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A morpho-histological analysis of the exoskeleton of *Clathrozoella medeae* (Cnidaria, Hydrozoa) reveals insights into the taxonomy of Clathrozoellidae and Hydroidolina.

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The taxonomic complexity of the Clathrozoidae and Clathrozoellidae families, rooted in early 20th-century hydroid descriptions, highlights the need for comprehensive and detailed morphological analyses. This study aimed to elucidate the histology of the polypoid stage of *Clathrozoella medeae*, with a particular focus on its exoskeletal structure. Specimens from the National Museum of Natural History were histologically examined using various staining techniques. The results revealed a three-layered mesoglea, diverse gland cells, and an exoskeleton comprising chitin and structural proteins, with notable differences from other anthoathecate hydroids. These findings have significant implications for the taxonomy and evolutionary relationships of Clathrozoellidae and Hydroidolina, as they underscore the importance of detailed histological data in understanding the unique exoskeletal architecture of *C. medeae*, termed “exoskeleton tube” which differentiates it from other hydroids and provide critical insights into the homology and phylogenetic position of Clathrozoellidae.

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Abstract

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Introduction

The taxonomic history of the families Clathrozoidae Stechow, 1921 and Clathrozoellidae Peña Cantero, Vervoort and Watson, 2003 is convoluted. It began with the description of the new genus and species *Clathrozoon wilsoni* Spencer, 1891, based on material from near Port Phillip Heads, Victoria (Australia). *Spencer* (*1891*) highlighted the unique morphology of the new taxon, comparing it to “Anthoathecata” (viz., Hydractiniidae and Solanderiidae, as “Ceratelladae” and “Hydrocorallinae”) due to the resemblance of the exoskeleton, and to

Leptothecata (*viz.*, Plumulariidae) due to the presence of nematothecae. He placed his new species and new genus in the new family Hydroceratinidae Spencer 1891, based on a "combination of characters, [that] together with the nature of the skeleton, serves to render the Hydroceratinidae distinct from any family of Hydroidea yet known" (*Spencer, 1890: 129*). Although *Spencer* (*1891*) used the term "hydrothecae" in describing the morphology of his new species, he did not establish anthoathecate or leptothecate assignments for Hydroceratinidae, contrary to the observation by *Vervoort & Watson* (*1996*: *119*).

Clathrozoon was not recorded again until *Vanhöffen (1910)* described a second species for the genus, based on material from the Davis Sea (Antarctica, 385 m deep), naming it "*Clathrozoon Drygalskii*" Vanhöffen 1910. Vanhöffen unequivocally placed his new species among the anthoathecates, still within the family Hydroceratinidae (*Vanhöffen, 1910*: 291), despite the generic use of polyp tubes (as "Polypenröhren", in German, *Vanhöffen, 1910*: 294). However, the family name Hydroceratinidae is known to be incorrect because it is not based on an existing genus (*WoRMS, 2024*).

Subsequently, *Stechow (1921)* observed differences between the exoskeleton of *C. wilsoni* and *C. drygalskii* and assigned Vanhöffen's species as the type species of his new genus *Clathrozoella* Stechow 1921. He also noted resemblances between both species with the anthoathecate genera *Nuttingia* and *Hydrodendrium* (currently *Hydractinia*) and the leptothecate genus *Keratosum* (presently *Lafoeina*), suggesting they form the subfamily Clathrozoinae Stechow 1921 or family Clathrozoidae Stechow 1921 (cf. *Stechow, 1921*; see also *Crowell, 1982* on Stechow's comments of regarding the uncertain position of *Keratosum*). Stechow used the term pseudotheca (in German, "pseudotheken") to describe the morphology of the species and retained the new family among the anthoathecates (*Stechow, 1921*).

Hirohito (1967) proposed the new genus *Pseudoclathrozoon* for a species related to *Clathrozoon*, regarding both as leptothecate hydroids. In the same study, Hirohito explicitly removed *Clathrozoella* from Clathrozoidae and considered its affinity with Leptothecata uncertain. This position was subsequently reaffirmed by *Vervoort & Watson* (*1996*), who noted Clathrozoella's uncertain affinity with "Anthoathecata", either with "Filifera" Hydractiniidae or Capitata Solanderiidae. Indeed, *Vervoort* (*2000*: 239) described the presence of unprotected developing female gonophores next to the hydranth body, communicating with the coenosarc of the tubules, as well as "desmones" (referring the desmonemes), both characters expected in an anthoathecate representative (see also *Peña Cantero, Vervoort & Watson, 2003* and *Calder, Choong & McDaniel, 2015*). However, he considered Clathrozoidae, including *Clathrozoon wilsoni*, to possess a "false hydrotheca" (*Vervoort, 2000*: 237).

Clathrozoella remained monospecific until *Peña Cantero, Vervoort & Watson* (*2003*) described three new species, *viz. Clathrozoella abyssalis*, *Clathrozoella bathyalis*, and *Clathrozoella medeae*. Those authors proposed the new family Clathrozoellidae, agreeing with its anthoathecate affinity and following *Stechow* (*1921*) in the use of the term "pseudohydrothecae", affirming it as a structure distinct from Leptothecatae hydrotheca (*Peña Cantero, Vervoort & Watson, 2003*: 282). Meanwhile, Clathrozoidae was retained as a separate

and valid family, still assigned to Leptothecata, including *Clathrozoon wilsoni* Spencer 1891 and *Pseudoclathrozoon cryptolarioides* Hirohito, 1967.

The taxonomic history of Clathrozoellidae highlights the benefits of broader and integrative data in systematics, including gonophore and exoskeletal morphology, cnidome, and sequence data (*Calder, Choong & McDaniel, 2015*). The exoskeleton is a crucial morphological character for Clathrozoellidae and Clathrozoidae. However, the variety of terms used in the studies, such as "hydrotheca" (e.g., *Spencer, 1891*), "polyp tubes" (as "Polypenrohren", *Vanhöffen, 1910*), and "Pseudotheken" or "false hydrotheca" or "pseudohydrothecae" (e.g., *Stechow, 1921*; *Vervoort, 2000; Peña Cantero, Vervoort & Watson, 2003*; respectively), indicates uncertainties in defining homologies and understanding the evolutionary relatinships of these groups.

Interestingly, the "pseudohydrothecae" structure mentioned above differs from the homonym described in other anthoathecate hydroids (cf. *Mendoza-Becerril et al., 2017*). Although the literature includes preliminary histological data on *C. drygalskii* (*Vervoort, 2000*), further histological studies on the tissue organization and chemical affinities of *Clathrozoella*, including the exoskeleton's nature, would improve be our understanding of the group's affinities and exoskeletal architecture among hydroids (*i.e.*, Leptothecata and the non-monophyletic "Anthoathecata"; see *Cartwright et al., 2008*; *Maronna et al., 2016; Mendoza-Becerril et al., 2018*). Unfortunately, this lack of knowledge is not restricted to these taxa – histological studies on hydroids are rare and generally focused on freshwater *Hydra* (e.g., *Parker, 1879*; *Siebert, Anton-Erxleben & Bosch, 2008*), with few studies on Leptothecata (e.g., *Shimabukuro & Marques, 2006*; *Pyataeva & Kosevich, 2008*) and "anthoathecate" non-calcareous polyps (e.g., *Warren, 1907*; *Wineera, 1968, 1972*; *Mendoza-Becerril et al., 2016; 2017*).

This study aims to describe the histology of the polypoid stage, including the exoskeleton, of *Clathrozoella medeae* Peña Cantero, Vervoort, and Watson, 2003. These data will be used to understand the taxonomic implications of exoskeleton's nature and organization for the group and hydroids in general.

Material & Methods

The studied material is part of the National Museum of Natural History collection, Smithsonian Institution, catalog number USNM1003100. Collected on March 13, 1964, in the Antarctic, South Shetland Islands (61°24.9'S, 56°30.1'W), at a depth of 300 m by the Department of Zoology from the University of Southern California, the material consists of a colony of polyps with gonophores attached to a rock, preserved in ethanol.

Fragments of the colony, including polyps and parts of the exoskeleton, were dehydrated and embedded in glycol methacrylate (GMA) resin (Leica Historesin Embedding Kit, Leica Microsystems Nussloch GmbH, Germany). Serial longitudinal and transversal sections (3 μm and 7 μm, respectively) of the exoskeleton were stained with various methods: toluidine blue (TB), hematoxylin and eosin (HE), periodic acid–Schiff (PAS, for identification of polysaccharides—P), alcian blue at pH 2.5 (AB, for identification of glycosaminoglycans—

GAGs), mercuric bromophenol blue, and naphthol yellow S (HgBPB and NYS, respectively, for identification of proteins) (*McManus, 1946*; *Deitch, 1955*; *Mowry, 1963*; *Pearse, 1985*). Staining methods (AB+PAS+H) and general staining procedures and times in GMA resin were combined for histological analyses as described by *Mendoza-Becerril et al.* (*2017*).

We also examined the cnidome using nematocyst terminology following Mariscal (1974). The histological slides produced are deposited in the National Museum of Natural History collection, Smithsonian Institution, under the same catalog number as the studied material.

Results

The analysis of the longitudinal sections of *C. medeae* polyps revealed three morphologically distinct regions (Fig. 1A), *viz.*, (a) the hypostome, characterized by the strongly developed gland cells in the gastrodermal layer; (b) the median body region, containing large vacuolated endodermal cells; and (c) the base of the polyp with gland cells in the epidermal layer.

The epidermal layer consists of muscular epithelial cells with sinuous surfaces and heterogeneous sizes (Fig. 1B). These cells are vacuolated, with some granulated glandular cells (PAS-positive) exhibiting a higher affinity for HgBPB and NYS (Table 1). Gland cells are more prominent at the basal part of the polyp, generally in the epidermis of the median and basal regions (Fig. 1B and 1C). Nematocysts are scarce in the median and basal regions but abundant in the hypostome (Fig. 1D) and tentacles (Fig. 1E). The most common cell type is the epitheliomuscular cell, which is thinner and presents a linear surface in the hypostome region (Fig. 1D). The tentacle epidermis is more uniform, with cubic cells containing a basal nucleus (Fig. 1F).

The mesoglea is acellular, prominent, and located immediately beneath the epidermis, projecting into folds at the base of the gastrodermis (Fig. 1G). The mesoglea was stained with HE, TB, and PAS (Table 1), dividing it into three main parallel layers, defined as msI, msII, and msIII. The msI layer is densely stained with HE and TB and has a strong PAS-positive reaction. This layer is located internally in the fibers (Fig. 1G). The two other mesogleal layers, msII and msIII, are more external and less densely stained (Fig. 1G). In the tentacles and hypostome, only the msII layer is observed (Fig. 1H and 1I), corresponding to a thin median layer.

The gastrodermis consists mainly of epitheliomuscular and gland cells (Fig. 1F). The hypostome contains two types of gland cells, both stained with the PAS procedure (Fig. 1H). One type, located distally in the oral region, stains intensely with AB (Fig. 1J, 1K), suggesting the presence of acidic GAGs. Additionallty, the solid tentacles have a core of vacuolated gastrodermal and some granular cells (Fig. 1H, 1L).

The stem comprises coenosarcal tubes surrounded by an exoskeleton, with long and curved exoskeletal elements, named “exoskeleton tubes”, originating irregularly around the stem (Fig. 2A-C). The coenosarcal epidermis contains vacuolated cells, separated from the gastrodermis by a thin, unstructured layer of mesoglea (Fig. 2D-F), which stained pink with HE (Fig. 2G). Large granulated gland cells, PAS-positive stained with HgBPB and NYS, indicate the

presence of proteins related to exoskeletal secretion by epidermal gland cells (Fig. 2H). In the gastrodermis, these cells have smaller globules (Fig. 2G).

The nematophore is enclosed in a perisarcal tube, formed by a pedicelar structure built by epidermis and gastrodermis, ending in a bundle of heterotrichous microbasic eurytele and microbasic mastigophore nematocysts (Fig. 2I, 2J). Nematocysts are PAS-positive and exhibit a strong affinity for AB dye and moderate affinity for HgBPB and NYS, especially those of the exoskeletal tube (Table 1). Three types of nematocysts have been identified, *viz*., heterotrichous microbasic eurytele (hme), desmoneme (d), and microbasic mastigophore (mm).

Undischarged heterotrichous microbasic euryteles stained with AB+PAS+H have magenta capsules with light purple spines and blue tubules (Fig. 2I, 2J); discharged nematocysts are generally light purple. Heterotrichous microbasic euryteles are present in the epidermis of the nematophore (25.4 x 9.2 μm) (Fig. 2J), tentacles (8.0 x 6.0 μm) (Fig. 2K), and hypostome (8.0 x 4.0 μm) (Fig. 2L), as well as isolated in the coenosarcal epidermis of the exoskeleton tubes (24.0 x 10.0 μm) (Fig. 2M). Desmonemes undischarged capsules, abundant in the apical part of tentacles, measure 4.0 x 2.0 μm, are PAS-positive compared with the Schiff-control, and stain purple with AB+PAS+H (Fig. 2K). Microbasic mastigophores are present in the coenosarc of exoskeleton tubes (53.6 x 14.6 μm) (Figs. 2M, 2N).

The exoskeleton consists of a main stem, from which surface tubes around the polyps arise, as well as nematophores surrounded by small tubes. The exoskeleton is bilayered, with an outer layer (exosarc) juxtaposed with the inner layer (perisarc). The exosarc is thin and irregularly shaped (Fig. 3A-H), extending from the base (18.1 μm thick) of the stem to the nematophore (nematotheca) (0.26 μm thick), composed of GAGs (Table 1) with abundant inorganic and few organic materials (Figs. 3B-D). Detecting the exosarc in some stem regions, such as in the inner wall of the exoskeleton tube (Fig. 3B), is challenging. The perisarc (inner layer), with a strong affinity for PAS (Fig. 3D, E), is in direct contact with the coenosarcal epidermis. The perisarc is homogeneous, extending from the base of the stem (31.81 μm thick) to the nematotheca (22.72 μm thick) (Figs. 3A, 3F). The stem comprises tight tubes of chitin and structural proteins (Table 1) (Figs. 3G, 3H), with a core of coenosarc containing gland cells with an affinity for HgBPB and NYS.

Discussion

The collected data underscores the importance of detailed morpho-histological and histochemical information on the exoskeleton for hypothesizing homology within Hydroidolina, particularly between Clathrozoellidae and other families. These characters are essential for inferring phylogenetic relationships or independently testing higher-level taxonomic proposals derived from molecular sequences. Observations on detailed histological analysis have demonstrated that it has phylogenetic implications in medusozoans (*e.g., Siebert et al., 2009; García-Rodríguez et al., 2023*) as well as other taxonomical data, for instance, morpho-molecular and fluorescence patterns (*Maggioni et al., 2020; Beckmann et al., 2024*).

198 The general tissue and cellular organization of *C. medeae* is similar to that described for
199 other anthoathecates, such as *Parawrightia robusta* (*Warren, 1907*), *Solanderia* spp. (*Wineera,*
200 *1968*), *Coryne eximia* (*Wineera, 1972*), and bougainvilliids (*Mendoza-Becerril et al., 2017*).
201 However, notable features include muscular epithelial cells with sinuous surfaces, the three-layer
202 mesoglea, and exoskeletal structure.
203 The arrangement of the anastomosed coenosarcal tubes resembles that of *Solanderia*
204 *misakinensis* (*Wineera, 1968*). Historically, affinities between Clathrozoidae or Clathrozoellidae
205 and Solanderiidae have been proposed since the original descriptions by *Spencer (1891)*,
206 *Vanhöffen* (*1910*), and more recently by *Vervoort & Watson* (*1996*). Nevertheless, the
207 arrangement of exoskeletal elements of *C. medeae* differs from all other Hydroidolina, including
208 Solanderiidae, by providing support and protection to the entire hydranth. Although *Solanderia*
209 spp. also have a rigid chitinous skeleton, it is arranged as an internal network of longitudinal and
210 transverse connecting fibers (*Wineera, 1968*), unlike the external tubes in *C. medeae*.
211 Additionally, the exosarc of Clathrozoellidae, recognized since early descriptions as a thick layer
212 of foreign bodies of tiny algae and diatoms (*Vervoort & Watson, 1996*), contrasts with the
213 external soft layer of *S. misakinensis* (*Wineera, 1968*), corresponding to the ectoderm. This
214 suggests that the origin of the exosarc in different anthoathecate clades may vary. Furthermore,
215 molecular analysis using the mitochondrial 16S marker corroborated the affinities of *C.*
216 *drygalskii* among "Anthoathecata" and "Filifera", and not Capitata, identifying it as the sister
217 group of Similiclavidae Calder, Choong & McDaniel 2015, within a more inclusive clade also
218 including ten other species of Eudendriidae (*Calder, Choong & McDaniel, 2015*).
219 A brief terminological discussion is necessary to avoid confusion regarding the nature of
220 exoskeletons. Few polypoid stages of Hydroidolina are entirely naked; most possess an
221 exoskeleton. In the non-monophyletic "Anthoathecata", the exoskeleton is present in the
222 hydrorhiza or in both the hydrorhiza and hydrocaulus, enclosing stolons and coenosarc, but the
223 hydranth is usually naked (*cf.*, *Millard, 1975*). However, some anthoathecate taxa have a
224 chitinous perisarc and exosarc composed of acid GAGs covering the colony, sometimes forming
225 a pseudohydrotheca when both layers cover the base of the hydranth (*Mendoza-Becerril et al.,*
226 *2016*; *2017*).
227 The exoskeleton of Leptothecata is formed by a continuous layer of chitin and structural
228 proteins, while some anthoathecates may have an exosarc as an additional layer (*Mendoza-*
229 *Becerril et al., 2017*). Conversely, the exoskeleton of *C. medeae* consists of a mesh of chitin and
230 structural proteins supplemented by a thin exosarc layer, both secreted by the ectoderm. The
231 morphology and histology of this exoskeletal structure do not correspond to the
232 pseudohydrotheca found in other hydroids, such as bougainvilliids, which are formed by a
233 corneus chitin-protein reinforced by a covering exosarc formed of GAGs (*Mendoza-Becerril et*
234 *al., 2016*). Therefore, the term "exoskeleton tube" is more appropriate for the exoskeleton of *C.*
235 *medeae*.
236
237 ## Conclusions

We demonstrated that detailed morpho-histological analysis of the exoskeleton is a useful tool for hypothesizing homology within Hydroidolina, particularly between Clathrozoellidae and other families. The tissue and cellular organization of *C. medeae* shares similarities with other anthoathecates but features unique elements such as muscular epithelial cells with sinuous surfaces, a three-layer mesoglea, and a distinctive exoskeletal structure. The exoskeleton of *C. medeae*, which provides support and protection to the entire hydranth, differs from the internal fiber network of *Solanderia* spp. This suggests that the exosarc may have originated in different anthoathecate clades. Additionally, the exoskeleton of *C. medeae*, consisting of a chitin-protein mesh and a thin exosarc layer, differs from the pseudohydrotheca observed in other hydroids, justifying the term "exoskeleton tube" for its description.

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Figure 1. Morphology and histology of the polyp of *Clathrozoella medeae*. A, Schematic representation of the polyp with three regions: (a) hypostome, (b) middle region, (c) base. B, Epitheliomuscular cells of the epidermis. C, Epidermis of the polyp base. D, Detail of the hypostomal epidermis. E, Epidermis of a tentacle. F, Detail of a tentacular epidermis. G, Detail of the three layers of mesoglea. H, Mesoglea exhibiting PAS-positivity. I, Mesoglea in the tentacles. J, Detail of the gastrodermis featuring gland cell type gcIII. K, Gastrodermis of the hypostome showing gland cell types gcI, gcII, and gcIII. L, Detail of a tentacular gastrodermis. Black arrowhead: gcI; blue arrowhead: gcII; orange arrowhead: gcIII; white arrowhead: nematocysts. Abbreviations: ep, epidermis; gc, gland cell; gt, gastrodermis; ms, mesoglea; msI, msII, msIII, three layers of mesoglea; t, tentacle. Scale bars: B, D, E, G, I—10 µm; C—50 µm; F, K—200 µm; H—1.0 mm; J, L—500 µm. Stain: B, D-G, I—HE; C, H, L—PAS; J—AB; K—AB+PAS+H.

Figure 2. Perisarc and coenosarc of the tubes of the stem and nematocysts. A-C, General schematic representation with details of the stem. A, General overview of the stem. B, Transversal section. C, Longitudinal section. D, Transversal section of skeletal tubes. E-F, Detailed views of panel D. G, Layers of coenosarc. H, Detail of the epidermis featuring type gcI gland cells. I, Coenosarc of the nematophore. J, Heterotrichous microbasic eurytele and microbasic mastigophores nematocysts in the nematophore. K, Desmoneme and heterotrichous microbasic eurytele nematocysts in the tentacle. L, Hypostome with heterotrichous microbasic eurytele nematocysts. M-N, coenosarc of the exoskeleton tubes with heterotrichous microbasic eurytele (only in M) and microbasic mastigophore nematocysts. Black arrowhead: gcI; white arrowhead: nematocysts. Abbreviations: cn, coenosarc; d, desmoneme nematocyst; ep, epidermis; et, exoskeleton tubes; gc, gland cell; gt, gastrodermis; hme, heterotrichous microbasic eurytele nematocyst; mm, microbasic mastigophore nematocyst; ms, mesoglea; n, nematotheca; p, polyp. Scale bars: D, H—500 µm; E-G—200 µm; I—25 µm; J—25 µm; K-N—10 µm. Stain: D-F, M—TB; G-I—AB+PAS+H; J—PAS; K-L, N—HE.

Figure 3. Exoskeleton. A, Schematic representation of the exoskeleton: (a) transversal view section, (b) longitudinal view section, (c) nematotheca. B, Layers of the exoskeleton of the stem. C, Detail of the exosarc. D, Detail of the exosarcal and perisarcal layers. E, Perisarc and type gcI gland cells. F, Detail of the nematothecal perisarc and exoskeleton tube. G-H, Perisarc and type gcI gland cells. Black arrowhead: gcI; white arrowhead: nematocysts. Abbreviations: cn, coenosarc; et, tubes of exoskeleton; ex, exosarc; gc, gland cell; n, nematotheca; pe, perisarc; st, stem. Scale equals: B, C, F—100 µm; D, E, G, H—50 µm. Stain: B, F—TB; C—AB; D—AB+PAS+H; E—PAS; G—HgBPB; H—NYS.

Figure 1

Morphology and histology of the polyp of *Clathrozoella medeae*

A, Schematic representation of the polyp with three regions: (a) hypostome, (b) middle region, (c) base. B, Epitheliomuscular cells of the epidermis. C, Epidermis of the polyp base. D, Detail of the hypostomal epidermis. E, Epidermis of a tentacle. F, Detail of a tentacular epidermis. G, Detail of the three layers of mesoglea. H, Mesoglea exhibiting PAS-positivity. I, Mesoglea in the tentacles. J, Detail of the gastrodermis featuring gland cell type gcIII. K, Gastrodermis of the hypostome showing gland cell types gcI, gcII, and gcIII. L, Detail of a tentacular gastrodermis. Black arrowhead: gcI; blue arrowhead: gcII; orange arrowhead: gcIII; white arrowhead: nematocysts. Abbreviations: ep, epidermis; gc, gland cell; gt, gastrodermis; ms, mesoglea; msI, msII, msIII, three layers of mesoglea; t, tentacle. Scale bars: B, D, E, G, I—10 µm; C—50 µm; F, K—200 µm; H—1.0 mm; J, L—500 µm. Stain: B, D-G, I—HE; C, H, L—PAS; J—AB; K—AB+PAS+H.

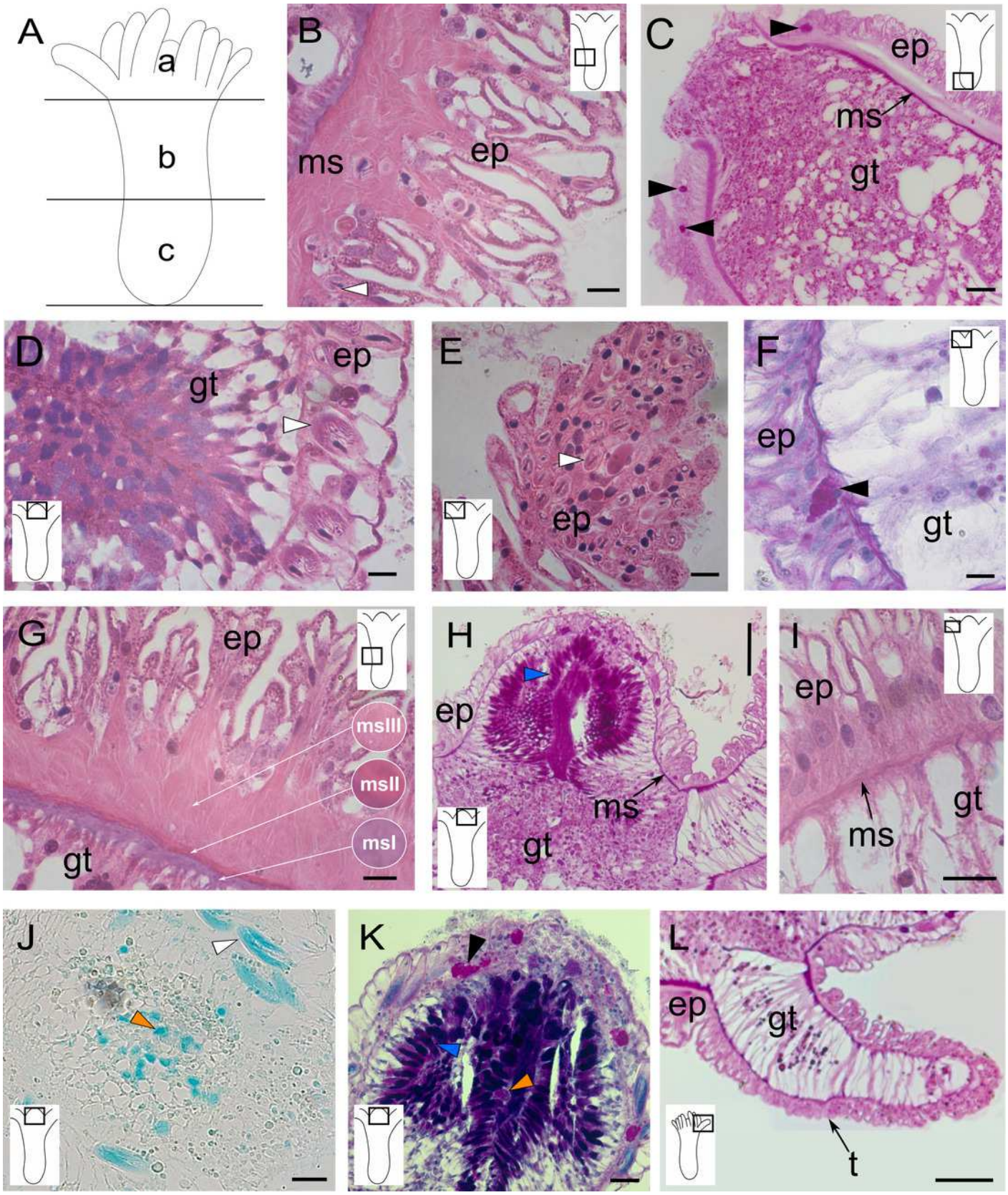
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Figure 2

Perisarc and coenosarc of the tubes of the stem and nematocysts

A-C, General schematic representation with details of the stem. A, General overview of the stem. B, Transversal section. C, Longitudinal section. D, Transversal section of skeletal tubes. E-F, Detailed views of panel D. G, Layers of coenosarc. H, Detail of the epidermis featuring type gcI gland cells. I, Coenosarc of the nematophore. J, Heterotrichous microbasic eurytele and microbasic mastigophores nematocysts in the nematophore. K, Desmoneme and heterotrichous microbasic eurytele nematocysts in the tentacle. L, Hypostome with heterotrichous microbasic eurytele nematocysts. M-N, coenosarc of the exoskeleton tubes with heterotrichous microbasic eurytele (only in M) and microbasic mastigophore nematocysts. Black arrowhead: gcI; white arrowhead: nematocysts. Abbreviations: cn, coenosarc; d, desmoneme nematocyst; ep, epidermis; et, exoskeleton tubes; gc, gland cell; gt, gastrodermis; hme, heterotrichous microbasic eurytele nematocyst; mm, microbasic mastigophore nematocyst; ms, mesoglea; n, nematotheca; p, polyp. Scale bars: D, H—500 µm; E-G—200 µm; I—25 µm; J—25 µm; K-N—10 µm. Stain: D-F, M—TB; G-I—AB+PAS+H; J—PAS; K-L, N—HE.

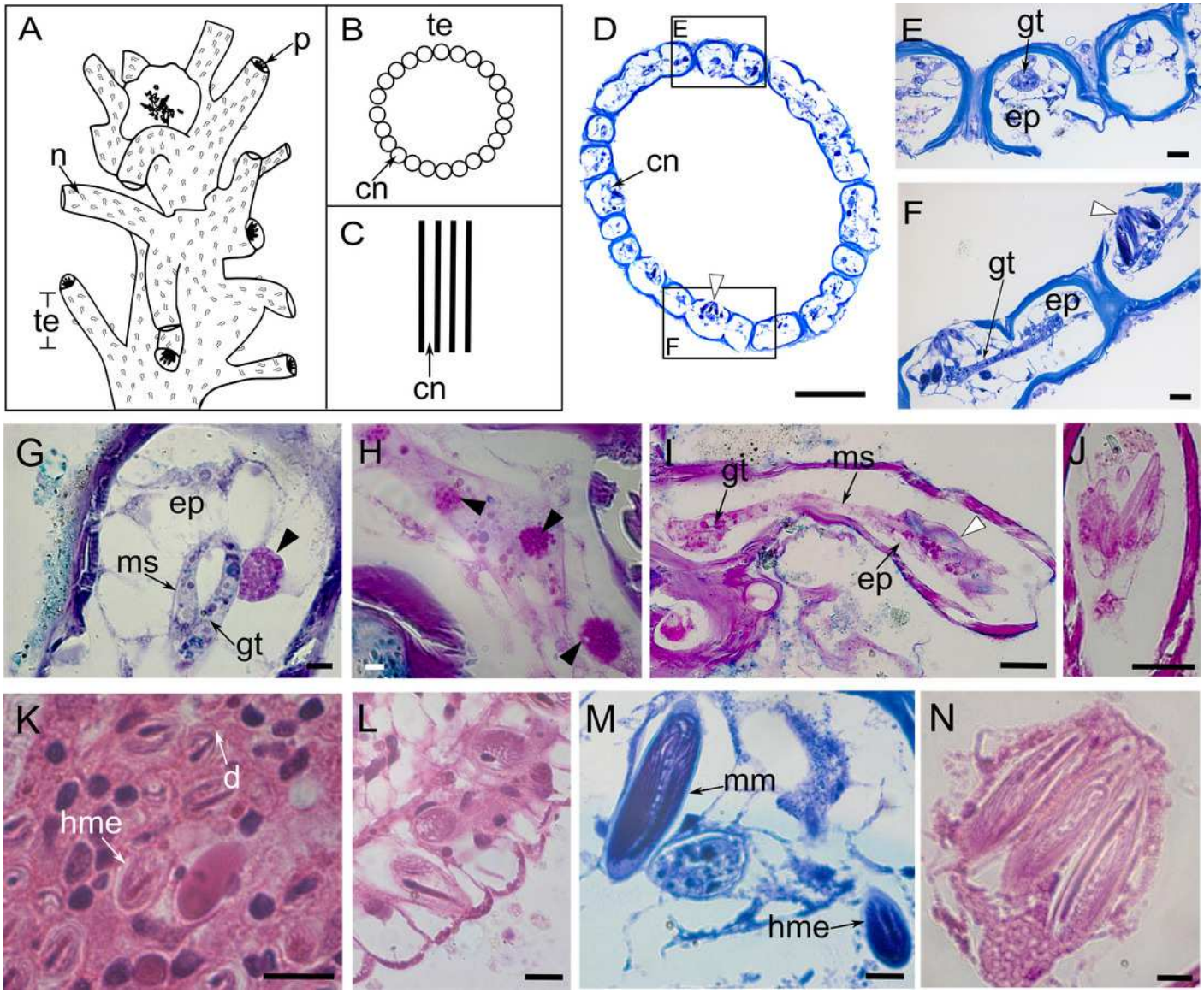
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Figure 3

Exoskeleton.

A, Schematic representation of the exoskeleton: (a) transversal view section, (b) longitudinal view section, (c) nematotheca. B, Layers of the exoskeleton of the stem. C, Detail of the exosarc. D, Detail of the exosarcal and perisarcal layers. E, Perisarc and type gcI gland cells. F, Detail of the nematothecal perisarc and exoskeleton tube. G-H, Perisarc and type gcI gland cells. Black arrowhead: gcI; white arrowhead: nematocysts. Abbreviations: cn, coenosarc; et, tubes of exoskeleton; ex, exosarc; gc, gland cell; n, nematotheca; pe, perisarc; st, stem. Scale equals: B, C, F—100 µm; D, E, G, H—50 µm. Stain: B, F—TB; C—AB; D—AB+PAS+H; E—PAS; G—HgBPB; H—NYS.

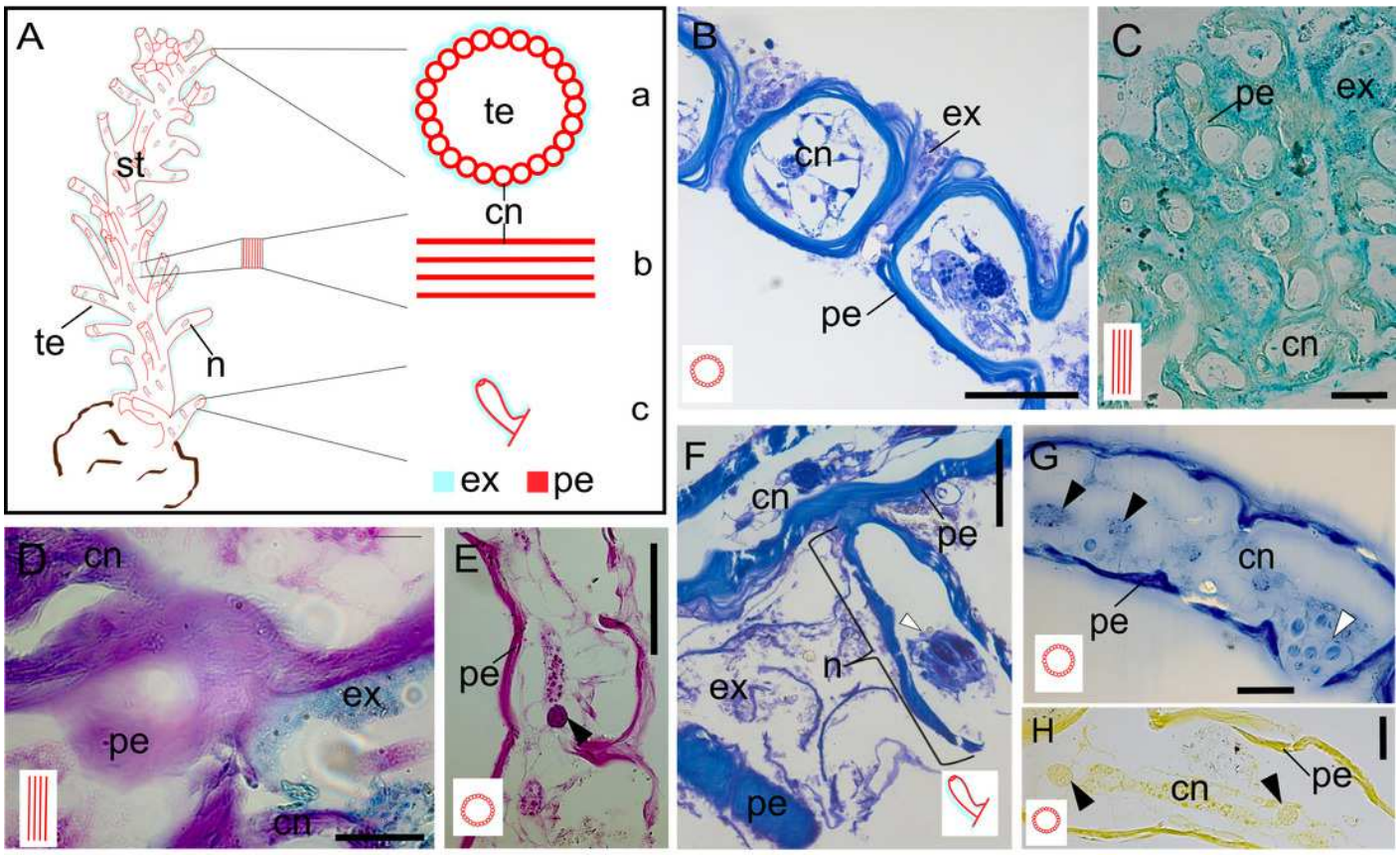

Table 1(on next page)

Reactions of the polyp and exoskeletal layers of to specific staining *Clathrozoella medeae* Peña Cantero, Vervoort & Watson, 2003.

-, not stained; <+ nearly unstained, + weakly stained; ++, moderately stained; +++, intensely stained. TB, Toluidina blue; Stain: HE, hematoxylin and eosin; Schiff, Schiff reagent applied without any pretreatment; PAS, Periodic Acid-Schiff; AB, Alcian blue pH 2.5; HgBPB, mercury-bromophenol blue; NYS, Naphtol yellow S.

Table 1 Reactions of the polyp and exoskeletal layers of to specific staining *Clathrozoella medeae* Peña Cantero, Vervoort & Watson, 2003

Structure	TB	HE	Schiff	PAS	AB	HgBPB	NYS
Polyp							
Epidermis	++ blue	+++ pink	<+ magenta	++ magenta	-	+ blue	++ yellow
Mesoglea	++ purple	+ purple	<+ magenta	+++ magenta	<+ alcian blue	+ blue	+ yellow
Gastrodermis	++ blue	++ purple	-	+++ magenta	+ alcian blue	++ blue	+++ yellow
Cnidome	+++ purple	+++ purple	<+ magenta	+++ purple	+++ alcian blue	+++ blue	++ yellow
Exoskeletal tube							
Epidermis	++ blue	+++ pink	-	+ magenta	-	+ blue	+ yellow
Mesoglea	+++ blue	+ pink	-	++ magenta	-	-	-
Gastrodermis	+++ purple	++ pink	-	+++ magenta	-	++ blue	++ yellow
Cnidome	+++ purple	++ purple	<+ magenta	++ magenta	+++ alcian blue	+++ blue	++ yellow
Exoskeleton							
Inner layer (=perisarc)	+++ blue	+++ pink	<+ magenta	+++ magenta	<+ alcian blue	+++ blue	+ yellow
Outer layer (=exosarc)	+++ purple	+ pink	-	++ magenta	+++ alcian blue	-	<+ yellow

-, not stained; <+ nearly unstained, + weakly stained; ++, moderately stained; +++, intensely stained. TB, Toluidina blue; ~~Stain:~~ HE, hematoxylin and eosin; Schiff, Schiff reagent applied without any pretreatment; PAS, Periodic Acid-Schiff; AB, Alcian blue pH 2.5; HgBPB, mercury-bromophenol blue; NYS, Naphtol yellow S.