to the Kurile-Kamchatka trench with description of *Garveia belyaevi* sp. nov. (Hydrozoa, Bougainvilliidae). Deep Sea Research Part II: Topical Studies in Oceanography 111: 44-48.

Vervoort, W. 2000. Additional notes on *Clathrozoella drygalskii* (Vanhöffen, 1910) (Cnidaria, Hydrozoa). Scientia Marina 64: 237-240.

Capítulo – 2

Synopsis on the knowledge and distribution of the family Bougainvilliidae (Hydrozoa, Hydroidolina)

María de los Angeles Mendoza-Becerril & Antonio C. Marques

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Resumo

A família Bougainvilliidae compreende um grupo de antoatecados hidrozoarios, taxonomicamente mal estruturado e biológica, ecológica e biogeograficamente pouco conhecido. Nesse contexto, o objetivo deste estudo é resumir o conhecimento atual da família, sob uma perspectiva histórica, e analisar seu potencial de distribuição em função da ecologia das espécies. Foi analisada toda a informação disponível da literatura sobre a família (em 303 artigos publicados e bases de dados), que compreende um total de 15 gêneros e 97 espécies válidos. Historicamente, as publicações apresentaram dois máximos importantes (1900 e 2000), predominando registros de espécies meroplanctônicas. As ocorrências mais frequentes correspondem à zona costeira. Os gêneros *Bimeria* e *Bougainvillia* são aqueles com maior distribuição latitudinal. A modelagem de nicho ecológico de 25 espécies, realizada com o algoritmo MaxEnt, revelou que a clorofila é a variável mais importante que afecta a distribuição da família. Cinco padrões possíveis de distribuição latitudinal são derivados da modelagem, dominando a distribuição subtropical-polar.

Palavras-chave: Filifera, Anthoathecata, Bougainvilliidae, nicho, taxonomia, biogeografia.

Abstract

The family Bougainvilliidae comprises a group of anthoathecate hydrozoans that is biologically, ecologically and biogeographically poorly understood, and consequently, poorly taxonomically organized. Here, our goal is to synthesize knowledge of the family from an historical perspective, and to analyze their potential distribution based on their ecology. We analyzed all the available information on the family (based on 303 articles and databases), comprising 15 genera and 97 valid species in five oceans. Two temporal peaks (1900 and

2000) in publications are dominated by records of meroplanktonic species. The coastal zone has the most frequently reported occurrences. The widest latitudinal ranges are found in the genera *Bimeria* and *Bougainvillia*. Ecological niche modeling of 25 species (MaxEnt algorithm) finds that chlorophyll is the most important variable that influences the distribution of the family. Five possible latitudinal distributional patterns are derived from the model, dominated by the subtropical-polar distribution.

Key words: Filifera, Anthoathecata, Bougainvilliidae, niche, taxonomy, zoogeography.

Introduction

The "hydroid" family Bougainvilliidae Lütken, 1850 pertains to a group of historically and conveniently (i.e., not monophyletically) described of cnidarians in the orders Leptothecata and "Anthoathecata" (the latter, also not monophyletic, Cartwright et al., 2008) in the class Hydrozoa. As with other anthoathecates, the family Bougainvilliidae has two phases in its life cycle: fixed to a substrate as a polyp and as free-swimming medusae (which may also have a reduced form that remains with the polyp, Russell, 1953). The pseudohydrotheca characterizes this family and it is defined as an external, glycosaminoglycan covering, with or without detrital incrustations, that envelops the hydrants and which can be reduced in some genera (cf., Calder, 1988; Schuchert, 2007).

The taxonomy of the family remains poorly structured, most likely as a consequence of the few studies of its biology, ecology and geographical distribution. Despite the 200 years since the description of the first species in the family (*Koellikerina fasciculata*, Péron & Lesueur, 1810), there are no compilations or syntheses of the family, its distribution or genera. Today, information is fragmented, from pioneer studies beginning ca. 150 years ago (Allman, 1871; Haeckel, 1879; Mayer, 1910; Kramp, 1961) to taxonomic lists in secondary sources (i.e., compilations) of Medusozoan species (Bouillon & Boero, 2000; Bouillon et al., 2006), or in data bases such as the World Register of Marine Species (WoRMS; Appeltans et al., 2011) and the relatively few ecological (Ballard & Myers, 1996; Frost et al., 2010; Genzano, 1994) and taxonomic studies (Segura-Puertas, 1991; Xu & Huang, 2004, 2006; Xu et al., 2007a, 2007b, 2009, Nogueira Jr. et al., 2013).

Here, we will summarize the deficiencies of understanding the Bougainvilliidae, so that we may then proceed to build a context in which we can better understand the group and suggest avenues for their future study in biogeography, ecology and taxonomy. More

specifically, we will synthesize current knowledge of the family based on historical and distribution perspectives, with which we will carry out an ecological analysis to predict potential occurrence of this interesting and diverse family.

Materials and methods

Basic taxonomy of the Bougainvilliidae and its place within the Hydrozoa follow Marques & Collins (2004), Bouillon et al. (2006); Schuchert (2007) and Cartwright et al. (2008). To determine historical series and distributions, we gathered 303 articles about the family published between 1890 and 2013. With this information, and databases, such as Genetic Sequence (GenBank) and WoRMS, we developed a data base for each genus and species, with a total of 1290 records having the following information: author, life-cycle phase, reproductive state, sex, location (body of water, country, state, region, latitude and longitude), date of collection, water depth (minimum, maximum), substrate type, salinity, temperature, synonymy and references cited. We then examined the historical and geographical information compiled.

To generate potential distribution models for valid species, we used the information from the data base described above, separating by life-cycle phase (polyp and medusa). Species were plotted using ArcGIS vers. 10 (ESRI, 2010). Environmental descriptive variables were: average surface water temperature, average surface salinity, and average concentration of nitrates, phosphates, oxygen and chlorophyll, from the BioOracle database (Tyberghein et al., 2011). We modeled using the Maxent algorithm (maximum entropy approach; Phillips et al., 2006). We used a threshold-independent measure, the area under the curve (AUC), to evaluate models and a Jackknife method (Sokal & Rohlf, 1995; Phillips et al., 2006) was used to examine the importance of each variable to the final model. The results of this evaluation are expressed as a measure known as gain, which shows the ecological requirements of each species, and probably determine their potential distribution. Classification of AUC values followed Metz (1986), as excellent (0.90-1.0), good (0.80-0.90), average (0.70-0.80), bad (0.60-0.70) and very bad (0.50-0.60).

Results

The Bougainvilliidae comprise a total of 19 genera and 170 named species, of which 54 species were synonymies. Most of these are the cosmopolitan species Bougainvillia muscus (Allman, 1863). Another 19 species were doubtful. Thus, a total of 15 genera and 97

species are valid (Table 1) representing about 3% of the Hydrozoa species in the world (3,140 species, cf. Bouillon et al., 2006).

Species in the Bougainvilliidae tend to be meroplanktonic genera (e.g., *Bougainvillia*, *Koellikerina* and *Nubiella*, Table 2), and are not the most studied. About 65% of publications are about the genera *Bimeria* (which is benthic) and *Bougainvillia*. While reports of new species has been continuous, the species accumulation curve shows that about six new species per decade are being described, and therefore the asymptotic number of species is far from being reached (Fig. 1).

Historically, studies of the Bougainvilliidae have had several temporal hiatuses. Beginning in 1900, with very few studies from 1920-1950, increasing in the 1950s and still doing so (Fig. 1), with most of these studies (3-10 papers each) being by Calder, Galea, Genzano, Marques, Pagés, Palma, Schuchert, Segura-Puertas and Xu. Most of these were studies in tropical and southern Atlantic, northeast and northwest Atlantic, southwestern and central eastern Pacific oceans, in contrast with previous studies, which were mostly in European and North American waters.

Geographically, valid species are found in all oceans, within a range of 155° of latitude, from 76.93oN to 78.49oS, and 360° of longitude (Fig. 2). Most species occur in the Pacific (73%), with 48% in the Atlantic, 26% in the Indian Ocean, 4% in the Arctic, and 5% in the Southern Ocean (Fig. 3). The Atlantic Ocean is the best studied, with 60% of all publications on the family, while the least studied are the Indian and Southern oceans. Latitudinally, most valid species were described between 30-40°N (44 species, Fig. 4).

The widest latitudinal distributions are found in the genera *Bimeria* and *Bougainvillia*. *Bimeria* is found from 56.1ºN to 76.1ºS (Fig. 5), mostly due to the species *Bimeria vestita*. The remaining species are more localized and usually in sublitoral waters (15-569 m, Vanhöffen, 1910; Fraser, 1938). *Bimeria vestita* is considered to be cosmopolitan (Ramil & Vervoort, 1992), although without records from the Arctic and Southern oceans (but see Marques et al., 2000), is eurythermic (16-31oC), euryhaline (salinity 29.0-36.5; Calder, 1993; Migotto, 1996), and in shallow (<25 m) to deep (358 m) waters (Vanhöffen, 1910, Wedler & Larson, 1986; Marques & Migotto, 2004; Genzano et al., 2009). The *Bougainvillia* are found between 76.86oN and 54.0oS (Fig. 5), in water temperatures of 0.8ºC to 20.7ºC (Vannucci, 1957; Calder, 1990; Petrova et al., 2011), and the greatest depth record was 7000 m (Kramp, 1965).

Species in the remaining genera have fragmented distributions (Fig. 5):

-*Chiarella* is in the Gulf of California (Brinton et al., 1986), and subequatorial zone of the Pacific Ocean;

-*Dicoryne* is antitropical at depths of 1-400 m (Hirohito, 1988; Schuchert, 1996);

-Garveia is most commonly reported from subequatorial and tropical regions in the northern hemisphere in the Atlantic, Indian and Pacific oceans and in deeper waters (2100 m, Ramil & Vervoort, 1992);

-*Koellikerina* has a tropical or subtropical distribution, with the exception of K. massi, whose type locality is the McMurdo Strait, in Antarctica, and apparently prefers cold waters (-0.4ºC to -1.5ºC; Browne, 1910; Moteki et al., 2010). The genus occurs in depths up to 100 m (e.g. Petersen & Vannucci, 1960; Kawamura & Kubota, 2005; Schuchert, 2007);

-*Millardiana* is restricted to Neotropical surface waters (<25 m; Calder, 1988) of the Caribbean Atlantic Ocean and ocean salinity (ca. 36.5, Calder, 1993);

-*Nemopsis* is found in subtropical and cold temperate waters of the north Atlantic and Pacific oceans (Calder, 1971; Schuchert, 2007; Mendoza-Becerril et al., 2009);

-*Nubiella* is intertropical in the Atlantic, Pacific and Indian oceans, usually less than 100 m depth (Segura-Puertas, 1980; Xu et al., 2009);

-*Pachycordyle* is found in the north Atlantic and Pacific oceans, except for P. navis found in the Indian Ocean near South Africa, at depths between the surface and 30 m (Calder, 1991; Kramp, 1959b);

-*Parawrightia* is found in northern and southern tropical waters of the western Atlantic and sub equatorially in the eastern Indian Ocean, at depths of 0-12 m (Kelmo & Santa-Isabel, 1998; Grohmann et al., 2003), and salinity of 36.5 (Calder, 1993);

-*Rhizorhagium* has a wide latitudinal distribution in all oceans, with the majority of records from sub polar regions, at depths to 890 m (Schuchert, 2007);

-*Silhouetta* is tropical in the Atlantic, Pacific and Indian oceans, and also in the Caribbean Sea, at a maximum depth of 30 m (Millard & Bouillon (1973), and salinity of 36.5 (Calder, 1993);

-*Thamnostoma* is common in warm tropical waters, including reefs in the Atlantic, Pacific and Indian Oceans, at depths of up to 100 m (Goy, 1979; Riera et al., 1986; Segura-Puertas, 1991);

-*Velkovrhia* is the only genus restricted to fresh waters and caverns at up to 1830 m altitude (Tvrtković & Veen, 2006) near the Adriatic Sea.

Modeling of ecological niche for a total of 25 species (with a minimum of 10 location records) resulted in AUC values >0.81, which indicates that classifications were good to excellent. In general, chlorophyll is the most important variable in 69% of the models, with phosphates important in 17%, temperature in 10% and salinity in 3%. The Jackknife test indicated that chlorophyll (implicating high primary productivity) is the greatest contributor (gain) to the models (Table 3).

These models suggest five possible patterns to describe potential latitudinal and longitudinal limits for species distributions: equatorial-tropical, tropical-subtropical, subtropical-subpolar, subtropical-polar and widespread (from the equator to subpolar waters; Table 3, Figs. 6-10). The subtropical-polar category dominates, with 31% of the species and includes peaks in species richness. Coastal species are also more abundant, with 90% of the species (Table 3). Predictions of distributions from the models concur with those observed in most life-phases in the Bougainvilliidae. However, for the species *B. vestita*, *B. carolinensis* (polyp), *B. fulva*, *B. muscus* (polyp), *B. rugosa*, *G. franciscana*, *N. alvarinoae* and *R. roseum* some occurrence points do not correspond to those predicted as most adequate.

Discussion

Considerations on global knowledge of the Bougainvilliidae

In the Atlantic Ocean, the most extensive studies were carried out in the northeast Atlantic and found a total of 28 species when including the Mediterranean (Motz-Kossowska, 1905; Stechow, 1919; Bouillon et al., 2004); Helgoland (Hartlaub, 1911), western Sweden (Rees & Rowe, 1969), Macaronesia, Mauritania, Morocco (Vervoort, 2006), Europe in general (Schuchert, 2007) and France, adjacent to the English Channel (Le Mao, 2009). In the northwest Atlantic, a total of 30 species were recorded in specific places, such as Canada (Fraser, 1944), the United States and Bermuda (Calder, 1971, 1988, 1993), the Bahamas (Bigelow, 1918), Puerto Rico (Wedler & Larson, 1986), Mexico (Segura-Puertas, 1992). It is evident, that this ocean has the greatest number of publications on this family, which is a reflection of the greater number of specialists in the region (e.g. Bigelow, Bouillon, Calder, Kramp, Mayer, Russell). The Bougainvilliidae are less well known in the southern Atlantic. Three species are reported from southwest Africa (Pagès et al., 1992). Comparatively, the southwestern Atlantic has been better studied, with inventories of hydromedusae (Ramírez & Zamponi, 1981; Bouillon, 1999; Migotto et al., 2002; Genzano et al., 2008, Nogueira Jr. et al., 2013) carried out from the primary literature (e.g. Haeckel, 1879; Vannucci, 1951, 1957;

Vannucci & Tundisi, 1962; Kramp, 1957, 1959a; Alvariño, 1968) and by examining plankton samples, with a total of 12 species being reported.

Despite the fewer studies in the Pacific Ocean, the number of species (82) is larger than any other area. There are 11 species in the Bismarck Sea (Bouillon, 1980), 12 in New Zealand (Schuchert, 1996) and 17 along the coast of China (Xu & Huang, 2004). In China, during the last decade, many more species have been identified, mostly in the genus *Nubiella*, as a consequence of which the diversity of Bougainvilliidae is increasing in a latitudinal band of 30-40°N. The best-studied regions of the Pacific are Canada (e.g. Foerster, 1923; Brinckmann-Voss, 1996), United States (e.g. Clarke, 1876; Nutting, 1901; Torrey, 1902; Calder, 2010), Ecuador (e.g. Fraser, 1938) and Chile (e.g. Galea, 2007; Palma et al., 2007a, 2007b; Villenas et al., 2009; Bravo et al., 2011; Palma et al., 2011).

In the Indian Ocean, two studies had the greatest sampled areas (Kramp, 1965; Millard 1975), in which 56% of the total, currently known fauna were reported. In the Arctic Ocean, the first studies were in the 1960s (Hand & Kan, 1961, EUA-Alaska; Naumov, 1969, Russia), followed by Zelickman (1972, Russia) and Ronowicz (2007, Norway). And finally, in the Southern, the expeditions of the 1900-1910's were most important (e.g. Hickson & Gravely, 1907; Browne, 1910; Vanhöffen, 1910).

Considerations about the distribution of the Bougainvilliidae

For a comprehensive analysis of aquatic species distributions, one must consider the three dimensions: latitude, longitude and depth (Miranda & Marques, 2011; Bentlage et al., 2013). In the case of the Bougainvilliidae, due to lack of information, those three dimensions are not always known, as most studies have focused on the epipelagic zone (0-200 m), as it is typical for Medusozoa (Marques et al., 2003; Segura-Puertas et al., 2003). The epipelagic is the zone in which we expect to find most species because it is known to be most diverse for zooplankton (Angel, 1994) as well as benthic organisms (Genzano et al., 2009).

Despite the lack of data, we carried out a distributional analysis in two dimensions. In latitude, the first dimension, the number of species declines (from 44 to 3) with increasing latitude, as is the case with respect to other groups of planktonic (Boltovskoy et al., 1999; Genzano et al., 2008) and benthic (Fautin et al., 2013) invertebrates. Longitudinally, the second dimension, we find that the majority of species are coastal, which shows the importance of substrates for development during the polyp phase, even for those species in which only the medusa phase is known. This suggests that the ancestral niche that was for

species with both polyp and medusa phases has been maintained, despite subsequent evolutionary modifications with respect to dispersal and development.

As mentioned, models were somewhat limited by the sparse available information, both about species distribution and environmental variables. Also, alternating generations (found in some genera during their life cycles) are a problem for this type of analysis, because there are no methods that consider the influence of dispersal along with biotic and abiotic factors on each developmental phase (Pearson & Dawson, 2003). Regardless of these potential problems, the distributions generated by the models appear to be robust, in that the predicted distributions are very similar to those observed today. In the few cases with apparent incongruence's between observed and predicted distributions, these may not be errors but they rather may represent samples from sink populations of Bougainvilliidae (as in the cubomedusa *Chironex fleckeri*, Bentlage et al., 2009). These kinds of populations tend to exist temporarily in marginal habitats where they may often disappear if dispersal does not supply more individuals, such as when mortality exceeds natality (Dias, 1996; Palmer et al., 1996).

Hydrozoans distribution are known to be influenced by a variety of environmental factors (Arai, 1992), yet most models suggest that chlorophyll is the most important. Chlorophyll plays an important role and is a proxy of high primary productivity and nutrient-rich waters for some regions, hence indicating abundance of food resources for pelagic and benthic communities (Acha et al., 2004). It is also evident that other biotic factors can be important, yet they were not considered in the analysis, such as epibiosis (e.g., Oliveira & Marques, 2007, 2011), and those associated with human activities (e.g., Rocha et al., 2013).

Temperature is important for the distribution of some species in the genera *Dicoryne* and *Garveia*. *Dicoryne* occurs where temperatures oscillate between 2.7-23.6ºC (Okolodkov, 2010), such as <20oC for D. conferta (Broch, 1916), while *Garveia* is found in waters with temperatures >25ºC (Okolodkov, 2010). Temperature is often considered a key factor in determining the distribution of benthic (Boltovskoy & Wright, 1976) and planktonic (Beaugrand et al., 2013) organisms. Of course, local and historical factors are considered to be important as well.

The potential distribution for some species includes regions in which important studies were carried out without noting the presence of those species. This may be due to the fact that modeling does not include biological interactions, geographic barriers and history, which clearly are also important and may explain why species seldom occupy all favorable

environments (Anderson & Martínez-Meyer, 2004). An example in point is found in the latitudinal distribution of *B. superciliaris* and *Nemopsis bachei*. Both have been found in the northern hemisphere and their potential distribution due to modeling includes both hemispheres, which suggests dispersal limitation. Specifically, *N. bachei* is considered to be euryhaline (Calder, 1971) (salinity 3-35, Denayer, 1973; Mendoza-Becerril et al., 2009) and usually present at temperatures <26°C (Cronin et al., 1962; Denayer, 1973; Marshalonis & Pinckney, 2007).

Of the species with sufficient information for polyp and medusa phases, models can be particular to the life phase. In two species (*B. carolinensis* and *B. muscus*) patterns are similar for both phases, and in others, one phase generally has a larger predicted distribution. In *B. muscus* the medusa phase has the greatest predicted distribution while in *B. superciliaris* it is the polyp phase. Similarly, in at least *B. superciliaris*, temperature is most important for the polyp phase, while chlorophyll is most important for the medusa phase. This reinforces the idea that the influence of the environment can vary by life phase and so coupling of phases is not trivial. As a consequence, modelling without respect to life phase, for organisms with distinct phases, may overlook very important and complex patterns. Adding to uncertainty is the dispersal potential of each phase, with some considering the medusa phase as the principal dispersal agent, which is reflected by a widespread distribution, including pelagic (Bouillon, 1981; Leclère et al., 2009). This is exemplified in padrões possíveis de distribuição latitudinal. On the other hand, some researchers suggest the contrary (Cornelius, 1992), as seen in B. superciliaris. Most likely is that while some patterns predominate, there will always be exceptions.

The lack of information from a variety of regions is also clear in this analysis, especially in the southeastern Atlantic, southern Gulf of Mexico, Central America, southwest Pacific and northeastern Indian Ocean. These regions also have relatively high AUC values (0.65-0.99) for several species and would be interesting places to increase sampling effort. However, the number of studies seems to be declining with respect to the polyp phase (Table 2). This trend and the species accumulation curves illustrate that more studies are needed, especially of benthic and planktonic faunas and at a variety of depths and aquatic environments, in addition to revisions based on morphological, reproductive and molecular characters to respond questions of evolutionary and geographic patterns.

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References

Acha, E.M., H. Mianzan, R.A. Guerrero, M. Favero & J. Bava. 2004. Marine fronts at the continental shelves of austral South America physical and ecological processes. J. Mar. Syst., 44: 83-105.

Allman, G.J. 1871. Of the Gymnoblastic or tubularian hydroids: I the hydroida in general, II The genera and species of the Gymnoblastea. The Ray Society, London, 450 pp.

Alvariño, A. 1968. Los quetognatos, sifonóforos and medusas en la región del atlántico ecuatorial bajo la influencia del Amazonas. An. Inst. Cienc. Mar Limnol. UNAM., 39: 41-76.

Anderson, R.P. & E. Martínez-Meyer. 2004. Modeling species geographic distribution for preeliminary conservation distribution for preliminary with the spiny pocket mice (Heteromys) of Ecuador. Biol. Conserv., 116: 167-179.

Angel, M.V. 1994. Biodiversity of the pelagic ocean. Conserv. Biol., 7: 760-762.

Appeltans, W., P. Bouchet, G.A. Boxshall, C. De Broyer, N.J. de Voogd, D.P. Gordon, B.W. Hoeksema, T. Horton, M. Kennedy, J. Mees, G.C.B. Poore, G. Read, S. Stöhr, T.C. Walter & M.J. Costello. 2011 World Register of Marine Species. [http://www.marinespecies.org/hydrozoa]. Reviewed: 3 November 2011.

Arai, M.N. 1992. Active and passive factors affecting aggregations of hydromedusae: a review. Sci. Mar., 56(2-3): 99-108.

Ballard, L. & A. Myers. 1996. Seasonal changes in the vertical distribution of five species of the family Bougainvilliidae (Cnidaria: Anthomedusae) at Lough Hyne, south-west Ireland. Sci. Mar., 60(1): 69-74.

Beaugrand, G., I. Rombouts & R. Kirby. 2013. Towards an understanding of the pattern of biodiversity in the oceans. Global Ecol. Biogeogr., 22: 440-449.

Bentlage, B., A.T. Peterson & P. Cartwright. 2009. Inferring distributions of chirodropid box-jellyfishes (Cnidaria: Cubozoa) in geographic and ecological space using ecological niche modeling. Mar. Ecol. Prog. Ser., 384: 121-133.

Bentlage, B., A.T. Peterson, N. Barve & P. Cartwright. 2013. Plumbing the depths: extending ecological niche modeling and species distribution modeling in three dimensions. Global Ecol. Biogeogr., DOI: 10:1111/geb.12049

Bigelow, H.B. 1918. Some medusae and siphonophora from the western Atlantic. Bull. Mus. Com. Zool., 62: 363-442.

Boltovskoy, E. & H. Wright. 1976. Recent Foraminifera. Junke Publishers, The Hague, 550 pp.

Boltovskoy, D., M.J. Gibbons, L. Hatchings & D. Binet. 1999. General biological features of the South Atlantic zooplankton. In: D. Boltovskoy (ed.). South Atlantic zooplankton. Backhuys Publishers Leiden, Leiden, pp. 869-1098.

Bouillon, J. 1980. Hydroméduses de la mer de Bismarck (Papouasie Nouvelle-Guinée). Partie III: Anthomedusae Filifera (Hydrozoa-Cnidaria). Cah. Biol. Mar., 21: 307-344.

Bouillon, J. 1981. Origine et phylogenèse des cnidaires et des hydropolypes-hydroméduses. Ann. Soc. Roy. Zool. Belgique, 1-4: 45-56.

Bouillon, J. 1999. Hydromedusae. In: D. Boltovskoy (ed.). South Atlantic zooplankton. Backhuys Publishers Leiden, Leiden, pp. 385-465.

Bouillon, J. & F. Boero. 2000. Phylogeny and classification of Hydroidomedusae. Thal. Salentina, 24: 5-296.

Bouillon, J., M.D. Medel, F. Pagès, J.M. Gili, F. Boero & C. Gravili. 2004. Fauna of the Mediterranean Hydrozoa. Sci. Mar., 68: 5-438.

Bouillon, J., C. Gravili, F. Pagès, J.M. Gili & F. Boero. 2006. An introduction to Hydrozoa. Publication Scientifiques du Museum, Paris, 591 pp.

Bravo, V., S. Palma & N. Silva. 2011. Seasonal and vertical distributional patterns of medusae in Aysén region, southern Chile. Lat. Am. J. Aquat. Res., 39(2): 359-377.

Brinckmann-Voss, A. 1996. Seasonality of hydroids (Hydrozoa, Cnidaria) from an intertidal pool and adjacent subtidal habitats at Race Rocks, off Vancouver Island, Canada. Sci. Mar., 60: 89-97.

Brinton, E., A. Fleminger & D. Siegel-Causey. 1986. The temperate and tropical planktonic biotas of the Gulf of California. CalCOFi. Invest. Rep., 27: 228-266.

Broch, H. 1916. Hydroida (Part I). The Danish Ingolf Exped., 5(6): 1-66.

Browne, E.T. 1910. Coelenterata V. Medusae. National Antarctic Expedition 1901-1904. Nat. Hist. Zool. Bot., 5: 1-62.

Calder, D.R. 1971. Hydroids and hydromedusae of southern chesapeake bay. Virginia Inst. Mar. Sci. Spec. papers Mar. Sci., 1: 1-109.

Calder, D.R. 1988. Shallow-water hydroids of Bermuda. The Athecatae. Life Sci. Contrib., 148: 1-107.

Calder, D.R. 1990. Seasonal cycles of activity and inactivity in some hydroids from Virginia and South Carolina, U.S.A. Can. J. Zool., 68(3): 442-450.

Calder, D.R. 1991. Abundance and distribution of hydroids in a mangrove ecosystem at Twin Cays, Belize, Central America. Hydrobiologia, 216/217: 221-228.

Calder, D.R. 1993. Local distribution and biogeography of the hydroids (Cnidaria) of Bermuda. Caribb. J. Sci., 29: 61-74.

Calder, D.R. 2010. Some anthoathecate hydroids and limnopolyps (Cnidaria, Hydrozoa) from the Hawaiian Archipelago. Zootaxa, 2590: 1-91.

Cartwright, P., N.M. Evans, C.W. Dunn, A.C. Marques, M.P. Miglietta, P. Schuchert & A.G. Collins. 2008. Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). J. Mar. Biol. Assoc. UK., 88(8): 1663-1672.

Clarke, S.F. 1876. The Hydroids of the Pacific Coast of the United States, south of Vancouver Island, with a report upon those in the museum of Yale College. Trans. Conn. Acad., 3: 249-268.

Cornelius, P.F.S. 1992. Medusa loss in leptolid Hydrozoa (Cnidaria), hydroid rafting, and abbreviated life-cycles among their remote-island faunae: an interim review. Sci. Mar., 56: 245-261.

Cronin, L.E., J.C. Daiber & E.M. Hulbert. 1962. Quantitative seasonal aspects of zooplankton in the delaware river estuary. Chesapeake Sci., 3(2): 63-93.

Denayer, J.C. 1973. Trois méduses nouvellles ou peu connues des côtes françaises: Maeotias inexpectata Ostroumov, 1896, Blackfordia virginica Mayer Nemopsis bachei Agassiz, 1849. Cah. Biol. Mar., 14: 285-294.

Dias, P.C. 1996. Sources and sinks in population biology. Trends Ecol. Evol., 11(8): 326-330.

ESRI. 2010. ArcGIS10. Environmental systems research institute, Inc. New York.

Fautin, D.G., L. Malarky & J. Soberón. 2013. Latitudinal diversity of sea anemones (Cnidaria: Actiniaria. Biol. Bull., 224: 89-98.

Foerster, R.E. 1923. The Hydromedusae of the west coast of North America, with special reference to those of the Vancouver Islands Region. Cont. Can. Biol., 1: 219-277.

Fraser, C. McLean. 1938. Hydroids of the 1934 Allan Hancock Pacific Expedition. Allan Hancock Pacific Exped., 4: 1-105.

Fraser, C. McLean. 1944. Hydroids of the Atlantic coast of North America. University of Toronto Press, Toronto, 320 pp.

Frost, J.R., C.A. Jacoby & M.J. Youngbluth. 2010. Behavior of Nemopsis bachei L. Agassiz, 1849 medusae in the presence of physical gradients and biological thin layers. Hydrobiologia, 645: 97-111.

Galea, H. 2007. Hydroids and hydromedusae (Cnidaria, Hydrozoa) from the fjords region of southern Chile. Zootaxa, 1597: 1-116.

Genzano, G.N. 1994. La comunidad hidroide del intermareal de Mar del Plata (Argentina), estacionalidad, abundancia and periodos reproductivos. Cah. Biol. Mar., 35: 289-303.

Genzano, G.N., H.W. Mianzan & J. Bouillon. 2008. Hydromedusae (Cnidaria: Hydrozoa) from the temperate southwestern Atlantic Ocean: a review. Zootaxa, 1750: 1-18.

Genzano, G.N., D. Giberto, L. Schejter, C. Bremec & P. Meretta. 2009. Hydroid assemblages from the southwestern Atlantic Ocean (34-42S). Mar. Ecol., 30: 33-46.

Goy, J. 1979. Campagne de la Calypso au large des côtes atlantiques de l'Amérique du Sud (1961-1962)-35. Méduses. Résult. Scient. Camp. Calypso, 11: 263-296.

Grohmann, P.A., C.C. Nogueira & V.M.P.A. da Silva. 2003. Hydroids (Cnidaria, Hydrozoa) collected on the continental shelf of Brazil during the Geomar X Oceanographic Operation. Zootaxa, 299: 1-19.

Haeckel, E. 1879. Das System der Medusen, Erster Theil einer Monographie der Medusen (Craspedotae). Denkschr. med. naturw. Ges. Jena, 1:1-360.

Hand, C. & L.B. Kan. 1961. The Medusae of the Chukchi and Beaufort Seas of the Arctic Ocean including the description of a new species of Eucodonium (Hydrozoa: Anthomedusae). Arctic. Inst. North Am. Tech. Pap., 6: 5-23.

Hartlaub, C. 1911. XII Craspedote Medusen. I. Teil. 2 Lief. Familie III Margelidae. Nord. Plankton 6: 137-235.

Hickson, S.J. & F.H. Gravely. 1907. Hydroid zoophytes, National Antarctic Expedition, 1901-1904. Nat. Hist., 3: 1-34.

Hirohito, Emperor of Japan. 1988. The hydroids of Sagami Bay. Biological Laboratory Imperial Household, Tokyo, pp. 1-179.

Kawamura, M. & S. Kubota. 2005. Two species of Koellikerina medusae (Cnidaria, Hydrozoa, Anthomedusae) from Japan. Publ. Seto Mar. Biol. Lab., 40(3-4): 121-130.

Kelmo, F. & L.M. Santa-Isabel. 1998. Theathecatae hydroids (Cnidaria, Hydrozoa) from Northern Bahía, Brazil. Rev. Biol. Trop., 46(Suppl. 5): 61-72.

Kramp, P.L. 1957. Hydromedusae from the Discovery collections. Discovery Rep., 29: 1-128.

Kramp, P.L. 1959a. The Hydromedusae of the Atlantic Ocean and adjacent waters. Dana Rep., 46: 1-283.

Kramp, P.L. 1959b. Some new and little-know Indo-Pacific Medusae. Vidensk. Meddr. Dansk. Naturh. Foren., 120: 223-259.

Kramp, P.L. 1961. Synopsis of the Medusae of the world. J. Mar. Biol. Assoc. UK, 40: 7-469.

Kramp, P.L. 1965. The hydromedusae of the Pacific and Indian Oceans. Dana Rep., 63: 1-162.

Leclère, L., P. Schuchert, C. Cruaud, A. Coloux & M. Manuel. 2009. Molecular phylogenetics of Thecata (Hydrozoa, Cnidarian) reveals long-term maintenance of life history traits despite high frequency of recent character changes. Syst. Biol., 58: 1-18.

Le Mao, P. 2009. Inventaire de la biodiversité marine dans le golfenormano-breton, Cnidaires. IFREMER, département des laboratoires environnementet ressources aquacoles, Finistére Bretagne Nord, 66 pp.

Marques, A.C. & A.G. Collins. 2004. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebr. Biol., 123(1): 23-42.

Marques, A.C., H. Mergner, R. Höinghaus & W. Vervoort. 2000. Bimeria vestita (Hydrozoa: Anthomedusae: Bougainvilliidae) senior synonym of Eudendrium vestitum (Hydrozoa: Anthomedusae: Eudendriidae). Zoöl. Meded. Leiden, 73(22): 321-325.

Marques, A.C. & A.E. Migotto. 2004. Hidrozoários (Cnidaria) marinhos bentônicos da estação ecológica Juréia-Itatins. In: O.A.V. Marques &W. Duleba (eds.). Estação ecológica Juréia-Itatins, ambiente físico, flora e fauna. Holos Editora, Ribeirão Preto, pp. 172-178.

Marques, A.C., A.C. Morandini & A.E. Migotto. 2003. Synopsis of knowledge on Cnidaria Medusozoa from Brazil. Biota Neotrop., 3: 1-18.

Marshalonis, D. & J.L. Pinckney. 2007. Respiration rates of dominant hydromedusae in the North Inlet tidal estuary during winter and summer. J. Plankton Res., 29(12): 1031-1040.

Mayer, A.G. 1910. Medusae of the world. Carnegie Institution Washington, pp. 1-735.

Mendoza-Becerril, M.A., A. Ocaña-Luna, M. Sánchez-Ramírez & L. Segura-Puertas. 2009. Primer registro de Phialella quadrata and ampliación del límite de distribución de ocho especies de hidromedusas (Hydrozoa) en el Océano Atlántico Occidental. Hidrobiológica, 19(3): 257-267.

Metz, C.E. 1986. ROC methodology in radiologic imaging. Invest. Radiol., 21: 720-733.

Migotto, A.E. 1996. Die benthic shallow-water hydroids (Cnidaria, Hydrozoa) of the Ocean of São Sebastião, Brazil, including a checklist of Brazilian hydroids. Zool. Verh., 306: 1-125.

Migotto, A.E., A.C. Marques, A.C. Morandini & F.L. Silveira. 2002. Checklist of the Cnidaria Medusozoa of Brazil. Biota Neotrop., 2: 1-31.

Millard, N.A.H. 1975. Monograph on the Hydroida of Southern Africa. Ann. S. Afr. Mus., 68: 1-513.

Millard, N.A.H. & J. Bouillon. 1973. Hydroids from the Seychelles (Coelenterata). Ann. Mus. R. Afr. Cent. Sci. Zool., 206(8): 1-106.

Miranda, T.P. & A.C. Marques. 2011. Abordagens atuais em biogeografia marinha. Rev. Biol., 7: 41-48.

Moteki, R.T.M., A.O.N. Horimoto, Y. Tanaka & T. Ishimaru. 2010. Structure of the pelagic cnidarian community in Lützow-Holm Bay in the Indian sector of the Southern Ocean. Polar Sci., 4: 387-404.

Motz-Kossowska, S. 1905. Contribution à la connaissance des hydraires de la Méditerranée occidentale. I. Hydraires gymnoblastiques. Arch. Zool. Exp. Gén. Sér., 4. T. pp. 39-98.

Naumov, D.V. 1969. Hydroids and Hydromedusae of the USSR. Isr. Prog. Sci. Trans. Jerusalem, 70: 1-631.

Nogueira Jr., M., C.S. Rodriguez, H. Mianzan, M.A. Haddad & G. Genzano. 2013. Description of a new hydromedusa from the southwestern Atlantic Ocean, Bougainvillia pagesi sp. nov. (Cnidaria, Hydrozoa, Anthoathecata). Mar. Ecol., 34(1): 113-122.

Nutting, C.C. 1901. Papers from the Harriman Alaska expedition. XXI. The Hydroids. Proc. Wash. Acad. Sci., 3: 157-216.

Okolodkov, Y.B. 2010. Biogeografía Marina. Universidad Autónoma de Campeche, Campeche, 217 pp.

Oliveira, O.M.P. & A.C. Marques. 2007. Epiphytic hydroids (Hydrozoa: Anthoathecata and Leptothecata) of the world. Check List, 3(1): 21-38.

Oliveira, O.M.P. & A.C. Marques. 2011. Global and local patterns in the use of macrophytes as substrata by hydroids (Hydrozoa: Anthoathecata and Leptothecata). Mar. Biol. Res., 7: 786-795.

Pagès, F., J.M. Gili & J. Bouillon. 1992. Medusae (Hydrozoa, Scyphozoa, Cubozoa) of the Benguela Current (southeastern Atlantic). Sci. Mar., 56: 1-64.

Palma, S., P. Apablaza & N. Silva. 2007a. Hydromedusae (Cnidaria) of the Chilean southern channels (from the Corcovado Gulf to the Pulluche-Chacabuco Channels). Sci. Mar., 71(1): 65-74.

Palma, S., P. Apablaza & D. Soto. 2007b. Diversity and aggregation áreas of planktonic cnidarians of the southern channels of Chile (Boca del Guafo to Pulluche Channel). Invest. Mar, Valparaíso, 5(2): 71-82.

Palma, S., N. Silva, M.C. Retamal & L. Castro. 2011. Seasonal and vertical distributional patterns of siphonophores and medusae in the Chiloé Interior Sea, Chile. Cont. Shelf Res., 31: 260-271.

Palmer, M.A., J.D. Allan & C.A. Butman. 1996. Dispersal as a regional process affecting the local dynamics of marine and stream benthic invertebrates. Trends Ecol. Evol., 11(8): 322-326.

Pearson, R.G. & T.P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Glob. Ecol. Biogeogr., 12: 361-371.

Péron, F. & C.A. Lesueur. 1810. Tableau des caractères génériques et spécifiques de toutes les espèces de méduses connues jusqu'à ce jour. Ann. Mus. Hist. Nat. Paris, 14: 325-366.

Petersen, K.W. & M. Vannucci. 1960. The life cycle of Koellikerina fasciculata (Anthomedusae, Bougainvilliidae). Pub. Stazione Zool. Napoli, 31(3): 473-492.

Petrova, E.A., T.N. Dautova & L.S. Shkoldina. 2011. Species composition, seasonal dynamics of quantities and spatial distribution of hydromedusae (Cnidaria: Hydrozoa) in Vostok Bay of the Sea of Japan. Russ. J. Mar. Biol., 37(2): 111-122.

Phillips, S.J., R.P. Anderson & R.E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model., 190: 231-259.

Ramil, F. & W. Vervoort. 1992. Report on the Hydroida collected by the "BALGIM" expedition in and around the Strait of Gibraltar. Zool. Verh., 277(7): 1-262.

Ramírez, F.C. & M.O. Zamponi. 1981. Hydromedusae. In: D. Boltovskoy (ed.). Atlas del zooplancton del Atlántico Sudoccidental and métodos de trabajo con el zooplancton marino. Publicación Especial del Instituto Nacional de Investigación and Desarrollo Pesquero, Mar de Plata, pp. 443-469.

Rees, W.J. & M. Rowe. 1969. Hydroids of the Swedish west coast. Acta R. Soc. Sci. Litt. Gothobg. Zool., 3: 1-23.

Riera, T., J.M. Gili & F. Pagès. 1986. Estudio cuantitativo y estacional de dos poblaciones de cnidarios planctónicos frente a las costas de Barcelona (Mediterráneo occidental): ciclos entre 1966-1967 and 1982-1983. Misc. Zool., 10: 23-32.

Rocha, R.M., L.M. Vieira, A.E. Migotto, A.C.Z. Amaral, C.R.R. Ventura, C.S. Serejo, F.B. Pitombo, K.C. Santos, L.R.L. Simone, M. Tavares, R.M. Lopes, U. Pinheiro & A.C. Marques. 2013. The need of more rigorous assessments of marine species introductions: a counter example from the Brazilian coast. Mar. Poll. Bull., 67: 241-243.

Ronowicz, M. 2007. Benthic hydroids (Cnidaria: Hydrozoa) from Svalbard waters-biodiversity and distribution. J. Mar. Biol. Assoc. UK., 87: 1089-1094.

Russell, F.S. 1953. The Medusae of the British Isles. Cambridge University Press, Cambridge, pp. 1-530.

Schuchert, P. 1996. The marine fauna of New Zealand: athecate hydroids and their medusae (Cnidaria: Hydrozoa). New Zeal. Oceanogr. Inst. Mem., 106: 1-159.

Schuchert, P. 2007. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 2. Rev. Suisse Zool., 114: 195-396.

Segura-Puertas, L. 1980. Two new species of Lizzia (Hydrozoa: Anthomedusae) from the eastern tropical Pacific. Proc. Biol. Soc. Wash., 93(3): 515-522.

Segura-Puertas, L. 1991. New records of two species of hydromedusae (Cnidaria) from the Mexican Caribbean. An. Inst. Cienc. Mar Limnol. Univ. Nac. Auton. Mex., 18: 133-135.

Segura-Puertas, L. 1992. Medusae (Cnidaria) from the Yucatan shelf and Mexican Caribbean. Bull. Mar. Sci., 51: 353-359.

Segura-Puertas, L., E. Suárez-Morales & L. Celis. 2003. A checklist of the Medusae (Hydrozoa, Scyphozoa and Cubozoa) of Mexico. Zootaxa, 194: 1-15.

Sokal, R. & J. Rohlf. 1995. Biometry. The principles and practice of statistics in biological research. State University of New York at Stony Brook, New York, 887 pp.

Stechow, E. 1919. Zur Kenntnis der hydroidenfauna des mittelmeeres, Amerikas und anderer Gebiete. Zool. Jahrb. Syst., 42: 1-172.

Torrey, H.B. 1902. The Hydroida of the Pacific coast of North America. Univ. Calif. Publ. Zool., 1: 1-104.

Tvrtković, N. & P. Veen. 2006. The dinaric alps rare habitats and species, a nature conservation Project in Croatia. Hrvatski prirodoslovnimuzej, Zagreb, pp. 1-67.

Tyberghein, L., H. Verbruggen, K. Pauly, C. Troupin, F. Mineur & O. De Clerck. 2011. Bio-ORACLE: a global environmental dataset for marine species distribution modeling. Global ecology and biogeography. [http://www.oracle.ugent.be/]. Reviewed: 20 octubre 2012.

Vanhöffen, E. 1910. Die Hydroiden der Deutschen Südpolar-Expedition 1901-1903. Deuts. Südpolar Exp., 11: 269-340.

Vannucci, M. 1951. Distribuição dos Hydrozoa até agora conhecidos nas costas do Brasil. Bol. Inst. Ocean., 2: 105-124.

Vannucci, M. 1957. On Brazilian Hydromedusae and their distribution in relation to different water masses. Bol. Inst. Ocean., 8: 23-109.

Vannucci, M. & J. Tundisi. 1962. Las medusas existentes en los museos de La Plata and Buenos Aires. Comun. Mus. Argent. Cienc. Nat. Bernardino Rivadavia, 3(8): 201-215.

Vervoort, W. 2006. Leptolida (Cnidaria: Hydrozoa) collected during the CANCAP and Mauritania-II expeditions of the National Museum of Natural History, Leiden, The Netherlands [Anthoathecata, various families of Leptothecata and addenda]. Zool. Meded. Leiden, 80-1: 181-318.

Villenas, F., D. Soto & S. Palma. 2009. Cambios interanuales en la biomasa y biodiversidad de zooplancton gelatinoso en aguas interiores de Chiloé, sur de Chile (primavera 2004 y 2005). Rev. Biol. Mar. Oceanogr., 44(2): 309-324.

Wedler, E. & R. Larson. 1986. Athecate hydroids from Puerto Rico and the Virgin Islands. Stud. Neotrop. Fauna E., 21: 69-101.

Xu, Z.Z. & J.Q. Huang. 2004. A survey on Anthomedusae (Hydrozoa: Hydroidomedusae) from the Taiwan Strait with description of new species and new combinations. Acta Oceanol. Sin., 23: 549-562.

Xu, Z.Z. & J.Q. Huang. 2006. On new genus, species and record of Laingiomedusae and Anthomedusae in Fujian coast (Cnidaria, Hydroidomedusae). J. Xiamen Univ. Nat. Sci., 45: 233-249.

Xu, Z.Z., J.Q. Huang & G. Liu. 2007a. On new species and records of Hydroidomedusae from the Changjiang River Estuary and its adjacent waters. Acta Oceanol. Sin., 26(1): 77-83.

Xu, Z.Z., J.Q. Huang & D. Guo. 2007b. A survey on Hydroidomedusae from the upwelling region of southern part of the Taiwan Strait of China I, On new species and records of Anthomedusae. Acta Oceanol. Sin., 26(5): 66-75.

Xu, Z.Z., J.Q. Huang, M. Lin & D. Guo. 2009. Study on genus Nubiella from the Taiwan Strait and its adjacent waters, China (Filifera, Bougainvilliidae). Acta Zoot. Sin., 34(1): 111-118

Zelickman, E.A. 1972. Distribution and ecology of the pelagic Hydromedusae, Siphonophores and Ctenophores of the Barents Sea, based on perennial plankton collection. Mar. Biol., 17: 256-264.

Table 1. Species described in the literature of the family Bougainvilliidae with life-cycle phase indicated and distribution.

Species	Phase	Distribution				
		Pacific	Atlantic	Indian	Arctic	Southern
Genus *Bimeria* Wright, 1859						
Bimeria australis Blackburn, 1937	P	x	x			
Bimeria belgicae (Vanhöffen, 1910)	P					x
Bimeria corynopsis Vanhöffen, 1910	P					x
Bimeria currumbinensis Pennycuik, 1959	P	x				
Bimeria fluminalis Annandale, 1915	P			x		
Bimeria pygmaea Fraser, 1938	P	x				
Bimeria rigida Warren, 1919	P			x		
Bimeria vestita Wright, 1859	P	x	x	x		
Genus *Bougainvillia* Lesson, 1830						
Bougainvillia aberrans Calder, 1993	P/M		x			
Bougainvillia aurantiaca Bouillon, 1980	M	x	x			
Bougainvillia bitentaculata Uchida, 1925	M	x		x		
Bougainvillia britannica (Forbes, 1841)	P/M	x	x			
Bougainvillia carolinensis (Mccrady, 1859)	P/M		x			
Bougainvillia chenyapingii Xu, Huang & Guo, 2007	M	x				
Bougainvillia crassa Fraser, 1938	P	x				
Bougainvillia dimorpha Schuchert, 1996	P/M	x				
Bougainvillia frondosa Mayer, 1900	M	x	x			
Bougainvillia fulva Agassiz & Mayer, 1899	M	x		x		
Bougainvillia inaequalis Fraser, 1944	P	x	x			
Bougainvillia involuta Uchida, 1947	M	x				
Bougainvillia lamellata Xu, Huang & Liu, 2007	M	x				
Bougainvillia longistyla Xu & Huang, 2004	M	x				
Bougainvillia macloviana Lesson, 1836	P/M	x	x	x		
Bougainvillia meinertiae Jäderholm, 1923	P			x		
Bougainvillia multitentaculata Förster, 1923	M	x				
Bougainvillia muscoides (M. Sars, 1846)	P/M	x	x	x		
Bougainvillia muscus (Allman, 1863)	P/M	x	x	x		
Bougainvillia nana Hartlaub, 1911	M		x			
Bougainvillia niobe Mayer, 1894	M	x	x			
Bougainvillia pagesi Nogueira et al., 2013	M		x			

Species	Phase	Distribution				
		Pacific	Atlantic	Indian	Arctic	Southern
Bougainvillia paraplatygaster Xu, Huang & Chen, 1991	M	x				
Bougainvillia platygaster (Haeckel, 1879)	M	x	x	x		
Bougainvillia principis (Steenstrup, 1850)	P/M	x	x		x	
Bougainvillia pyramidata (Forbes & Goodsir, 1853)	P/M	x	x			
Bougainvillia reticulata Xu & Huang, 2006	M	x				
Bougainvillia rugosa Clarke, 1882	P/M		x			
Bougainvillia superciliaris (L. Agassiz, 1849)	P/M	x	x		x	
Bougainvillia vervoorti Bouillon, 1995	P/M	x				
Genus *Chiarella* Maas, 1897						
Chiarella centripetalis Maas, 1897	M	x				
Genus *Dicoryne* Allman, 1859						
Dicoryne conferta (Alder, 1856)	P		x	x	x	
Dicoryne conybearei (Allman, 1864)	P	x	x			
Genus *Garveia* Wright, 1859						
Garveia annulata Nutting, 1901	P	x				
Garveia arborea (Browne, 1907)	P	x	x			
Garveia cerulea (Clarke, 1882)	P		x			
Garveia clevelandensis Pennycuik, 1959	P	x		x		
Garveia crassa (Stechow, 1923)	P			x		
Garveia franciscana (Torrey, 1902)	P	x	x	x		
Garveia gracilis (Clark, 1876)	P	x	x			
Garveia grisea (Motz-Kossowska, 1905)	P		x			
Garveia nutans Wright, 1859	P	x	x			
Genus *Koellikerina* Kramp, 1939						
Koellikerina bouilloni Kawamura & Kubota, 2005	M	x				
Koellikerina constricta (Menon, 1932)	M	x		x		
Koellikerina diforficulata (Xu & Zhang, 1978)	M	x				
Koellikerina elegans (Mayer, 1900)	M		x	x		
Koellikerina fasciculata (Péron & Lesueur, 1810)	P/M	x	x			
Koellikerina heteronemalis Xu, Huang & Chen, 1991	M	x				
Koellikerina maasi (Browne, 1910)	M	x	x	x		x
Koellikerina multicirrata (Kramp, 1928)	M	x		x		
Koellikerina octonemalis (Maas, 1905)	M	x		x		
Koellikerina ornata Kramp, 1959	M	x		x		
Koellikerina staurogaster Xu & Huang, 2004	M	x				

Species	Phase	Distribution				
		Pacific	Atlantic	Indian	Arctic	Southern
Koellikerina taiwanensis Xu, Huang & Chen, 1991	M	x				
Genus *Millardiana* Wedler & Larson, 1986						
Millardiana logitentaculata Wedler & Larson, 1986	P		x			
Genus *Nemopsis* Agassiz, 1849						
Nemopsis bachei L. Agassiz, 1849	P/M	x	x			
Nemopsis dofleini Maas, 1909	M	x				
Nemopsis hexacanalis Huang & Xu, 1994	M	x				
Genus *Nubiella* Bouillon, 1980						
Nubiella alvarinoae (Segura-Puertas, 1980)	M	x	x			
Nubiella atentaculata Xu & Huang, 2004	M	x				
Nubiella claviformis Xu, Huang & Lin, 2009	M	x				
Nubiella intergona Xu, Huang & Lin, 2009	M	x				
Nubiella macrogastera Xu, Huang & Lin, 2009	M	x				
Nubiella macrogona Xu, Huang & Guo, 2009	M	x				
Nubiella mitra Bouillon, 1980	M	x	x			
Nubiella oralospinella Xu, Huang & Guo, 2009	M	x				
Nubiella papillaris Xu, Huang & Guo, 2009	M	x				
Nubiella paramitra Xu, Huang & Guo, 2007	M	x				
Nubiella sinica Huang, Xu, Liu & Chen, 2009	M	x				
Nubiella tubularis Xu, Huang & Guo, 2007	M	x				
Genus *Pachycordyle* Weismann, 1883						
Pachycordyle kubotai Stepanjants, Timoshkin, Anokhin & Napara, 2000	P	x				
Pachycordyle mashikoi (Itô, 1952)	P	x				
Pachycordyle michaeli (Berrill, 1948)	P		x			
Pachycordyle napolitana Weismann, 1883	P		x			
Pachycordyle navis (Millard, 1959)	P		x	x		
Pachycordyle pusilla (Motz-Kossowska, 1905)	P		x			
Genus *Parawrightia* Warren, 1907						
Parawrightia robusta Warren, 1907	P		x	x		
Genus *Rhizorhagium* M. Sars, 1874						
Rhizorhagium antarcticum (Hickson & Gravely, 1907)	P	x				x
Rhizorhagium arenosum (Alder, 1862)	P	x	x			
Rhizorhagium formosum (Fewkes, 1889)	P	x				
Rhizorhagium palori Mammen, 1963	P			x		

Species	Phase	Distribution				
		Pacific	Atlantic	Indian	Arctic	Southern
Rhizorhagium roseum Sars, 1874	P	x	x		x	
Rhizorhagium sagamiense Hirohito, 1988	P	x				
Genus *Silhouetta* Millard & Bouillon, 1973						
Silhouetta uvacarpa Millard & Bouillon, 1973	P	x	x	x		
Genus *Thamnostoma* Haeckel, 1879						
Thamnostoma dibalium (Busch, 1851)	M		x			
Thamnostoma eilatensis Schmidt, 1972	M		x			
Thamnostoma macrostomum Haeckel, 1879	M			x		
Thamnostoma tetrellum (Haeckel, 1879)	M		x			
Genus *Velkovrhia* Matjasic & Sket, 1971						
Velkovrhia enigmatica Matjasic & Sket, 1971	P		x			

P: polyp, M: medusa

Table 2. List of valid species in the genera of Bougainvilliidae, 1809 to 2013.

Genus (phase)	Time interval (each column is since the year of the previous column)										
	1809-1830	1850	1870	1890	1910	1930	1950	1970	1990	2013	Total
Bimeria (P)			1		2	2	2	1			8
Bougainvillia (P/M)		5	3	2	3	4	3		1	9	30
Chiarella (M)					1						1
Dicoryne (P)			2								2
Garveia (P)			1	2	4	1		1			9
Koellikerina (P/M)	1				3	1	1	1	1	4	12
Millardiana (P)									1		1
Nemopsis (P/M)		1			1					1	3
Nubiella (M)									2	10	12
Pachycordyle (P)				1	1		1	2		1	6
Parawrightia (P)					1						1
Rhizorhagium (P)			1	2	1			1	1		6
Silhouetta (P)									1		1
Thamnostoma (M)			1	2					1		4
Velkovrhia (P)									1		1
Total	1	6	9	9	17	8	7	6	9	25	

M: medusa, P: polyp

Table 3. Modeled classification for distribution of the 25 valid species with >10 records.

Species	AUC	Most important variable	Life phase	Potential distribution from model	
				Latitudinal	Longitudinal
Bougainvillia platygaster	0.95	Phosphates	M	Equatorial	Oceanic
Bougainvillia niobe	0.99	Phosphates	M	Equatorial	Oceanic
Koellikerina multicirrata	0.9	Temperature	M	Equatorial	Coastal, Oceanic
Nubiella alvarinoae	0.93	Temperature	M	Equatorial	Coastal, Oceanic
Parawrightia robusta	0.98	Chlorophyll	P	Equatorial	Coastal
Bougainvillia fulva	0.93	Chlorophyll	M	Equatorial	Coastal, Oceanic
Bougainvillia muscus	0.97	Chlorophyll	M	Tropical, Subtropical	Coastal, Oceanic
Bougainvillia muscus	0.98	Chlorophyll	P	Tropical, Subtropical	Coastal, Oceanic
Nemopsis bachei	0.99	Chlorophyll	M	Tropical, Subtropical	Coastal
Bougainvillia principis	0.99	Chlorophyll	M	Subtropical, Subpolar	Coastal
Bougainvillia pyramidata	0.99	Chlorophyll	P	Subtropical, Subpolar	Coastal
Bougainvillia macloviana	0.98	Chlorophyll	M	Subtropical, Subpolar	Coastal, Oceanic
Bougainvillia carolinensis	0.95	Chlorophyll	M	Subtropical, Subpolar	Coastal, Oceanic
Bougainvillia carolinensis	0.98	Chlorophyll	P	Subtropical, Subpolar	Coastal
Bougainvillia muscoides	0.86	Chlorophyll	M	Subtropical, Polar	Coastal, Oceanic
Bougainvillia muscoides	0.99	Chlorophyll	P	Subtropical, Polar	Coastal, Oceanic
Bougainvillia britannica	0.91	Chlorophyll	M	Subtropical, Polar	Coastal, Oceanic
Bougainvillia superciliaris	0.98	Chlorophyll	M	Subtropical, Polar	Coastal
Bougainvillia superciliaris	0.93	Temperature	P	Subtropical, Polar	Coastal, Oceanic
Dicoryne conferta	0.99	Chlorophyll	P	Subtropical, Polar	Coastal
Dicoryne conybearei	0.97	Phosphates	P	Subtropical, Polar	Coastal, Oceanic
Garveia nutans	0.89	Chlorophyll	P	Subtropical, Polar	Coastal, Oceanic
Rhizorhagium roseum	0.97	Phosphates	P	Subtropical, Polar	Coastal, Oceanic
Bimeria vestita	0.97	Chlorophyll	P	Equatorial to Subpolar	Coastal, Oceanic
Bougainvillia rugosa	0.98	Chlorophyll	P	Equatorial to Subpolar	Coastal, Oceanic
Garveia franciscana	0.99	Chlorophyll	P	Equatorial to Subpolar	Coastal
Garveia gracilis	0.91	Chlorophyll	P	Equatorial to Subpolar	Coastal, Oceanic
Koellikerina fasciculata	0.82	salinidad	M	Equatorial to Subpolar	Coastal, Oceanic
Pachycordyle napolitana	0.96	Phosphates	P	Equatorial to Subpolar	Oceanic

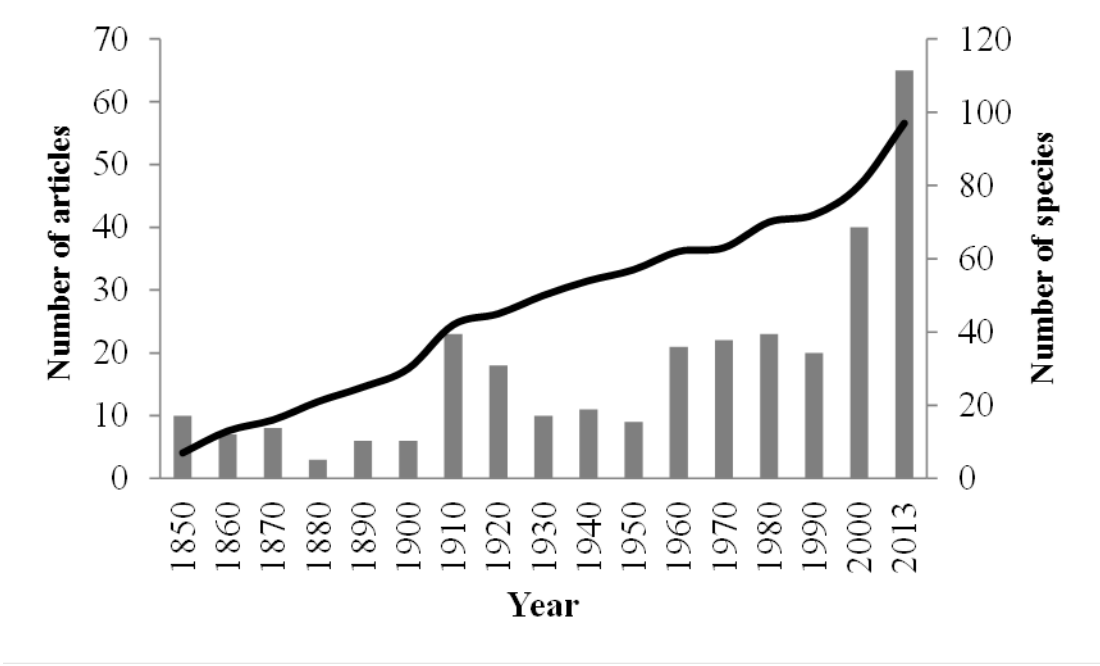

Figure 1. Cumulative number of species described since 1809 to date (2013) and number of studies that include the family Bougainvilliidae since 1809, by decade. The line indicates the cumulative number of species and the bars indicate of number the articles.

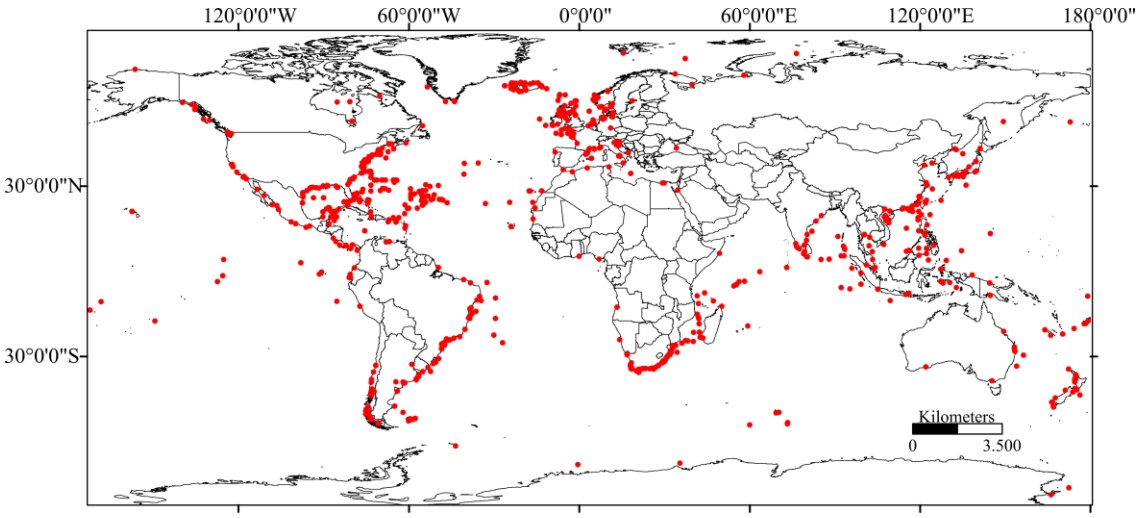

Figure 2. Current global distribution of all known valid species in the family Bougainvilliidae.

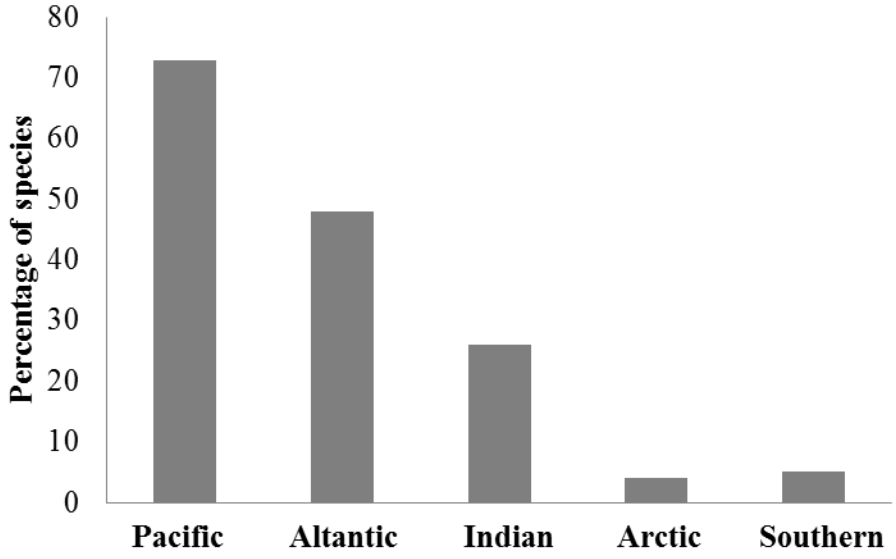

Figure 3. Percent of the total number of currently valid species in the family Bougainvilliidae found in each ocean. The total adds to greater than 100% because several species are found in more than one ocean.

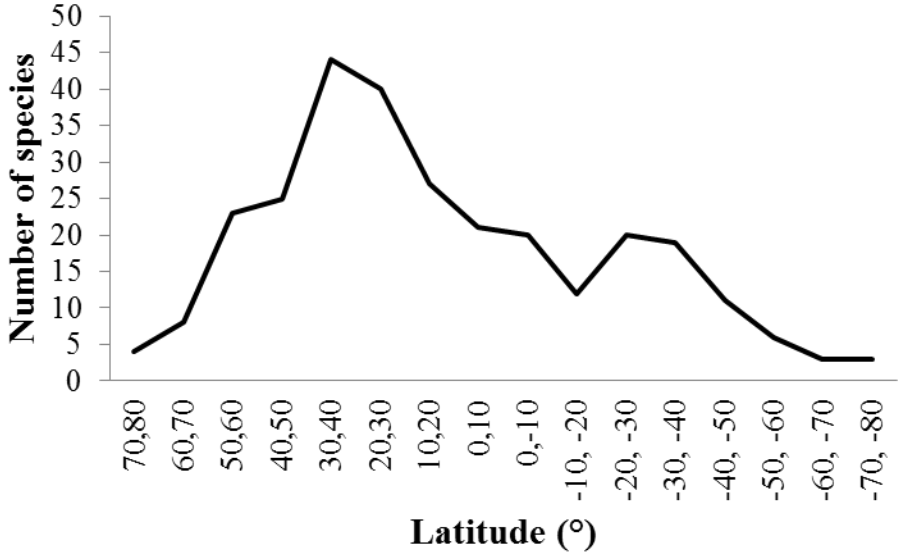

Figure 4. Frequency distribution over latitude of the number of currently valid species in the family Bougainvilliidae.

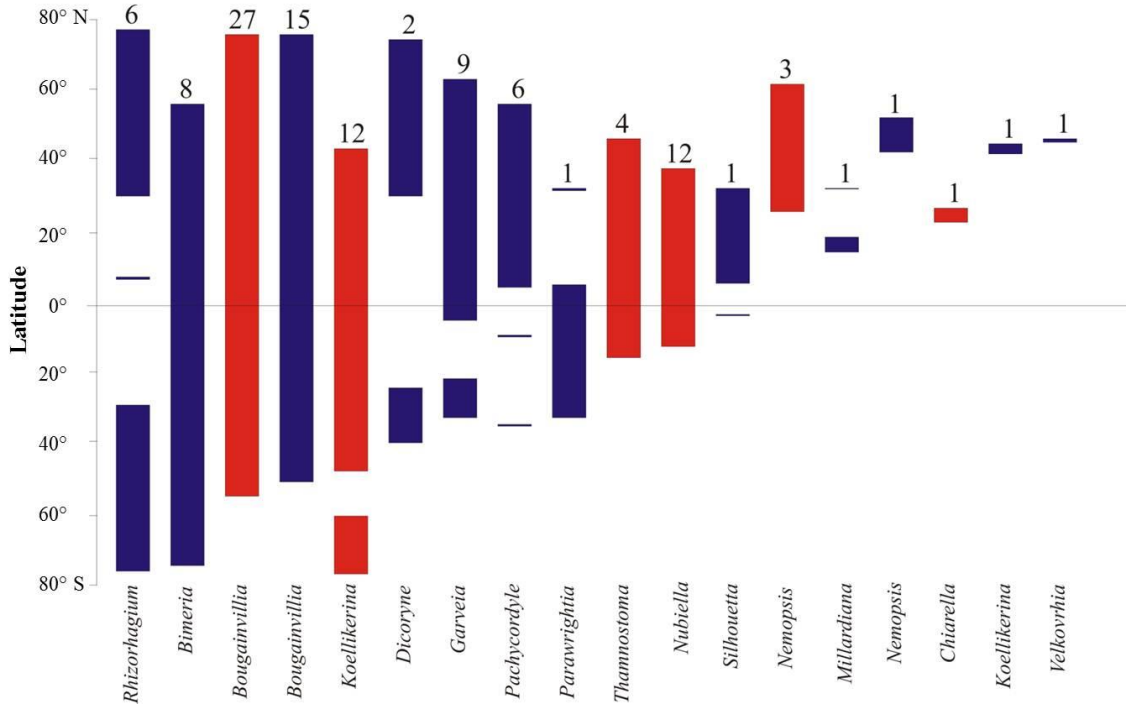

Figure 5. Latitudinal range for genera in the family Bougainvilliidae. ■ indicates genera in polyp phase, ■ indicates genera in medusa phase. The number above the bars is the number of valid species for each genus. Fragmented bars indicate that the latitudinal distribution is not continuous.

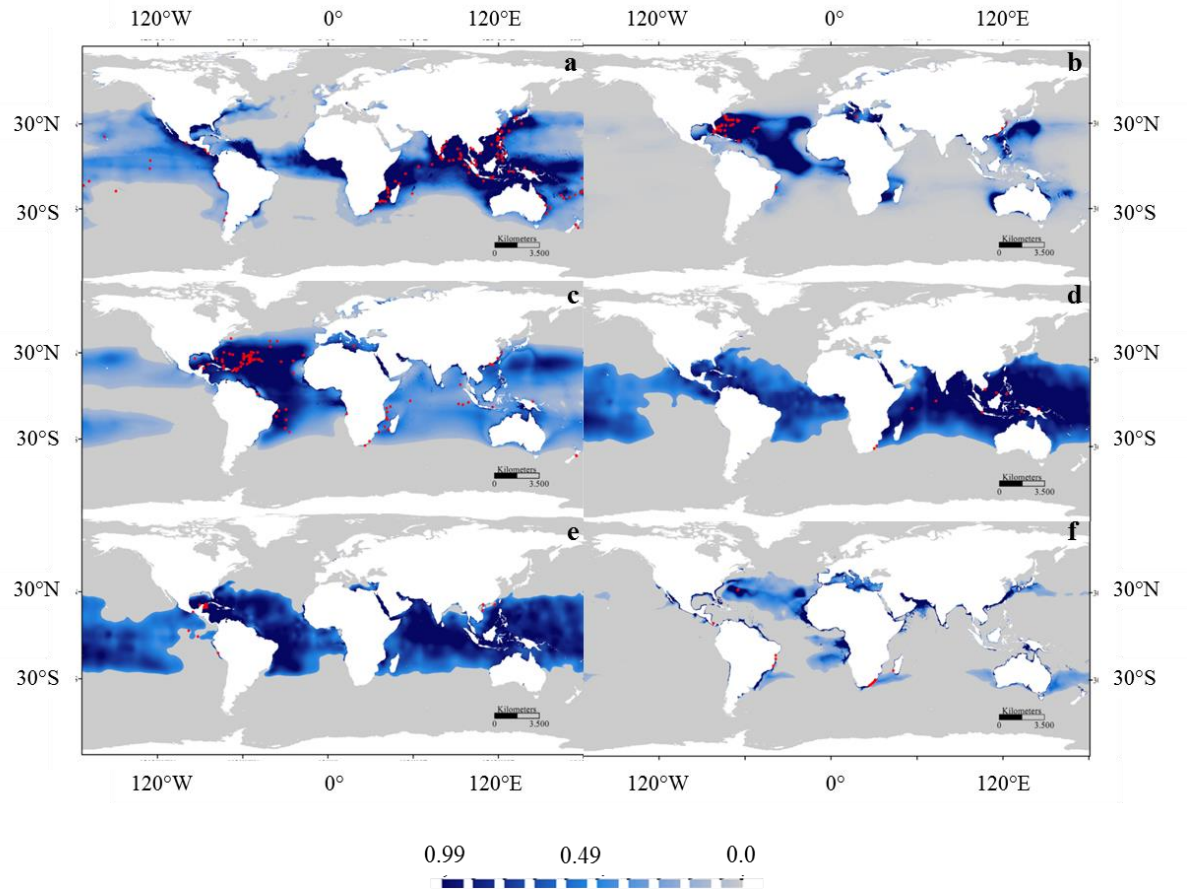

Figure 6. Potential distributions (as shades of blue indicating probabilities) from the model for equatorial-tropical species: a) *Bougainvillia fulva*-medusa, b) *Bougainvillia niobe*-medusa, c) *Bougainvillia platygaster*-medusa, d) *Nubiella alvarinoae*-medusa, e) *Koellikerina multicirrata*-medusa and f) *Parawrightia robusta*-polyp. Sampling locations are indicated by red dots.

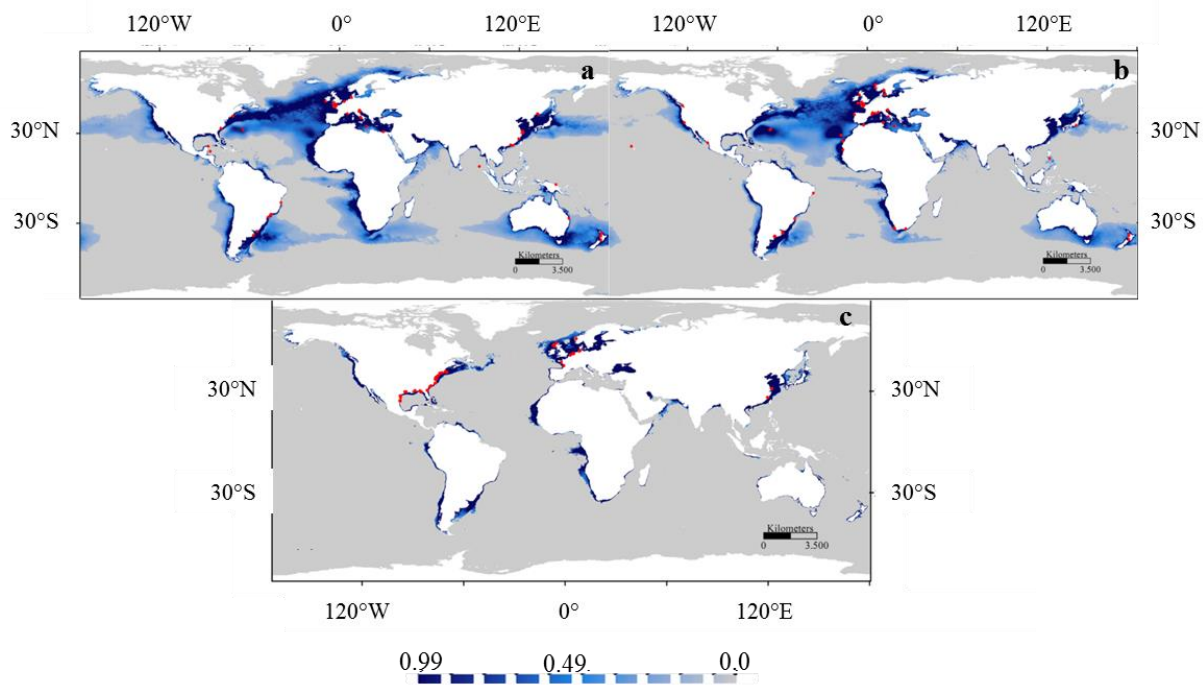

Figure 7. Potential distributions (as shades of blue indicating probabilities) from the model for tropical-subtropical species: a) *Bougainvillia muscus*-medusa, b) *Bougainvillia muscus*-polyp and c) *Nemopsis bachei*-medusa. Sampling locations are indicated by red dots.

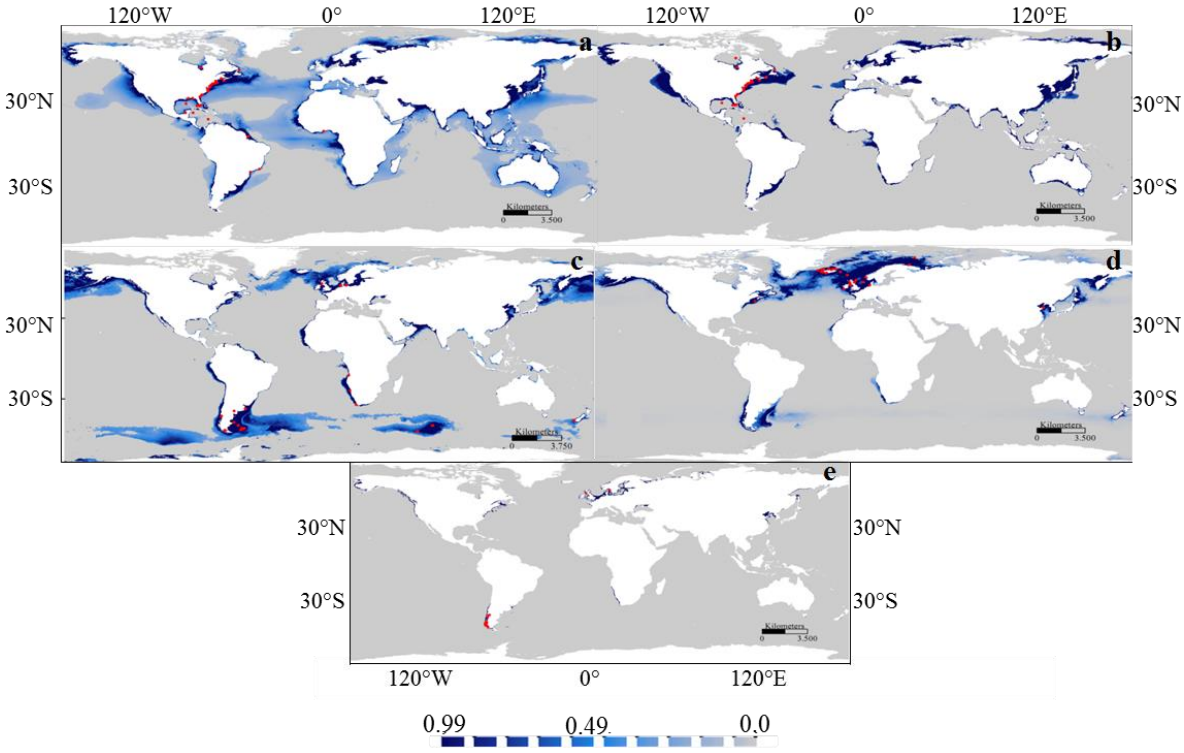

Figure 8. Potential distributions (as shades of blue indicating probabilities) from the model for subtropical-subpolar species: a) *Bougainvillia carolinensis*-medusa, b) *Bougainvillia carolinensis*-polyp, c) *Bougainvillia macloviana*-medusa, d) *Bougainvillia principis*-medusa and e) *Bougainvillia pyramidata*-polyp. Sampling locations are indicated by red dots.

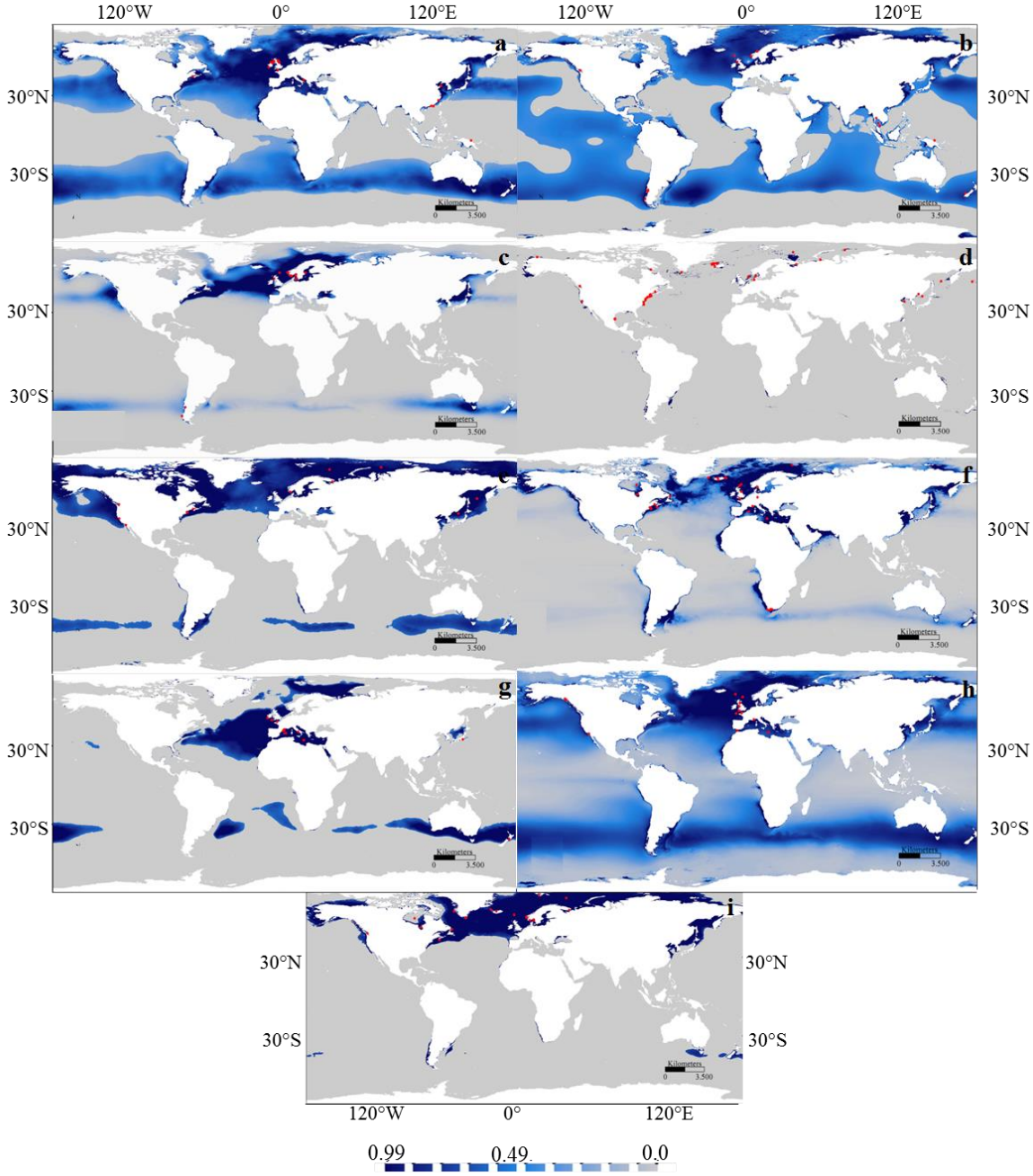

Figure 9. Potential distributions (as shades of blue indicating probabilities) from the model for subtropical-polar species: a) *Bougainvillia britannica*-medusa, b) *Bougainvillia muscoides*-medusa, c) *Bougainvillia muscoides*-polyp, d) *Bougainvillia superciliaris*-medusa, e) *Bougainvillia superciliaris*-polyp, f) *Dicoryne conferta*-polyp, g) *Dicoryne conybearei*-polyp, h) *Garveia nutans*-polyp and i) *Rhizorhagium roseum*-polyp. Sampling locations are indicated by red dots.

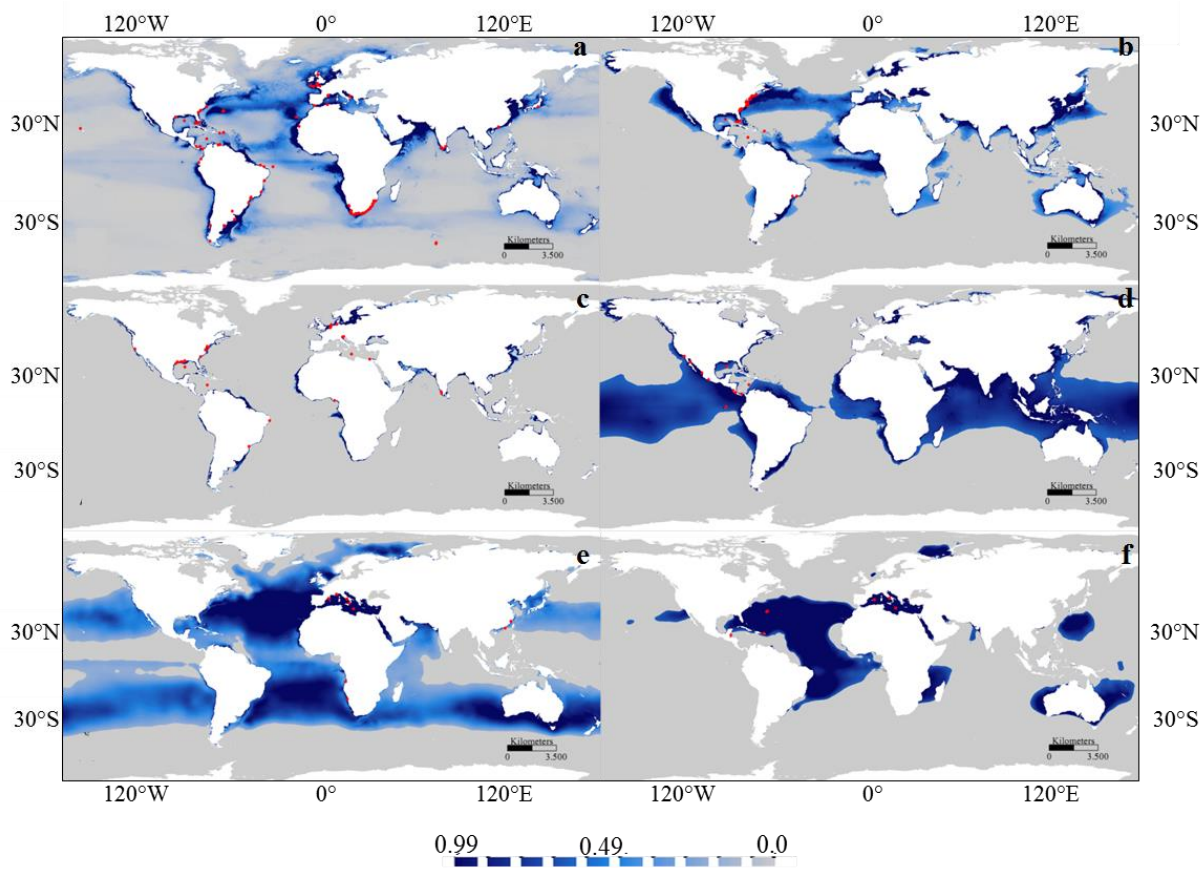

Figure 10. Potential distributions (as shades of blue indicating probabilities) from the model for widely distributed species: a) *Bimeria vestita*-polyp, b) *Bougainvillia rugosa*-polyp, c) *Garveia franciscana*-polyp, d) *Garveia gracilis*-polyp, e) *Koellikerina fasciculata*-medusa and f) *Pachycordyle napolitana*-polyp. Sampling locations are indicated by red dots.

Capítulo – 3

An evolutionary, comparative analysis of the medusozoan (Cnidaria) exoskeleton

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Resumo

A fase bentônica das espécies de Medusozoa (Staurozoa, Cubozoa, Scyphozoa e Hydrozoa) possui sistemas de suporte endoesqueléticos ou exoesqueléticos, mas grandes hiatos sobre o conhecimento de sua composição, desenvolvimento e evolução ainda existem. O objetivo deste estudo foi analisar e comparar as variações de síntese, estruturais e funcionais, e propor hipóteses para a origem e diversificação dos tipos exoesqueléticos nos diversos grupos medusozoários fósseis e atuais. Para tal, fizemos revisões sobre dados da literatura e adicionamos várias informações procedentes de análises histológicas e microestruturais de alguns grupos. A quitina é o componente característico no exoesqueleto dos Medusozoa, atuando como uma estrutura extracelular de suporte, proteção e reserva de íons e diversos tipos de moléculas orgânicas e inorgânicas, que possivelmente definem a fase orgânica do processo de biomineralização, resultando em exoesqueletos rígidos. A esqueletogênese dos Medusozoa retrocede ao Ediacarano, quando processos bióticos, abióticos e fisiológicos atuaram potencialmente como sinergéticos, derivando o padrão de enrijecimento do exoesqueleto. O exoesqueleto do tipo axial córneo predomina nos pólipos de medusozoários atuais, apresentando maior variação e complexidade estrutural nos pólipos de Hydroidolina, grupo para o qual descrevemos um novo tipo de exoesqueleto bicamada, onde o exossarco complementa o perisarco.

Palavras-haves: quitina, glicosaminoglicanos, Hydroidolina, perissarco, pseudo-hidroteca, filogenia

Abstract

The benthic, polyp, phase of Medusozoa (Staurozoa, Cubozoa, Scyphozoa and Hydrozoa) has endoskeletal or exoskeletal support systems, but their composition,

development, and evolution is poorly known. In this contribution the variation in synthesis, structure and function of the medusozoan exoskeleton were examined. In addition, an evolutionary hypothesis for its origin and diversification is proposed for both extinct and extant medusozoans. We also critically reviewed the literature and included data from our own histological and microstructural analyses of some groups. Chitin among the characteristic components of exoskeletons in Medusozoa, functioning as support, protection, and a reserve for various ions and inorganic and organic molecules, which may persuade biomineralization, resulting in rigid biomineralized exoskeletons. Skeletogenesis in Medusozoa dates back to the Ediacaran, when potentially synergetic biotic, abiotic, and physiological processes resulted in development of rigid structures that became the exoskeleton. Of the many types of exoskeletons that evolved, the corneous (chitin-protein) exoskeleton predominates today in polyps of medusozoans, with its greatest variation and complexity in the polyps of Hydroidolina. Here we describe a new type of bilayered exoskeleton in which there is an exosarc complementing the perisarc construction is here described.

Additional keywords: chitin, glycosaminoglycan, Hydroidolina, perisarc, phylogenetics, Pseudohydrotheca

Intriduction

Cnidarians are an early branch of diploblastic animals, which diverged from the shared ancestor of the Bilateria ~600 million years ago (Ryan *et al*., 2013), with some fossil records in the Ediacaran (Van Iten *et al*., 2013a, 2014; Liu *et al.*, 2014) and most groups already present in the Cambrian (Zhao & Bengtson, 1999; Hughes, Gunderson & Weedon, 2000; Cartwright *et al*., 2007). Cnidaria comprise two main clades: Anthozoa and Medusozoa (Ruggiero *et al*., 2015). Anthozoa is typically benthic and marine polyps, while Medusozoa have a greater diversity of forms and habits, including pelagic, free-swimming (usually medusae), benthic and sessile (usually polyps), all mostly marine, but with a few fresh-water species. Medusozoa encompasses the classes Staurozoa, Cubozoa, Scyphozoa, and Hydrozoa, whose phylogenetic relationships have been explored using morphology, life cycles, and nuclear and mitochondrial molecular markers (Marques & Collins, 2004; Collins *et al*., 2006; Van Iten *et al*., 2006, 2014).

A fundamental evolutionary feature of Cnidaria is the skeleton that may be present as an endoskeleton, exoskeleton, or hydrostatic skeleton. This is a consequence of the two epithelial layer *bauplan*. The internal gastrodermis delimits the gastrovascular cavity from the tentacles to the pedal disc and functions in absorption of nutrients as well as contraction. The external epidermis functions in protection from the environment and responds to external stimuli (Chapman, 1974). These two epithelial layers are separated by mesoglea, which is an extracellular matrix primarily containing collagen and that may or may not contain cells (Chapman, 1974; Tucker, Shibata & Blankenship, 2011).

The epidermis is fundamental because of the many cell types it presents, including epithelio-muscular, interstitial, glandular, nervous, and cnidae cells, as well as determining how the animals will interact with their aquatic environment (Mackie, 1984). The skeleton of Cnidaria is a key feature providing protection, ion storage, fixation to substrates, swimming, flexibility, floating/drifting/dispersal, as well as other aspects of cnidarian life (Garstang, 1946; Pyefinch & Downing, 1949; Chapman, 1968, 1974; Fields & Mackie, 1971; Blanquet, 1972; Hündgen, 1984; Tidball, 1984; Thomas & Edwards, 1991; Marques & Migotto, 2001; Fraune *et al*., 2010; Di Camillo *et al*., 2012).

Anthozoan skeletons are reasonably well-studied (e.g. Barnes, 1970; Fukuda *et al*., 2003; Ramos-Silva *et al*., 2013), while Medusozoa, with their different exoskeletons, are much less understood. Indeed, after studies of composition and development, a long hiatus ensued before additional study of the role of the exoskeleton in biology and evolution of the group, despite its basal position in the evolution of animals (Marques, Morandini & Migotto, 2003; Collins *et al*., 2006). Due to this gap in our knowledge, our goals here are the detailed analyzes of Medusozoa skeleton highlighting the variation in origin, structure, function, and how disparity in these features have accompanied the evolution and diversification of this group. To achieve these goals, we bring together the literature with published and unpublished data from fossil cnidarians as well as current histological information from extant groups of Medusozoa.

The Metazoan exoskeleton - synthesis

Macromolecule evolution resulted in the development of extracellular structures with many functions, such as support, osmoregulation, defense, biofilms, cell and tissue morphogenesis and so on (Sentandreu, Mormeneo & Ruiz-Herrera, 1994; Ruiz-Herrera &

Ortiz-Castellanos, 2010). The most significant structural macromolecules in multicellular organisms are carbohydrates of the group of polysaccharides as cellulose in plants (Richmond & Somerville, 2000), chitin in fungi and animals, and the protein collagen, which is important for internal support in the Metazoa (Ehrlich, 2010a).

Chitin often makes up a significant fraction of structural support. For example, chitin accounts for 10-30% of the total skeletal components in some hydrozoans and 3-15% in bryozoans (Jeuniaux & Voss-Foucart, 1991; Kaya *et al*., 2015). Chitin is a polymer with repeating units of N-acetyl-D-glycosamine (NAG; Muzzarelli & Muzzarelli, 2009), usually with a visible fibrous organization at different hierarchical levels (nanofibrils, microfibrils or fibers; Ehrlich *et al*., 2010), and in three alternative forms: anti-parallel α (the most common), parallel β, and alternate γ (Pillai, Paul & Sharma, 2009). Biosynthesis of chitin includes synthesis and degradation catalyzed by enzymes found in all living organisms (Ruiz-Herrera, González-Prieto & Ruiz-Medrano, 2002; Merzendorfer & Zimoch, 2003; Tang *et al.*, 2015). These expression, processes, and function of chitin have been most studied in fungi and arthropods (e.g. Ruiz-Herrera & Ortiz-Castellanos, 2010; Merzendorfer, 2011; Souza *et al*., 2011).

Chitin synthetase (Chs) are the most important enzymes that form chitin, and their genes are found in several Medusozoa (e.g. *Hydractinia echinata* (Fleming, 1828), Mali *et al*., 2004; *Hydra vulgaris* Pallas, 1766, GenBank database, Table 1) and are shared between the Choanoflagellata and some Metazoa (Porifera, Anthozoa, Deuterostomia; Zakrzewski *et al*., 2014). Additionally, other genes involved in the biosynthesis of chitin in other groups of metazoans (e.g. Arthropoda; Merzendorfer & Zimoch, 2003) are also found in Medusozoa (Table 1). These genes suggest that the basic components are conserved and functional since a particular moment in animal evolution. Yet, the Chs genes have not been found in the genomes of non-chitinous organisms (Willmer, 1990; Wagner, 1994), like *Trichoplax adhaerens* Schulze, 1883 (Placozoa; Dellaporta *et al*., 2006; Signorovitch, Buss & Dellaporta, 2007) nor *Mnemiopsis leidyi* A. Agassiz, 1865 (Ctenophora; Table 1; Ryan *et al*., 2013; Bolte *et al*., 2014).

Chitinases (family 18 of glycosyl hydrolases) are the most important enzymes that degrade chitin and are functional at different life stages in different organisms (Dahiya, 2009). Chitinase functions are typically associated with organism growth and immunity (in organisms with chitin), and in digestion and immunity (in organisms without chitin; Mali *et al*., 2004). In the Metazoa, chitinase genes are present and variable in several lineages (Table

1), although chitinase evolution and function are still poorly known, especially in organisms that do not produce chitin (e.g. the hydrozoan *Hydra vulgaris*; Mali *et al*., 2004).

Chitin and alternative chitin-like molecules (e.g. chitooligosaccharides) are recorded in a few prokaryotes, some protists and algae (Gooday, 1990; Cohen, 2010), and in several lineages of Opisthokonta (Fungi + Metazoa + some unicellular lineages; Paps *et al*., 2013). Chitin is found in at least 19 phyla of the Metazoa (Willmer, 1990) and is common in Cnidaria (Anthozoa and Medusozoa; Table 1). Phylogenetic and developmental evidence show a relationship between animal and fungal chitin systems and they share some Chs (Wagner, 1993; Ruiz-Herrera *et al*., 2002).

Chitin is not merely a neutral extracellular structural component, but rather can interact with a variety of inorganic and organic molecules (polysaccharides, lipids, pigments, non-collagen chitin-binding protein, minerals (magnesium carbonate) and chemical compound (calcium carbonate); Shen & Jacobs-Lorena, 1999; Ehrlich *et al*., 2010). This interaction forms a structural backbone that defines the organic phase in extracellular biomineralization, acting as a mold, nucleation niche, and orientation modifier for crystalline and amorphous minerals, thereby forming a rigid exoskeleton that served as defense against chitinases and as an important reserve for ions or chemical compounds (cf. Ehrlich, 2010b). Silica (e.g. Porifera, Crustacea, Copepoda, Mollusca Docoglossa), and calcium carbonates (e.g. Porifera Calcarea, Cnidaria Anthozoa, some Hydrozoa, Bryozoa, Arthropoda Crustacea, Mollusca and Brachiopoda) are among the most characteristic compound and element that participate in biomineralization of metazoan exoskeletons (Ehrlich, 2010b).

In addition to chitin, cnidarian skeletons have calcium carbonate in the crystalline forms of aragonite and calcite, silicates, magnesium hydroxides, other chemical compounds, and calcium phosphate minerals in lower concentrations (Table 2; Milliman, 1974). Also, glycosaminoglycans (GAGs) in the form of chondroitin sulfate and heparin sulfate can be found as elements in hydrozoan exoskeletons (Yamada *et al*., 2007; Böttger *et al*., 2012). Cnidarians are the basal animal branch with GAGs, which have been conserved in other animal groups (Medeiros *et al*., 2000; Yamada, Sugahara & Özbek, 2011).

The exoskeleton in Medusozoa is derived from the ectoderm, which secretes the macromolecules (e.g. structural proteins and enzymes, phenols, polysaccharides) that combine to form rigid exoskeletons (Fig. 1; Knight, 1970; Kossevitch, Herrmann & Berking, 2001). Nevertheless, there are still relatively few studies of the composition and the

concentration of the macromolecular components of the cells and skeletons of Medusozoa (Hwang *et al*., 2013).

Origin and evolution of animal exoskeletons - hypotheses

It is well-known that oxygen and mineral (phosphates, carbonates, silicates, among others) concentrations have varied over geological time in the water column (Cook & Shergold, 1986; Brasier, 1992; Lenton & Watson, 2004; Papineau, 2010; Wood, 2011; Och & Shields-Zhou, 2011; Sperling *et al.*, 2013; Lenton *et al*., 2014). The causes favoring the development of an exoskeleton are poorly understood, but they seem to be associated with time intervals during which concentrations of these nutrients were greater in Proterozoic marine waters. These chemical changes in the oceans are related with the increase in animal biomass and biomineralized skeletons (Brasier, 1992; Cook, 1992; Erwin & Tweedt, 2012; Wood & Zhuravlev, 2012; Kazmierczak, Kempe & Kremer, 2013; Wood *et al.*, 2015).

At the end of the Ediacaran, oceans had greater concentrations of salt (NaCl), a neutral pH and other ions more similar to Phanerozoic seas. During this time, Ca_2^+ concentrations reached ~36 meq l-1 (Hardie, 2003). These conditions, together with possible increases in predation risk and general species diversification (Warren *et al*., 2012), resulted in the appearance of skeletons and exploration of new habitats (such as shallow waters) by a variety of taxa, causing increased trophic web complexity (Stanley, 1973; Conway Morris & Robison, 1986; Grant, 1990; Bengtson, 1994; Grotzinger, Watters & Knoll, 2000; Wood, Grotzinger & Dickson, 2002; Bambach, Bush & Erwin, 2007; Wood, 2011; Penny *et al*., 2014). Thus, the different skeleton types found in distinct lineages would have arisen through homoplasy, even with phylogenetic conservation of some molecular pathways. Hence, through deep homology in the Opisthokonta (Scotland, 2010), there may have been an ancestral condition in the genetic components of chitin production that was followed by different evolutionary pathways taken by the various taxa that resulted in the current variety of exoskeleton types.

Therefore, we may consider various hypotheses to explain the origin and evolution of the exoskeleton, based on a trade-off between survival (the cost of the exoskeleton as protection) and the cost of reproduction. This is suggested by the synchronous appearance of exoskeletons and the infauna in the fossil record (Dzik, 2007). Hence, the origin of the exoskeleton would be associated with biotic (i.e. predation; Warren *et al*., 2012), abiotic (mechanical/chemical changes in the environment; Brasier, 1992; Cook, 1992; Cohen, 2005),

and physiological changes as a consequence of evolution (Vermeij, 1989; Knoll, 2003; Dzik, 2007, Wood & Zhuravlev, 2012).

Biotic, abiotic, and physiological processes must be considered in synergy, the result of which was an increasing rigidity and biomineralization of the exoskeleton. In sessile phases of life in organisms, this development would have to maintain a degree of flexibility and so would require a lower energetic cost than that in vagile organisms (Warren *et al*., 2012). Also, exoskeletons would have originated *de novo* in the infauna (Dzik, 2007). Regardless of how it originated, once present, the exoskeleton would have resulted in a restructuring of the interactions among organisms, especially with respect to predation (Dzik, 2007; Penny *et al*., 2014), possibly involved with the loss of the domination by the algal mats (stromatolites) that were typical of the Ediacaran oceans (cf. Pratt, 1982; Warren *et al*., 2013).

The Verongida sponges of the Middle Cambrian were the first animals with chitin (Ehrlich *et al*., 2010, 2013). However, skeletogenesis would have begun by the Neoproterozoic, with the record of possible spicules of "parazoan" ancestors (Brain *et al.*, 2012; Wallace *et al*., 2014), continuing in the early Cambrian (Stage 2, Tommotian, 521 Mya), with the appearance in the fossil record of the Small Shelly Fauna (SSF), in which rigid bodies are present in Archaeocyatha (Antcliffe, Callow & Brasier, 2014), exoskeletal structures are found in the fossil *Coronacollina acula* Clites *et al*., 2012 and in the spicules in the Cambrian sponge in the genus *Choia* (Clites, Droser & Gehling, 2012). The chemical structure of these fossil spicules is unknown, but may have been chitin and silica, or calcium carbonate, and their radial organization in the body suggests a support, rather than protective, function (Clites *et al*., 2012). The presence of *C. acula* also demonstrates that biomineralization did not have an abrupt beginning in the Cambrian (cf. Vermeij, 1989), but rather diversification of animals with biomineralized exoskeletons occurred during the last evolutionary phase of Ediacara Biota (~543 Myr) (Xiao & Laflamme, 2008).

The exoskeleton in Medusozoa

Fossil records

The exoskeleton in Cnidaria occurred at least since the Ediacaran (~635-551 Myr) (Liu *et al.*, 2008; Xiao, Yuan & Knoll, 2010; Leme *et al*., 2013; Van Iten *et al*., 2013a; Pacheco *et al.*, 2015), concomitant with the radiation of other animal groups also capable of building exoskeletons (Xiao & Laflamme, 2008) or support systems based on aggregated mineral particles (Serezhnikova, 2014), which then continued taxonomically and geologically during

the Cambrian (Vermeij, 1989; Van Iten *et al*., 2006, 2014). The oldest exoskeletal fossils of metazoans already documented include the conical calcitic shells of *Cloudina*, a problematic genus that is now considered a cnidarian (Vinn & Zatón, 2012); the tubular annulated and assigned as chitinous scyphozoan polyps of *Olivooides* (Zhao & Bengtson, 1999; Dong *et al*., 2013; Yasui *et al*., 2013); the late Ediacaran chitin-mineralized fossil *Corumbella* (Pacheco *et al*., 2015); and the possible mineralized phosphate type of Conulatae scyphozoans (Leme *et al*., 2013; Van Iten *et al*., 2013a). Phosphate Conulatae exoskeleton is proposed as a synapomorphy of the Conulatae (Leme *et al*., 2008a) and is homologous with the sister group Coronatae, in which the exoskeleton is not mineralized (Werner, 1966, 1967; Leme *et al*., 2008a, b; Leme, Simões & Van Iten, 2010).

Initial discussion of the composition and microstructure of the exoskeleton (= theca, in the literature) of the Conulatae proposed that the exoskeleton present ribs covered by integument (Babcock & Feldmann, 1986). The ribs would have been solid, narrow, long and subcircular in cross-section and the integument fine and flexible, formed by several lamellae of calcium phosphate and protein (Fig. 2A; Table 3). In the exoskeleton were semidiscontinuous thickenings (nodes) and small projections (= spines, in the literature; Babcock & Feldmann, 1986; Fig. 2A). However, upon examination of cross-sections of the conularian exoskeleton with scanning electron microscopy, the exoskeleton was shown to be continuous, of individual lamella of calcium phosphate (apatite), thicker in some regions (Van Iten, 1992a). Thicker regions were structural supports, externally as ribs, nodes, and spines, and internally as septa and carina (Van Iten, 1992a). Detailed microstructure of the exoskeleton, showing pores in the lamellae, can be found in Van Iten *et al*. (2005b).

The affinity of the Conulatae with Coronatae is supported by exoskeleton construction and growth, with the centripetal increase in the lamellae, external ornamentation (longitudinal and transverse corrugations), repair by apical wall formation, internal perradial and interradial, with carina and septa in the conulariids (Van Iten, 1991; 1992a, b; Van Iten, Fitzke & Cox, 1996; Jerre, 1994; Hughes *et al*., 2000; Van Iten *et al*., 2006, 2014; Leme *et al*., 2008a, b, 2010). In addition to the Conulatae, Corumbellata and *Cloudina*, several other groups of Cnidaria are found only at the end of the Ediacaran (Hahn *et al*., 1982; Grotzinger *et al*., 1995; Amthor *et al*., 2003; Knoll *et al*., 2006; Warren *et al*., 2012, 2013; Pacheco *et al*., 2015). *Cloudina* is cosmopolitan and found on rocks that are younger than 555 Myr (Amthor *et al*., 2003). Its exoskeleton was formed by a layer of calcium carbonate (Grant, 1990; Hua *et al*., 2005) that, in some cases, has vertical perforations that have been suggested to be caused

by predation, thus indicating predator prey dynamics established by the end of the Ediacaran (Bengtson & Zhao, 1992; Hua, Pratt & Zhang, 2003).

The scyphozoan fossil *Corumbella werneri* (Hahn *et al*., 1982), from the Ediacaran in the USA, Brazil, and Paraguay (Hagadorn & Waggoner, 2000; Warren *et al*., 2012, 2013; Pacheco *et al*., 2015), is also among the first metazoans with, possibly biomineralized, exoskeleton synthesis (Pacheco, Leme & Machado, 2011; Warren *et al*., 2012; Pacheco *et al*., 2015). Its ultrastructure (and that of the Ordovician scyphozoan *Sphenothallus* – Van Iten *et al*., 2005b) differs from the chitin-protein complex of the exoskeleton (= tegument in the literature) of Cambrian scyphozoans, such as *Byronia robusta* Matthew, 1899 (Mierzejewska & Mierzejewski, 1979; Mierzejewski, 1986), due to its exoskeleton (= carapace in the literature) formed by polygonal plates (of unknown organic composition) as microlamellae with pores and papillae (Fig. 2B; Table 3), as described for conulariids with chitin-mineralized exoskeletons (Van Iten *et al*., 2005a, b; Warren *et al*., 2012, Pacheco *et al*., 2015). This morphology permits flexibility of the exoskeleton of *C. werneri*, allowing some deformation, but which may break, demonstrating less elasticity than that found in extant Coronatae (Chapman & Werner, 1972) and in the fossil scyphozoan *B. robusta* (Mierzejewska & Mierzejewski, 1979), which is functionally similar to modern arthropod exoskeletons (Pacheco *et al*., 2015).

Fossil records of exoskeleton of the chitin-protein type of Leptothecata of the Ordovician *Sinobryon elongatum* Balinski *et al.* 2014 (Balinski, Sun & Dzik, 2014), or the biomineralized carbonate exoskeletons of anthozoan corals of the Cambrian (Stanley & Fautin, 2001), and Hydrozoa Milleporidae (~150 Myr, Jablonski, 2005), Stylasteridae (~65 Myr, Lindner, Cairns & Cunningham, 2008) and Hydractiniidae (~50 Myr, Miglietta, McNally & Cunningham, 2010) are conserved among living groups.

Living groups: major trends and a new exoskeleton type in Hydrozoa

Exoskeletal composition is similar in all Cnidaria and is predominantly chitin-protein, and proteins associated with quinones or calcium carbonate (Knight, 1970; Chapman, 1974). However, some exceptions exist, such as the prevalence of collagen in gorgonian anthozoans (Tidball, 1984).

Siebold (1874) defined three types of skeleton in Cnidaria, despite some structural variation: corneous, calcareous, and coriaceous. Corneous types occur in several groups of anthozoans (Pennatulacea, Antipatharia; Siebold, 1874), hydrozoans and some scyphozoans.

The corneous exoskeleton with a composition of chitin-protein predominates in medusozoan polyps (Fig. 3; Table 3). Calcareous types, with sclerites that fit tightly together, forming a rigid structure, are typical of octocorals (Siebold, 1874; Grillo, Goldberg & Allemand, 1993). Coriaceous types are formed from biomineralization of calcium carbonate and are typical of some anthozoans (stony and blue corals) and hydrozoans (hydrocorals; Siebold, 1874).

Staurozoa has some indications of an exoskeleton (= periderm, in the literature) of uncertain chemical structure, at the base of the body during the larval (planula) and stauromedusa stages. Planula larvae of the genus *Haliclystus* secrete substances that cover them as they move, perhaps associated with adhesion to the substrate, but also likely serving as the substrate itself (Wietrzykowski, 1910, 1912; Otto, 1976). During *Haliclystus* planula settlement, the cells in the base of the larva apparently secrete a chitinous layer, covering the lower half of the larva (Wietrzykowski, 1912). After settling, the larva is surrounded by an amorphous sheath, and plaques of hexagonally packed subunits can enclose the planula (Otto, 1978). These plaques are also visible in epidermal cell cytoplasmic vesicles, where they are likely being formed and then transported to the exterior, and are apparently distinct from other extracellular enveloping described for Cnidaria, probably associated with an overwintering phase (Otto, 1978). Settled larvae of *Haliclystus antarcticus* Pfeffer, 1889 ("microhydrula" stage; Jarms & Tiemann, 1996; Miranda, Collins & Marques, 2010) have a thin exoskeleton produced by the cells of the basal epidermis, forming a circular disc but never a cup (Jarms & Tiemann, 1996). Besides, planulae of *Lucernariopsis campanulata* (Lamouroux, 1815) secrete a gelatinous substance that can encyst the larva (Kowalevsky, 1884; Hanaoka, 1934), forming a resting larval stage (Otto, 1978; Miranda, Morandini & Marques, 2012). Stauropolyps have not yet been found with an exoskeleton.

The basal disc in the stauromedusa *Haliclystus* is covered by a filamentous and adhesive layer (Fig. 3A; Otto, 1978; Miranda, Collins & Marques, 2013). Stauromedusae of *Haliclystus* have four kinds of basal epidermal cells: support, adhesive secretory, mucous secretory, and cnidoblasts (Singla, 1976). Support cells have contractile elements and secretory vesicles, similar to the glandulomuscular cells of *Hydra* (Singla, 1976). These cells, on the other hand, are morphologically and structurally similar to desmocytes of *Aurelia*, whose function is usually the anchoring of tissues to the exoskeleton (Singla, 1976; Lesh-Laurie & Suchy, 1991). Apparently, secretions of adhesive cells, supportive cells and mucous cells form an extracellular layer (~60-100 μm) in the basal epidermis of *Haliclystus* (Fig. 3A) (Singla, 1976; Lesh-Laurie & Suchy, 1991). Even though this layer appears homogenous,

fibril components can be found at fixation points, which are probably formed by polymerization of the adhesive secretions and mucous from the epidermal cells (Singla, 1976; Lesh-Laurie & Suchy, 1991). In addition, a continuous and individualized chitinous layer has also been reported for the stauromedusa stage of *Haliclystus* between the basal disc and the substrate (Fig. 3A; Fig. 1, Migot 1922a), which would be responsible for the fixation of the animal to the substrate (Migot, 1922a) In other parts of the body of a stauromedusa, there is only a thin mucous covering (Migot, 1922a, b). However, the presence of chitin at the pedal disc was not confirmed in subsequent studies (Singla, 1976; Miranda, Collins & Marques, 2013), and understanding the links between the chitinous layer and the different secretions, in the different stages of development, requires further study.

In Scyphozoa, polyps (scyphistomae) have an exoskeleton (= periderm in the literature) with one or more layers of chitin without becoming rigid as in other medusozoans (Lesh-Laurie & Suchy, 1991). The exoskeleton in the Coronatae completely covers the polyp body (Jarms, 1991) and is formed by an internal, thick (~38 µm), wrinkled and fibrous at the base, chitin-protein layer that becomes thinner (~4 µm) and uniform towards the top. Additionally, there is an external thin and continuous GAG layer (pers. obs.; Fig. 3B). The Discomedusae ("Semaeostomeae" + Rhizostomeae) have a reduced chitinous exoskeleton at the basal portion of the polyp or, rarely, in the form of resistant structures called podocysts (Chapman, 1966; Chapman & Werner, 1972). We observed and confirmed the presence of an exoskeleton (~4 µm thick) in the podocysts of *Chrysaora fuscescens* Brandt, 1835 (Fig. 3C).

Polyps in the Cubozoa, based on the scarce information available, are described as having an exoskeleton (= periderm, in the literature) of two layers (~5 µm each) that are restricted to the base (Fig. 3D; Table 3; Chapman, 1978). Cysts may also occur in the planulae (Toshino *et al*., 2013) and around degenerate polyps (Carrette, Straehler-Pohl & Seymour, 2014). Our histological analysis of the polyp of *Carybdea* sp. found the two-layer exoskeleton of chitin and proteins (each ~12 µm thick). The first layer is in contact with the epidermis and the second in contact with the environment, the second layer is covered by a mucous membrane (when reared in the laboratory). We found that polyps of *Carybdea* sp. also have fibrous anchoring structures that join the mesoglea with the homogenous layer of the skeleton, similar to the desmocytes in scyphozoans and leptothecate hydrozoans (cf. Chapman, 1969; Knight, 1970; Lesh-Laurie & Suchy, 1991).

Hydrozoa has the greatest exoskeleton variability and structural complexity, especially in Hydroidolina (Fig. 3E-I). In Leptothecata, the homogeneous chitin-proteic exoskeleton (=

perisarc in the literature) covers the colony from the hydrorhiza to the hydranth. The exoskeleton forms a hydrotheca around of the hydranth and a gonotheca around of gonozooids, and both exoskeletal structures represent a synapomorphy of the group (Fig. 3E; Table 3; Marques, 2001; Marques & Collins, 2004; Van Iten *et al*., 2006). Rigidity and hardening of the exoskeleton is a result of a reaction of the enzyme phenoloxidase with a dopamine substrate that is secreted by epidermal cells (tanning cells) and liberated in spherules in the extracellular matrix. There they react, forming a quinone that, in turn, forms strong connections when in contact with the proteins of the matrix (Knight, 1970). This process of secretion is greater in growth regions where the exoskeleton remains elastic and extendible (Knight, 1970).

In the order Siphonophora, the chitinous component (= pneumatocyst, in the literature) is reduced to an internal covering of the pneumatophore, also formed by lipids (Mackie, 1960). In "Anthoathecata", (a non-monophyletic group, cf. Marques & Collins 2004; Cartwright *et al*., 2008; Van Iten *et al*., 2014) it is generally assumed that the exoskeleton (= perisarc, in the literature) only covers to the base or pedicel of the hydranth (Tidball, 1984; Fig. 3F), with some exceptions. In the pelagic Porpitidae, the exoskeleton is reduced to an internal layer of the basal disc of the float chamber (Garstang, 1946; Chapman, 1974), and is not strictly an exoskeleton in the same way as in Siphonophora (Garstang, 1946; Fields & Mackie, 1971). In the suborder Aplanulata, the fibrous exoskeleton (= cuticle, in the literature) of *Hydra* has GAGs and putative peroxidase proteins (exclusive to this group) (Yamada *et al*., 2007). Structurally, the exoskeleton is five-layered (1.5 µm thick), covering from the base of the polyp to the hydranth, except for the tentacles (Fig. 3G; Table 3; Böttger *et al*., 2012). In Solanderiidae, the exoskeleton is an internal, rigid, network formed by vertical and horizontal chitin fibers, surrounding the central tissues (= coenosarc) with which the endoskeleton is in contact (Wineera, 1968). Our observations in Bougainvilliidae and Eudendriidae revealed a chitin-protein exoskeleton (Table 3), usually laminated and vertically striated (1-11 µm thick), from the hydrorhiza to the peduncle of the hydranth. Some Bougainvilliidae may be thinly covered (~1 µm thickness and not striated) to the whorl of tentacles (classically called pseudohydrotheca). In general, the exoskeleton in the base of the hydrocaulus and branches may be ringed or irregularly wrinkled along the entire colony, such as in the genus *Pachycordyle* (Stepanjants *et al*., 2000).

In some "Anthoathecata", the exoskeleton may be reinforced by the process of biomineralization (mineral deposition; Le Tissier, 1991), such as in the families Milleporidae,

Stylasteridae and Hydractiniidae (Cairns & Macintyre, 1992; Lindner *et al*., 2008, Miglietta *et al*., 2010). Biologically, secretions (e.g. of glycoproteins) from epidermal cells (= calycoblasts) constitute the extracellular matrix that modulates ion ingression to form spheres of aragonite or calcite that, once joined, make a firm, rigid, skeletal structure that is somewhat more fibrous and porous in Milleporidae (Fig. 3H; Table 3; Sorauf, 1980; Lewis, 2006).

Biomineralization is not the only way to reinforce the exoskeleton, and in other groups, such as Bougainvilliidae, there is a covering of GAGs (pers. obs.) with incrustations of inorganic (e.g. small sand grains) or organic (e.g. diatoms) or both particles. We propose that this type of covering should be called the exosarc (Table 3, Fig. 3I). The exosarc is the most external layer, radial in relation to the chitin-proteic layer (= perisarc) of the exoskeleton, which may vary in extent and thickness (3.9-132.5 µm). The exosarc may cover all colonial structures, including those not covered by a chitin-protein layer. For example, *Bougainvillia rugosa* Clarke, 1882 and *Parawrightia robusta* Warren, 1907 have an exosarc that extends from the hydrorhiza to the tentacular whorl, together with the chitin-proteic layer. On the other hand, *Bimeria vestita* Wright, 1859 and *B. rigida* have an exosarc that covers the hypostome and the base of the tentacles. Therefore, with this evidence we proposed that some "Anthoathecata" have exoskeletons formed by two layers (chitin-protein and GAGs) with a granular appearance and different from other cnidarians (corneous, calcareous, coriaceous), and here it is designated as bilayered.

The exosarc has received little or no attention and is often called by generic terms restricted to hydranths of some families of "Anthoathecata": a cuticle (Brown, 1975), a gelatinous-looking investment (Allman, 1871), a gelatinous structure (Warren, 1919; Cartwright *et al*., 2008), external secretions (Thomas & Edwards, 1991), mucous-like perisarc (Stepanjants *et al*., 2000), or a pseudohydrotheca (Calder, 1988; Schuchert, 2007). A detailed examination of the exoskeleton of Bougainvilliidae shows that the exosarc is not limited to the hydranth. Thus, we suggest that the name pseudohydrotheca continue to be used exclusively for the part of the exosarc covering the hydranth. Detailed morphological, histological, histochemical, and genetic examination of the exosarc will be necessary to resolve questions of homology (whether around hydranths, branches, hydrorhiza, or gonophores) and thereby its evolutionary history.

Phylogenetic patterns of exoskeletons in Medusozoa

Diversification in the corneous, calcareous, coriaceous, and bilayered exoskeletons reflects particular evolutionary histories in Medusozoa (Fig. 4). Phylogenetically, the

exoskeleton is found in all medusozoans, with uncertainties in Staurozoa, and is reasonable to consider that it would be present in the medusozoan ancestral lineage (Van Iten *et al*., 2006).

Exoskeletal structure and composition in Hydrozoa is more variable in the clades of Hydroidolina (Leptothecata and "Anthoathecata"), it is modified in Siphonophora, or reduced in some "Anthoathecata" (Aplanulata and Capitata), or absent in Trachylina. Biomineralized exoskeletons (coriaceous) may be a synapomorphy for the monophyletic group "Filifera III," because it appears in the sister groups Hydractiniidae and Stylasteridae (Miglietta *et al*., 2010). However, this type of exoskeleton would be a homoplastic character, because it is also represented in Milleporidae (Capitata).

The bilayered exoskeleton (perisarc and exosarc), although variable, is perhaps a synapomorphy in the "Filifera IV" (sensu Cartwright *et al*., 2008; Van Iten *et al*., 2014), even if referred to as a pseudohydrotheca in Bougainvilliidae and Pandeidae, or if only present on the hydrorhiza to the base of the hydranth in Oceaniidae and Rathkeidae. The exosarc is also homoplastic in other groups, such as the anthoathecate Clathrozoellidae (as a pseudohydrotheca; not included in Cartwright *et al*., 2008). Therefore, the exosarc requires further study, especially of its composition, to propose hypotheses of its homology as well as to understand its biological and ecological function and evolutionary history.

A crucial step to resolve these evolutionary considerations lies in species phylogeny itself. Nowadays there is no consensus about major patterns among the main hydroidolinan clades (see Fig. 4 for a current hypothesis). Improvements on this subject will be important to future discussions about the evolutionary processes related to Medusozoan exoskeleton.

Exoskeletal structure: cause and effect in morphological diversification in Medusozoa

In Medusozoa there is a clear interaction between abiotic factors (e.g. waves and/or currents), and the organization and composition of the exoskeleton to resolve the needs of simple protection to structural rigidity (Murdock, 1976; Hughes, 1980). This trend is preserved in the fossil record. For example, in the Ponta Grossa Formation (Devonian), Paraná Basin, Brazil, sedimentological, stratigraphical and taphonomic evidences show the influence of deep water currents in the distribution of some conulariid species (Simões *et al*., 2000; Rodrigues Simões & Leme, 2003; Van Iten *et al*., 2013b). In these rocks, the simple (without septa or carina) and thin exoskeleton of *Conularia quichua* Ulrich, 1890, would have been transported and reworked prior to its final deposition (Rodrigues *et al*., 2003; Leme *et al*., 2004). Normally, when preserved *in situ* in the Ponta Grossa Formation, its exoskeleton is

three dimensional, completely inflated, with the aperture region turned upward, as in life position. These fossils were preserved below fair-weather wave base (Simões *et al*., 2000; Rodrigues *et al.*, 2003; Van Iten *et al*., 2013b). In contrast, the exoskeleton of *Eoconularia loculata* (Wiman, 1895) (Silurian in Sweden) is robust, with strongly mineralized septa and internally thick corner groove (Jerre, 1994). Because these fossils were split apart above the insertion of the septa, or at the base, we can infer that these features were reinforcements of the exoskeleton as an adaptation to life in a high-energy marine environment (Jerre, 1994).

It has been proposed that hydroids subjected to stronger currents have a tendency to produce a more annular exoskeleton, especially in regions of flexing or attachment to substrates, such as at branches and at the peduncles that support the hydranths (Murdock, 1976; Hughes, 1980). Also, growth and branching patterns may be influenced by currents, such as the transverse axis being perpendicular to the direction of current, to increase feeding efficiency (Tidball, 1984), or the increase in thickness that confers greater resistance (Kosevich, 2012).

Structurally and ecologically, the development of a more rigid exoskeleton has consequences for colony organization, as observed in Aplanulata *Ectopleura* (cf. Nawrocki & Cartwright, 2012). Therefore the exosarc thickness should be a consequence of the habitat in which it is found, as well as of resource availability (Rees, 1956, referring to the pseudohydrotheca). Thickness has been shown, experimentally, to change due to the application of chemical reagents (e.g. changing external mucosal secretions due to detergents and changes in pH; Schlichter, 1984).

Skeletogenesis was undoubtedly a key factor in animal evolution and ecological interactions, perhaps first due to structure and the environment, and then as an exaptation for predation avoidance (e.g. Knoll, 2003). Radiation of metazoans with skeletons was both cause and effect of diversity due to the many benefits arising from a support structure in a variety of environments. Hence, skeletons generated a restructuring of ancient ecosystems that led to dramatic changes in evolution and ecological interactions (Jones, Lawton & Shachak, 1994, 1997; Seilacher, 2007; Wright & Jones, 2006; Erwin, 2008; Erwin & Twedt, 2012).

Cnidarian diversification took place during the Cambrian (or earlier) and was simultaneous with, and a consequence of, the evolution of the exoskeleton (cf. Glaessner, 1971). Due to the age of diversification and if modern patterns indicate past history, then already, in the Cambrian, Medusozoans had colonized probably all the same environments that they thrive in the Present-day (Gili & Hughes, 1995). This adaptive capacity and

diversification was also linked to the life cycle, with the medusa and polyp stages in their life cycles, with asexual reproduction and regeneration (Piraino *et al*., 2004). Specifically during the benthic polyp phase in several groups, diversification associated with the development of the exoskeleton, allowed the exploitation of the many habitats occupied in Modern oceans. Therefore, the varying compositions, structures and functions of the exoskeleton likely contributed to the diversification and species richness of Leptothecata, which has the greatest species richness of Hydrozoa (Cornelius, 1982) and of "Anthoathecata", such as Stylasteridae and Bougainvilliidae.

Diversification in Stylasteridae and Milleporidae (not sister taxa) was indeed associated with the composition of the rigid exoskeleton, with its associated increase in survival likelihood and dispersal and survival of polyp fragments as a result of asexual reproduction or breakage (Cairns & Macintyre, 1992; Lewis, 2006). Other predation-avoidance strategies became available, such as protection of the gastrozooids and dactylozooids that can retract in Milleporidae (Kruijf, 1975), and the skeletal operculum that can close in the gastrozooids of Stylasteridae (Lindner *et al*., 2008). While physiological response to the environment is similar in Milleporidae and Stylasteridae, the latter has nearly 18 times more species than the former (268 versus 15; Cairns, 2011; Schuchert, 2014). Stylasteridae is also much more widespread, from the Arctic to the Antarctic (Cairns 2007), while Milleporidae is tropical (Milliman, 1974). In addition to mutualism of Stylasteridae with zooxanthellae (Milliman, 1974), we suggest that skeletal structure may have also been important to its huge diversification. Similarly, Bougainvilliidae, with 97 species, may owe its current widespread distribution and tolerance to varying salinity (Mendoza-Becerril & Marques, 2013) to its bilayered exoskeleton (perisarc and exosarc).

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References

Allman GJ. 1871. *A monograph of the Gymnoblastic or Tubularian hydroids*. London: Published for the Ray Society.

Amthor JE, Grotzinger JP, Schröder S, Bowring SA, Ramezani J, Martin MW, Matter A. 2003. Extinction of *Cloudina* and *Namacalathus* at the Precambrian-Cambrian boundary in Oman. *Geology* 31: 431-434.

Antcliffe JB, Callow RHT, Brasier MD. 2014. Giving the early fossil record of sponges a squeeze. *Biological Reviews* 89: 972-1004.

Babcock LE, Feldmann RM. 1986. Devonian and Mississipian conulariids of North America. Part A. General description and *Conularia*. *Annals of Carnegie Museum* 55: 349-410.

Balinski A, Sun Y, Dzik J. 2014. Probable advanced hydroid from the Early Ordovician of China. *Paläontologische Zeitschrift* 88: 1-10. DOI: 10.1007/s12542-013-0169-1.

Bambach RK, Bush AM, Erwin DH. 2007. Autecology and the filling of ecospace: key metazoan radiations. *Palaeontology* 50: 1-22.

Barnes DJ. 1970. Coral skeletons: An explanation of their growth and structure. *Science* 170: 1305-1308.

Bengtson S. 1994. The advent of animal skeletons. In: Bengston S, ed. *Early life on Earth*. New York: Columbia University Press, 412-425.

Bengtson S, Zhao Y. 1992. Predatorial borings in late Precambrian mineralized exoskeletons. *Science* 257: 367-369.

Blanquet RS. 1972. Structural and chemical aspects of the podocyst cuticle of the Scyphozoan medusa, *Chrysaora quinquecirrha*. *The Biological Bulletin* 142: 1-10.

Bolte S, Roth O, Philipp EER, Saphörster J, Rosenstiel P, Reusch BH. 2014. Specific immune priming in the invasive ctenophore *Mnemiopsis leidyi*. *Biology Letters* 9: 20130864. DOI: org/10.1098/rsbl.2013.0864.

Böttger A, Doxey AC, Hess MW, Pfaller K, Salvenmoser W, Deutzmann R, Geissner A, Pauly B, Alstätter J, Münder S, Heim A, Gabius H, McConkey B, David CN. 2012. Horizontal gene transfer contributed to the evolution of extracellular surface structures: the freshwater polyp *Hydra* is covered by a complex fibrous cuticle containing glycosaminoglycans and proteins of the PPOD and SWT (Sweet Tooth) families. *PLoS ONE* 7: e52278. DOI: 10.1371/journal.pone.0052278.

Brain CK, Prave AR, Hoffmann KH, Fallick AE, Botha A, Herd DA, Sturrock C, Young I, Condon DJ, Allison SG. 2012. The first animals: ca. 760–million–year–old sponge-like fossils from Namibia. *South African Journal of Science* 108: 1-8. DOI: 10.4102/sajs.v108i1/2.658.

Brasier MD. 1992. Nutrient-enriched waters and the early skeletal fossil record. *Journal of the Geological Society, London* 149: 621-629.

Brown CH. 1975. *Structural materials in animals*: *Coelenterata*. New York: John Wiley & Sons.

Cairns SD. 2007. Deep-water corals: an overview with special reference to diversity and distribution of deep-water scleractinian corals. *Bulletin of Marine Science* 81: 311-322.

Cairns SD. 2011. Global Diversity of the Stylasteridae (Cnidaria: Hydrozoa: Athecatae). *PLoS ONE* 6: e21670. DOI: 10.1371/journal.pone.0021670.

Cairns SD, Macintyre I. 1992. Phylogenetic implications of calcium carbonate mineralogy in the Stylasteridae (Cnidaria: Hydrozoa). *Society for Sedimentary Geology* 7: 96-107.

Cairns SD, Grant-Mackie JA. 1993. Review of the fossil Stylasteridae (Cnidaria: Hydrozoa) from the New Zealand region, New Zealand. *Journal of Geology and Geophysics* 36: 1-8.

Calder DR. 1988. Shallow-Water Hydroids of Bermuda: The Athecatae. *Life Sciences Contributions* 148: 1-107.

Carrette T, Straehler-Pohl I, Seymour J. 2014. Early life history of *Alatina* cf. *moseri* populations from Australia and Hawaii with implications for taxonomy (Cubozoa: Carybdeida, Alatinidae). *PLo S ONE* 9: e84377. DOI: 10.1371/journal.pone.0084377.

Cartwright P, Halgedahl SL, Hendricks JR, Jarrard RD, Marques AC, Collins AG, Lieberman BS. 2007. Exceptionally preserved jellyfishes from the Middle Cambrian. *PLoS ONE* 2: e1121. DOI: 10.1371/journal.pone.0001121.

Cartwright P, Evans NM, Dunn CW, Marques AC, Miglietta MP, Schuchert P, Collins AG. 2008. Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). *Journal of the Marine Biological Association of the United Kingdom* 88: 1663-1672.

Chapman DM. 1966. Evolution of the scyphistoma. In: Rees WJ, ed. *The Cnidaria and their evolution*. London: Academic Press, 51-75.

Chapman DM. 1968. Structure, histochemistry and formation of the podocyst and cuticle of *Aurelia aurita*. *Journal of Marine Biological Association of the United Kingdom* 48: 187-208.

Chapman DM. 1969. The nature of cnidarian desmocytes. *Tissue & Cell* 1: 619-632.

Chapman DM. 1974. Cnidarian histology. In: Muscatine L, Lenhoff HM, eds. *Coelenterate Biology, Reviews and New Perspectives*. London: Academic Press, 1-92.

Chapman DM. 1978. Microanatomy of the cubopolyp, *Tripedalia cystophora* (Class Cubozoa). *Helgoländer wissenschaftliche Meeresuntersuchungen* 31: 128-168.

Chapman DM, Werner B. 1972. Structure of a solitary and colonial species of *Stephanoscyphus* (Scyphozoa, Coronate) with observations on periderm repair. *Helgoländer wissenschaftliche Meeresuntersuchungen* 23: 393-421.

Clites E, Droser ML, Gehling JG. 2012. The advent of hard-part structural support among the Ediacara biota: Ediacaran harbinger of a Cambrian mode of body construction. *Geology* 40: 307-310.

Cohen BL. 2005. Not armour, but biomechanics, ecological opportunity and increased fecundity as keys to the origin and expansion of the mineralized benthic metazoan fauna. *Biological Journal of the Linnean Society* 85: 483-490.

Cohen E. 2010. Chitin Biochemistry: Synthesis, Hydrolysis and Inhibition. *Advances in Insect Physiology* 38: 5-74.

Collins AG. 2009. Recent Insights into Cnidarian Phylogeny. *Smithsonian Contributions to the Marine Sciences* 38: 139-149.

Collins AG, Schuchert P, Marques AC, Jankowski T, Medina M, Schierwater B. 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. *Systematic Biology* 55: 97-115.

Conway Morris S, Robison RA. 1986. Middle Cambrian priapulids and other soft-bodied fossils from Utah and Spain. *University of Kansas Paleontological Contributions* 1: 1-22.

Cook F. 1992. Racklan orogeny. *Canadian Journal of Earth Sciences* 29: 2490-2496.

Cook PJ, Shergold JH. 1986. Proterozoic and Cambrian phosphorites - nature and origin. In: Cook PJ, Shergold JH, eds. *Phosphate Deposits of the World, Proterozoic and Cambrian Phosphorites*. New York: Cambridge University Press, 369-390.

Cornelius PFS. 1982. Hydroids and medusa of the family Campanulariidae recorded from the eastern North Atlantic, with a world synopsis of the genera. *Bulletin of the British Museum* (*Natural History*), *Zoology* 42: 37-148.

Daly M, Brugler MR, Cartwright P, Collins AG, Dawson MN, Fautin DG, France SC, McFadden CS, Opresko DM, Rodriguez E, Romano SL, Stake JL. 2007. The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. *Zootaxa* 1668: 127-182.

Dahiya N. 2009. Role of chitinase in nature. In Musumeci S, Paoletti MG, eds. *Binomium chitin-chitinase*: *recent issues*. New York: Nova Biomedical Books, 27-44.

Dellaporta SL, Xu A, Sagasser S, Jakob W, Moreno MA, Buss LW, Schierwater B. 2006. Mitochondrial genome of *Trichoplax adhaerens* supports placozoa as the basal lower metazoan phylum. *Proceedings of the National Academy of Sciences* 103: 8751-8756.

Di Camillo CG, Luna GM, Bo M, Giordano G, Corinaldesi C, Bavestrello G. 2012. Biodiversity of prokaryotic communities associated with the ectoderm of *Ectopleura crocea* (Cnidaria, Hydrozoa). *PLoS ONE* 7(6): e39926. DOI: 10.1371/journal.pone.0039926.

Dong XP, Cunningham JA, Bengtson S, Thomas CW, Liu J, Stampanoni M, Donoghue PCJ. 2013. Embryos, polyps and medusae of the early cambrian scyphozoan *Olivooides*. *Proceedings of Royal Society* 280: 20130071.

Dzik J. 2007. The Verdun Syndrome: Simultaneous origin of protective armour and infaunal shelters at the Precambrian-Cambrian transition. In: Vickers-Rich P, Komarower P. eds. *The Rise and Fall of the Ediacaran Biota*. London: Geological Society of London, 405-414.

Ehrlich H. 2010a. *Biological Materials of Marine Origin, Invertebrates*. New York: Springer.

Ehrlich H. 2010b. Chitin and collagen as universal and alternative templates in biomineralization. *International Geology Review* 52: 661-699.

Ehrlich H, Ilan M, Maldonado M, Muricy G, Bavestrello G, Kljajic Z, Carballo JL, Schiaparelli S, Ereskovsky A, Schupp P, Born R, Worch H, Bazhenov VV, Kurek D, Varlamov V, Vyalikh D, Kummer K, Sivkov VV, Molodtsov SL, Meissner H, Richterp G, Steck E, Richter W, Hunoldt S, Kammer M, Paasch S, Krasokhin V, Patzke G, Brunner E. 2010. Three-dimensional chitin-based scaffolds from Verongida sponges (Demospongiae: Porifera). Part I. Isolation and identification of chitin. *International Journal of Biological Macromolecules* 47: 132-140.

Ehrlich H, Rigby JK, Botting JP, Tsurkan MV, Werner C, Schwille P, Petrásek Z, Pisera A, Simon P, Sivkov VN, Vyalikh DV, Molodtsov SL, Kurek D, Kammer M, Hunoldt S, Born R, Stawski D, Steinhof A, Bazhenov VV, Geisler T. 2013. Discovery of 505-million-year old chitin in the basal demosponge *Vauxia gracilenta*. *Scientific Reports* 3: 3497.

Erwin DH. 2008. Macroevolution of ecosystem engineering, niche construction and diversity. *Trends in Ecology and Evolution* 23: 304-310.

Erwin DH, Tweedt S. 2012. Ecological drivers of the Ediacaran-Cambrian diversification of Metazoa. *Evolutionary Ecology* 26: 417-433.

Fields WD, Mackie GO. 1971. Evolution of the Chondrophora: Evidence from behavioural studies on *Velella*. *Journal of Fisheries Research Board of Canada* 28: 1595-1602.

Fraune S, Augustin R, Anton-Erxleben F, Wittlieb J, Gelhaus C, Klimovich VB, Samoilovich MP, Bosch TCG. 2010. In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. *Proceedings of the National Academies of Science* 107: 18067-18072.

Fryer G, Stanley Jr.GD. 2004. A silurian porpitoid hydrozoan from Cumbria, Engand, and a note on porpitoid relationships. *Paleontology* 45: 1109-1119.

Fukuda I, Ooki S, Fujita T, Murayama E, Nagasawa H, Isa Y, Watanabe T. 2003. Molecular cloning of a cDNA encoding a soluble protein in the coral exoskeleton. *Biochemical and Biophysical Research Communications* 304: 11-17.

Garstang W. 1946. The morphology and relations of the Siphonophora. *Quarterly Journal of Microscopical Science* 87: 103-193.

Gili JM, Hughes RG. 1995. The ecology of marine benthic hydroids. *Oceanography and Marine Biology*: *an Annual Review* 33: 351-426.

Glaessner MFA. 1971. The genus *Conomedusites* Glaessner & Wade and the diversification of the Cnidaria. *Paläontologische Zeitschrift* 45: 7-17.

Gooday GW. 1990. Physiology of microbial degradation of chitin and chitosan. *Biodegradation* 1: 177-190.

Grant SWF. 1990. Shell structure and distribution of *Cloudina*, a potential index fossil for the terminal Proterozoic. *American Journal of Science* 290: 261-294.

Grillo MC, Goldberg WM, Allemand D. 1993. Skeleton and sclerite formation in the precious red coral *Corallium rubrum*. *Marine Biology* 117: 119-128.

Grotzinger JP, Bowring SA, Saylor BZ, Kaufman AJ. 1995. Biostratigraphic and geochronologic constraints on early animal evolution. *Science* 270: 598-604.

Grotzinger JP, Watters W, Knoll AH. 2000. Calcified metazoans in thrombolite-stromatolite reefs of the terminal Proterozoic Nama Group, Namibia. *Paleobiology* 26: 334-359.

Hagadorn JW, Waggoner B. 2000. Ediacaran fossils from the southwestern Great Basin, United States. *Journal of Paleontology* 74: 349-359.

Hahn G, Hahn R, Leonardos OH, Pflug HD, Walde DHG. 1982. Kfrperlich erhaltene Scyphozoen-Reste aus dem Jungprekambrium Brasiliens. *Geologica et Paleontologica* 16: 1-18.

Hanaoka KI. 1934. Notes on the early development of a stalked medusa. *Proceedings of the Imperial Academy* 10: 117-120.

Hardie LA. 2003. Secular variations in Precambrian seawater chemistry and the timing of Precambrian aragonite seas and calcite seas. *Geology* 31: 785-788.

Hua H, Chen Z, Yuan X, Zhang L, Xiao S. 2005. Skeletogenesis and asexual reproduction in the earliest biomineralizing animal *Cloudina*. *Geology* 33: 277-280.

Hua H, Pratt BR, Zhang LY. 2003. Borings in *Cloudina* shells: complex predator−prey dynamics in the terminal Neoproterozoic. *Palaios* 18: 454-459. DOI: 10.1669/0883-1351(2003)018<0454:BICSCP>2.0.CO;2.

Hughes RG. 1980. Current induced variations in the growth and morphology of hydroids. In: Tardent P, Tardent R, eds. *Developmental and cellular biology of Coelenterates*. North Holland, Amsterdam: Elsevier, 179-184.

Hughes NC, Gunderson GO, Weedon MJ. 2000. Late Cambrian conulariids from Wisconsin and Minnesota. *Journal of Paleontology* 74: 828-838.

Hündgen M. 1984. Cnidarian: Cells Types. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, eds. *Biology of the integument I-Invertebrates*. New York: Springer Verlag, 47-56.

Hwang DS, Masic A, Prajatelistia E, Iordachescu M, Waite H. 2013. Marine hydroid perisarc: A chitin- and melanin-reinforced composite with DOPA-iron (III) complexes. *Acta Biomaterialia* 9: 8110-8117.

Jablonski D. 2005. Evolutionary innovations in the fossil record: The intersection of ecology, development, and macroevolution. *Journal of Experimental Zoology* 304B: 504-519.

Jarms G. 1991. Taxonomic characters from the polyp tubes of coronate meduse (Scyphozoa, Coronate). *Hydrobiologia* 216/217: 463-470.

Jarms G, Tiemann H. 1996. On a new hydropolyp without tentacles, *Microhydrula limopsicola* n. sp., epibiotic on bivalve shells from the Antarctic. *Scientia Marina* 60: 109-115.

Jerre F. 1994. Anatomy and phylogenetic significance of *Eoconularia loculata*, a conulariid from the Silurian of Gotland. *Lethaia* 27: 97-109.

Jeuniaux C, Voss-Foucart MF. 1991. Chitin biomass and production in the marine environment. *Biochemical Systematics and Ecology* 19: 347-356.

Jones CG, Lawton JH, Shachak M. 1994. Organisms as ecosystem engineers. *Oikos* 69: 373-386.

Jones CG, Lawton JH, Shachak M. 1997. Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* 78: 1946-1957.

Kaya M, Baublys V, Šatkauskiené I,˙ Akyuz B, Bulut E, Tubelyté V. 2015. First chitin extraction from *Plumatella repens* (Bryozoa) with comparison to chitins of insect and fungal origin. *International Journal of Biological Macromolecules* DOI: 10.1016/j.ijbiomac.2015.04.066

Kazmierczak J, Kempe S, Kremer B. 2013. Calcium in the early evolution of living systems: A biohistorical approach. *Current Organic Chemistry* 17: 1738-1750.

Knight DP. 1970. Sclerotization of the perisarc of the caliptoblastic hydroid, *Laomedea flexuosa*. 1 The identification and localization of dopamine in the hydroid. *Tissue Cell* 2: 467-477.

Knoll AH. 2003. Biomineralization and Evolutionary History. *Reviews in Mineralogy and Geochemistry* 54: 329-356.

Knoll AH, Javaux EJ, Hewitt D, Cohen P. 2006. Eukaryotic organisms in Proterozoic oceans. *Philosophical Transactions of the Royal Society* 361: 1023-1038.

Kosevich IA. 2012. Morphogenetic foundations for increased evolutionary complexity in the organization of thecate hydroids shoots (Cnidaria, Hydromedusa, Leptomedusae). *Biology Bulletin* 39: 172-185.

Kossevitch IA, Herrmann K, Berking S. 2001. Shaping of colony elements in *Laomedea flexuosa* Hinks (Hydrozoa, Thecaphora) includes a temporal and spatial control of skeleton hardening. *The Biological Bulletin* 201: 417-423.

Kowalevsky A. 1884. Zur Entwicklungsgeschichte der *Lucernaria*. *Zoologischer Anzeiger* 7: 712-719.

Kruijf HAM. 1975. General morphology and behavior of gastrozoids and dactylozoids in two species of *Millepora* (Milleporina, Coelenterata). *Marine Behaviour and Physiology* 3: 181-192.

Le Tissier MD'AA. 1991. The nature of the skeleton and skeletogenic tissues in the Cnidaria. *Hydrobiologia* 216/217: 397-402.

Leme JM, Rodrigues SC, Simões MG, Van Iten H. 2004. Sistemática dos Conulários (Cnidaria) da Formação Ponta Grossa (Devoniano), do Estado do Paraná, Bacia do Paraná, Brasil. *Revista Brasileira de Paleontologia* 7: 213-222.

Leme JM, Simões MG, Marques AC, Van Iten H. 2008a. Cladistic analysis of the suborder Conulariina Miller and Gurley, 1896 (Cnidaria, Scyphozoa; Vendian-Triassic). *Palaeontology* 51: 649-662.

Leme JM, Simões MG, Rodrigues SC, Van Iten H, Marques AC. 2008b. Major developments in conulariid research: Problems of interpretation and future perspectives. *Ameghiniana* 45: 407-420.

Leme JM, Simões MG, Van Iten H. 2010. *Phylogenetic Systematics and evolution of conulariids*. Germany: Lap Lambert Academic Publishing Gmb H & Co, Saarbrücken.

Leme JM, Van Iten H, Simões MG, Fairchild TR, Rodrigues F, Galante D, Pacheco MLAF. 2013. A new Ediacaran conulariid from the Tamengo Formation, Corumbá Group, Brazil, and the deep Precambrian evolutionary history of Cnidarians. *The Neoproterozoic Paraguay Fold Belt (Brazil)*: *glaciations, iron-manganese formation and biota*. Brazil: Abstracts Corumbá Meeting, Mato Grosso do Sul.

Lenton TM, Watson AJ. 2004. Biotic enhancement of weathering, atmospheric oxygen and carbon dioxide in the Neoproterozoic. *Geophysical Research Letters* 31: 1-5. DOI: 10.1029/2003GL018802.

Lenton TM, Boyle RA, Poulton SW, Shields-Zhou GA, Butterfield NJ. 2014. Co-evolution of eukaryotes and ocean oxygenation in the Neoproterozoic era. *Nature Geoscience* 7: 257-265.

Lesh-Laurie GE, Suchy PE. 1991. Cnidaria: Scyphozoa and Cubozoa. In: Harrison FW, Westfall JA, eds. *Microscopic Anatomy of Invertebrates II – Placozoa, Porifera, Cnidaria and Ctenophora*. New York: Wiley-Liss, 185-266.

Lewis JB. 2006. Biology and ecology of the hydrocoral *Millepora* on coral reefs. *Advances in Marine Biology* 50: 1-55.

Lindner A, Cairns SD, Cunningham CW. 2008. From offshore to onshore: Multiple origins of shallow-water corals from deep-sea ancestors. *PloS ONE* 3: e2429. DOI: 10.1371/journal.pone.0002429.

Liu AG, Matthews JJ, Menon LR, McIlroy D, Brasier MD. 2014. *Haootia quadriformis* n. gen., n. sp., interpreted as a muscular cnidarian impression from the Late Ediacaran period (approx. 560 Ma). *Proceedings of the Royal Society* 281: 20141202. DOI: 10.1098/rspb .2014.1202.

Liu P, Xiao S, Yin C, Zhou C, Gao L, Tang F. 2008. Systematic description and phylogenetic affinity of tubular microfossils from the Ediacaran Doushantuo Formation at Weng'an, South China. *Palaeontology* 51: 339-366.

Mackie GO. 1960. Studies on *Physalia physalis* (L.) Part II, Behaviour and histology. *Discovery Report* 30: 371-407.

Mackie GO. 1984. Introduction to the dipoblastic level. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, eds. *Biology of the integument I-invertebrates*. New York: Springer Verlag, 43-46.

Mali B, Möhrlen F, Frohme M, Frank U. 2004. A putative double role of a chitinase in a cnidarian: pattern formation and immunity. *Developmental & Comparative Immunology* 28: 973-981.

Marques AC. 2001. Simplifying hydrozoan classification: Inappropriateness of the group Hydroidomedusae in a phylogenetic context. *Contributions to Zoology* 70: 175-179.

Marques AC, Collins AG. 2004. Cladistic analysis of Medusozoa and cnidarian evolution. *Invertebrate Biology* 123: 23-42.

Marques AC, Migotto AE. 2001. Cladistic analysis and new classification of the family Tubulariidae (Hydrozoa, Anthomedusae). *Papéis Avulsos de Zoologia, Museo de Zoologia da Universidade de São Paulo* 41: 465-488.

Marques AC, Morandini AC, Migotto AE. 2003. Synopsis of knowledge on Cnidaria Medusozoa from Brazil. *Biota Neotropica* 3: 1-18.

Medeiros GF, Mendes A, Castro RAB, Baú EC, Nader HB, Dietrich CP. 2000. Distribution of sulfated glycosaminoglycans in the animal kingdom: Widespread occurrence of heparina-like compounds in invertebrates. *Biochimica et Biophysica Acta* 1475: 287-294.

Mendoza-Becerril MA, Marques AC. 2013. Synopsis on the knowledge and distribution of the family Bougainvilliidae (Hydrozoa, Hydroidolina). *Latin American Journal of Aquatic Research* 41: 908-924.

Merzendorfer H. 2011. The cellular basis of chitin synthesis in fungi and insects: common principles and differences. *European Journal of Cell Biology* 90: 759-769.

Merzendorfer H, Zimoch L. 2003. Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. *The Journal of Experimental Biology* 206: 4393-4412.

Mierzejewska G, Mierzejewski P. 1979. Traces of bacterial activity on the Ordovician polychaete jaws. *Acta Medica Polona* 20: 35-36.

Mierzejewski P. 1986. Ultrastructure, taxonomy and affinities of some Ordovician and Silurian organic microfossils. *Palaeontologia Polonica* 47: 129-220.

Miglietta MP, McNally L, Cunningham CW. 2010. Evolution of calcium-carbonate skeletons in the Hydractiniidae. *Integrative and Comparative Biology* 50: 428-435.

Migot A. 1922a. Sur le mode de fixation des Lucernaires à leur support. *Comptes Rendues des*

Séances de la Société de Biologie et de ses filiales 86: 827-829.
Migot A. 1922b. A propos de la fixation des Lucernaires. *Comptes Rendues des Séances de la Société de Biologie et de ses filiales* 87: 151-153.
Milliman JD. 1974. *Marine carbonates-part 1*. New York: Springer-Verlag.
Miranda LS, Collins AG, Marques AC. 2010. Molecules clarify a cnidarian life cycle – the "hydrozoan" *Microhydrula limopsicola* is an early life stage of the staurozoan *Haliclystus antarcticus*. *PLoS ONE* 5: e10182. DOI: 10.1371/journal.pone.0010182.
Miranda LS, Morandini AC, Marques AC. 2012. Do Staurozoa bloom? A review of stauromedusan population biology. *Hydrobiologia* 690: 57-67.
Miranda LS, Collins AG, Marques AC. 2013. Internal anatomy of *Haliclystus antarcticus* (Cnidaria, Staurozoa) with a discussion on histological features used in staurozoan taxonomy. *Journal of Morphology* 274: 1365-1383. DOI: 10.1002/jmor.20185.
Murdock GR. 1976. Hydroid skeletons and fluid flow. In: Mackie GO, ed. *Coelenterate ecology and behavior*. London: Plenum Press, 33-40.
Muzzarelli RAA, Muzzarelli C. 2009. Chitin and chitosan hydrogels. In: Phillips GO, Williams PA, eds. *Handbook of hydrocolloids*. USA: CRC Press, 850-876.
Nawrocki AM, Cartwright P. 2012. A novel of colony formation in a hydrozoan through fusion of sexually generated individuals. *Current Biology* 22: 825-829.
Och LM, Shields-Zhou GA. 2011. The Neoproterozoic oxygenation event: Environmental perturbations and biogeochemical cycling. *Earth-Science Reviews* 110: 26-57.
Otto JJ. 1976. Early development and planula movement in *Haliclystus* (Scyphozoa, Stauromedusae) In: Mackie GO, ed. *Coelenterate Ecology and Behavior*. New York: Plenum Press 319-329.
Otto JJ. 1978. The settlement of *Haliclystus* planulae. In: Chia FS, Rice M, eds. *Settlement and Metamorphosis of Marine Invertebrate Larvae*. New York: Elsevier-North Holland, 13-22.
Pacheco MLAF, Leme J, Machado A. 2011. Taphonomic analysis and geometric modelling for the reconstitution of the Ediacaran Metazoan *Corumbella werneri* Hahn *et al.*, 1982 (Tamengo Formation, Corumbá Basin, Brazil). *Journal of Taphonomy* 9: 269-283.
Pacheco MLAF, Leme JM, Galante D, Pidassa B, Hagadorn W, Pfeiffer F, Marques AC. 2015. Insights into the skeletonization, lifestyle, and affinity of the bizarre Ediacaran fossil *Corumbella*. *PLoS ONE* 10: e0114219. DOI: 10.1371/journal.pone.0114219.
Papineau D. 2010. Global Biogeochemical Changes at Both Ends of the Proterozoic: Insights from Phosphorites. *Astrobiology* 2: 165-181.
Paps J, Medina-Charcón LA, Marshall W, Suga H, Ruiz-Trillo I. 2013. Molecular phylogeny of unikonts insights into the position of apusomonads and ancyromonads and the internal relationships of opisthokonts. *Protist* 164: 2-12.
Penny AMR, Wood A, Curtis F, Bowyer R, Tostevin K, Hoffman H. 2014. Ediacaran metazoan reefs from the Nama Group, Namibia. *Science* 344: 1504-1506.
Pillai CKS, Paul W, Sharma CP. 2009. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Progress in Polymer Science* 34: 641-678.
Piraino S, De Vito D, Schmich J, Bouillon J, Boero F. 2004. Reverse development in Cnidaria. *Canadian Journal of Zoology* 82: 1748-1754.
Pratt BR. 1982. Stromatolite decline - A reconsideration. *Geology* 10: 512-515.
Pyefinch KA, Downing FS. 1949. Notes on the general biology of *Tubularia larynx* Ellis & Solander. *Journal of the Marine Biological Association of the United Kingdom* 28: 21-43.
Ramos-Silva P, Kaandorp J, Huisman L, Marie B, Zanella-Cleón I, Guichard N, Miller DJ, Marin F. 2013. The Skeletal Proteome of the Coral Acropora millepora: The Evolution of Calcification

by Co-Option and Domain Shuffling. *Molecular Biology and Evolution*. 30: 2099-2112. DOI: 10.1093/molbev/mst109.

Rees WM. 1956. A revision of the hydroid genus *Perigonimus* M. Sars 1846. *Bulletin* of the *British Museum* (Natural History), *Zoology* 3: 337-350.

Richmond TA, Somerville CR. 2000. The cellulose synthase superfamily. *American Society of Plants Physiologists* 124: 495-498.

Rodrigues SC, Simões MG, Leme JM. 2003. Tafonomia Comparada dos Conulatae (Cnidaria), Formação Ponta Grossa, Bacia do Paraná, Estado do Paraná. *Revista Brasileira de Geociências* 33: 1-10.

Ruggiero MA, Gordon DP, Orrell TM, Bailly N, Bourgoin T, Brusca RC, Cavalier-Smith, T, Guiry MD, Kirk PM. 2015. A Higher Level Classification of All Living Organisms. *PLoS ONE* 10: e0119248. DOI: 10.1371/journal.pone.0119248.

Ruiz-Herrera J, Ortiz-Castellanos L. 2010. Analysis of the phylogenetic relationships and evolution of the cell walls from yeasts and fungi. *FEMS Yeast Research* 10: 225-243.

Ruiz-Herrera J, González-Prieto JM, Ruiz-Medrano R. 2002. Evolution and phylogenetic relationships of chitin synthases from yeasts and fungi. *Yeast Research* 1: 247-256.

Ryan JF, Pang K, Schnitzler CE, Nguyen AD, Moreland RT, Simmons DK, Koch BJ, Francis WR, Havlak P. NISC Comparative Sequencing Program Smith SA, Putnam NH, Haddock SH, Dunn CW, Wolfsberg TG, Mullikin JC, Martindale MQ, Baxevanis AD. 2013. The genome of the Ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* 342: 1242592. DOI: 10.1126/science.1242592.

Schlichter D. 1984. Cnidaria: Permeability, epidermal transport and related phenomena. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, eds. *Biology of the integument I-invertebrates*. New York: Springer Verlag, 79-95.

Schuchert P. 2007. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 2. *Reveu Suisse de Zoologie* 114: 195-396.

Schuchert P. 2014. Milleporidae Fleming, 1828. Accessed through: Schuchert P. 2014 World Hydrozoa database at http://www.marinespecies.org/hydrozoa/aphia.php?p=taxdetails &id=196235 on 2015-01-02.

Scotland RW. 2010. Deep homology: a view from systematics. *BioEssays* 32: 438-449.

Seilacher A. 2007. The nature of vendobionts. *Geological Society of London Special Publications* 286: 387-397.

Sentandreu R, Mormeneo S, Ruiz-Herrera J. 1994. Biogenesis of the fungal cell wall. In: Wessels JGH, Meinhardt F, eds. *The Mycota I*: *Growth, differentiation and sexuality*. New York: Springer Berlin Heidelberg, 111-124.

Serezhnikova EA. 2014. Skeletogenesis in problematic Late Proterozoic Lower Metazoa. *Paleontological Journal* 48: 1457-1472.

Shen Z, Jacobs-Lorena M. 1999. Evolution of chitin-biding proteins in invertebrates. *Journal of Molecular Evolution* 48: 341-347.

Siebold CTH. 1874. *Anatomy of the Invertebrata*. Boston: J Campbell.

Signorovitch AY, Buss LW, Dellaporta SL. 2007. Comparative genomics of large mitochondria in placozoans. *PLoS Genet* 12: e13. DOI: 10.1371/journal.pgen.0030013

Simões M, Mello L, Rodrigues SC, Leme J, Marques A. 2000. Conulariid taphonomy as a tool in paleoenvirontal analysis. *Revista Brasileira de Geociências* 30: 757-762.

Singla CL. 1976. Ultrastructure and attachment of the basal disk of *Haliclystus.* In: Mackie GO, ed. *Coelenterate ecology and behavior*. New York: Plenum Press, 533-540.

Sorauf JE. 1980. Biomineralization, structure and diagenesis of the Coelenterate skeleton. *Acta Palaentologica Polonica* 25: 327-343.

Souza CP, Almeida BC, Colwell RR, Rivera IN. 2011. *The importance of chitin in the marine environment*. New York: Marine Biotechnology, 13: 823-830.

Sperling EA, Frieder CA, Raman AV, Girguis PR, Levin LA, Knoll AH. 2013. Oxygen, ecology, and the Cambrian radiation of animals. *Proceedings of the National Academy of Sciences of the United States of America* 110: 13446-13451.

Stanley SM. 1973. An ecological theory for sudden origin of multicellular life in the Late Precambrian. *Proceedings of the National Academy of Sciences of the United States of America* 70: 1486-1489.

Stanley Jr.GD, Fautin DG. 2001. The Origins of Modern Corals. *Science* 291: 1913-1914.

Stepanjants SD, Timoshkin OA, Anokhin BA, Napara TO. 2000. A new species of *Pachycordyle* (Hydrozoa, Clavidae) from Lake Biwa (Japan), with remarks on this and related Clavid genera. *Scientia Marina* 64: 225-236.

Tang WJ, Fernandez JG, Sohn JJ, Amemiya CT. 2015. Chitin is endogenously produced in vertebrates. *Current Biology* 25: 897-900.

Thomas MB, Edwards NC. 1991. Cnidaria: Hydrozoa. In: Harrison, FW, Westfall JA, eds. *Microscopic Anatomy of Invertebrates II – Placozoa, Porifera, Cnidaria and Ctenophora*. New York: Wiley-Liss, 91-183.

Tidball JG. 1984. Cnidaria: Secreted surface. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, eds. *Biology of the integument I-invertebrates*. New York: Springer Verlag, 69-78.

Toshino S, Miyake H, Otsuka S, Okuizumi K, Adachi A, Hamatsu Y, Urata M, Nakaguchi K, Yamaguchi S. 2013. Development and polyp formation of the giant box jellyfish *Morbakka virulenta* (Kishinouye, 1910) (Cnidaria: Cubozoa) collected from the Seto Inland Sea, Western Japan. *Plankton and Benthos Research* 8: 1-8.

Tucker RP, Shibata B, Blankenship TN. 2011. Ultrastructure of the mesoglea of the sea anemone *Nematostella vectensis* (Edwardsiidae). *Invertebrate Biology* 130: 11-24.

Van Iten H. 1991. Anatomy, patterns of occurrence, and nature of the conulariid schott. *Palaeontology* 34: 939-954.

Van Iten H. 1992a. Morphology and phylogenetic significance of the corners and midlines of the conulariid test. *Paleontology* 35: 335-358.

Van Iten H. 1992b. Microstructure and growth of the conulariid test: implications for conulariid affinities. *Paleontology* 35: 359-372.

Van Iten H, Fitzke JA, Cox RS. 1996. Problematical fossil cnidarians from the Upper Ordovician of the North-Central USA. *Palaeontology* 39: 1037-1064.

Van Iten H, Leme JM, Rodrigues SC, Simões MG. 2005a. Reinterpretation of a conulariid-like fossil from the Vendian of Russia. *Palaeontology* 48: 619-622.

Van Iten H, Vyhlasova Z, Zhu MY, Yi Q. 2005b. Widespread occurrence of microscopic pores in conulariids. *Journal of Paleontology* 79: 400-407.

Van Iten H, Leme JM, Simões MG, Marques AC, Collins AG. 2006. Reassessment of the phylogenetic position of conulariids (?Ediacaran-Triassic) within the subphylum Medusozoa (Phylum Cnidaria). *Journal of Systematic Palaeontology* 4: 109-118.

Van Iten H, Leme JD, Marques AC, Simões MG. 2013a. Alternative interpretations of some earliest Ediacaran fossils from China. *Acta Palaeontologica Polonica* 58: 111-113.

Van Iten H, Tollerton VP, Ver Straeten CA, Leme JM, Simões MG, Rodrigues SC. 2013b. Life mode of in a Middle Devonian epibole. *Palaeontology* 56: 29-48.

Van Iten H, Marques AC, Leme JM, Pacheco MLAF, Simões MG. 2014. Origin and early diversification of the phylum Cnidaria Verrill: major developments in the analysis of the taxon's Proterozoic-Cambrian history. *Palaeontology* 57: 677-690.

Vermeij GJ. 1989. The origin of skeletons. *Palaios* 4: 585-589.

Vinn O, Zatón M. 2012. Inconsistencies in proposed annelid affinities of early biomineralized organism *Cloudina* (Ediacaran): structural and ontogenetic evidences. *Carnets de Geologie* CG2012_A03: 39-47.

Wagner GP. 1993. Evolution and multi-functionality of the chitin system. *Experientia Revue Mensuelle Des Sciences Pures Et Appliquées* 49: 559-577.

Wagner GP. 1994. Evolution and multi-functionality of the chitin system In: Schierwater B, Streit B, Wagner GP, DeSalle R, editors. Molecular Ecology and Evolution: Approaches and Applications. Switzerland: Birkhäuser Verlag Basel, 560-577.

Wallace MW, Hood AvS, Woon EMS, Hoffmann KH, Reed CP. 2014. Enigmatic chambered structures in Cryogenian reefs: The oldest sponge-grade organisms? *Precambrian Research* 255: 109-123.

Warren E. 1919. On the anatomy of a New South African hydroid, *Bimeria rigida* sp. n. *Annals of the Natal Museum* 4: 1-18.

Warren LV, Pacheco, MLAF, Fairchild TR, Simões MG, Riccomini C, Boggiani PC, Cáceres AA. 2012. The dawn of animal skelotogenesis: Ultrastructural analysis of the Ediacaran metazoan *Corumbella werneri. Geology* 40: 691-694.

Warren LV, Simões MG, Fairchild TR, Riccomini C, Gaucher C, Anelli LE, Freitas BT, Boggiani PC, Quaglio F. 2013. Origin and impact of the oldest metazoan bioclastic sediments. *Geology* 41: 507-510.

Werner B. 1966. *Stephanoscyphus* (Scyphozoa Coronate) und seine direkte Abstammung von den fossilen Conulata. *Helgoländer wissenschaftliche Meeresuntersuchungen* 15: 317-347.

Werner B. 1967. *Stephanoscyphus* Allman (Scyphozoa Coronatae), ein rezenter Vertreter der Conulata? *Paläontologische Zeitschrift* 41: 137-153.

Wietrzykowski W. 1910. Sur le développement des Lucernaridés (note préliminaire). *Archives de Zoologie Expérimentale et Générale. Tome V, Notes et Revue* 2: 10-27.

Wietrzykowski W. 1912. Recherches sur le développement des Lucernaires. *Archives de Zoologie Expérimentale et Générale, 5th series* 10: 1-95.

Willmer P. 1990. *Invertebrate relationships*. New York: Cambridge University Press.

Wineera JS. 1968. The histology of a species of *Solanderia* Duchassaing & Michelin, 1846. *Zoology Publications from Victoria of Wellington* 43: 1-11.

Wood RA. 2011. Paleoecology of the earliest skeletal metazoan communities: Implications for early biomineralization. *Earth-Science Reviews* 106: 184-190.

Wood R, Zhuravlev AY. 2012. Escalation and ecological selectively of mineralogy in the Cambrian Radiation of skeletons. *Earth-Science Reviews* 115: 249-261.

Wood RA, Grotzinger JP, Dickson JAD. 2002. Proterozoic modular biomineralized metazoan from the Nama Group, Namibia. *Science* 296: 2383-2386.

Wood RA, Poulton SW, Prave AR, Hoffmann KH, Clarkson MO, Guilbaud R, Lyne JW, Curtis A, Kasemann SA. 2015. Dynamic redox conditions control late Ediacaran ecosystems in the Nama Group, Namibia. *Precambrian Research* 261: 252-271. DOI: 10.1016/j.precamres.2015.02.004.

Wright JP, Jones CG. 2006. The Concept of Organisms as Ecosystem Engineers Ten Years On: Progress, Limitations, and Challenges. *BioScience* 56: 203-209.

Xiao S, Laflamme M. 2008. On the eve of animal radiation: Phylogeny, ecology and evolution of the Ediacara biota. *Trends in Ecology & Evolution* 24: 31-40.

Xiao S, Yuan X, Knoll A.H. 2010. Eumetazoan fossil in terminal Proterozoic phosphorites? *Proceedings of the National Academy of Sciences of the United States of America* 97: 13684-13689.

Yamada S, Sugahara K, Özbek S. 2011. Evolution of glycosaminoglycans. *Communicative & Integrative Biology* 4: 150-158.

Yamada S, Morimoto H, Fujisawa T, Sugahara K. 2007. Glycosaminoglycans in *Hydra magnipapillata* (Hydrozoa, Cnidaria): demonstration of chondroitin in the developing nematocyst, the sting organelle, and structural characterization of glycosaminoglycan. *Glycobiology* 17: 886-894.

Yasui K, Reimer JD, Liu H, Yao X, Kubo D, Shu D, Li Y. 2013. A diploblastic radiate animal at the dawn of Cambrian diversification with a simple body plan: distinct from Cnidaria? *PLoS ONE* 8: e65890.DOI: 10.1371/journal.pone.0065890.

Zakrzewski AC, Weigert A, Helm C, Adamski M, Adamska M, Bleidorn C, Raible F, Hausen H. 2014. Early divergence, broad distribution, and High diversity of Animal Chitin Synthases. *Genome Biology and Evolution* 6: 316-325.

Zhao Y, Bengtson S. 1999. Embryonic and postembryonic development of the early Cambrian cnidarian *Olivooides*. *Lethaia* 32: 181-195.

Table 1. Enzymes involved in the synthesis of chitin in the basal Metazoa.

Enzyme		Code Pfam	Placozoa *Trichoplax adhaerens*	Porifera *Amphimedon queenslandica*	Ctenophora *Mnemiopsis leidyi*	Anthozoa *Nematostella vectensis*	Medusozoa *Hydra vulgaris*
1	Trehalase	PF01204	B3RZE81	I1FUB91	*	EZ0207921♠	T2MCD41
2	Hexokinase-I	PF00349	B3S8Y61	I1F4T51	ML069127a3	A7RZJ91	T2MH691
3	Glucose-6-phosphate isomerase	PF00342#	B3RLE21	I1G9281	ML11532a3	A7SGU11	T2MHV31
4	Glutamine: fructose-6-phosphate aminotransferase	PF00310	•4	XM_0033865051	ML035810a3	Nv.T1.6461.42	T2MHY01
5	Glucosamine-6-phosphate N-acetyltransferase	PF13508	•4	*	*	EZ0454821♠	GNPNAT11
6	Phosphoacetylglucosamine mutase-I		B3S2Y21	I1GBD61	ML033212a3	A7S2H71	T2MHA61
7	UDP-N-acetylglucosamine pyrophosphorylase	PF01704	•4	•4	ML008012a3	*	*
8	Chitin synthase	PF03142	*	XP_0033854414	*	XP_0016370594	XP_0021625044
9	Chitinase	PF00704-I PF02010	B3RWQ51	B3RWQ51	ML368913a3	A7RFM31	T2M6D91
10	Tyrosinase+	PF00264	*	I1E6461	ML070211a3	A7RQY21	XP_0021559904
12	Tyrosine hydroxilase	PF00351	B3SC111	XM_0033833394	ML154513a3	Nv.T1.7540.22	T2MHI21

Enzymes 1-8, chitin synthesis in insects (Merzendorfer & Zimoch, 2003); enzymes 9-12 participate in, or associate with, synthesis (Knight, 1970; Kossevitch *et al*., 2001). * unidentified enzymes; # enzyme also known as Phosphoglucose isomerase (http://pfam.xfam.org/search/keyword?query=Glucose-6-phosphate+isomerase); • enzymes of uncertain presence; + dopamine synthysized via tyrosinase; ♠ enzyme found only in *Acropora millepora*. Enzyme families follow Pfam (Protein family database, http://pfam.xfam.org/), presence is inferred from databases: 1 - UniProt (Universal Protein Resource - www.uniprot.org), 2 - *Nematostella vectensis* Genomics Database (StellaBase cnidarians.bu.edu/stellabase), 3 - *Mnemiopsis* Genome Project Portal (research.nhgri.nih.gov/mnemiopsis/), 4 - NCBI (nucleotide - www.ncbi.nlm.nih.gov/).

Table 2. Chemicals and minerals included in the composition of Cnidarian exoskeletons (Milliman, 1974; Warren *et al*., 2012).

Chemical element/compound	**Anthozoa**	**Scyphozoa**	**Hydrozoa**
Calcium carbonate [$CaCO_3$]	X	X	X
Calcium phosphate [$Ca_3(PO_4)_2$]	X	X*	
Silicate (Si_xO_y)	X	X	
Magnesium hydroxide [$Mg(OH)_s$]	X		
Strontium (Sr)	X		X
Iron (Fe)	X		X
Manganese (Mn)	X		
Potassium (K)	X		
Barium (Ba)	X		X
Copper (Cu)	X		
Zinc (Zn)	X		
Lead (Pb)	X		
Phosphorous (P)	X		X
Boron (B)	X		
Uranium (U)	X		
Nickel (Ni)	X		
Chromium (Cr)	X		
Cobalt (Co)	X		

*Fossil Conulatae.

Table 3. Types of exoskeleton in Medusozoa. GAGs – glycosaminoglycans.

Class	Subtaxa	Layers	Chemical composition	Type	Regions with exoskeleton	Common name in literature	Figure
Staurozoa	Stauromedusae	*1	*Chitin and mucus	corneous	Lower half of the planulae larvae, larval cysts and basal disc of stauromedusa	Periderm	3A
Scyphozoa	Discomedusae	1	Chitin	corneous	Podocysts	Periderm	3C
	Coronatae	2	Chitin-protein and GAGs	corneous	Polyp body	Periderm	3B
	Conulatae[1]	2	Calcium phosphate	coriaceous	Polyp body	Theca	2A
	Corumbellata[1]	1	Calcium carbonate	coriaceous	Polyp body	Carapace	2B
Cubozoa	Carybdeida	2	Chitin-protein	corneous	Basal portion of polyp	Periderm	3D
Hydrozoa	"Anthoathecata"	1	Calcium carbonate	coriaceous	Polyp body	Perisarc	3H
		1	Chitin-protein	corneous	Hydrorhiza and hydrocaulus	Perisarc	3F
		2	Chitin-protein and GAGs	bilayered	Hydrorhiza, hydrocaulus and base of hydranth	Perisarc or pseudohydrotheca	3I
	Leptothecata	1	Chitin-protein	corneous	Polyp body and reproductive structures	Perisarc	3E
	Hydridae	5	Glycosaminoglycan Chondroitin sulfate and putative peroxidase proteins	fibrous	Polyp body, except tentacles	Cuticle	3G

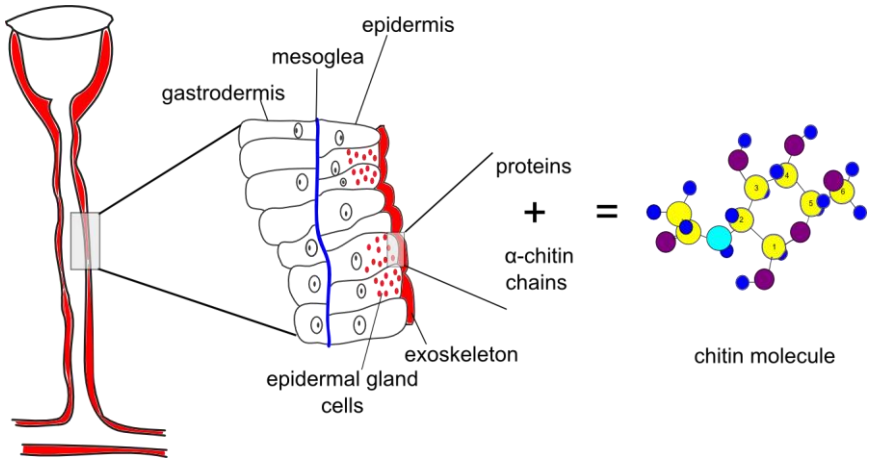

Figure 1. Model of the chitin-protein (corneous) exoskeleton and cell tissues in Medusozoa. Red line refers to the exoskeleton, yellow circles are carbon atoms, blue circles hydrogen, purple circles oxygen, cyan circles nitrogen (circle cyan showing of the N-acetyl group).

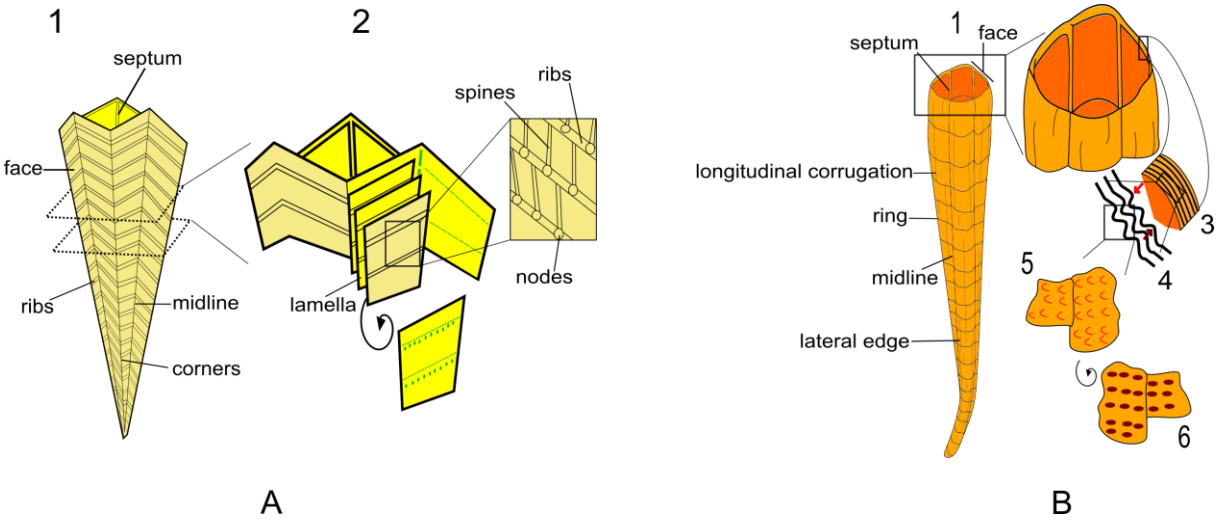

Figure 2. Schematic of the exoskeleton in fossil groups in Medusozoa. A: Conulatae, A1: hypothetical Conulatae, general morphology with main features (modified from Leme *et al*., 2004), A2: lamellar ornament details of the exoskeleton. B: *Corumbella*, B1: *Corumbella werneri*, B2: oral region, B3: cross section of the exoskeleton with microlamellae, B4: lamellar detail showing pores (brown arrow) and papillae (red arrow), B5: underside view example of two of the polygonal plates that comprise the lamellae with papillae (as red "u"), B6: topside view of the same plates as in B, showing pores (as brown oval) (modified from Pacheco *et al*., 2011). Color-coded chemical composition and structure of the exoskeleton: yellow – calcium phosphate, orange – calcium carbonate.

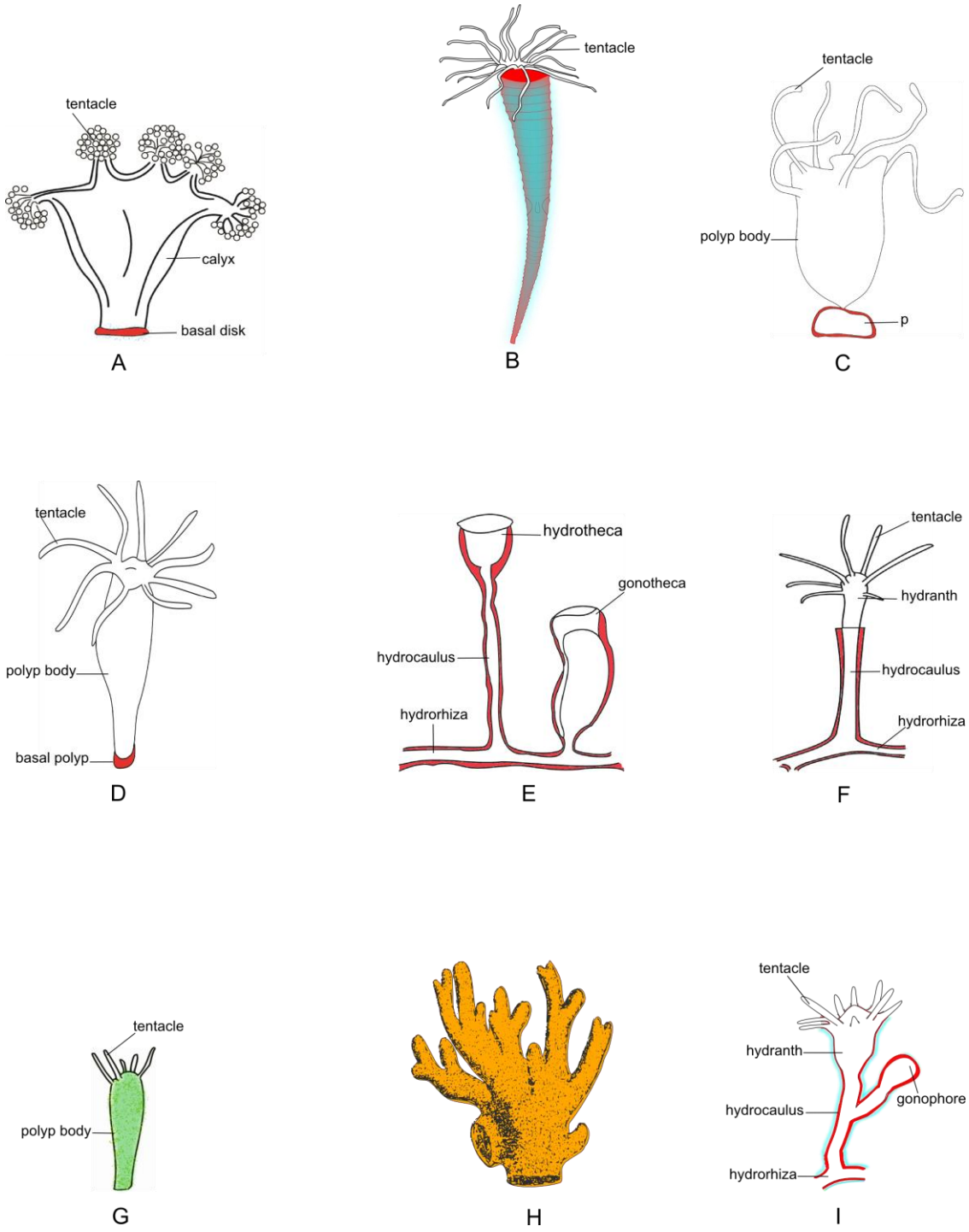

Figure 3. Schematic view of the exoskeleton in extant groups of Medusozoa. A: Staurozoa, Stauromedusae, *Haliclystus*; B-C: Scyphozoa, B: Coronatae, C: Discomedusae; D: Cubozoa, Carybdeida; E-I: Hydrozoa, E: Leptothecata, F: "Anthoathecata", G: Hydridae, *Hydra vulgaris*, H: *Millepora* sp.: I: *Bimeria vestita*. Chemical composition and structures of the exoskeleton indicated in different colors: red – chitin-protein, cyan – glycosaminoglycans, orange – calcium carbonate, green – Glycosaminoglycan, Chondroitin sulfate and putative peroxidase proteins.

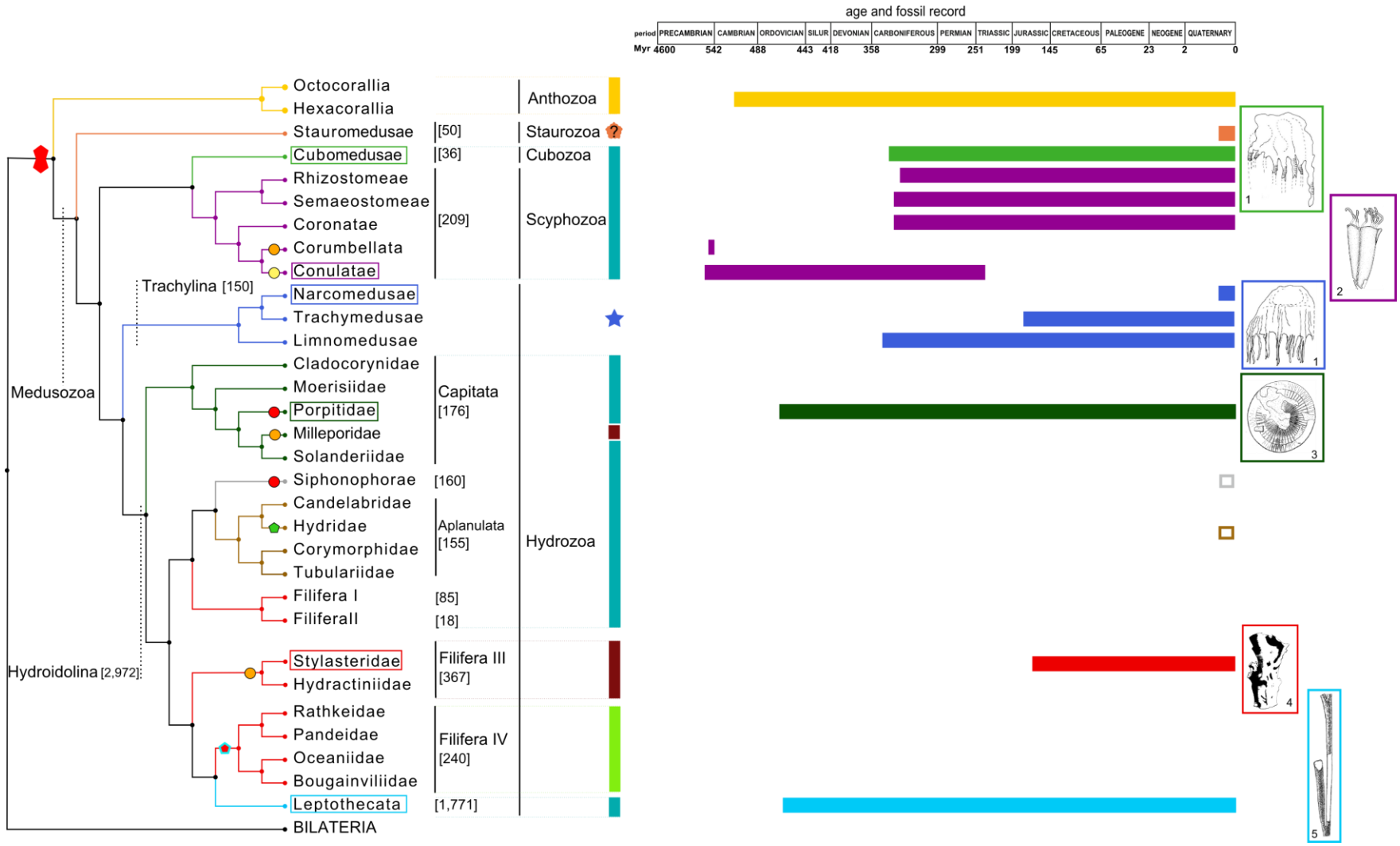

Figure 4. Legend on next page.

Figure 4. Phylogenetic hypothesis of the exoskeleton in Cnidaria, with fossil Medusozoa, with optimization for different skeleton types: calcareous, corneous, coriaceous; bilayered; unknown, without exoskeleton. Skeleton composition: chitin, calcium carbonate, calcium phosphate, GAGs and putative peroxidase proteins, chitin-protein and GAGs. Lineages are by color: yellow – Anthozoa; orange – Staurozoa, purple – Scyphozoa, blue – Trachylina, green – Capitata, gray – Siphonophora, brown – Aplanulata, red – "Anthoathecata," cyan – Leptothecata. Numbers in brackets indicate the total number of extant species, based on Daly *et al*. (2007) and Collins (2009). Solid bars indicate fossils, open squares do not have fossil records. Red circles indicate groups with internal, chitinous skeletons. This hypothesis combines the phylogeny in Collins *et al*. (2006) with, for the position of the Conulatae, Van Iten *et al*. (2006; 2014), Cartwright *et al*. (2008). Hypothetical relations for Hydroidolina are based on unpubl. data (M.M. Maronna & A.C. Marques). Images of fossils: 1 – Cubozoa and Narcomedusae (Cartwright *et al*., 2007); 2 – Conulatae (Van Iten *et al*., 2013a); 3 - *Pseudodiscophylum windermerensis* (Fryer & Stanley, 2004); 4 – *Lepidopora* sp. (modified from Cairns & Grant-Mackie, 1993); 5 - *Sinobryon elongatum* (Balinski *et al*., 2014).

Capítulo – 4

Exoskeletal system of Bougainvilliidae and other Hydroidolina (Cnidaria, Hydrozoa)

María A. Mendoza-Becerril & Antonio C. Marques

Resumo

O exoesqueleto é uma fonte importante de caracteres na taxonomia de Hydroidolina. Ele é originado por secreções epidérmicas e protege o cenossarco dos pólipos, além de apresentar outras funções. Entretanto, estudos comparativos sobre sua origem tecidual, desenvolvimento e características químicas e estruturais, bem como sua evolução e homologia, são escassos e fragmentados. Neste estudo a composição e estrutura do cenossarco e exoesqueleto em pólipos de alguns Leptothecata e "Anthoathecata" (principalmente Bougainvilliidae) foi analisada, comparada e discutida entre os táxons. Também foi estudado o desenvolvimento do exoesqueleto sob condições experimentais. Identificamos três tipos de células epidérmicas glandulares relacionadas com a origem e secreção de polissacarídeos, molécula característica do exoesqueleto. O tipo de exoesqueleto nas espécies estudadas é bicamada (perissarco e exossarco, especialmente em bougainvilídeos) ou axial córneo (perissarco). O exoesqueleto varia em composição química, rigidez estrutural, espessura, extensão e cobertura de diferentes regiões da colônia. No tipo bicamada, o exossarco é a primeira camada a se formar, sugerindo ser um passo chave na formação de um exoesqueleto rígido. Desmócitos e "extensões do perissarco" são estruturas especializadas, com função de ancoragem, associadas ao exoesqueleto. Nossos resultados incorporam aspetos relevantes ao conhecimento do sistema exoesquelético em hidrozoarios e indicam que as pesquisas abrangentes sobre o exoesqueleto podem fornecer dados importantes para entender suas implicações evolutivas e ecológicas em Hydroidolina.

Palavras-chave: Anthoathecata, exossarco, histologia, Leptothecata, perissarco

Abstract

The exoskeleton is an important source of characters for the taxonomy of Hydroidolina. The exoskeleton is originated by epidermal secretions and protects the coenosarc of the polypoid stage, among other functions. However, comparative studies on the exoskeletal tissue origin, development, chemical and structural characteristics, as well as its evolution and homology, are few and fragmented. This study compared the structure and composition of the exoskeleton and underlying coenosarc in members of "Anthoathecata" and some Leptothecata, mainly in bougainvilliid polyps. We also studied the development of the exoskeleton under experimental conditions. We identified three types of glandular epidermal cells related to the origin of the exoskeleton and the secretion of its component polysaccharides. The exoskeleton of the species studied is bilayered (perisarc and exosarc, especially in bougainvilliids) or corneous (perisarc). The exoskeleton varies in chemical composition, structural rigidity, thickness, extension and coverage of different regions of the colony. In bilayered exoskeletons, the exosarc is produced first, and can be a key step in the formation of the rigid exoskeleton. We identified specialized exoskeletal structures such as desmocytes and "perisarc extensions", both used as anchoring structures associated with the exoskeleton. Our study added to the knowledge of the hydrozoan exoskeleton and demonstrated that comprehensive exoskeletal surveys may provide important data to understand the evolutionary and ecological implications for Hydroidolina.

Key words: Anthoathecata, exosarc, histology, Leptothecata, perisarc

Introduction

It has long been accepted that the exoskeleton in Hydroidolina is originated by epidermal secretions (Knight, 1970; Sorauf, 1980; Kossevitch et al., 2001). The epithelial epidermal layer of the coenosarc of benthic colonial or solitary polyps participates in the development of the exoskeleton, by giving rise to diverse cell types (e.g., epithelio-muscular, interstitial, glandular, nervous and cnidocytes; Chapman, 1974; Mackie, 1984), and ultimately helps in the interaction of the polyp with its surroundings.

The glandular epithelial cells are responsible for secreting compounds (e.g., structural proteins and enzymes, phenols, polysaccharides) that are associated with the exoskeleton of

Hydroidolina (Knight, 1970; Kossevitch et al., 2001; Böttger et al., 2012; Hwang et al., 2013; Mendoza-Becerril et al., 2015). Other organisms possess the same compounds identified in hydroidoline exoskeletons (e.g., chitin in fungi; Wagner, 1994), indicating that the exoskeletal system can be decomposed into a molecular matrix (MM) and a molecular synthesis system (MSS). The MM is the extracellular substance containing the molecules, and is located at the outer surface of the epithelium, while the MSS is the biosynthetic apparatus that produces the genetically encoded molecules.

The exoskeleton is involved in the formation of colonial elements such as the stolon and internodes, hydranth, and growing tips (Kosevich, 2013), in addition to participating in other aspects of hydrozoan life (cf. Mendoza-Becerril et al., 2015). Structurally, the exoskeletons has been considered a key morphological diagnostic character at different taxonomic levels for Hydroidolina (cf. Cornelius, 1995; Schuchert, 2012), although some characteristics, such as the thickness and presence of annulations, and the development of hydrotheca, gonotheca and nematotheca are taxonomically more important for Leptothecata (e.g., Cornelius, 1982, 1995; Cunha et al., 2015) than for "Anthoathecata" (a non-monophyletic group, cf. Collins et al., 2006; Cartwright et al., 2008; Van Iten et al., 2014).

Little is known about the nature of the exoskeleton in Hydroidolina, and few studies have investigated the tissue origin, development, chemical and structural characteristics. The majority of these studies have focused on Leptothecata (e.g., Berrill, 1950; Knight, 1970; Kossevitch et al., 2001; Tretenichenko et al., 2006; Kosevich, 2013; Hwang et al., 2013), with few and patchy studies for "Anthoathecata" (e.g., Congdon, 1906; Berrill, 1949; Cowden, 1965; Wineera, 1968, 1972; Yamada et al., 2007), even though the exoskeleton is important in the systematics and biology of some anthoathecate families (e.g., Bougainvilliidae, Allman, 1864; Petersen, 1979; Calder, 1988; Schuchert, 2007; Milleporidae, Razak and Hoeksema, 2003; Stylasteridae, Cairns, 2011; and Hydractiniidae, Miglietta et al., 2010).

"Anthoathecata" is the group with the widest exoskeletal variability and structural complexity, with corneous, coriaceous or bilayered exoskeletons (Mendoza Becerril et al., 2015). Bilayered exoskeletons, such as those of the pseudo-hydrotheca of Bougainvilliidae, are formed by a corneous chitin-protein reinforced by a covering exosarc formed by glycosaminoglycans (GAGs) with incrusting inorganic and/or organic particles (Mendoza-Becerril et al., 2015).

Morphological, histological and histochemical studies of bilayered exoskeletons are needed in order to understand the evolution and homology of these structures. We compared the structure and composition of the coenosarc and exoskeleton in polyps of five families of "Anthoathecata" and two families of Leptothecata, focusing on the poorly known Bougainvilliidae Lütken, 1850 (Hydroidolina, "Anthoathecata"). Additionally, we investigated the development of the exoskeleton under different experimental conditions for five species of Bougainvilliidae, Pandeidae, and Oceaniidae.

Material and Methods

Taxa sampled and histology

Our sample included specimens from the collection of the Museum of Zoology, University of São Paulo (MZUSP); Laboratory of Marine Evolution, Institute of Biosciences, University of São Paulo (LEM-IBUSP); Nagera Station, University of Mar del Plata, Argentina (UNMdP); and the National Museum of Natural History, Smithsonian Institution (USNM). Materials were fixed in 4% or 10% formalin solution (in seawater), or in 92% ethanol. Samples were dehydrated and embedded in glycol methacrylate resin (Leica Historesin Embedding Kit, Leica Microsystems Nussloch GmbH, Germany). Serial longitudinal sections (3 to 7 μm) (the few exceptions referring to transverse sections are noted in the figure captions) were stained with toluidine blue (TB), hematoxylin-hosin (HE), periodic acid-Schiff (PAS, for identification of polysaccharides - P), alcian blue pH 2.5 (AB, for identification of glycosaminoglycans - GAGs), mercury-bromophenol blue (HgBpB, for identification of proteins), and naphthol yellow S (NYS, for identification of proteins) (cf. McManus, 1946; Deitch, 1955; Mowry, 1963; Pearse, 1985). When present, the thickness of each exoskeletal layer of the hydrorhiza, hydrocaulus, hydranth and gonophore was measured. The different thicknesses of the exoskeletal layers were classified as thin (≤ 5 μm), moderately thick (5.1 – 10 μm), thick (10.1 – 50 μm), or very thick (≥ 50.1 μm). Histological slides are deposited in the collection of the Laboratory of Marine Evolution, Institute of Biosciences, University of São Paulo. Specimens in ethanol are deposited in the Museum of Zoology, University of São Paulo (MZUSP 1673, 1740, 1832, 4210, 4217, 4332, 4379, 5201), and the National Museum of Natural History, Smithsonian Institution (USNM 43967, 20234, 29449, 42339, 43330, 43496, 43497, 71026, 89229).

Culture of colonies

Infertile colonies of *Bimeria vestita*, *Bougainvillia muscus*, *Leuckartiara octona*, *Parawrightia robusta* and *Turritopsis nutricula* were collected from the intertidal zone in São Sebastião, São Paulo State, Brazil. The colonies were carefully cut into small pieces and maintained on glass plates in plastic boxes containing aerated seawater at room temperature (22.3 ±1.4 °C) with artificial lighting (15-16 h light, 9-8 h dark). Seawater was changed every three days, and animals were fed twice a day with plankton or nauplii of the brine shrimp (*Artemia salina*).

In order to study the development of the exoskeleton, the colonies were maintained under two different conditions: A, with unfiltered sea water (21.9 ± 0.8 °C); and B, with filtered sea water (22.2 ± 0.6 °C). In the latter experiment, the seawater was filtered using a <25 µm filter, and the animals were fed individually in small finger-bowls to avoid contamination with organic and inorganic particles. In this experiment, the seawater was changed and the glass plates cleaned each day.

Results

Longitudinal sections of polyps revealed three morphologically distinct regions, the basal hydrorhiza (formed by stolons), median hydrocaulus (= stem, stalk), and distal hydranth (Fig. 1A,B). Early stages of colonial development already have five regions, the free stolon/branch, hydrorhiza, side-branch, stolonal hydranth/developing polyp, and terminal hydranth (Fig. 1C).

The epidermal layer consists of muscular epithelial cells, nerve cells, glandular cells, cnidoblasts, and cnidae (Fig. 2). The epidermal layer is thin in the region of the tentacles and gonophores. The thin, acellular mesoglea extensively underlies the polyp epidermis (Fig. 2), and is thinner at the tentacles, although this thickness varies greatly when the polyp contracts. The gastrodermis is a thick layer, mainly containing vacuolated digestive-muscle cells, but also zymogen glandular cells, mucous glandular cells, interstitial (i-cells), and sensory cells (Fig. 2), as well as some cells that most likely correspond to zooxanthellae. The gastrodermis of the tentacles is solid, formed by two layers of muscular epithelial cells tightly adhered to each other; the nuclei of these cells are distally placed and thus close to each other.

We provide below a detailed histological/histochemical description of the exoskeleton in Bougainvilliidae and other members of Hydroidolina, as well as the epidermal cells that may be associated with the exoskeleton and adjacent structures (Tables 1, 2).

The general organization of the exoskeleton in Bougainvilliidae Lütken, 1850

The general pattern in bougainvilliid polyps is the presence of three types of epidermal glandular cells, i.e., vacuolated glandular cells (PAS-positive cells; Fig. 3A); highly granulated glandular cells (HgBpB and NYS-positive cells; Fig. 3B,C); and mucous glandular cells (TB, PAS, HgBpB, and AB-positive cells; Fig. 3D,E). The exoskeleton of almost all species studied has inner (= perisarc) and outer (= exosarc) layers, and is therefore termed bilayered (cf. Mendoza-Becerril et al., 2015).

The inner layer is continuous from the hydrorhiza to the base of the hydrocaulus (Fig. 4A), rigid and laminated, sometimes reticulated or with a gelatinous ("non-rigid") appearance (4B). This layer may sometimes extend over the hydranth and even the tentacles (Fig. 4B,C). The inner layer has an affinity for TB (with a blue staining) (Fig. 4D), eosin (pink) (Fig. 4E), PAS (Fig. 4F), HgBpB (Fig. 4G) and NYS (Fig. 4H), and the intensity of the staining varies throughout the polyp (Table 1). This histochemistry suggests a chemical composition of AP associated with proteins. The inner layer of some species has an affinity for AB at the region of the hydrocaulus, while in others this layer has no affinity for PAS.

The outer layer is usually thick and rugose, extending from the hydrorhiza to the hydranth (Fig. 4A,C). This layer has an affinity for TB (with a purple staining) (Fig. 4D), and is PAS- (Fig. 4F) and AB-positive (Fig. 4I), suggesting a chemical composition of GAGs (Table 1). The outer layer is easily distinguished from the inner layer when treated with TB and AB techniques. However, it is difficult to identify when the inner layer is thin or when this outer layer has no external material attached. The two layers may be connected by an anchoring system formed by extensions from the inner layer ("perisarc extensions"; Fig. 4E).

***Bimeria vestita* Wright, 1859**

Material examined: LEM-IBUSP, Brazil, São Paulo, São Sebastião, Yacht Club Ilhabela, 23°46.37 S, 045°21.35 W, 2013, on shell, without gonophores, water depth <1 m, coll. M.A. Mendoza-Becerril. MZUSP 5201, Brazil, Paraná, Paranaguá, Ilha do Mel, 25°34.00 S, 048°18.00

W, 02.1997, on octocoral *Carijoa riisei*, with gonophores, coll. M.A. Haddad. UNMdP Hd3-38, Argentina, Mar del Plata, 38º4.55 S, 057º32.32 W, 10.08.1990, intertidal, without gonophores.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions". Inner layer rigid and laminated (Fig. 5A), moderately thick (hydrorhiza 14.2 µm, hydrocaulus 6.7 µm, side-branch 4.0 µm, hydranth 2.9 µm, gonophore 24.0 µm), continuous from hydrorhiza to tentacle base (Fig. 5B-C), frequently annulated on pedicels, or spirally corrugated at origin of side-branches (Fig. 5B,D), also covering gonophore but in this case not rigid (Fig. 5E). Inner layer stains with PAS, with higher positivity for HgBpB and NYS, and a small content of structural proteins weakly stained with HgBpB and NYS (Table 1). Outer layer thick (hydrorhiza 31.10 µm, hydrocaulus 34.6 µm, side-branch 3.1 µm, hydranth 11.0 µm, gonophore 21 µm), rugose and encrusted with thin organic and inorganic material (diatoms, sand grains, mud), therefore appearing granular and rigid (Fig. 5A,D). Outer layer extends from hydrorhiza to tentacle base (Fig. 5C), also on gonophore (Fig. 5E), becoming thinner (as a sheath) on tentacular bases and hypostome. Outer layer with affinity for TB, PAS and AB (Table 1).

Development: Polyps maintained in culture with both filtered and unfiltered seawater have the exoskeleton developed, with both layers (Fig. 5F). Inner layer thinner at apical hydranth region close to tentacular whorl and at tentacular base (Fig. 5F), weakly stained with HgBpB and NYS compared to hydrorhizal and hydrocauline regions. Stolonal hydrorhiza grows with thin inner and thick outer layers (Fig. 5G). Exoskeleton of developing polyp not detectable under stereomicroscope (Fig. 5H), requiring histological preparations for detection.

"*Bougainvillia glorietta*" Torrey, 1904

Material examined: USNM 43497, USA, California, San Pedro, Duffey's Float, 33°41.72' N, 118°18.47'W, 30.12.1901, with gonophores.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" (Fig. 6D). Inner layer laminated, irregularly corrugated (Fig. 6A), thick (hydrocaulus 13.9 µm, side-branch 7.3 µm, hydranth 12.8 µm, gonophore 7.1 µm); continuous from hydrorhiza to whorl of tentacles

(Fig. 6A,B), not laminated at gonophore (Fig. 6E-G). Inner layer stains with PAS, HgBpB and NYS (Table 1). Outer layer thick (hydrocaulus 11.83 µm, side-branch 21.7 µm, hydranth 74.6 µm, gonophore 0.8 µm), extending from hydrorhiza to tentacle base and encrusted with detritus (Fig. 6A-D). Outer layer exhibits affinities with TB and PAS, and higher affinity for AB (Table 1).

***Bougainvillia muscus* (Allman, 1863)**

Material examined: LEM-IBUSP, Brazil, São Paulo, São Sebastião, Yacht Club Ilhabela, 23°46.37 S, 045°21.35 W, Segredo Beach 23°49.68 S, 045°25.36 W, 2013, on shell and artificial substrate, without gonophores, water depth <1 m, coll. M.A. Mendoza-Becerril. MZUSP 4217, Brazil, Santa Catarina, Bombinhas, Tainha Beach, 27°12.97 S, 048°30.61 W, 02.12.2006, on Hydrozoa *Eudendrium* sp., with gonophores, coll. A.C. Marques and E. Ale. UNMdP Hd11-128, Argentina, Comodoro Rivadavia, 45°52.93 S, 067°29.06 W, 01.2013, without gonophores.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" (Fig. 7A-B). Inner layer smooth, laminated and reticulated, thick (hydrorhiza 25.2 µm, hydrocaulus 20.5 µm, side-branch 4.6 µm, hydranth 1.4 µm), continuous from hydrorhiza to whorl of tentacles (Fig. 7C-F) and stains with PAS, HgBpB and NYS (Table 1). Outer layer thin (hydrorhiza 5.4 µm, hydrocaulus 3.1 µm, side-branch 8.4 µm, hydranth 1.8 µm), undulated and encrusted with organic and inorganic material (diatoms, sand grains, mud), therefore with granular appearance (Fig. 7D,F). Outer layer extends from hydrorhiza to whorl of tentacles (Fig. 7E), partially covering the tentacles or fully covering them in contracted hydranth (Fig. 7F). Outer layer with affinity for TB, PAS and AB (Table 1).

Development: Polyps maintained in culture with both filtered and unfiltered seawater develop an evident exoskeleton, even in polyps 240 µm in height (Fig. 7G). Developing stolonal hydranth and developing stolon with only outer layer (Fig. 7H-J), encrusted with little external material (Fig. 7G-J).

***Bougainvillia rugosa* Clarke, 1882**

Material examined: MZUSP 4332, Brazil, Santa Catarina, Penha, Enseada da Armação do Itaporoy, 26°46.26 S, 048°36.48 W, 24.06.2005, polyp on *Perna perna*, without gonophores, water depth 2 m, coll. E.C. Bornancin.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" (Fig. 8A). Inner layer, smooth, laminated and reticulated (Fig. 8B), thick (hydrorhiza, 11.1 µm, hydrocaulus 14.7 µm, hydranth 1.6 µm, gonophore 2.9 µm), irregularly corrugated at origin of side-branches and at base of hydranth (Fig. 8B,C), continuous from hydrorhiza to whorl of tentacles (Fig. 8D), also covering gonophores (Fig. 8E). Inner layer stains weakly with PAS, moderately with HgBpB, and intensely with NYS (Table 1). Outer layer fairly thick (hydrocaulus 132.5 µm, side-branch 15.0 µm, hydranth 5.0 µm, gonophore 3.9 µm), undulating and encrusted with inorganic material (detritus), therefore with granular and rigid appearance (Fig. 8B). Outer layer extends from hydrorhiza to whorl of tentacles (Fig. 8D), also covering gonophore (Fig. 8E). Outer layer with affinity for TB, PAS and AB (Table 1).

***Dicoryne conferta* Alder, 1856**

Material examined: USNM 20234, USA, Massachusetts, Gloucester Harbor, 42°35.43' N, 070°40.40 W, 16.08.1878, on Mollusca, with gonophores, water depth 110 m, coll. United States Fish Commission. USNM 2499, no data. USNM 43967, Canada, Newfoundland, south of Peter's Bank, 47°19.45' N, 056°46.91' W, 04.06.1885, with gonophores, water depth 368 m.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" (Fig. 8F). Inner layer irregularly corrugated and thick (hydrocaulus 18.9 µm, side-branch 9.6 µm, gonophore 9.8 µm), continuous from hydrorhiza to lower part of hydranth (Fig. 8G-H), also on gonophore (Fig. 8I), although not rigid. Inner layer strongly PAS-positive, stains weakly with AB, HgBpB and NYS (Table 1). Outer layer thick (hydrocaulus 16.3 µm, side-branch 9.4 µm, gonophore 5.6 µm), wrinkled and with inorganic material (detritus). Outer layer extends from hydrorhiza to lower part of hydranth (Fig. 8F-H), also on gonophore (Fig. 8I). Outer layer with affinity for TB, PAS and AB (Table 1). Blastostyle without exoskeleton.

***Garveia annulata* Nutting, 1901**

Material examined: USNM 71026, USA, California, Monterey Bay, Carmel Point, 36°32.31 N, 122°0.77 W, 31.10.1978, with gonophores, intertidal, coll. J. Cooper.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" (Fig. 9A), forming 'exoskeletal connections' between layers mainly at hydrocaulus and side-branches (Fig. 9B). Inner layer strongly laminated and rigid, corrugated, thick (hydrorhiza 42.7 µm, hydrocaulus 11.7 µm, hydranth 14.1 µm, gonophore 28.5 µm), continuous from hydrorhiza to whorl of tentacles (Fig. 9C,D), also covering hydranth (Fig. 9C) and gonophore (Fig. 9E-G) but in this case not rigid. Inner layer stains with PAS and weakly with AB, but intensely with HgBpB and NYS (Table 1). Outer layer moderately thick (hydrocaulus 9.2 µm, hydranth 2.7 µm, gonophore 4.0 µm), encrusted with organic (diatoms on base of hydrocaulus) and inorganic material (Fig. 9A,B,D), therefore with granular appearance. Outer layer extends from hydrorhiza to whorl of tentacles, being discontinuous in hydrocaulus. Outer layer stains very weakly with AB (Table 1). Gonophore with desmocytes (Fig. 9G).

***Garveia franciscana* (Torrey, 1902)**

Material examined: USNM 43496, USA, California, Martinez, 38°0.99 N, 122°24.71 W, with gonophores. USNM 89229, Panama, Gulf of Panama, 8°11.26 N, 079°33.42 W, 26.08.1974, with gonophores.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" (Fig. 9H). Inner layer laminated and reticulated, thick (hydrocaulus 24.9 µm, side-branch 14.9 µm, hydranth 3.6 µm, gonophore 6.0 µm), continuous from hydrorhiza to whorl of tentacles, irregularly corrugated, annulated on gonophore pedicels and at origins of side-branches (Fig. 9I,J), also covering hydranth and gonophore but in this case not rigid (Fig. 9K). Inner layer stains intensely with PAS, HgBpB and NYS, and weakly with AB (Table 1). Outer layer granular, thick (hydrocaulus 17.0, side-branch 11.8 µm, hydranth 9.9 µm, gonophore 6.2 µm), encrusted with organic and inorganic material (Fig. 9H,I), also covering gonophores. Outer layer stains with AB.

***Garveia gracilis* (Clark, 1876)**

Material examined: USNM 43330, Panama, Gulf of Panama, 8°11.26 N, 079°33.42 W, 29.03.1973, without gonophores, S. Hildebrand.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" and exoskeleton with desmocytes (Fig. 10A). Inner layer laminated and reticulated, thick (hydrocaulus 20.9 µm, side-branch 4.2 µm, hydranth 3.6 µm), continuous from hydrorhiza to whorl of tentacles (Fig. 10B), annulated at origin of side-branches (Fig. 10C,D), not rigid at hydranth. Inner layer stains with PAS and intensely with NYS (Table 1). Outer layer thin (hydrocaulus 3.1 µm, side-branch 11.9 µm, hydranth 28.0 µm), densely encrusted with detritus (Fig. 10A,B), continuous from hydrorhiza to whorl of tentacles, also may fully cover contracted hydranth and tentacles (Fig. 10B). Outer layer shows affinity for TB and PAS, and moderate affinity for AB (Table 1).

***Garveia nutans* Wright, 1859**

Material examined: USNM 29449, United Kingdom, England, Plymouth Sound, 50°20.77' N, 004°8.87' W, with gonophores, coll. G.E. Bullen.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" and exoskeleton with desmocytes (Fig. 10E). Inner layer laminated, irregularly corrugated (Fig. 10F) and thick (hydrocaulus 16.4 µm, hydranth 3.6 µm, side-branch 12.4 µm, gonophore 1.6 µm), continuous from hydrorhiza to whorl of tentacles, also covering gonophore but in this case not rigid, also not rigid on hydranth (Fig. 10G,H). Inner layer moderately to intensely stained with PAS, HgBpB and NYS (Table 1), with strong affinity for AB (unlike other species). Outer layer thick (hydrocaulus 10.5 µm, side-branch 5.8 µm, hydranth 8.8 µm, gonophore 1.8 µm), continuous from hydrorhiza to whorl of tentacles, also covering gonophore (Fig. 10G,H), encrusted with detritus, therefore with rigid granular appearance (Fig. 10F). Outer layer with affinity for TB and PAS, and strong affinity for AB (Table 1).

***Pachycordyle michaeli* (Berrill, 1948)**

Material examined: MZUSP 1832, USA, Maine, Port Harbor Marine, 43°38.46' S, 070°13.34' W, 28.07.2007, on rock, with gonophores, coll. A.C. Marques.

Description: Corneous exoskeleton (chitin-protein) thick (hydrocaulus base 15.4 µm, hydrocaulus 13.7 µm, gonophore 9.8 µm), laminated with distinct series of sheets (Fig. 11A), corrugated, continuous from hydrorhiza to base of hydranth, irregularly corrugated at hydranth (Fig. 11B,C), also covering gonophore where laminae are more consolidated (Fig. 11D). Exoskeleton encrusted with diatoms, particularly at hydrorhiza (Fig. 11E). Layer with strong affinity for PAS and moderate affinity for HgBpB and NYS (Table 1).

***Parawrightia robusta* Warren, 1907**

Material examined: LEM-IBUSP, Brazil, Pará, Atalaia Beach, 00°35.60' S, 047°18.71' W, 04.07.2012, on rock, without gonophores, intertidal zone, coll. A.F. Cunha and M.A. Mendoza-Becerril. LEM-IBUSP, Brazil, São Paulo, São Sebastião, Yacht Club Ilhabela, 23°46.37 S, 045°21.35 W, 2013, on ascidian, without gonophores, water depth <1 m, coll. M.A. Mendoza-Becerril. MZUSP 4379, Brazil, Santa Catarina, Itapoá, 26°07.016' S, 048°36.967' W, 25.10.2003, on ascidian, without gonophores, intertidal zone, coll. M.A. Haddad.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" (Fig. 12A,B). Inner layer laminated and reticulated, irregularly corrugated, moderately thick (hydrocaulus 7.0 µm, hydranth 5.1 µm), continuous from hydrorhiza to whorl of tentacles (Fig. 12A-C), not rigid on hydranth (Fig. 12C). Inner layer stains with PAS and intensely with HgBpB and NYS (Table 1). Outer layer rugose and fairly thick (hydrocaulus 96.6 µm, hydranth 15.4 µm), continuous from hydrorhiza to whorl of tentacles (Fig. 12A-D), encrusted with inorganic and organic material, therefore with rigid granular appearance. Outer layer with affinity for TB, PAS and AB (Table 1).

Development: Polyps maintained in culture with filtered and unfiltered seawater have the developed exoskeleton with both layers. Inner layer thinner, not rigid, with vertical divisions in

some regions of hydrocaulus (Fig. 12E). Inner layer with secretory granules positive for HgBpB (Fig. 12F,G). Hydranth with thin exoskeleton (Fig. 12H) and developing polyps with secretory granules in epidermal glandular cells (Fig. 12I).

***Rhizorhagium* sp.**

Material examined: USNM 42339, USA, Washington, Puget Sound, 47°42.05' N, 122°28.18' W, without gonophores.

Description: Bilayered exoskeleton. Inner layer rigid and laminated, occasionally corrugated at hydrocaulus (Fig. 13A), thick except at tentacles (hydrorhiza 18.5 µm, hydrocaulus base 14.9 µm, hydrocaulus 10.3 µm, hydranth 2.1 µm, tentacle 2.1 µm), continuous from hydrorhiza to tentacles (Fig. 13B-D). Inner layer with agglutinated organic particles (e.g., diatoms; Fig. 13A). Inner layer stains intensely with hematoxylin and PAS and moderately with HgBpB and NYS (Table 1). Outer layer not rigid, generally thin (hydrorhiza 18.6 µm, hydrocaulus base 8.5 µm, hydrocaulus 1.4 µm, hydranth 2.7 µm), except hydrocaulus base covered with detritus and diatoms. Outer layer continuous from hydrorhiza to hydranth (Fig. 13A-C); stains with TB, PAS and AB.

Exoskeleton organization in some other anthoathecate families

Eudendriidae L. Agassiz, 1862

***Eudendrium carneum* Clarke, 1882**

Material examined: MZUSP 1673, Brazil, Alagoas, Barra de São Miguel, 09°50.00' S, 035°53.08' W, 22.10.2006, water depth 0-3 m, coll. A.C. Marques.

Epidermal cells: Hydrocaulus with vacuolated glandular cells with affinity for TB and PAS (Fig. 13E), with mucous glandular cells, abundant at side-branches, stain positively for H and AB (Fig. 13F).

Description: Bilayered exoskeleton, layers rigid, slightly corrugated at side-branch origin (Fig. 13E-H), with invagination at hydranth base (Fig. 13I). Inner layer laminated and reticulated (Fig.

13G), moderately thick (hydrocaulus base 27.4 µm, hydrocaulus 8.9 µm, side-branch 2.9 µm), continuous from hydrorhiza to hydranth base, also covering gonophore (Fig. 13J). Inner layer positive for PAS, with higher affinity for HgBpB and NYS (Table 1). Outer layer homogeneous, moderately thick (hydrocaulus base 4.4 µm, hydrocaulus 5.3 µm, side-branch 1.2 µm), continuous from hydrorhiza to hydranth base (Fig. 13G,I). Outer layer stains intensely with AB, similar to outer layer of other bougainvilliids although with different coverage, i.e., not extending over hydranth. Hydrocaulus base encrusted with few organic and inorganic particles, similar to a third layer.

Oceaniidae Eschscholtz, 1829

***Turritopsis nutricula* McCrady, 1857**

Material examined: LEM-IBUSP, Brazil, São Paulo, São Sebastião, Segredo Beach, 23°49.68'S, 045°25.36' W, 03.11.2013, on shell, water depth <1 m, without gonophores, coll. M.A. Mendoza-Becerril.

Epidermal cells: Comprising glandular cells with affinity for PAS, stain intensely with HgBpB and weakly with NYS (Fig. 14A,B); i-cells (Fig. 14C); and possibly undifferentiated cells (Fig. 14C-E).

Description: Exoskeleton semi-transparent in developing polyps, pale cream-colored in developed polyps. Exoskeleton corneous (chitin-protein), moderately thick (hydrorhiza 5.4 µm, hydrocaulus 9.1 µm, hydranth 1.4 µm), continuous from hydrorhiza to lower part of hydranth (below tentacles) (Fig. 14F), occasionally corrugated in older polyps (Fig. 14G) and encrusted with organic and inorganic material under natural conditions, especially at hydrorhiza and hydrocaulus base. Exoskeleton stains intensely with PAS and HgBpB, weakly with NYS (Table 1). Exoskeleton covered with membrane (Fig. 14G-I), which stains weakly with AB, suggesting low concentration of GAGs. Membrane encrusted with abundant external material, with granular appearance, irregularly continuous from hydrorhiza to lower part of hydranth.

Development: Polyps maintained in culture with both filtered and unfiltered seawater have exoskeleton not rigid, thin (developing polyp 2.1 µm) (Fig. 14J). Exoskeleton with affinity for PAS but not for AB, suggesting only AP present. Polyps maintained in culture with unfiltered seawater developed membrane over exoskeleton but without encrusted material attached; polyps maintained in culture with filtered seawater developed no membrane. Therefore, this membrane not equivalent to outer layer present in Bougainvilliidae.

Pandeidae Haeckel, 1879

Leuckartiara octona (Fleming, 1823)

Material examined: LEM-IBUSP, Brazil, São Paulo, São Sebastião, Segredo Beach, 23°49.68' S, 045°25.36' W, 13.11.2013, on shell of *Strombus* sp., water depth 4 m, without gonophores, coll. J.M. Oliveira and A.A.W. Monteiro.

Epidermal cells: Hydrocaulus with i-cells (Fig. 15A), epithelio-muscular cells in hydranth with thin granular film apically (Fig. 15B), similar granules observed in developing stolon and developing polyp.

Description: Bilayered exoskeleton, layers connected by "perisarc extensions" (Fig. 15C,D), exoskeleton with desmocytes (Fig. 15E). Inner layer rigid and laminated, moderately thick (hydrorhiza 9.6 µm, hydrocaulus 9.2 µm, hydranth base 2.8 µm, hydranth 1.6 µm, tentacle 1.1 µm), irregularly corrugated at hydrocaulus (Fig. 15A,C-E) and continuous from hydrorhiza to tentacles (Fig. 15E,F). Inner layer stains with PAS, but more intensely with HgBpB and NYS (Table 1). Outer layer thick (hydrorhiza 29.4 µm, hydrocaulus 16.8 µm, hydranth base 9.0 µm, distal part of hydranth 67.5 µm), undulateing (Fig. 15C), encrusted with organic (diatoms) and inorganic material (detritus) (Fig. 15C-J). Outer layer extends from hydrorhiza to whorl of tentacles (Fig. 15E,F). Outer layer with affinity for TB, PAS and AB (Table 1). Exoskeleton at free stolon/branch (Fig. 15I), developing stolon (Fig. 15G), and developing polyp (Fig. 15H) with single "non-rigid" layer, encrusted with external material.

Development: Polyps maintained in culture with both filtered and unfiltered seawater with exoskeleton developed with both layers. Outer layer with slightly different staining intensity with TB and AB compared to material developed under natural conditions (Table 1).

Pennariidae McCrady, 1859

***Pennaria disticha* Goldfuss, 1820**

Material examined: LEM-IBUSP, Brazil, São Paulo, São Sebastião, Pitangueiras Beach, 23°49.48'S, 045°25.19' W, 23.09.2013, on rock, water depth 1 m, without gonophores, coll. M.A. Mendoza-Becerril.

Epidermal cells: Epidermis without evident glandular cells in developed polyps.

Description: Bilayered semi-transparent or opaque exoskeleton. Inner layer laminated, fairly thick (hydrorhiza 75.5 µm, hydrocaulus 59.0 µm, annulation region 81.9 µm, side-branch 14.7 µm), continuous from hydrorhiza to hydranth base then ending abruptly, annulated in basal regions (hydrocaulus and side-branches) and throughout hydrocaulus at more or less regular intervals (Fig. 16A,B). Inner layer stains intensely with HgBpB, NYS and PAS (Table 1). Outer layer not rigid, thin (hydrorhiza 2.4 µm, hydrocaulus 25.1 µm, annulation region 12.2 µm, side-branch 1.2 µm, lower part of hydranth 1.1 µm), continuous from hydrorhiza to hydranth base (Fig. 16C). Outer layer stains intensely with AB (Fig. 16A,B). Apical region of developing side-branch covered by outer layer, not rigid, thin (1.2 µm), stains with AB, therefore with same chemical composition (GAGs) as outer layer of hydranth base. Developing side-branches with thin inner layer, annulated in distal region (Fig. 16D-F), perhaps indistinctly laminated in proximal part (Fig. 16D).

Exoskeleton organization in leptothecates

Campanulariidae Johnston, 1836

Epidermal cells: Hydrorhiza and hydrocaulus with glandular cells, with affinity for TB, PAS, HgBpB and NYS (Table 1; Fig. 17A,B), abundant at hydrocaulus and gonophore base (Fig. 17C). I-cells rarely observed (Fig. 17B).

Description: Exoskeleton corneous (chitin-protein), laminated, covering different regions of polyp (Fig. 17A,C-F), sometimes with associated organic (diatoms) and inorganic material (Fig. 17A). Exoskeleton with affinities for TB, PAS, HgBpB and NYS in some species, but with weak affinity for AB (Table 1). Association with diatoms may depend on environmental conditions.

***Clytia gracilis* (M. Sars, 1850)**

Material examined: MZUSP 4210, Brazil, Santa Catarina, Bombinhas, Tainha Beach, 27°12.97' S, 048°30.61' W, 02.12.2006, on *Eudendrium carneum*, water depth 5-7 m, with gonophores, coll. A.C. Marques and E. Ale.

Description: Exoskeleton corneous (chitin-protein), semi-transparent, laminated, smooth, thick (hydrorhiza 11.6 µm, hydrocaulus 4.9 µm, annular thickening 7.5 µm, hydrotheca 3.7 µm, diaphragm 6.4 µm, gonotheca 7.5 µm), annulated in distal and proximal regions of hydrocaulus (Fig. 18A-C); hydrotheca with transverse diaphragm and triangular cusps (Fig. 18A). Exoskeleton stains weakly with PAS, HgBpB, NYS and AB (Table 1). External region (in contact with environment) stains more intensely with AB than internal part (in contact with coenosarc), showing larger amount of GAGs or other substances produced either by diatoms associated with exoskeleton or by natural substrates (e.g., mollusks). Polyps of *Clytia* sp. maintained in culture developed thin, AB-negative exoskeleton.

***Obelia dichotoma* (Linnaeus, 1758)**

Material examined: LEM-IBUSP, Brazil, Pará, Salinópolis, Farol Velho Beach, 00°35.46’ S, 047°19.48’ W, 03.07.2012,with gonophores, on rock, at water surface, coll. A.F. Cunha and M.A. Mendoza-Becerril. Slovenia, Piran, Farol Amarelo, 45°35.66’ N, 013°42.28’ E, 01.08.2011, without gonophores, coll. A.C. Morandini and L.S. Miranda. USA, Massachusetts, Westport, 41°30.21’ N, 076°3.77’ W, 26.07.2010, without gonophores, coll. A.C. Marques.

Description: Exoskeleton corneous (chitin-protein), laminated, moderately thick (hydrorhiza 9.7 µm, hydrocaulus 9.7 µm, diaphragm 2.2 µm, annular thickening 13.8 µm, hydrotheca 2.9 µm,

gonotheca 2.5 µm), continuous from hydrorhiza to hydranth, annulated on internodes and origin of side-branches (Fig. 19A-C). Exoskeleton with oblique diaphragm and smooth rim at distal region of hydrocaulus, entirely covering hydranth when retracted (= hydrotheca) and gonophore (= gonotheca; Fig. 19A,D). Exoskeleton stains with TB, HE, PAS, HgBpB and NYS (Table 1). Exoskeleton of specimens from USA with vertical epidermis-parallel marks (Fig. 19E) and lateral fold on each side at diaphragm level (Fig. 19F). Exoskeleton of specimens from Pará and Slovenia with diatoms attached, mainly at hydrorhiza, with rigid appearance. Diatoms stain weakly with PAS and intensely with AB (Fig. 19G,H).

***Orthopyxis sargassicola* (Nutting, 1915)**

Material examined: MZUSP 1740, Brazil, Alagoas, Maceió, 09°40.84' S, 035°42.67' W, 25.10.2006, water depth 0-2 m, with gonophores, coll. A.C. Marques.

Description: Exoskeleton corneous (chitin-protein), rigid and laminated, moderately thick (hydrorhiza 11.6 µm, hydrocaulus 9.1 µm, spherule 6.1 µm, annular thickening 19.5 µm, hydrotheca 3.0 µm, gonotheca 6.7 µm, annulation of gonotheca 45.2 µm), continuous from hydrorhiza to hydranth, annulated or sinuous throughout or only at proximal and distal ends of polyp (Fig. 20A-D). Distal region of hydrocaulus with spherule (= subhydrothecal spherule), with basal annular thickening immediately above spherule (Fig. 20B). Contracted hydranth fully covered by exoskeleton (=hydrotheca), with cusps at border (Fig. 20B). Gonophore covered by exoskeleton (= gonotheca), with marked annulations (Fig. 20E). Exoskeleton stains with TB, HE, PAS, HgBpB and NYS (Table 1). Some regions of polyp, especially hydrocaulus base, with thin exoskeletal membrane covering, with associated diatoms (Fig. 20F). Membrane stains weakly with PAS and AB.

Haleciidae Hincks, 1868

***Halecium bermudense* Congdon, 1907**

Material examined: LEM-IBUSP, Brazil, São Paulo, São Sebastião, 23°45.17' S, 045°24.41 W, 08.2011, on artificial substrate, at water surface, with gonophores, coll. M. Fernandez.

Epidermal cells: Hydrocaulus with vacuolated glandular cells with affinity for TB, PAS, and HgBpB (Fig. 21A), abundant in developing polyps.

Description: Exoskeleton corneous (chitin-protein), rigid but not laminated, thin (hydrocaulus base 15.2 µm, hydrocaulus 4.3 µm, side-branch 2.0 µm, hydrotheca 1.3 µm, gonotheca 12.9 µm), continuous from hydrorhiza to lower part of hydranth (Fig. 21B), also on gonophores (= gonotheca) (Fig. 21C). Exoskeleton stains intensely with PAS and HgBpB, weakly with NYS (Table 1). Exoskeleton forming internodes throughout polyp, primary hydrotheca, secondary hydrotheca and pedicel of secondary hydrotheca at hydranth base and lower part of hydranth (Fig. 21D-F). Hydrotheca with thin diaphragm and desmocytes (Fig. 21F).

Discussion

Organization of epithelia and their cells

General aspects of the histology of the coenosarc concord with previous observations (e.g., Congdon, 1906; Cowden, 1965; Chapman, 1974; Thomas and Edwards, 1991), although we are able to contribute new interpretations and data.

The nature of some cells remains to be confirmed. Cells of uncertain nature are present in the gastrodermis of *Clytia* sp. and *Halecium bermudense*. They may correspond to zooxanthellae, widely distributed symbionts of cnidarians, which are present in hard and soft corals, sea anemones, jellyfish, cubozoan polyps, and hydrocorals (cf. Taylor, 1968; Calder, 1982; Marques et al., 2000; Simon et al., 2012; Straehler-Pohl and Jarms, 2011; Weng et al., 2014). These dinoflagellates are typically associated with the gastrodermal epithelium of the host cnidarians (Muscatine, 1974; Marques et al., 2000).

The coenosarc of *Turritopsis nutricula* also contains an unidentified cell type that might correspond to undifferentiated cells, such as those observed in *Hydra* (Siebert et al., 2008). They are present from the hydrorhiza (abundantly) to the hydranth, and also in the epidermis of developing polyps. These cells are intensely basophilic when stained with HE, possibly due to free ribosomes, which are characteristic of cells that are about to differentiate. For other taxa, i-cells are commonly found grouped at the base of the hydrocaulus, where they differentiate into nematocysts or glandular cells, the latter participating in the production of different substances

forming the exoskeleton. Therefore, the base of the hydrocaulus may be important for both cnidogenesis and skeletogenesis in Hydrozoa in general.

The three types of glandular cells (vacuolated, granulated and mucous) were more abundant in developing polyps of the majority of the species examined here, but were not observed in polyps of *Dicoryne conferta* and *Pennaria disticha.* Histochemical tests indicated that the gastrodermal glandular cells of the hypostome also continually produce and contain GAGs [cf. *Syncoryne tenella* (accepted as *Coryne tenella)* (Wineera, 1972); *Hydra* (Wood, 1979)]. This is a different condition from the GAGs produced by epidermal glandular cells. These substances may correspond to enzymes (Cowden, 1965), although we were unable to test this hypothesis.

Different enzymatic types and activities are specific for each function and region of the polyps, and the enzymes are PAS- and/or AB-positive. The enzyme acid phosphatase has also been recorded in species of Leptothecata (Östman, 1982). Other important enzymes participating in exoskeleton formation have been recorded in several hydrozoans (see Mendoza-Becerril et al., 2015). Chitin synthetase (Chs) is found in *Hydractinia echinata* (Mali et al., 2004). Chitinase is restricted to the gastrodermis of the hydrocaulus and absent in the epidermis and tentacles of *Podocoryna carnea* (accepted as *Hydractinia carnea*) and *Hydra attenuata* (accepted as *Hydra circumcincta*) (Klug et al., 1984). Phenoloxidase, produced in epidermal cells of *Laomedea flexuosa*, is involved in cross-linking of perisarc components (Knight, 1970; Kossevitch et al., 2001).

Our results corroborate the hypothesis that the coenosarc of hydrozoan polyps does not have a fixed composition of cell types. During development, different types of cells constantly migrate from specific areas of cell differentiation and proliferation to their final location (Chapman, 1974; Thomas and Edwards, 1991; Kosevich, 2013). Thus, cell action depends on a definite sequence of events, including cell multiplication, cell differentiation, and cell migration (see Kosevich, 2013 for *Gonothyraea loveni*). Also, the different epidermal glandular cells involved in exoskeletal development can change the type and secretion of one or several chemical components as the polyp grows (Kosevich, 2013).

Organization of the exoskeleton

Our findings confirm that polysaccharides are the basic and predominant chemical component of the exoskeleton (Knight, 1970; Kossevitch et al., 2001, Mendoza-Becerril et al., 2015) (Fig. 22A), produced by epidermal i-cells (Fig. 22B). These polysaccharides can combine with units of amino-sugars, forming aminopolysaccharides (AP) (Ruiz-Herrera and Ortiz-Castellanos, 2010) (PAS-positive); amino-sugars and hexuronic acid, forming glycosaminoglycans (GAGs) (Frazier et al., 2008; Yamada et al., 2011) (PAS-positive and AB-positive); or only with structural proteins (glycoproteins) (BeMiller, 2008) (HgBpB and NYS-positive) (Table 1). The predominant exoskeletal component is the AP, in the form of chitin (cf. Mendoza-Becerril et al., 2015), but there are variations in the chemical composition and in the physical properties related to adaptations to particular conditions and physiological changes in the organism, such as the tanning process (Chapman, 1973).

We documented two types of structural exoskeleton, (a) the bilayered exoskeleton formed by an inner layer of perisarc surrounding the coenosarc and covered by an outer layer of exosarc in contact with the environment (Fig. 22C), and (b) a single coriaceous exoskeleton formed exclusively by the perisarc. The perisarc and exosarc vary in their chemical composition (AP or GAGs; Table 2), structural rigidity (Fig. 22D), and thickness, extension and coverage of different regions of the colony (Fig. 22E). A thick exosarc is generally derived from the aggregation of extraneous inorganic (sand and mud grains) and organic material (tests of radial centric and araphid pinnate diatoms). These extraneous materials lend a rigid granular appearance to the exoskeleton of Bougainvilliidae and Pandeidae (Fig. 22F).

All the species of Bougainvilliidae that we studied have a bilayered exoskeleton. The only, and doubtful, exception was *Pachycordyle michaeli*, although we found a discontinuous thin layer in its hydrorhiza and hydrocaulus, perhaps corresponding to a tenuous exosarc. However, descriptions of *P. michaeli* consider the "pseudohydrotheca" (= exosarc on the hydranth) absent in this species (Schuchert, 2007).

Gonophores of the bougainvilliids studied (except *P. michaeli*) are completely enclosed by a bilayered exoskeleton, even in the species that have been described with an exoskeleton that covers only the gonophore pedicels, such as *Garveia annulata* (Nutting, 1901). The exoskeleton

completely surrounding the gonophore is described in the literature as a filmy perisarc, loose filmy perisarc, or thin perisarc membrane (Schuchert, 2007).

Epidermal glandular cells of the colonies of Bougainvilliidae (*Bimeria vestita*, *Bougainvillia muscus*, and *Parawrightia robusta*) and Pandeidae (*Leuckartiara octona*), forming the MM, apparently are differentiated at the developing border of the free stolons/branch, hydrorhiza, side-branch, stolonal and terminal hydranths. Developing extremities of growing polyps and hydranths of some bougainvilliids were covered by a "non-rigid" layer of GAGs, while regions near their origin were covered by a "non-rigid" exoskeleton formed by an exosarc, constituted predominantly by GAGs, and a perisarc with AP and proteins. This exoskeleton also had particles and thin filaments extending outward. Therefore, the "non-rigid" layer may correspond to MM, and this "non-rigid" exoskeleton may be involved in the process of formation of a rigid, bilayered, exoskeleton (perisarc and/or exosarc). Particles and thin filaments present in the exoskeleton would serve to harden this structure.

The exosarc is produced first, and may be an important step in the formation of the rigid perisarc. The exosarc can interact with other molecules (e.g., AP, structural proteins) functioning as a single layer in developing polyps but as two separate layers in developed polyps. This hypothesis is supported by the presence of AB-positive granules in the epidermal glandular cells at the base of the hydrocaulus of *Garveia franciscana*, and of TB- and PAS-positive granules in the skeletal outer layer and epidermal glandular cells of the developing hydranth of *L. octona*. The MM with acid polysaccharides is an important element in the mineralization process in the stony coral *Mycetophyllia reesi* (Goldberg, 2001).

Variations in staining intensity suggest the presence of different concentrations of the chemical components, depending on the developmental stage of the polyps. The presence of GAGs in the perisarc of *Garveia annulata*, *Eudendrium carneum*, and *Clytia gracilis* indicates that acidic GAGs are trapped within the inner layer, sclerotized in the presence of proteins. The mix of GAGs and proteins (glycoproteins) is common in mollusk shells (Marxen et al., 1998), functioning as possible calcium-binding sites or playing a role in the nucleation and growth inhibition of the mineral (Marin et al., 1996).

A perisarc was formed in all species studied, although the perisarc was sometimes weakly stained with PAS and intensely with NYS in bougainvilliids (especially *Bougainvillia rugosa*).

Chitin reacts negatively with PAS, but positively in protein tests if it is present as protein complexes (Pearse, 1985). Chitin does not occur in its pure form in nature, but always mixed with protein and/or other chemical substances (Pearse, 1985). However, the absence of chitin in a tissue sample does not necessarily indicate that the species is not able to produce chitin, because it may be modified (deacetylated or sulfated) and undetectable by classical histochemical methods (Wagner, 1994). Indeed, chitin has been found in the perisarc as part of the hydroidoline exoskeleton (e.g., *Laomedea flexuosa*, Knight, 1970; *Aglaophenia latirostris*, Hwang et al., 2013). Wagner (1994) proposed that the most straightforward ways to address this problem are to test for chitin synthase activity or to search for genes with sequences similar to chitin synthases.

The perisarc has been described as multilayered in the hydrorhiza and hydrocaulus (Wineera, 1972), although the laminae can be either continuous or discontinuous (Fig. 4A, F). The laminated perisarc (Fig. 4B) has multiple discontinuous fibers, extending in parallel through the length of the thick, rigid perisarc. In addition, the laminar perisarc can be reticulated, with grooves perpendicular to the fibers (Fig. 4B). The laminated and reticulated appearance is most likely a consequence of different degrees of stabilization and hardening during the polymerization (Berrill, 1949) or of sclerotization process (Knight, 1970), and it is more frequent in developed polyps because of the higher concentration and interaction of molecules incorporated into the MM (Congdon, 1906). However, our understanding of the chemistry of exoskeleton sclerotization has not much improved since Knight (1970), who suggested that "tanning cells" (observed in the Leptothecate *Laomedea flexuosa*) are essential to the process. Therefore, many important questions remain unanswered, especially regarding the precise regional and temporal regulation of the various steps in the process.

We observed secretory granules (HgBpB-positive) scattered in the hydrocaulus of developing polyps of *P. robusta*, and glandular cells (PAS-positive) in new side-branches of *Pennaria disticha*, as well as in developing polyps of *Turritopsis nutricula* and the hydrocaulus of *Clytia* sp. The presence of secretory granules and glandular cells, and their reaction to different chemical tests are indicative of a sclerotization process in “Anthoathecata”, although specific studies are necessary to test this hypothesis.

The "non-rigid" exosarc is detected by AB pH 2.5, suggesting a chemical composition of GAGs (carboxylic groups). However, only some species (e.g., *B. rugosa*, *L. octona*) have

epidermal glandular cells with an affinity for AB. Therefore, the origin of the exoskeletal acidic GAGs is not clear. Some data support different hypotheses regarding their origin and variable composition in specific groups, such as the reactivity of AB pH 2.5 with GAGs influenced by specific GAG-associated properties (structure, purity, and other factors), and the failure to detect them among major heparin and unsulfated types of GAGs (Frazier et al., 2008).

Developing polyps have only an exosarc, suggesting that this is the first layer in the skeletal ontogenesis. Subsequently, epidermal cells differentiate, producing other specialized glandular cells and therefore changing the nature of the secreted compounds over time. Such changes may have prevented us from observing mucous glandular cells in some species, which would be capable of rapidly eliminating their secretions and developing into different cell types, as in *Hydra pseudoligactis* (accepted as *Hydra canadensis*), a species with epidermal cells that release acid GAGs (Burnett and Lambruschi, 1973).

The thin, discontinuous outermost cover observed in the exoskeleton of some leptothecates (*Obelia dichotoma* and *Orthopyxis sargassicola*), with an affinity for AB, has adhered diatoms (Fig. 19G,H). However, this cover is not part of the exoskeleton, and is not equivalent to the exosarc of bougainvilliids and some other members of "Anthoathecata". It is formed by diatoms, which secrete acid sugars in the form of uronic acids and sulfated sugars (Staats et al., 1999), whereas leptothecate polyps are incapable of producing GAGs independently.

We observed that the exoskeleton develops under culture with filtered seawater, and therefore its secretion is genetically encoded and innate (a putative MSS), and does not depend on age or environmental conditions, although some of its characteristics may change according to the habitat (Rees, 1956; Murdock, 1976; Hughes, 1980). Exoskeleton thickness, especially of the exosarc, depends on the quantity and type of material available under natural conditions, and food particles may also be a source of external material to be agglutinated into the exoskeleton. Some species of *Garveia* spp. have a polymorphic expression of the exosarc, and its presence or absence may be associated with preservation of the material or with differences in its habitat. The morphology of the exoskeleton can be modified by environmental conditions (Murdock, 1976; Hughes, 1980) and the amount of agglutinated external organic or inorganic material (Rees, 1956; Schuchert, 2007; Mendoza-Becerril et al., 2015).

Structural differences among exoskeletons result from the biological activity of living polyps, e.g., cellular activities related to nucleation, formation of new chemical components, growth, morphology, chemical deposition, and the transformation of the main stem of the hydranth into a stolon; and from the degree of contraction of the polyps. Different levels of contraction were observed in living and fixed polyps. A contracted hydranth of some species, such as *B. muscus* and *G. franciscana*, appears to be fully covered by the exoskeleton, even though their exoskeleton extends only from the hydrorhiza to the tentacular base of the hydranth; while other species, such as *G. nutans* and *P. robusta*, appear to be covered up to the whorl of tentacles, as delimited by a fold just below that, although the extended body clearly has free tentacles. Species such as *B. vestita* may have the hydranth completely covered by the exoskeleton even when fully extended, but may appear to have exoskeleton coverage similar to contracted hydranths of *B. muscus* or *G. franciscana*.

Another structural difference is the thickness of the exoskeletal layer, which varies intraspecifically in some "Filifera" from different locations (e.g., *G. franciscana*, Vervoort, 1964). Consequently, this structural variation may lead to misidentifications of the species, especially when other diagnostic characters are absent (e.g., reproductive structures), and should not be used as diagnostic characters for the taxonomy of groups such as Pandeidae and Bougainvilliidae (Millard, 1975; Calder, 1988). Nevertheless, the variation in thickness of the chitin-protein exoskeleton is considered a useful diagnostic character for some families of Leptothecata (e.g., Campanulariidae, Cunha et al., 2015).

Desmocytes (Fig. 22G) are specialized cells that are found along the upright hydrocauline coenosarc of the polyps and side branches of colonies of Bougainvilliidae, Eudendriidae, Pandeidae, and Haleciidae. They are characterized by a dense accumulation of chitin and protein filaments (Knight, 1970; Chapman, 1974), and therefore have a high affinity for PAS, HgBpB and NYS. These filaments aggregate into dense rods and reach the exoskeletal perisarc at the apical end of the desmocyte (Fig. 22G), and form rigid connections with the mesoglea at the basal end of the desmocyte (Fig. 22G).

Desmocytes (also termed "rivets" by Knight, 1970 and "anchors" by Buss et al., 2013) function similarly to other anchoring devices in Hydrozoa (e.g., Cordylophoridae *Cordylophora caspia*, Marcum and Diehl, 1978; Zancleidae *Zanclea margarita*, Pantos and Hoegh-Guldberg,

2011; Hydractiniidae *Podocoryna carnea*, Buss et al., 2013; Campanulariidae *Laomedea flexuosa*, Knight, 1970), Scyphozoa (Ulmariidae *Aurelia* sp., Lesh-Laurie and Suchy, 1991), and Cubozoa (Carybdeidae *Carybdea* sp., Mendoza-Becerril et al., 2015). However, these cells show structural differences that are directly related to the pattern of exoskeleton extension, colony growth, and symbiotic relationships. The reason for the apparent absence of these cells in some Hydroidolina that we studied might be that they are more evident when they are functional desmocytes, which bear many filaments and possess connections to both the mesoglea and the exoskeleton (Chapman, 1974; Marcum and Diehl, 1978). Moreover, considering that *Z. margarita* possesses desmocytes but apparently not an exoskeleton, we hypothesize that these structures are conserved in the Medusozoa as a possible reduction of the exoskeletal system of a MM but not of the MSS, therefore retaining the information to produce chemical substances (AP, GAGs and structural proteins) by the epidermal glandular cells. We suggest that the "perisarc extensions" are a still-undescribed type of anchoring system, acting in the adherence between the perisarc and exosarc. The “perisarc extensions” increase the rigidity of the exoskeleton, and are present along the upright hydrocaulus of the polyps. These structures were also described as “lamellar membranes” in the bougainvilliid *Garveia grisea* (Schuchert, 2007).

In conclusion, our study added to the knowledge of the hydrozoan exoskeleton, helping to understanding hydroidolinan evolution, but also left unanswered several questions on its structure and chemical composition: Which specific components are present within in the exoskeleton (e.g., glycoproteins, proteoglycans and hexuronic acids, more specifically, chondroitin sulfate and heparan sulfate)? What are the concentrations of the different chemical components and what are their chemical interactions? What are the biomechanical properties related to the different types of exoskeletons and their biological consequences? Further investigations applying immunohistochemistry (e.g., to identify the type of GAGs), confocal microscopy (e.g., using congo red as a fluorescence marker for chitin), and transmission electron microscopy and X-ray diffraction may help to answer these questions.

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References

Allman GJ. 1864. On the construction and limitation of genera among the Hydroida. Ann Mag Nat Hist 13:345-380.

BeMiller JN. 2008. Polysaccharides: Ocurrence, significance, and properties. In: Fraser-Reid B, Tatsuta K, Thiem J, editors. Glycoscience. Berlin: Springer-Verlag. p 1413-1435.

Berrill NJ. 1949. Growth and form in gymnoblastic hydroids. I. Polymorphic development in Bougainvillia and Aselomaris. J Morphol 84:1-30.

Berrill NJ. 1950. Growth and form in Calyptoblastic hydroids. II. Polymorphism within the Campanularidae. J Morphol 87:1-26.

Böttger A, Doxey AC, Hess MW, Pfaller K, Salvenmoser W, Deutzmann R, Geissner A, Pauly B, Alstätter J, Münder S, Heim A, Gabius H, McConkey B, David CN. 2012. Horizontal gene transfer contributed to the evolution of extracellular surface structures: the freshwater polyp *Hydra* is covered by a complex fibrous cuticle containing glycosaminoglycans and proteins of the PPOD and SWT (Sweet Tooth) families. PLoS ONE 7:e52278. DOI: 10.1371/journal.pone. 0052278.

Burnett AL, Lambruschi PG. 1973. Regeneration of a *Hydra* containing no interstitial cells from an isolated basal disc. In: Burnett AL, editor. Biology of *Hydra*. London: Academic Press. p 239-247.

Buss LW, Anderson C, Bolton EW. 2013. Muscular Anatomy of the *Podocoryna carnea* Hydrorhiza. PLoS ONE 8:e72221. doi:10.1371/journal.pone.0072221.

Cairns SD. 2011. Global Diversity of the Stylasteridae (Cnidaria: Hydrozoa: Athecatae). PLos ONE 6:e21670. DOI:10.1371/journal.pone.0021670.

Calder DR. 1982. Life history of the cannonball jellyfish *Stomolophus meleagris* L. Agassiz, 1860 (Scyphozoa, Rhizostomida). Biol Bull 162:149-162.

Calder DR. 1988. Shallow-Water Hydroids of Bermuda: The Athecatae. R Ont Mus Life Sci Contrib 148:1-107.

Cartwright P, Evans NM, Dunn CW, Marques AC, Miglietta MP, Schuchert P, Collins AG. 2008. Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). J Mar Biol Ass UK 88:1663-1672.

Chapman DM. 1974. Cnidarian histology. In: Muscatine L, Lenhoff HM, editors. Coelenterate Biology, Reviews and New Perspectives. New York: Academic Press. p 1-92.

Chapman G. 1973. A note on the composition of some coelenterate exoskeletal materials. Comp Biochem Physiol 45B:279–282.

Collins AG, Schuchert P, Marques AC, Jankowski T, Medina M, Schierwater B. 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. Syst Biol 55:97-115.

Congdon ED. 1906. Notes on the morphology and development of two species of Eudendrium. Biol Bull Mar Biol Lab Woods Hole 11:27-46.

Cornelius PFS. 1982. Hydroids and medusae of the family Campanulariidae recorded from the eastern North Atlantic, with a world synopsis of genera. Bull Br Mus Nat Hist Zool 42:37-148.

Cornelius PFS. 1995. North-west European thecate hydroids and their medusae. Part 1. Introduction, Laodiceidae to Haleciidae. London: Linnean Society of London and The Estuarine and Coastal Sciences Association. 386 p.

Cowden RR. 1965. A cytological and cytochemical study of hydranths of the hydroid coelenterate, *Pennaria tiarella*. Z Zellforsch 65:869-883.

Cunha AF, Genzano GN, Marques AC. 2015. Reassessment of morphological diagnostic characters and species boundaries requires taxonomical changes for the genus *Orthopyxis* L. Agassiz, 1862 (Campanulariidae, Hydrozoa) and some related campanulariids. PLoS ONE 10:e0117553. D OI:10.1371/journal.pone.0117553.

Deitch AD. 1955. Microspectrophotometric study of the binding of the anionic dye, naphthol yellow S, by tissue sections and by purified proteins. Lab Invest 4:324-351.

Frazier SB, Roodhouse KA, Hourcade DE, Zhang L. 2008. The quantification of glycosaminoglycans: A comparison of HPLC, carbazole, and alcian blue methods. Open Glycosci 1:31-39.

Goldberg WM. 2001. Acid polysaccharides in the skeletal matrix and calicoblastic epithelium of the stony coral *Mycetophyllia reesi*. Tissue Cell 33:376-387.

Hughes RG. 1980. Current induced variations in the growth and morphology of hydroids. In: Tardent P, Tardent R, editors. Developmental and Cellular Biology of Coelenterates. Amsterdam: Elsevier. p 179-184.

Hwang DS, Masic A, Prajatelistia E, Iordachescu M, Waite HJ. 2013. Marine hydroid perisarc: A chitin- and melanin-reinforced composite with DOPA-iron (III) complexes. Acta Biomater 9:8110-8117.

Klug M, Tardent P, Smid I, Holstein T. 1984. Presence and localization of chitinase in *Hydra* and *Podocoryne* (Cnidaria, Hydrozoa). J Exp Zool 229:69-72.

Knight DP. 1970. Sclerotization of the perisarc of the caliptoblastic hydroid, *Laomedea flexuosa*. 1 The identification and localization of dopamine in the hydroid. Tissue Cell 2:467-477.

Kosevich IA. 2013. Cell migration during growth and morphogenesis in thecate hydroids. Mar Ecol 34:83-95.

Kossevitch IA, Herrmann K, Berking S. 2001. Shaping of colony elements in *Laomedea flexuosa* Hinks (Hydrozoa, Thecaphora) includes a temporal and spatial control of skeleton hardening. Biol Bull 201:417-423.

Lesh-Laurie GE, Suchy PE. 1991. Cnidaria: Scyphozoa and Cubozoa. In: Harrison FW, Westfall JA, editors. Microscopic Anatomy of Invertebrates II – Placozoa, Porifera, Cnidaria and Ctenophora. New York: Wiley-Liss. p 185-266.

Mackie GO. 1984. Introduction to the diploblastic level. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, editors. Biology of the Integument: I - Invertebrates. New York: Springer Verlag. p 43-46.

Mali B, Möhrlen F, Frohme M, Frank U. 2004. A putative double role of a chitinase in a cnidarian: pattern formation and immunity. Dev Comp Immunol 28:973-981.

Marcum BA, Diehl FA. 1978. Anchoring cells (desmocytes) in the hydrozoan polyp *Cordylophora*. Tissue Cell 10:113-124.

Marin F, Mar S, Isa Y, Muyzer G, Westbroek P. 1996. Skeletal matrices, muci, and the origin of invertebrate calcification. Proc Natl Acad Sci USA 93:1554-1559.

Marques AC, Peña Cantero AL, Vervoort W. 2000. Mediterranean species of *Eudendrium* Ehrenberg, 1834 (Hydrozoa, Anthomedusae, Eudendriidae) with the description of a new species. J Zool Lond 252:197-213.

Marxen JC, Hammer M, Gehrke T, Becker W. 1998. Carbohydrates of the organic shell matrix and shell-forming tissue of the snail *Biomphalaria glabrata* (Say). Biol Bull 194:231-240.

McManus JF. 1946. Histological demonstration of mucin after periodic acid. Nature 158:202.

Mendoza-Becerril MA., Maronna MM, Pacheco MLAF, Simões MG, Leme J, Miranda LS, Morandini AC, Marques AC. 2015b. An evolutionary, comparative analysis of the medusozoan (Cnidaria) exoskeleton. [Capítulo 2 - submitted].

Miglietta MP, McNally L, Cunningham CW. 2010. Evolution of calcium-carbonate skeletons in the Hydractiniidae. Integr Comp Biol 50:428-435.

Millard NAH. 1975. Monograph on the Hydroida of Southern Africa. Ann S Afr Mus 68:1-513.

Mowry RW. 1963. The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins, with revised directions for the colloidal iron stain, and the use of alcian blue 8GX and their contributions with the periodic acid-Schiff reaction. Ann NY Acad Sci 106:402-423.

Murdock GR. 1976. Hydroid Skeletons and Fluid Flow. In: Mackie, GO, editor. Coelenterate Ecology and Behavior. London: Plenum Press. p 33-40.

Muscatine L. 1974. Endosymbiosis of cnidarians and algae. In: Muscatine L, Lenhoff HM, editors. Coelenterate Biology: Reviews and New Perspectives. New York: Academic Press. p 359-395.

Nutting CC. 1901. Papers from the Harriman Alaska expedition. XXI. The hydroids. Proc Wash Acad Sci 3:157-216.

Östman C. 1982. Isoenzymes and Taxonomy in Scandinavian Hydroids (Cnidaria, Campanulariidae). Zool Scr 11:155-163.

Pantos O, Hoegh-Guldberg O. 2011. Shared skeletal support in a coral-hydroid symbiosis. PLoS ONE 6:e20946. DOI:10.1371/journal.pone.0020946

Pearse AGE. 1985. Histochemistry: Theoretical and Applied. Vol. 2: Analytical Technology. Edinburgh: Churchill-Livingstone. 1055 p.

Petersen KW. 1979. Development of coloniality in Hydrozoa. In: Larwood G, Rosen BR, editors. Biology and Systematics of Colonial Organisms. New York: Academic Press. p 105-139.

Razak TB, Hoeksema BW. 2003. The hydrocoral genus *Millepora* (Hydrozoa: Capitata: Milleporidae) in Indonesia. Zool Verh Leiden 345:313-336.

Rees WM. 1956. A Revision of the Hydroid Genus *Perigonimus* M. Sars 1846. Bull Br Mus Nat Hist (Zool) 3:337-350.

Ruiz-Herrera J, Ortiz-Castellanos L. 2010. Analysis of the phylogenetic relationships and evolution of the cell walls from yeasts and fungi. FEMS Yeast Res 10:225-243.

Schuchert P. 2007. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 2. Rev Suisse Zool 114:195-396.

Schuchert P. 2012. North-west European Athecate Hydroids and Their Medusae. London: Linnean Society of London and The Estuarine and Coastal Sciences Association. 364 p.

Siebert S, Anton-Erxleben F, Bosch TCG. 2008. Cell type complexity in the basal metazoan *Hydra* is maintained by both stem cell based mechanisms and transdifferentiation. Dev Biol 313:13-24.

Simon KD, Allemand D, Weis VM. 2012. Cell biology of cnidarian-dinoflagellate symbiosis. Microbiol Mol Biol Rev 72:229-261.

Sorauf JE. 1980. Biomineralization, structure and diagenesis of the Coelenterate skeleton. Acta Palaeontol Pol 25:327-343.

Staats S, De Winder B, Stal LJ, Mur LR. 1999. Isolation and characterization of extracellular polysaccharides from the epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. Eur J Phycol 34:161-169.

Straehler-Pohl I, Jarms G. 2011. Morphology and life cycle of *Carybdea morandinii*, sp. nov. (Cnidaria), a cubozoan with zooxanthellae and peculiar polyp anatomy. Zootaxa 2755:36-56.

Taylor DL. 1968. In situ studies on the cytochemistry and ultrastructure of a symbiotic marine dinoflagellate. J Mar Biol Ass UK 48:349-366.

Thomas MB, Edwards NC. 1991. Cnidaria: Hydrozoa. In: Harrison FW, Westfall JA, editors. Microscopic Anatomy of Invertebrates II – Placozoa, Porifera, Cnidaria and Ctenophora. New York: Wiley-Liss. p 91-183.

Tretenichenko EM, Datsun VM, Ignatyuk LN, Nud'ga LA. 2006. Preparation and properties of chitin and chitosan from a hydroid polyp. Russ J Appl Chem 79:1341-1346.

Van Iten H, Marques AC, Leme JM, Pacheco MLAF, Simões MG. 2014. Origin and early diversification of the phylum Cnidaria Verrill: major developments in the analysis of the taxon's Proterozoic-Cambrian history. Palaeontology 57:677-690.

Vervoort W. 1964. Note on the distribution of *Garveia franciscana* (Torrey, 1902) and *Cordylophora caspia* (Pallas, 1771) in the Netherlands. Zool Meded Leiden 39:125-146.

Wagner GP. 1994. Evolution and multi-functionally of the chitin system. In: Schierwater B, Streit B, Wagner GP, DeSalle R, editors. Molecular and Ecology and Evolution: Approaches and Applications. Basel: Birkhäuser Verlag. p 559-577.

Weng LC, Pasaribu B, Lin P, Tsai CH, Chen CS, Jiang PL. 2014. Nitrogen deprivation induces lipid droplet accumulation and alters fatty acid metabolism in symbiotic dinoflagellates isolated from *Aiptasia pulchella*. Sci Rep 4:5777. DOI: 10.1038/srep05777.

Wineera JS. 1968. The histology of a species of *Solanderia* Duchassaing & Michelin, 1846 from Auckland Harbour, New Zealand, with special reference to the internal skeleton of the Solanderiidae (Coelenterata, Hydrozoa). Zool Publs Vict Univ Wellington 43:1-12.

Wineera JS. 1972. The body wall of the hydroid *Syncoryne tenella* (Farquhar, 1895). Part I: Histological and histochemical observations. Zool Publs Vict Univ Wellington 61:1-29.

Wood RL. 1979. The fine structure of the hypostome and mouth of *Hydra*. II Transmission electron microscopy. Cell Tissue Res 199:319-338.

Yamada S, Morimoto H, Fujisawa T, Sugahara K. 2007. Glycosaminoglycans in *Hydra magnipapillata* (Hydrozoa, Cnidaria): demonstration of chondroitin in the developing nematocyst, the sting organelle, and structural characterization of glycosaminoglycan. Glycobiology 17:886-894.

Yamada S, Sugahara K, Özbek S. 2011. Evolution of glycosaminoglycans. Commun Integr Biol 4:150-158.

Table 1. Reactions of the exoskeletal layers of several hydroidolinan species to specific staining.

Species	Layer	region/staining	TB	HE	Schiff	PAS	AB	HgBpB	NYS
"Anthoathecata"									
Bougainvilliidae									
Bimeria vestita	inner layer	hydrorhiza	+++ blue	++ magenta	-	++ magenta	-	+++ blue	+++ yellow
		hydrocaulus	+++ blue	++ magenta	-	++ magenta	-	+++ blue	+++ yellow
		hydranth	+ blue	+ magenta	-	+ magenta	-	<+ blue	<+ yellow
		gonophore	+ blue	+ magenta	-	+ magenta	-	<+ blue	<+ yellow
	outer layer	hydrorhiza	+++ purple	+ magenta	<+ magenta	+ magenta	++ alcian blue	-	-
		hydrocaulus	+++ purple	++ magenta	<+ magenta	+ magenta	++ alcian blue	-	-
		hydranth	+++ purple	++ magenta	<+ magenta	+ magenta	+++ alcian blue	-	-
		gonophore	+++ purple	+ magenta	-	+ magenta	++ alcian blue	-	-
"*Bougainvillia glorietta*"	inner layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	+++ blue	+++ magenta	<+ magenta	+++ magenta	-	+++ blue	+++ yellow
		hydranth	++ blue	+ magenta	<+ magenta	+ magenta	<+ alcian blue	<+ blue	-
		gonophore	+ blue	+ magenta	<+ magenta	+ magenta	<+ alcian blue	<+ blue	-
	outer layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	+++ purple	+ magenta	-	++ magenta	+++ alcian blue	-	-
		hydranth	+++ purple	+ magenta	-	++ magenta	+ alcian blue	-	-
		gonophore	+++ purple	+ magenta	-	+++ magenta	+ alcian blue	-	-
Bougainvillia muscus	inner layer	hydrorhiza	+++ blue	++ magenta	-	+++ magenta	-	+++ blue	+++ yellow
		hydrocaulus	+++ blue	++ magenta	-	+++ magenta	-	+++ blue	+++ yellow
		hydranth	+ blue	<+ magenta	-	+ magenta	-	<+ blue	<+ yellow
		gonophore	+ blue	+ magenta	-	+ magenta	-	<+ blue	<+ yellow
	outer layer	hydrorhiza	+++ purple	++ magenta	<+ magenta	++ magenta	++ alcian blue	-	-
		hydrocaulus	+++ purple	++ magenta	<+ magenta	++ magenta	++ alcian blue	-	-
		hydranth	+++ purple	++ magenta	<+ magenta	++ magenta	+++ alcian blue	-	-
		gonophore	+++ purple	+ magenta	-	++ magenta	++ alcian blue	-	-
Bougainvillia rugosa	inner layer	hydrorhiza	+ blue	+ magenta	-	+ magenta	-	++ blue	+++ yellow

Species	Layer	region/staining	TB	HE	Schiff	PAS	AB	HgBpB	NYS
		hydrocaulus	++ blue	+ magenta	-	+ magenta	-	++ blue	+++ yellow
		hydranth	+ blue	+ magenta	-	+ magenta	-	<+ blue	+ yellow
		gonophore	+ blue	<+ magenta	-	++ magenta	-	<+ blue	<+ yellow
	outer layer	hydrorhiza	+++ purple	++ magenta	<+ magenta	+++ magenta	+++ alcian blue	-	-
		hydrocaulus	+++ purple	++ magenta	<+ magenta	+++ magenta	+++ alcian blue	-	-
		hydranth	+++ purple	++ magenta	<+ magenta	+++ magenta	+++ alcian blue	-	-
		gonophore	+++ purple	++ magenta	-	++ magenta	++ alcian blue	-	-
Dicoryne conferta	inner layer	hydrorhiza	+++ blue	++ purple	+ magenta	++ magenta	<+ alcian blue	+ blue	+ yellow
		hydrocaulus	+++ blue	++ purple	+ magenta	++ magenta	<+ alcian blue	+ blue	+ yellow
		hydranth	+ blue	+ magenta	+ magenta	+ magenta	+ alcian blue	+ blue	<+ yellow
		gonophore	+ blue	+ magenta	<+ magenta	++ magenta	+ alcian blue	+ blue	-
	outer layer	hydrorhiza	+++ purple	+ purple	<+ magenta	++ magenta	+++ alcian blue	-	+ brown
		hydrocaulus	+++ purple	+ purple	<+ magenta	+++ magenta	+++ alcian blue	-	+ brown
		hydranth	++purple	+ magenta	<+ magenta	+++ magenta	+++ alcian blue	-	-
		gonophore	++purple	<+ magenta	<+ magenta	+ magenta	+++ alcian blue	-	-
Garveia annulata	inner layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	+++ blue	+++ magenta	-	+++ magenta	-	+++ blue	+++ yellow
		hydranth	+++ blue	+++ magenta	-	+++ magenta	-	+++ blue	+++ yellow
		gonophore	+++ blue	?	-	+++ magenta	-	+ blue	<+ yellow
	outer layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	++ purple	++ magenta	-	+++ magenta	++ alcian blue	-	-
		hydranth	++ purple	++ magenta	-	++ magenta	++ alcian blue	-	-
		gonophore	++ purple	++ magenta	-	+++ magenta	+ alcian blue	-	-
Garveia franciscana	inner layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	+ blue	++ magenta	<+ magenta	++ magenta	-	+++ blue	+++ yellow
		hydranth	<+ blue	+ magenta	-	+ magenta	-	-	-
		gonophore	+ blue	+ magenta	<+ magenta	+ magenta	-	+ blue	+ yellow
	outer layer	hydrorhiza	*	*	*	*	*	*	*
		side-branch	+++ purple	+++ magenta	<+ magenta	+++ magenta	+++ alcian blue	++ blue	-

Species	Layer	region/staining	TB	HE	Schiff	PAS	AB	HgBpB	NYS
		hydranth	+++ purple	++ magenta	-	+++ magenta	+++ alcian blue	-	+ brown
		gonophore	+++ purple	++ magenta	-	++ magenta	+++ alcian blue	-	+ brown
Garveia gracilis	inner layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	+++ blue	+++ magenta	-	+ magenta	-	+++ blue	+++ yellow
		hydranth	+ blue	+ magenta	-	+ magenta	-	-	-
	outer layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	++ purple	<+ magenta	<+ magenta	+++ magenta	++ alcian blue	-	<+ brown
		hydranth	+++ purple	+ magenta	+ magenta	+++ magenta	++ alcian blue	-	+ brown
Garveia nutans	inner layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	+++ blue	++ magenta	<+ magenta	+++ magenta	+++ alcian blue	+++ blue	++ yellow
		hydranth	+ blue	++ magenta	-	+ magenta	+ alcian blue	-	-
		gonophore	++ blue	++ magenta	-	++ magenta	-	<+ blue	-
	outer layer	hydrorhiza	x	x	x	x	x	x	x
		hydrocaulus	++ purple	++ magenta	-	+++ magenta	+++ alcian blue	-	+ brown
		hydranth	++ purple	+++ magenta	-	+++ magenta	+++ alcian blue	-	+ brown
		gonophore	++ purple	+++ magenta	-	+ magenta	++ alcian blue	-	+ brown
Pachycordyle michaeli	inner layer	hydrorhiza	++ blue	+ magenta	-	+++ magenta	+ alcian blue	++ blue	+ yellow
		hydrocaulus	++ blue	+ magenta	-	+++ magenta	-	++ blue	+ yellow
		hydranth	ø	ø	ø	ø	ø	ø	ø
		gonophore	+ blue	+ magenta	-	++ magenta	-	++ blue	<+ yellow
	Outer layer	hydrorhiza	ø	ø	ø	ø	ø	ø	ø
		hydrocaulus	ø	ø	ø	ø	ø	ø	ø
		hydranth	ø	ø	ø	ø	ø	ø	ø
		gonophore	ø	ø	ø	ø	ø	ø	ø
Parawrightia robusta	inner layer	hydrorhiza	++ blue	++ magenta	-	++ magenta	-	+++ blue	++ yellow
		hydrocaulus	++ blue	++ magenta	-	++ magenta	-	++ blue	++ yellow
		hydranth	+ blue	++ magenta	-	++ magenta	-	+ blue	+ yellow
	outer layer	hydrorhiza	+++ purple	+++ magenta	<+ magenta	+++ magenta	+++ alcian blue	-	-
		hydrocaulus	+++ purple	+++ magenta	<+ magenta	+++ magenta	+++ alcian blue	-	-

Species	Layer	region/staining	TB	HE	Schiff	PAS	AB	HgBpB	NYS
		hydranth	+++ purple	+++ magenta	-	+++ magenta	+++ alcian blue	-	-
Rhizorhagium sp.	inner layer	hydrorhiza	+++ blue	+++ magenta	<+ magenta	+++ magenta	-	+++ blue	+++ yellow
		hydrocaulus	+++ blue	+++ magenta	<+ magenta	+++ magenta	-	+++ blue	+++ yellow
		hydranth	+ blue	<+ magenta	-	+ magenta	<+ alcian blue	<+ blue	-
	outer layer	hydrorhiza	+++ purple	+ magenta	<+ magenta	++ magenta	+++ alcian blue	-	-
		hydrocaulus	+++ purple	+ magenta	-	++ magenta	+++ alcian blue	-	-
		hydranth	+++ purple	+ magenta	-	++ magenta	++ alcian blue	-	-
Eudendriidae									
Eudendrium carneum	inner layer	hydrorhiza	++ blue	++ magenta	-	++ magenta	-	+++ blue	+++ yellow
		hydrocaulus	++ blue	++ magenta	-	++ magenta	-	+++ blue	+++ yellow
		hydranth	ø	ø	ø	ø	ø	ø	ø
		gonophore	+ blue	+ magenta	-	+ magenta	-	+ blue	<+ yellow
	outer layer	hydrorhiza	+++ blue	+ magenta	<+ magenta	+++ magenta	+++ alcian blue	-	-
		hydrocaulus	+++ blue	+ magenta	<+ magenta	+++ magenta	+++ alcian blue	-	-
		hydranth	ø	ø	ø	ø	ø	ø	ø
		gonophore	ø	ø	ø	ø	ø	ø	ø
Oceaniidae									
Turritopsis nutricula	inner layer	hydrorhiza	+++ blue	+++ magenta	-	+++ magenta	-	+++ blue	+++ yellow
		hydrocaulus	+++ blue	+++ magenta	-	+++ magenta	-	+++ blue	+++ yellow
		hydranth	+ blue	+ magenta	-	+ magenta	-	+ blue	?
	Membrane	hydrorhiza	+++ purple	<+ magenta	-	++ magenta	++ alcian blue	-	-
		hydrocaulus	+++ purple	<+ magenta	-	++ magenta	++ alcian blue	-	-
		hydranth	+ purple	<+ magenta	-	++ magenta	+ alcian blue	-	-
Pandeidae									
Leuckartiara octona	inner layer	hydrorhiza	+++ blue	+++ magenta	-	+++ magenta	-	+++ blue	++ yellow
		hydrocaulus	+++ blue	+++ magenta	-	+++ magenta	-	+++ blue	++ yellow
		hydranth	+ blue	+ magenta	-	+ magenta	-	++ blue	-
	outer layer	hydrorhiza	+++ purple	++ magenta	-	++ magenta	+++ alcian blue	-	-
		hydrocaulus	+++ purple	++ magenta	-	++ magenta	+++ alcian blue	<+ blue	-

Species	Layer	region/staining	TB	HE	Schiff	PAS	AB	HgBpB	NYS
		hydranth	+++ purple	++ magenta	-	++ magenta	+++ alcian blue	<+ blue	-
Pennariidae									
Pennaria disticha	inner layer	hydrorhiza	+++ blue	+++ magenta	-	+++ magenta	-	+++ blue	+++ yellow
		hydrocaulus	+++ blue	+++ magenta	-	+++ magenta	-	+++ blue	+++ yellow
		hydranth	ø	ø	ø	ø	ø	ø	ø
	outer layer	hydrorhiza	+++ purple	+ magenta	<+ magenta	++ magenta	++ alcian blue	-	-
		hydrocaulus	+++ purple	+ magenta	<+ magenta	++ magenta	++ alcian blue	-	-
		hydranth	ø	ø	ø	ø	ø	ø	ø
Leptothecata									
Campanulariidae									
Clytia gracilis	inner layer	hydrorhiza	+++ blue	+++ magenta	-	+ magenta	++ alcian blue	+++ blue	+++ yellow
		hydrocaulus	+++ blue	+++ magenta	-	+ magenta	++ alcian blue	+++ blue	+++ yellow
		hydranth	++ blue	++ magenta	-	<+ magenta	++ alcian blue	++ blue	++ yellow
	outer layer	hydrorhiza	ø	ø	ø	ø	ø	ø	ø
		hydrocaulus	ø	ø	ø	ø	ø	ø	ø
		hydranth	ø	ø	ø	ø	ø	ø	ø
Obelia dichotoma	inner layer	hydrorhiza	+++ blue	+++ magenta	<+ magenta	+++ magenta	-	+++ blue	++ yellow
		hydrocaulus	+++ blue	+++ magenta	<+ magenta	+++ magenta	-	+++ blue	++ yellow
		hydranth	+++ blue	++ magenta	-	+++ magenta	-	++ blue	++ yellow
		gonophore	+++ blue	++ magenta	-	+++ magenta	-	+++ blue	++ yellow
	outer layer	hydrorhiza	ø	ø	ø	ø	ø	ø	ø
		hydrocaulus	ø	ø	ø	ø	ø	ø	ø
		hydranth	ø	ø	ø	ø	ø	ø	ø
		gonophore	ø	ø	ø	ø	ø	ø	ø
Orthopyxis sargassicola	inner layer	hydrorhiza	+++ blue	++ magenta	<+ magenta	++ magenta	-	++ blue	+ yellow
		hydrocaulus	++ blue	+ magenta	<+ magenta	++ magenta	-	++ blue	+ yellow
		hydranth	++ blue	+ magenta	-	++ magenta	-	++ blue	+ yellow
		gonophore	++ blue	+ magenta	<+ magenta	++ magenta	-	++ blue	+ yellow
	outer layer	hydrorhiza	ø	ø	ø	ø	ø	ø	ø

Species	Layer	region/staining	TB	HE	Schiff	PAS	AB	HgBpB	NYS
		hydrocaulus	ø	ø	ø	ø	ø	ø	ø
		hydranth	ø	ø	ø	ø	ø	ø	ø
		gonophore	ø	ø	ø	ø	ø	ø	ø
Haleciidae									
Halecium bermudense	inner layer	hydrorhiza	+++ blue	++ magenta	-	++ magenta	-	+++ blue	+ yellow
		hydrocaulus	+++ blue	++ magenta	-	++ magenta	-	+++ blue	+ yellow
		hydranth	+++ blue	++ magenta	-	++ magenta	-	+++ blue	+ yellow
		gonophore	+++ blue	++ magenta	-	++ magenta	-	+++ blue	+ yellow
	outer layer	hydrorhiza	ø	ø	ø	ø	ø	ø	ø
		hydrocaulus	ø	ø	ø	ø	ø	ø	ø
		hydranth	ø	ø	ø	ø	ø	ø	ø
		gonophore	ø	ø	ø	ø	ø	ø	ø

–, not stained; <+, nearly unstained; +, weakly stained; ++, moderately stained; +++, intensely stained; x, not analyzed histologically; ø, without structure; * structure not identified; ?, doubtful reaction.

Table 2. Chemical and morphological types of exoskeleton.

Species/Exoskeleton	inner layer (= perisarc)			outer layer (= exosarc)	Type of exoskeleton
	AP	**Proteins**	**GAGs**	**GAGs**	
"Anthoathecata"					
Bougainvilliidae					
Bimeria vestita	+	+	x	+	bilayered
"*Bougainvillia glorietta*"	+	+	x	+	bilayered
Bougainvillia muscus	+	+	x	+	bilayered
Bougainvillia rugosa	+	+	x	+	bilayered
Dicoryne conferta	+	+	+	+	bilayered
Garveia annulata	+	+	+	+	bilayered
Garveia franciscana	+	+	+	+	bilayered
Garveia gracilis	+	+	x	+	bilayered
Garveia nutans	+	+	+	+	bilayered
Pachycordyle michaeli	+	+	x	ø	corneous
Parawrightia robusta	+	+	x	+	bilayered
Rhizorhagium sp.	+	+	x	+	bilayered
Eudendriidae					
Eudendrium carneum	+	+	x	+	bilayered
Oceaniidae					
Turritopsis nutricula	+	+	x	ø	corneous
Pandeidae					
Leuckartiara octona	+	+	x	+	bilayered
Pennariidae					
Pennaria disticha	+	+	x	+	bilayered
Leptothecata					
Campanulariidae					
Clytia gracilis	+	+	+	ø	corneous
Obelia dichotoma	+	+	x	ø	corneous
Orthopyxis sargassicola	+	+	x	ø	corneous
Haleciidae					
Halecium bermudense	+	+	x	ø	corneous

AP, aminopolysaccharides; GAGs, glycosaminoglycans; +, present; x, absent; ø, without layer.

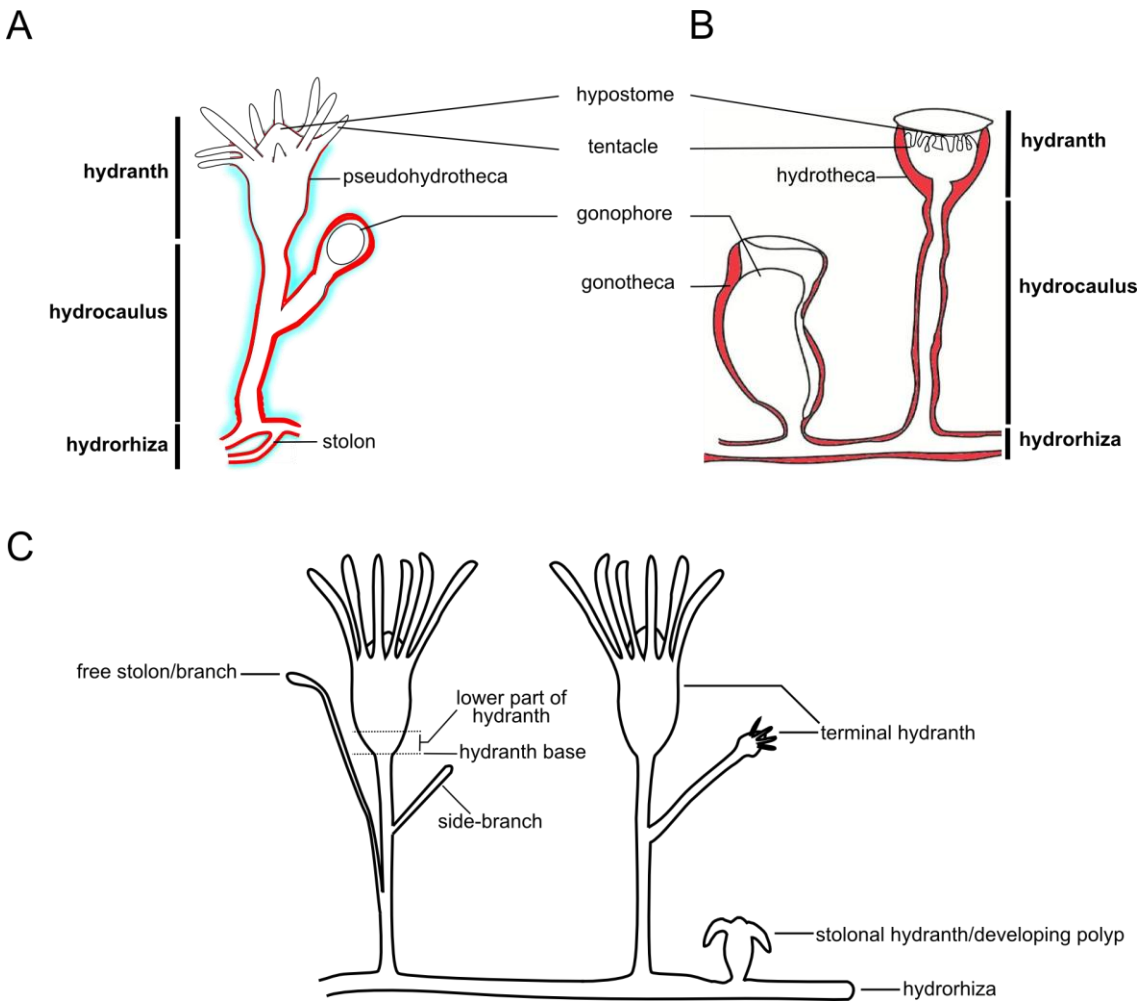

Figure 1. Anatomy of polyps. A: polyp of "Anthoathecata"; B: polyp of Leptothecata; C: Regions described for the exoskeleton.

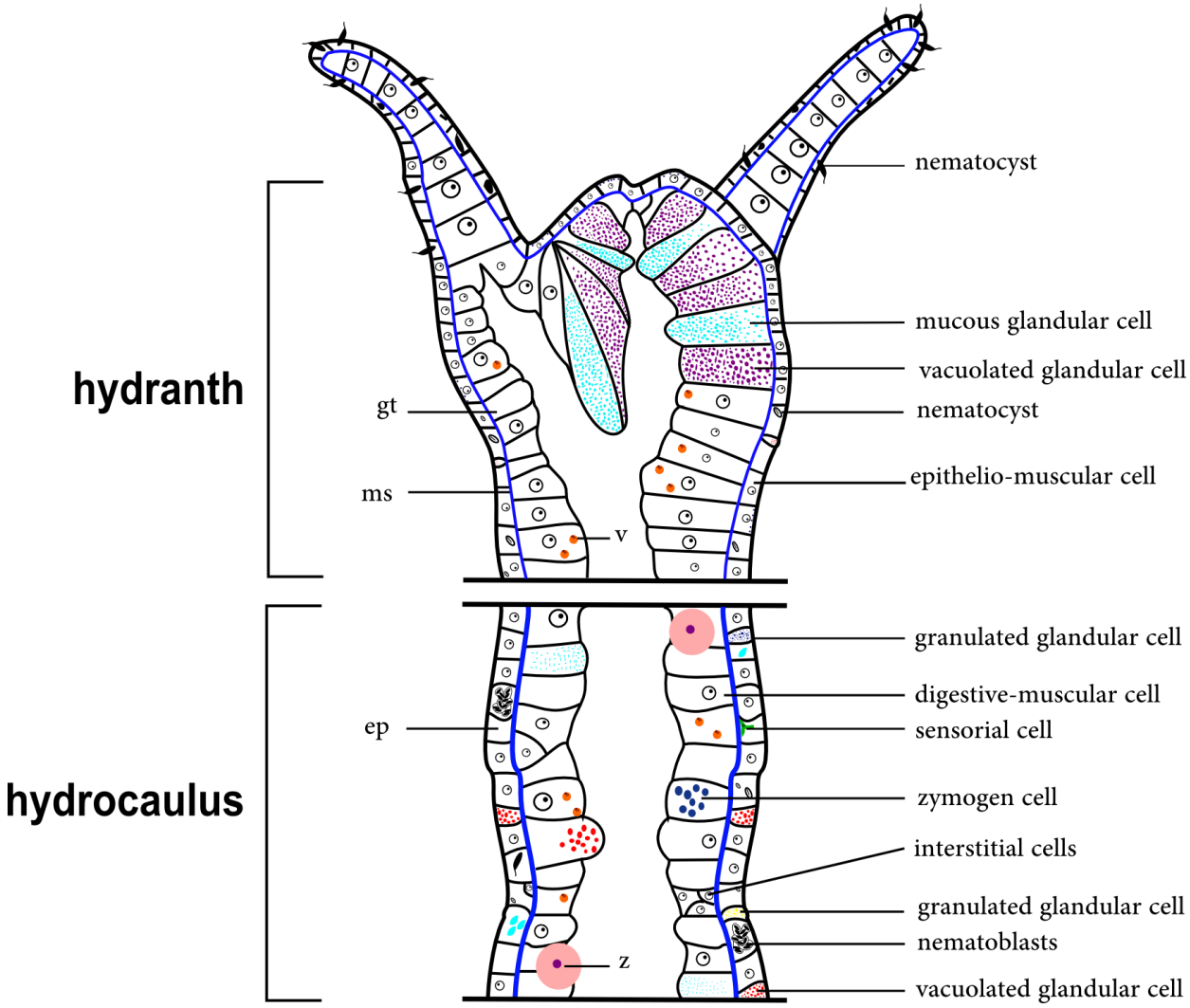

Figure 2. Schematic drawing of the coenosarc of a generalized polyp of Hydroidolina. Abbreviations: z, zooxanthellae; ep, epidermis; gt, gastrodermis; ms, mesoglea; v, vacuole.

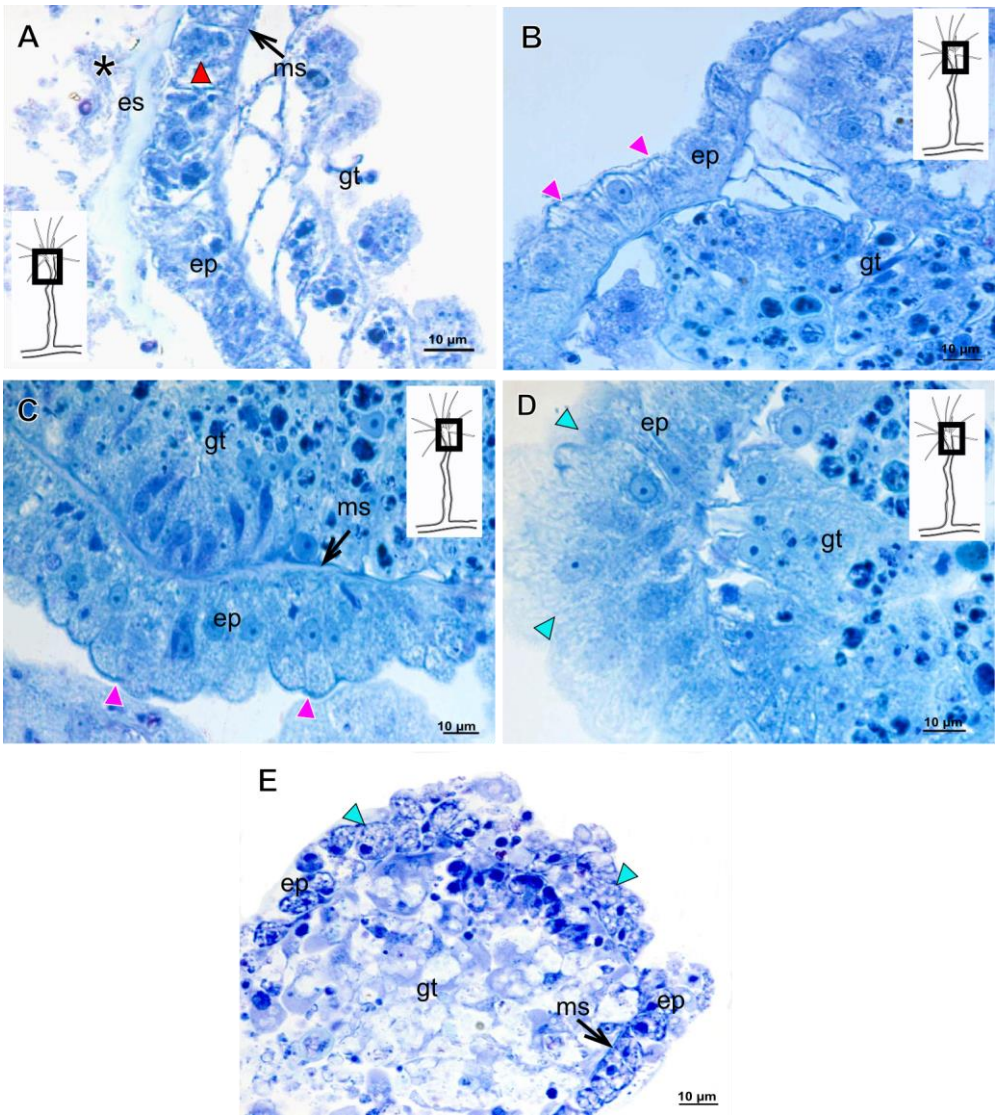

Figure 3. Glandular cells of Bougainvilliidae Lütken, 1850. A-D: glandular cells in the epidermis of *Parawrightia robusta*; E: developing stolonal hydranth of *Bougainvillia muscus*. Alcian blue arrowhead indicates mucous glandular cells, pink arrowhead indicates highly granulated glandular cells, red arrowhead indicates vacuolated glandular cells, asterisk indicates "perisarc extensions". Abbreviations: ep, epidermis; es, exoskeleton; gt, gastrodermis; ms, mesoglea.

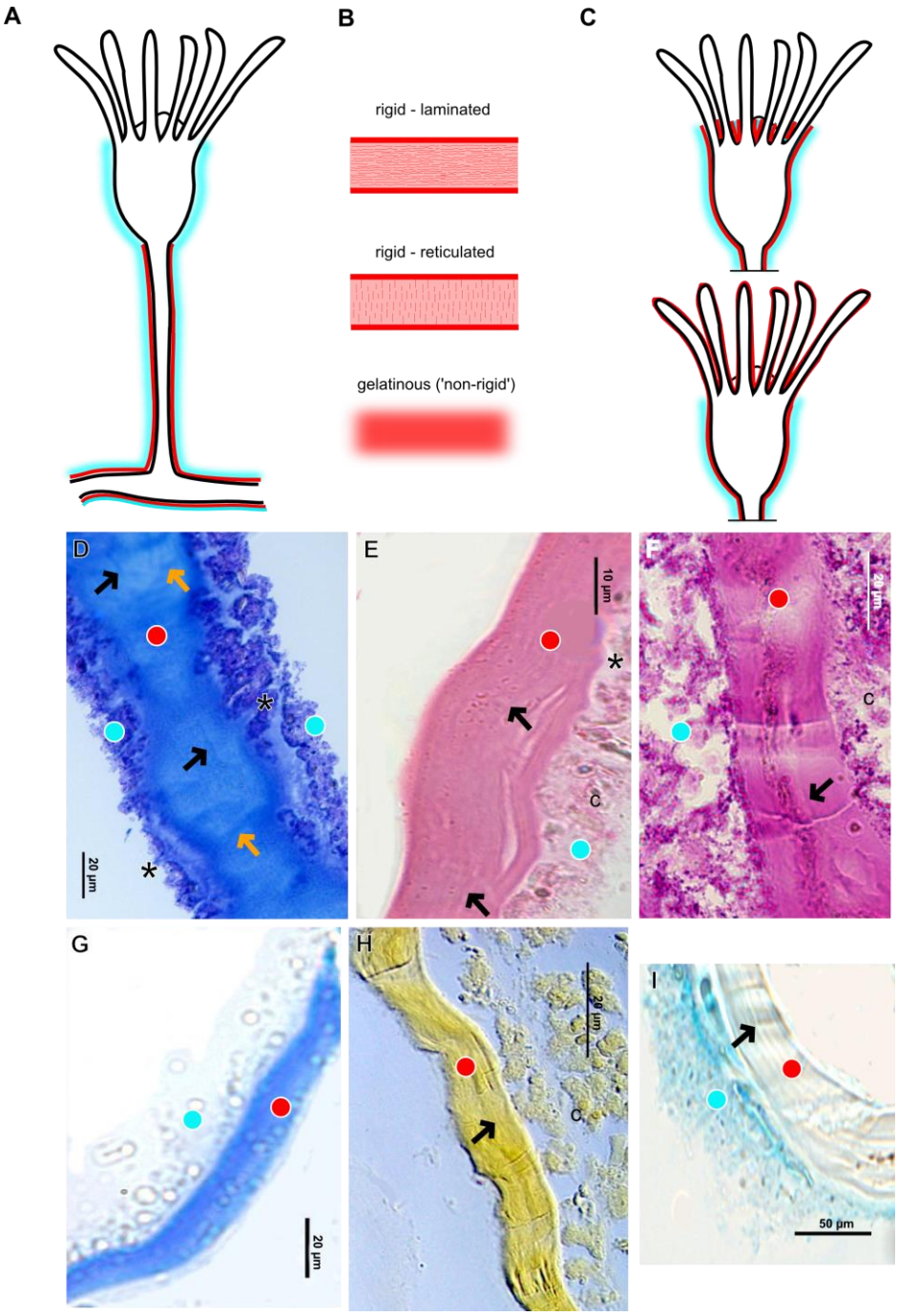

Figure 4. Exoskeletal structure of Bougainvilliidae Lütken, 1850. A: Coverage of exoskeletal layers over hydrocaulus; B: Morphological structure of the inner layer; C: Coverage of exoskeletal layers over hydranth; D-I: Affinity for chemical tests and details of exoskeleton; D: Toluidine blue; E: Eosin; F: Periodic acid-Schiff; G: Mercury-bromophenol blue; H: Naphthol yellow S; I: Alcian blue pH 2.5. Alcian blue line and circle indicate the outer layer of the exoskeleton, red line and circle indicate the inner layer of the exoskeleton, black arrow indicates laminae, orange arrow indicates transverse marks, asterisk indicates "perisarc extensions". Abbreviation: c, coenosarc.

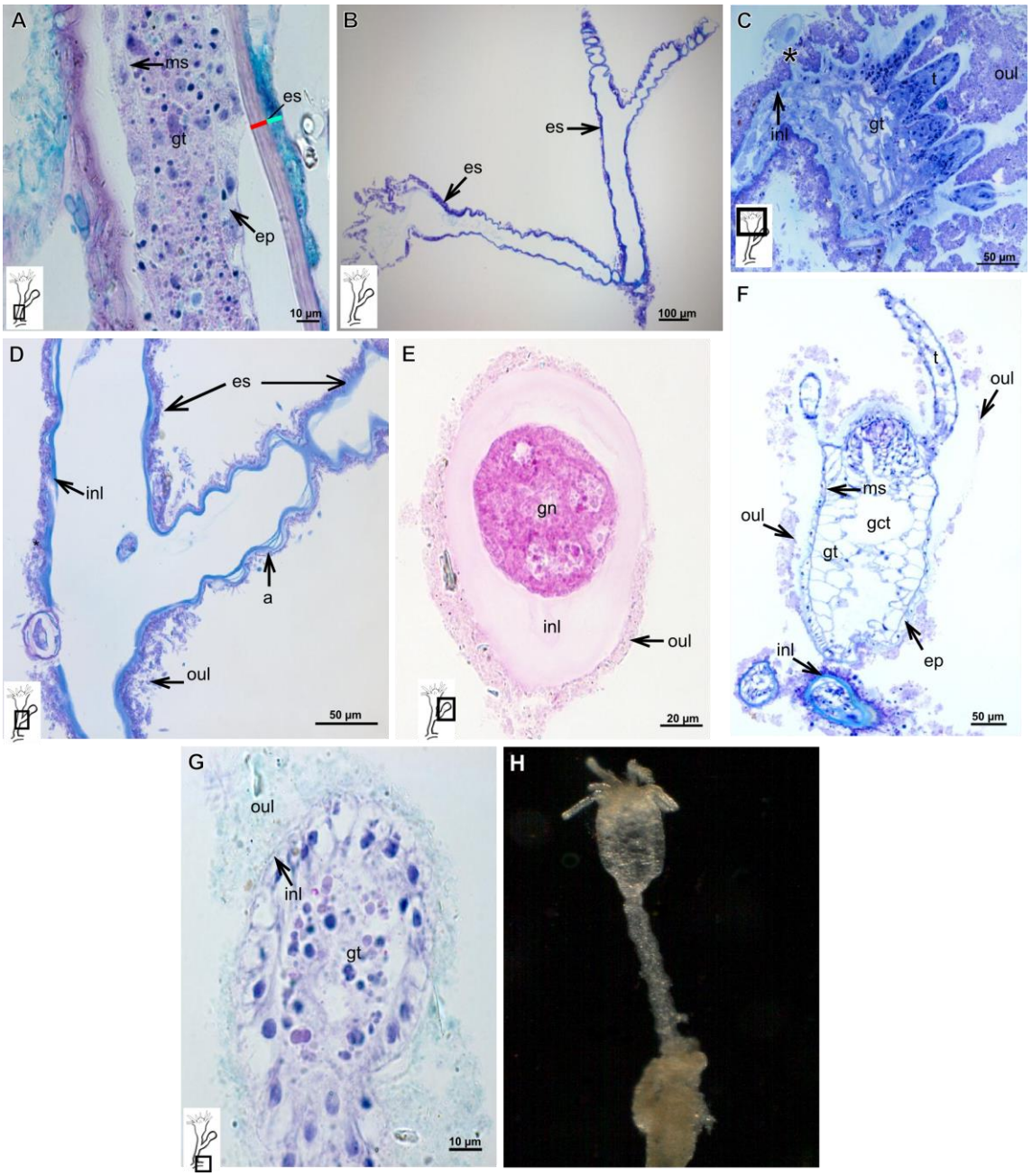

Figure 5. Exoskeletal structure of *Bimeria vestita* Wright, 1859. A: detail of hydrocauline exoskeleton; B: general organization of the exoskeleton of part of the colony; C: exoskeleton of the hydranth; D: detail of exoskeleton at side-branch and hydrocaulus; E: detail of exoskeleton in female gonophore during development; F: developing polyp in culture with filtered water; G: developing stolon; H: external view of a polyp in culture with unfiltered water. Alcian blue line indicates the outer the layer of the exoskeleton, red line indicates the inner layer of the exoskeleton, asterisk indicates "perisarc extensions". Abbreviations: a, annulation; ep, epidermis; es, exoskeleton; gn, gonadal cell cluster; gct, gastrovascular cavity; gt, gastrodermis; inl, inner layer; ms, mesoglea; oul, outer layer; t, tentacle.

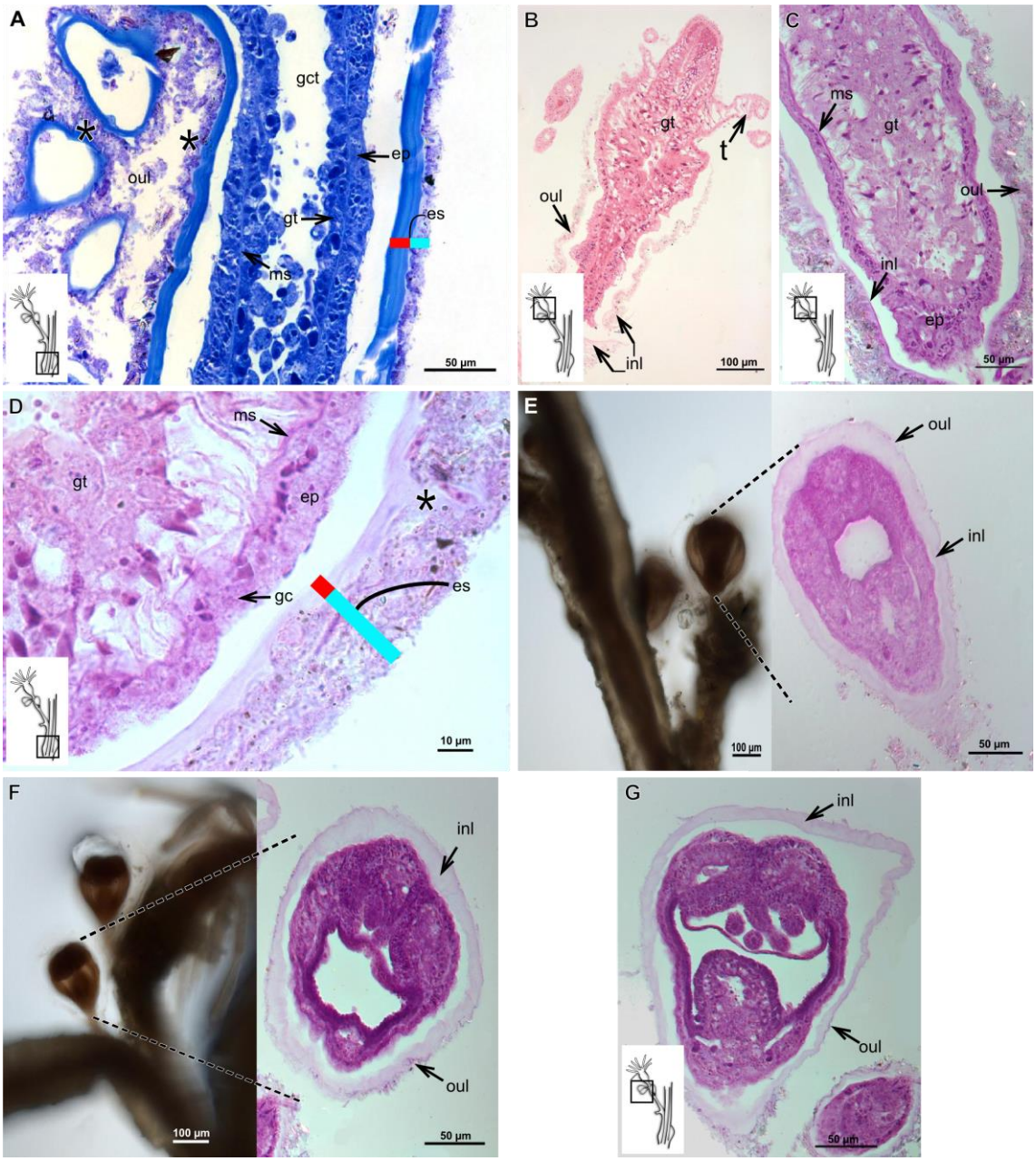

Figure 6. Exoskeletal structure of "*Bougainvillia glorietta*" Torrey, 1904. A: detail of hydrocauline exoskeleton; B: exoskeleton of the hydranth; C: detail of the exoskeleton of the hydranth; D: transverse section of hydrocauline exoskeleton; E: formation of subumbrellar lining from the entocodon; F: formation of subumbrellar lining and manubrium from the entocodon; G: complete medusa with manubrium and marginal tentacle units with bulb. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton, asterisk indicates "perisarc extensions". Abbreviations: ep, epidermis; es, exoskeleton; gc, glandular cells; gct, gastrovascular cavity; gt, gastrodermis; inl, inner layer; ms, mesoglea; oul, outer layer; t, tentacle.

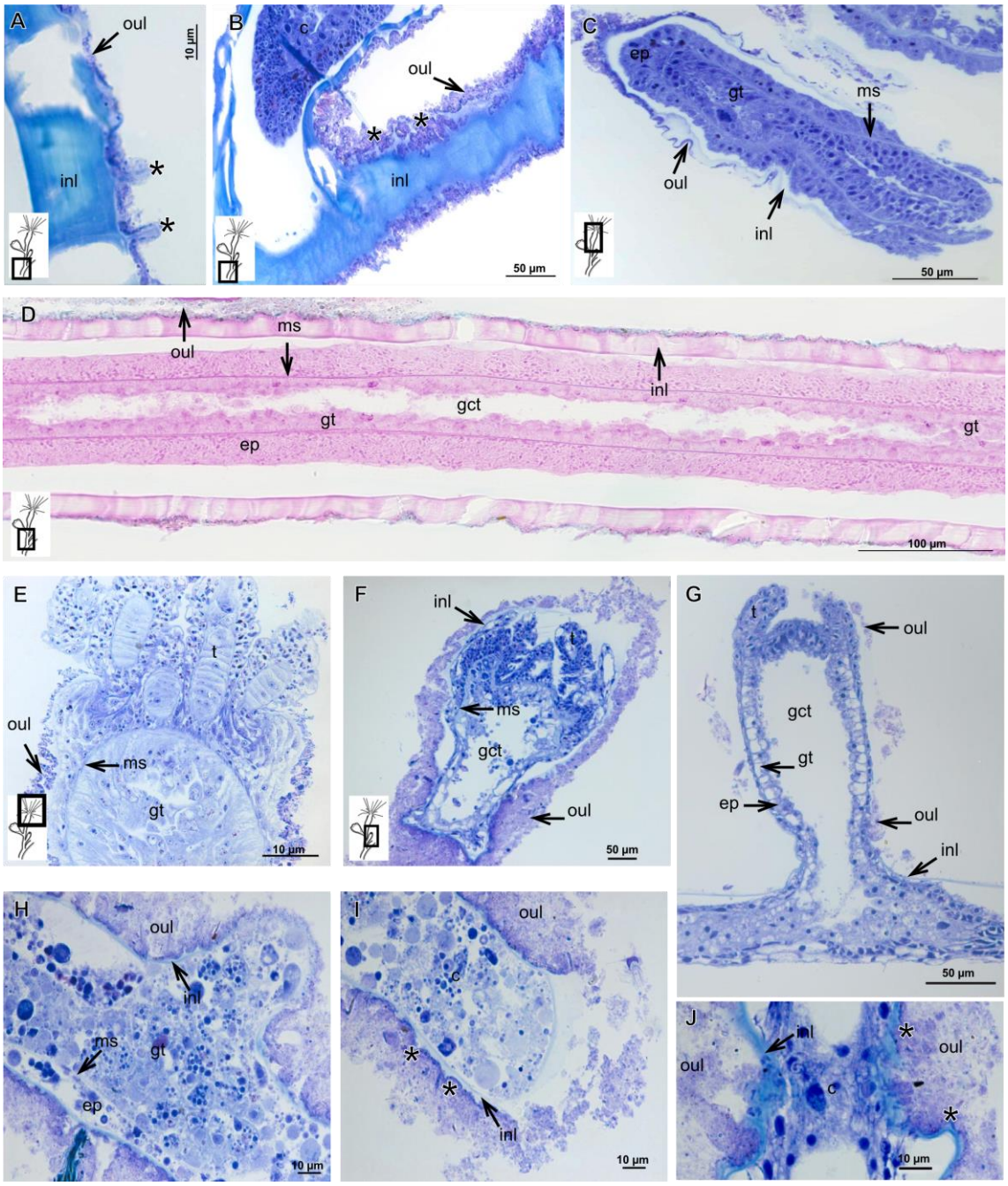

Figure 7. Exoskeletal structure of *Bougainvillia muscus* (Allman, 1863). A-B: detail of 'perisarc extensions' in the inner layer of the exoskeleton; C: exoskeleton of the hydranth; D: general internal and external structure of the hydrocaulus; E-F: contracted hydranth; G: developing stolonal hydranth; H: stolon of developing hydranth; I: development of stolon of the hydrorhiza; J: hydrocaulus of a new polyp. Asterisk indicates "perisarc extensions". Abbreviations: c, coenosarc; ep, epidermis; gt, gastrodermis; inl, inner layer; ms, mesoglea; oul, outer layer; t, tentacle.

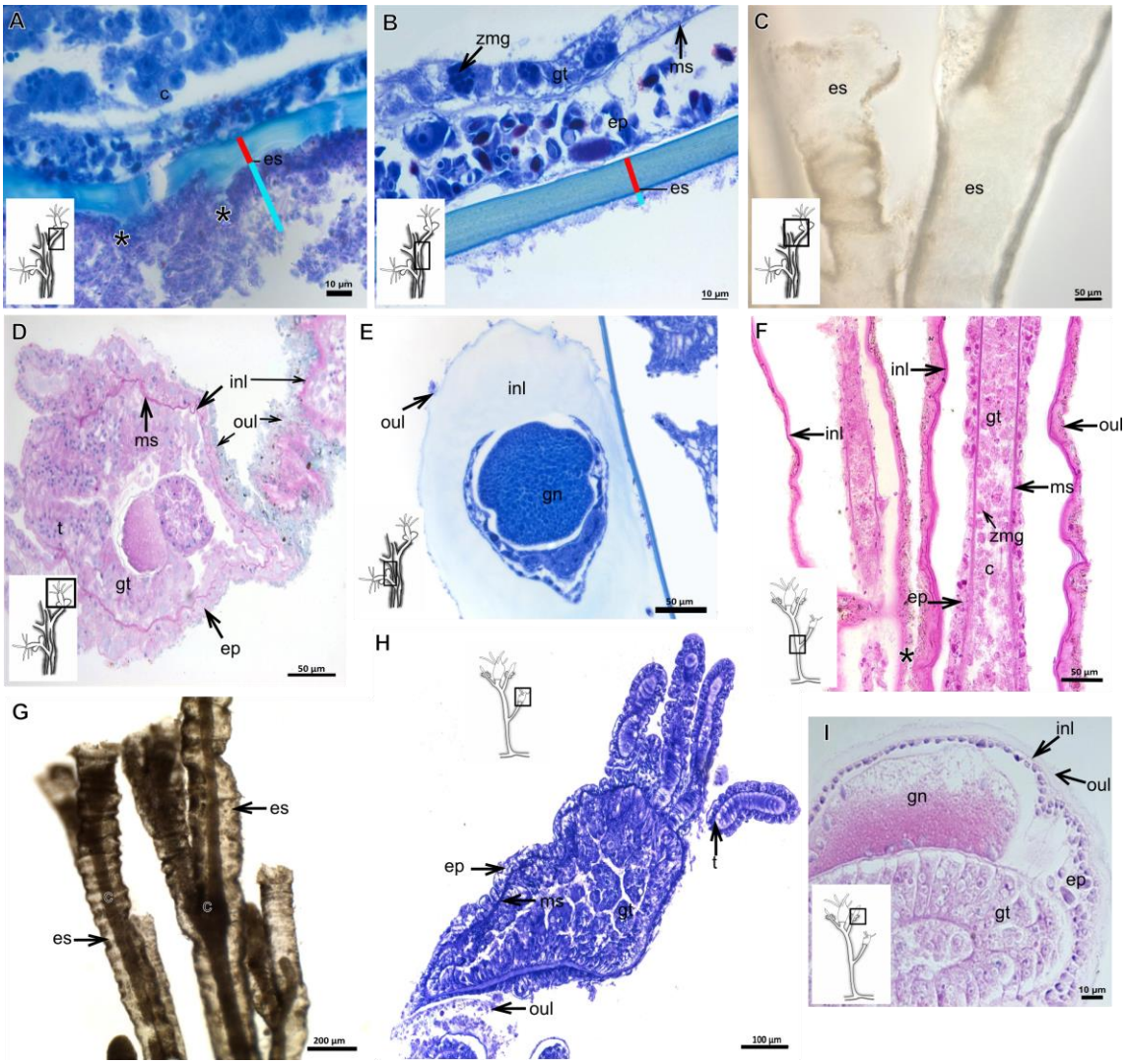

Figure 8. A-E. Exoskeletal structure of *Bougainvillia rugosa* Clarke, 1882. A: exoskeleton of the hydrocaulus; B: exoskeleton of the side-branch; C: general exoskeleton of the polyp; D: exoskeleton of the hydranth; E: exoskeleton of mature female gonophore. F-I. Exoskeletal structure of *Dicoryne conferta* Alder, 1856. F: hydrocaulus of the central region of the polyp; G: external appearance of the exoskeleton; H: exoskeleton of the basal part of the hydranth; I: mature female gonophore with sporosacs of styloid type. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton, asterisk indicates "perisarc extensions". Abbreviations: c, coenosarc; ep, epidermis; es, exoskeleton; gn, gonadal cell cluster; gt, gastrodermis; inl, inner layer; ms, mesoglea; oul, outer layer; t, tentacle; zmg, zymogen glandular cell.

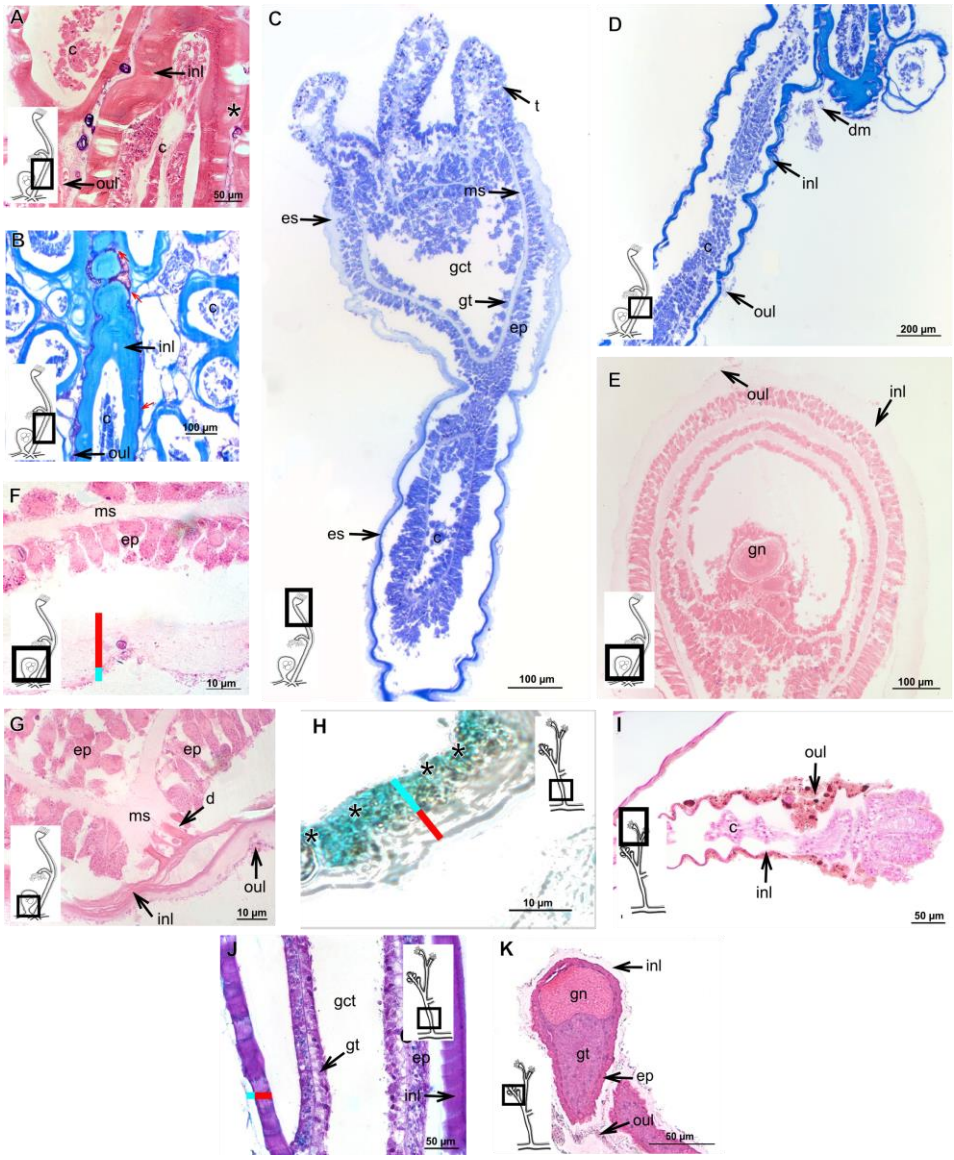

Figure 9. A-G: Exoskeletal structure of *Garveia annulata* Nutting, 1901. A-B: exoskeleton; C: hydrocauline exoskeleton; D: corrugated exoskeleton; E: female gonophore showing sporosacs of the heteromedusoid type; F: exoskeleton of female gonophore; G: desmocytes in the female gonophore with sporosacs of the heteromedusoid type; H-K: Exoskeletal structure of *Garveia franciscana* (Torrey, 1902). H: exoskeleton; I: exoskeleton of the hydranth; J: exoskeleton of the hydrocaulus; K: exoskeleton of the female gonophore with sporosacs of the styloid type. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton, asterisk indicates "perisarc extensions", red arrow indicates the "exoskeletal connections" among the hydrocauline tubes. Abbreviations: c, coenosarc; d, desmocyte; ep, epidermis; es, exoskeleton; gn, gonadal cell cluster; gct, gastrovascular cavity; gt, gastrodermis; inl, inner layer; ms, mesoglea; oul, outer layer; t, tentacle.

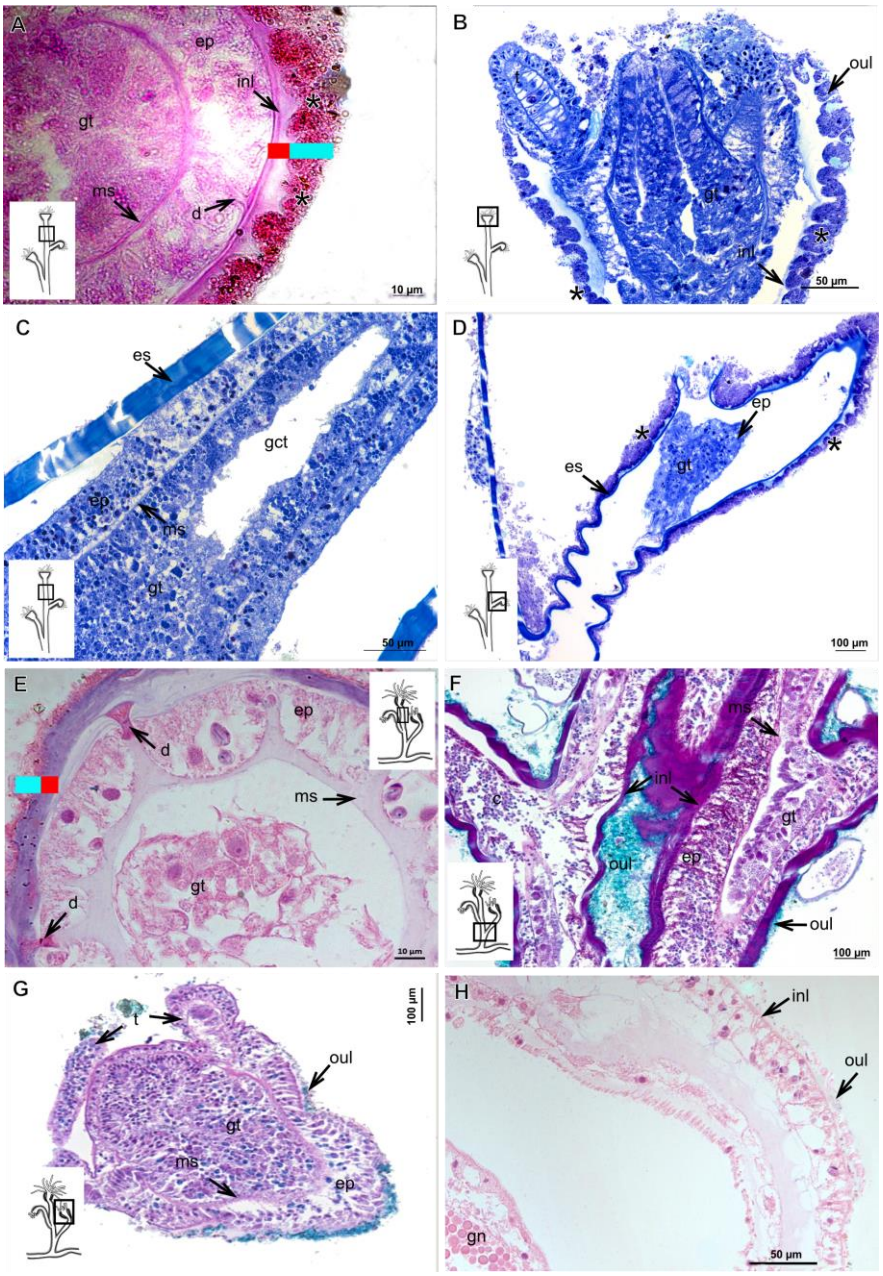

Figure 10. A-D: Exoskeletal structure of *Garveia gracilis* (Clark, 1876). A: transverse section of the exoskeleton of the hydrocaulus; B: layers of the exoskeleton of the hydranth; C: exoskeleton of the hydrocaulus; D: exoskeleton of the side-branch. E-H: Exoskeletal structure of *Garveia nutans* Wright, 1859. E: transverse section of the exoskeleton of the hydrocaulus; F: exoskeleton of the hydrocaulus; G: exoskeleton of the hydranth; H: exoskeleton of the female gonophore. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton, asterisk indicates "perisarc extensions". Abbreviations: c, coenosarc; d, desmocyte; ep, epidermis; es, exoskeleton; gct, gastrovascular cavity; gn, gonadal cell cluster; gt, gastrodermis; inl, inner layer; ms, mesoglea; oul, outer layer; t, tentacle.

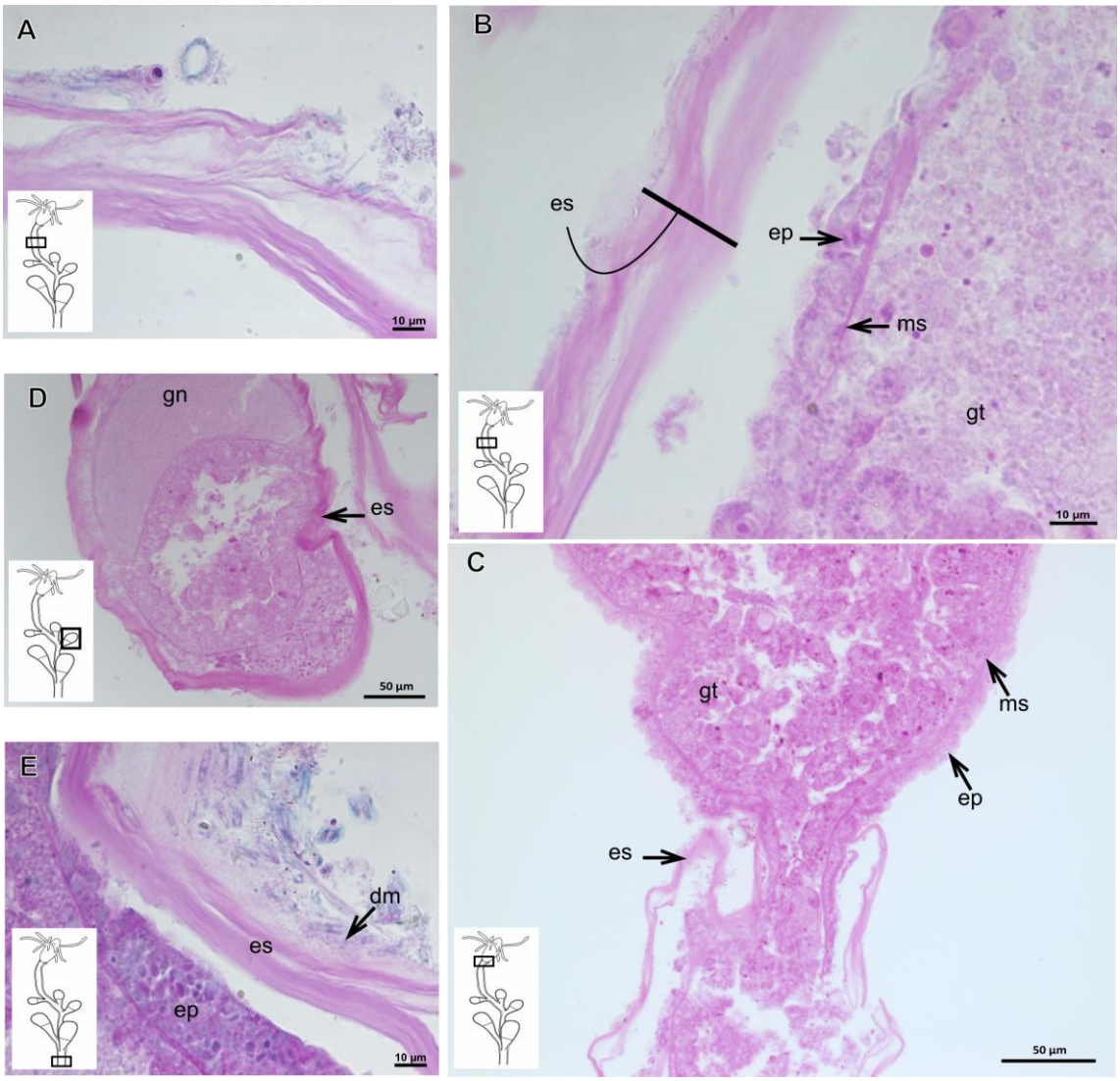

Figure 11. Exoskeletal structure of *Pachycordyle michaeli* (Berrill, 1948). A: exoskeleton; B: hydrocauline exoskeleton; C: exoskeleton at the base of the hydranth; D: exoskeleton of the male gonophore with entocodon development; E: exoskeleton with external material encrusted. Abbreviations: dm, diatoms; ep, epidermis; es, exoskeleton; gn, gonadal cell cluster; gt, gastrodermis; ms, mesoglea.

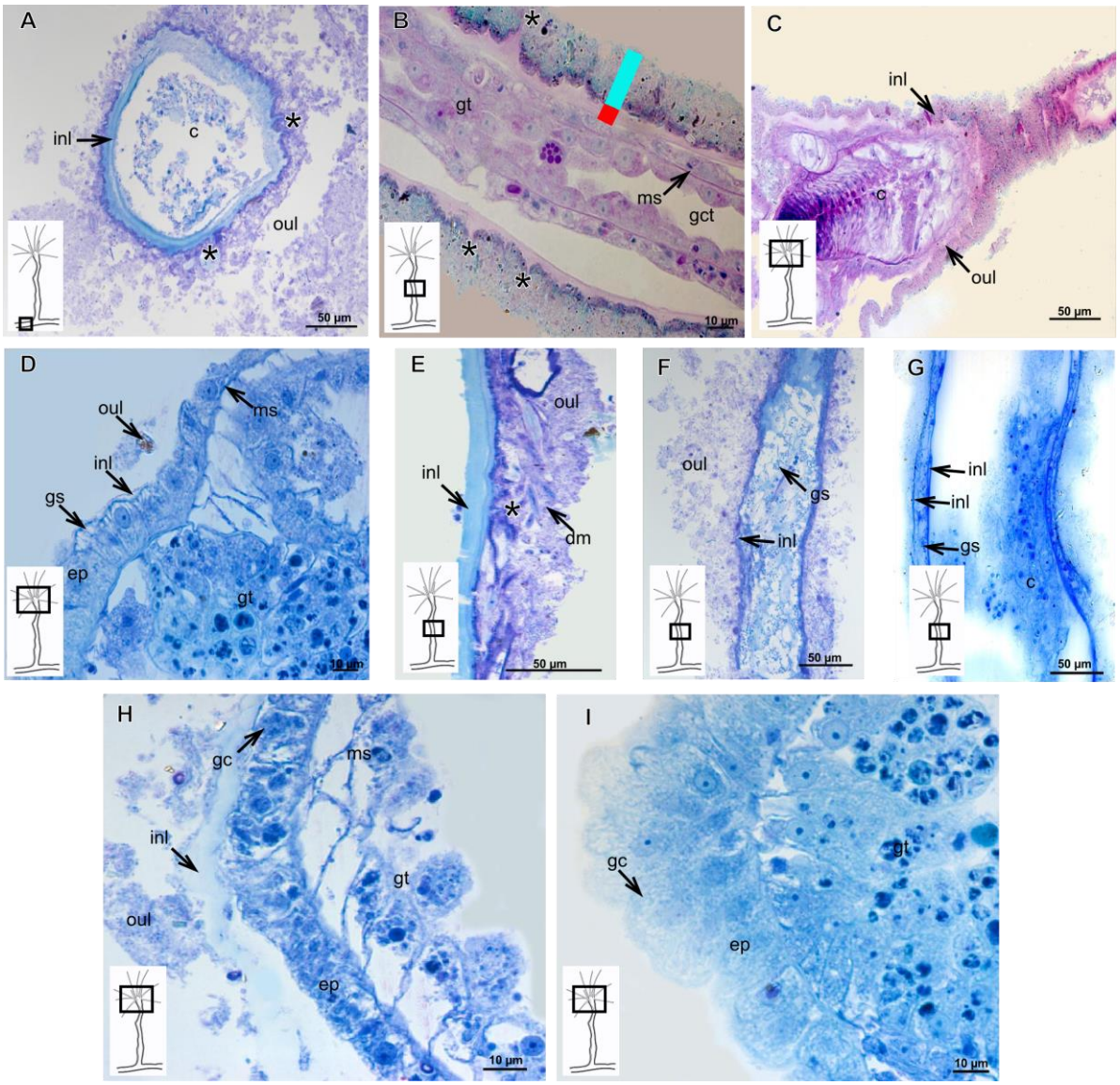

Figure 12. Exoskeletal structure of *Parawrightia robusta* Warren, 1907. A: transverse section of the exoskeleton of the hydrorhiza; B: hydrocauline exoskeleton; C: exoskeleton of the hydranth; D: exoskeleton at the tentacular base; E-G: hydrocauline exoskeleton; E: hydrocauline exoskeleton of specimens maintained in culture conditions (unfiltered water); F-G: hydrocauline exoskeleton of specimens maintained in culture conditions (filtered water); H-I: glandular cells in the epidermis of the hydranth. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton, asterisk indicates "perisarc extensions". Abbreviations: c, coenosarc; dm, diatoms; ep, epidermis; gct, gastrovascular cavity; gs, secretory granules; gt, gastrodermis; inl, inner layer; ms, mesoglea; oul, outer layer.

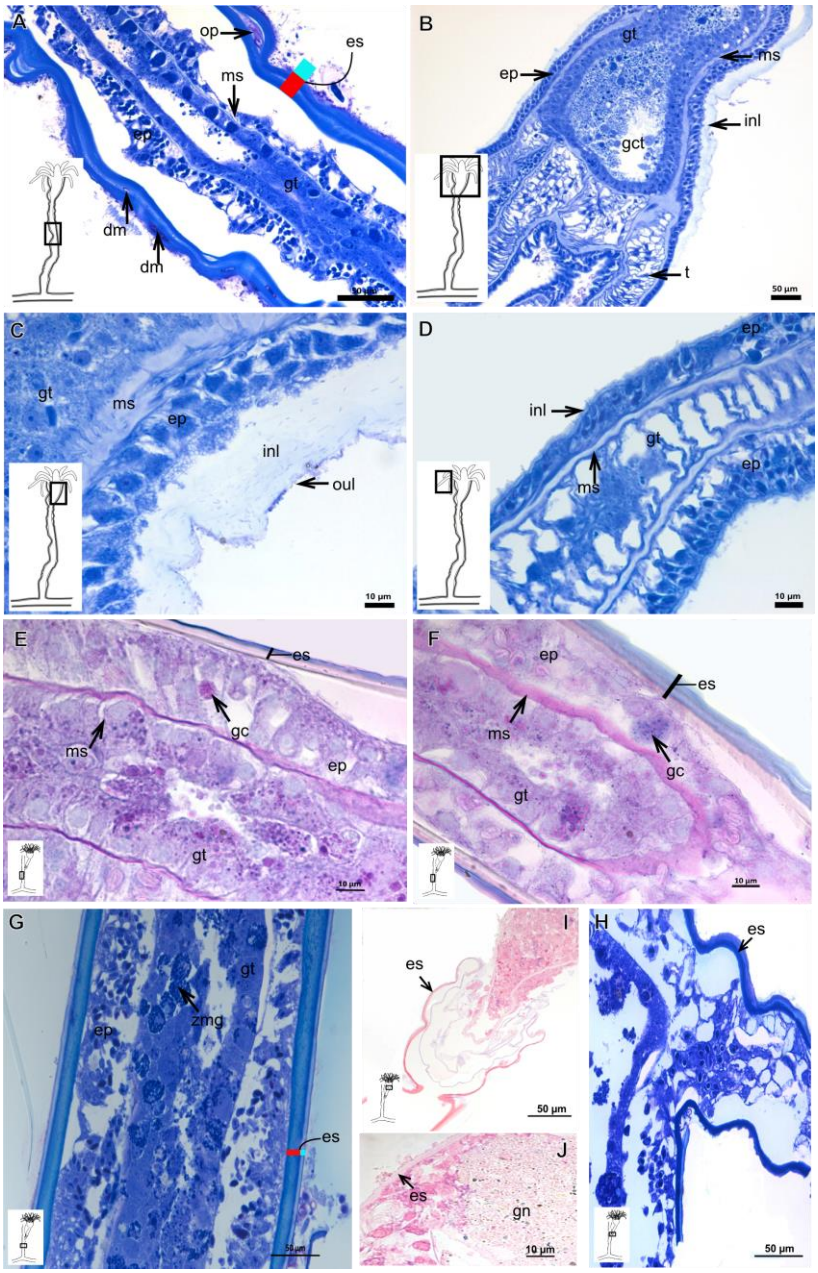

Figure 13. A-D: Exoskeletal structure of *Rhizorhagium* sp. A: hydrocaulus in the central region of the polyp; B: hydranth; C: exoskeleton of the hydranth; D: exoskeleton of the tentacle. E-J: Exoskeletal structure of *Eudendrium carneum* Clarke, 1882. E-G: glandular cells and exoskeleton of the central region of the hydrocaulus; H: slightly corrugated exoskeleton of side-branch; I: invagination of exoskeleton at the base of the hydranth; J: exoskeleton in the gonophore. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton. Abbreviations: dm, diatoms; ep, epidermis; es, exoskeleton; gc, glandular cells; gct, gastrovascular cavity; gn, gonadal cell cluster; gt, gastrodermis; inl, inner layer; ms, mesoglea; op, organic particle; oul, outer layer; t, tentacle; zmg, zymogen glandular cell.

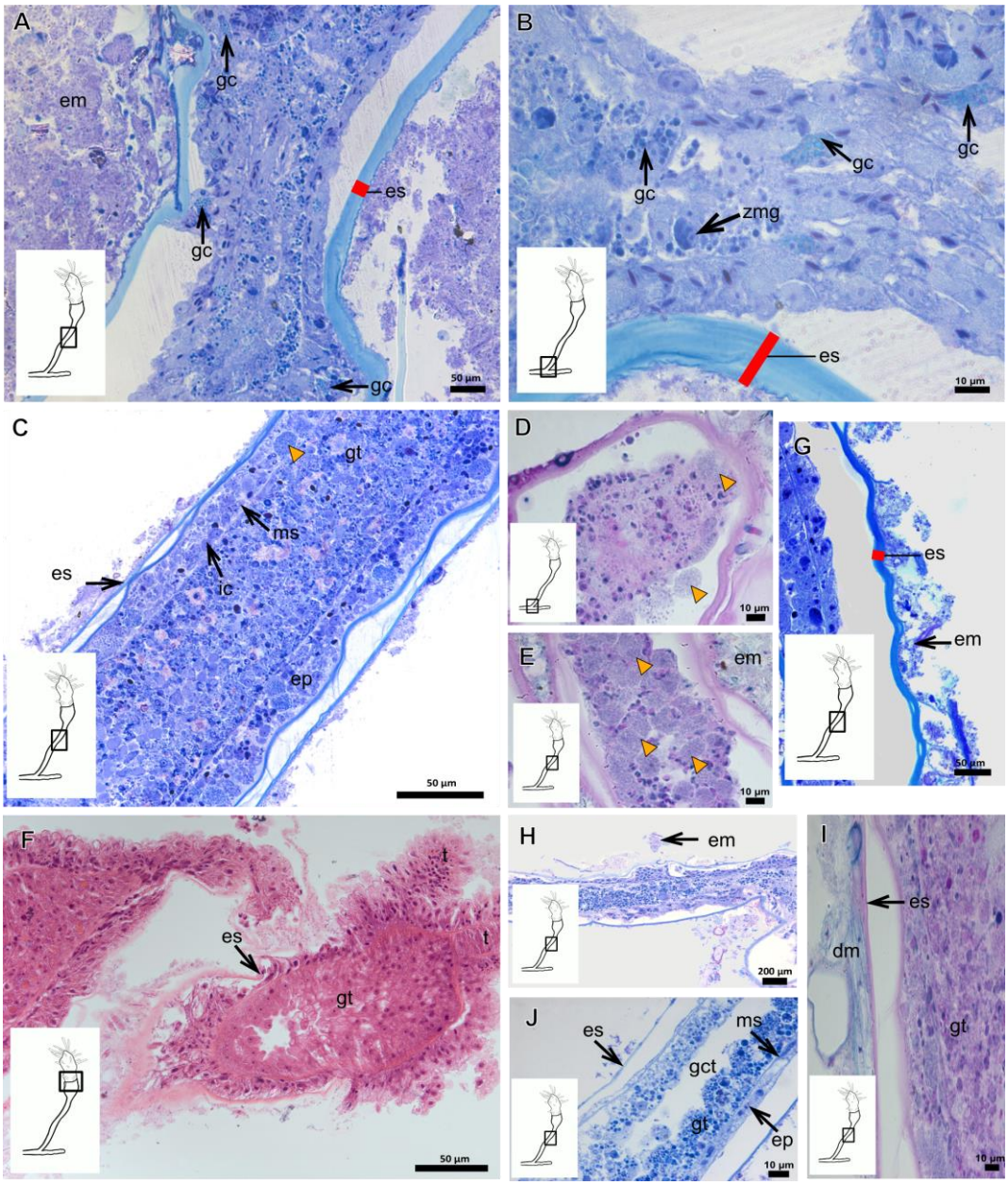

Figure 14. Internal and exoskeletal structure of *Turritopsis nutricula* McCrady, 1857. A: hydrocaulus with external material encrusted on the central region of the polyp; B: coenosarc and exoskeleton in the basal region of polyp; C: epithelial layers and exoskeleton of the hydrocaulus; D-E: epidermis; F: exoskeleton of the lower part of the hydranth; G: irregularly corrugated exoskeleton of polyps; H: granular membrane of the hydrocaulus; I: exoskeleton with diatoms; J: exoskeletal and coenosarc details of the hydrocaulus in a developing polyp. Orange arrowhead indicates undifferentiated cells, red line indicates the exoskeleton. Abbreviations: dm, diatoms; ep, epidermis; em, external material; es, exoskeleton; gc, glandular cells; gct, gastrovascular cavity; gt, gastrodermis; ic, interstitial cells; ms, mesoglea; t, tentacle; zmg, zymogen glandular cell.

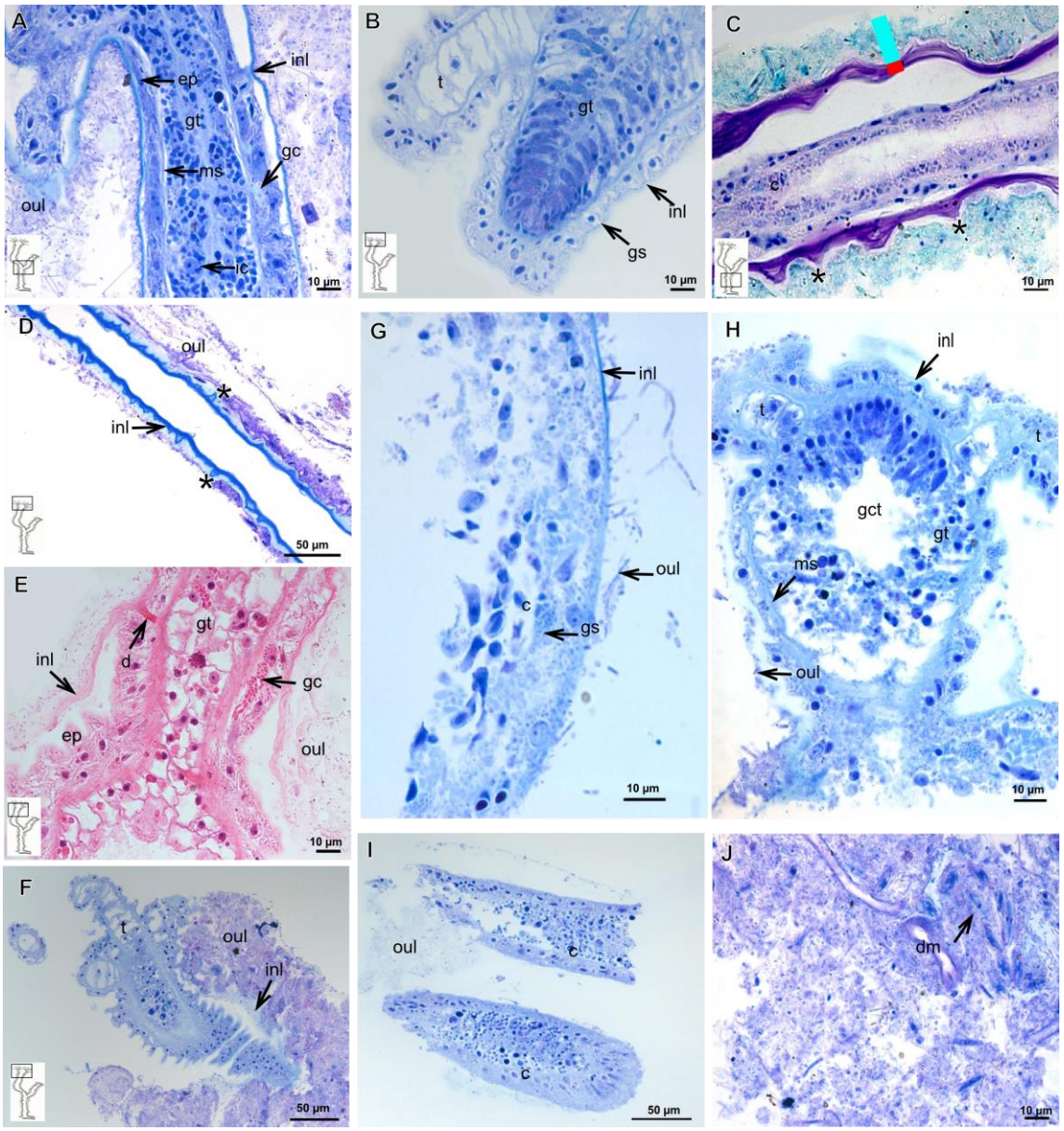

Figure 15. Internal and exoskeletal structure of *Leuckartiara octona* (Fleming, 1823). A: hydrocaulus and side-branch of the central region of the polyp; B: apical region of the hydranth; C: hydrocauline exoskeleton; D: general exoskeleton of a developing polyp; E: basal region of the hydranth; F: exoskeleton of the hydranth; G: structure of the developing stolon; H: developing polyp; I: anatomy of the free stolon/branch; J: outer layer with organic and inorganic material. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton, asterisk indicates "perisarc extensions". Abbreviations: c, coenosarc; d, desmocyte; dm, diatoms; ep, epidermis; gc, glandular cells; gct, gastrovascular cavity; gs, secretory granules; gt, gastrodermis; inl, inner layer; ms, mesoglea; oul, outer layer; t, tentacle.

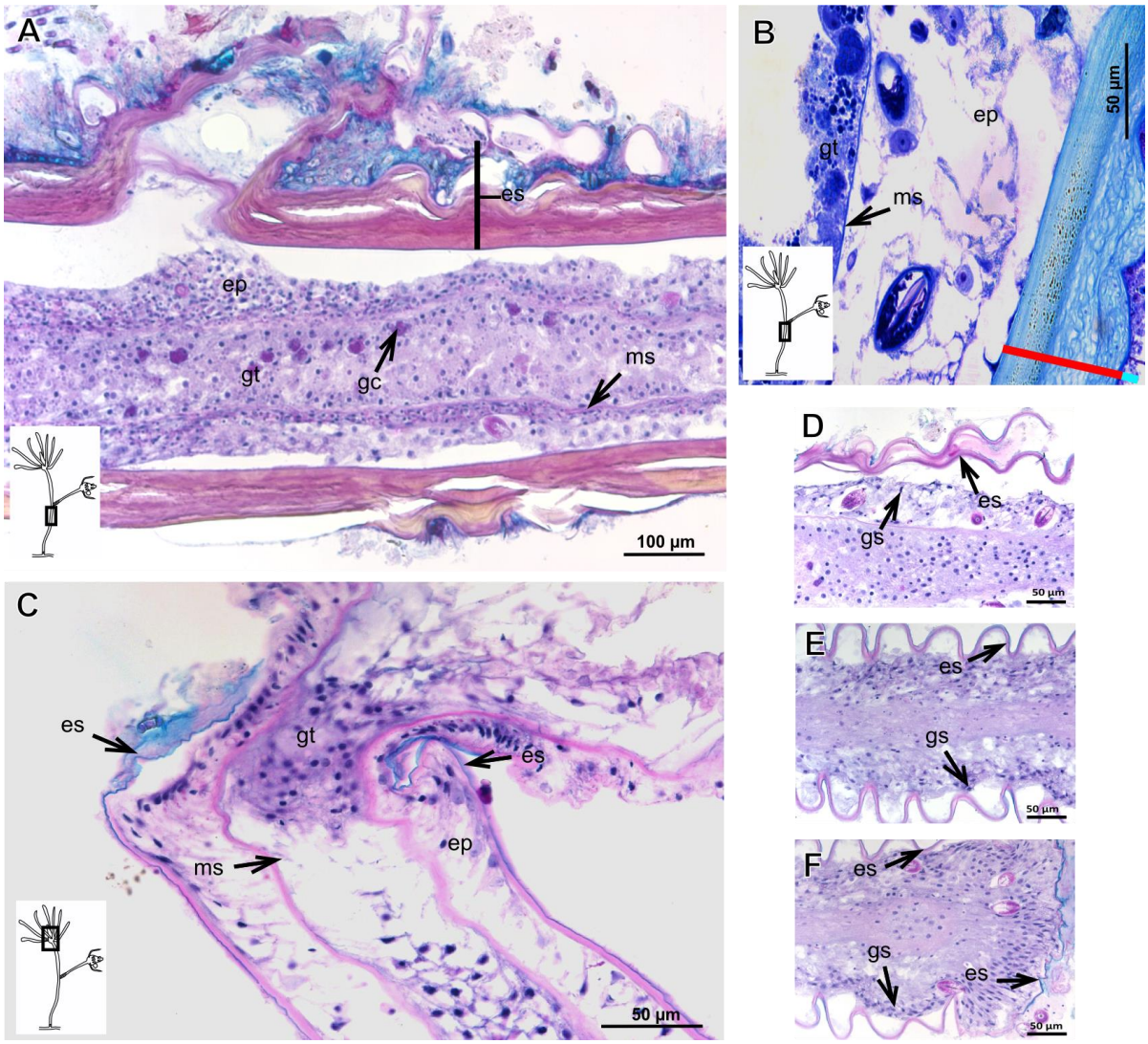

Figure 16. Internal and exoskeletal structure of *Pennaria disticha* Goldfuss, 1820. A: hydrocaulus and side-branch of the central region of the polyp; B: epidermis in the central region of the polyp; C: exoskeleton in the lower part of the hydranth; D-F: developing side-branch. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton. Abbreviations: ep, epidermis; es, exoskeleton; gc, glandular cells; gct, gastrovascular cavity; gs, secretory granules; gt, gastrodermis; ms, mesoglea.

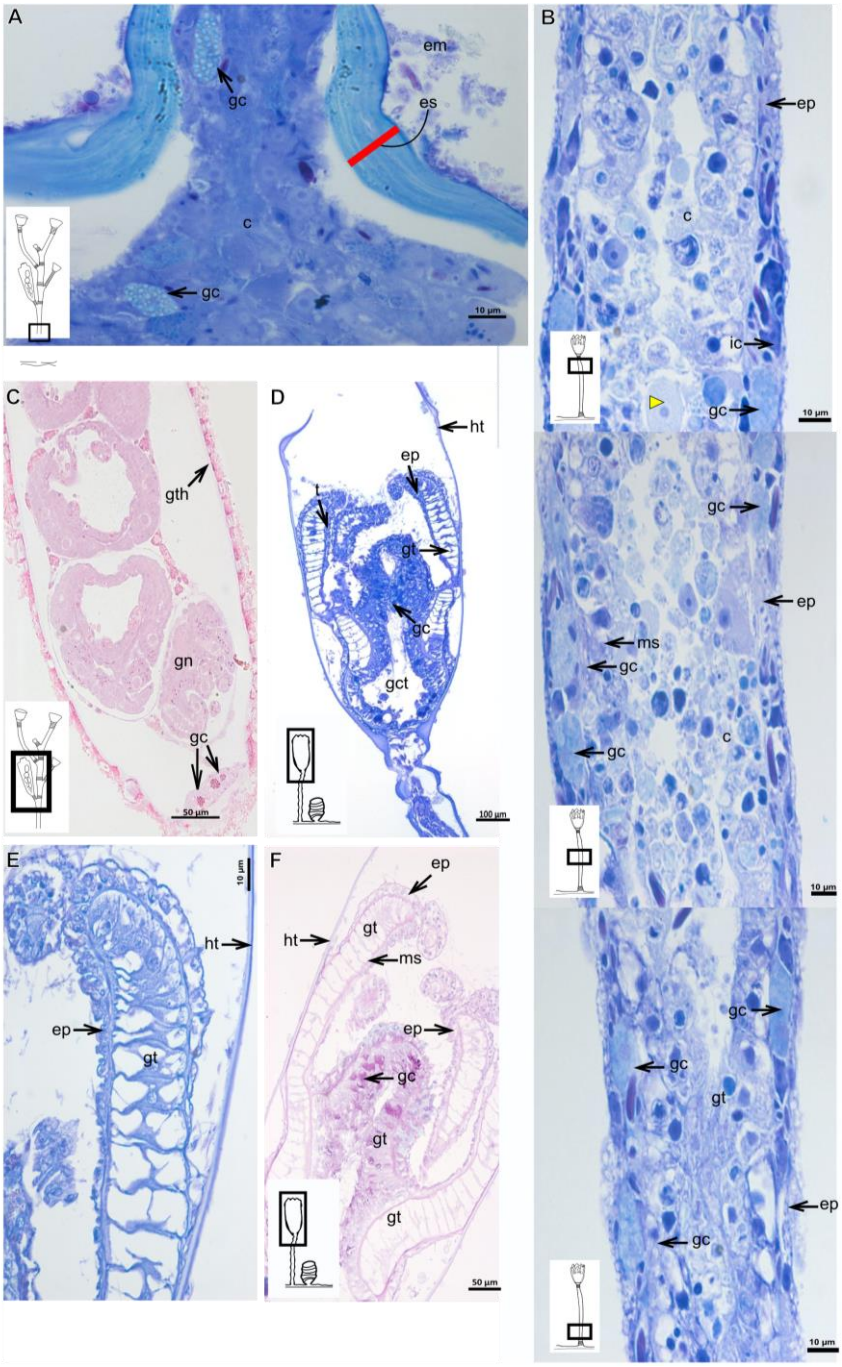

Figure 17. Coenosarc of species of Campanulariidae Johnston, 1836. A: epithelial glandular cells of *Orthopyxis sargassicola*; B: coenosarc of the hydrocaulus from apical to basal region in a polyp of *Clytia* sp.; C: epidermal glandular cells of the gonotheca of *Obelia dichotoma*; D: hydranth of *Orthopyxis sargassicola*; E: coenosarc of the tentacle in *Orthopyxis sargassicola*; F: hydranth of *Orthopyxis sargassicola*. Yellow arrowhead indicates zooxanthellae, red line indicates the exoskeleton. Abbreviations: c, coenosarc; em, external material; ep, epidermis; es, exoskeleton; gc, glandular cells; gct, gastrovascular cavity; gt, gastrodermis; ht, hydrotheca; ic, interstitial cells; ms, mesoglea; t, tentacle.

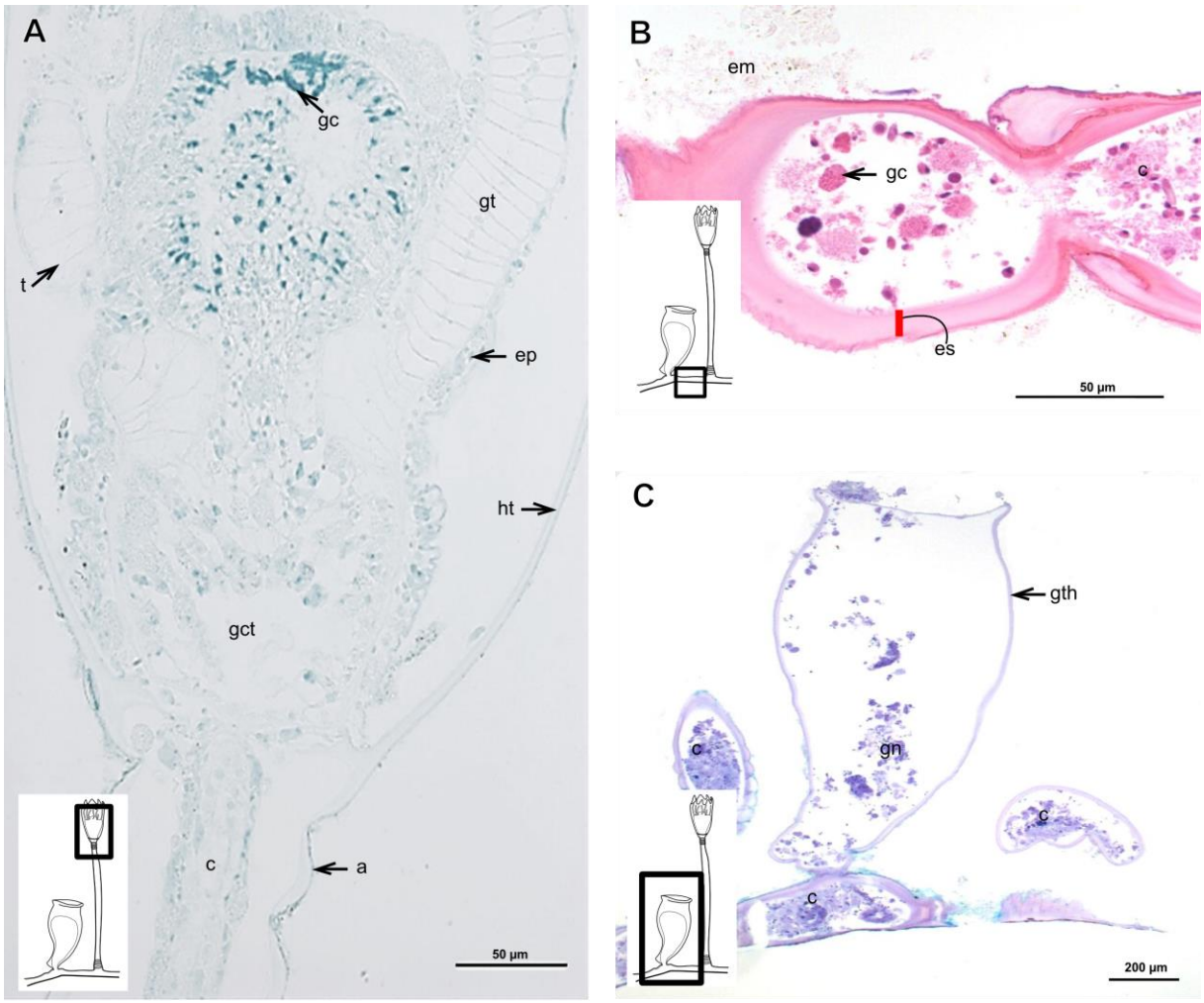

Figure 18. Exoskeletal structure in specimens of *Clytia gracilis* (M. Sars, 1850). A: exoskeleton of the hydranth; B: exoskeleton of the hydrorhiza; C: exoskeleton of gonotheca. Red line indicates the layer of exoskeleton. Abbreviations: a, annulation; c, coenosarc; em, external material; ep, epidermis; es, exoskeleton; gc, glandular cells; gct, gastrovascular cavity; gt, gastrodermis; gth, gonotheca; ht, hydrotheca; t, tentacle.

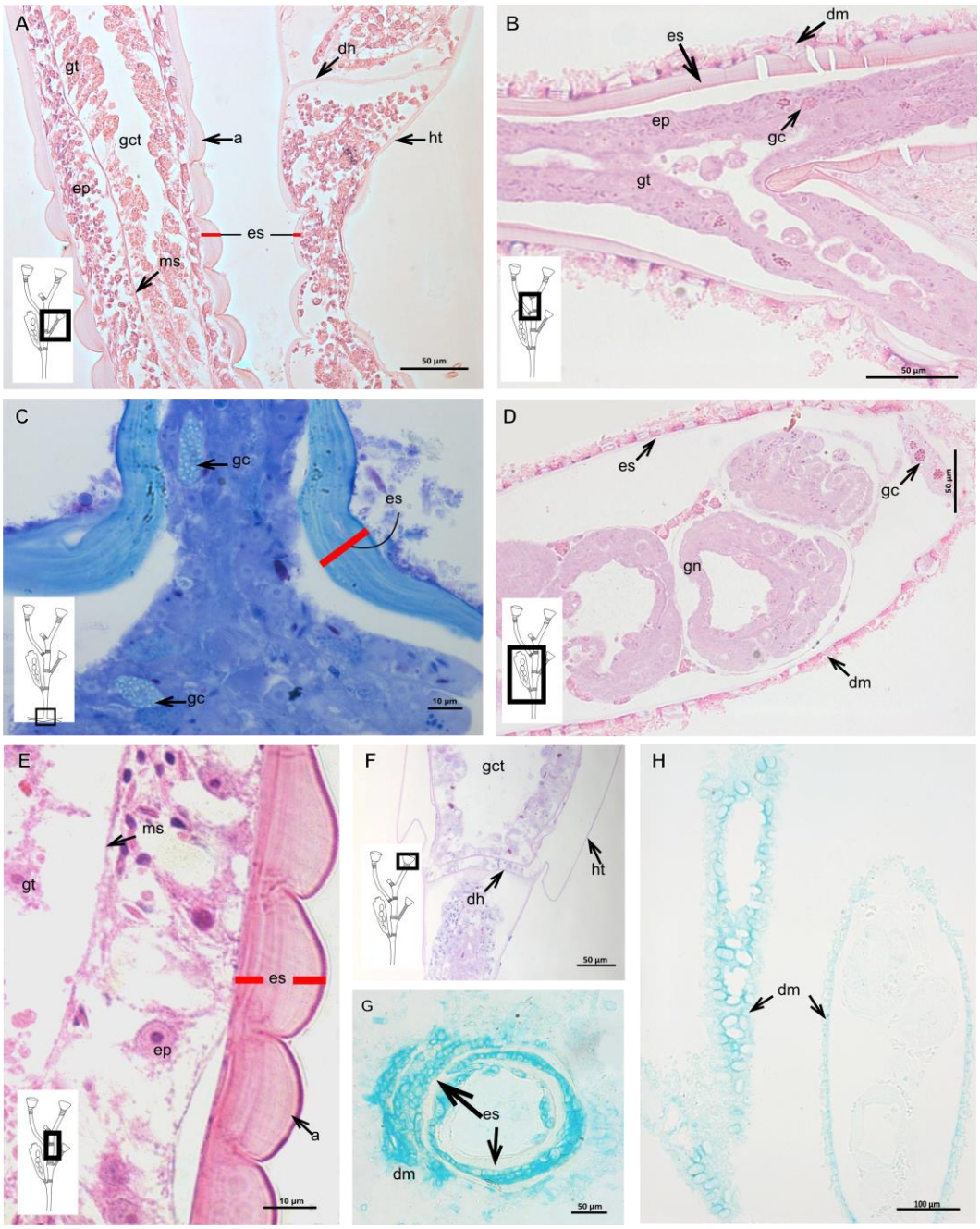

Figure 19. Exoskeletal structure of *Obelia dichotoma* (Linnaeus, 1758). A: exoskeleton of hydrocaulus and hydranth; B, exoskeleton of the hydrocaulus; C: hydrorhiza with glandular cells and exoskeleton; D: exoskeleton with attached diatoms in the gonophore; E: exoskeletal layer in the hydrocaulus; F: hydrotheca with lateral folds; G: transverse section of the exoskeleton of the hydrorhiza; H: general exoskeletal structure with associated diatoms. Red line indicates the exoskeleton. Abbreviations: a, annulation; dh, diaphragm; dm, diatoms; ep, epidermis; es, exoskeleton; gc, glandular cell; gct, gastrovascular cavity; gn, gonadal cell cluster; gt, gastrodermis; ht, hydrotheca; ms, mesoglea.

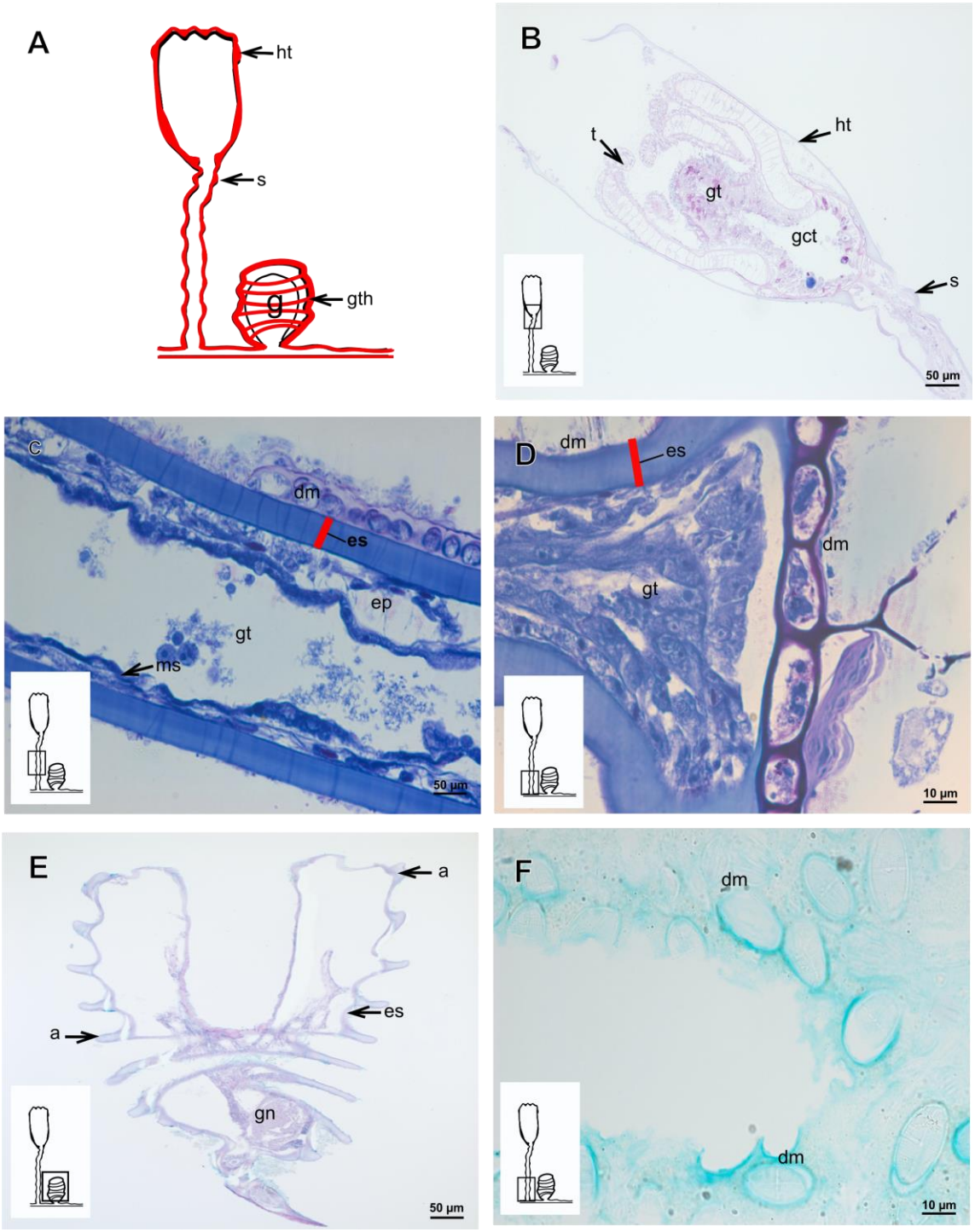

Figure 20. Exoskeletal structure of *Orthopyxis sargassicola* (Nutting, 1915). A: general morphology of the polyp; B, exoskeleton of the hydrotheca and subhydrothecal spherule; C: hydrocaulus; D: exoskeleton with diatoms at the base of the hydrocaulus; E: gonotheca; F: diatoms on the exoskeleton of the hydrocaulus. Red line indicates the exoskeleton. Abbreviations: a, annulation; dm, diatoms; ep, epidermis; es, exoskeleton; g, gonophore; gct, gastrovascular cavity; gn, gonadal cell cluster; gt, gastrodermis; gth, gonotheca; ht, hydrotheca; ms, mesoglea; s, spherule; t, tentacle.

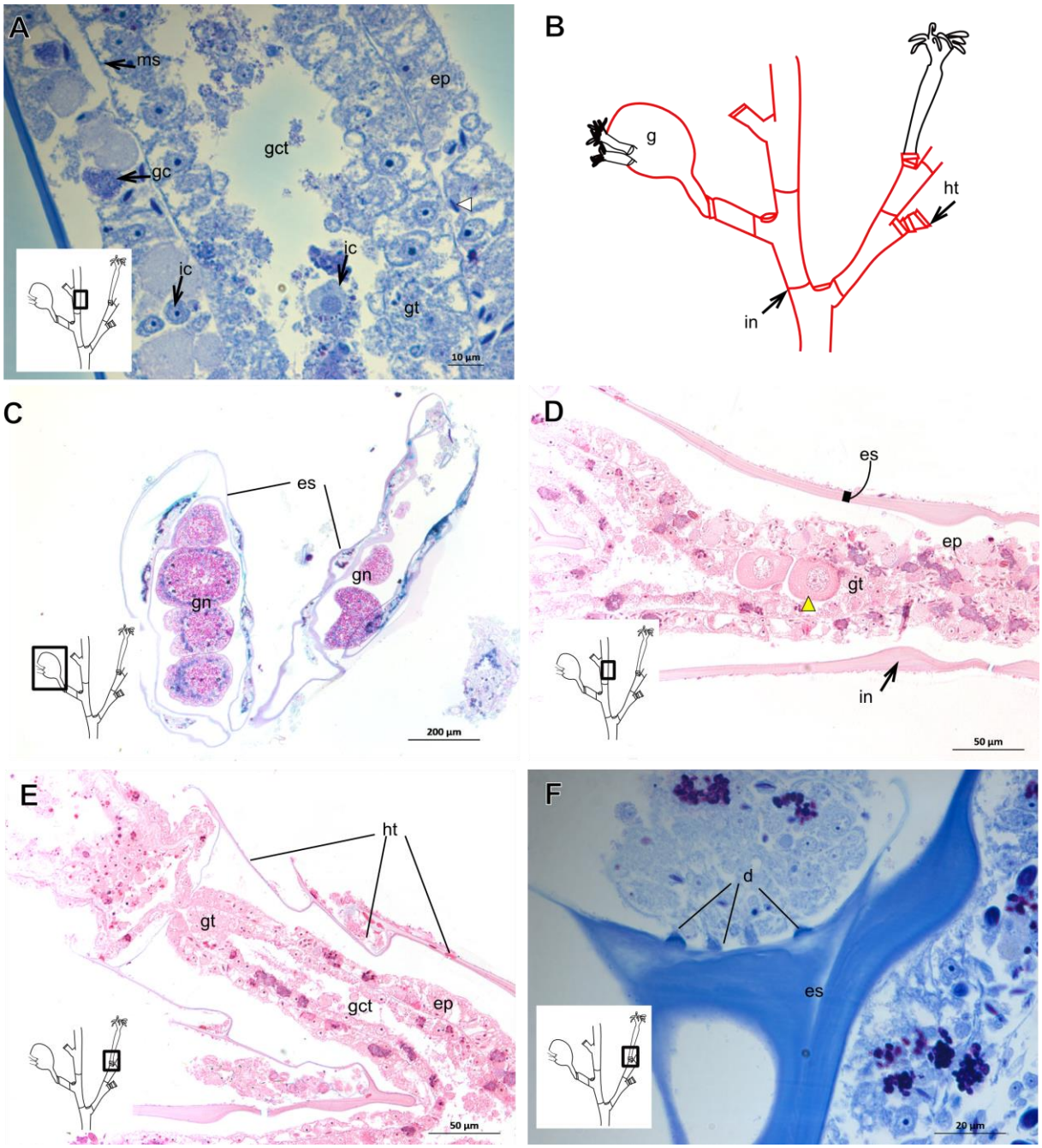

Figure 21. Coenosarc and exoskeletal structure of *Halecium bermudense* Congdon, 1907. A: hydrocauline coenosarc; B: general morphology of the polyp; C: exoskeleton of the female gonophore; D: hydrocauline exoskeleton; E: primary and secondary hydrothecae; F: primary hydrothecae. Yellow arrowhead indicates the zooxanthellae, red line indicates the exoskeleton. Abbreviations: d, desmocytes; ep, epidermis; es, exoskeleton; g, gonophore; gc, glandular cells; gct, gastrovascular cavity; gn, gonadal cell cluster; gt, gastrodermis; ht, hydrotheca; ic, interstitial cells; in, internode; ms, mesoglea; t, tentacle.

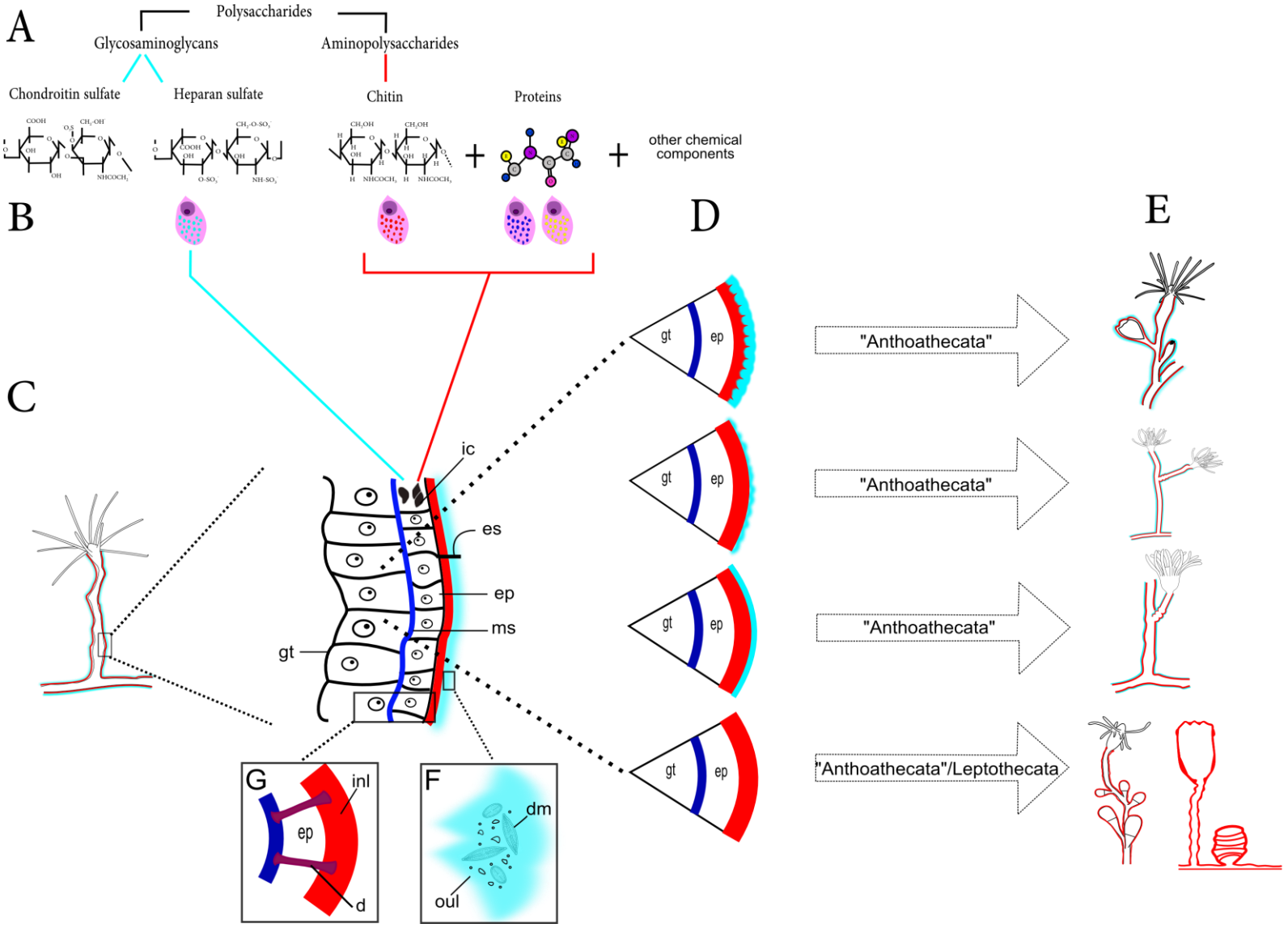

Figure 22. Legend on next page

Figure 22. Schematic drawing of different chemical and structural types of the exoskeleton in the Hydroidolina. A: chemical components; B: scheme of glandular and interstitial cells; C: coenosarc and exoskeleton; D: structural types of the exoskeleton; E: exoskeleton extension in polyps of "Anthoathecata" and Leptothecata; F: outer layer encrusted with inorganic and organic material; G: desmocytes connecting inner layer with mesoglea. Alcian blue line indicates the outer layer of the exoskeleton, red line indicates the inner layer of the exoskeleton. Abbreviations: d, desmocyte; dm, diatoms; ep, epidermis; es, exoskeleton; gt, gastrodermis; ic, interstitial cells; inl, inner layer; ms, mesoglea; oul, outer layer.

Capítulo – 5

Relações filogenéticas e cenários da evolução morfológica em Bougainvilliidae (Cnidaria, Hydrozoa)

María A. Mendoza-Becerril, Adrian José Jaimes-Becerra & Antonio C. Marques

Resumo

Este estudo traz uma análise filogenética (parcimônia, máxima verossimilhança e inferência Bayesiana) para 69 linhagens de "Anthoathecata" com base em 18 caracteres morfológicos (12 dos quais são novos) e de marcadores moleculares mitocondriais (16S e COI) e nucleares (18S e 28S). O objetivo dessa análise foi inferir o monofiletismo e a posição filogenética de Bougainvilliidae, e investigar cenários evolutivos relacionados à sua morfologia, especialmente os caracteres ligados ao exoesqueleto. Os resultados corroboram algumas incertezas já publicadas sobre as relações filogenéticas entre Hydroidolina, ao mesmo tempo que delimitam oito linhagens monofiléticas bem suportadas: Hydroidolina, Siphonophorae, Leptothecata, Aplanulata, Filifera II, Filifera III, Capitata e Pseudothecata *taxon novum*. Vários clados no nível de família (Bougainvilliidae, Cordylophoridae, Oceaniidae, Rathkeidae e Pandeidae) resultaram como não monofilético. Os gêneros classicamente assumidos para "Bougainvilliidae" (exceto por *Dicoryne*) estão incluídos em Pseudothecata, que tem uma relação de grupo irmão com Leptothecata suportada por quatro caracteres morfológicos. Um aspecto chave nos padrões de diversificação dentre Hydroidolina é a evolução exoesquelética, especialmente no que se refere à transição de um corpo do hidrante não envolto por exoesqueleto até hidrantes totalmente envoltos. Neste cenário, membros de "Bougainvilliidae" apresentam estados intermediários na forma de um exossarco. Nossos resultados também sugerem que o desenvolvimento exoesquelético de Hydroidolina pode ser decomposto em três sistemas ("síntese molecular", "matriz molecular" e "expressão morfológica"), e seu uso concatenado pode ser útil na inferência de relações filogenéticas e, consequentemente, no entendimento da implicação evolutiva e ecológica do exoesqueleto nos Medusozoa.

Palavras-chave: exoesqueleto, filogenia, Hydroidolina, pólipos.

Abstract

This study provides a phylogenetic analysis (parsimony, maximum likelihood and Bayesian inference) of 69 taxa of "Anthoathecata" based on 18 morphological characters (from which 12 are new) and on a combined dataset of molecular data, including mitochondrial (16S and COI) and nuclear markers (18S and 28S). The aim of this analysis was to infer the monophyletic and phylogenetic position of Bougainvilliidae, and investigate evolutionary scenarios related to its morphology, specially concerning exoskeletal characters. The results support some uncertainties that have been published on the phylogenetic relationships of the Hydroidolina, while delimiting eight well supported monophyletic lineages: Hydroidolina, Siphonophorae, Leptothecata, Aplanulata, Filifera II, Filifera III, Capitata and Pseudothecata *taxon novum*. Several clades on the family level (Bougainvilliidae, Cordylophoridae, Oceaniidae, Rathkeidae and Pandeidae) are not monophyletic. Genera traditionally referred to "Bougainvilliidae" (except *Dicoryne*) are included in Pseudothecata, which is sister group to Leptothecata, a relationship supported by four morphological characters. Exoskeletal evolution is a key aspect in the patterns of diversity within Hydroidolina, especially concerning the transition from a hydrant body not completely covered by the exoskeleton to one completely covered. In this context, members of "Bougainvilliidae" have intermediate states, as the exosarc. Our results also suggest that the exoskeletal development of Hydroidolina can be decomposed into three systems ("molecular synthesis system", "molecular matrix" and "morphological expression"), which may be helpful to infer phylogenetic relationships when used in conjunction, and, as a consequence, to understand the evolutionary and ecological implications of the exoskeleton in the Medusozoa.

Key words: exoskeleton, phylogenetics, Hydroidolina, polyps.

Introdução

A família Bougainvilliidae Lütken, 1850 (Hydrozoa, Hydroidolina), incluída na ordem não-monofilética “Anthoathecata” (cf. Cartwright et al., 2008), tem ampla distribuição latitudinal em águas marinhas e salobras (Mendoza-Becerril & Marques, 2013), e conta inclusive com um gênero de água doce, *Velkovrhia* Matjašiĉ & Sket, 1971 (Schuchert, 2007).

Como outros Hydroidolina, Bougainvilliidae apresenta duas fases em seu ciclo de vida, um pólipo que vive fixo a um substrato e uma medusa que pode ser livre natante ou reduzida a um gonóforo aderido ao pólipo (Russell, 1953). O desconhecimento sobre essas fases e os ciclos de vida de Bougainvilliidae originou um sistema de classificação dual, em que pólipos e medusas das mesmas espécies eram nomeados independentemente. Por exemplo, o gênero *Bougainvillia* Lesson, 1830 foi originalmente usado para descrever uma medusa cujo pólipo equivalente foi primeiramente descrito no gênero *Perigonimus* M. Sars, 1846. Como consequência, *Bougainvillia*, por exemplo, tem atualmente seis nomes sinônimos (ver Calder, 1988; Schuchert, 2015). Evidentemente, estes obstáculos taxonômicos e a dualidade de formas do ciclo de vida dificultam a inferência das relações filogenéticas do grupo e são refletidas nas escassas filogenias com base morfológica para Hydrozoa como um todo (e.g., Petersen, 1990; Peña Cantero & Marques, 1999; Marques & Migotto, 2001; Marques et al., 2006).

A maioria das espécies de Bougainvilliidae possui morfologias simples, variáveis e similares entre si, dificultando o estabelecimento de caracteres diagnósticos em sua taxonomia morfológica (cf. Lütken, 1850; Mayer, 1910: 131; Fraser, 1944: 47; Russell, 1953: 143-144; Kramp, 1961:74, Vannucci & Rees, 1961: 57-58; Millard 1975: 88-91; Calder, 1988: 12-13; Schuchert, 1996: 27, 2007: 196-197). Até mesmo a delimitação de Bougainvilliidae em relação a outras famílias com morfologias semelhantes (e.g., Cordylophoridae, Cytaeididae, Oceaniidae, Pandeidae, Russelliidae) é difícil (e.g., Millard, 1975; Calder, 1988, Schuchert, 2007, 2012).

Estudos taxonômicos em Bougainvilliidae foram majoritariamente limitados ao nível específico e a áreas geográficas limitadas, e sua taxonomia supraespecífica foi alvo de poucos estudos. A classificação mais completa divide Bougainvilliidae em quatro subfamílias (Bimeriinae, Bougainvilliinae, Pachycordylinae, Rhizorhagiinae), com base em caracteres morfológicos dos pólipos, tais como a extensão da cobertura da pseudo-hidroteca, forma do hidrante e hipostômio, arranjo dos tentáculos, posição e tipo de gonóforo (Calder, 1988). Porém, a classificação mais utilizada atualmente não divide a família em subgrupos (Schuchert, 2007, 2012).

Dados moleculares para o grupo são recentes (Collins, 2000; Collins et al., 2006) e há apenas uma inferência molecular focada na família (Schuchert, 2007), embora uma melhor amostragem taxonômica e de marcadores exista no contexto da posição de bougainvilídeos dentre os Gonoproxima, táxon definido pela posição do gonóforo no hidrocaule (Cartwright et

al., 2008). De fato, o monofiletismo e a posição filogenética de Bougainvilliidae entre os "Anthoathecata" ainda são ambíguos (Cartwright & Nawrocki, 2010; Schuchert, 2012; Maronna, 2014).

Classicamente, a pseudo-hidroteca, uma cobertura externa com ou sem detritos que envolve os hidrantes (Allman, 1871; Calder, 1988), tem sido central na taxonomia tradicional de Bougainvilliidae e alguns outros táxons antoatecados. Porém, estudos histológicos e do desenvolvimento do sistema exoesquelético em vários Hydroidolina, considerando a pseudo-hidroteca, são recentes, e desvelaram que essa estrutura é parte de uma das camadas de um exoesqueleto bicamada. Essa camada mais externa (= exossarco) envolve a camada quitino-proteica do exoesqueleto, e tem implicações evolutivas e ecológicas importantes (Mendoza-Becerril et al., 2015a, 2015b).

O exoesqueleto, no que diz respeito à sua variação morfológica, química e de origem, tem sido pouco discutido no contexto das relações filogenéticas nos Hydrozoa. Há poucas análises com hipóteses evolutivas que incluem caracteres exoesqueléticos (e.g., Petersen, 1990, caracteres 15, 17 e 22; Marques & Migotto, 2001, caracteres 5, 14, 32, 35, 38 e 40). Abordagens mais recentes têm reconstruído filogeneticamente caracteres morfológicos, mas não têm utilizado esses caracteres nas inferências filogenéticas *per se* (cf. Cartwright & Nawrocki, 2010; Miglietta & Cunningham, 2012), e apenas um estudo inclui caracteres exoesqueléticos (Miglietta et al., 2010).

A partir do exposto, o objetivo desse estudo é inferir o monofiletismo e a posição filogenética de Bougainvilliidae dentre algumas linhagens de "antoatecados" utilizando dados morfológicos e moleculares, e investigar cenários de evolução dos caracteres morfológicos, principalmente aqueles da estrutura exoesquelética.

Material e métodos

Terminais e caracteres

A análise incluiu 69 terminais, sendo 10 "Bougainvilliidae" (representando 10% das espécies e 47% dos gêneros válidos da família) e 59 representantes dos grupos Capitata (5 espécies), Aplanulata (3 espécies), Siphonophorae (3 espécies), "Filifera" (27 espécies) e Leptothecata (16 espécies). Os terminais foram selecionados pela disponibilidade de sequências de DNA, descrições morfológicas mais detalhadas na literatura e presença de diferentes tipos exoesqueléticos. Cinco espécies de Trachylina, grupo monofilético irmão de

Hydroidolina, foram usadas no enraizamento das hipóteses (Collins et al., 2006; Cartwright & Nawrocki, 2010).

Os dados morfológicos da matriz foram baseados em Cartwright & Nawrocki (2010), mas seus caracteres 2 e 3 foram recodificados e divididos nos caracteres 2-3 e 5-6, respectivamente, e seu caráter 4 foi recodificado. Além disso, adicionamos 12 novos caracteres (7-18) com base em descrições inequívocas das espécies (e.g., Calder, 1988; Cornelius, 1995a, b; Schuchert, 2010, 2012; Mendoza-Becerril et al., 2015a). A matriz final inclui 11 caracteres morfológicos binários e 7 multiestados não-aditivos (Tabela 1; Fig. 1; Material suplementar A).

Utilizamos como marcadores moleculares dois genes mitocondriais (16S e COI) e dois nucleares (18S e 28S), obtidos a partir de sequências disponíveis no GenBank (especialmente de Cartwright et al., 2008; Cartwright & Nawrocki, 2010) e outras do Laboratório de Evolução Marinha (Tabela 2).

Análise filogenética

As sequências foram alinhadas com o software MAFFT v6 (Katoh & Toh, 2008) usando os parâmetros *default* e a estratégia E-INS-i. As regiões de baixa qualidade nas extremidades 5' e 3' foram eliminadas usando Gblocks versão 0.91b (Castresana, 2000; Talavera & Castresana, 2007), com parâmetros permitindo blocos finais menores, e menos rigor nas posições de indel dentro dos blocos finais e extremos.

Duas análises filogenéticas foram realizadas: (a) uma para a matriz denominada molecular, com os marcadores 28S, 18S, 16S e COI; e (b) outra denominada combinada, em que se adicionou os dados morfológicos. As sequências foram concatenadas por meio do software SequenceMatrix (Vaidya et al., 2010). Na busca da maior eficiência possível para cada análise, a maioria dos táxons terminais possui sequências para todos os marcadores, sendo alguns terminais "quimeras", mas, também foram incluídos táxons com um mínimo de 50% dos marcadores e 1.133bp (Tabela 2, 3). Todos os dados foram tratados como não aditivos e sem pesagem. Os critérios para as análises foram os seguintes:

Parcimônia (P) – as matrizes foram processadas no programa TNT (Goloboff et al., 2008) e analisadas utilizando-se algoritmos de *New Technology* sob os parâmetros: *Max. Trees*=10.000; *Random addition sequence*=1.000; *Ratchet*=100 interações com 20 árvores retidas cada, com 4% de probabilidade de *upweighting* e *downweighting*; *Drifting*=100 ciclos; *Tree fusing*=100 rodadas. *Indels* foram tratados como quinto estado. O suporte dos ramos foi calculado no TNT, por meio de *bootstrap* com 100 réplicas e do suporte de Bremer (Bremer,

1988, 1994) a partir da retenção de árvores subótimas com até 25 passos extras e obtidas por *Random addition sequence* (1000 réplicas, 10 árvores retidas por réplica) e TBR (*Tree bisection reconection*). Árvores de consenso estrito foram calculadas no TNT.

Métodos estatísticos – realizamos análises de máxima verossimilhança (ML, *Maximum Likelihood*) e inferência Bayesiana (BI, *bayesian inference*) para testar e/ou corroborar os clados obtidos a partir da análise de parcimônia, sendo alguns eventualmente insensíveis aos diferentes algoritmos de cada método (i.e., grupos pretensamente bem suportados) e outros sensíveis (i.e., grupos pretensamente mal suportados).

O modelo evolutivo para cada um dos genes foi calculado com o jModelTest 2.1.1 (Darriba et al., 2012) sob o critério de informação Akaike (AIC) (Posada & Buckley, 2004) (Tabela 3) e os dados foram analisados por critérios de ML e BI.

A análise de ML foi realizada apenas para a matriz molecular, no programa RaxML 7.0.4 (Stematakis, 2006), com os parâmetros em *default* de distribuição gama sem tratamento de dados invariáveis (modelo "GTR+GAMMA"). Cada marcador foi considerado como uma partição para que o cálculo de verossimilhança específico de cada marcador não influísse no cálculo dos demais, evitando artefatos relacionados a diferenças nas taxas de evolução dos diferentes genes. O suporte foi inferido por *bootstrap* (100 réplicas).

A análise de BI foi realizada apenas para a matriz molecular, no programa Mr. Bayes versão 3.2.4 (Huelsenbeck & Ronquist, 2001), com um modelo evolutivo para cada gene utilizando uma estratégia particionada com duas corridas independentes de cada quatro cadeias *Metropolis-Coupled Markov* (MCMC) para 5 milhões de gerações (começando com uma árvore aleatória), sendo as cadeias amostradas a cada 1.000 gerações. A convergência da análise foi válida quando as frequências atingiram um desvio padrão de $p<0,01$. Os parâmetros posteriores e os *outputs* da análise BI foram analisados no software Tracer 1.5 (Rambaut & Drummond, 2007), desconsiderando-se 25% das árvores como *burn-in* para atingir a estabilidade dos valores de *likelihood* na fase de aquecimento. Após as análises obtivemos a árvore de consenso de maioria (nível de corte de 50%) com valores de probabilidade posterior.

O potencial de "informatividade" filogenética (sítios informativos para parcimônia) das sequências para cada marcador foi calculado no programa MEGA 6.0 (Kumar et al., 2001), enquanto para os dados morfológicos foi usado o TNT. As topologias obtidas foram comparadas entre si pela distância SPR *distances between the trees* no TNT, que calcula o

número mínimo de movimentos SPR requeridos para transformar uma árvore na árvore de referência (Goloboff, 2008).

Reconstrução dos estados de caráter ancestral – Adotamos como hipótese-base para a reconstrução dos estados ancestrais a filogenia molecular obtida por parcimônia. Essa topologia assegura o mesmo critério (parcimônia) para os métodos de inferência filogenética e otimização da reconstrução dos 18 caracteres morfológicos, que foi realizada no programa Mesquite 2.75 (Maddison & Maddison, 2011). A maioria dos estados dos caracteres nos ramos internos da topologia é inequívoca e, no caso de ambiguidades, adotamos a otimização ACCTRAN usando o WINCLADA 1.00.08 (Nixon, 1999-2004; ver Agnarsson & Miller, 2008; Gainett et al., 2014). Desconsideramos reconstruções de estado de caráter nos ramos internos espúrios e derivados por dados desconhecidos, não estudados ou inaplicáveis ("?" e "N") (cf. Agnarsson & Miller, 2008). Calculamos índices de consistência (CI) (Kluge & Farris, 1969), índice de retenção (RI) (Farris, 1989), índice de consistência reescalonado (Farris, 1989) e índice de homoplasia (IH) para cada caráter morfológico e sequências de cada marcador molecular.

Resultados

Hipóteses filogenéticas

A hipótese de trabalho adotada foi o consenso estrito de quatro topologias igualmente parcimoniosas (19.933 passos) resultante da análise com dados moleculares totais (Tabela 3). Esse consenso tem dez linhagens com valores de bootstrap>70 e Bremer>8 (Fig. 2; Tabela 4) – Eudendriidae foi a única linhagem com menor suporte, embora seu monofiletismo conte com diversas autapomorfias (cf. Marques et al., 2000; Marques, 1996, 2001). Análises de ML e BI resultaram nos mesmos clados, com suportes igualmente altos (Figs. MSB1-MSB2; Tabela 4).

Relações filogenéticas entre as linhagens diferem nas topologias de P, ML e BI, sendo P e ML mais congruentes com hipóteses anteriores (e.g., Collins et al., 2006; Cartwright et al., 2008; Maronna, 2014), com uma distância SPR de oito entre elas (Tabela 5). As topologias de P e ML mostram um clado bem suportado de filíferos, que denominamos Pseudothecata *taxon novum*, como grupo irmão de Leptothecata (Figs. 1, MSB1). Embora Pseudothecata + Leptothecata tenha suportes baixos para P e ML, sua hipótese é relevante para a discussão de homologias morfológicas dentre os Hydroidolina.

A análise de parcimônia com a matriz combinada (dados moleculares e morfológicos) resultou em oito árvores igualmente parcimoniosas (20.356 passos), cujo consenso estrito apresenta as mesmas dez linhagens das análises de dados moleculares, mas com uma grande politomia basal, tendo como único grupo definido Pseudothecata + Leptothecata (Fig. MSB3). Ao executar o *jacknife* de primeira ordem dos caracteres, observamos que a exclusão do caráter 18 (cobertura do exossarco sobre o hidrante) não altera a topologia em relação àquela obtida para a matriz molecular (Fig. 2). A otimização do caráter 18 demonstra sua ocorrência homoplástica dentre os Hydroidolina, embora tenha poder de resolução em alguns táxons em níveis menos inclusivos da topologia. O fato desta topologia ser menos resolvida que a obtida a partir da análise de P da matriz molecular corrobora a adoção da última como hipótese de trabalho.

Hydroidolina, Siphonophorae, Leptothecata, Aplanulata, Filifera III (senso Cartwright et al., 2008) e Capitata resultaram como monofiléticas na hipótese de trabalho (Fig. 2). À exceção de Eudendriidae, clados menos inclusivos de filíferos, viz. Bougainvilliidae, Cordylophoridae, Oceaniidae, Rathkeidae e Pandeidae, resultaram não monofiléticos. Pseudothecata *taxon novum* está dividido em dois clados principais bem suportados, que rejeitam o monofiletismo de famílias tradicionalmente representadas: o clado A inclui apenas espécies da família Oceaniidae e o clado B inclui espécies de Bougainvilliidae (exceto *Dicoryne conybearei*, cuja relação permanece ambígua), Cordylophoridae e Rathkeidae. Por sua vez, o clado B está dividido em duas linhagens: C (*Koellikerina fasciculata, Podocorynoides minima, Bougainvillia carolinensis* e *Bougainvillia fulva*) e D (*Nemopsis bachei, Garveia grisea, Cordylophora caspia, Bimeria vestita, Bougainvillia muscus, Pachycordyle michaeli* e *Pachycordyle pusilla*). Os gêneros de Bougainvilliidae com mais de uma espécie na análise resultaram monofiléticos, exceto por *Bougainvillia* (Fig. 2).

Reconstrução dos estados ancestrais dos caracteres morfológicos

Dos 18 caracteres morfológicos (Tabela 1), 16 eram informativos para parcimônia, mas geralmente com altos valores de homoplasia (Tabela 6), o que é refletido nas reconstruções dos estados ancestrais (Tabela 7). Os caracteres não informativos para parcimônia foram considerados na reconstrução por sua relevância no estabelecimento das relações entre grupos menos inclusivos. Abaixo segue a exposição desses caracteres morfológicos e suas reconstruções (Material suplementar 2, Figs. MSB4-MSB12).

Caráter 1 – Desenvolvimento do gonóforo até maturidade sexual (Fig. MBS4). 0 – medusa; 1 – medusoide; 2 – esporossaco; 3 – sem gonóforo.

Medusa é o estado ancestral para os Hydroidolina. Há reduções independentes para esporossaco e medusoide em alguns terminais, mas também há reversões. Em Pseudothecata houve redução ao esporossaco a partir da divergência do clado D.

2 – Organização da fase polipoide (Fig. MSB4). 0 – solitário; 1 – colonial.

Pólipo solitário seria a forma ancestral para Hydroidolina. Entretanto, um clado é colonial à exceção de uma reversão pontual no Bougainvilliidae *Nemopsis bachei*.

3 – Organização colonial da fase polipoide (Fig. MSB5). 0 – colônia incrustante; 1 – colônia vertical; 2 – colônia pelágica.

Colônias incrustantes seriam a forma ancestral em Hydroidolina. Colônias pelágicas teriam evoluído independentemente em Siphonophorae e Capitata. Já o surgimento independente de colônias incrustantes e verticais aparentemente ocorreu várias vezes na evolução de Hydroidolina, talvez relacionado a um componente ambiental e adaptativo. Entretanto, para Pseudothecata + Leptothecata o estado vertical resultou como o estado ancestral.

4 – Posição do gonóforo (Fig. MSB5). 0 – sobre hidrante; 1 – sobre hidrocaule; 2 – sobre hidrorriza.

Gonóforos no hidrocaule seriam o estado ancestral em Hydroidolina, com o gonóforo no hidrante surgindo independentemente em Aplanulata, Eudendriidae e alguns terminais específicos. O gonóforo na hidrorriza surge de forma independente em diversos terminais. Gonóforo no hidrocaule é a condição ancestral e diagnóstica para Pseudothecata (exceto para *N. bachei*).

5. – Gonozooide como um tipo de pólipo na colônia (Fig. MSB6). 0 – ausente; 1 – presente.

A ausência de gonozooides como um dos possíveis tipos polimórficos de pólipos na colônia é o estado ancestral para Hydroidolina. Sua presença surge a partir de Filifera II. Porém, um grado com parte dos filíferos, capitados e com Pseudothecata não possuiria gonozooide, condição revertida em Leptothecata. "Bougainvilliidae" não possuem gonozooide, à exceção de *D. conybearei*, cuja posição filogenética necessita ser melhor investigada.

6 – Dactilozooide como um tipo de pólipo na colônia (Fig. MSB6). 0 – ausente; 1 – presente.

Dactilozooides ocorrem homoplasticamente em diversas linhagens, como Filifera II, Siphonophorae e em alguns Capitata e filíferos, mas não presentes no grupo Pseudothecata + Leptothecata. É uma sinapomorfia no nível de Filifera III.

7 – Tipo de tentáculos orais (Fig. MSB7). 0 – filiforme; 1 – capitado.

A condição filífera é ancestral em Hydroidolina e melhor representada nos grupos Aplanulata e "Filifera".

8 – Tentáculos aborais nos gastrozooides (Fig. MSB7). 0 – ausente; 1 – presente.

Tentáculos aborais ausentes é o estado ancestral em Hydroidolina, com surgimento independente em terminais das linhagens Aplanulata e Capitata.

9 – Arranjo dos tentáculos orais (Fig. MSB8). 0 – coroa simples; 1 – duas ou mais coroas; 2 – dispersos ou não em coroas.

Tentáculos organizados em uma coroa é o estado ancestral em Hydroidolina, com a dispersão ou a ausência de coroas definidas ocorrendo homoplasticamente em diversos terminais. Vale notar que o clado B de Pseudothecata é caracterizado pela presença de tentáculos em duas ou mais coroas.

10 – Presença de quitina como parte da estrutura esquelética (Fig. MSB8). 0 - interna; 1 – externa; 2 – ausente.

Quitina como parte da estrutura exoesquelética é o estado ancestral em Hydroidolina, mas há surgimentos independentes de endoesqueleto quitinoso em Siphonophorae e em um clado de Capitata.

11 – Esqueleto com estrutura anastomosada (Fig. MSB9). 0 – ausente; 1 – presente.

Como autopomorfia de *Solanderia secunda*, o caráter não é informativo no atual contexto, mas pode ser relevante em análises que incluam mais espécies com estrutura esquelética anastomosada (e.g., espécies de Clathrozoellidae, Hydractiniidae, Rosalindidae e Zancleidae) que se relacionariam aos clados "Filifera", Filifera III e Capitata (cf. Cartwright & Nawrocki, 2010; Miglietta et al., 2010; Calder et al., 2015).

12 – Esqueleto com estrutura em forma de disco (Fig. MSB9). 0 – ausente; 1 - presente.

Estruturas endoesqueléticas discoides, ausentes no ancestral de Hydroidolina, surgiram independentemente em subgrupos de Capitata e Siphonophorae.

13 – Esqueleto de carbonato de cálcio (Fig. MSB10). 0 – ausente; 1 – presente.

Esqueletos de carbonato de cálcio surgem independentemente em táxons de Filifera III e Capitata (cf. Miglietta et al., 2010).

14 – Tipo de esqueleto quitinoso (Fig. MSB10). 0 – sem GAGs; 1 – com GAGs.

Quitina com GAGs (glicosaminoglicanos) é o estado ancestral em Hydroidolina, com perda ambígua de GAGs em Pseudothecata + Leptothecata seguida por reversão no clado B-D de Pseudothecata. Porém, deve haver cautela em se assumir a reconstrução do estado ancestral para Pseudothecata como "ausência de GAGs". Há várias espécies de pseudotecados que possuem GAGs em sua estrutura esquelética (e.g., *Bougainvillia rugosa, Garveia gracilis, Garveia franciscana, Garveia nutans Parawrightia robusta*; Mendoza-Becerril et al., 2015), mas que não foram incluídas nesta análise por seus dados moleculares não serem conhecidos.

15 – Estrutura morfológica do perissarco e exossarco (Fig. MSB11). 0 – laminar; 1 – heterogênea.

Há ambiguidade se a estrutura exoesquelética laminar ou heterogênea seria a condição ancestral em Hydroidolina, mas a condição heterogênea é predominante a partir da divergência dos Filifera III, com subsequente reversão em Leptothecata (Mendoza-Becerril et al., 2015a).

16 – Conexão entre perissarco e exossarco (Fig. MSB11). 0 – ausente; 1 – presente.

Caráter não informativo no atual contexto, devido aos dados ausentes para diversos terminais. Entretanto, a presença das estruturas de conexão pode ser testada como uma possível sinapomorfia para o clado D de Pseudothecata.

17 – Cobertura do perissarco sobre o hidrante (Fig. MSB12). 0 – ausente; 1 – parcial; 2 – total.

Reconstrução basal ambígua , mas a otimização ACCTRAN indica ausência de perissarco sobre o hidrante como condição ancestral em Hydroidolina. É evidente, entretanto, que hidrantes totalmente cobertos por perissarco ocorrem em Pseudothecata + Leptothecata, e há origens independentes ocasionais (em *L. octona*). Coberturas parciais são reversões observadas em alguns táxons específicos de Leptothecata.

18 – Cobertura do exossarco sobre o hidrante (Fig. MSB12). 0 – ausente; 1 – parcial; 2 – total.

A cobertura total do hidrante pelo exossarco (definido em Mendoza Becerril et al., 2015b) é o estado ancestral de Pseudothecata, e distingue seus clados A e B. Embora informativo em Pseudothecata, este caráter tem um padrão incerto para o resto das grandes linhagens. Em Leptothecata, por exemplo, a condição ancestral por otimização ACCTRAN seria a cobertura total do hidrante, mas há terminais em que o caráter não é aplicável (seg. Mendoza-Becerril et al., 2015a).

Discussão

Reconstrução filogenética

Incongruências nas relações dos clados nas diferentes topologias refletem que há incertezas na filogenia de Hydroidolina, mas parte é resultado de incongruências inerentes ao uso de diferentes conjuntos de dados e algoritmos (Lemmon et al., 2009; Simmons & Goloboff, 2013). Assim, a exploração de alternativas na análise auxiliaram a constatar padrões importantes. Por exemplo, ao se considerar *gaps* como um quinto estado, encontra-se resolução para os nós internos em Hydroidolina e caracterização de possíveis relações ambíguas, como a de Capitata e "Filifera". Esse padrão contrasta com estudos prévios que não trataram *gaps* como fonte da informação (cf. Cartwright et al., 2008; Maronna, 2014), e pode explicar a posição basal de Capitata na topologia de ML.

Comparando-se a estudos anteriores, há corroboração para o monofiletismo de Hydroidolina, Siphonophorae, Leptothecata, Aplanulata, Filifera III e Capitata (Cartwright et al., 2008; Cartwright & Nawrocki, 2010; Nawrocki et al., 2010, 2013; Maronna, 2014). Porém, há discrepâncias em relação à posição filogenética de alguns "Filifera", como *Hydrichthella epigorgia*, aqui próxima a Eudendriidae, o que pode ser uma relação espúria. Outro ponto interessante é que nossa hipótese filogenética rejeita o monofiletismo de Gonoproxima (Cartwright et al., 2008), já que uma parte dos terminais que pertenceriam a esse táxons resultaram incluídos em Pseudothecata, com quem compartilham o arranjo dos tentáculos orais em duas ou mais coroas e a presença de um exossarco no hidrante, embora essas características aparentemente tenham sido perdidas em membros do clado A.

Um aspecto evolutivo chave é a relação de grupos irmãos de Pseudothecata e Leptothecata. Ambos apresentam uma estrutura exoesquelética o que leva a induzir a existências de um série evolutiva de transição entre o corpo do hidrante não envolto por exoesqueleto (e.g., como em capitados) até um exoesqueleto rígido e laminar como ocorre nos Leptothecata, passando por um estágio intermediário formado por exossarco (e.g., como em "Bougainvilliidae"). De fato, uma eventual relação de "Bougainvilliidae" com Haleciidae já havia sido sugerida (Stechow, 1909), ou mesmo diretamente com Leptothecata (Maronna, 2014). Entretanto, uma vez que a família, tal como em sua concepção original, não é monofilética, alguns padrões comparativos ainda não são claros.

Espécies com exossarco no hidrante (= pseudo-hidroteca) incluem as que foram classicamente consideradas em "Bougainvilliidae" e outras de diferentes famílias, diversas vezes consideradas relacionadas entre si (cf. Rees, 1956; Calder, 1988; Cartwright et al.,

2008; Maronna, 2014). Entretanto, nossos resultados mostram que a presença de exossarco é mais abrangente e plesiomórfica dentro de "Filifera", e o exossarco sobre o hidrante não seria um caráter diagnóstico para linhagens no nível de família (Tabela 8).

De fato, a família "Bougainvilliidae" precisa ser redefinida, inclusive com a incorporação de mais táxons e dados de outras naturezas nas análises, como os da morfologia da medusa, por exemplo. Uma das diferenças mais conspícuas para clados que possuem membros associados classicamente à família (exceto *D. conybearei*) é o arranjo dos tentáculos orais, e a posição e desenvolvimento dos gonóforos. Igualmente, é necessário também redefinir táxons em níveis menos inclusivo, como gênero, principalmente para táxons não monofiléticos como "*Bougainvillia*".

Como dito, o gênero "*Bougainvillia*" foi inicialmente proposto para a fase medusoide (cf. Lesson, 1830), e são facilmente distinguíveis quando suas medusas estão maduras (Vannucci & Rees, 1961). Porém, sua fase de hidroide é difícil de ser determinada por causa da similaridade com outros gêneros nos caracteres diagnósticos tradicionalmente utilizados (cf. Millard, 1975; Calder, 1988). Portanto, os hidroides que não possuem gonóforos ou brotos de medusa/medusoides suficientemente desenvolvidos são de difícil determinação. Além disso, a cobertura do exossarco sobre hidrante, caráter diagnóstico referente à morfologia externa, é influenciado pelo grau de contração do organismo (Schuchert, 2007; Mendoza-Becerril et al., 2015b) e pelas condições ambientais (Vannucci & Rees, 1961; Mendoza-Becerril et al., 2015b).

O gênero *Dicoryne* e classicamente considerado como "Bougainvilliidae" devido à presença de um exossarco no hidrante e gonóforo (que é uma plesiomorfia), pela semelhança de seu trofossomo com o de *Bougainvillia*, e de seu desenvolvimento gonadal com *Bougainvillia superciliaris* (Ashworth & Ritchie, 1915). Entretanto, nossa topologia mostra que o grupo não tem relação de parentesco com a família. Além disso, as espécies de *Dicoryne* possuem características únicas, não compartilhadas com outros "Bougainvilliidae" e Pseudothecata (e.g., presença de blastóstilos e esporossacos ciliados e livres natantes; Schuchert, 2007). Essas hipóteses deverão ser testadas no futuro, mas cremos que, no atual estágio de conhecimento, é mais adequado adotar família Dicorynidae, como proposto por Allman (1864).

Reconstrução dos estados de caráter ancestral

Alguns caracteres têm consequência direta para a biologia dos grupos e, portanto, compreender sua evolução auxilia a entender a ecologia, abundância e diversificação desses

táxons. Um exemplo é a presença de medusa, que muda o ciclo e, por consequência, as estratégias de vida das espécies, que passam a interagir com fatores bióticos e abióticos do ambiente planctônico, além do bentônico ancestral. Sua presença tem sido considerada como estado ancestral em Medusozoa e em grupos menos inclusivos (cf. Marques & Collins, 2004; Van Iten et al., 2006; Cartwright & Nawrocki, 2010), embora haja diversas perdas ou transformações em estágios não natantes (Leclère et al., 2007). É interessante notar que nosso padrão de reconstrução indicaria uma transformação para a expressão de esporossacos em determinado momento da evolução dos Hydroidolina, posteriormente revertido para uma re-expressão da medusa (Fig. MSB4, caráter 1). Esse padrão, entretanto, deve ser interpretado com prudência, devido à posição ainda instável de *Dicoryne conybearei*.

A presença/ausência de medusa no ciclo de vida interage com a colonialidade das espécies (Fig. MSB4, caráter 2), outro caráter que modula fortemente a ecologia e biologia das espécies. Organismos modulares ocorrem em diversos níveis de Cnidaria, especialmente em Anthozoa, e parecem ser a condição ancestral no filo. Entretanto, a perda de colonialidade pode estar subestimada dentre os Hydroidolina, porque há vários "Anthoathecata" solitários que ainda não foram incluídos nas análises (e.g., pólipos de Halimedusidae, *Brinckmannia hexactinellidophila*; Cartwright & Nawrocki, 2010) e que, inclusive, justificam a hipótese de que pólipos solitários seriam ancestrais nos grupo (Rees, 1957).

O tipo de tentáculos tem sido ampla- e classicamente utilizado para dividir os grupos "atecados" em "Filifera" e Capitata, mesmo que o estado filiforme seja considerado como plesiomórfico (Petersen, 1990). De fato, nossos resultados mostram que a condição capitada é apomórfica porém homoplástica de dois grupos (Fig. MSB7 caráter 7). Em relação ao arranjo dos tentáculos (Fig. MSB8, caráter 9), nossa otimização rejeita a hipótese de ancestralidade de tentáculos espalhados ao longo do corpo para "Capitata" e "Filifera" (Rees, 1957; Millard, 1975; Petersen, 1979, 1990).

A relação de Pseudothecata com Leptothecata, suportada por quatro dos 18 caracteres morfológicos, permite construir uma hipótese de morfologia ancestral do clado que teve a maior diversificação dentre os Medusozoa. A posição do gonóforo no hidrocaule, arranjo dos tentáculos em duas ou mais coroas, presença de estruturas de conexão entre camadas exoesqueléticas (perissarco e exossarco) e a cobertura total do pólipo pelo exossarco são a condição ancestral dos Pseudothecata. Desses caracteres, apenas a posição do gonóforo já havia sido utilizada para agrupar algumas linhagens de Bougainvilliidae no grupo Gonoproxima (Cartwright et al., 2008).

Estudos sobre componentes do exoesqueleto (e.g., quitina, Wagner, 1994; carbonato de cálcio, Miglietta et al., 2010; aminopolissacarídeos (AP), glicoproteínas (GP) e glucosaminoglicanos (GAGs), Mendoza-Becerril et al., 2015b), aliados aos nossos resultados sugerem que o desenvolvimento exoesquelético de Hydroidolina sea definido por uma série de sistemas. O primeiro é um "sistema de síntese molecular" (MSS – *molecular synthesis system*, em inglês), que inclui o mecanismo biossintético que produz as moléculas e que está geneticamente codificado. O segundo sistema é a "matriz molecular" (MM – *molecular matrix*, em inglês), que é a substância extracelular formada a partir do MSS e localizada na superfície epidérmica. Há ainda o sistema de "expressão morfológica" (ME - *morphological expression*, em inglês), referente à estrutura morfológica do exoesqueleto que é possivelmente o resultado da interação entre as moléculas da MM e com substâncias do seu entorno.

Os padrões da reconstrução dos caracteres do exoesqueleto corroboram hipótese baseada em uma filogenia composta de estudos anteriores (Mendoza-Becerril et al., 2015), e ainda permitem inferir uma hipótese para a evolução do sistema esquelético em Hydroidolina (o mais complexo dentre os Medusozoa) no que tange os sistemas MM e ME (Fig. 3). Há origens múltiplas e independentes entre os tipos exoesqueléticos, com possíveis estados de transição entre eles. O estado ancestral do MM em Hydroidolina é composto por aminopolissacarídeos (AP), glicoproteínas (GP) e glucosaminoglicanos (GAGs), enquanto seu ME é uma estrutura que cobre a base e o hidrocaule dos pólipos (Fig. 3).

A presença de GAGs e AP em grupos basais de Hydroidolina (e.g., Aplanulata), pode representar uma transição no sentido do desenvolvimento de exoesqueletos rígidos (e.g., quitinosos e carbonáticos). Exoesqueletos com maior concentração de AP e baixo conteúdo de GP, característicos de táxons solitários, são moles e podem ser perdidos com facilidade. Porém, quando há um incremento na produção de GP, esse padrão pode ser a base para a formação de filamentos de ancoragem, adicionando um passo no processo de quitinização exoesquelética e da colonialidade (Vervoort, 1966). Nos exoesqueletos calcários, especificamente, o incremento de AP funciona como uma matriz orgânica para a deposição de cálcio (Vervoort, 1966), um padrão observado ao menos duas vezes na história dos Hydroidolina.

Por outro lado, alguns terminais de "Filifera" aparentemente perdem a cobertura exoesquelética de quitina (e.g., *Corymorpha nutans* e espécies com esqueleto calcário de Filifera III e Capitata). Essa ausência da ME não implica estritamente na falta de um MSS para a produção de quitina, ou de outras sustâncias que participam no desenvolvimento

esquelético. Espécies de Hydractiniidae (e.g., *Hydrissa sodalis*, *Hydractinia symbiolongicarpus*, *Schuchertinia conchicola*, *Podocoryna hayamaensis*) possuem grânulos de carbonato, ainda que não haja produção esquelética calcária (Miglietta et al., 2010). Similarmente, a perda de quitina não indica, necessariamente, a perda do MSS para a quitina, já que a quitina tem uma expressão multifuncional nos diferentes grupos dos Metazoa e Medusozoa (cf. Wagner, 1994; Mendoza-Becerril et al., 2015b). Portanto, os resultados sugerem que o MSS ancestral é conservado, mas há modificações independentemente nas linhagens dos Hydroidolina. Esqueletos de carbonato de cálcio e quitina, interna ou externa, ocorrem em terminais de Capitata e Filifera III. As otimizações sugerem que a ME interna na forma de disco surgiria independentemente em Siphonophorae e Capitata, e a ME interna do tipo anastomosado em alguns Capitata pode ter se originado a partir de um padrão evolutivo ancestral (Fig. 3).

O clado Pseudothecata + Leptothecata possui uma estrutura esquelética possivelmente restrita a um exoesqueleto quitinoso com base de GAGs, esses últimos não evidentes externamente em alguns táxons de Oceaniidae e Leptothecata, mas que podem estar presentes no MSS e MM, como sugerido pela presença de GAGs em *Clytia gracilis* e nas primeiras fases de desenvolvimento de *Turritopsis nutricula* (cf. Mendoza-Becerril et al., 2015a). Leptothecata é o grupo onde há um aumento de AP e GP, e menor produção de GAGs na MM, o que resulta no desenvolvimento de uma ME rígida, embora haja diferentes níveis de cobertura, de basal e incipiente (Haleciidae) até total (Fig. 3). As diferentes expressões entre o exoesqueleto de Pseudothecata e Leptothecata podem ter a mesma origem, derivadas de processos anagenéticos próprios na história de cada uma das linhagens.

Nossas inferências são uma primeira aproximação para padrão maiores, e apontam para uma série de questões a serem investigadas, como a origem da quitinização nos Hydroidolina, os mecanismos de desenvolvimento subjacentes às transições de esqueletos com grânulos de carbonato de cálcio, GAGs ou outro tipo químico de esqueletos, o teste se há uma interrupção da expressão de MSS no ancestral ou se há perda genética da capacidade de produção de alguns dos componentes envolvidos neste MSS, e a implicação evolutiva e ecológica das modificações na extensão da cobertura sobre o pólipo, e o tipo de estrutura esquelética.

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Literatura citada

Agnarsson, I. & Miller, J. A. 2008. Is ACCTRAN better than DELTRAN? Cladistics 24: 1-7.

Allman, G. J. 1864. On the construction and limitation of genera among the Hydroida. Annals and Magazine of Natural History (3) 13: 345-380.

Allman, G. J. 1871. A monograph of the gymnoblastic or tubularian hydroids. Conclusion of Part I, and Part II, containing descriptions of the genera and species of Gymnoblastea. London: Ray Society.

Ashworth, J. & Ritchie, H. J. 1915. The morphology and development of the free-swimming sporosacs of the hydroid genus *Dicoryne*. Transactions of the Royal Society of Edinburgh 51: 257-284.

Bouillon, J. 1985. Essai de classification des Hydropolypes Hydroméduses (Hydrozoa-Cnidaria). Indo-Malayan Zoology 2: 29-243.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795-803.

Bremer, K. 1994. Branch support and tree stability. Cladistics 10: 295-304.

Calder, D. R. 1988. Shallow-Water Hydroids of Bermuda The Athecatae. Life Sciences Contributions 148: 1-107.

Calder, D. R., Choong, H. H. & McDaniel, N. 2015. *Similiclava nivea* (Cnidaria: Hydrozoa: Similiclavidae): a new family, genus and species of athecate hydroid from the Pacific coast of North America. Journal of Natural History DOI: 10.1080/ 00222933.2014.979261.

Cartwright, P. & Nawrocki, A. M. 2010. Character evolution in Hydrozoa (phylum Cnidaria). Integrative and Comparative Biology 50: 456-472.

Cartwright, P., Evans, N. M., Dunn, C. W., Marques, A. C., Miglietta, M. P., Schuchert P. & Collins, A. G. 2008. Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). Journal of the Marine Biological Association of the United Kingdom 88: 1663-1672.

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

Collins A. G. 2000. Towards understanding the phylogenetic history of Hydrozoa: Hypothesis testing with 18S gene sequence data. Scientia Marina 64: 5-22.

Collins, A. G., Schuchert, P., Marques, A. C., Jankowski, T., Medina, M. & Schierwater, B. 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit

rDNA data and an assessment of the utility of phylogenetic mixture models. Systematic Biology 55: 97-115.

Cornelius, P. F. S. 1995a. North-west European thecate hydroids and their medusae. Part 1. Introduction, Laodiceidae to Haleciidae. Synopses of the British Fauna (New Series) 50: 1-347.

Cornelius, P. F. S. 1995b. North-west European thecate hydroids and their medusae. Part 2. Sertulariidae to Campanulariidae. Synopses of the British Fauna (New Series) 50: 1-386.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. DOI:10.1038/nmeth.2109.

Farris, J. S. 1989. The retention index and rescaled consistency index. Cladistics 5: 417-419.

Fraser, C. McLean. 1944. Hydroids of the Atlantic coast of North America. Canada: The University of Toronto Press.

Gainett, G., Sharma, P. P., Pinto-da-Rocha, R., Giribet, G. & Willemart, R. H. 2014. Walk it off: predictive power of appendicular characters toward inference of higher-level relationships in Laniatores (Arachnida: Opiliones). Cladistics 30: 120-138.

Goloboff, P. A. 2008. Calculating SPR distances between trees. Cladistics 24: 591-597.

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

Hickson, S. J. & Gravely, F. H. 1907. II Hydroid Zoophytes.National Antarctic Expedition 34: 1-33.

Huelsenbeck, J. P. & Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754-755.

Katoh, K. & Toh, H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286-298.

Kluge, A. G. & Farris, J. S. 1969. Quantitative phyletics and the evolution of anurans. Systematic Zoology 18: 1-32.

Kramp, P. L. 1926. Medusae. Part II. Anthomedusae. Danish Ingolf Expedition 5: 1-102.

Kramp, P. L. 1961. Synopsis of the Medusae of the world. Journal of the Marine Biological Association of the United Kingdom 40: 7-469.

Kumar, S. Tamura, K., Jakobsen, I. B. & Nei, M. 2001. MEGA 2: molecular evolutionary genetics analysis software. Bioinformatics 17: 1244-1245.

Leclère, L., Schuchert, P. & Manuel, M. 2007. Phylogeny of the Plumularioidea (Hydrozoa, Leptothecata): evolution of colonial organization and life cycle. Zoologica Scripta 36: 371-394.

Lemmon, A. R., Brown, J. M., Stanger-Hall, K. & Lemmon, E. M. 2009. The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. Systematic Biology 58: 130-145.

Lesson, R. P. 1830. Voyage autour du monde, exécuté par ordre du roi, sur la corvette de Sa Majesté, la Coquille, pendant les années 1822, 1823, 1824 et 1825. In L. I. Duperrey (Ed) Zoologie (pp. 1794-1849). Paris: Arthus Bertrand.

Lütken, C. 1850. Nogle Bemaerkninger om Medusernes systematiske Inddeling, navnlig med Hensyn til Forbes's History of British Naked-eyed Medusae. Videnskabelige Meddelelser Dansk Naturhistorisk Forening 1850: 15-35.

Maddison, W. P. & Maddison, D. R. 2011. Mesquite: a modular system for evolutionary analysis. Version 3.02 [Computer software].

Maronna, M. M. 2014. Análise filogenética de Leptothecata (Hydrozoa; Hydroidolina) e evolução da metagênese. Tese – Doutorado, Instituto de Biociências da Universidade de São Paulo, Departamento de Genética e Biologia Evolutiva. 188p.

Marques, A. C. 1996. A critical analysis of a cladistic study of the genus *Eudendrium* (Cnidaria: Hydrozoa), with some comments on the family Eudendriidae. Journal of Comparative Biology 1: 153-162.

Marques, A. C. 2001. O gênero *Eudendrium* (Hydrozoa, Anthomedusae, Eudendriidae) no Brasil. Papéis Avulsos de Zoologia 41: 329-405.

Marques, A. C. & Collins, A. 2004. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebrate Biology 123: 23-42.

Marques, A. C. & Migotto, A. E. 2001. Cladistic analysis and new classification of the family Tubulariidae (Hydrozoa, Anthomedusae). Papéis Avulsos de Zoologia 41: 465-488.

Marques, A. C., Mergner, H., Höinghaus, R., Santos, C. M. D & Vervoort, W. 2000. Morphological study and taxonomical notes on Eudendriidae (Cnidaria: Hydrozoa: Athecatae/Anthomedusae). Zoologische Mededelingen 74: 75-118.

Marques, A. C., Peña Cantero, A. L. & Migotto, A. E. 2006. An overview of the phylogeny of the families Lafoeidae and Hebellidae (Hydrozoa, Leptothecata), their composition and classification. Invertebrate Systematics 20: 43-58.

Matjašiĉ J. & Sket B. 1971. Jamski hidroids slovenskaga krasa. A cave hydroid from Slovene karst. Biološki Vestnik 19: 139-145.

Mayer, A. G. 1910. Medusae of the world. Carnegie Institution Washington 1: 1-735.

Mendoza-Becerril, M. A. & Marques, A. C. 2013. Synopsis on the knowledge and distribution of the family Bougainvilliidae (Hydrozoa, Hydroidolina). Latin American Journal Aquatic Research 41: 908-924.

Mendoza-Becerril, M. A. & Marques, A. C. 2015a. Capítulo 3: Exoskeletal system of Bougainvilliidae and other Hydroidolina (Cnidaria, Hydrozoa). In: Padrões evolutivos de diversificação na família Bougainvilliidae (Hydrozoa, Hydroidolina). Tese de Doutorado, Universidade de São Paulo, São Paulo, Brasil.

Mendoza-Becerril, M. A., Maronna, M. M., Pacheco, M. L. A. F, Simões, M. G., Leme, J., Miranda, L. S., Morandini, A. C. & Marques, A. C. 2015b. An evolutionary, comparative analysis of the medusozoan (Cnidaria) exoskeleton. [Capítulo 3 - submetido].

Miglietta, M. P. & Cunningham, C. W. 2012. Evolution of life cycle, colony morphology, and host specificity in the Family Hydractiniidae (Hydrozoa, Cnidaria). Evolution 66: 3876-3901.

Miglietta, M. P., McNally, L. & Cunningham, C. W. 2010. Evolution of Calcium-carbonate skeletons in the Hydractiniidae. Integrative and Comparative Biology 50: 428-435.

Millard, N. A. H. 1975. Monograph on the Hydroida of Southern Africa. Annals of the South African Museum 68: 1-513.

Nawrocki, A. M., Collins, A. G., Hirano, Y. M., Schuchert, P. & Cartwright, P. 2013. Phylogenetic placement of *Hydra* and relationships within Aplanulata (Cnidaria: Hydrozoa). Molecular Phylogenetics and Evolution 67: 60-71.

Nawrocki, A. M., Schuchert, P. & Cartwright, P. 2010. Phylogenetics and evolution of Capitata (Cnidaria: Hydrozoa), and the systematics of Corynidae. Zoologica Scripta 39: 290-304.

Nixon, K.C. 1999–2004. Winclada (BETA) ver. Asado 1.89. Publicado pelo autor, Ithaca, NY.

Nixon, C. K. & Carpenter, J. M. 1993. On outgroups. Cladistics 9: 413-426.

Peña Cantero, A. L. & Marques, A. C. 1999. Phylogenetic analysis of the Antarctic genus *Oswaldella* Stechow, 1919 (Hydrozoa, Leptomedusae, Kirchenpaueriidae). Contributions to Zoology 68: 83-93.

Petersen, K. W. 1979. Development of coloniality in Hydrozoa. In G. Larwood & B. R. Rosen. (Eds) Biology and systematics of colonial organisms (pp. 105-139). New York: Academic Press.

Petersen, K. W. 1990. Evolution and taxonomy in capitate hydroids and medusae (Cnidaria: Hydrozoa). Zoological Journal of the Linnean Society 100: 101-231.

Posada, D., Buckley, T. R. 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Systematic Biology 53: 793-808.

Rambaut, A. & Drummond, A. J. 2007 (outubro, 2014). Tracer ver.1.5. Available via http://tree.bio.ed.ac.uk/software/tracer/.

Rees, W. 1956. A revision of the hydroid genus *Perigonimus* M Sars 1846. Bulletin of the British Museum, Natural History 3: 337-350.

Rees, W. 1957. Evolutionary trends in the classification of capitate hydroids and medusae. Bulletin of The British Museum (Natural History) Zoology 4: 453-534.

Russell, F. S. 1953. The medusae of the British Isles. Anthomedusae, Leptomedusae, Limnomedusae, Trachymedusae and Narcomedusae. 1-530.

Sars, M. 1846. Fauna littoralis Norvegiae, I Heft: Ueber die Fortpflanzunsweise der Polypen. Johann Dahl, Christiania.

Schuchert, P. 1996. The marine fauna of New Zealand: athecate hydroids and their medusae (Cnidaria: Hydrozoa). New Zealand Oceanographic Institute Memoir 106: 1-159

Schuchert, P. 2007. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 2. Revue Suisse de Zoologie 114: 195-396.

Schuchert, P. 2010. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Capitata part 2. Revue suisse de Zoologie 117: 337-555.

Schuchert, P. 2012. North-West European Athecate Hydroids and their Medusae. Synopses of the British Fauna (New Series) 59. London: The Linnean Society of London.

Schuchert, P. 2015 (march, 2015). *Bougainvillia* Lesson, 1830. In: Schuchert, P. (Ed.) World Hydrozoa database. Available via: http://www.marinespecies.org/hydrozoa/aphia.php?p=taxdetails&id=117015 on 2015-05-13.

Simmons, M. P. & Goloboff, P. A. 2013. An artifact caused by undersampling optimal trees in supermatrix analyses of locally sampled characters. Molecular Phylogenetics and Evolution 69: 265-275.

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

Stechow, E. 1909. Hydroidpolypen der japanischen Ostküste. I. Teil: Athecata und Plumularidae. In F. Doflein (Ed) Beiträge zur Naturgeschichte Ostasiens (pp. 1-111). Abhandlungen der Mathematisch-Phyikalische Klasse der Königlichen Bayerischen Akademie der Wissenschaften.

Talavera, G. & Castresana, J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56: 564-577.

Vaidya, G., Lohman, D. & Meier, R. 2010. SequenceMatrix: concat-enation software for the fast assembly of multi-gene data sets with character set and codon information. Cladistics 27: 171-180.

Van Iten, H., Leme, J. M., Simões, M. G., Marques, A. C. & Collins, A. G. 2006. Reassessment of the phylogenetic position of conulariids (?Ediacaran-Triassic) within the subphylum Medusozoa (Phylum Cnidaria). Journal of Systematic Palaentology 4: 109-118.

Vannucci, M. & ReeS, W. J. 1961. A revision of the genus Bougainvillia (Anthomedusae). Boletim do Instituto Oceanográfico, São Paulo 11: 57-100.

Vervoort, W. 1962. A redescription of *Solanderia gracilis* Duchassaing & Michelin, 1846, and general notes on the family Solanderiidae (Coelenterata: Hydrozoa). Bulletin of Marine Science of the Gulf and Caribbean 12: 508-542.

Vervoort, W. 1966. Skeletal structure in the Solanderiidae and its bearing on hydroid classification. In Rees, W.J. (Ed) The Cnidaria and their Evolution (pp. 372-396). London: The Proceedings of a Symposium held at The Zoological Society of London.

Wagner, G.P. 1994. Evolution and multi-functionally of the chitin system. In Schierwater, B., Streit, B., Wagner, G.P. & DeSalle, R. (Eds). Molecular and Ecology and Evolution: Approaches and Applications (pp. 559-577). Boston: Birkhäuser, Basel-Boston.

Tabela 1. Matriz de caracteres morfológicos utilizado nas inferências filogenéticas.

Táxon	Família	Espécie	Desenvolvimento do gonóforo até maturidade sexual	Organização da fase polipoide	Organização colonial da fase polipoide	Posição do gonóforo	Gonozooide como um tipo de pólipo na colônia	Dactilozooide como um tipo de pólipo na colônia	Tipo de tentáculos orais	Tentáculos aborais nos gastrozooides	Arranjo dos tentáculos orais	Presença de quitina como parte da estrutura esquelética	Esqueleto com estrutura anastomosada	Esqueleto com estrutura em forma de disco	Esqueleto de carbonato de cálcio	Tipo de esqueleto quitinoso	Estrutura morfológica do perissarco e exossarco	Conexão entre perissarco e exossarco	Cobertura do perissarco sobre o hidrante	Cobertura do exossarco sobre o hidrante
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Trachylina																				
	Rhopalonematidae	*Aglantha digitale*	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rhopalonematidae	*Aglaura hemistoma*	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Geryoniidae	*Liriope tetraphylla*	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Olindiidae	*Olindias sambaquiensis*	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
	Aeginidae	*Solmundella bitentaculata*	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hydroidolina																				
Capitata	Solanderiidae	*Solanderia secunda*	1	1	1	1	0	0	1	0	2	0	1	0	0	N	N	N	N	N
	Porpitidae	*Porpita porpita*	0	1	2	1	1	1	N	N	N	0	0	1	0	N	N	N	N	N
	Porpitidae	*Velella velella*	0	1	2	1	1	1	N	N	N	0	0	1	0	N	N	N	N	N
	Milleporidae	*Millepora* sp.	1	1	1	1	0	1	1	0	0	2	0	0	1	N	N	N	N	N
	Pennariidae	*Pennaria disticha*	1	1	1	0	0	0	1	1	2	1	0	0	0	1	1	?	0	0
"Anthoathecata"																				
"Filifera"	Ptilocodiidae	*Hydrichthella epigorgia*	1	1	0	1	1	1	0	0	0	1	0	0	0	?	?	?	?	?
	Eudendriidae	*Eudendrium carneum*	2	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0
	Eudendriidae	*Eudendrium glomeratum*	2	1	1	0	1	0	0	0	0	1	0	0	0	?	?	?	?	?
	Eudendriidae	*Eudendrium californicum*	2	1	1	0	1	0	0	0	0	1	0	0	0	?	?	?	?	?
	Magapiidae	*Fabienna sphaerica*	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
	Proboscidactylidae	*Proboscidactyla flavicirrata*	0	1	0	1	1	1	0	0	N	1	0	0	0	?	?	?	?	?
	Proboscidactylidae	*Proboscidactyla ornata*	0	1	0	1	1	0	0	0	N	1	0	0	0	?	?	?	?	?

Táxon	Família	Espécie	1 Desenvolvimento do gonóforo até maturidade sexual	2 Organização da fase polipoide	3 Organização colonial da fase polipoide	4 Posição do gonóforo	5 Gonozooide como um tipo de pólipo na colônia	6 Dactilozooide como um tipo de pólipo na colônia	7 Tipo de tentáculos orais	8 Tentáculos aborais nos gastrozooides	9 Arranjo dos tentáculos orais	10 Presença de quitina como parte da estrutura esquelética	11 Esqueleto com estrutura anastomosada	12 Esqueleto com estrutura em forma de disco	13 Esqueleto de carbonato de cálcio	14 Tipo de esqueleto quitinoso	15 Estrutura morfológica do perissarco e exossarco	16 Conexão entre perissarco e exossarco	17 Cobertura do perissarco sobre o hidrante	18 Cobertura do exossarco sobre o hidrante
Aplanulata	Corymorphidae	*Corymorpha nutans*	0	0	N	0	0	0	0	1	1	1	0	0	0	?	?	?	?	?
	Candelabridae	*Candelabrum cocksii*	2	0	N	0	0	0	1	0	2	?	0	0	0	?	?	?	?	?
	Hydridae	*Hydra vulgaris*	3	0	N	N	0	0	0	0	0	2	0	0	0	N	N	N	N	N
Siphonophorae	Physaliidae	*Physalia physalis*	1	1	2	1	1	1	N	N	N	0	0	1	0	N	N	N	N	N
	Hippopodiidae	*Hippopodius hippopus*	1	1	2	1	0	0	N	N	N	0	0	0	0	N	N	N	N	N
	Physophoridae	*Physophora hydrostatica*	1	1	2	1	1	0	N	N	N	0	0	0	0	N	N	N	N	N
"Filifera"	Stylasteridae	*Lepidopora microstylus*	2	1	1	1	1	1	0	0	0	2	0	0	1	N	N	N	N	N
	Stylasteridae	*Pseudocrypthelia pachypoma*	2	1	1	1	1	1	0	0	0	2	0	0	1	N	N	N	N	N
	Hydractiniidae	*Clava multicornis*	2	1	0	0	0	0	0	0	2	1	0	0	0	?	?	?	?	?
	Hydractiniidae	*Podocoryna exigua*	0	1	0	0	1	0	0	0	0	1	0	0	0	?	?	?	?	?
	Hydractiniidae	*Janaria mirabilis*	2	1	0	1	1	1	0	0	0	2	0	0	1	N	N	N	N	N
	Hydractiniidae	*Hydractinia symbiolongicarpus*	2	1	0	0	1	1	0	0	1	1	0	0	0	?	?	?	?	?
	Bougainvilliidae	*Dicoryne conybearei*	2	1	0	0	1	0	0	0	1	1	0	0	0	?	1	?	?	1
	Pandeidae	*Leuckartiara octona*	0	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1	2	2
	Pandeidae	*Hydrichthys boycei*	0	1	0	0	1	0	N	N	N	1	0	0	0	?	?	?	?	?
	Pandeidae	*Pandea* sp.	0	1	0	2	0	1	0	0	1	1	0	0	0	?	?	?	?	?
	Pandeidae	*Neoturris breviconis*	0	1	0	1	0	0	0	0	0	1	0	0	0	?	?	?	?	?
	Cytaeididae	*Perarella schneideri*	1	1	0	1	0	0	0	0	0	1	0	0	0	?	?	?	?	?
	Pandeidae	*Amphinema dinema*	0	1	0	2	0	0	0	0	0	1	0	0	0	?	?	?	?	?
	Oceaniidae	*Rhizogeton nudus*	2	1	0	2	0	0	0	0	2	1	0	0	0	?	?	?	?	?
	Rathkeidae	*Rathkea octopunctata*	0	1	0	2	0	0	0	0	0	1	0	0	0	?	?	?	?	?

Táxon	Família	Espécie	1 Desenvolvimento do gonóforo até maturidade sexual	2 Organização da fase polipoide	3 Organização colonial da fase polipoide	4 Posição do gonóforo	5 Gonozooide como um tipo de pólipo na colônia	6 Dactilozooide como um tipo de pólipo na colônia	7 Tipo de tentáculos orais	8 Tentáculos aborais nos gastrozooides	9 Arranjo dos tentáculos orais	10 Presença de quitina como parte da estrutura esquelética	11 Esqueleto com estrutura anastomosada	12 Esqueleto com estrutura em forma de disco	13 Esqueleto de carbonato de cálcio	14 Tipo de esqueleto quitinoso	15 Estrutura morfológica do perissarco e exossarco	16 Conexão entre perissarco e exossarco	17 Cobertura do perissarco sobre o hidrante	18 Cobertura do exossarco sobre o hidrante
	Rathkeidae	*Lizzia blondina*	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
	Oceaniidae	*Turritopsis lata*	0	1	1	1	0	0	0	0	2	1	0	0	0	?	?	?	?	?
	Oceaniidae	*Turritopsis dohrnii*	0	1	1	1	0	0	0	0	2	1	0	0	0	?	?	?	?	?
	Oceaniidae	*Turritopsis nutricula*	0	1	1	1	0	0	0	0	2	1	0	0	0	0	N	N	2	N
	Bougainvilliidae	*Koellikerina fasciculata*	0	1	0	1	0	0	0	0	1	1	0	0	0	?	1	?	?	2
	Rathkeidae	*Podocorynoides minima*	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
	Bougainvilliidae	*Bougainvillia carolinensis*	0	1	1	1	0	0	0	0	0	1	0	0	0	?	1	?	?	1
	Bougainvilliidae	*Bougainvillia fulva*	0	1	?	1	0	0	0	0	0	1	0	0	0	?	?	?	?	?
	Bougainvilliidae	*Nemopsis bachei*	0	0	N	0	0	0	0	0	0	1	0	0	0	?	?	?	?	2
	Bougainvilliidae	*Garveia grisea*	2	1	1	1	0	0	0	0	1	1	0	0	0	?	1	1	?	2
	Cordylophoridae	*Cordylophora caspia*	2	1	1	1	0	0	0	0	2	1	0	0	0	?	?	?	?	?
	Bougainvilliidae	*Bimeria vestita*	2	1	1	1	0	0	0	0	1	1	0	0	0	1	1	1	2	2
	Bougainvilliidae	*Bougainvillia muscus*	0	1	1	1	0	0	0	0	1	1	0	0	0	1	1	1	2	2
	Bougainvilliidae	*Pachycordyle michaeli*	2	1	0	1	0	0	0	0	1	1	0	0	0	?	?	?	0	0
	Bougainvilliidae	*Pachycordyle pusilla*	2	1	0	1	0	0	0	0	1	1	0	0	0	?	1	1	0	0
Leptothecata																				
	Lafoeidae	*Lafoea dumosa*	2	1	1	1	1	0	0	0	0	1	0	0	0	?	?	?	2	?
	Melicertidae	*Melicertum octocostatum*	0	1	0	1	0	0	0	0	0	1	0	0	0	?	?	?	0	?
	Tiarannidae	*Modeeria rotunda*	0	1	0	2	1	0	0	0	0	1	0	0	0	?	?	?	2	?
	Tiarannidae	*Stegopoma plicatile*	2	1	1	1	1	0	0	0	0	1	0	0	0	?	?	?	2	?
	Campanulariidae	*Clytia gracilis*	0	1	0	2	1	0	0	0	0	1	0	0	0	1	0	N	2	N

Táxon	Família	Espécie	1 Desenvolvimento do gonóforo até maturidade sexual	2 Organização da fase polipoide	3 Organização colonial da fase polipoide	4 Posição do gonóforo	5 Gonozooide como um tipo de pólipo na colônia	6 Dactilozooide como um tipo de pólipo na colônia	7 Tipo de tentáculos orais	8 Tentáculos aborais nos gastrozooides	9 Arranjo dos tentáculos orais	10 Presença de quitina como parte da estrutura esquelética	11 Esqueleto com estrutura anastomosada	12 Esqueleto com estrutura em forma de disco	13 Esqueleto de carbonato de cálcio	14 Tipo de esqueleto quitinoso	15 Estrutura morfológica do perissarco e exossarco	16 Conexão entre perissarco e exossarco	17 Cobertura do perissarco sobre o hidrante	18 Cobertura do exossarco sobre o hidrante
	Campanulariidae	*Obelia dichotoma*	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	N	2	N
	Campanulariidae	*Orthopyxis sargassicola*	1	1	0	2	1	0	0	0	0	1	0	0	0	0	0	N	2	N
	Campanulinidae	*Calycella syringa*	0	1	0	2	1	0	0	0	0	1	0	0	0	?	?	?	2	?
	Mitrocomidae	*Mitrocomella niwai*	0	1	0	2	1	0	0	0	0	1	0	0	0	?	?	?	2	?
	Phialellidae	*Phialella quadrata*	0	1	1	2	1	0	0	0	0	1	0	0	0	?	?	?	2	?
	Eirenidae	*Eutima curva*	0	1	?	1	1	0	0	0	0	1	0	0	0	?	?	?	1	?
	Eirenidae	*Eutima gegenbauri*	0	1	0	1	1	0	0	0	0	1	0	0	0	?	?	?	1	?
	Lovenellidae	*Hydranthea margarica*	1	1	0	2	1	0	0	0	0	1	0	0	0	?	?	?	1	?
	Haleciidae	*Halecium labrosum*	2	1	1	1	1	0	0	0	0	1	0	0	0	?	?	?	1	?
	Haleciidae	*Halecium lenticulare*	2	1	1	1	1	0	0	0	0	1	0	0	0	?	?	?	1	?
	Haleciidae	*Halecium muricatum*	2	1	1	1	1	0	0	0	0	1	0	0	0	?	?	?	1	?

? - não conhecidos/não estudados; N - não aplicáveis.

Tabela 2. Espécies de Hydrozoa incluídas na análise, com a informação sobre os número de acesso do GenBank por marcador, ou se foram cedidas por colegas do Laboratório de Evolução Marinha (LEM).

			Número de acesso no GenBank			
Táxon	**Família**	**Espécie**	**28S**	**18S**	**16S**	**COI**
Trachylina						
	Rhopalonematidae	*Aglantha digitale**	AY920791	EU247821	EU999230	GQ120073
	Rhopalonematidae	*Aglaura hemistoma**	EU247803	EU247818	EU293984	GQ120074
	Geryoniidae	*Liriope tetraphylla**	LEM	LEM	LEM	LEM
	Olindiidae	*Olindias sambaquiensis**	EU247809	EU247814	LEM	LEM
	Aeginidae	*Solmundella bitentaculata**	EU247795	EU247812	EU293998	JQ716088
Hydroidolina						
Capitata	Solanderiidae	*Solanderia secunda**	EU305533	AJ133506	EU305484	JX121599
	Porpitidae	*Porpita porpita**	EU883551	GQ424319	AY935322	GQ120060
	Porpitidae	*Velella velella**	EU879949	EU876576	EU305487	KC706685
	Milleporidae	*Millepora* sp.	EU879950	x	EU876551	x
	Pennariidae	*Pennaria disticha**	EU272581	GQ424342	AM088481	x
"Anthoathecata"						
"Filifera"	Ptilocodiidae	*Hydrichthella epigorgia*	EU272569	EU272622	EU305478	JX121601
	Eudendriidae	*Eudendrium carneum*	LEM	LEM	LEM	LEM
	Eudendriidae	*Eudendrium glomeratum**	LEM	LEM	AM991302	x
	Eudendriidae	*Eudendrium californicum*	EU305513	EU305492	EU305475	x
	Magapiidae	*Fabienna sphaerica*	AY920797	AY920767	AM183133	x
	Proboscidactylidae	*Proboscidactyla flavicirrata**	EU305527	AY920768	AM183137	JX121600
	Proboscidactylidae	*Proboscidactyla ornata**	EU272587	EU272631	EU305481	JQ716080
Aplanulata	Corymorphidae	*Corymorpha nutans**	EU879931	EU876558	EU876532	KC440103
	Candelabridae	*Candelabrum cocksii*	AY920796	AY920758	EU876535	GU812438
	Hydridae	*Hydra vulgaris**	EU879941	EU876570	EU876543	EF059934
Siphonophorae	Physaliidae	*Physalia physalis**	EU448095	AF358065	AY935284	GQ120033
	Hippopodiidae	*Hippopodius hippopus**	EU305517	AF358069	AY935299	GQ119993
	Physophoridae	*Physophora hydrostatica**	EU272582	AF358072	AY935300	GQ120035
"Filifera"	Stylasteridae	*Lepidopora microstylus*	EU272572	EU272644	EU645329	JX121603

Táxon	Família	Espécie	Número de acesso no GenBank			
			28S	18S	16S	COI
	Stylasteridae	*Pseudocrypthelia pachypoma*	EU272589	EU272643	EU645280	x
	Hydractiniidae	*Clava multicornis**	JQ410722	EU272609	EU305471	x
	Hydractiniidae	*Podocoryna exigua**	AY920802	AF358092	FJ214470	x
	Hydractiniidae	*Janaria mirabilis*	JQ410717	JQ407363	x	x
	Hydractiniidae	*Hydractinia symbiolongicarpus**	EU272568	JQ407377	FJ214380	x
	Bougainvilliidae	*Dicoryne conybearei*	EU272559	EU272614	AM183141	x
	Pandeidae	*Leuckartiara octona**	EU272573	EU272624	AM411421	KC440110
	Pandeidae	*Hydrichthys boycei*	EU272570	EU305496	EU448102	x
	Pandeidae	*Pandea* sp.*	EU272580	AY920765	x	x
	Pandeidae	*Neoturris breviconis*	EU305524	EU448097	EU448103	x
	Cytaeididae	*Perarella schneideri*	HM357628	HM357626	AM411414	x
	Pandeidae	*Amphinema dinema**	x	x	AM183136	JQ716085
	Oceaniidae	*Rhizogeton nudus**	EU272592	EU272635	AY787883	x
	Rathkeidae	*Rathkea octopunctata**	EU272591	EU272634	EU305483	FJ602540
	Rathkeidae	*Lizzia blondina**	EU272574	EU272625	AM411423	KC440074
	Oceaniidae	*Turritopsis lata**	KF962372	x	KF962531	KF962182
	Oceaniidae	*Turritopsis dohrnii*	EU272596	EU272638	AY787889	x
	Oceaniidae	*Turritopsis nutricula**	x	x	EU624349	JQ716082
	Bougainvilliidae	*Koellikerina fasciculata**	EU272571	EU272623	AM183129	x
	Rathkeidae	*Podocorynoides minima*	EU883552	EU883546	EU883541	x
	Bougainvilliidae	*Bougainvillia carolinensis**	EU272549	LEM	LEM	LEM
	Bougainvilliidae	*Bougainvillia fulva*	EU305507	EU305490	EU305470	x
	Bougainvilliidae	*Nemopsis bachei**	x	x	JQ715898	KC440112
	Bougainvilliidae	*Garveia grisea*	EU272588	EU272632	AM183131	x
	Cordylophoridae	*Cordylophora caspia**	EU272556	EU272612	EU305472	KC489509
	Bougainvilliidae	*Bimeria vestita*	LEM	LEM	LEM	LEM
	Bougainvilliidae	*Bougainvillia muscus*	LEM	LEM	LEM	LEM
	Bougainvilliidae	*Pachycordyle michaeli*	LEM	LEM	LEM	x
	Bougainvilliidae	*Pachycordyle pusilla*	EU272579	EU272627	AM183132	x

Táxon	Família	Espécie	Número de acesso no GenBank			
			28S	18S	16S	COI
Leptothecata						
	Lafoeidae	*Lafoea dumosa**	EU305520	LEM	FN424137	x
	Melicertidae	*Melicertum octocostatum**	FJ550451	AY920757	FJ550510	GQ120071
	Tiarannidae	*Modeeria rotunda*	FJ550396	FJ550540	FJ550476	x
	Tiarannidae	*Stegopoma plicatile*	FJ550454	FJ550598	FJ550513	x
	Campanulariidae	*Clytia gracilis*	LEM	LEM	LEM	LEM
	Campanulariidae	*Obelia dichotoma*	LEM	LEM	LEM	LEM
	Campanulariidae	*Orthopyxis sargassicola*	LEM	LEM	KM405629	KM405523
	Campanulinidae	*Calycella syringa**	FJ550372	FJ550519	FJ550460	AY789916
	Mitrocomidae	*Mitrocomella niwai*	FJ550392	FJ550536	FJ550473	x
	Phialellidae	*Phialella quadrata*	FJ550393	FJ550537	FJ550474	x
	Eirenidae	*Eutima curva*	FJ550455	FJ550599	FJ550514	x
	Eirenidae	*Eutima gegenbauri**	FJ550456	FJ550600	FJ550515	KC440033
	Lovenellidae	*Hydranthea margarica*	FJ550424	FJ550567	DQ855932	x
	Haleciidae	*Halecium labrosum*	FJ550407	FJ550550	AY787916	x
	Haleciidae	*Halecium lenticulare*	FJ550387	FJ550532	FJ550469	x
	Haleciidae	*Halecium muricatum*	FJ550408	EU272619	AY787915	x

* - marcadores obtidos de espécimes diferentes (“quimeras”). X - ausência de para o marcador molecular.

Tabela 3. Informações descritivas e estatísticas básicas sobre os marcadores moleculares utilizados nas análises.

	Marcadores moleculares				
	16S	**18S**	**28S**	**COI**	**Total**
Número de bases alinhadas	616	1759	3334	593	6302
Sítios informativos para parcimônia	372	394	925	311	2002
% sítios informativos por gene	60	22	27	52	-
% sítios informativos em relação ao total	19	20	46	16	-
Número de espécies com dados moleculares	67	64	66	36	69
Modelo de evolução molecular (ML)	GTR+GAMMA	GTR+GAMMA	GTR+GAMMA	GTR+GAMMA	-
Modelo de evolução molecular (BI)	TVM+I+G	GTR+I+G	GTR+I+G	GTR+G	-

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Tabela 4. Suporte para os principais grupos resultantes nas análises de Parcimônia (P), Máxima Verossimilhança (ML) e Inferência Bayesiana (BI).

	Topologia	**P_matriz combinada (18 caracteres)**		**P_matriz combinada (17 caracteres)**		**P_matriz molecular**		**ML_matriz molecular**	**BI_matriz molecular**
		Bootstrap	Bremer	*Bootstrap*	Bremer	*Bootstrap*	Bremer	*Bootstrap*	*Probabilidade posterior*
	Clados								
1	Hydroidolina	100	51	100	52	100	51	100	1
2	Aplanulata	99	29	100	30	100	30	100	1
3	Filifera II	100	65	100	68	100	66	100	1
4	Siphonophorae	100	35	100	37	100	36	100	1
5	Eudendriidae *	66	3	69	4	60	4	73	0.99
6	Filifera III	97	13	98	14	97	14	100	1
7	Pandeidae	100	21	100	22	100	23	100	1
8	Capitata	94	18	98	19	95	19	100	1
9	Pseudothecata *taxon novum*	70	8	79	6	78	9	95	1
10	Leptothecata	95	17	97	29	94	30	100	1
11	Pseudothecata + Leptothecata *	-	1	-	1	-	1	38	X

* - grupos com baixo valor de suporte; X - clados não resgatados nas topologias.

Tabela 5. Estatística descritiva e matriz de valores de SPR das topologias das análises de Parcimônia (P), Máxima Verossimilhança (ML) e Inferência Bayesiana (BI).

Estatística descritiva / Topologia	**P_matriz combinada**	**P_matriz molecular**	**ML_matriz molecular**	**BI_matriz molecular**
Número de nós	58	65	68	66
Número de nós possíveis	68	68	68	68
Resolução das árvores (ICF)	0.85	0.96	1.00	0.97
Índice de consistência (CI)	0.39	0.40	ø	ø
Índice de retenção (RI)	0.47	0.49	ø	ø
Índice de homoplasia (HI)	0.61	0.60	ø	ø
Índice de consistência reescalonado (RCI)	0.18	0.19	ø	ø
Comprimento	20356	19933	ø	ø
Total de dados usados	6320	6302	6302	6302
Dados informativos	2720	2002	ø	ø
Dados não informativos	3600	4300	ø	ø
Matriz de valores SPR				
P_matriz combinada				
P_matriz molecular	0 / 1.0			
ML_matriz molecular	4 / 0.94	8 / 0.88		
BI_matriz molecular	6 / 0.91	10 / 0.85	4 / 0.94	

ø - estatística não aplicável para o tipo de análise. Valores de SPR: número de movimentos / valores de similaridade.

Tabela 6. Índices filogenéticos para os caracteres morfológicos.

	Caracteres morfológicos	**no. de passos**	**CI**	**RI**	**RCI**	**IH**
1	Desenvolvimento do gonóforo até maturidade sexual	19	0.15	0.41	0.06	0.85
2	Organização da fase polipoide	2	0.50	0.67	0.33	0.50
3	Organização colonial da fase polipoide	13	0.15	0.58	0.09	0.85
4	Posição do gonóforo	16	0.13	0.33	0.04	0.88
5	Gonozooide como um tipo de pólipo na colônia	8	0.09	0.64	0.06	0.91
6	Dactilozooide como um tipo de pólipo na colônia	8	0.13	0.30	0.04	0.88
7	Tipo de tentáculos orais	2	0.50	0.67	0.33	0.50
8	Tentáculos aborais nos gastrozooides	2	0.50	0.00	0.00	0.50
9	Arranjo dos tentáculos orais	17	0.14	0.29	0.04	0.86
10	Presença da quitina como parte da estrutura esquelética	6	0.33	0.56	0.19	0.67
11	Esqueleto com estrutura anastomosada	X	X	X	X	X
12	Esqueleto com estrutura em forma de disco	2	0.50	0.50	0.25	0.50
13	Esqueleto de carbonato de cálcio	3	0.33	0.33	0.11	0.67
14	Tipo de esqueleto quitinoso	3	0.33	0.00	0.00	0.67
15	Estrutura morfológica do perissarco e exossarco	2	0.50	0.67	0.33	0.50
16	Conexão entre perissarco e exossarco	X	X	X	X	X
17	Cobertura do perissarco sobre o hidrante	6	0.33	0.56	0.19	0.67
18	Cobertura do exossarco sobre o hidrante	5	0.40	0.25	0.10	0.60

CI - índice de consistência; RI - índice de retenção; RCI - índice de consistência reescalonado; IH - índice de homoplasia; X - caracteres não informativos.

Tabela 7. Reconstrução dos estados ancestrais dos caracteres nas linhagens definidas a partir da topologia de parcimônia usando dados moleculares.

Táxon / # do caráter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Hydroidolina	medusa	solitário	colônia incrustante	hidrocaule	ausente	ausente	filiforme	ausente	coroa simples	externa	ausente	ausente	ausente	c/GAGs	Laminar	ausente	ausente	ausente
Aplanulata	medusa	solitário	N	hidrante	ausente	ausente	filiforme	ausente	coroa simples	externa	ausente	ausente	ausente	c/GAGs	Laminar	ausente	ausente	ausente
Filifera II	medusa	colonial	colônia incrustante	hidrocaule	presente	ausente	filiforme	ausente	coroa simples	externa	ausente	ausente	ausente	c/GAGs	Laminar	ausente	ausente	ausente
Siphonophorae	medusoide	colonial	colônia pelágica	hidrocaule	presente	ausente	N	N	N	interna	ausente	ausente	ausente	N	N	N	N	N
Eudendriidae *	esporossaco	colonial	colônia vertical	hidrante	presente	ausente	filiforme	ausente	coroa simples	externa	ausente	ausente	ausente	c/GAGs	Laminar	ausente	ausente	ausente
Filifera III	esporossaco	colonial	colônia incrustante	ambíguo•	presente	presente	filiforme	ausente	coroa simples	externa	ausente	ausente	ausente	c/GAGs	heterogêneo	presente	total	parcial
Pandeidae	medusa	colonial	colônia incrustante	hidrocaule	ausente	ausente	filiforme	ausente	coroa simples	externa	ausente	ausente	ausente	c/GAGs	heterogêneo	presente	total	total
Capitata	medusoide	colonial	colônia vertical	hidrocaule	ausente	ausente	capitado	ausente	dispersos ou não em coroas	externa	ausente	ausente	ausente	c/GAGs	heterogêneo	presente	ausente	ausente
Pseudothecata *taxon novum*	medusa	colonial	colônia vertical	hidrocaule	ausente	ausente	filiforme	ausente	2 ou mais coroas	externa	ausente	ausente	ausente	s/GAGs	heterogêneo	presente	total	total
Leptothecata	medusa	colonial	colônia vertical	hidrocaule	presente	ausente	filiforme	ausente	coroa simples	externa	ausente	ausente	ausente	s/GAGs	Laminar	presente	total	total
Pseudothecata+Leptothecata*	medusa	colonial	colônia vertical	hidrocaule	ausente	ausente	filiforme	ausente	coroa simples	externa	ausente	ausente	ausente	s/GAGs	heterogêneo	presente	total	total
Pseudothecata - clado A	medusa	colonial	colônia vertical	hidrocaule	ausente	ausente	filiforme	ausente	dispersos ou não em coroas	externa	ausente	ausente	ausente	s/GAGs	heterogêneo	presente	total	total
Pseudothecata - clado B	medusa	colonial	colônia vertical	hidrocaule	ausente	ausente	filiforme	ausente	2 ou mais coroas	externa	ausente	ausente	ausente	c/GAGs	heterogêneo	presente	total	total
Pseudothecata - clado C	medusa	colonial	colônia vertical	hidrocaule	ausente	ausente	filiforme	ausente	2 ou mais coroas	externa	ausente	ausente	ausente	c/GAGs	heterogêneo	presente	total	total
Pseudothecata - clado D*	esporossaco	colonial	colônia vertical	hidrocaule	ausente	ausente	filiforme	ausente	2 ou mais coroas	externa	ausente	ausente	ausente	c/GAGs	heterogêneo	presente	total	total

N - não aplicável; * - grupo com baixo suporte; • - ambíguo (hidrante/hidrocaule/hidrorriza); 1- Desenvolvimento do gonóforo até maturidade sexual; 2 - Organização da fase polipoide; 3 - Organização colonial da fase polipoide; 4 - Posição do gonóforo; 5 - Gonozooide como um tipo de pólipo na colônia; 6 - Dactilozooide como um tipo de pólipo na colônia; 7 - Tipo de tentáculos orais; 8 - Tentáculos aborais nos gastrozooides; 9 - Arranjo dos tentáculos orais; 10 - Presença de quitina como parte da estrutura esquelética; 11 - Esqueleto com estrutura anastomosada; 12 - Esqueleto com estrutura em forma de disco; 13 - Esqueleto de carbonato de cálcio; 14 - Tipo de esqueleto quitinoso; 15 - Estrutura morfológica do perissarco e exossarco; 16 - Conexão entre perissarco e exossarco; 17 - Cobertura do perissarco sobre o hidrante; 18 - Cobertura do exossarco sobre o hidrante; células em cinza: reconstrução com o uso da otimização ACCTRAN; células em cinza com borda: reconstruções possivelmente incorretas.

Tabela 8. Histórico de classificações propostas para famílias com gêneros que foram ou são considerados como parte de "Bougainvilliidae", além de outras famílias com pseudo-hidroteca.
* - táxons com pseudo-hidroteca; (?) - fase de pólipo desconhecida.

Estudo	Subordem	Superfamília	Família	Subfamília	Gênero
Allman, 1864			Eudendriidae*		*Bimeria**
					*Bougainvillia**
					*Garveia**
					*Rhizorhagium**
			Dicorynidae*		*Dicoryne**
			Tubulariidae		*Nemopsis*
Allman, 1871			Bimeriidae*		*Bimeria**
					*Garveia**
			Bougainvilliidae*		*Bougainvillia**
			Dicorynidae*		*Dicoryne**
			Nemopsidae		*Nemopsis*
Hickson & Gravely, 1907			Bougainvilliidae*	Bougainvilliidae*	*Rhizorhagium**
				Margelinae	
				Dicorynae*	
				Eudendriinae*	
				Bimeriinae*	
Kramp, 1926			Margelidae*		*Bougainvillia**
					Lizzia (?)
					Rhatkea
			Tiaridae*		*Leuckartiara**
Petersen, 1979	Pandeidae	Bougainvillioidea*	Cytaeidae		
			Bougainvillidae*		
			Heterotentaculidae		
		Pandeoidea*	Pandeidae*		
Calder, 1988			Bougainvilliidae*	Bimeriinae*	*Bimeria**
				Pachycordylinae	*Millardiana*
					Pachycordyle
					Silhoueta
				Rhizorhagiinae*	*Parawrightia**
					*Rhizorhagium**
				Bougainvilliinae*	*Bougainvillia**
					Nemopsis
					*Dicoryne**
					*Garveia**
			Pandeoidea*	Pandeidae*	
Schuchert, 2007			Bougainvilliidae*		*Bougainvillia**
					*Dicoryne**
					*Koellikerina**
					Nemopsis
			Pandeidae*		*Leuckartiara**

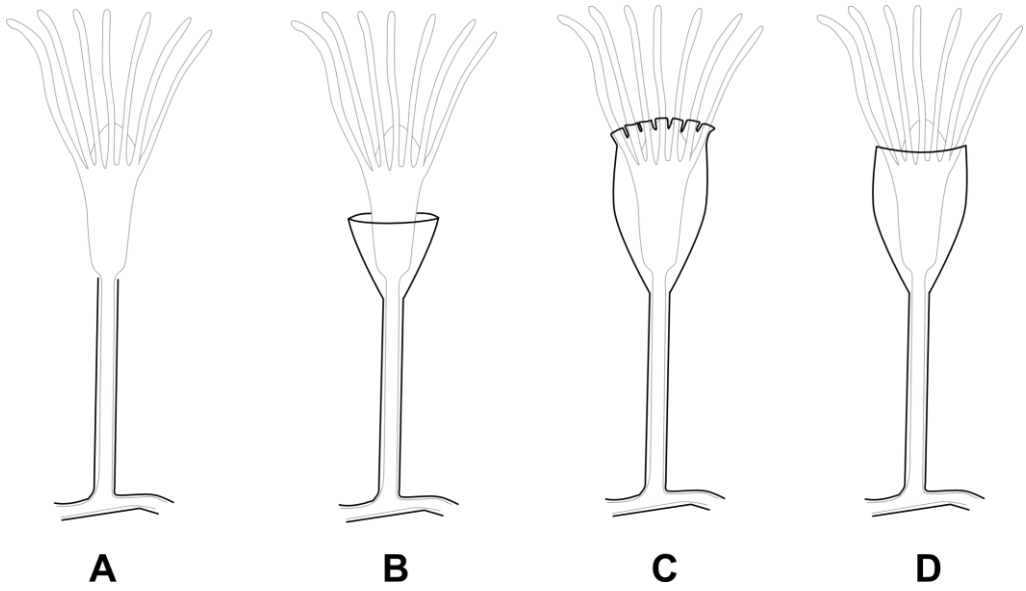


Figura 1. Representação esquemática da extensão da cobertura do esqueleto sobre o hidrante. A: nenhuma cobertura; B: cobertura parcial; C e D: cobertura total.

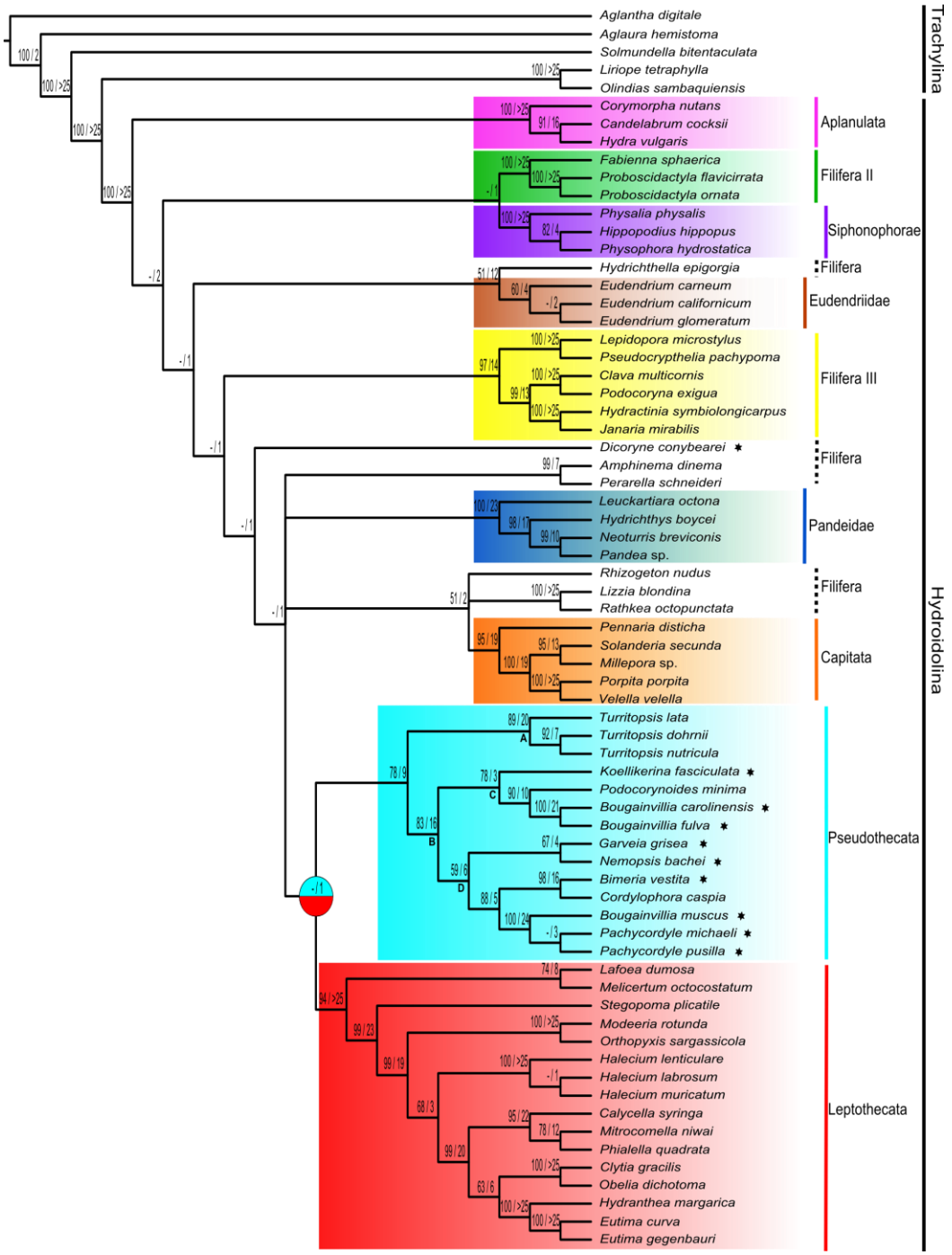


Figura 2. Hipótese filogenética resultante de análise de Parcimônia (P), a partir de dados moleculares concatenados (marcadores - 28S, 18S, 16S e COI). Números acima dos ramos indicam os valores de *bootstrap* / Bremer. Grupos monofiléticos principais diferenciados por cores. ✱ - espécies classicamente atribuídos à família Bougainvilliidae; ◒ - grupo Pseudothecata + Leptothecata.

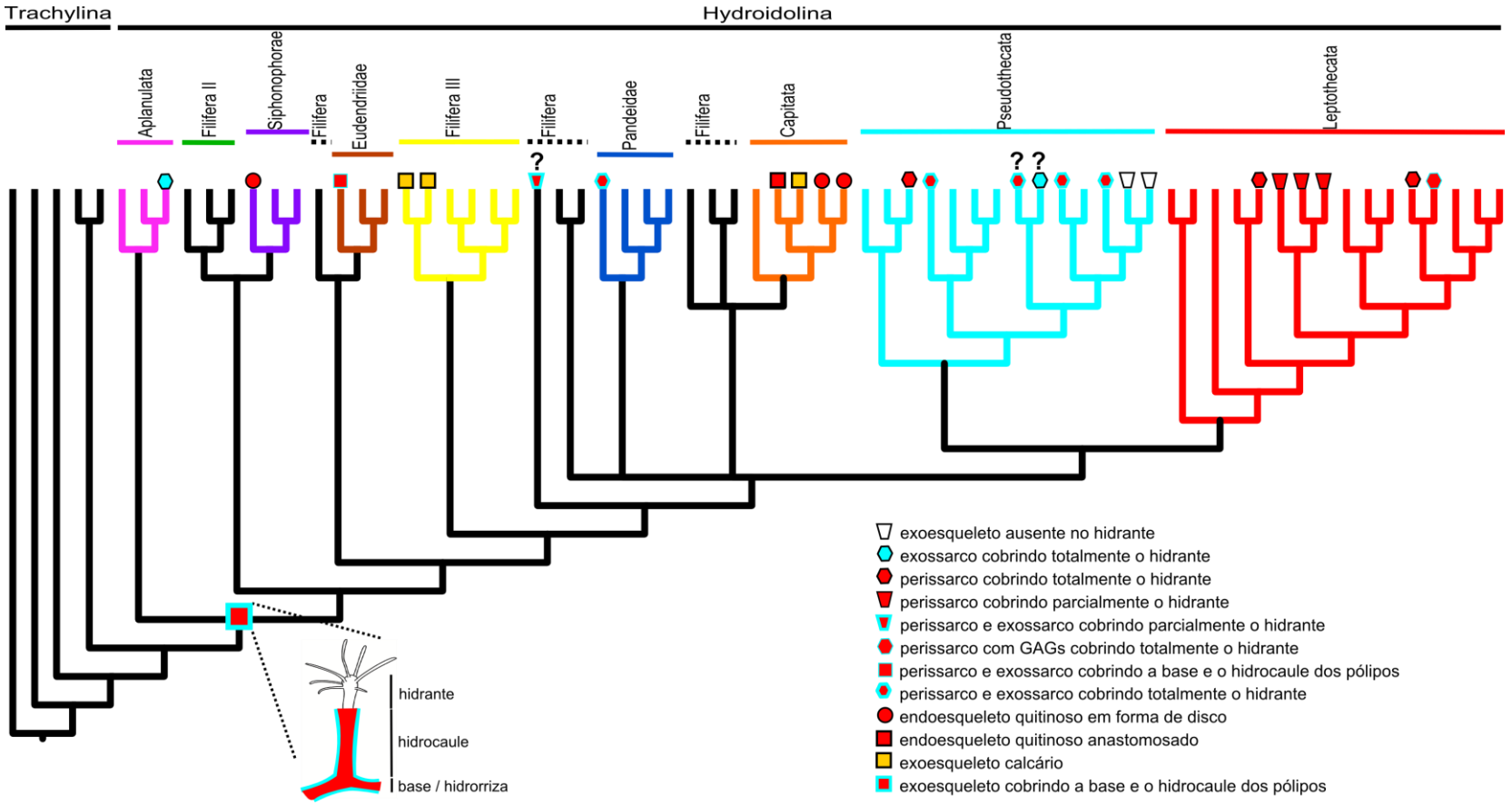


Figura 3. Representação de uma hipótese de evolução do esqueleto dentre os grandes grupos de Hydroidolina, considerando a "matriz molecular" do esqueleto (MM) e a "expressão morfológica" do esqueleto (ME). "?" - ausência de dados do SSE para os terminais. Cores nos ramos definem seus grupos na topologia e nos símbolos, azul ciano: com algum tipo de GAGs como componente da estrutura esquelética; laranja: componente esquelético principal carbonato de cálcio; vermelha: componente esquelético principal quitina.

Material suplementar - A. Caracteres morfológicos usados na análise filogenética e reconstrução de caracteres.

Fase polipoide

Espécies sem a fase de pólipo no ciclo de vida, ou que não possuem estruturas morfológicas específicas desta fase, tiveram os caracteres relacionados codificados como "não-aplicável" (N); espécies com a fase de pólipo desconhecida tiveram os caracteres relacionados codificados como “desconhecido” (?). No entanto, para a fase de pólipo de algumas espécies, foram consideradas as características gerais do gênero para a codificação dos caracteres.

1 – Desenvolvimento do gonóforo até maturidade sexual. 0 – medusa; 1 – medusoide; 2 – esporossaco; 3 – sem gonóforo (segundo Cartwright & Nawrocki, 2010).

A medusa é livre-natante, com boca funcional, sendo liberada após o brotamento do pólipo; medusoides (também conhecido como eumedusoides, cf. Kühn, 1913) não possui boca e sistema de canais radiais funcionais que, nas medusas, fazem parte do sistema digestivo; esporossacos (também conhecidos como criptomedusoides e heteromedusoides, cf. Kühn, 1913), não possuem boca nem sistema de canais radiais, mesmo durante a maturidade sexual (Cartwright & Nawrocki, 2010). A maioria dos medusoides e esporossacos permanecem fixos ao pólipo, formando as gônadas. Entretanto, os eumedusoides (e.g., *Perarella schneideri*) e as medusas abortivas (e.g., *Millepora* spp.), podem ser liberados na coluna água e, portanto, foram codificados como medusoides. Já os esporossacos natantes (e.g., *Dicoryne conybearei*), foram codificados como esporossacos, e classificados segundo a definição acima (Cartwright & Nawrocki, 2010). As espécies de hidrozoários sem gonóforos, mas com desenvolvimento dos gametas representado apenas por uma evaginação epitelial, onde as células sexuais amadurecem (e.g., *Hydra* spp.), foram codificadas como “sem gonóforo” (Cartwright & Nawrocki, 2010).

2 – Organização da fase polipoide. 0 – solitário; 1 – colonial.

As espécies foram codificadas com o estado de caráter solitário se os pólipos não formam conexões permanentes com outro pólipos, por meio de estolão ou hidrocaule, mesmo que transitoriamente, e compartilhem uma cavidade gastrovascular (e.g., *Candelabrum cocksii*) (Cartwright & Nawrocki, 2010).

3 – Organização colonial da fase polipoide. 0 – colônia incrustante; 1 – colônia vertical; 2 – colônia pelágica.

As colônias foram codificadas como incrustantes se crescem horizontalmente sobre um substrato, a partir de um estolão ou hidrorriza. Como verticais, se as colônias estão fixas ao substrato, mas com crescimento ereto oposto à superfície. As colônias verticais incluem colônias ramificadas (e.g., *Pennaria disticha*), arbustivas (e.g., *Eudendrium carneum*) e eretas (e.g., *Solanderia secunda*). Colônias pelágicas são aquelas com indivíduos polipoides interligados, atuando como um "indivíduo" planctônico na coluna de água, e incluem os sifonóforos e espécies pelágicas de Capitata (*Velella* sp. e *Porpita* sp.).

4 – Posição do gonóforo. 0 – sobre o hidrante; 1 – sobre o hidrocaule; 2 – sobre a hidrorriza.

Nas colônias polipoides, os gonóforos podem brotar sobre os hidrantes íntegros ou reduzidos, sobre o hidrocaule ou sobre a hidrorriza. Em alguns pólipos, no entanto, é difícil distinguir o limite entre o hidrante e o hidrocaule, como ocorre nos gonozooides sem tentáculos (e.g., *Hydrichthella epigorgia*) – nesses casos consideramos a posição do brotamento como estado 0. O brotamento de gonóforos localizado sobre pedículos ou ramos (e.g., *Obelia* spp.) e o brotamento de gonóforos em Siphonophora foram codificados como sendo sobre o hidrocaule (cf. Cartwright & Nawrocki, 2010). Igualmente, os gonóforos desenvolvidos em cavidades chamadas ampolas (e.g., Stylasteridae) (Cairns, 1983), foram codificados com o estado de caráter 1, pois sua arquitetura está imersa dentro do cenósteo (= cenossarco de outros Hydrozoa) do hidrocaule e dos ramos (cf. Cairns, 1983). Um estado não comparável seria o que ocorre em *Hydra* spp., pois as espécies desse gênero não produzem gonóforo.

Pólipos polimórficos

Colônias de Hydrozoa podem ser polimórficas e possuir gastrozooides, gonozooides e/ou dactilozooides. Esses pólipos são diferentes morfologicamente entre si e possuem funções específicas, representando um homologia seriada. Desta forma, foram codificados como caracteres independentes. Gastrozooides não foram incluídos porque sua presença é universal e não-informativa.

5. – Gonozooide como um tipo de pólipo na colônia. 0 – ausente; 1 – presente.

Gonozooides são pólipos reprodutivos da colônia dos quais brotam os gonóforos (brotos medusoides ou medusas reduzidas). Eles são geralmente gastrozooides modificados com vários graus de redução das estruturas reprodutivas (e.g., *Eudendrium*). Às vezes não há nenhum sinal de tentáculos em qualquer fase e o corpo do hidrante forma um eixo oco ou talo conhecido como o blastóstilo (e.g., *Dicoryne* spp.) (Millard, 1975).

6 – Dactilozooide como um tipo de pólipo na colônia. 0 – ausente; 1 – presente.

Dactilozooides são pólipos reduzidos, com função defensiva, de forma variada e fortemente armados com baterias de nematocistos. Não possuem as funções de alimentação e reprodução (Millard, 1975). *Pandea* sp. foi codificada por Cartwright & Nawrocki (2010) como possuindo dactilozooides porque algumas espécies do gênero possuem tentaculozooides e/ou dactilozooides.

Tentáculos

7 – Tipo de tentáculos orais. 0 – filiforme; 1 – capitado.

Os tentáculos orais estão geralmente localizados acima da zona de brotamento, onde os gonóforos se originam, enquanto, os tentáculos aborais estão localizados na zona de brotamento ou imediatamente abaixo desta (Petersen, 1990). Gastrozooides sem tentáculos (e.g., *Porpita porpita*) e hidrozoários sem a fase de pólipo (e.g., alguns Trachylina) foram codificados com o estado "não aplicável".

8 – Tentáculos aborais nos gastrozooides. 0 – ausente; 1 – presente.

Os tentáculos aborais são do tipo filiforme, exceto em *Coryne eximia*.

9 – Arranjo dos tentáculos orais. 0 – coroa simples; 1 – duas ou mais coroas; 2 – dispersos ou não em coroas.

O caráter não é comparável para os pólipos pelágicos de Porpitidae e para *Proboscidactyla flavicirrata*, que possui apenas dois tentáculos orais, não sendo clara se sua disposição é em coroa ou dispersa. *Hydractinia symbiolongicarpus* Buss & Yund, 1989, foi descrita com tentáculos dispostos em uma coroa simples. Entretanto, seus tentáculos podem se apresentar em um arranjo de duas coroas, aparentemente muito próximas uma da outra, o que pode estar relacionado com a contração do pólipo. Desta forma, a espécie foi codificada com estado de caráter 1.

Estrutura esquelética

O exoesqueleto pode ser do tipo axial córneo, com escleritos calcários, maciço, ou bicamada. O exoesqueleto axial córneo é composto principalmente por quitina, enquanto que o maciço por carbonato de cálcio. O bicamada está formado de uma camada mais interna quitino-proteica (= perissarco) e de outra mais externa de glicosaminoglicanos (= exossarco) (Mendoza-Becerril et al., 2015a,b).

10 – Presença de quitina como parte da estrutura esquelética (Fig. MS8). 0 – interna; 1 – externa; 2 – ausente.

A presença de quitina interna refere-se a um endoesqueleto rígido ou flexível. A quitina é considerada como ausente em espécies com esqueleto composto principalmente por carbonato de cálcio (e.g., *Millepora* spp.), ou por glicosaminoglicanos (GAGs) (e.g., *Hydra vulgaris*; Yamada et al., 2007).

11 – Esqueleto com estrutura anastomosada . 0 – ausente; 1 – presente.

Esqueleto formado de fibras quitinosas anastomosadas, cobertas e intercaladas por cenossarco (e.g., *Solanderia* spp., Vervoort, 1962). Hidrozoários com fibras quitinosas anastomosadas, mas não cobertas por cenossarco (e.g., *Schuchertinia antonii*, espécie não considerada neste estudo) foram codificados com o estado 1.

12 – Esqueleto com estrutura em forma de disco. 0 – ausente; 1 – presente.

Esqueleto presente em pólipos de colônias pelágicas. A câmara de flutuação contém um disco basal quitinoso interno ou um revestimento quitinoso interno. Este endosqueleto é característico de *Porpita* e *Velella*, bem como do sifonóforo *Physalia physalis* (Mendoza-Becerril et al., 2015a).

13 – Esqueleto de carbonato de cálcio. 0 – ausente; 1 – presente.

Exoesqueleto calcário está presente em Stylasteridae, Milleporidae e em algumas espécies de Hydractiniidae.

14 – Tipo de esqueleto quitinoso. 0 – sem GAGs; 1 – com GAGs.

O esqueleto quitino-proteico é o exoesqueleto da maioria dos Leptothecata. No entanto, às vezes pode estar coberto por uma camada de glicosaminoglicanos (GAGs) que

formam o exossarco, ou simplesmente conter esses GAGs como parte da sua estrutura química (e.g., *Clytia gracilis*; Mendoza-Becerril et al., 2015b). O caráter é codificado como não comparável para as espécies com endoesqueleto, e para as espécies com exoesqueleto composto principalmente de carbonato de cálcio ou GAGs.

15 – Estrutura morfológica do perissarco e exossarco. 0 – laminar; 1 – heterogênea.

O estado laminar refere-se aos pólipos com exoesqueleto (perissarco e exossarco) de aparência homogênea, enquanto o estado heterogêneo refere-se à morfologia do exossarco, cuja aparência está associada a sua consistência mucosa de GAGs e ao material orgânico ou inorgânico aderido (detritos). O caráter é codificado como não comparável para as espécies sem exoesqueleto.

16 – Conexão entre perissarco e exossarco. 0 – ausente; 1 – presente.

Em alguns pólipos, o perissarco e o exossarco estão ligados por estruturas de ancoragem. Essas estruturas são canais formados pelo perissarco no interior do exossarco (mais detalhes sobre estas estruturas em Mendoza-Becerril et al., 2015b). O caráter é codificado como não comparável para as espécies sem exossarco.

17 – Cobertura do perissarco sobre o hidrante. 0 – ausente; 1 – parcial; 2 – total.

O perissarco pode envolver o hidrante, total ou parcialmente (= hidroteca).

18 – Cobertura do exossarco sobre o hidrante. 0 – ausente; 1 – parcial; 2 – total.

O exossarco pode envolver o hidrante de maneira total ou parcial (= pseudo-hidroteca).

Para os caracteres 18 e 19 os pesquisadores podem generalizar os nomes específicos de ‘hidroteca’ e ‘pseudo-hidroteca’ por ‘perissarco’ e ‘exossarco’, respectivamente. Neste estudo, esses nomes específicos referem-se exclusivamente à porção de exoesqueleto que cobre o hidrante. É importante separar o perissarco do exossarco e considerá-los como caracteres diferentes, porque possuem uma composição química distinta. Além disso, a região coberta que envolve o hidrante é variável em espessura e cobertura. O exoesqueleto sobre o hidrante pode estar em contato com a epiderme, como observado nas espécies de “Anthoathecata” (e.g., *Bimeria vestita, Koellikerina fasciculata*). Também pode ser livre, sendo que, nesse caso, os pólipos têm capacidade de retrair-se para o interior da teca, o que

lhes confere uma proteção relevante, como ocorre na maioria de Leptothecata. O estado de caráter parcial refere-se aos pólipos com perissarco e exossarco reduzido à região basal do hidrante. O estado de caráter total corresponde aos pólipos com perissarco e exossarco cobrindo inclusive os tentáculos quando expandidos.

Literatura citada

Buss, L. W. & Yund, P. O. 1989. A sibling species group of Hydractinia in the north-eastern United States. Journal of the Marine Biological Association of the United Kingdom 69: 857-874.

Cairns, S. D. 1983. A generic revision of the Stylasterina (Coelenterata: Hydrozoa). Part 1. Description of the genera. Bulletin of Marine Science 33: 427-508.

Cartwright, P. & Nawrocki, A. M. 2010. Character evolution in Hydrozoa (phylum Cnidaria). Integrative and Comparative Biology 50: 456-472.

Kühn, A. 1913. Entwicklungsgeschichte und Verwandtsschaftsbeziehungen der Hydrozoen. I Theil. Die Hydroiden. Ergebnisse und Fortschritte der Zoologie, 4: 1-284.

Mendoza-Becerril, M. A., Maronna, M. M., Pacheco, M. L. A. F, Simões, M. G., Leme, J., Miranda, L. S., Morandini, A. C. & Marques, A. C. 2015a. An evolutionary, comparative analysis of the medusozoan (Cnidaria) exoskeleton. [Capítulo 3 - submetido].

Mendoza-Becerril, M. A., & Marques, A. C. 2015b. Capítulo 3: Exoskeletal system of Bougainvilliidae and other Hydroidolina (Cnidaria, Hydrozoa). In: Padrões evolutivos de diversificação na família Bougainvilliidae (Hydrozoa, Hydroidolina). Tese de Doutorado, Universidade de São Paulo, São Paulo, Brasil.

Millard, N. A. H. 1975. Monograph on the Hydroida of Southern Africa. Annals of the South African Museum 68: 1-513.

Petersen, K. W. 1990. Evolution and taxonomy in capitate hydroids and medusae (Cnidaria: Hydrozoa). Zoological Journal of the Linnean Society 100: 101-231.

Vervoort, W. 1962. A redescription of *Solanderia gracilis* Duchassaing & Michelin, 1846, and general notes on the family Solanderiidae (Coelenterata: Hydrozoa). Bulletin of Marine Science of the Gulf and Caribbean 12: 508-542.

Yamada, S., Morimoto, H., Fujisawa, T. & Sugahara., K. 2007. Glycosaminoglycans in Hydra magnipapillata(Hydrozoa, Cnidaria): demonstration of chondroitin in the developing nematocyst, the sting organelle, and structural characterization of glycosaminoglycan. Glycobiology 17: 886-894.

Material suplementar - B

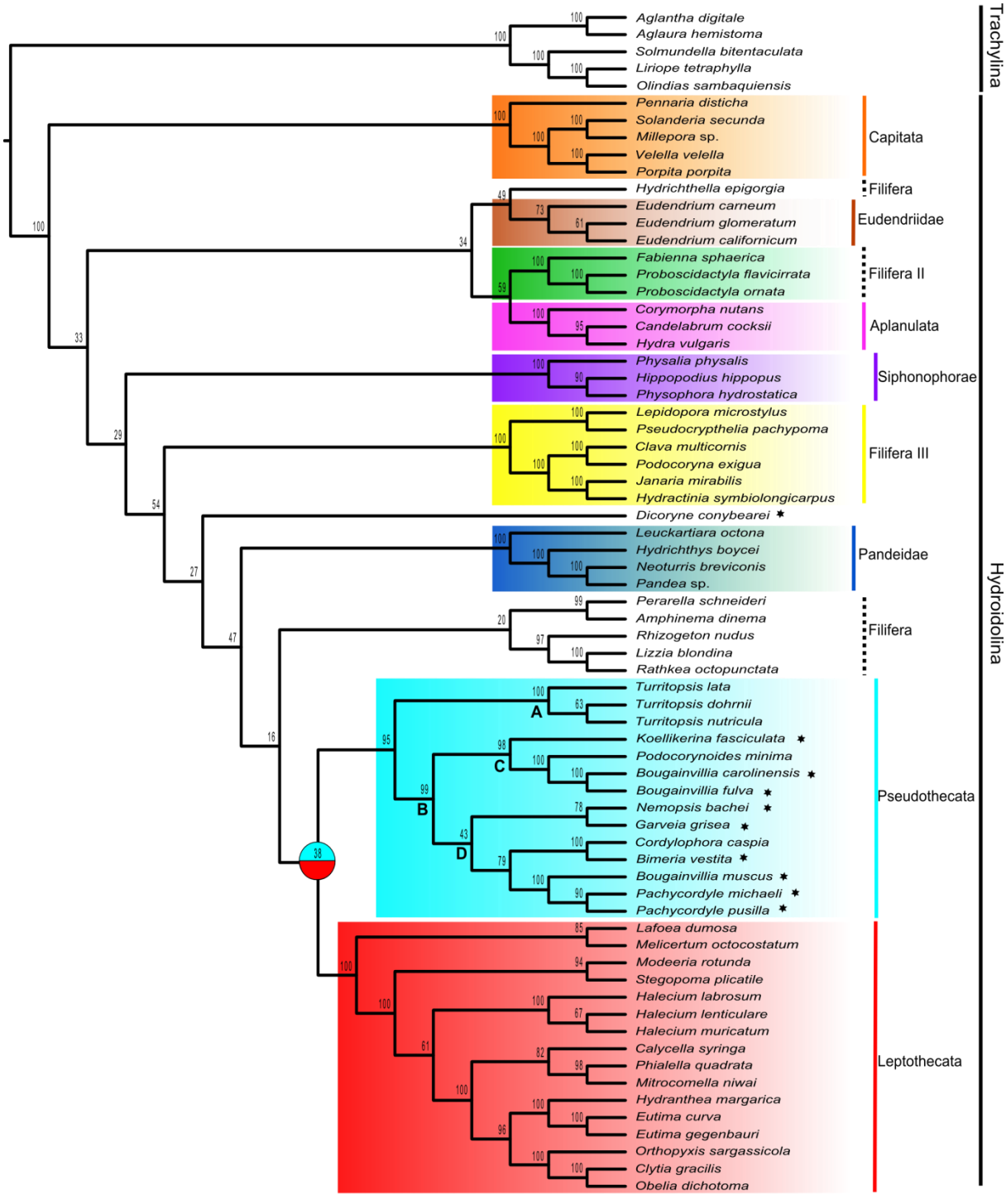
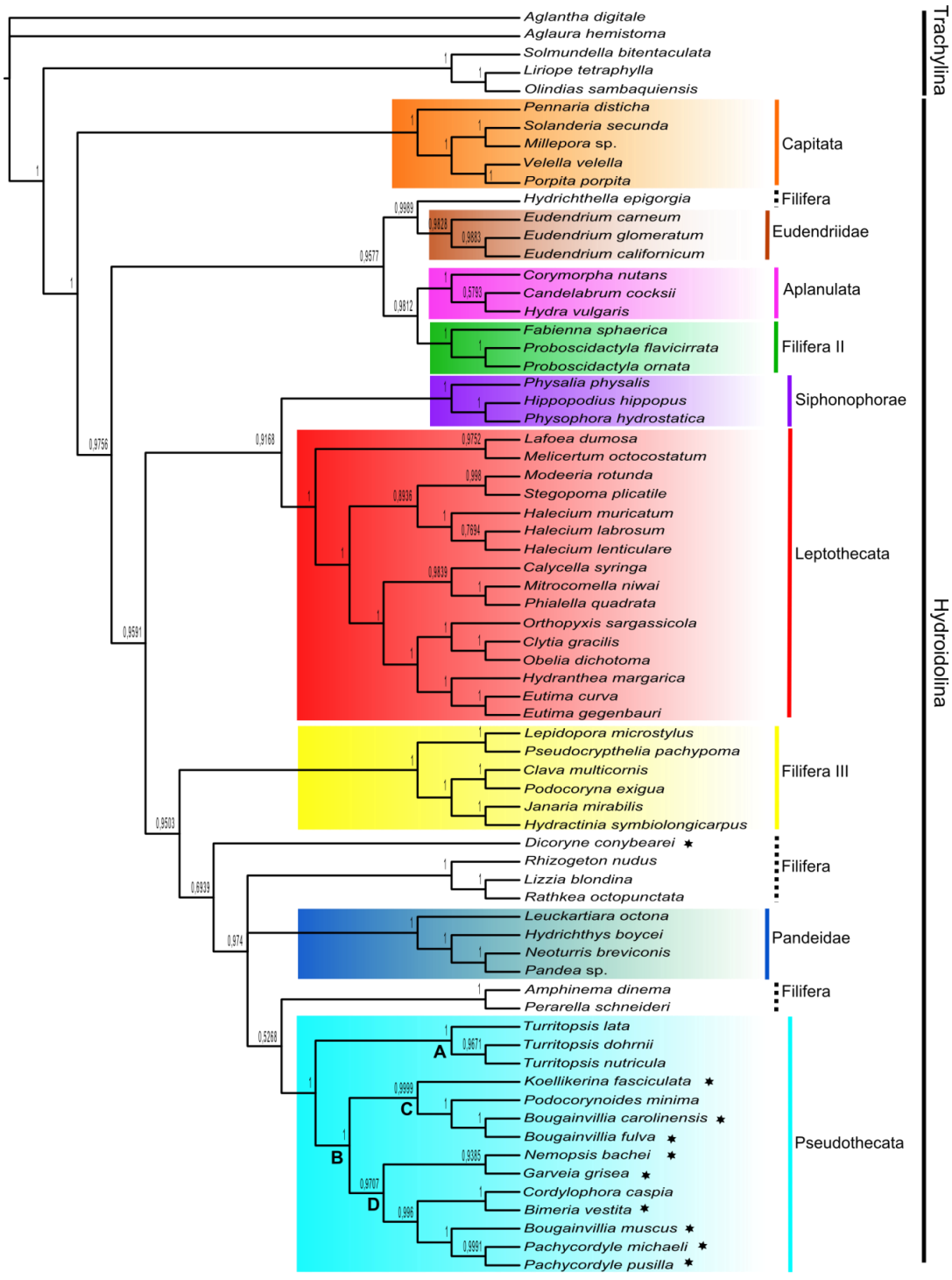


Figura MSB1. Hipótese filogenética resultante de análise de Máxima Verossimilhança (ML), a partir de dados moleculares concatenados (marcadores 28S, 18S, 16S e COI). Números acima dos ramos indicam os valores de *bootstrap*. Grupos monofiléticos principais diferenciados por cores. * - espécies classicamente atribuídos à família Bougainvilliidae; ● - grupo Pseudothecata + Leptothecata.



Figura

MSB2. Hipótese filogenética resultante de análise de Inferência Bayesiana (BI), a partir de dados moleculares concatenados (marcadores 28S, 18S, 16S e COI). Números acima dos ramos indicam os valores de probabilidade posterior. Grupos monofiléticos principais diferenciados por cores. * espécies classicamente atribuídos à família Bougainvilliidae.

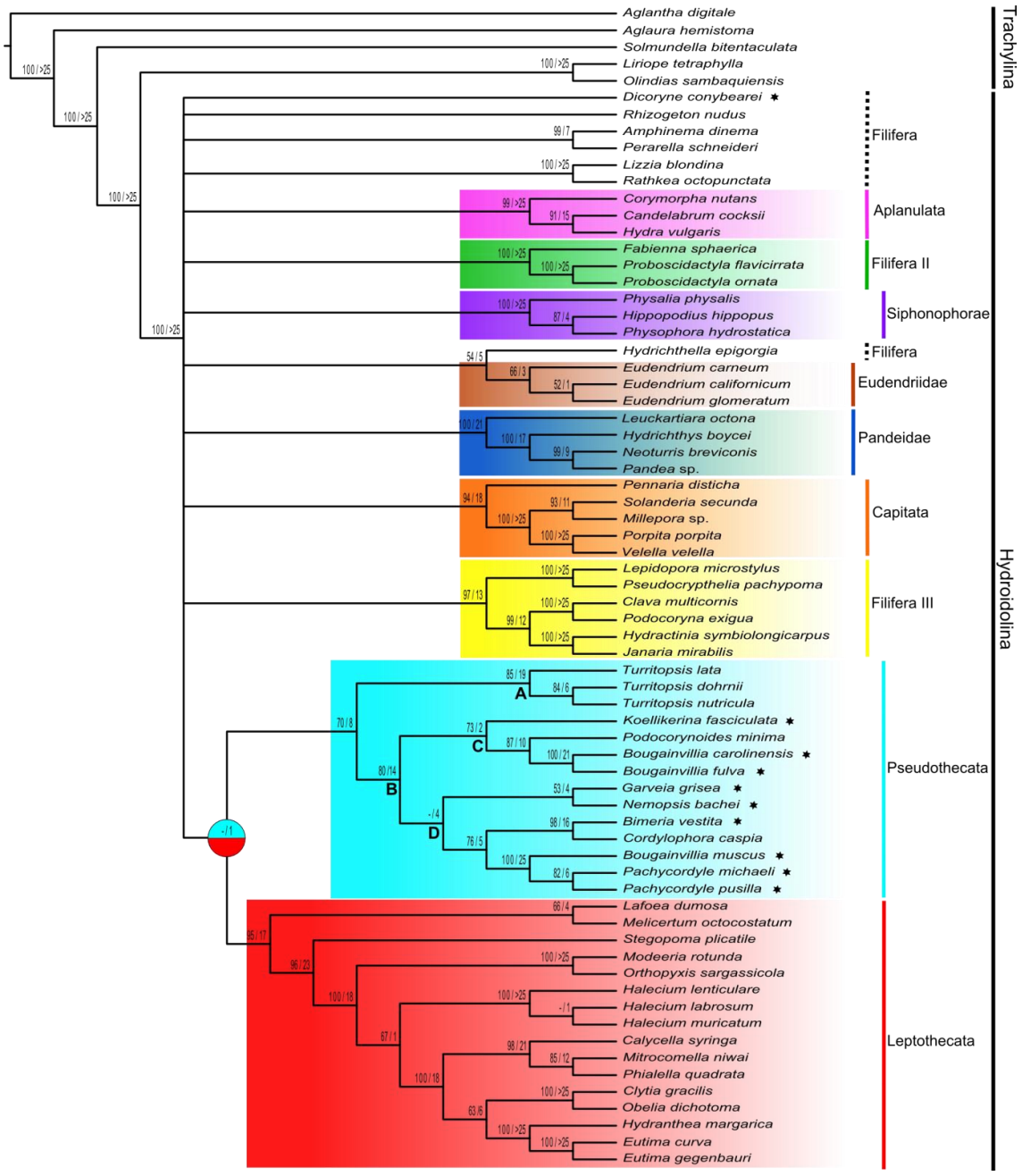


Figura MSB3. Hipótese filogenética resultante de análise de Parcimônia (P) a partir de dados moleculares (marcadores 28S, 18S, 16S e COI) e morfológicos (18 caracteres). Números acima dos ramos indicam os valores de *bootstrap* / Bremer. Grupos monofiléticos principais diferenciados por cores. * - espécies classicamente atribuídos à família Bougainvilliidae; ● - grupo Pseudothecata + Leptothecata

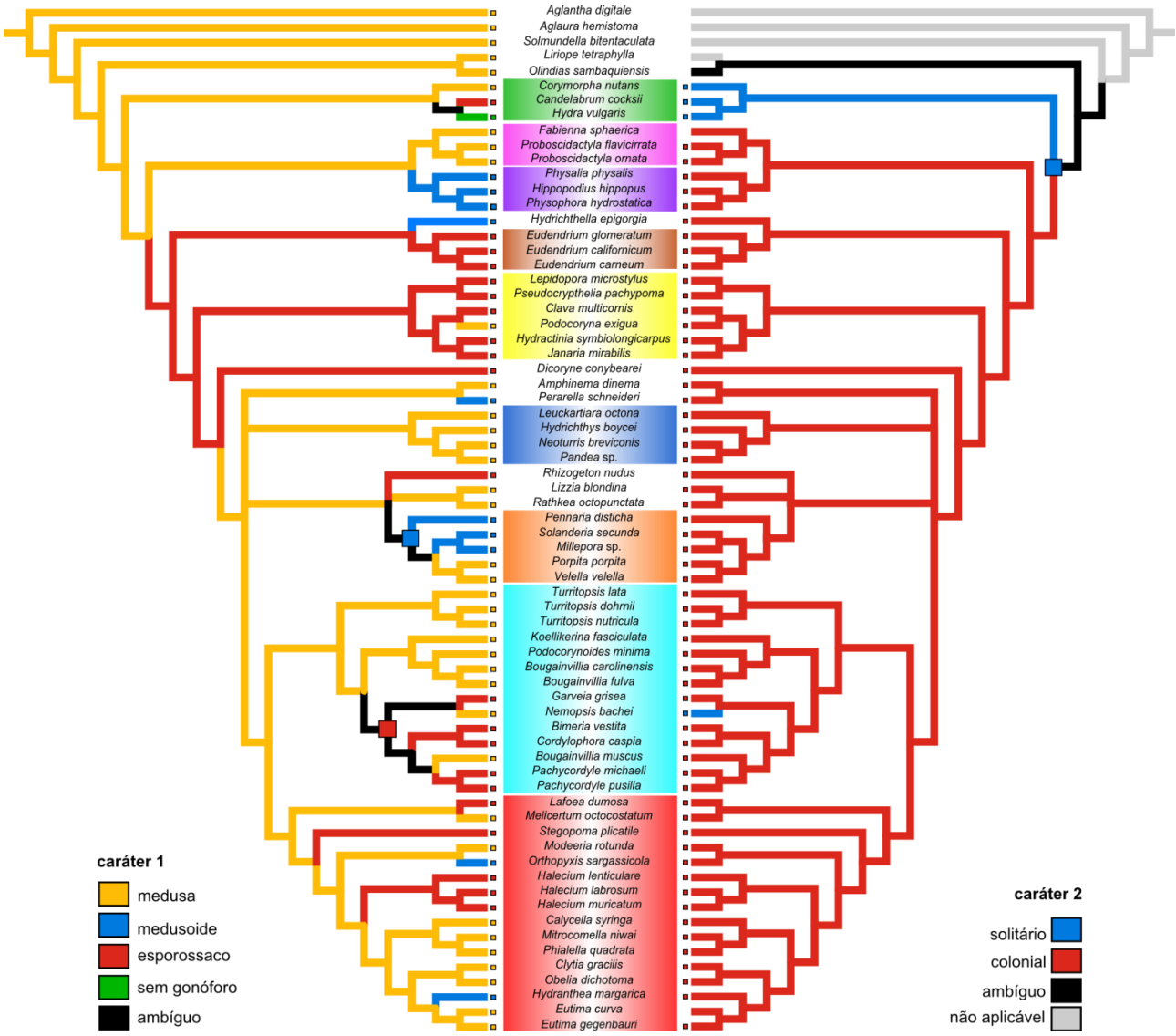


Figura MSB4. Reconstrução dos estados ancestrais dos caracteres morfológicos 1-2 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir de uma otimização ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

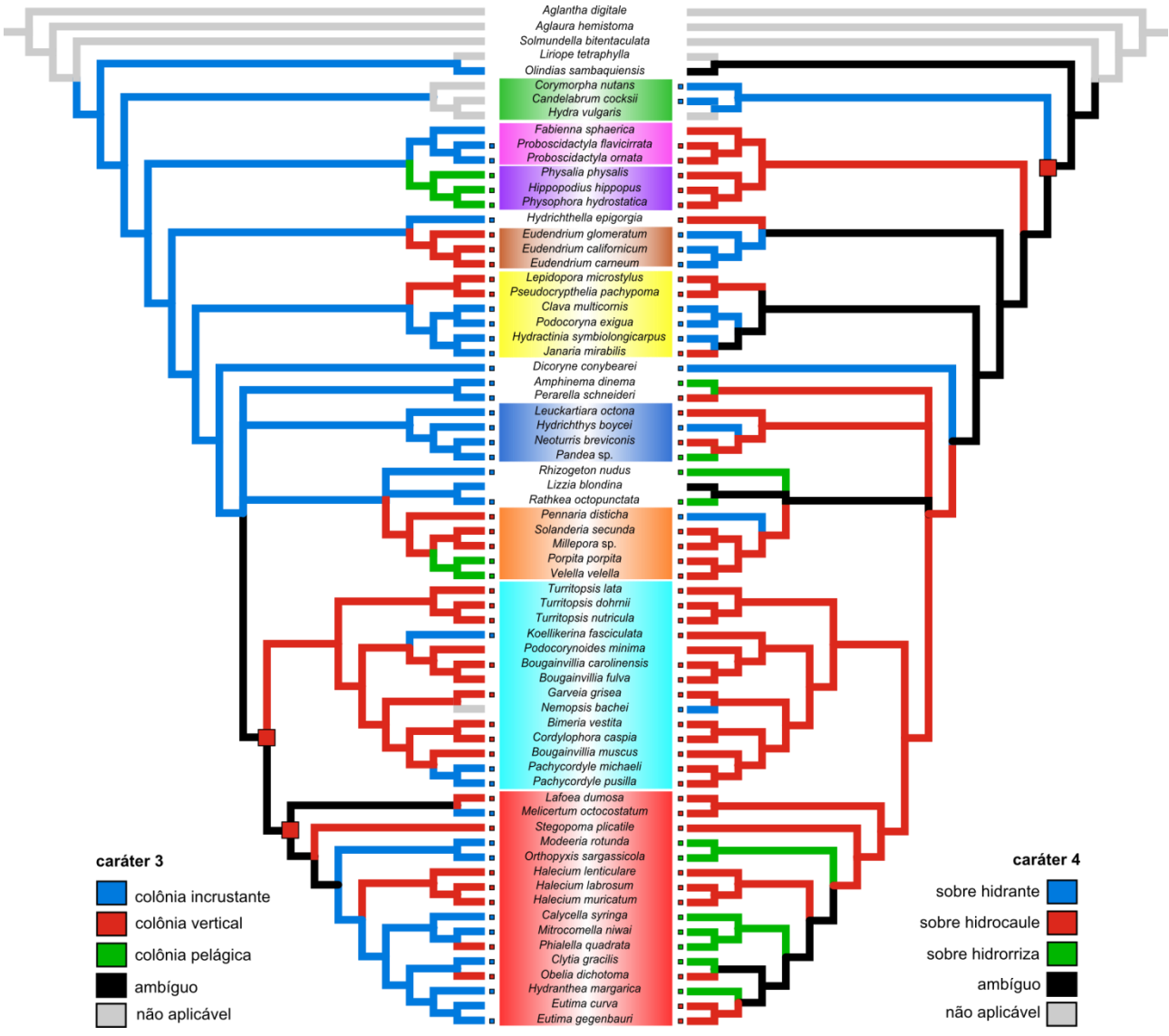


Figura MSB5. Reconstrução dos estados ancestrais dos caracteres morfológicos 3-4 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir da metodologia ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

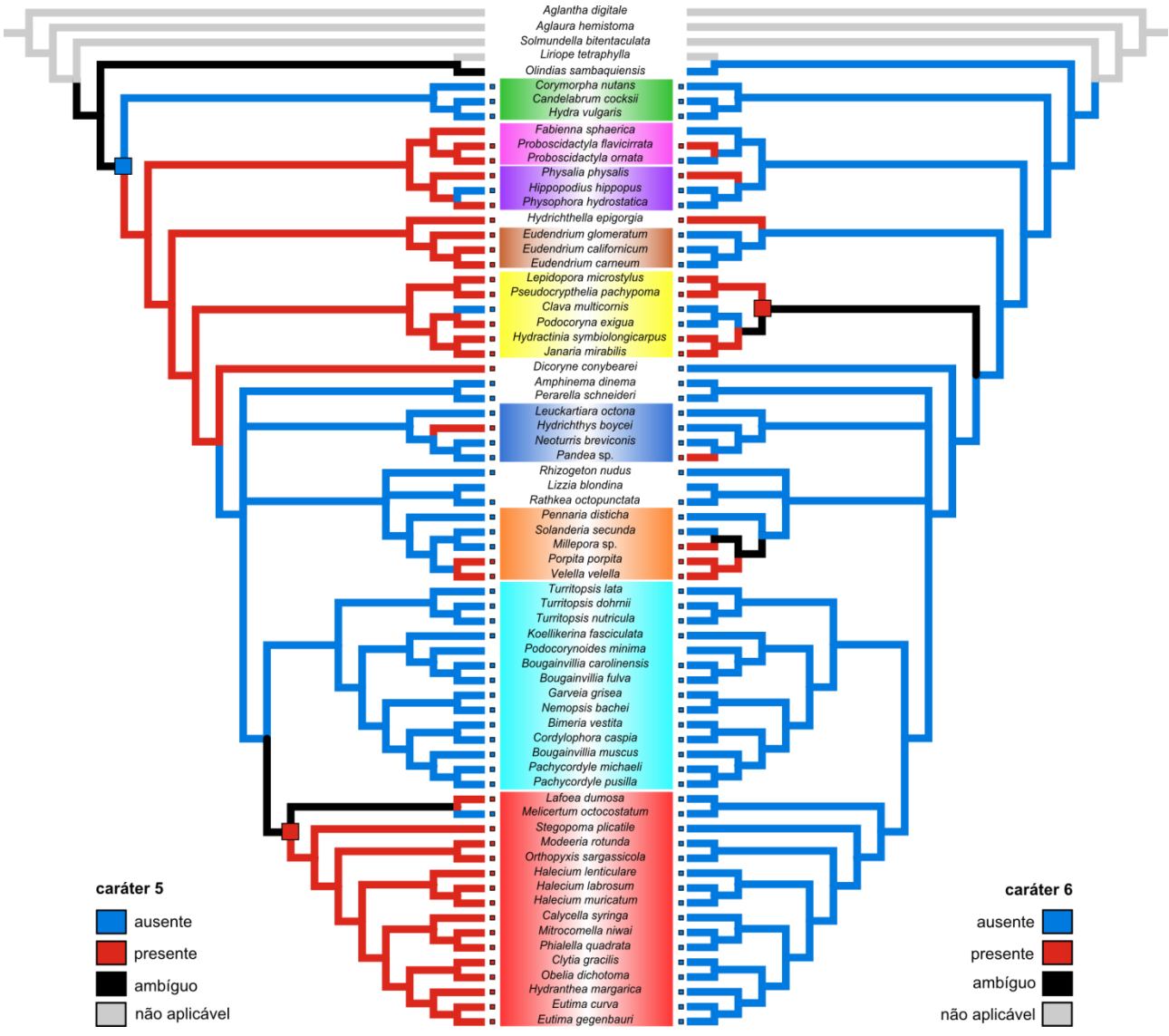


Figura MSB6. Reconstrução dos estados ancestrais dos caracteres morfológicos 5-6 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir de uma otimização ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

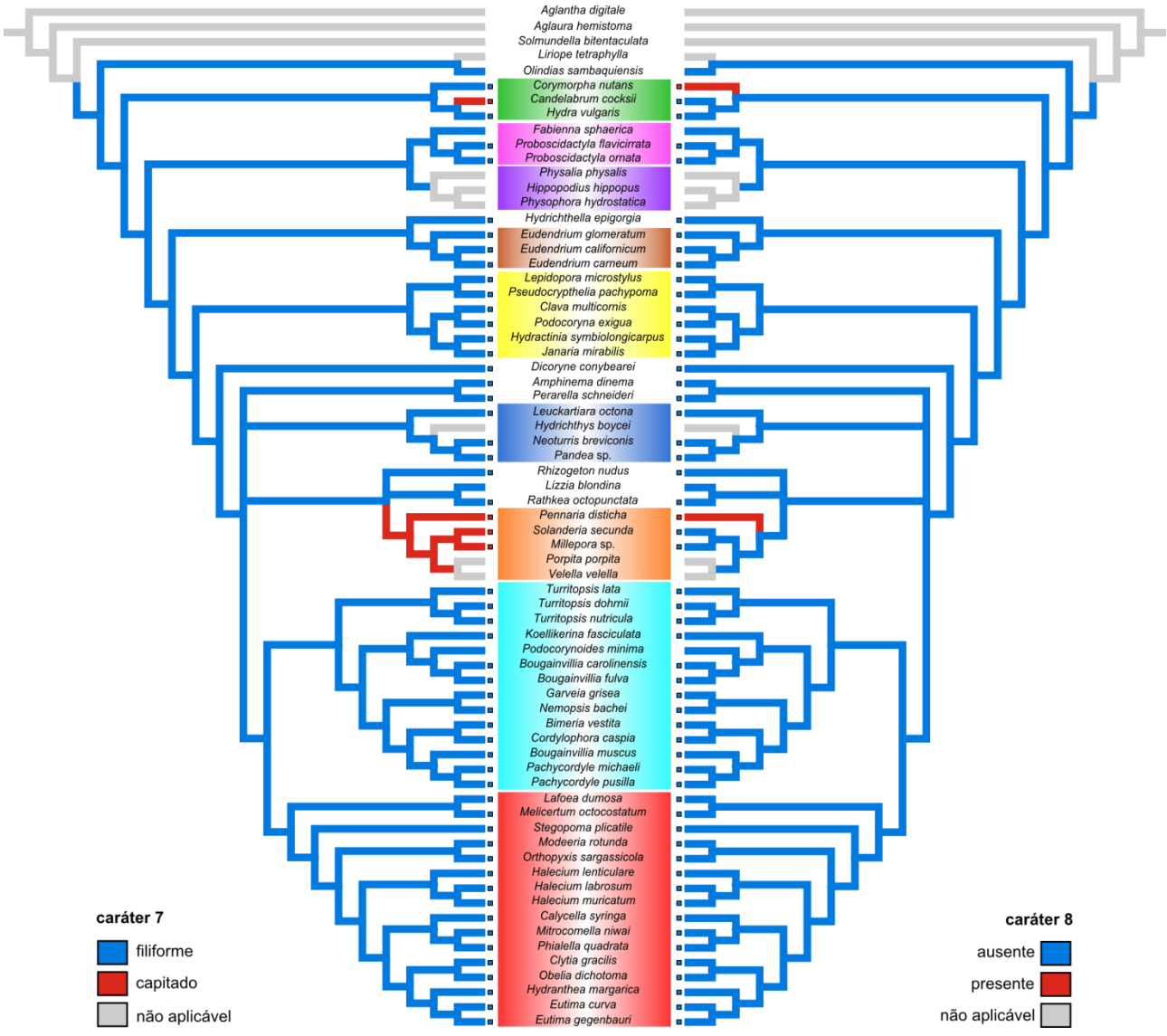


Figura MSB7. Reconstrução dos estados ancestrais dos caracteres morfológicos 7-8 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir de uma otimização ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

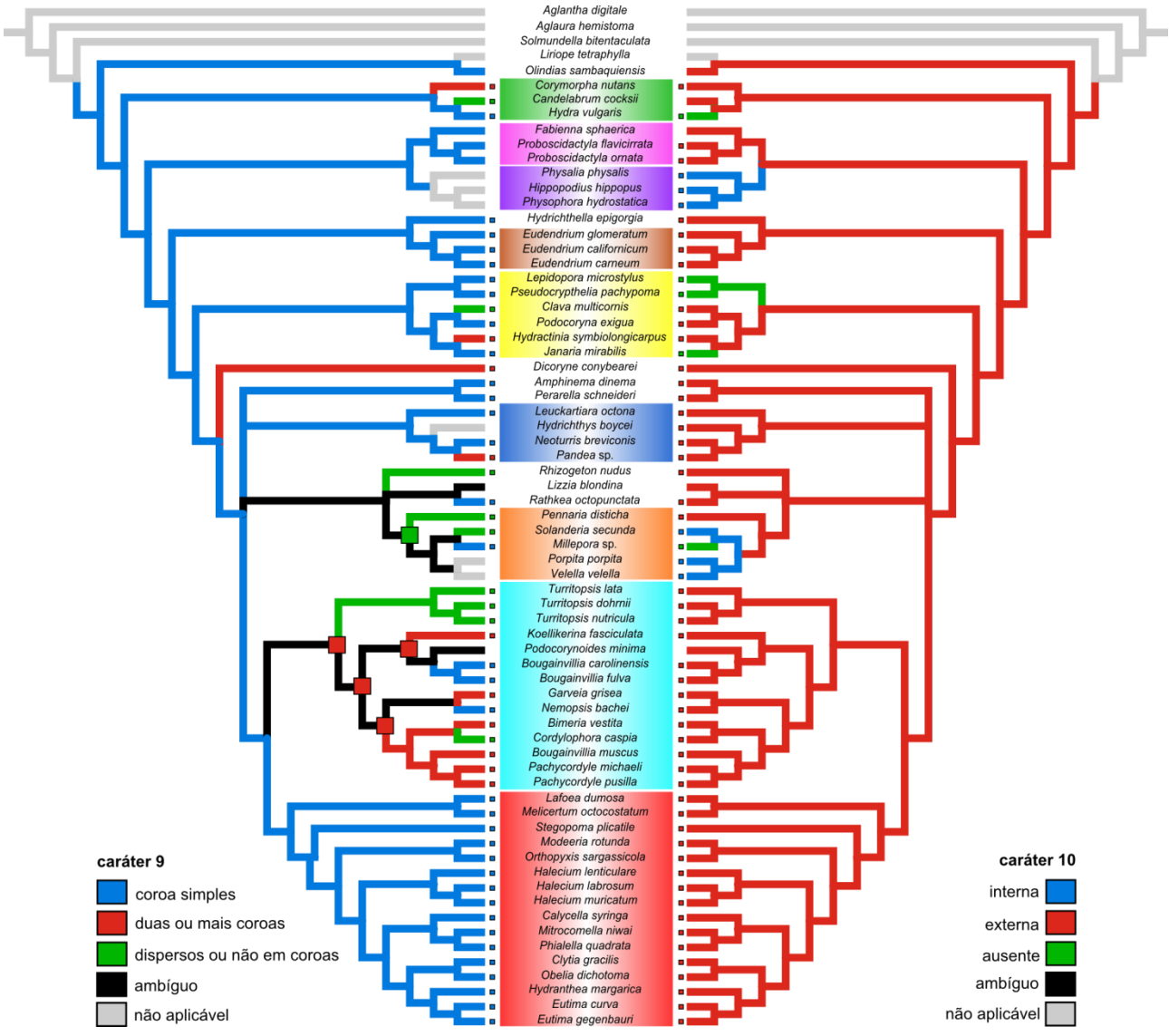


Figura MSB8. Reconstrução dos estados ancestrais dos caracteres morfológicos 9-10 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir de uma otimização ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

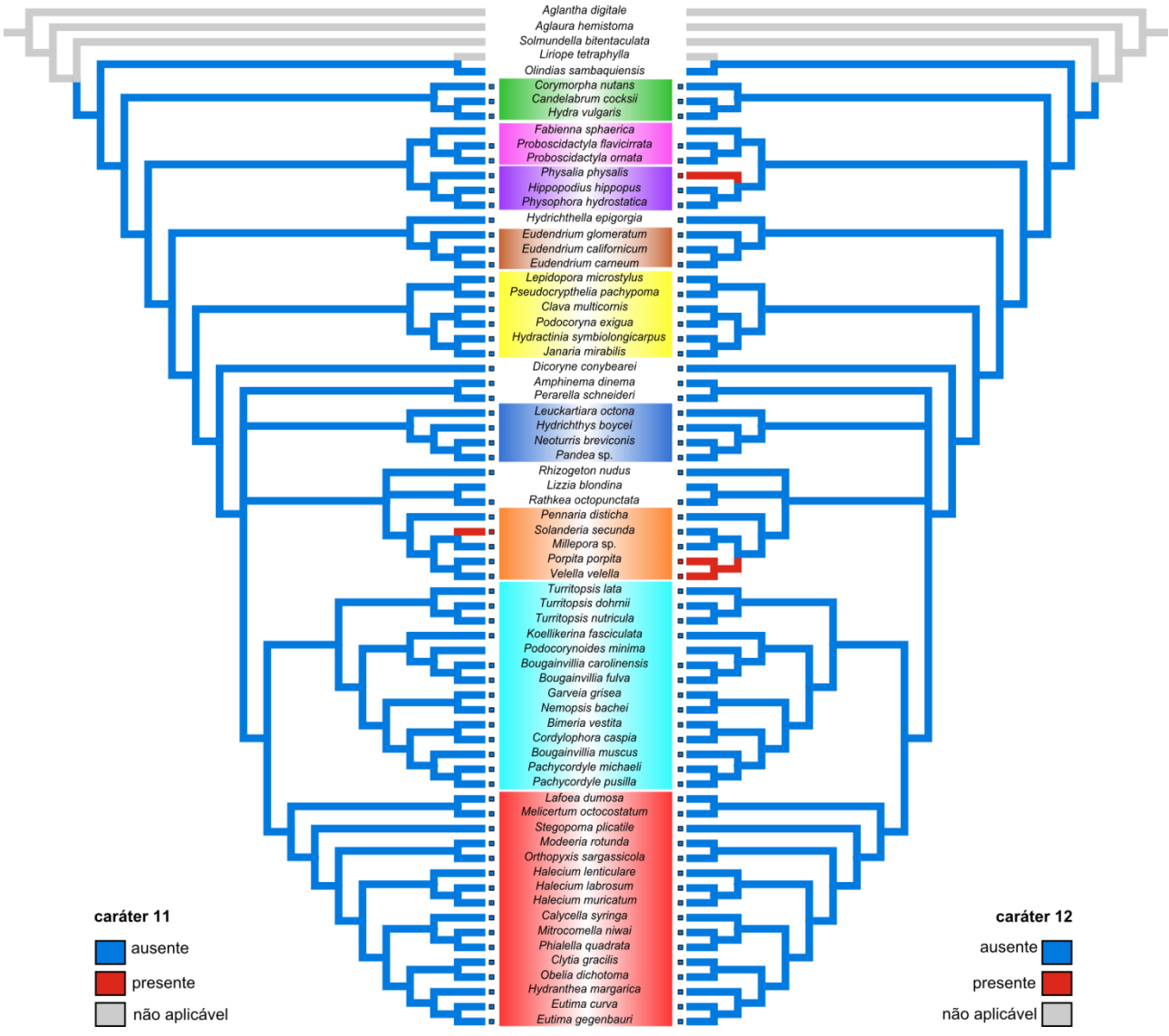


Figura MSB9. Reconstrução dos estados ancestrais dos caracteres morfológicos 11-12 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir de uma otimização ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

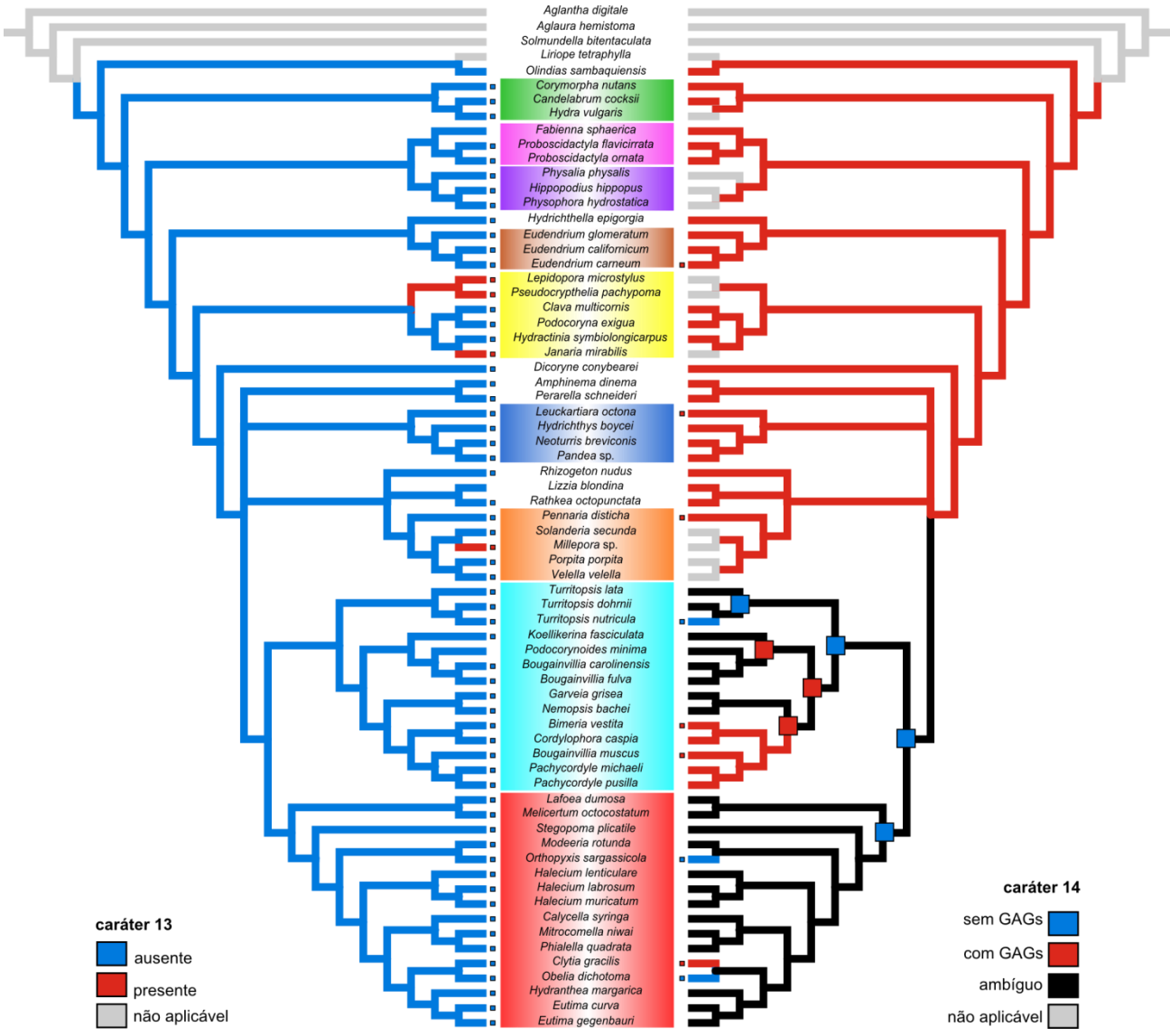


Figura MSB10. Reconstrução dos estados ancestrais dos caracteres morfológicos 13-14 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir de uma otimização ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

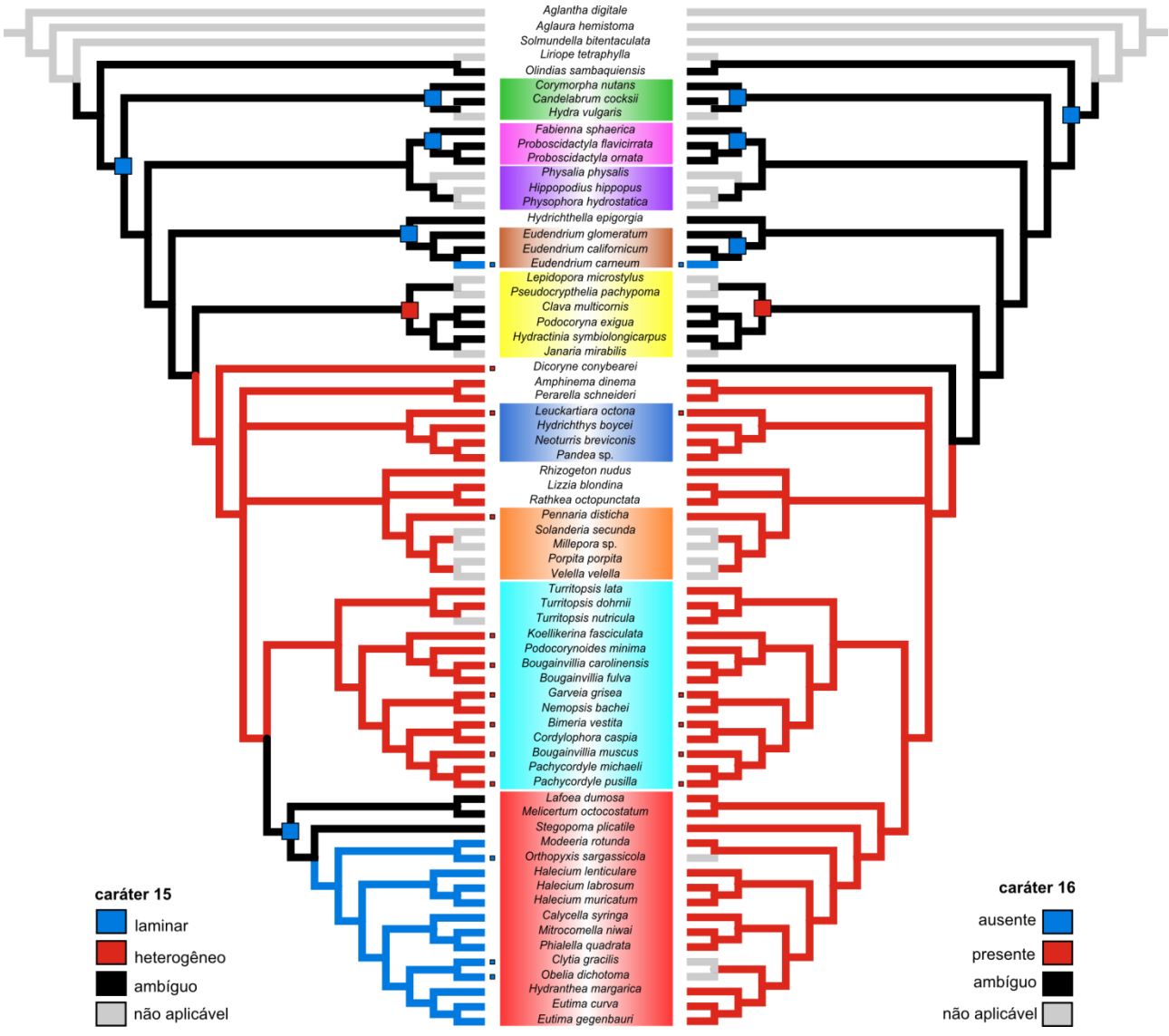


Figura MSB11. Reconstrução dos estados ancestrais dos caracteres morfológicos 15-16 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir de uma otimização ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

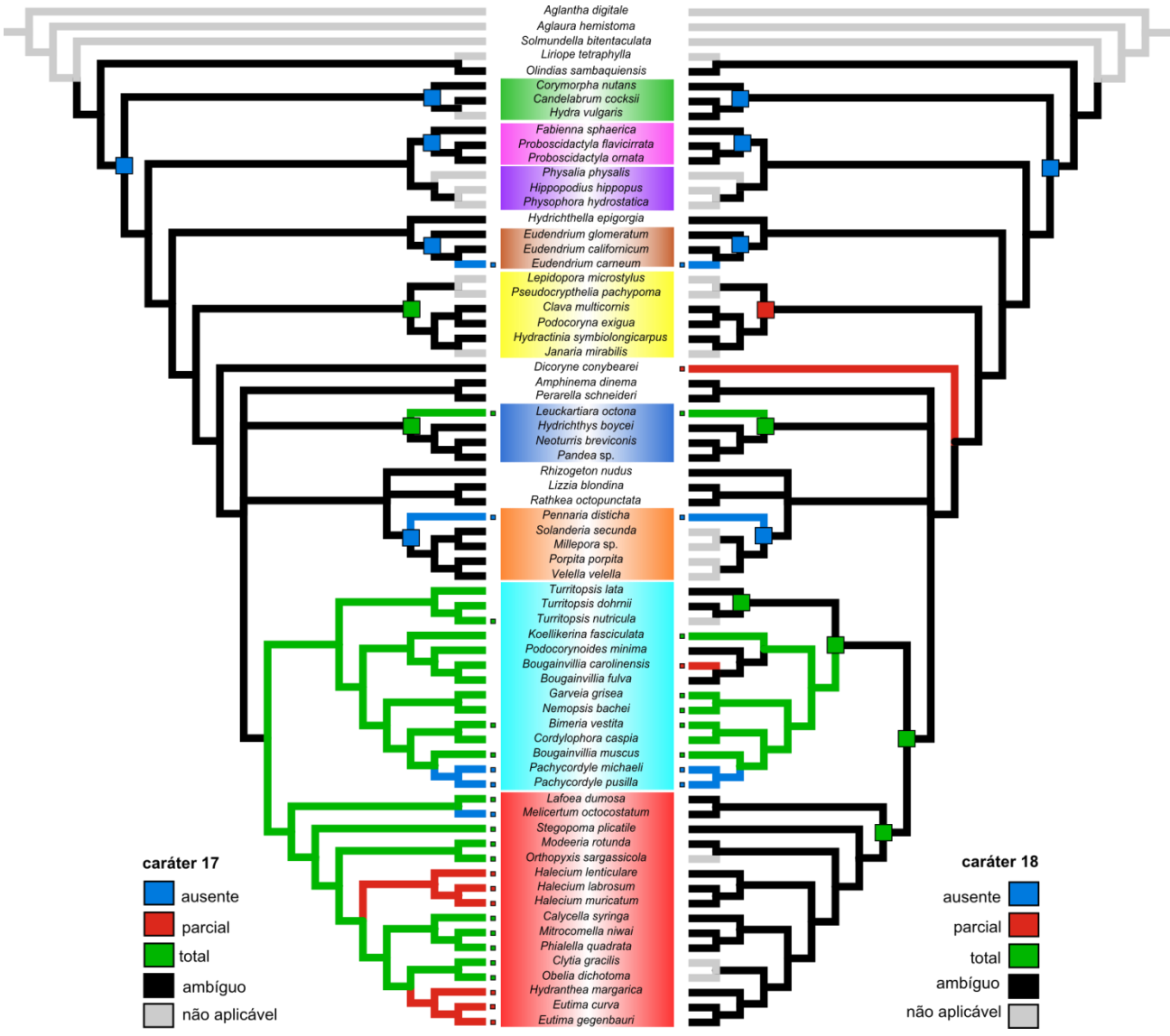


Figura MSB12. Reconstrução dos estados ancestrais dos caracteres morfológicos 17-18 baseada na hipótese filogenética resultante de análise de Parcimônia (P). As cores dos ramos representam os estados da legenda e as cores nas espécies definem seus grupos na topologia. Quadrados na base de alguns clados indicam o estado ancestral obtido a partir de uma otimização ACCTRAN e os quadrados menores ao lado do nome de cada terminal indicam seu estado usado na análise (ausência indica estado de caráter codificado como ?).

Capítulo – 6

Discussão geral e conclusões

O conhecimento prévio sobre diversos aspectos de Bougainvilliidae (Hydrozoa, Anthoathecata) era limitado, demandando a necessidade de estudar aspectos sobre sua morfologia e biologia, permitindo formular e/ou testar hipóteses de padrões evolutivos de diversificação e riqueza do grupo.

Uma revisão bibliográfica de seus táxons (baseada na classificação seguida por Schuchert, 2007), sob uma perspectiva histórica, caracterizou gêneros e espécies válidos, e desvendou o potencial de distribuição em função da ecologia das espécies mais bem representadas, resultando em cinco padrões possíveis de distribuição latitudinal para pólipos e medusas de Bougainvilliidae (Mendoza-Becerril & Marques, 2013). Conjuntamente, identificamos caracteres diagnósticos usados nas classificações tradicionais da fase polipoide (cf. Millard, 1975; Calder, 1988; Schuchert, 2007), em que o tipo de gonóforo é essencial para caracterização dos táxons e, ante a ausência de gonóforos, os hidroides podem ser aproximadamente determinados pela cobertura de sua pseudo-hidroteca, que é bastante variável e também presente em outras famílias de "Anthoathecata".

A pseudo-hidrotheca é, de fato, um caráter chave na taxonomia de Bougainvilliidae, mas há grandes hiatos sobre sua origem, desenvolvimento, composição e variação morfológica. Além disso, baseado na semelhança morfológica, há pelo menos oito nomes diferentes para a estrutura na literatura (Allman, 1871; Warren, 1919; Brown, 1975; Thomas & Edwards, 1991; Calder, 1988; Stepanjants et al., 2000; Vervoort, 2000; Schuchert, 2007; Cartwright et al., 2008), causando uma inexatidão e ambiguidade na delimitação entre os táxons.

A pseudo-hidroteca faz parte do sistema geral de suporte dos hidroides e, como tal, a universalidade e evolução do exoesqueleto fornecem informações cruciais para compreender a evolução em Medusozoa em si. Nossas análises histológicas e microestruturais de diversos grupos mostrou que a pseudo-hidroteca faz parte do sistema exoesquelético, sendo melhor representada em Bougainvilliidae. Uma análise comparada dos dados de grupos fósseis e atuais deixa claro que há variação na síntese, estrutura e função do exoesqueleto dentre os medusozoários, e que a esqueletogênese retrocede ao Ediacarano, quando processos bióticos, abióticos e fisiológicos atuaram conjuntamente resultando em um exoesqueleto rígido e/ou biomineralizado. No entanto, o exoesqueleto axial córneo (complexo quitina-proteico)

predomina nos pólipos atuais, apresentando a maior variação e complexidade estrutural entre os pólipos de Hydroidolina, grupo para o qual foi descrito um novo tipo de exoesqueleto bicamada.

O exoesqueleto bicamada, carateristico da maioria de Bougainvilliidae, é formado de uma camada externa de glicosaminoglicanos (= exossarco), disposta radialmente em relação a uma camada interna quitino-proteica (= perissarco) e considerada um reforço exoesquelético. Consequentemente, a pseudo-hidroteca, classicamente uma cobertura externa com ou sem detritos que envolve os hidrantes (Allman, 1871; Calder, 1988), é redefinida como um exossarco que envolve o hidrante em diferentes graus e que pode ou não estar associado a material orgânico ou inorgânico.

Concluímos que é essencial estudar o exoesqueleto de forma ampla, não se limitando a estruturas específicas. Com essa perspectiva, e focando em estudos sobre seu desenvolvimento, morfologia e histologia entre diferentes espécies de Bougainvilliidae e de outros Hydroidolina, caracterizamos o desenvolvimento exoesquelético em três sistemas, viz. "síntese molecular", "matriz molecular" e "expressão morfológica", que têm implicações evolutivas e ecológicas importantes.

Os dados levantados permitiram um estudo integrado da evolução de Bougainvilliidae considerando-se dados moleculares e morfológicos, incluindo os novos caracteres exoesqueléticos. As análises identificaram "Bougainvilliidae" e "Bougainvillia" como não-monofiléticos. Por outro lado, propusemos o grupo monofilético Pseudothecata *taxon novum* incluindo os gêneros classicamente assumidos em "Bougainvilliidae" (exceto *Dicoryne*) e algumas outras família. Pseudothecata é grupo irmão de Leptothecata, grupo com exoesqueleto rígido amplamente desenvolvido nas colônias na maioria de seus representantes. Vimos ainda que membros de "Bougainvilliidae" possuem estados exoesqueletais intermediários, na forma de um exossarco.

As evidências apontadas ampliam uma linha de pesquisa sobre a origem e composição em diferentes níveis estruturais, o sinal filogenético do exoesqueleto e possíveis influências de variações batimétricas sobre o mesmo, além de métodos para compreender seu sistema molecular. Entretanto, estudos evolutivos sobre o grupo são imprescindíveis para uma melhor compreensão dos caracteres compartilhados entre "Bougainvilliidae" e outros Medusozoa, que permitirá correlacionar a áreas como conservação e filogeografia.

Literatura citada

Allman, G.J. 1871. A monograph of the Gymnoblastic or Tubularian hydroids. London: The Ray Society Monograph 450 p.

Brown, C.H. 1975. Structural materials in animals: Coelenterata. New York: John Wiley & Sons 448 p.

Calder, D.R. 1988. Shallow-water hydroids of Bermuda the Athecatae. Life Sciences Contributions 148: 1-107.

Cartwright, P., Evans, N.M., Dunn, C.W., Marques, A.C., Miglietta, M.P., Schuchert, P. & Collins, A.G. 2008. Phylogenetics of Hydroidolina (Hydrozoa: Cnidaria). Journal of the Marine Biological Association of the United Kingdom 88: 1663-1672.

Mendoza-Becerril, M.A. & Marques, A.C. 2013. Synopsis on the knowledge and distribution of the family Bougainvilliidae (Hydrozoa, Hydroidolina). Latin American Journal Aquatic Research 41: 908-924.

Millard, N.A.H. 1975. Monograph on the Hydroida of Southern Africa. Annals of the South African Museum 68: 1-513.

Schuchert, P. 2007. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 2. Revue Suisse de Zoologie 114: 195-396.

Stepanjants, S.D., Timoshkin, O.A., Anokhin, B.A. & Napara T.O. 2000. A new species of Pachycordyle (Hydrozoa, Clavidae) from Lake Biwa (Japan), with remarks on this and related Clavid genera. Scientia Marina 64: 225-236.

Thomas, M.B. & Edwards, N.C. 1991. Cnidaria: Hydrozoa. In: Harrison, F.W. & Westfall, J.A. (Eds.). Microscopic anatomy of invertebrates II – Placozoa, Porifera, Cnidaria and Ctenophora 91-183 p.

Warren, E. 1919. On the anatomy of a New South African hydroid, *Bimeria rigida* sp. n. Annals of the Natal Museum 4: 1-18.

Vervoort, W. 2000. Additional notes on *Clathrozoella drygalskii* (Vanhöffen, 1910) (Cnidaria, Hydrozoa). Scientia Marina 64: 237-240.

Resumo

A família Bougainvilliidae é um grupo de hidrozoários "Anthoathecata" "Filifera" pouco conhecido. Nesse estudo, diversos aspectos da biologia do grupo e de táxons relacionados a ele foram analisados e discutidos. Nossas análises incluem: uma revisão bibliográfica dos táxons de Bougainvilliidae, a partir de um embasamento histórico e geográfico sobre seu conhecimento atual; uma síntese sobre sua estrutura exoesquelética, abrangendo informações de outros medusozoários fósseis e atuais; análises histológicas e microestruturais de pólipos de diversos grupos de Medusozoa; e um estudo integrado da evolução de Bougainvilliidae, considerando-se dados moleculares e morfológicos. Os resultados desvendaram gêneros e espécies válidos, padrões possíveis de distribuição latitudinal para pólipos e medusas de Bougainvilliidae, assim como a universalidade e evolução do exoesqueleto como fonte de informação para compreender padrões de diversificação dentro de Bougainvilliidae e em relação a outros Medusozoa. Além disso, os resultados evidenciam a variação na síntese, estrutura e função do exoesqueleto dentre os medusozoários, apontando que a esqueletogênese retrocede ao Ediacarano, sendo que o exoesqueleto axial córneo (complexo quitina-proteico) predomina nos pólipos atuais e atua como uma estrutura de suporte e proteção, entre outras funções. O exoesqueleto apresenta maior variação e complexidade estrutural entre os pólipos de Hydroidolina, grupo para o qual foi descrito um novo tipo de exoesqueleto bicamada, que é encontrado na maioria dos Bougainvilliidae. Resultados das análises filogenéticas identificam a "Bougainvilliidae" e "*Bougainvillia*" como táxons não-monofiléticos, e demonstram que o grupo monofilético Pseudothecata *taxon novum* inclui os gêneros classicamente assumidos em "Bougainvilliidae" (exceto *Dicoryne*), entre outras famílias de "Anthoathecata". Neste estudo, ampliamos o nosso entendimento sobre a natureza química e física do exoesqueleto em Medusozoa, estrutura com um valor subestimado na taxonomia do grupo. Concluímos que estudar a "síntese molecular", "matriz molecular" e "expressão morfológica" do exoesqueleto é essencial para inferências evolutivas e ecológicas, as quais podem ser intrinsecamente correlacionadas com outras áreas biológicas, tais como biologia de conservação e filogeografia.

Abstract

The family Bougainvillidae is a poorly known group of hydrozoans “Anthoathecata” “Filifera”. In this study, several aspects of the biology of this group and other related taxa are analyzed and discussed. Our analyzes include: a bibliographic revision of the taxa comprising the Bougainvilliidae, based on its current historical and geographical knowledge; a synthesis regarding its exoskeletal structure, including information on other extinct and extant medusozoans; histological and microstructural analyzes of polyps of several groups of Medusozoa; and an integrated study on the evolution of the Bougainvilliidae, considering molecular and morphological data. The results validated several genera and species and possible latitudinal distributional patterns for polyps and medusae of Bougainvilliidae, as well as the universality and evolution of the exoskeleton as a source of information to understand its role in the diversification patterns in Bougainvilliidae and with relation to other Medusozoa. Additionally, the results reveal the existence of variation on the synthesis, structure and function of the exoskeleton among the Medusozoa, showing that the exoskeletogenesis dates back to the Ediacaran, since the corneus exoskeleton (chitin-protein complex) predominates today in current polyps and acts as a supporting structure and protection, among other functions. The skeleton has higher variation and structural complexity among polyps of Hydroidolina, taxon from which we described a new type of bilayer exoskeleton, which is found in most of the species of Bougainvilliidae. Results of phylogenetic analyzes identificate “Bougainvilliidae” and “*Bougainvillia*” as non-monophyletic taxa, and showed that the monophyletic group Pseudothecata *taxon novum* includes the classical genera usually inserted in the “Bougainvilliidae” (excluding *Dicoryne*), and other families of “Anthoathecata”. In this study, we increased our understanding of the chemical and physical nature of the exoeskeleton of Medusozoa, a structure whith an underestimate role in the taxonomy of the group. We concluded that the study of the “molecular synthesis”, the “molecular matrix” and the “morphological expression” of the exoskeleton is necessary for evolutionary and ecological inferences, which are intrinsically related to other biological areas, such as conservation biology and phylogeography.