

Internal Anatomy of *Haliclystus antarcticus* (Cnidaria, Staurozoa) with a Discussion on Histological Features Used in Staurozoan Taxonomy

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ABSTRACT Stauromedusae have relatively few macromorphological characters, making both their taxonomy and identification difficult. For this reason, histological characters are also employed in the taxonomy of the group. This study presents a detailed description of the histomorphology of *Haliclystus antarcticus* Pfeffer, 1889 (Cnidaria, Staurozoa). We make new observations for the species and for the class, and address functional, taxonomical, and evolutionary aspects of staurozoan histo-anatomy. A complete reconstruction of *H. antarcticus* body plan is used to guide a more detailed observation, based on light microscopy, of structures rarely cited in the literature, such as the intertentacular lobules, the ostia between adjacent per-radial pockets, and the male and female gonadal vesicles. Two possible regions of nematocyst formation are hypothesized and discussed. We also provide a review of the current use of histological characters in the taxonomy of the group. Understanding the body plan of stauromedusae is a challenge, because each single individual presents characters found in medusae and in polyps of other medusozoans. Comprehensive histological descriptions are important to establish relations of homology within Staurozoa and Cnidaria, providing crucial data on their evolution. *J. Morphol.* 000:000–000, 2013. © 2013 Wiley Periodicals, Inc.

KEY WORDS: Stauromedusae; Medusozoa; body plan; evolution; nematogenesis

INTRODUCTION

The Staurozoa (Marques and Collins, 2004) is a small clade of the phylum Cnidaria, encompassing about 50 species of the so-called stalked jellyfishes (Collins et al. 2006; Daly et al., 2007). Species of this clade do not produce pelagic medusae which are a synapomorphy of the clade Scyphozoa+Cubozoa+Hydrozoa (Collins et al., 2006; Van Iten et al., 2006). Instead, the stauropolyp apically metamorphoses into an adult stauromedusa, which lives attached to the substrate by a peduncle (Mayer, 1910). The apical half of the metamorphosed stauromedusa bears similar characters to those present in adult medusae of scyphozoans and cubozoans, e.g., hollow structures of tentacular origin (rhopalioids/rhopalia), circular coronal muscles,

gastric filaments, and gonads (Collins, 2002; Collins et al., 2006). The aboral region, on the other hand, retains polypoid characters such as gastric septa associated with four longitudinal muscles (Collins, 2002; Stangl et al., 2002). Therefore, the particular life cycle of stauromedusae is tightly connected to their unique anatomy, in which polypoid and medusoid characters are present in a single individual (Collins et al., 2006), making detailed knowledge of this group essential for the correct understanding of life cycle evolution in Cnidaria.

Stauromedusae possess relatively few macromorphological characters useful for taxonomy (Hirano, 1997). Moreover, most of these characters exhibit significant intraspecific variation (Miranda et al., 2009), making identifications of staurozoan species difficult. For this reason, internal/histological characters are additionally employed to characterize species. For instance, the presence of four intramesogleal muscles of the peduncle (e.g., present in *Haliclystus*, but absent in *Kishinouyea*; Uchida, 1929), the number of chambers in the peduncle (e.g., four in *Haliclystus* and one in *Lucernaria*; Kramp, 1961), and the presence of the claustrum (a tissue that transversely divides the

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four gastric pockets, present in the suborder Cleistocarpida and absent in the suborder Eleuthero-
carpida; Clark, 1863; Gross, 1900; Mayer, 1910) are used to distinguish different species or higher taxa within Staurozoa.

Although internal anatomy is used for the taxonomy of Staurozoa and helpful for understanding the group's relationships with other medusozoans, histological accounts for staurozoan species are rare. A few studies employ general histological characters (e.g., sections of the peduncle) in the taxonomy of the class (e.g., Larson, 1980, 1988; Larson and Fautin, 1989; Collins and Daly, 2005), but there are examples in which even the general characters adopted were misunderstood or misinterpreted, e.g., the number of chambers in the peduncle of *Kishinouyea hawaiiensis* is actually one chamber subdivided into four, and not four chambers as described by Edmondson (1930). Similarly, efforts to understand the body plan evolution of stauromedusae through comparative analysis are scarce (Berrill, 1963; Thiel, 1966). Nevertheless, some detailed histological descriptions exist (Clark, 1878; Wietrzykowski, 1912; Uchida, 1929; Uchida and Hanaoka, 1933, 1934; Ling, 1939), and these studies provide observations that have proven important for understanding the evolution of characters in the group. Collins and Daly (2005), for example, suggested that the presence/absence of a claustrum and the taxonomic division into Cleistocarpida and Eleuthero-
carpida, respectively, was not consistent with a preliminary molecular-based phylogenetic hypothesis for the group.

Miranda et al. (2009) redescribed *Haliclystus antarcticus* Pfeffer, 1889, providing data on intraspecific variation and highlighting the use of some internal characters in the taxonomy of the genus *Haliclystus*. For instance, intertentacular lobules have been used to distinguish some *Haliclystus* species (Hirano, 1997), but have not broadly been verified across different species, or elsewhere in the class, highlighting the importance of additional histological studies. The aims of the present study are to augment the description of *Haliclystus antarcticus*, provide new data on the histo-morphology of *H. antarcticus* and Staurozoa more generally, and review the current usage of histological features as taxonomic characters of the group.

MATERIALS AND METHODS

Specimens of *Haliclystus antarcticus* were collected manually during low tide on two beaches in Admiralty Bay, King George Island, Antarctic Peninsula: 1) Pieter Lenie, in Copacabana (North American Refuge, 62°10'S, 58°26'W) and 2) Shag Point, in Arctowski (Polish Station, 62°10'S, 58°31'W), during the XXV Brazilian Antarctic Program (February–March 2007). Materials were fixed directly in 4% formaldehyde solution with seawater. Routine histological procedures for cnidarians were carried out (modified from Humanson, 1962; Mahoney, 1966:

cleaning in distilled water, dehydration in a graded ethanol series (70–100%), clearing in xylene (three steps), infiltration and embedding in paraffin, sectioning with a microtome, clearing in xylene (two steps), rehydration in a graded ethanol series (100–70%), cleaning in distilled water, and staining) for six specimens of different sizes, with immature and mature gonads, both for transverse and longitudinal sections (7.0–10.0 µm thick), stained with the multistep trichrome acid dyes: acid fuchsin (15') (Mallory; Humanson, 1962:147), chromotrope 2R-aniline blue (5') (Masson trichrome stain; modified from Humanson, 1962:158) and acetic aniline blue (3') (Mallory; modified from Humanson, 1962:231), intercalated with distilled water to improve the contrast between structures. The body plan of *H. antarcticus* was studied and photographed using the microscope Axio Imager M2, Carl Zeiss (Germany). The main terminology of morphological features followed Uchida (1929) and Uchida and Hanaoka (1934). The slides are deposited in the collection of the Laboratory of Marine Evolution of the Institute of Biosciences, University of São Paulo (boxes LEM_01-08, five specimens), Brazil, and in the collection of the National Museum of Natural History, Smithsonian Institution (USNM 1207980, one specimen), and are available for loan.

RESULTS

General Body Anatomy

Aboral region formed by pedal disk of peduncle (epidermis) with increased surface area by having many invaginations (Figs. 1–3A). Peduncle with four perradial chambers (delimited by gastrodermis), alternating with four interradial longitudinal muscle bands embedded in mesoglea (Figs. 2A,B, 4A), circular in cross section; chambers and muscles developed throughout peduncle except at pedal disk. Perradial chambers with some invaginations basally, mainly at central region, becoming more oval and smooth toward oral region; perradial chambers fusing at junction of peduncle and calyx (Fig. 2A–C), connecting with gastrovascular cavity by a median aperture (Fig. 2B). Gastrodermis envelops muscle bands at basal region of calyx, forming four interradial gastric septa: a thin layer of mesoglea surrounded by two layers of gastrodermis (Figs. 2E, 5A,B).

Four infundibula (peristomal pits) funnel-shaped with blind end, delimited by epidermis (Fig. 6), deeply developed up to base of calyx, widening orally, with broad apertures on subumbrella (Figs. 1B, 2F–J). At base of infundibulum, muscle becomes compressed and flattened, then becomes V-shaped apically (Figs. 2F,G, 6A–C).

Adjacent septal gastrodermis fuses forming four perradial regions that divide the gastrovascular cavity (Fig. 2I). Fusion of septal gastrodermis forms basal region of manubrium and perradial pockets (i.e., central part of gastrodermis of each septum join forming four-sided manubrial gastrodermis while lateral parts of adjacent septa join forming perradial pockets; Fig. 2I). Similarly, the infundibular epidermis also progressively fuses apically (Fig. 2J): central part of each infundibular epidermis becomes manubrial epidermis; epidermis of adjacent infundibula forms epidermis of perradial pockets (Fig. 2J,K). In other words, each

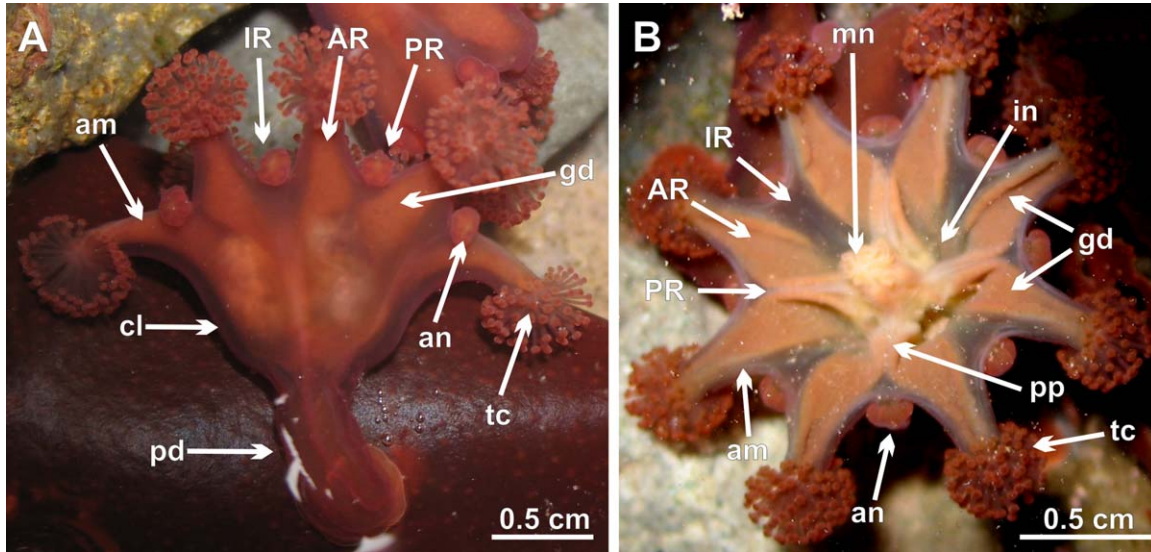


Fig. 1. **A:** Lateral view of living specimen of *Haliclystus antarcticus*, attached to the red alga *Iridaea cordata*. **B:** Oral view of living specimen of *H. antarcticus*. Abbreviations: am, arm; an, anchor; AR, adradial; cl, calyx; gd, gonad; in, infundibulum; IR, interradial; mn, manubrium; pd, peduncle; pp, perradial pocket; PR, perradial; tc, tentacles. Pictures from AC Morandini.

perradial pocket is formed by fusion of gastrodermis and epidermis of adjacent septa, and the manubrium is formed by fusion of all four septa. Four perradial gastric pockets laterally separated from each other by septa (Fig. 2K), only directly connected by means of a small ostium at margin of calyx (Figs. 2L); each perradial pocket connected to gastrovascular cavity (Fig. 2H–M). Gastrovascular cavity without claustrum.

Anchors (or rhopaloids) hollow, with a hollow stem, formed by evagination of body surface (Figs. 2K–N, 7–9). Eight large anchors organized as four perradial and four interradial, each anchor located between adjacent arms at calyx margin (Figs. 1, 2K–N). Gastrodermis of perradial anchors directly connected to gastrodermis of perradial pockets through stem (Fig. 7). At interradial regions, presence of septa could prevent direct connection of gastrodermis of interradial anchors to that of calyx. However, at interradial junctions, septa are detached from gastrodermis and small ostia connect adjacent perradial pockets along margin of calyx (Fig. 8C–I), allowing for gastrodermis of anchors to be contiguous with that of calyx (Fig. 8H,I).

Each perradial pocket extending throughout calyx margin, aborally continuing into two adradial arms (Fig. 2N–Q) and respective tentacular clusters (Fig. 1). Subumbrellar epidermis (continuous with epidermis of infundibula) marginally merges with exumbrellar epidermis, dividing perradial pockets at origin of arms (Fig. 2P,Q). Gonads (Fig. 1) formed by numerous vesicles (follicles), which are evaginations of gastrodermal layer of septa, perradial pockets and arms

(Figs. 2H–Q, 10); vesicles of same perradial pocket formed by gastrodermis of two different interradial septa (two adjacent septa). Gastric filaments formed by evagination of gastrodermal layer of septa at base of manubrium, concentrated in the perradial (Figs. 2H, 11).

Large interradial muscle bundles located at septa between adjacent perradial pockets branch into smaller and diffuse muscle bundles, going toward arms and respective secondary tentacles. Eight sections of coronal muscles at calyx margin, each between adjacent arms (Fig. 12).

Cell and Tissue Description

Pedal disk and peduncle. Aborally, epidermis of pedal disk comprising high and thick epithelial cells, characterized by glandular cells highly granulated, stained with fuchsin (red); vacuolated glandular cells and supporting cells less common (Fig. 3A–D). In distal region of peduncle (more orally) epidermis thinner and with sinuous surface because of differentiated size of its cells; supporting cells become more conspicuous, vacuolated and granulated glandular cells rarer, few nematocysts present (Fig. 4).

Gastrodermis of peduncle chambers mainly comprising vacuolated cells (external, juxtaposed to mesoglea) and granulated glandular cells (juxtaposed to chamber; Fig. 4G). Granulated glandular cells organized in two differing layers: one intermediate layer densely stained with fuchsin, one internal (closer to chamber) stained with aniline blue (blue; Fig. 4C,G). Gastrodermis almost uniform throughout peduncle, but thinner at aboral end of peduncle at level in which four chambers are not

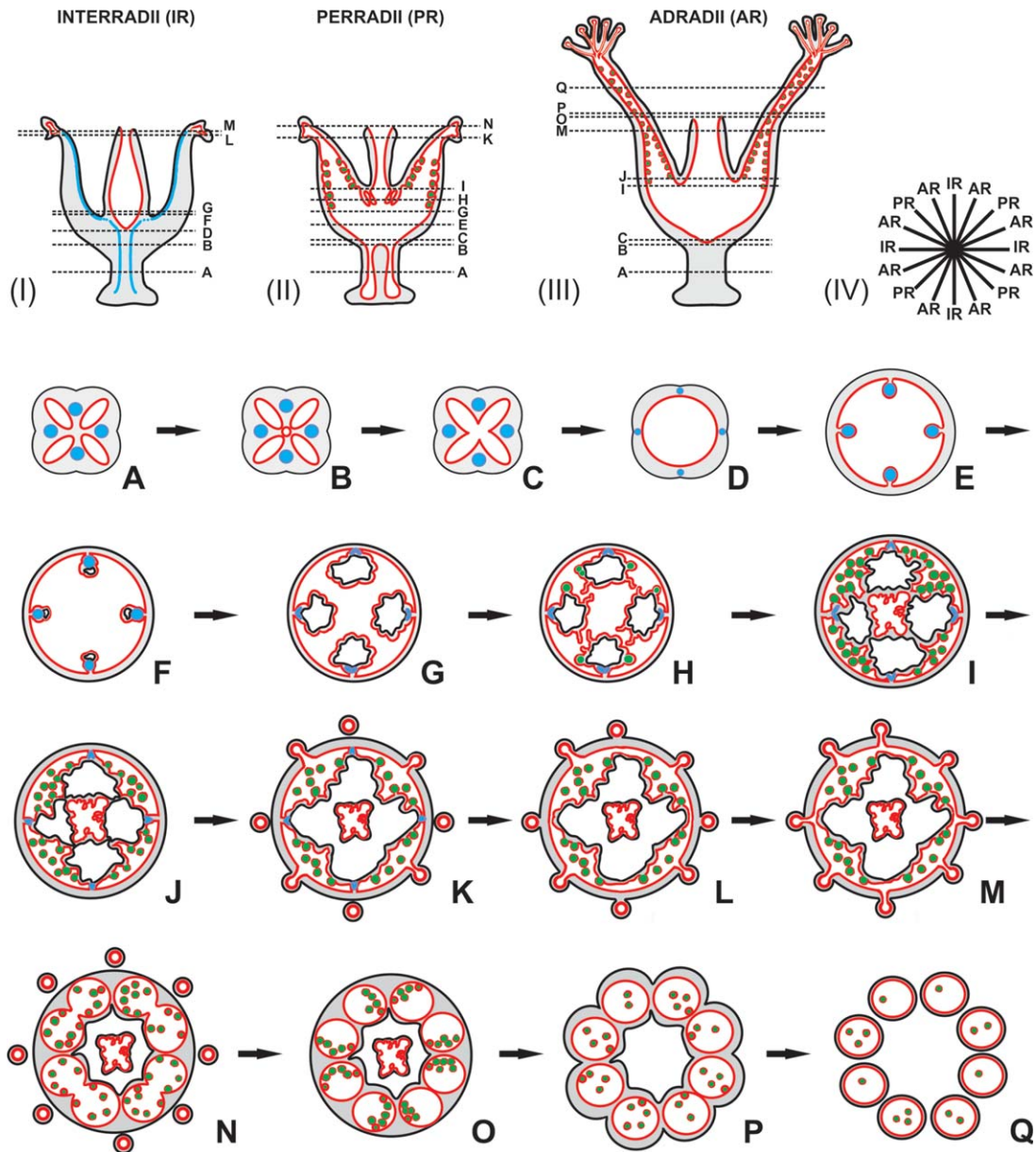


Fig. 2. Internal anatomy of *Haliclystus antarcticus*. I–III: Longitudinal sections of the body, showing the sites where the cross sections were made. I: interradii; II: perradii; III: adradial; IV: orientation of the cross sections (A–Q). A–Q: Cross sections of the body, from the basal region (A) to the apical region (Q). A: peduncle with four perradial chambers and four longitudinal interradial muscles; B: four chambers start to fuse, and connect to a median aperture; C: apical region of peduncle, with the four chambers fused; D: connection region between calyx and peduncle; E: basal region of calyx with gastrodermis enveloping the interradial longitudinal muscle, forming four septa; F: basal region of four infundibula, within septa; G: enlargement of infundibula, which divides interradial longitudinal muscles into two regions in each septum; H: evagination of gastrodermis forming gastric filaments and gonads; I: gastrodermis of adjacent septa fuse, forming gastrodermis of manubrium and perradial pockets; J: epidermis of adjacent septa start to fuse; K: epidermis of adjacent septa fuse, completing the formation of manubrium and perradial pockets, and anchors are visible (perradial anchors connected to perradial pockets, and interradial anchors not connected, because of septa); L: perradial anchors connected to perradial pockets and ostia at interradii, connecting two adjacent perradial pockets; M: perradial and interradial anchors connected to gastrodermis of calyx, and adjacent perradial pockets connected by ostia; N: division of perradial pockets into eight regions, toward the eight arms, and anchors are visible; O–Q: perradial pockets fully divided toward the eight arms, fusion of subumbrellar epidermis with exumbrellar epidermis at the arms region. Legend: epidermis- black; gastrodermis- red; mesoglea- gray; longitudinal interradial muscles- blue; vesicles of gonads- green.

fully developed (Fig. 3E,F), where they contain only a few granulated and vacuolated cells. Interradial longitudinal musculature composed by myoepithelial cells stained with fuchsin, interspersed by thin

layers of mesoglea stained with aniline blue (Fig. 4A,C,F). Bundles of myoepithelial muscle fibers have reticulated appearance because of presence of empty spaces among them (Fig. 4C,F).

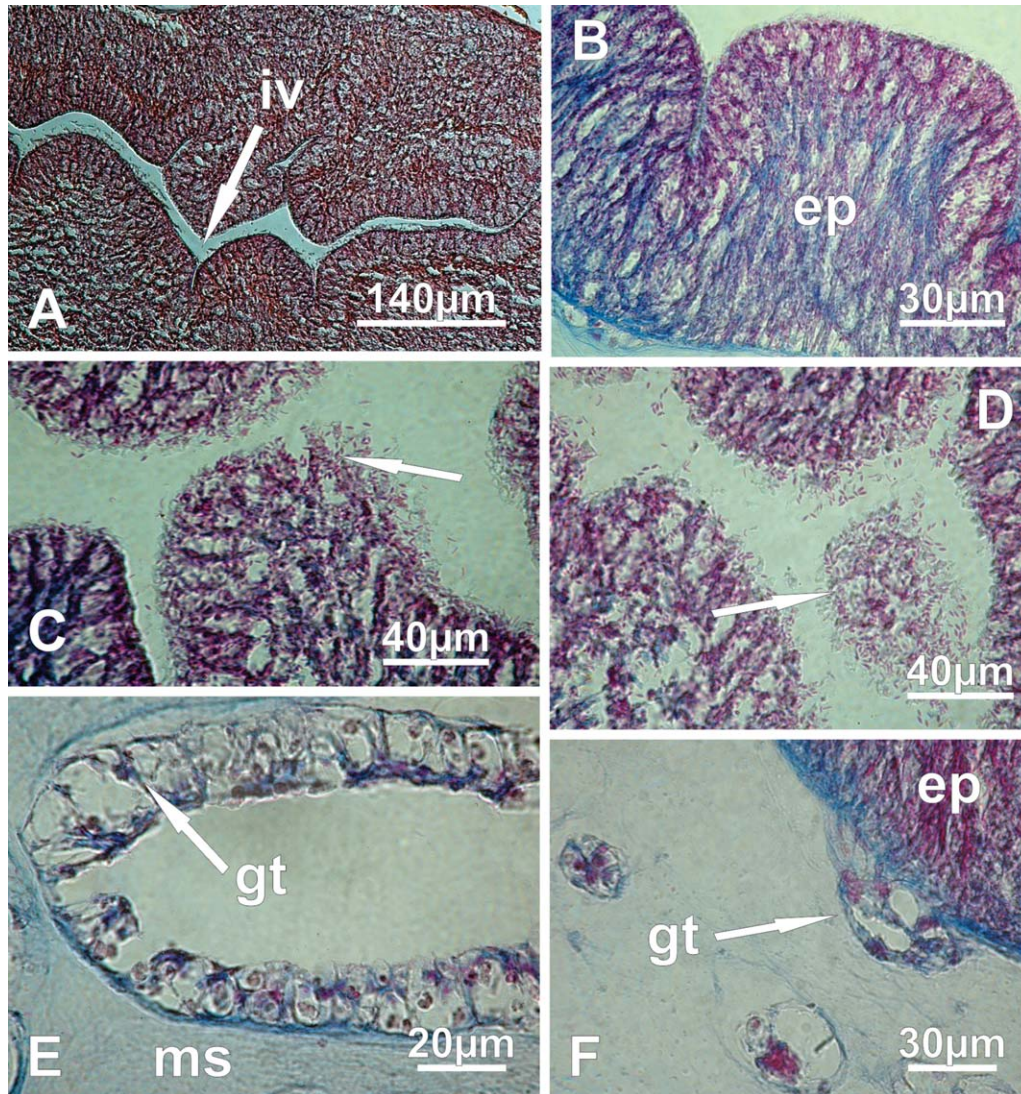


Fig. 3. Basal region of peduncle; A–F: cross sections. A: invagination in pedal disk (indicated by an arrow); B: tall epidermis of pedal disk; C–D: granules produced by glandular cells at pedal disk (indicated by an arrow); E: gastrodermis lining perradial chamber; F: gastrodermis at the region close to mesoglea and epidermis. Abbreviations: ep, epidermis; gt, gastrodermis; iv, invagination; ms, mesoglea.

Mesoglea strongly stained at central portion of peduncle (Fig. 4A), peripheral region divided into two main layers: one densely stained region, located internally close to perradial chambers, one less densely stained with aniline blue region, more external (Fig. 4A). Another strongly stained mesogleal layer juxtaposed to epidermis only visible in higher magnification (Fig. 4B).

Calyx and infundibula. Epidermis of aboral region of calyx similar to that of upper region of peduncle, although the number of nematocysts progressively increases (Fig. 5). Septal mesoglea strongly stained with aniline blue, respective gastrodermis vacuolated, muscles embedded in septa surrounded by layer of dense mesoglea (Fig. 5A,B). Septal mesoglea becomes thinner orally (Fig. 6A–C). Epidermis of infundibula divided into two

main regions: lateral side, facing perradii, composed by thicker cells including many granulated glandular cells and nematocysts; interradii side facing muscles and central region comprised by thinner cells (Fig. 6D–G). Gastrodermis presents similar thickness throughout the infundibula, therefore gastrodermis comparatively thinner or thicker than epidermis at infundibula (Fig. 6C–G). Thin layer of mesoglea between the gastrodermis and epidermis of infundibula (Fig. 6). Externally white spots of nematocysts not present on the subumbrella.

Gastric filaments. Perradial gastric filaments elliptical in cross section, originated by gastrodermal evagination, with internal mesoglea (Fig. 11). Nematocysts present, as well as glandular cells with well-stained granules (Fig. 11D,E).

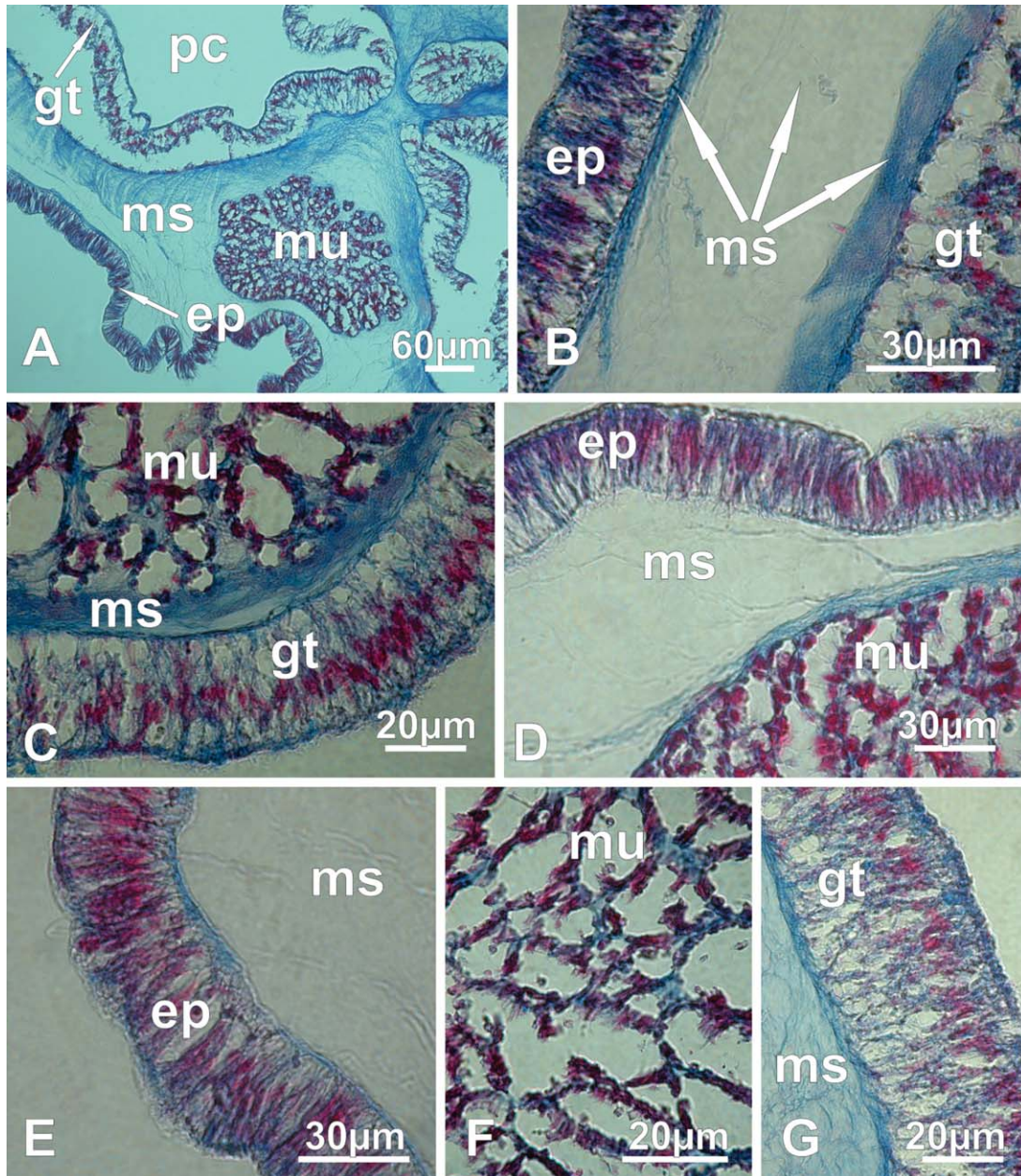


Fig. 4. Median region of peduncle; **A–G**: cross sections. **A**: epidermis, interradial longitudinal muscle embedded in mesoglea, and chambers lined by gastrodermis; **B**: mesoglea organized into three layers; **C**: gastrodermis lining chambers close to muscle; **D**: epidermis close to muscle; **E**: detail of epidermis; **F**: detail of interradial longitudinal muscle; **G**: detail of gastrodermis with three layers (vacuolar cells juxtaposed to mesoglea; glandular cells stained with fuchsin; and glandular cells stained with aniline blue juxtaposed to perradial chamber). Abbreviations: ep, epidermis; gt, gastrodermis; ms, mesoglea; mu, interradial longitudinal muscle; pc, perradial chamber.

Gonads. Gonads formed by numerous vesicles, which are serial gastrodermal evaginations at lateral regions of interradial septa (Fig. 10), therefore younger ones located near interradia and older ones in perradia. Thin layer of mesoglea separates outer gastrodermis from inner vesicular content (Fig. 10). The specimens examined are dioecious. Testicular vesicles with two main internal layers: peripheral layer densely stained with fuchsin, composed by many lobules with spermatocytes;

internal layer weakly stained with fuchsin, composed by spermatozoa (Fig. 10E,F). Ovarian vesicles with two main layers: peripheral layer composed by immature oocytes, in different stages of development, and by follicle cells, which surround the oocytes; internal layer with mature oocytes, which contain scattered yolk granules (Fig. 10G,H) Oocytes with a large nuclei and single nucleoli (deeply stained with fuchsin) (Fig. 10H).

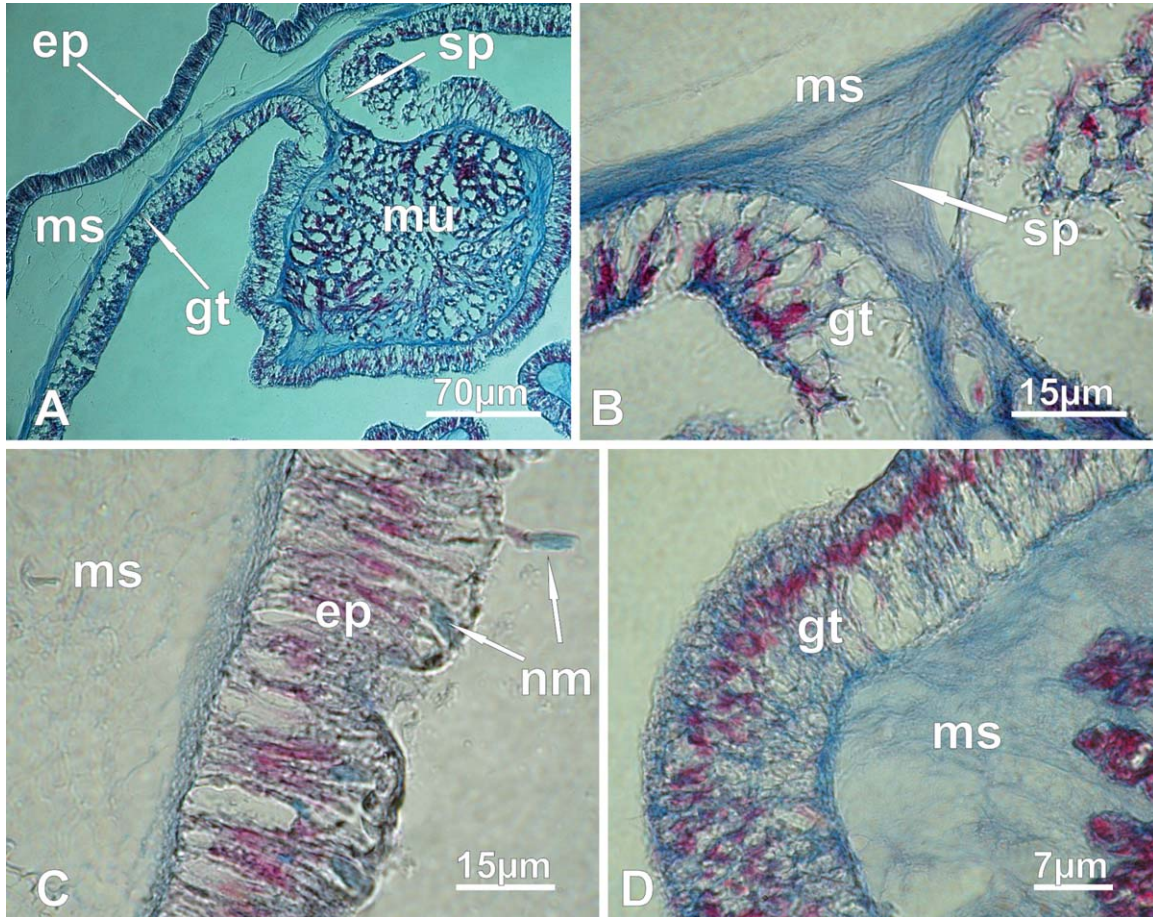


Fig. 5. Basal region of calyx; **A–D**: cross sections. **A**: gastrodermis envelops interradial longitudinal muscle, forming the septum; **B**: detail of septal region; **C**: detail of epidermis with nematocysts; **D**: detail of gastrodermis, with cells organized in three layers. Abbreviations: ep, epidermis; gt, gastrodermis; ms, mesoglea; mu, interradial longitudinal muscle; nm, nematocyst; sp, septum.

Manubrium and perradial pockets. Manubrium internally composed by gastrodermis, externally by epidermis (Fig. 13A). Manubrial gastrodermis thick, continuous and similar to that of central region of septa, but manubrial corner at perradii with taller granulated glandular cells (Fig. 13B–D). Manubrial epidermis thin, continuous and similar to that of infundibula. Perradial pockets formed by lateral regions of septa (Figs. 8A, 10D), whose gastrodermis forms the vesicles of the gonads, and by thick and granular epidermis, with nematocysts. Epidermis of perradial pockets (basally continuous with epidermis of infundibula) thicker at base of manubrium, where an internal layer of nematocysts begins (Fig. 14E–I). Epidermal nematocyst layer of perradial pockets continuous apically with subumbrellar epidermis of arms (Figs. 2K–P, 14A–D). Externally white spots of nematocysts not present on subumbrella.

Anchors. Anchors epidermis formed by tall supporting cells, additionally with vesiculated glandular cells (Fig. 9). Median region of anchor with an invagination, probably in association with

ontogenetic reduction of primary tentacle (Fig. 9E,F). Nematocysts not present. Mesogleal layer between epidermis and gastrodermis thinner at apex of anchor, relatively thicker on lateral and basal regions (Figs. 7–9). Gastrodermis thick with many dense stained granules (Fig. 9), lining a hollow chamber in anchor and its stem (Figs. 7–9), directly connected to gastrodermis of perradial pockets in perradial anchors, or connected to ostia separating adjacent perradial pockets in interradial anchors (Figs. 7, 8).

Arms. Arms with thick and granulated subumbrellar epidermis, similar and continuous to lateral epidermis of infundibula, and epidermis of perradial pockets; thinner exumbrellar epidermis with numerous supporting cells. Subumbrellar epidermis contains an internal layer of nematocysts (Fig. 14A–D); which is continuous with that of infundibula and perradial pockets (Fig. 14E–I). Vesicles of gonads present inside arms, formed by gastrodermal evaginations, continuous to gastrodermis of perradial pockets. Distal end of arm with intertentacular lobules containing thin layer

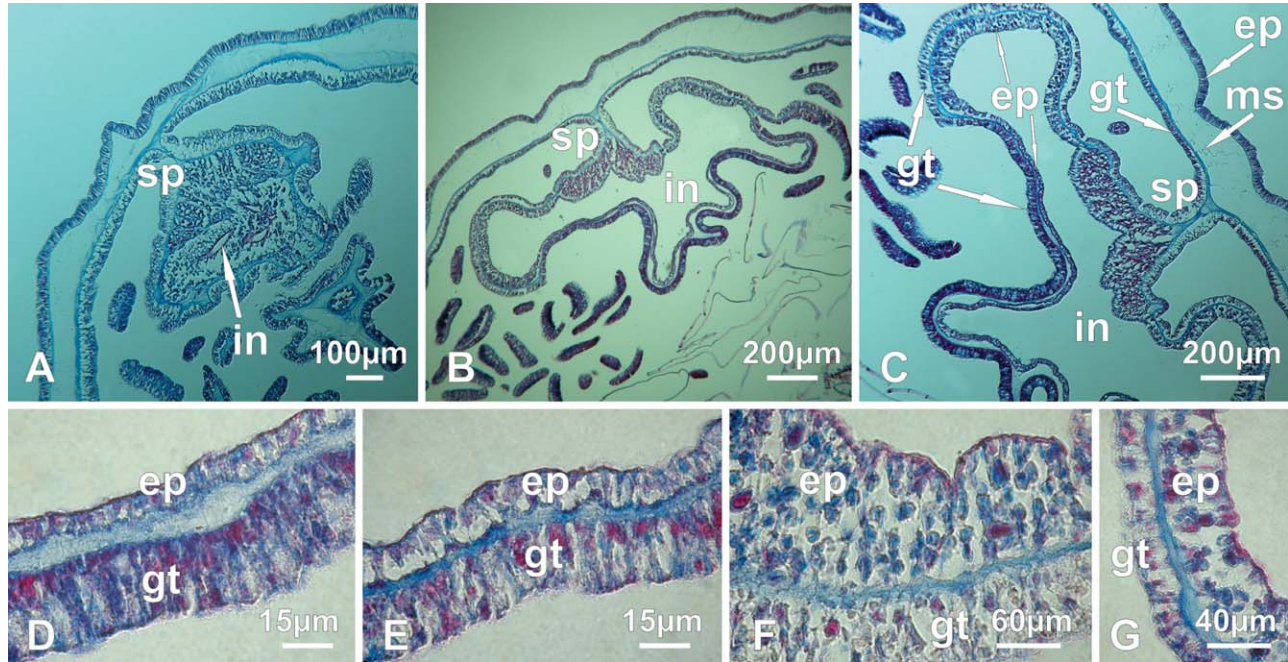


Fig. 6. Infundibulum; A–G: cross sections. A: basal region of infundibulum; B: infundibulum at the median region of calyx; C: epidermis of infundibulum, thicker at lateral region and thinner in front of muscle and central region; D–E: detail of gastrodermis, thicker than epidermis at central region and in front of muscle; F–G: detail of gastrodermis thinner than epidermis (with many granules and nematocysts) at lateral sides. Abbreviations: ep, epidermis; gt, gastrodermis; in, infundibulum; ms, mesoglea; sp, septum.

of mesoglea and gastrodermis intensely granulated (Figs. 12E, 15K,L).

Secondary tentacles. Secondary hollow tentacles composed by two parts, knob and stem (Figs. 15, 16). At stem base, tentacles tightly joined, separated only by thin layer of mesoglea, with a beehive appearance in cross section (Fig.

15G–I). Each tentacle with loose inner layer of gastrodermis with highly vacuolated cells (Fig. 15I,J). Epidermis of stem interspersed by longitudinal muscles (Figs. 15J, 16F,G), stained with fuchsin, extending throughout tentacular stem in longitudinal sections (Fig. 16A,B,D,E), visible as close together dots in cross section at basal region

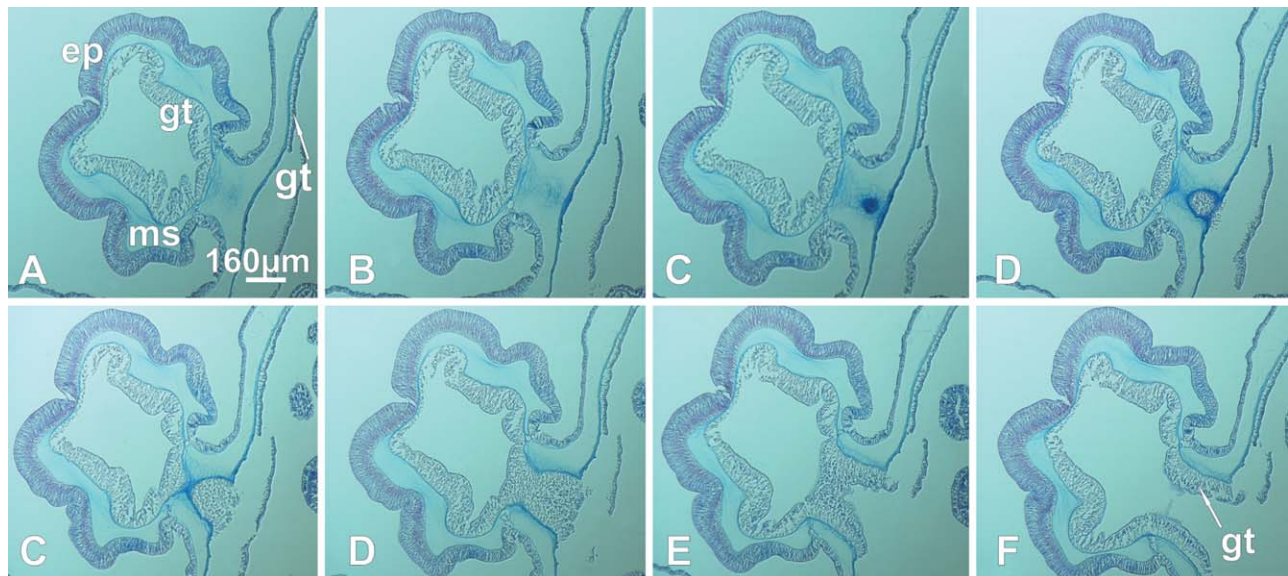


Fig. 7. Perradial anchor; A–F: longitudinal sections of the anchor (cross sections of the animal). A–F: gastrodermis of the hollow perradial anchor connecting with gastrodermis of calyx, through the stem of the anchor. Abbreviations: ep, epidermis; gt, gastrodermis; ms, mesoglea.

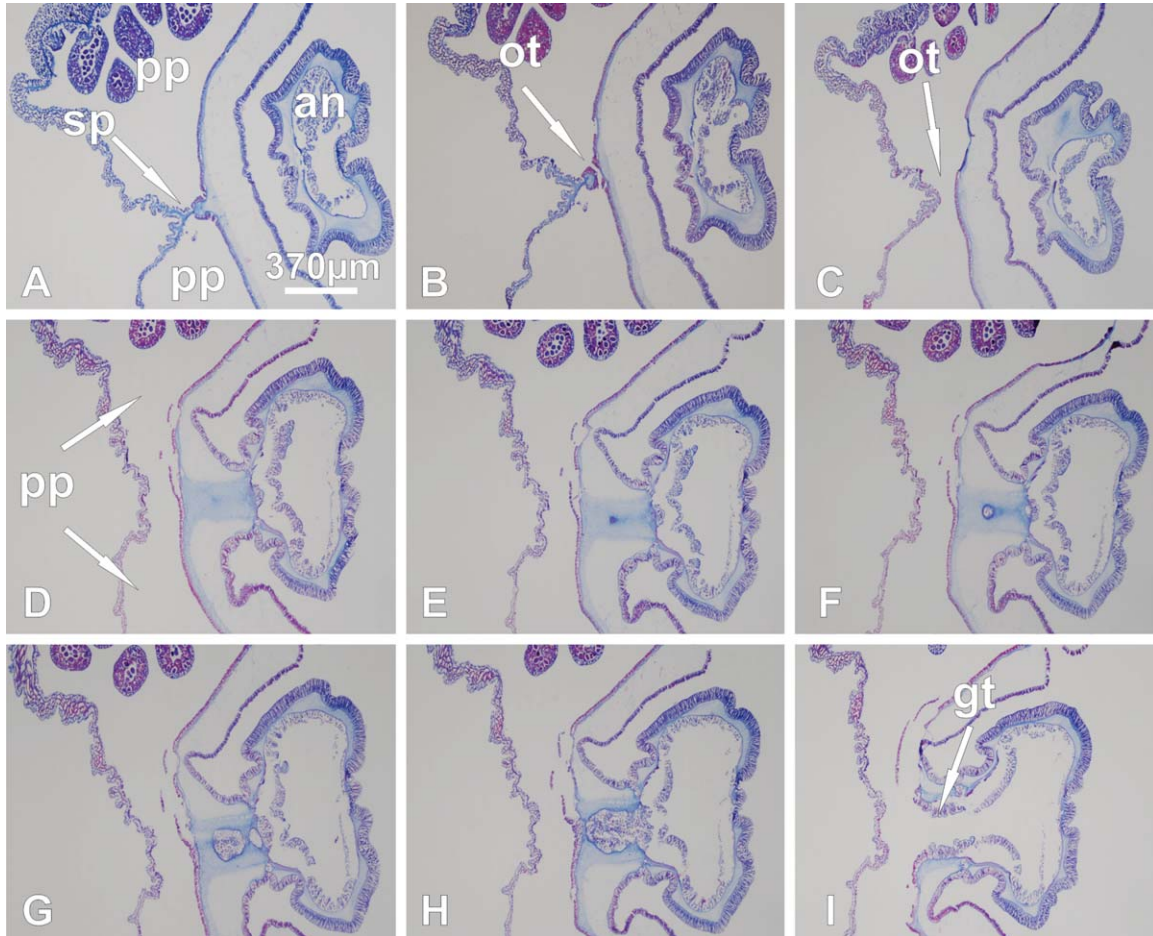


Fig. 8. Interradial anchor; **A–I**: longitudinal sections of the anchor (cross sections of the animal). **A–I**: gastrodermis of hollow interradial anchor connecting with gastrodermis of calyx, through the stem of the anchor, by means of small ostia. **A**: interradial anchor, and septum between two adjacent perradial pockets; **B**: beginning of ostium, where septum detaches from layer of gastrodermis of calyx; **C**: ostium; **D**: two adjacent perradial chambers connected by ostium; **E–I**: gradual communication of the gastrodermis of hollow interradial anchor with gastrodermis of calyx. Abbreviations: an, anchor; gt, gastrodermis; ot, ostia; pp, perradial pocket; sp, septum.

of stem (Fig. 16G). Groups of nematocysts at tentacular base, between epidermis and mesoglea, with different sizes and types, not organized (Fig. 14J–M); these groups are continuous with the layer of nematocysts found in internal region of subumbrellar epidermis of arms, epidermis of perradial pockets and infundibula (Fig. 14A–I). Nematocysts also found in different regions of stem of tentacles, between epidermis and mesoglea (Fig. 14M,N). Tentacular knob region with very tall epidermal cells (Fig. 15A–F), especially supporting and glandular cells, with numerous nematocysts (isorhizas abundant, and microbasic heterotrichs scarce) on its apex, organized in one row (Fig. 15A,C,F); nematocysts strongly stained with fuchsin and aniline blue. Nematocysts also found in internal region of knob, among supporting cells (Fig. 14O). Knob epidermis with vacuoles at basal region (Fig. 15F). Knob gastrodermis more densely stained than that of stem, containing many granules (Fig. 15E).

DISCUSSION

Functional Aspects of Cellular and Tissue Data

The cellular composition and microscopic anatomy is directly related to the natural history and life cycle of the stauromedusa. General tissue and cellular organization of *Haliclystus antarcticus* is qualitatively similar to those described for its congeners (cf. Clark, 1878; Wietrzykowski, 1912; Uchida, 1929; Uchida and Hanaoka, 1933, 1934; Berrill, 1963).

The numerous invaginations of the pedal disk as well as the presence of numerous nonvacuolated gland cells containing granules at the basal portion of the peduncle (Fig. 3) likely enhance attachment to the surface of the substrate, usually algae and rocks for *H. antarcticus* (cf. Miranda et al., 2009) and many other stauromedusae (Mayer, 1910; Uchida, 1929; Edmondson, 1930; Uchida and Hanaoka, 1934; Larson, 1980; Larson and Fautin, 1989). Indeed, adherence is even more difficult for

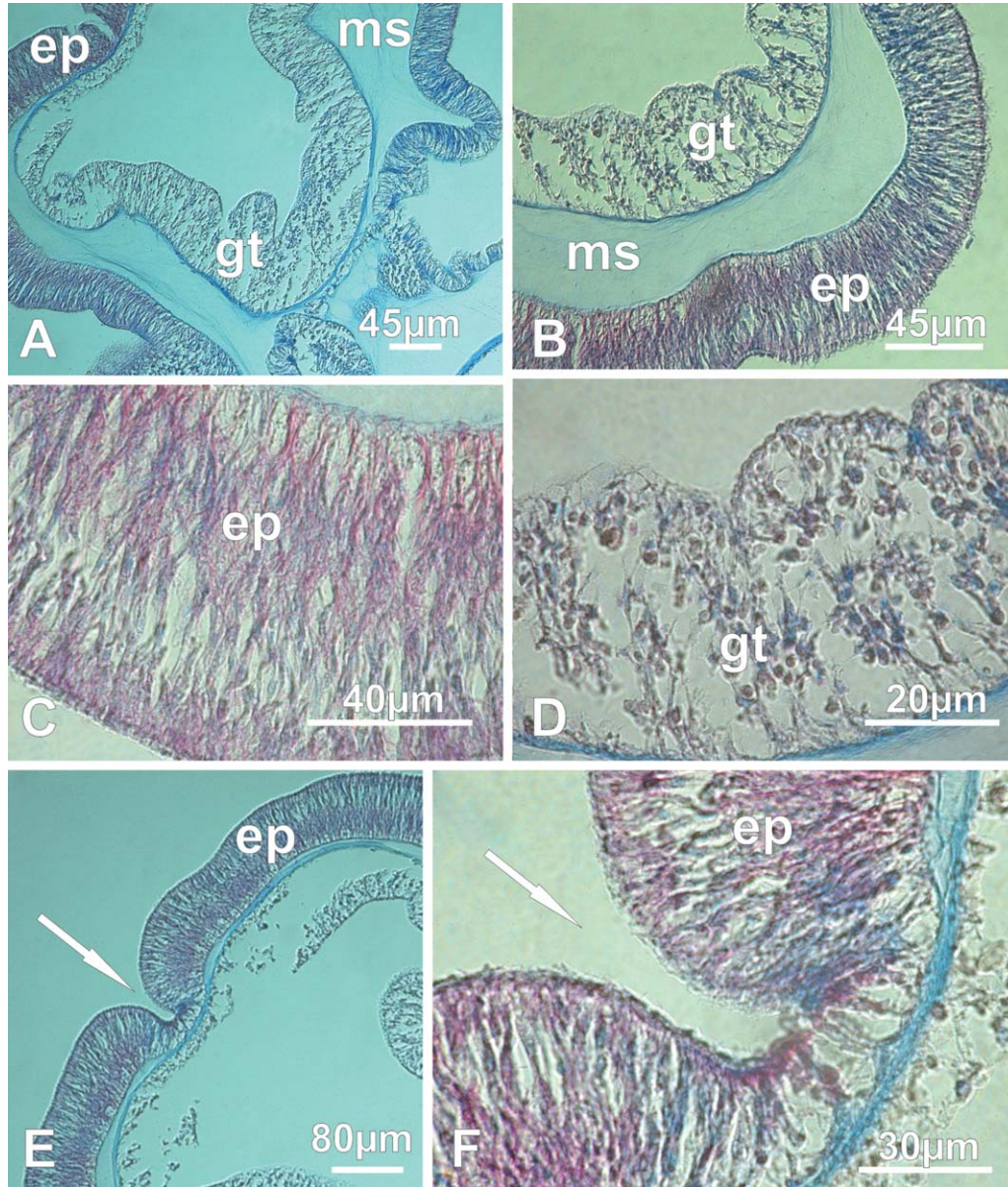


Fig. 9. Anchor; **A–F**: longitudinal sections (cross sections of the animal). **A**: general structure; **B**: detail of epidermis, mesoglea, and gastrodermis; **C**: detail of epidermis with tall cells; **D**: detail of gastrodermis with granules inside cells; **E–F**: region of reduction of primary tentacle (indicated by an arrow). Abbreviations: ep, epidermis; gt, gastrodermis; ms, mesoglea.

those species living in the intertidal zone, where hydrodynamic stress from current and wave action is high (Franc, 1994). Similar gland cells containing granules has been described in the peduncle for other *Haliclystus* species (Uchida, 1929), and a detailed study of the pedal disk of one species of *Haliclystus* (as *H. stejnegeri*) characterized it as highly specialized for attachment, with adhesive and mucous cells, whose secretions may polymerize after being released and become fibrillar components at the region of contact with the substrate (Singla, 1976).

The muscular system (Fig. 12) of sessile animals capable of movement, such as staurozoans, must

be regarded as important (Uchida, 1929). The developed longitudinal musculature of *H. antarcticus* likely helps the medusa attaching to loose substrates such as the fronds of macrophytes. Additionally, the contraction of the coronal musculature (responsible for calyx movement) causes a reduction of the calyx opening. Contraction of the coronal muscles of the calyx can be simultaneous with contraction of the longitudinal muscles of the peduncle and arms, making the arms fold over the manubrium and keeping the animal tightly fixed to the substrate when disturbed (Hyman, 1940). The combination of these movements considerably reduces the total volume of the animal, probably

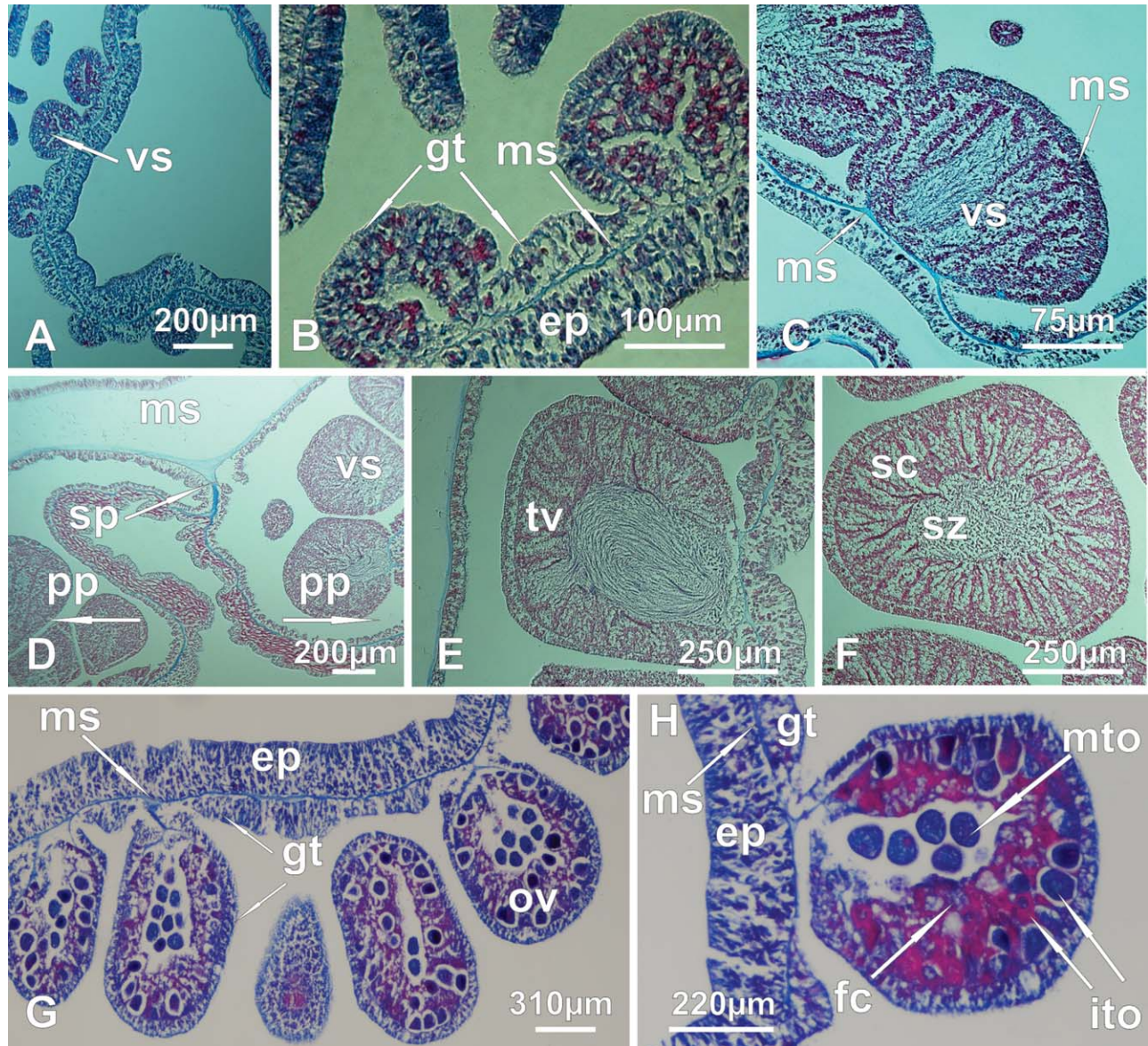


Fig. 10. Vesicles of gonads; **A–H**: cross sections. **A–B**: vesicles formation by evagination of gastrodermis (immature gonads); **C**: thin layer of mesoglea separating gastrodermis and epidermis from gonadal content; **D**: perradial pockets separated by septum, with vesicles in their interior; **E**: developed testicular vesicle; **F**: testicular vesicle, with a peripheral layer constituted by spermatocytes, and with a central layer constituted by spermatozoa; **G**: developed ovarian vesicle; **H**: ovarian vesicle, with a peripheral layer constituted by follicle cells and immature oocytes, and a central layer with mature oocytes. Abbreviations: ep, epidermis; fc, follicle cells; gt, gastrodermis; ito, immature oocytes; ms, mesoglea; mto, mature oocytes; ov, ovarian vesicle; pp, perradial pockets; sc, spermatocytes; sp, septum; sz, spermatozoa; tv, testicular vesicle; vs, vesicle.

making its adherence in highly hydrodynamic habitats more efficient. The myoepithelial cells longitudinally organized along the arms are also likely related to the feeding behavior, contracting to bring the food to the manubrium (Hyman, 1940).

As for most other cnidarians, the tentacles (Figs. 15, 16) act in protecting the animal and capturing prey, with the urticant and adhesive cnidae. Touching the tentacles starts the feeding reaction, consisting of quick bending of one arm so that the tentacle cluster touches the manubrium (Hyman,

1940). The nematocysts and the glandular cells of the gastrodermis, present in the gastric filaments and at the region of the manubrium, are also important to subdue prey and to help in digestion by releasing enzymes (Heeger and Möller, 1987).

Additionally tentacles also play a role in the locomotion of the medusa, adhering to the substrate, serving as a counterpart of the pedal disk. In this respect, tentacles work together with the anchors, glandular structures that also allow momentary adhesion to the substrate through the

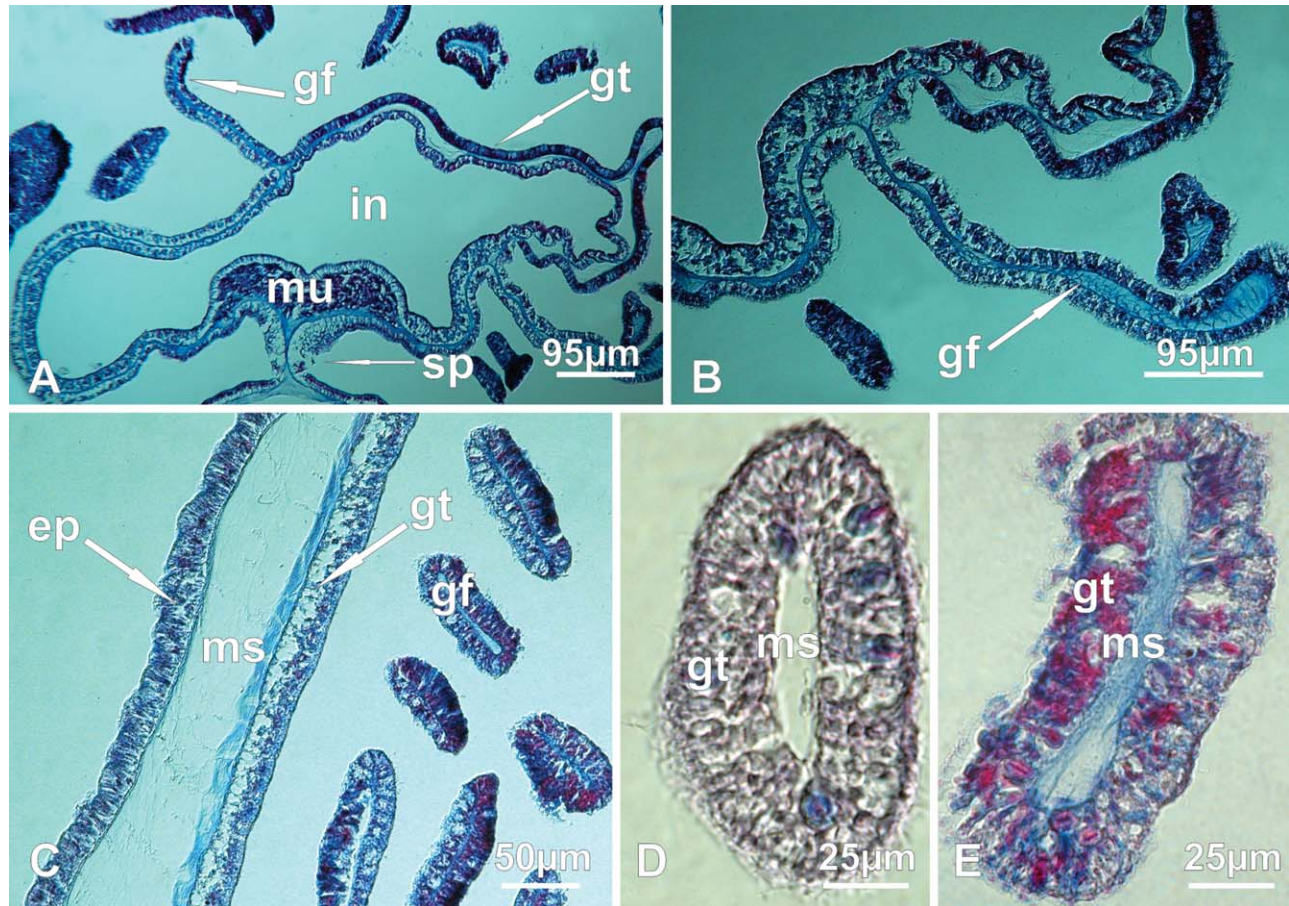


Fig. 11. Gastric filaments; A–E: cross sections. A: formation of gastric filament by evagination of septal gastrodermis; B: detail of gastric filament formation; C: sections of gastric filaments; D–E: detail of gastric filament with nematocysts in gastrodermis and internal mesoglea. Abbreviations: ep, epidermis; gf, gastric filament; gt, gastrodermis; in, infundibulum; ms, mesoglea; mu, interradian longitudinal muscle; sp, septum.

abundant adhesive and supporting cells of their epithelium (Hyman, 1940; Franc, 1994:662). Not coincidentally, all adhesive regions of the body of a stauromedusa, viz. peduncle and pedal disk (Fig. 3), anchors (Figs. 7–9) and tentacular knob (Figs. 15, 16), have the epidermis composed by tall cells, mainly with glandular and supporting functions.

In this study, we identified a region at the base of secondary tentacles, internal to the epidermis and just opposite the intertactular lobules, containing clusters of unorganized nematocysts of differing types (Fig. 14J–M). These nematocysts have no contact with the external environment, suggesting that they are not functional. We propose two main hypotheses: 1) this could be a region where nematocysts originate, or 2) a region where they accumulate. According to the first hypothesis, this region at the base of secondary tentacles is where the nematocysts originate, and once they mature they migrate to different parts of the body, especially to the tentacular knobs, where they are organized in one row, in contact with the environment and functional. A similar process has been

described in *Clytia hemisphaerica* (Hydrozoa), with nematogenesis at tentacular bases, and mature nematocysts at the tips of tentacles (Denker et al., 2008). Such a system would provide the animal with a constant and dynamic supply of nematocysts for defense and prey capture.

The second hypothesis supports the proposal by Weill (1925) and Uchida and Hanaoka (1934) on the developmental origin of the tentacular nematocysts for species of *Haliclystus*. According to this hypothesis, the nematocysts of the tentacular knobs are “derived from the ectodermal wall of the infundibula and have wandered upwards (orally) to the tip of the tentacle” (Uchida and Hanaoka, 1934:222). Indeed, we observed a continuous layer of nematocysts at the internal region of the subumbrellar epidermis of the arms, epidermis of the perradial pockets and infundibula, from the base of the manubrium until the tip of the arms (Fig. 14A–I) (see also Weill, 1925: Figs. 1, 2; Uchida and Hanaoka, 1934: Figs. 5, 9, 11, 19, 20). At this internal layer, the nematocysts are not in contact with the external environment, which strengthens

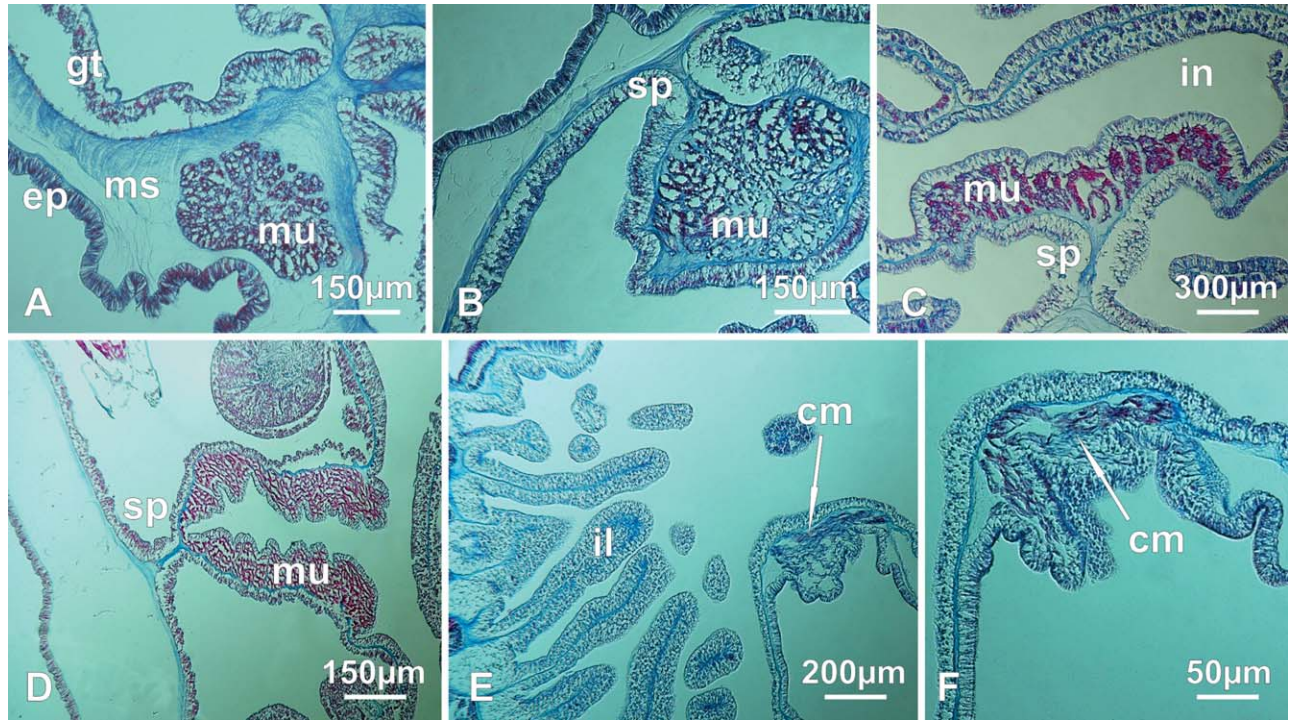


Fig. 12. Musculature; **A–D**: cross sections; **E–F**: longitudinal sections. **A**: interradial longitudinal muscle in peduncle, embedded in mesoglea; **B**: gastrodermis involves interradial longitudinal muscle, forming the septum; **C**: compression of interradial longitudinal muscle at infundibulum; **D**: interradial longitudinal muscle at calyx divided into two regions separated by infundibulum; **E–F**: coronal musculature, close to the arms. Abbreviations: cm, coronal musculature; ep, epidermis; gt, gastrodermis; il, intertentacular lobules; in, infundibulum; ms, mesoglea; mu, interradial longitudinal muscle; sp, septum.

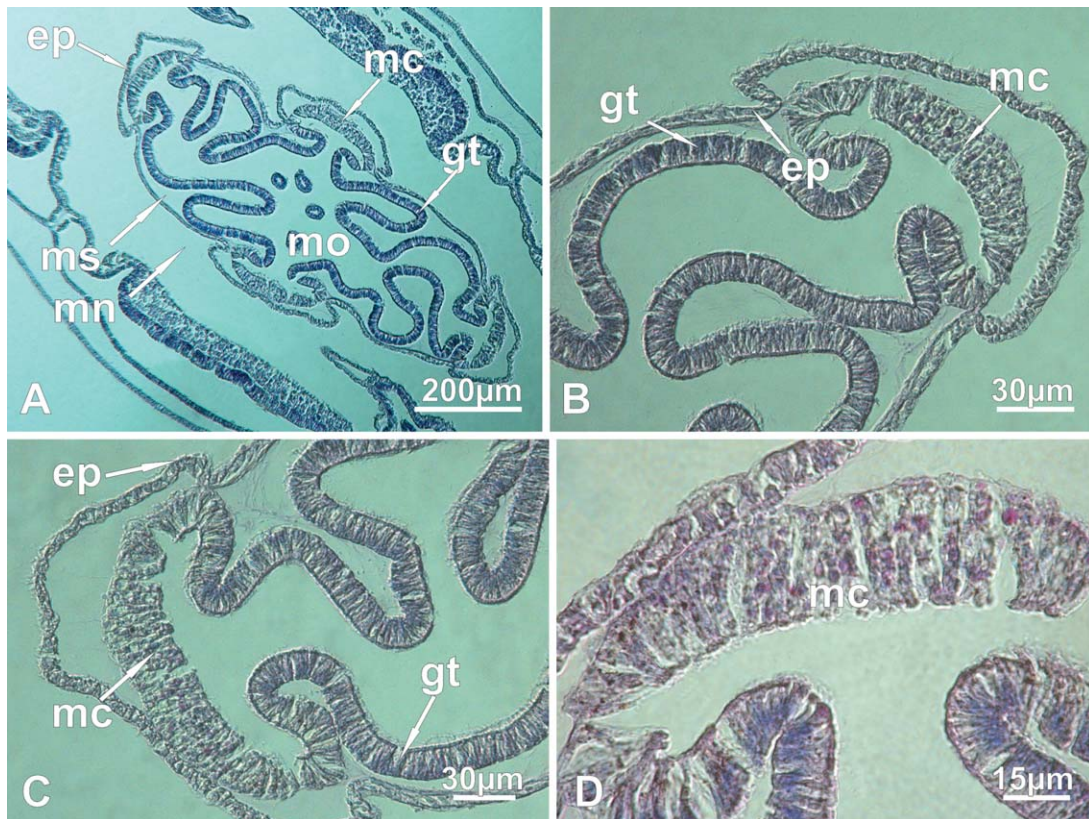


Fig. 13. Manubrium; **A–D**: cross sections. **A**: general view of manubrium; **B–D**: detail of perradial corner of manubrium. Abbreviations: ep, epidermis; gt, gastrodermis; mc, manubrial corner; mn, manubrium; mo, mouth; ms, mesoglea.

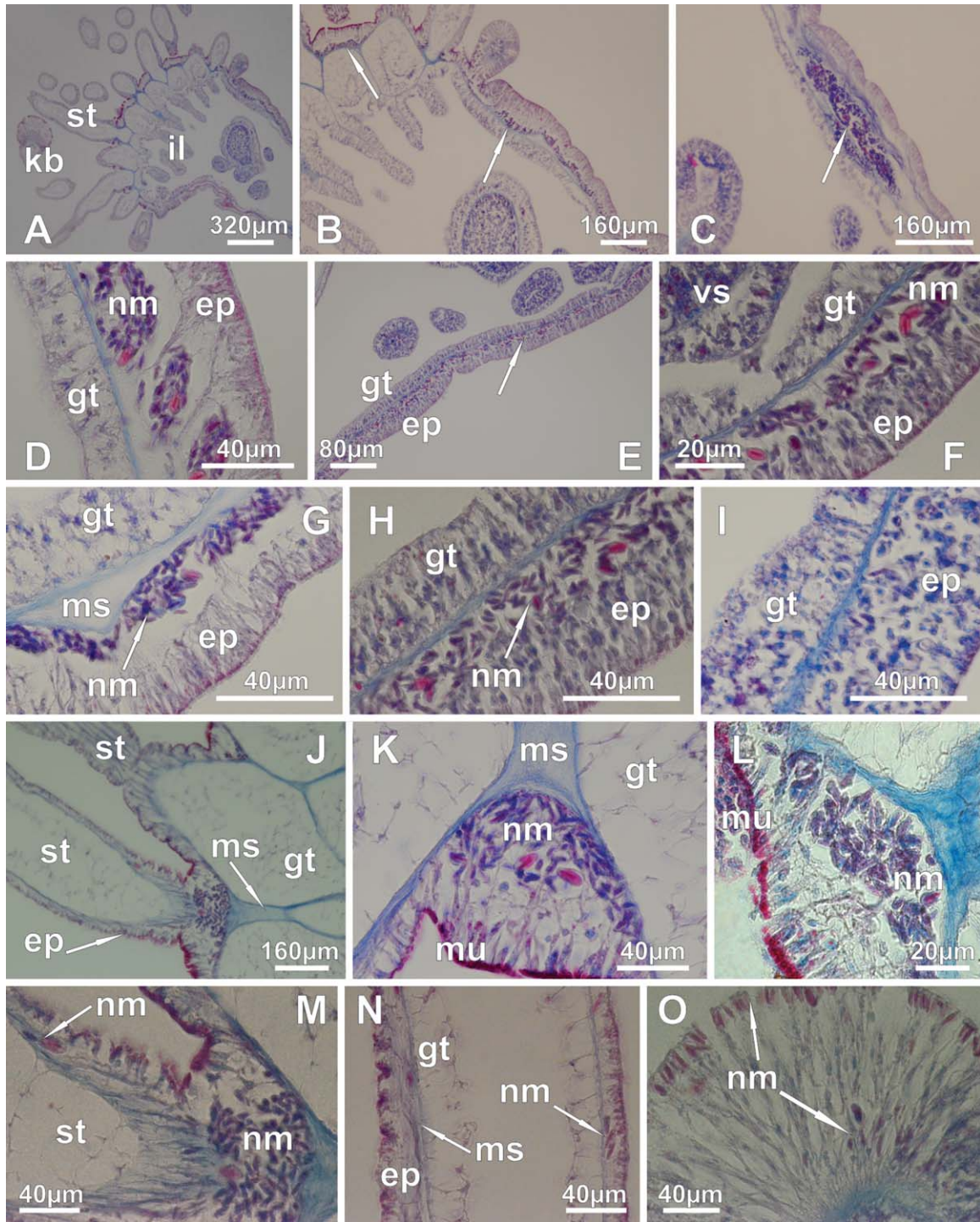


Fig. 14. Nematocysts; **A–D, J–O**: longitudinal sections; **E–I**: cross sections. **A**: tip of arm, with tentacles (stem and knob) and intertentacular lobules; **B**: layer of nematocysts in the internal region of subumbrellar epidermis (indicated by an arrow); **C**: detail of layer of nematocysts at the tip of the arm (indicated by an arrow); **D**: detail of layer of nematocysts at the base of the arm; **E**: epidermis of perradial pockets (layer of nematocysts indicated by an arrow); **F**: layer of nematocysts in the epidermis of perradial pockets; **G**: epidermis of infundibula, at base of manubrium and perradial pockets, with a layer of nematocysts; **H**: epidermis at median region of infundibula, with layer of nematocysts; **I**: epidermis at basal region of infundibula, without a differentiated layer of nematocysts; **J**: base of tentacular stem, with an unorganized group of nematocysts; **K–L**: detail of the unorganized group of nematocysts, between mesoglea and epidermis; **M**: base of stem, with nematocysts between epidermis and mesoglea of stem; **N**: detail of nematocysts between epidermis and mesoglea of stem; **O**: nematocysts in the knob, externally organized in a row, and internally among the supporting cells. Abbreviations: ep, epidermis; gt, gastrodermis; il, intertentacular lobules; kb, knob; ms, mesoglea; mu, muscle; nm, nematocysts; st, stem; vs, vesicle.

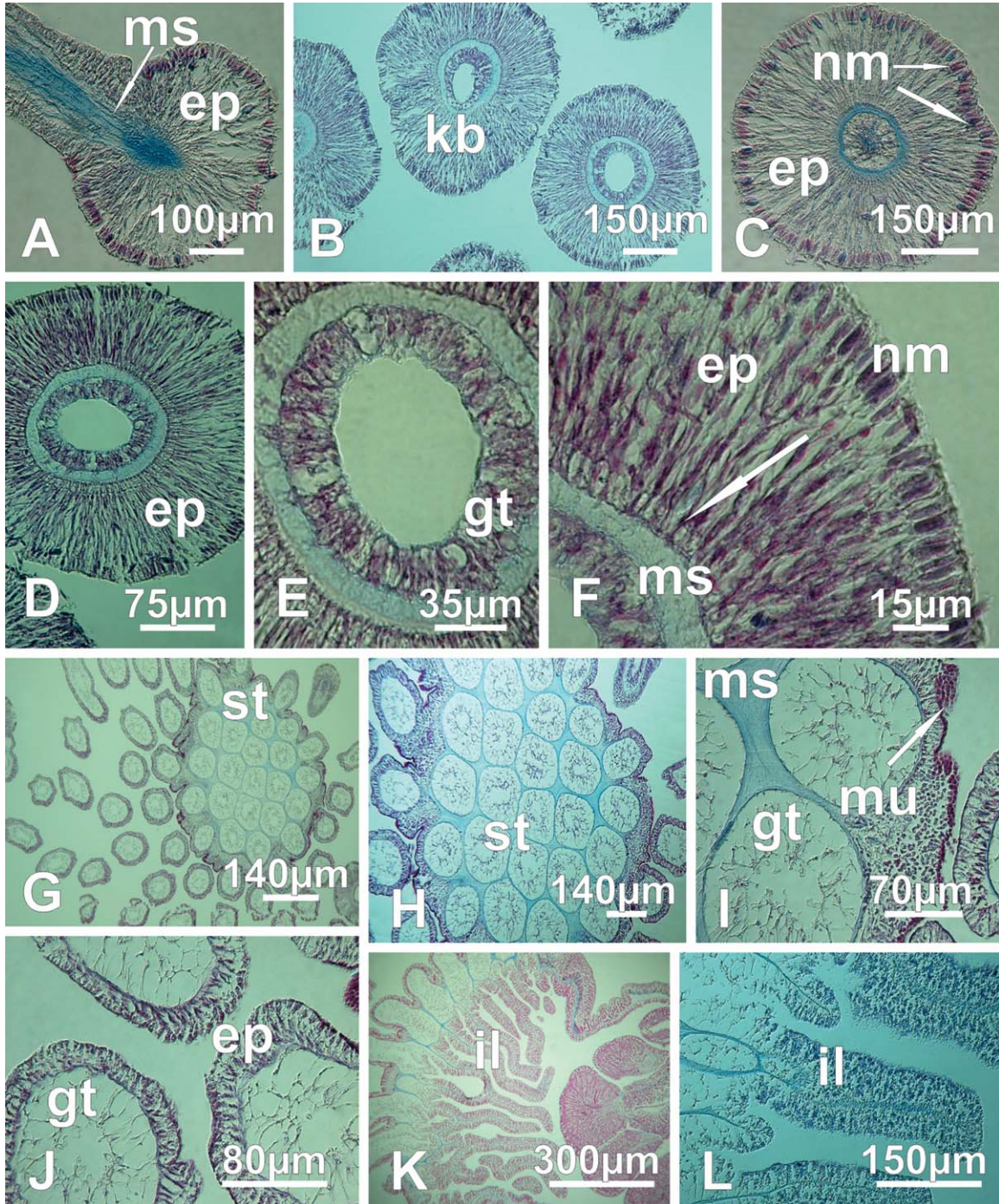


Fig. 15. Tentacles; **A, K, L**: longitudinal sections; **B–J**: cross sections. **A**: knob tentacle, highlighting the tall epidermis and mesoglea inside tentacle; **B**: tentacular knobs; **C–D**: tentacular knob with tall epidermis and nematocysts on its apex; **E**: gastrodermis of tentacular knob with vacuolated glandular cells; **F**: basal region of epidermis with vacuoles (indicated by an arrow); **G–H**: tentacular stem basis; **I–J**: tentacular stem base highlighting the longitudinal muscle and gastrodermis with vacuolated cells; **K–L**: longitudinal section of tentacular base, showing intertentacular lobules. Abbreviations: ep, epidermis; gt, gastrodermis; il, intertentacular lobules; kb, knob; ms, mesoglea; mu, muscle; nm, nematocyst; st, stem.

the hypothesis that they are just migrating through the body (Weill, 1925). Uchida and Hanaoka (1934) also found many nematocysts “wandering in the ectoderm of the secondary tentacles, sometimes forming round clusters.” We

observed different types of nematocysts in the tentacular stem, between epidermis and mesoglea, which probably are migrating toward the knob (Fig. 14M–O). Most likely, their “round clusters” of nematocysts are equivalent to the groups of

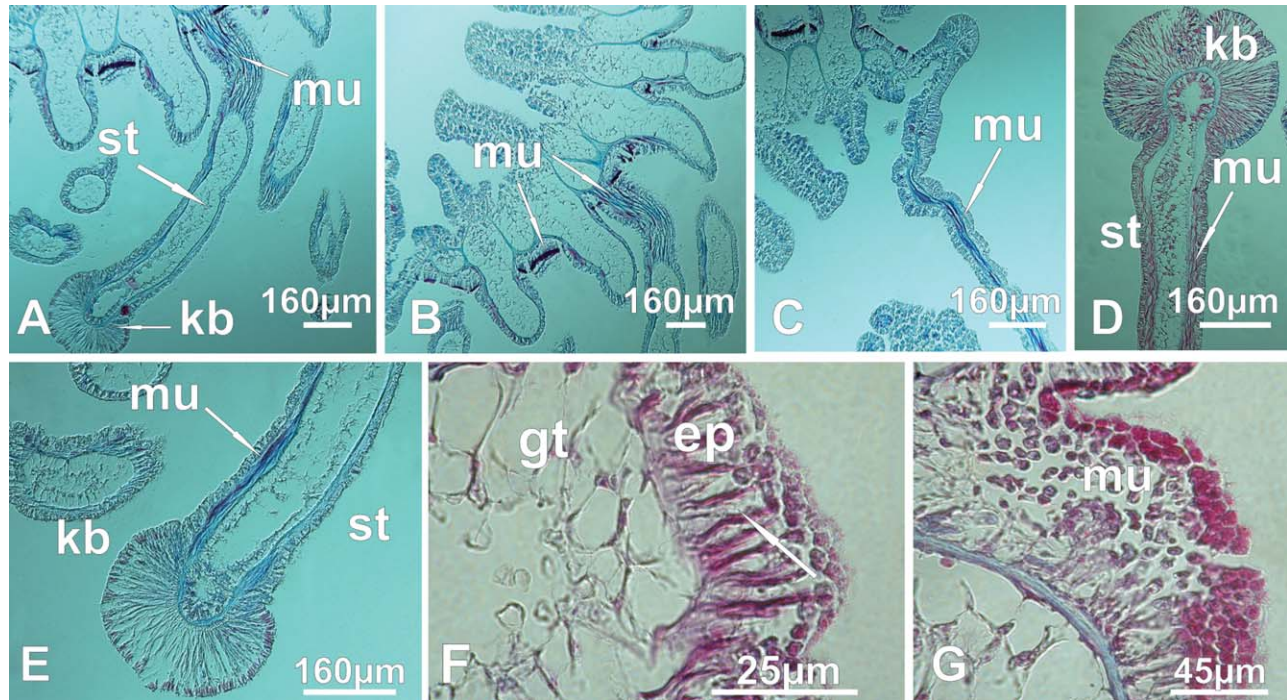


Fig. 16. Tentacles; **A–E**: longitudinal sections; **F,G**: cross sections. **A**: longitudinal muscle throughout tentacular stem; **B**: longitudinal and cross section of longitudinal muscles (dots stained with fuchsin); **C**: longitudinal muscle along arms, toward tentacular tips; **D–E**: detail of longitudinal muscle of stem; **F**: cross section of base of stem showing epidermis interspersed by longitudinal muscles; **G**: cross section of longitudinal muscle at tentacular base. Abbreviations: ep, epidermis; gt, gastrodermis; kb, knob; mu, muscle; st, stem.

nematocysts that we found between epidermis and mesoglea, at the tentacular base (Fig. 14J–M), which, according to this hypothesis, is an accumulation, also likely involved in providing a constant supply of nematocysts to the tentacular knob.

We found no evidence that would rule out one hypothesis or the other, and we cannot conclude on a directionality of nematocyst movement within the subumbrellar epidermis. Further testing is needed to determine whether the tentacular stem is a region of formation or accumulation/maturation of nematocysts in *H. antarcticus*. Answering this question is important because it will facilitate understanding of variation in nematogenesis across Staurozoa and Medusozoa more broadly. White nematocyst clusters are not found on the subumbrella in *H. antarcticus*, but it seems that the formation of the nematocysts in these white spots is different from that of the tentacles in *Manania distincta* (Uchida and Hanaoka, 1933:146–147), suggesting at least two regions of origin in this species (although see Weill, 1925 for a different opinion).

In Staurozoa the sexes are separate, can generally only be recognized with a microscope (Mills and Hirano, 2007), and most species mature in the summer (Eckelbarger and Larson, 1993; Miranda et al., 2012). In an environment with favorable conditions, most stauromedusae apparently spawn daily for a month or more (Corbin, 1979; Mills and

Hirano, 2007). Two species of *Haliclystus* have been shown to spawn in response to light after a period of darkness (Otto, 1976). The gametes are released into the gastrovascular cavity, and expelled through the mouth (Otto, 1976). The gonads of *H. antarcticus* presented the same structures recorded in the literature for other species of *Haliclystus*. In the testicular vesicles were observed two layers, the spermatocytes externally and the spermatozoa internally (Fig. 10E,F), as recorded to *Haliclystus tenuis* (as *H. auricula*; Uchida, 1929). The structure of ovarian vesicles matches the description for *Haliclystus octoradiatus* (Eckelbarger and Larson, 1993; Eckelbarger, 1994), which has a complex organization, with immature oocytes in the periphery, surrounded by follicle cells, and mature oocytes in the central region (Fig. 10G,H). However, Eckelbarger and Larson (1993) erroneously labeled a layer at the base of the vesicle as gastrodermis, of their Figure 2. In fact, the layer at the base of the vesicle is epidermis (Fig. 10), as also indicated by previous authors (e.g., Uchida, 1929; Berrill, 1963).

An ostium was observed between two adjacent perradial pockets, which enables the connection of the gastrodermis of the interradian anchor, with the gastrodermis of the calyx (Fig. 8). This structure has only been mentioned before in *Haliclystus salpinx* and *Manania atlantica* (Berrill, 1963). According to Berrill (1963:742), these ostia can be of little use, as they are too small to allow effective

TABLE 1. Review of the histo-morphological characters used in the taxonomy of Staurozoa (Kishinouye, 1910; Mayer, 1910; Uchida, 1929; Kramp, 1961; Larson, 1988; Larson and Fautin, 1989; Kikinger and Salvini-Plawen, 1995)

Genera/ Characters	Claustrum	Muscles in peduncle	Chambers in peduncle	Coronal muscle
<i>Craterolophus</i>	Present	0	4	?
<i>Depastromorpha</i>	Present	4	4	Entire
<i>Depastrum</i>	Present	4	4	Entire
<i>Haliclystus</i>	Absent	4	4	Divided
<i>Halimocyathus</i>	Present	4	4	Entire
<i>Kishinouyea</i>	Absent	0	4-1*	Divided
<i>Kyopoda</i>	Absent	4	4	Entire
<i>Lipkea</i>	Absent	4	1	Entire
<i>Lucernaria</i>	Absent	4	1	Divided
<i>Lucernariopsis</i>	Absent	0	1	Divided
<i>Manania</i>	Present	4	1/4/1-4 [†]	Entire
<i>Sasakiella</i>	Absent	0	4-1*	Divided
<i>Stenoscyphus</i>	Absent	4	4	Entire
<i>Stylocoronella</i>	Absent	4	1	Absent [‡]

*Four chambers in lower position and 1 chamber in upper position

[†]One chamber throughout the peduncle, or 4 chambers throughout the peduncle, or 1 chamber in lower position and 4 chambers in upper position.

[‡]Coronal muscle could not be distinguished in *Stylocoronella*, and it was considered vestigial or absent in the genus (Kikinger and Salvini-Plawen, 1995).

circulation of fluids between two adjacent perradial pockets; their only function would be to permit a nutritional extension into the four interradial anchors (or retained primary tentacles, e.g., *Manania atlantica*; Berrill, 1963). Based on this hypothesis, we conjecture that species without anchors or retained primary tentacles will not have the ostium connecting pouches if they really only serve to allow for the connection to the anchor/primary tentacle. Therefore, the extension of its occurrence in other genera of Staurozoa needs to be assessed, mainly in species without anchors.

Histological Characters and the Taxonomy of Staurozoa

A small number of internal characters of Staurozoa, for example the presence of four intramesogleal muscles of the peduncle, the number (one or four) of chambers in the peduncle, and the pres-

ence and anatomy of the claustrum have been considered useful in the taxonomy of the group (Uchida, 1929; Kramp, 1961; Collins and Daly, 2005; Table 1). Histo-anatomical studies seem particularly valuable in this regard, especially because of the few macromorphological characters available for the identification of species (Hirano, 1997). Uchida and Hanaoka (1934:211) also emphasized the importance of histological analysis, stating that “the specific identification of Stauromedusae is very difficult without examining living specimens and without cutting sections.” However, the use of histo-morphological characters in the literature may be also misguided. For instance, Mayer (1910:536) argued that *Haliclystus* is closely related to *Lucernaria* because *H. antarcticus* has one chamber in the peduncle, like species of *Lucernaria*. However, we (as well as Pfeffer, 1889 and Carlgren, 1930) observed that this remark is incorrect and *H. antarcticus* has four chambers in the peduncle. Indeed, a phylogeny based on molecular markers showed that the genera *Lucernaria* and *Haliclystus* are not closely related (Collins and Daly, 2005).

The genus *Haliclystus* has been the subject of many taxonomic discussions (Uchida, 1929; Gwilliam, 1956; Hirano, 1997; Miranda et al., 2009; Kahn et al. 2010). Species were synonymized, particularly with *Haliclystus auricula*, and later validated (Uchida, 1929; Gwilliam, 1956; Kramp, 1961; Hirano, 1986; Hirano, 1997), generating uncertainty about the current status of its taxonomy. In order to solve some of these problems, Hirano (1997) proposed the use of two new characters in the taxonomy of the group: intertentacular lobules and white nematocyst spots on the subumbrella (Table 2). According to her observations of *Haliclystus* specimens from boreal regions, the species could be divided into two main groups based on the morphology of the base of the tentacle clusters: species with intertentacular lobules, and species without intertentacular lobules (U-shaped space). In addition, the species could be subdivided based on the presence/absence of white nematocysts spots, resulting in four distinct morphotypes (Hirano, 1997; Table 2). If specimens of *H. antarcticus* were classified in this way, they

TABLE 2. Morphotypes of *Haliclystus* proposed by Hirano (1997), based on the shape of the base of the tentacle clusters, and the presence of white nematocysts spots on subumbrella

Morphotypes		White nematocysts spots on subumbrella		
		Absent	Perradii and Interradii	Perradii
Base of tentacle clusters	Intertentacular lobules	Morphotype 1 <i>H. auricula</i> <i>H. antarcticus</i> *	Morphotype 2 <i>H. octoradiatus</i>	Morphotype 3 <i>H. “sanjuanensis”</i> <i>H. stejnegeri</i>
	U-shaped space	–	Morphotype 4 <i>H. borealis</i> <i>H. tenuis</i>	–

*This study.

would fit morphotype 1, as *H. auricula* (Table 2), which could explain misidentifications of some stauromedusae found in Chile and Argentina (Amor, 1962; Mianzan, 1989; Zagal, 2004a, 2004b, 2008; Miranda et al., 2009, 2010).

Haliclystus antarcticus has four chambers in the peduncle, as described to the others species of *Haliclystus* (Kramp, 1961; Table 1). However, this character can present different states: four-chambered peduncle (e.g., *Haliclystus* and *Depastromorpha*), single-chambered peduncle (e.g., *Lucernaria* and *Lucernariopsis*), single-chambered peduncle with four chambers in lower position (e.g., *Kishinouyea* and *Sasakiella*), and four-chambered peduncle with one chamber in lower position (e.g., some *Manania*; Mayer, 1910; Carlgren, 1935; Kramp, 1961; Larson and Fautin, 1989; Table 1). In order to avoid misinterpretations, this character should be cautiously employed in the taxonomy of the group, because one chamber can be subdivided in four (e.g., *Kishinouyea hawaiiensis*, Edmondson, 1930). The species of *Haliclystus* have four muscles in the peduncle (Kramp, 1961), in contrast to the absence of muscle in the peduncle, presented by some genera of Staurozoa (e.g., *Craterolophus* and *Lucernariopsis*; Kramp, 1961; Table 1). In addition, *Haliclystus* has divided coronal muscle (Kramp, 1961), different from the unbroken coronal muscle, observed for example in *Manania* (Larson and Fautin, 1989) and in *Lipkea* (Pisani et al., 2007; Table 1).

As the preceding discussion shows, histomorphological characters have long been employed in classifications of Staurozoa (Clark, 1863; Mayer, 1910; Uchida, 1929; Kramp, 1961; Uchida, 1973), albeit incompletely. The study of Collins and Daly (2005), even with limited taxon sampling, suggests that further knowledge of the distribution of histomorphological characters across Staurozoa could be important for defining natural groups. For instance, the family Lucernariidae does not appear to be monophyletic. This is perhaps not so surprising when considering the number of dualities in character states included in its definition, as provided by Kramp (1961:292): “faintly or well developed marginal lobes; [...] peduncle single-chambered or with four-chambers [...]. Coronal muscle unbroken or divided into eight separate sectors.” On the other hand, a sharp dichotomy into taxonomic groups based on a single character might also be incongruent with evolutionary history. For instance, Stauromedusae has long been divided into two primary groups, Cleistocarpida and Eleutherocarpida (Clark, 1863), on the basis of presence or absence of a claustrum, respectively (Clark, 1863; Gross, 1900; Daly et al., 2007; Table 1). However, if the working hypothesis of Collins and Daly (2005) is correct, then this structure either evolved homoplastically in different groups or was lost several times. Fully assessing the evolution and taxonomic usefulness of histomorphological characters in Stauromedusae

will require a robust phylogenetic framework with much more complete taxon sampling and detailed histological accounts, such as that presented here, for species representing the full breadth of staurozoan diversity. Many characters have not yet been broadly explored for the class (e.g., intertentacular lobules, ostia), nor appropriately applied in its taxonomy (e.g., muscles and chambers in the peduncle, claustrum). However, studying body plan evolution in Staurozoa is an exciting challenge. With their unusual life cycles (Wietrzykowski, 1912; Kikinger and Salvini-Plawen, 1995; Miranda et al., 2010) and features that are thought of as “polypoid” or “medusoid” (e.g., Collins et al., 2006), staurozoans are difficult to compare with other cnidarians, but doing so should provide indispensable information for understanding the evolution in the phylum.

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