



An evolutionary comparative analysis of the medusozoan (Cnidaria) exoskeleton

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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 was 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 is a characteristic component of exoskeleton 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. A new type of bilayered exoskeleton in which there is an exosarc complementing the perisarc construction is here described.

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INTRODUCTION

Cnidarians are an early branch of diploblastic animals, which diverged from the shared ancestor of the Bilateria ~600 Mya (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 are typically benthic and marine polyps, whereas Medusozoa have a greater diversity of forms and habits, including pelagic, free-swimming (usually medusae), benthic and sessile (usually

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polyps), all mostly marine, but with a few freshwater 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). Recently, some molecular phylogenetic analyses indicated that Myxozoa is a cnidarian group (Chang *et al.*, 2015; Foox & Siddall, 2015; Foox *et al.*, 2015).

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 *bauplan* of two epithelial layers. The internal gastrodermis delimits the gastrovascular cavity from the tentacles to the pedal disc and functions in the 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 contains, including epithelio-muscular, interstitial, glandular, nervous, and cnidae cells, as well as determining how the animal interacts with its aquatic environment (Mackie, 1984). The skeleton of Cnidaria is a key feature that plays roles in protection, ion storage, fixation to substrates, swimming, flexibility, and floating/drift/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), whereas the exoskeletons of Medusozoa 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 the biology and evolution of the group, despite its basal position in the evolution of animals (Marques, Morandini & Migotto, 2003; Collins *et al.*, 2006). Owing to this gap in our knowledge, our goals here were to conduct detailed analyses of medusozoan skeletons, highlighting the variation in origin, structure, and function, and how disparities in these features have accompanied the evolution and diversification of this group. To achieve these goals, we have brought together published and unpublished data for fossil cnidarians and modern histological information for extant groups of Medusozoa.

THE METAZOAN EXOSKELETON – SYNTHESIS

Macromolecule evolution has resulted in the development of extracellular structures with many functions, such as support, osmoregulation, defence, 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 polysaccharide carbohydrates, such as cellulose in plants (Richmond & Somerville, 2000) and 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 structures. 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-glucosamine (Muzzarelli & Muzzarelli, 2009), usually with a visible fibrous organization at different hierarchical levels (nanofibrils, microfibrils, or fibres; Ehrlich *et al.*, 2010) and in three alternative forms: antiparallel α (the most common), parallel β , and alternate γ (Pillai, Paul & Sharma, 2009). Biosynthesis of chitin includes synthesis and degradation catalysed by enzymes found in all living organisms (Ruiz-Herrera, González-Prieto & Ruiz-Medrano, 2002; Merzendorfer & Zimoch, 2003; Tang *et al.*, 2015). Processes of expression and functions of chitin have been most studied in fungi and arthropods (e.g. Ruiz-Herrera & Ortiz-Castellanos, 2010; Merzendorfer, 2011; Souza *et al.*, 2011).

Chitin synthetases (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 present between some other Metazoa (Porifera, Anthozoa, Deuterostomia) and the Choanoflagellata (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). The presence of these genes in different groups suggests that the basic components are conserved and these are functional since a particular moment in animal evolution. Yet, *Chs* genes have not been found in the genomes of nonchitinous organisms (Willmer, 1990; Wagner, 1994), e.g. *Trichoplax adhaerens* Schulze, 1883 (Placozoa; Dellaporta *et al.*, 2006; Signorovitch, Buss & Dellaporta, 2007) and *Mnemiopsis leidyi* A. Agassiz, 1865 (Ctenophora; Table 1; Ryan *et al.*, 2013; Bolte *et al.*, 2014).

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

Enzyme	Pfam code	Placozoa <i>Trichoplax adhaerens</i>	Porifera <i>Amphimedon queenstandica</i>	Ctenophora <i>Mnemiopsis leidy</i>	Anthozoa <i>Nematostella vectensis</i>	Medusozoa <i>Hydra vulgaris</i>
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	B3RWQ51	B3RWQ51	ML368913a3	A7RFM31	T2M6D91
	PF02010					
10. Tyrosinase [§]	PF00264	*	I1E6461	ML070211a3	A7RQY21	XP_0021559904
11. Tyrosine hydroxylase	PF00351	B3SC111	XM_0033833394	ML154513a3	Nv.T1.7540.22	T2MHI21

Enzymes 1–8 participate in chitin synthesis in insects (Merzendorfer & Zimoch, 2003) and enzymes 9–11 participate or associate in chitin synthesis in other taxa (Knight, 1970; Kossevitch et al., 2001). Species name under higher taxon are examples for each taxon.

Enzyme families follow Protein family database (Pfam, <http://pfam.xfam.org/>); presence is inferred from databases: 1, Universal Protein Resource (UniProt, www.uniprot.org); 2, *Nematostella vectensis* Genomics Database (StellaBase cnidarians.bu.edu/stellabase/); 3, *Mnemiopsis* Genome Project Portal (www.research.nhgri.nih.gov/mnemiopsis/); 4, Nucleotide follow National Center for Biotechnology Information database (NCBI, www.ncbi.nlm.nih.gov/).

*Unidentified enzymes; †enzyme also known as phosphoglucose isomerase (<http://pfam.xfam.org/search/keyword?query=Glucose-6-phosphate+isomerase>); ‡enzymes of uncertain presence; §dopamine synthesized via tyrosinase; ¶enzyme found only in *Acropora millepora*.

Chitinases (family 18 of the 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) have been 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 shows 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, noncollagen chitin-binding protein, minerals (e.g. magnesium carbonate), and chemical compounds (e.g. calcium carbonate); Shen & Jacobs-Lorena, 1999; Ehrlich *et al.*, 2010]. These interactions help to form a structural backbone that defines the organic phase in extracellular biomineralization, acting as a mould, nucleation niche, and orientation modifier for crystalline and amorphous minerals, thereby forming a rigid exoskeleton that serves as a defence 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 amongst the most characteristic compounds and elements that participate in the 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). Furthermore, glycosaminoglycans (GAGs) in the form of chondroitin sulphate and heparin sulphate can be found as elements in hydrozoan exoskeletons (Yamada *et al.*, 2007; Böttger *et al.*,

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]	×	×	×
Calcium phosphate [Ca ₃ (PO ₄) ₂]	×	×*	
Silicate (Si _x O _y)	×	×	
Magnesium hydroxide [Mg(OH) _s]	×		
Strontium (Sr)	×		×
Iron (Fe)	×		×
Manganese (Mn)	×		
Potassium (K)	×		
Barium (Ba)	×		×
Copper (Cu)	×		
Zinc (Zn)	×		
Lead (Pb)	×		
Phosphorous (P)	×		×
Boron (B)	×		
Uranium (U)	×		
Nickel (Ni)	×		
Chromium (Cr)	×		
Cobalt (Co)	×		

*Fossil Conulatae.

2012). Cnidarians are the basal animal branch to possess GAGs, which are conserved in other animal groups (Medeiros *et al.*, 2000; Yamada, Sugahara & Ozbek, 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 published 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, amongst others) concentrations have varied over geological time in the water column (Cook & Shergold, 1986; Brasier, 1992; Lenton & Watson, 2004; Papineau, 2010; Och & Shields-Zhou, 2011; Wood, 2011; Sperling *et al.*, 2013; Lenton *et al.*, 2014). The factors favouring the

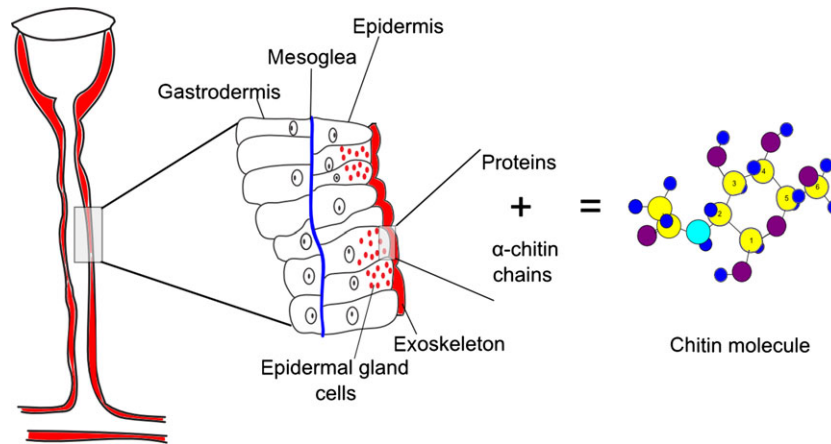


Figure 1. Model of the chitin-protein (corneous) exoskeleton and cell tissues in Medusozoa. Red line refers to the exoskeleton, yellow circles are carbon atoms, blue circles hydrogen, purple circles oxygen, and cyan circle N-acetyl group.

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 were related to increases in animal biomass and biomineralized skeletons (Brasier, 1992; Cook, 1992; Erwin & Tweedt, 2012; Wood & Zhuravlev, 2012; Kazmierczak, Kempe & Kremer, 2013; Wood *et al.*, 2015).

By the end of the Ediacaran, the oceans experienced an increase in the concentration of NaCl and other ions together with neutral pH conditions, all of which resulted in a general state similar to that of the Phanerozoic seas. During this time, Ca^{2+} concentrations reached 18 mmol L^{-1} (Hardie, 2003; converted from the original $\sim 36 \text{ mEq L}^{-1}$), compared with 10.6 mmol L^{-1} nowadays. 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, in spite of with phylogenetic conservation of some molecular pathways. Hence, through deep homology in the Opisthokonta (Scotland, 2010), there would be a common 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 have been 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 as synergistic factors, which increase the rigidity and biomineralization of the exoskeleton. In sessile phases of life in organisms, this exoskeleton 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) and 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 amongst organisms, especially with respect to predation (Dzik, 2007; Penny *et al.*, 2014), possibly being involved with the loss of 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 records of possible spicules of 'parazoan' ancestors (Brain *et al.*, 2012; Wallace *et al.*, 2014). It then continued 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) and exoskeletal structures are found in the fossil *Coronacollina acula* Clites *et al.*, 2012 and in the spicules of the Cambrian sponge in the genus *Choia* (Clites, Droser & Gehling, 2012). The chemical structure of these fossil spicules is unknown, but they may have contained 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 the Ediacara Biota (~543 Mya; Xiao & Laflamme, 2008).

THE EXOSKELETON IN MEDUSOZOA

FOSSIL RECORDS

The exoskeleton in Cnidaria occurred at least since the Ediacaran (~635–551 Mya; 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 & Zátón, 2012); the chitinous and tubular annulated polyps of scyphozoa *Olivoooides* (Zhao & Bengtson, 1999; Dong *et al.*, 2013; Yasui *et al.*, 2013); the late Ediacaran chitin-mineralized fossil *Corumbella* (Pacheco *et al.*, 2015); and the possibly mineralized phosphate type of Conulatae scyphozoans (Leme *et al.*, 2013; Van Iten *et al.*, 2013a). Conulatae are also recorded in the Ediacaran, as exemplified by the indisputable occurrence of *Paraconularia* sp. in the Tamengo Formation, Brazil (Van Iten *et al.*, 2014). The phosphatic 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 pre-

sented 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 there 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, consisting of individual lamella of calcium phosphate (apatite) that were 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). The 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, their exoskeletons are characterized by the centripetal increase in the lamellae, external ornamentation (longitudinal and transverse corrugations), repair by apical wall formation, internal perradial and inter-radial, with carina and septa in the conulariids (Van Iten, 1991, 1992a,b; Jerre, 1994; Van Iten, Fitzke & Cox, 1996; Hughes *et al.*, 2000; Van Iten *et al.*, 2006, 2014; Leme *et al.*, 2008a,b, 2010). In addition to several other groups of Cnidaria as the Conulatae, Corumbellata and *Cloudina* 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 old (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 that were established by the end of the Ediacaran (Bengtson & Zhao, 1992; Hua, Pratt & Zhang, 2003).

The scyphozoan fossil *Corumbella weneri* (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 amongst the first metazoans with biomineralized (phosphatic) exoskeletons (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; Muscente & Xiao, 2015; Vinn & Kirsimäe, 2015) 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,

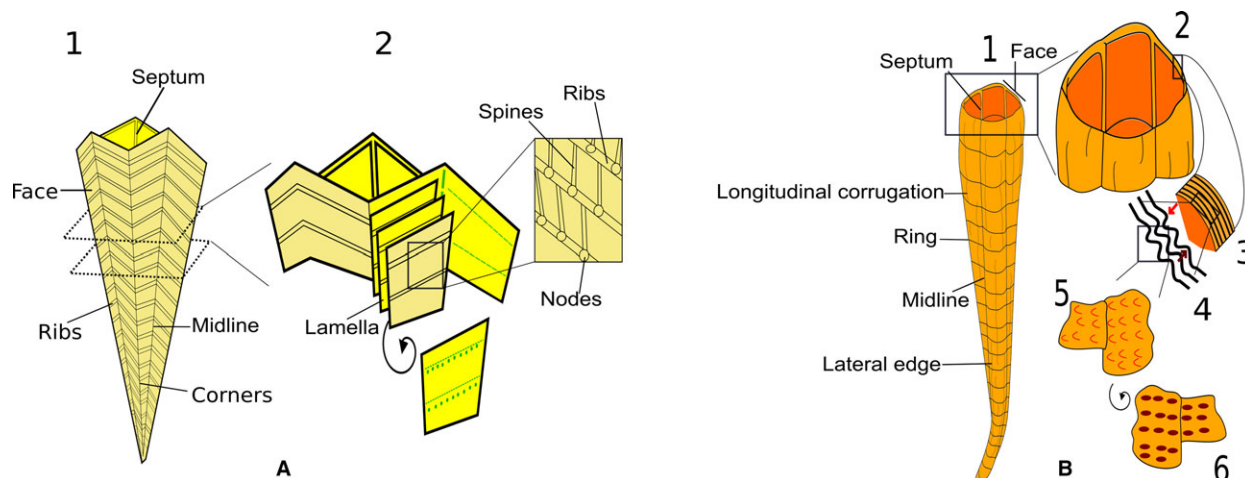


Figure 2. Schematic of the exoskeleton in fossil groups in Medusozoa. A, Conulatae: A1, hypothetical Conulatae, general morphology with main features (modified from Leme *et al.*, 2004); A2, lamellar ornament details of the exoskeleton. B, *Corumbella*: B1, *Corumbella weneri*; B2, oral region; B3, cross-section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

1986), owing to its exoskeleton (=carapace in the literature) which is 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 confers flexibility to the exoskeleton of *C. weneri*, 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 record evidence show that the types of exoskeleton are conserved amongst living groups. For example, of the chitin-protein type of Leptothecata of Ordovician *Sinobryon elongatum* Balinski, Sun & Dzik, 2014 (Balinski *et al.*, 2014) the biomineralized carbonate exoskeletons of anthozoan corals of the Cambrian (Stanley & Fautin, 2001), and Hydrozoa Milleporidae (~150 Mya, Jablonski, 2005), Stylasteridae (~65 Mya, Lindner, Cairns & Cunningham, 2008), and Hydractiniidae (~50 Mya, Miglietta, McNally & Cunningham, 2010).

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 type predominant in medusozoan polyps is composed of chitin-protein (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 have 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 probably 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

Table 3. Types of exoskeleton

	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†	2	Calcium phosphate	Coriaceous	Polyp body	Theca	2A
	Corumbellata†	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 sulphate and putative peroxidase proteins	Fibrous	Polyp body, except tentacles	Cuticle	3G

GAGs, glycosaminoglycans.

*Uncertain; †fossil groups.

subunits can enclose the planula (Otto, 1978). These plaques are also visible in epidermal cell cytoplasmic vesicles, where they probably formed before transport to the exterior, and are apparently distinct from other extracellular covering described for Cnidaria, probably associated with an overwintering phase (Otto, 1978). Settled larvae of *Halicylistus 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). By contrast, 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 *Halicylistus* is covered by a filamentous and adhesive layer (Fig. 3A; Otto, 1978; Miranda, Collins & Marques, 2013). Stauromedusae of *Halicylistus* 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, however, 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). Secretions of adhesive, supportive, and mucous cells appear to form an extracellular layer (~60–100 µm) in the basal epidermis of *Halicylistus* (Fig. 3A; Singla, 1976; Lesh-Laurie & Suchy, 1991). Even though this layer appears homogenous, fibril components can be found at fixa-

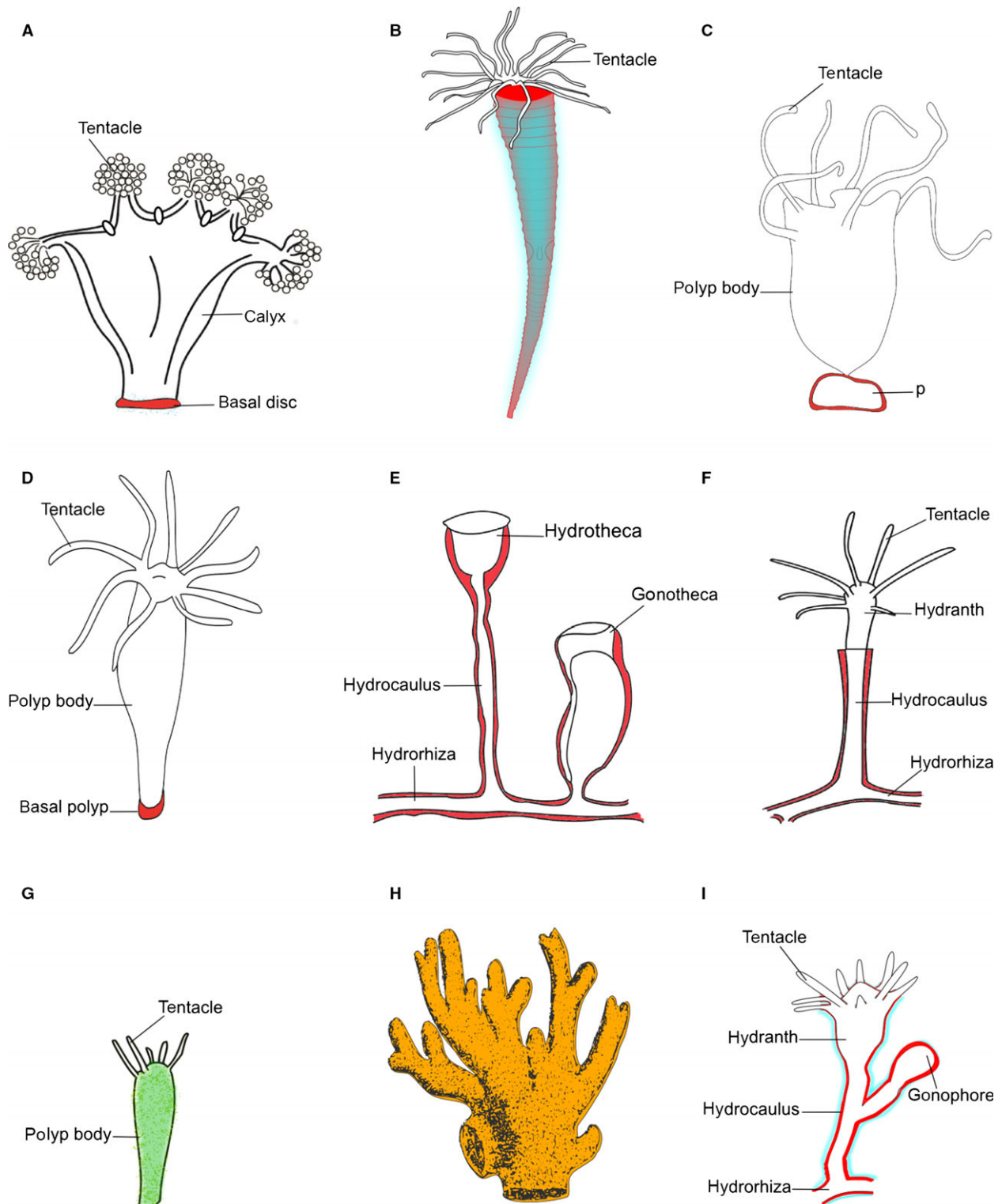


Figure 3. Schematic view of the exoskeleton in extant groups of Medusozoa. A, Staurozoa, Stauromedusae, *Haliclystus*; B, C, Scyphozoa: B, Coronatae, C, Discomedusae; D, Cubozoa, Carybdeida; E–I, Hydrozoa: E, Leptothecata, F, ‘Anthoathecata’, G, Hydridae, *Hydra vulgaris*, H, *Millepora* sp., I, *Bimeria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

tion 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; Migot, 1922a: fig 1), 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 *et al.*, 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, but this does not become 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 (M.A. Mendoza-Becerril, pers. observ.; Fig. 3B). The Discomedusae ('Semaestomeae' + 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 a two-layer exoskeleton consisting 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 have the greatest exoskeleton variability and structural complexity, especially in Hydroidolina

(Fig. 3E–I). In Leptothecata, the homogeneous chitin-protein exoskeleton (=perisarc in the literature) covers the colony from the hydrorhiza to the hydranth. The exoskeleton forms a hydrotheca around the hydranth and a gonotheca around the 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 are 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 nonmonophyletic 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 invisible 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 fibres, 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 at 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) and

other Hydroidolina, for example the genus *Eudendrium* (Marques *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 (=calyco blasts) constitute the extracellular matrix that modulates ion ingress to form spheres of aragonite or calcite that, once joined, make a firm, and rigid skeletal structure that is more fibrous and porous in Milleporidae than in other families (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 gelatinous covering of GAGs (M.A. Mendoza-Becerril, pers. observ.) 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-protein layer (=perisarc) of the exoskeleton, and 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-protein layer. By contrast, *Bimeria vestita* Wright, 1859, and *Bimeria rigida* have an exosarc that covers the hypostome and the base of the tentacles. Therefore, with this evidence we propose that some 'Anthoathecata' have an exoskeleton that is formed by two layers (chitin-protein and GAGs), has a granular appearance, and is different from that of other cnidarians (corneous, calcareous, coriaceous), and designate it here as bilayered.

The exosarc has received little or no previous research 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 continues to be used exclusively for the part of the exosarc covering the hydranth. Detailed morphological, histo-

logical, histochemical, and genetic examination of the exosarc will be necessary to resolve questions of homology (whether around hydranths, branches, hydrorhiza, or gonophores).

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 it is thus reasonable to consider that it would be present in the medusozoan ancestral lineage (Van Iten *et al.*, 2006).

Exoskeletal structure and composition in Hydrozoa are most variable in the clades of Hydroidolina (Leptothecata and 'Anthoathecata'); it is modified in Siphonophora, reduced in some 'Anthoathecata' (Aplanulata and Capitata), and it is absent in Trachylina. Biomineralized exoskeletons (coriaceous) may be a synapomorphy for the monophyletic group 'Filifera III', because they appear 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 though it is referred to as a pseudohydrotheca in Bougainvilliidae and Pandeidae, and is 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 to understand its biological and ecological function and evolutionary history.

A crucial step to resolving these evolutionary considerations lies in species phylogeny itself. Nowadays there is no consensus about major patterns amongst 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 exoskeletons.

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

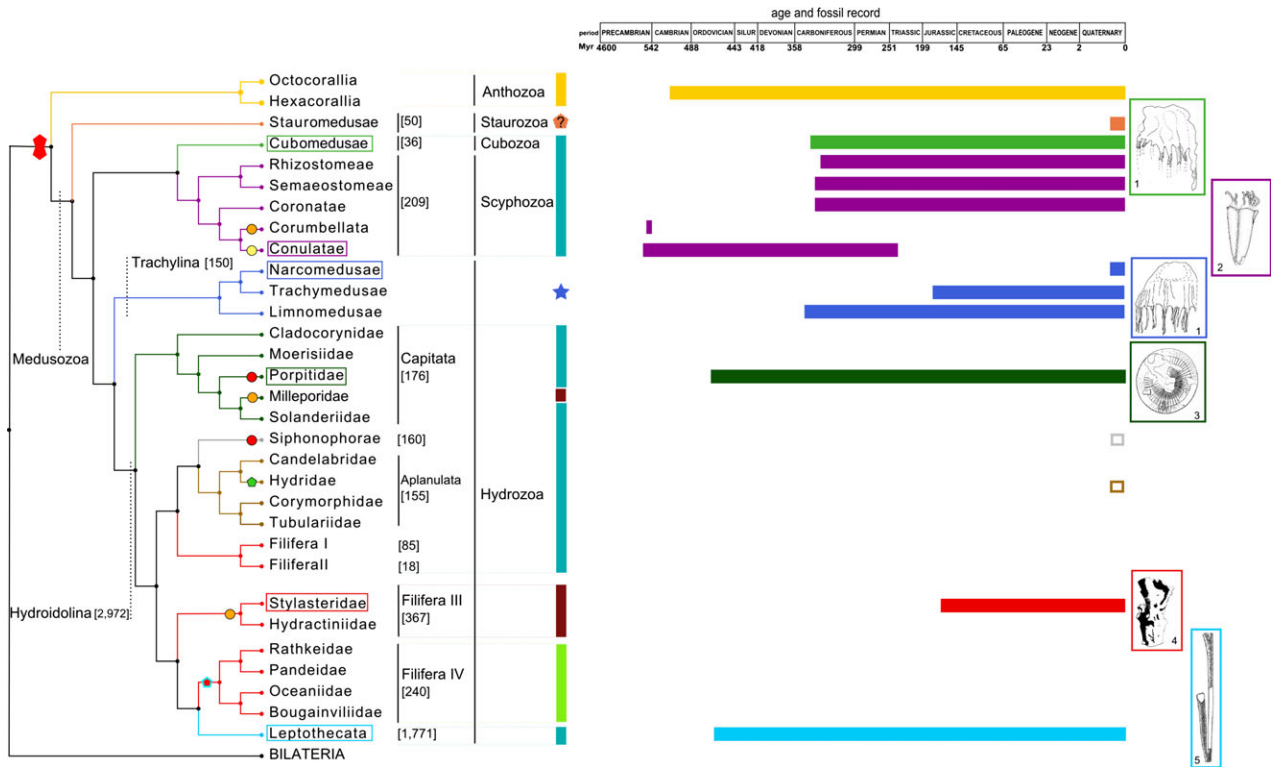


Figure 4. Phylogenetic hypothesis of the exoskeleton in Cnidaria, with fossil Medusozoa, with optimization for different skeleton types: ■ calcareous, ■ corneous, ■ coriaceous; ■ bilayered; ♦ unknown, ★ without exoskeleton. Skeleton composition: ♦ chitin, ● calcium carbonate, ● calcium phosphate, ● glycosaminoglycans (GAGs) and putative peroxidase proteins, ♦ chitin-protein and GAGs. Lineages are by colour: yellow, Anthozoa; orange, Staurozoa; green Cubozoa; purple, Scyphozoa; blue, Trachylina; dark green, Capitata; grey, Siphonophora; brown, Aplanulata; red, ‘Anthoathecata’; cyan, Leptothecata. Numbers in parentheses 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) and Cartwright *et al.* (2008). Hypothetical relations for Hydroidolina are based on unpublished 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 windermereensis* (Fryer & Stanley, 2004); 4, *Lepidopora* sp. (modified from Cairns & Grant-Mackie, 1993); 5, *Sinobryon elongatum* (Balinski *et al.*, 2014).

organization and composition of the exoskeleton with the function of this, either as a simple protection or as a rigid structure (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 evidence shows the influence of deep-water currents upon 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. These fossils were preserved below fair-weather wave base (Simões *et al.*, 2000; Rodrigues *et al.*, 2003; Van Iten *et al.*, 2013b). By contrast, the exoskeleton of *Eoconularia oculata* (Wiman, 1895) (Silurian in Sweden) is robust, with strongly mineralized septa and an 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). In addition, growth and branching patterns may be influenced by currents, such as the transverse axis being perpendicular to the direction of the current to increase feeding efficiency (Tidball, 1984) or an increase in thickness, which confers greater resistance (Kosevich, 2012).

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

Skeletogenesis was undoubtedly a key factor in animal evolution and ecological interactions, perhaps first owing to structure and the environment, and then as an exaptation for predation avoidance (e.g. Knoll, 2003). The radiation of metazoans with skeletons was both a cause and an 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; Wright & Jones, 2006; Seilacher, 2007; Erwin, 2008; Erwin & Tweedt, 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). Owing to the age of their diversification, and if modern patterns indicate past history, then, in the Cambrian, medusozoans had already colonized probably all of the same environments in which they thrive in the present-day (Gili & Hughes, 1995). This adaptive capacity and diversification was also linked to their life cycle (e.g. the medusa and polyp stages), 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 still occupied in modern oceans. Therefore, the varying compositions, structures, and functions of the exoskeleton probably contributed to the diversification and species richness of Hydrozoa 'Anthoathecata', such as Stylasteridae, Bougainvilliidae, and Eudendriidae (Cairns, 2011; Mendoza-Becerril & Marques, 2013; Schuchert, 2015), and Leptothecata, which has the greatest species richness within Hydrozoa (Cornelius, 1982).

Diversification in Stylasteridae and Milleporidae (not sister taxa) was indeed associated with the composition of the rigid exoskeleton, which is associated to increase in survival and dispersal likelihood of polyp fragments, produced by 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 (Kruijff, 1975), and the skeletal operculum that can close in the gastrozooids of Stylasteridae (Lindner *et al.*, 2008). Although the physiological response to the environment is similar in Milleporidae and Stylasteridae, the latter has nearly 18 times more species than the former (268 vs. 15; Cairns, 2011; Schuchert, 2015). Stylasteridae is also much more widespread, from the Arctic to the Antarctic (Cairns, 2007), whereas Milleporidae is tropical (Milliman, 1974). In addition to the 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).

CONCLUSIONS

Skeletogenesis in Medusozoa dates back to the Ediacaran period, having over 600 Myr of evolutionary history. Depending on the phylogenetic framework adopted, the process of skeleton formation would be present in the ancestor of medusozoans, although it is not present in the basal Staurozoa. It appears that, since the origin of skeletogenesis in this taxon, polysaccharides, glycosaminoglycans, enzymes, and other chemical and mineral compounds may have participated in exoskeleton synthesis, and a combination of these compounds results in the complex diversity presently observed, i.e. corneous, coriaceous, fibrous, and the new type described in this study, the bilayered exoskeleton. The origin and transformation of the medusozoan exoskeleton in general are associated with biotic, abiotic, and physiological/ontogenetic changes in habitats and animals, and an exoskeleton is undoubtedly a key factor in Medusozoa evolution and ecology. Future investigations on the subject should focus on the developmental programmes involved in skeletogenesis, but basic (even histological) knowledge on several taxa is still much needed.

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