The LINNEAN SOCIETY of London ZOOLOGICAL Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2016, **178**, 206–225. With 4 figures

An evolutionary comparative analysis of the medusozoan (Cnidaria) exoskeleton

MARÍA A. MENDOZA-BECERRIL[1]*, MAXIMILIANO M. MARONNA[1], MÍRIAN L. A. F. PACHECO[2], MARCELLO G. SIMÕES[3], JULIANA M. LEME[4], LUCÍLIA S. MIRANDA[1], ANDRÉ C. MORANDINI[1] and ANTONIO C. MARQUES[1,5]

[1]*Department of Zoology, Institute of Biosciences, University of São Paulo, Rua do Matão, Trav. 14, 101, 05508-090 São Paulo, Brazil*
[2]*Department of Biology, Federal University of Sao Carlos, Rodovia João Leme dos Santos - até km 104.000 Parque Reserva Fazenda Imperial, 18052780 Sorocaba, São Paulo, Brazil*
[3]*Department of Zoology, Laboratory of Paleozoology, São Paulo State University Botucatu, Jardim Santo Inácio (Rubião Junior), 18618970 Botucatu, São Paulo, Brazil*
[4]*Department of Sedimentary and Environmental Geology, Institute of Geosciences, University of São Paulo, Rua do Lago, 562, 05508-080 São Paulo, Brazil*
[5]*Center for Marine Biology, University of São Paulo, Rodovia Manoel H. Do Rego km 131.5, CEP 11600-000 São Sebastião, São Paulo, Brazil*

Received 16 June 2015; revised 3 January 2016; accepted for publication 7 February 2016

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.

doi: 10.1111/zoj.12415

KEYWORDS: chitin – exoskeleton – glycosaminoglycan – Hydroidolina – Medusozoa – perisarc – phylogenetics – Pseudohydrotheca.

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

*Corresponding author. E-mail: m_angelesmb@hotmail.com

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/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), 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-glycosamine (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 *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
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.*, 2012). Cnidarians are the basal animal branch to possess GAGs, which are conserved in other animal groups (Medeiros *et al.*, 2000; Yamada, Sugahara & Özbek, 2011).

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_xO_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.

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

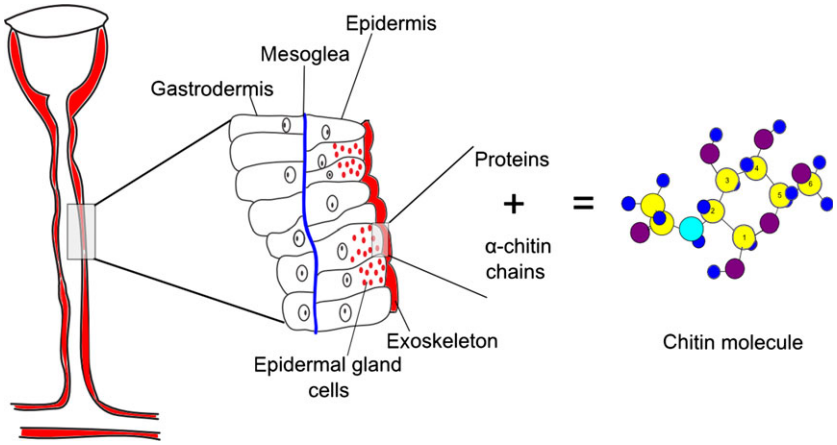

Figure 1. Model of the chitin-protein (corneous) exoskeleton and cell tissues in Medusozoa. Red line refers to the exoskeleton, yellow circles are carbon atoms, blue circles hydrogen, purple circles oxygen, and cyan circle N-acetyl group.

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 ~36 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 & Zatón, 2012); the chitinous and tubular annulated polyps of scyphozoa *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 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 presented 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 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 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,

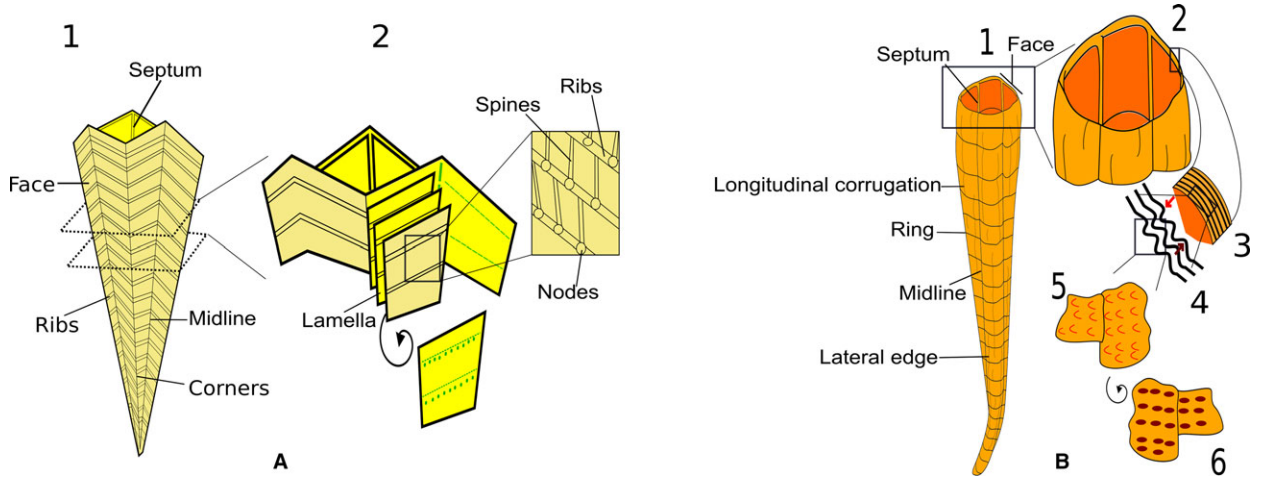

Figure 2. Schematic of the exoskeleton in fossil groups in Medusozoa. A, Conulatae: A1, hypothetical Conulatae, general morphology with main features (modified from Leme *et al.*, 2004); A2, lamellar ornament details of the exoskeleton. B, *Corumbella*: B1, *Corumbella werneri*; B2, oral region; B3, cross-section of the exoskeleton with microlamellae; B4, lamellar detail showing pores (brown arrow) and papillae (red arrow); B5, underside view 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. 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 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 *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). 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 *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, 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 *Haliclystus* (Fig. 3A; Singla, 1976; Lesh-Laurie & Suchy, 1991). Even though this layer appears homogenous, fibril components can be found at fixa-

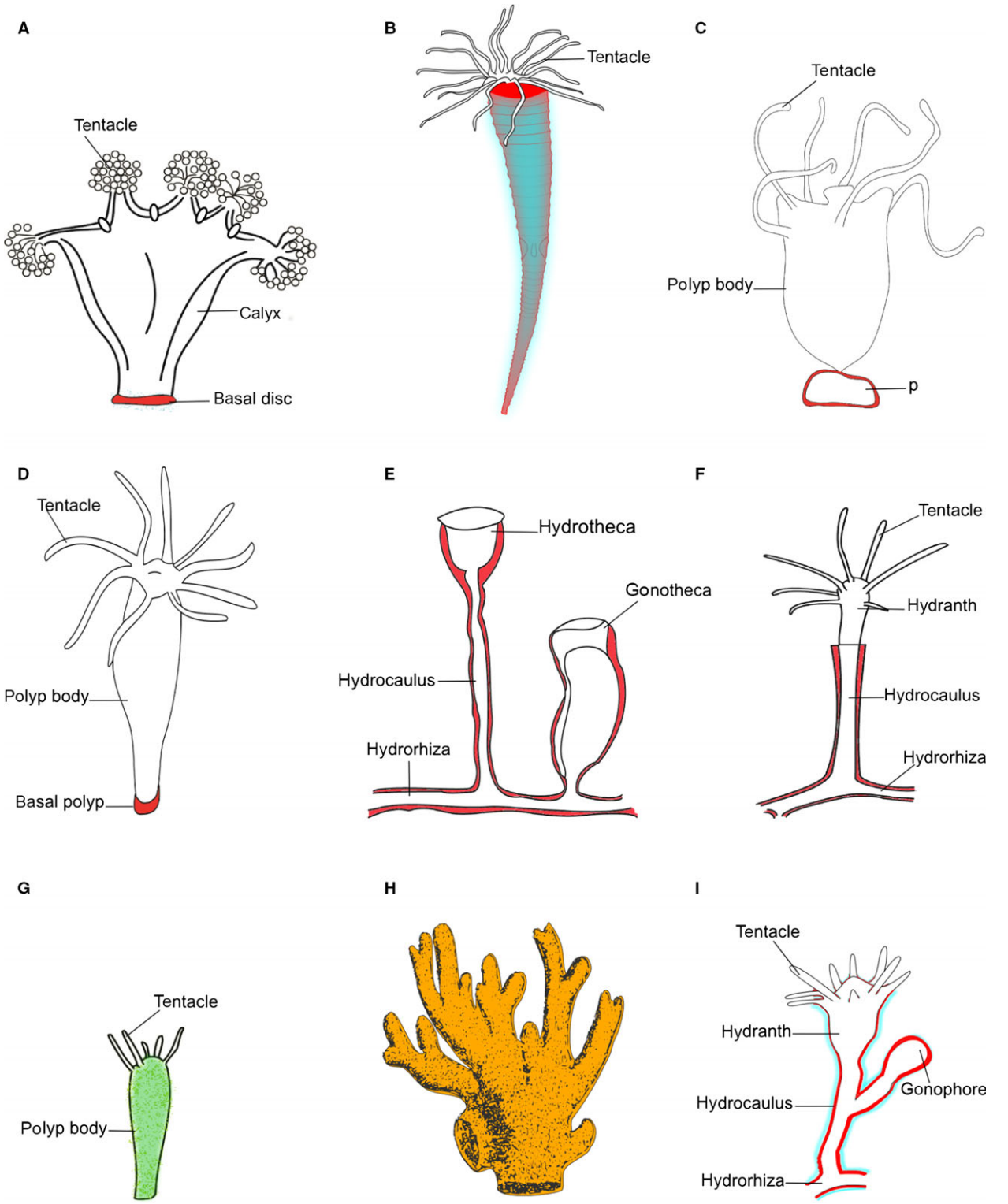

Figure 3. Schematic view of the exoskeleton in extant groups of Medusozoa. A, Staurozoa, Stauromedusae, *Haliclystus*; B, C, Scyphozoa: B, Coronatae, C, Discomedusae; D, Cubozoa, Carybdeida; E–I, Hydrozoa: E, Leptothecata, F, 'Anthoathecata', G, Hydridae, *Hydra vulgaris*, H, *Millepora* sp., I, *Bimeria 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 ('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 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 (=calycoblasts) constitute the extracellular matrix that modulates ion ingression 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, histological, 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

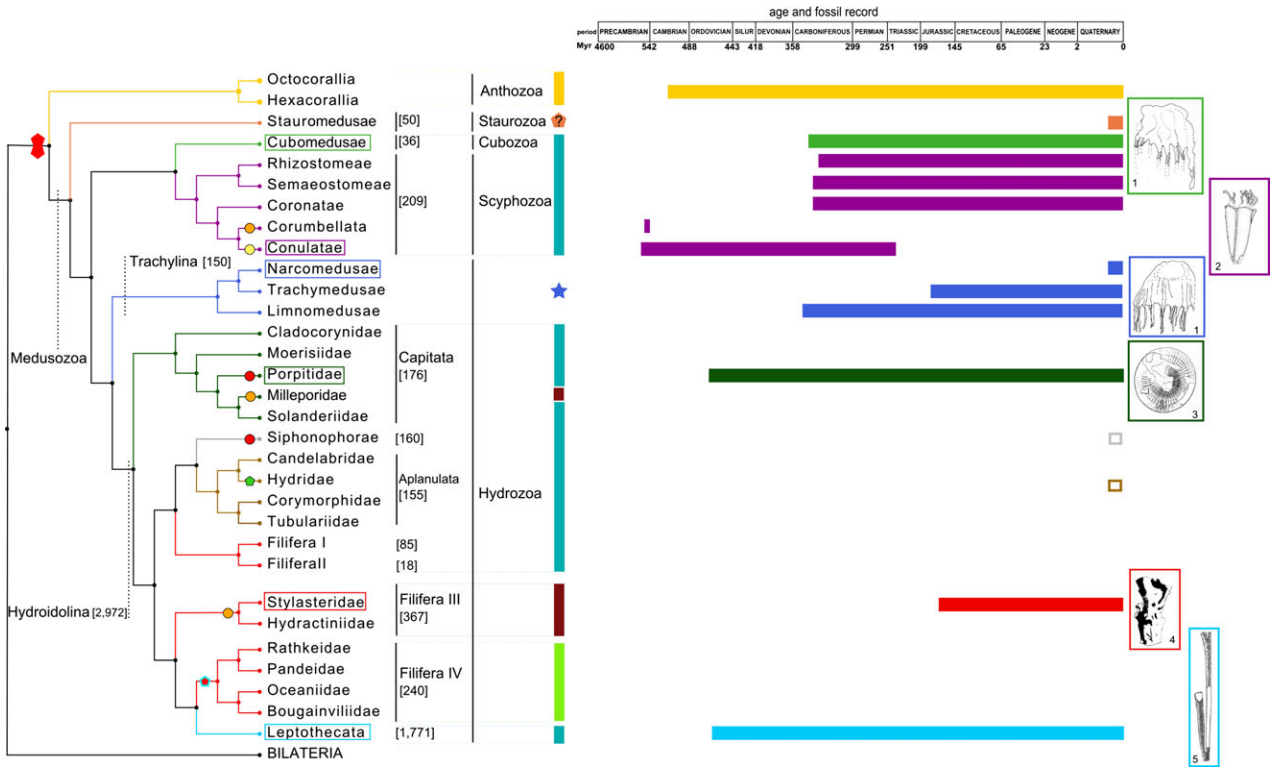

Figure 4. Phylogenetic hypothesis of the exoskeleton in Cnidaria, with fossil Medusozoa, with optimization for different skeleton types: ■ calcareous, ■ corneous, ■ coriaceous; ■ bilayered; ● unknown, ★ without exoskeleton. Skeleton composition: ◆ chitin, ● calcium carbonate, ○ calcium phosphate, ⬟ glycosaminoglycans (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 windermerensis* (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 loculata* (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 (Kruijf, 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.

ACKNOWLEDGEMENTS

We thank colleagues in the Marine Evolution Laboratory, University of São Paulo, for their suggestions,

José E. A. R. Marian for sharing his knowledge on histology, Fabio Rodrigues for his help with chemical conversions, and James J. Roper and two anonymous referees for their valuable suggestions on the text. This study was supported by fellowships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/Conselho Nacional de Desenvolvimento Científico e Tecnológico - Instituto Euvaldo Lodi – IEL National – Brazil (process 6101100-2011) and Programa Nacional de Cooperação Acadêmica, National Council for Scientific and Technological Development (CNPq) (process 490348/2006-8, 304720/2009-7, 562143/2010-6, 563106/2010-7, 477156/2011-8, 305805/2013-4, 301039/2013-5, 445444/2014-2) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (process 2004/09961-4, 2006/58226-0, 2009/02312-4; 2010/52324-6, 2010/06927-0, 2010/50174-7, 2011/50242-5, 2013/50484-4). This is a contribution of the Núcleo de Pesquisa em Biodiversidade Marinha da Universidade de São Paulo (NP-BioMar, USP).

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 Mississippian 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.

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.

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.

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.

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.

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.

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

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). *PLoS One* **9:**e84377.

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.

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.

Chang ES, Neuhof M, Rubinstein ND, Diamant A, Philippe H, Huchon D, Cartwright P. 2015. Genomic insights into the evolutionary origin of Myxozoa within Cnidaria. *Proceedings of the National Academy of Sciences, USA* **112:** 14912–14917.

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 and 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.

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.

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.

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.

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 the Royal Society of London B: Biological Sciences* **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.

Foox J, Siddall ME. 2015. The road to Cnidaria: history of phylogeny of the Myxozoa. *The Journal of Parasitology* **101:** 269–274.

Foox J, Ringuette M, Desser SS, Siddall ME. 2015. In silico hybridization enables transcriptomic illumination of the nature and evolution of Myxozoa. *BMC Genomics* **16:** 840.

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 Academy of Sciences, USA* **107:** 18067–18072.

Fryer G, Stanley Jr GD. 2004. A Silurian porpitoid hydrozoan from Cumbria, England, and a note on porpitoid relationships. *Palaeontology* **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. Körperlich erhaltene Scyphozoen-Reste aus dem Jungpräkambrium 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, Pratt BR, Zhang LY. 2003. Borings in *Cloudina* shells: complex predator–prey dynamics in the terminal Neoproterozoic. *Palaios* **18:** 454–459.

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.

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 medusae (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* **79:** 126–132.

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 calyptoblastic hydroid, *Laomedea flexuosa*. 1 The identification and localization of dopamine in the hydroid. *Tissue and 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* (Mille-

porina, 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 Formacão 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. In: *The Neoproterozoic Paraguay Fold Belt (Brazil): glaciations, iron–manganese formation and biota*. Brazil: Abstracts Corumbá Meeting, Mato Grosso do Sul, 15 pp.

Lenton TM, Watson AJ. 2004. Biotic enhancement of weathering, atmospheric oxygen and carbon dioxide in the Neoproterozoic. *Geophysical Research Letters* **31:** 1–5.

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.

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.

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 of London B: Biological Sciences* **281:**20141202.

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

Mackie GO. 1984. Introduction to the diploblastic 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, Museu de Zoologia da Universidade de São Paulo* **41:** 465–488.

Marques AC, Mergner H, Höinghaus R, Santos CMD, Vervoort W. 2000. Morphological study and taxonomical notes on Eudendriidae (Cnidaria: Hydrozoa: Athecatae/Anthomedusae). *Zoologische Mededelingen Leiden* **74:** 75–118.

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 Rendus 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 Rendus 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.

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.

Murdock GR. 1976. Hydroid skeletons and fluid flow. In: Mackie GO, ed. *Coelenterate ecology and behavior*. London: Plenum Press, 33–40.

Muscente AD, Xiao S. 2015. New occurrences of *Sphenothallus* in the lower Cambrian of South China: implications for its affinities and taphonomic demineralization of shelly fossils. *Palaeogeography, Palaeoclimatology, Palaeoecology* **437:** 141–164.

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.

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.

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 Plant Physiologists* **124:** 495–498.

Rodrigues SC, Simões MG, Leme JM. 2003. Tafonomia comparada dos Conulatae (Cnidaria), Formacão Ponta Grossa, Bacia do Paraná, Estado do Paraná. *Revista Brasileira de Geociéncias* **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.

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, 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.

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. *Revue Suisse de Zoologie* **114:** 195–396.

Schuchert p. 2015. World Hydrozoa Database. Accessed through 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 Genetics* **12:** e13.

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 Palaeontologica Polonica* **25:** 327–343.

Souza CP, Almeida BC, Colwell RR, Rivera IN. 2011. The importance of chitin in the marine environment. *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, USA* **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, USA* **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. *Palaeontology* **35:** 335–358.

Van Iten H. 1992b. Microstructure and growth of the conulariid test: implications for conulariid affinities. *Palaeontology* **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, Kirsimäe K. 2015. Alleged cnidarian *Sphenothallus* in the Late Ordovician of Baltica, its mineral composition and microstructure. *Acta Palaeontologica Polonica* **60:** 1001–1008.

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, eds. *Molecular ecology and evolution: approaches and applications*. Switzerland: Birkhäuser Verlag Basel, 560–577.

Wallace MW, AvS Hood, 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 skeletogenesis: 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.

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 AH. 2010. Eumetazoan fossil in terminal Proterozoic phosphorites? *Proceedings of the National Academy of Sciences, USA* **97:** 13684–13689.

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. *Communicative & Integrative Biology* **4:** 150–158.

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.

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.