

Evolution of the claustrum in Cnidaria: comparative anatomy reveals that it is exclusive to some species of Staurozoa and absent in Cubozoa

Lucília S. Miranda¹ · Jimena García-Rodríguez¹ · Allen G. Collins² · André C. Morandini¹ · Antonio C. Marques^{1,3}

Received: 21 April 2017 / Accepted: 5 September 2017
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Abstract The claustrum in Cnidaria is a tissue in the gastrovascular cavity delimited by a central layer of mesoglea surrounded by gastrodermis (i.e., gastrodermis-mesoglea-gastrodermis), without communication with epidermis. By dividing the gastrovascular cavity, the four claustra provide an additional level of complexity. The presence of claustra in Cubozoa and Staurozoa has been used as evidence supporting a close relationship between these two cnidarian classes. However, the detailed anatomy of the claustrum has never been comparatively analyzed, rendering the evolution of this character among Cnidaria and its homology in Staurozoa and Cubozoa uncertain. This study provides a comparative investigation of the internal anatomy of the claustrum in Staurozoa and Cubozoa, addressing its evolutionary history based on recent phylogenetic hypotheses for Cnidaria. We conclude that the claustrum is a character exclusive to some species of Staurozoa, with a homoplastic evolution in the class, and that the structure called the “claustrum” in Cubozoa corresponds to the valve of gastric ostium, a structure at the base of the manubrium, which is also present in Staurozoa with and without claustrum. Thus, the claustrum cannot be a synapomorphy of a hypothetical clade uniting Staurozoa and Cubozoa, nor can

its hypothetical presence in enigmatic fossils be used to support cubozoan affinities.

Keywords Medusozoa · Stauromedusae · Stalked jellyfish · Box jellyfish · Histology

Introduction

Although the establishment of homologies is crucial in evolutionary studies, many hypotheses of homology in cnidarian structures remain untested. One example is the hypothetical homology of the claustrum in stauromedusae and cubomedusae (cf. Thiel 1966). Originally, the claustrum was used as a character to separate two taxa of stalked jellyfishes (Staurozoa): Eleutherocarpida (without claustrum) and Cleistocarpida (with claustrum) (Clark 1863). According to Clark (1863: 535, 536), the gastrovascular cavity of members of Eleutherocarpida is divided into four radial gastric pockets (pouches), whereas those of Cleistocarpida have a “transverse horizontal membrane, which divides each of the four quadrant camerae [four radial gastric pockets] of the disc into two superposed spaces, the oral one of which forms a cul-de-sac, or claustrum opening at the axial end, and includes the genitalia.” Therefore, the more complex structure of Cleistocarpida (Collins and Daly 2005; Miranda et al. 2016a, b) has a membrane dividing the gastrovascular cavity resulting in four external radial pockets and four oral (internal) radial pockets (Gross 1900; Berrill 1963; Miranda et al. 2016b).

Although “claustrum” explicitly referred to the oral radial pocket in Cleistocarpida that confines the gonads (Clark 1863), a homonym “claustrum” has been used to designate a different structure in Staurozoa. Haeckel (1879) and Gross (1900) called “claustrum” the membrane that divides the gastrovascular cavity in Cleistocarpida and designated the oral

✉ Lucília S. Miranda
mirandals@ib.usp.br

¹ Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, travessa 14, 101, Cidade Universitária, São Paulo, São Paulo 05508-090, Brazil

² National Systematics Laboratory, National Marine Fisheries Service (NMFS), National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

³ Centro de Biologia Marinha, Universidade de São Paulo, São Sebastião, São Paulo, Brazil

radial pockets as “Mesogontaschen” (i.e., mesogon, Haeckel 1879; Thiel 1966) and the external radial pockets as “Exogontaschen” (i.e., exogon, Gross 1900; Thiel 1966). The first detailed study of the anatomy of claustra compared *Haliclystus octoradiatus* Clark, 1863 (lacking claustrum) with *Craterolophus convolvulus* (Johnston, 1835) (with claustrum) (Gross 1900). The claustrum was then described as the tissue connecting adjacent interradial septa, comprised of a double layer of gastrodermis separated by a layer of mesoglea (i.e., gastrodermis-mesoglea-gastrodermis) (Gross 1900). Numerous subsequent authors (Mayer 1910; Uchida 1929; Uchida and Hanaoka 1933; Carlgren 1935; Berrill 1963; Thiel 1966; Collins and Daly 2005; Miranda et al. 2013, 2016a, b) have used claustrum in the sense of Haeckel (1879) and Gross (1900), and which we follow herein to avoid confusion.

Within Cnidaria, the presence of claustra has also been proposed for Cubozoa (Krumbach 1925; Thiel 1966). Based on these assertions, and assuming their veracity, the claustrum has been cited as evidence of a close evolutionary relationship between Cubozoa and Staurozoa (Thiel 1966; Collins 2002; Marques and Collins 2004). Although hypotheses of phylogenetic relationships among cnidarian classes are still under debate (Collins et al. 2006; Van Iten et al. 2006, 2014; Kayal et al. 2013; Zapata et al. 2015), the claustrum plays a fundamental role in evolutionary interpretations of Cnidaria. For instance, a hypothetical claustrum was used to argue for a cubozoan affinity within Cnidaria for early Cambrian pentamerous fossils from South China (Han et al. 2013).

Recent evolutionary studies of Staurozoa, however, suggest that claustra may be homoplastic within Staurozoa because species possessing claustra (“cleistocarpids”) form a polyphyletic assemblage (Collins and Daly 2005; Miranda et al. 2016a, b). In addition, the questionable presence of claustra in Cubozoa (Thiel 1966: 95) has never been properly detailed and compared with those of staurozoans, making their homology uncertain. The aim of this study is to test the homology of the structures called claustra in Staurozoa and Cubozoa by comparing their internal anatomy in light of recent phylogenetic hypotheses for Cnidaria.

Material and methods

We analyzed different species of Staurozoa (stauromedusa stage, with and without claustrum) and Cubozoa (cubopolyp and young cubomedusa stages), either from laboratory cultures or from museum collections (Table 1). Materials were preserved directly in 4% formaldehyde solution in seawater. The histological techniques followed two procedures due to the size of the animals: paraffin (larger specimens, used for Staurozoa) and resin (smaller specimens, used for Cubozoa).

The paraffin technique follows Miranda et al. (2013; modified from Humason 1962; Mahoney 1966). Specimens were cleaned in distilled water; dehydrated in a graded ethanol series (70–100%); cleared in xylene (three steps); infiltrated and embedded in paraffin; serially sectioned transversely (7.0–10.0 μm thick) with a microtome Leica RM2025; cleared in xylene (twice); rehydrated in a graded ethanol series (100–70%); cleaned in distilled water; and stained, using acid fuchsin (15') (Humason 1962: 147), and acetic aniline blue (3') (modified from Humason 1962: 231), intercalated with distilled water to improve the contrast between structures.

In the resin technique, the samples were dehydrated and embedded in glycol methacrylate following the instructions provided with the kit (“Leica HistoResin Embedding Kit”, Leica Microsystems Nussloch GmbH, Germany). Thin sections (3.0–5.0 μm) were cut with a Leica RM2255 microtome and stained with hematoxylin-eosin and toluidine blue for 3.0 μm sections (for general morphology), periodic acid-Schiff + hematoxylin (identification of neutral polysaccharides), and Gomori’s trichrome (identification of collagen) for 5.0 μm sections (for histochemistry) (according to Humason 1962; Behmer et al. 1976; Bancroft and Stevens 1982; Pearse 1985; Junqueira 1995; Mendoza-Becerril et al. 2016).

Slides were observed and photographed under a Zeiss microscope AXIO Imager M2. The slides are deposited in the collection of the Laboratory of Marine Evolution of the Institute of Bioscience, University of São Paulo, Brazil (Table 1). The complete list of abbreviations used in the figures are provided in Table 2.

Table 1 Cnidarian species examined in this study (one specimen for each species), with respective life cycle stage, voucher catalog number, and slides catalog number

Class	Species	Stage	Voucher catalog number	Slides catalog number
Staurozoa	<i>Craterolophus convolvulus</i> (Johnston, 1835)	Stauromedusa (with claustrum)	USNM 54321	LEM 17
	<i>Manania uchidai</i> (Naumov, 1961)	Stauromedusa (with claustrum)	USNM 1106645	LEM 10
	<i>Haliclystus tenuis</i> Kishinouye, 1910	Stauromedusa (without claustrum)	USNM 1106652	LEM 09
Cubozoa	<i>Carybdea xaymacana</i> Conant, 1897	Cubopolyp	Laboratory culture	LEM 18
	<i>Copula sivickisi</i> (Stiasny, 1926)	Young cubomedusa	Laboratory culture	LEM 19

LEM: Laboratory of Marine Evolution of the Institute of Biosciences, University of São Paulo, Brazil; USNM: National Museum of Natural History, Smithsonian Institution, USA

Table 2 Abbreviations of structures reported in the figures

Abbreviations	Structures
ar	Accessory radial pocket
ax	Auxiliary radial pocket
cl	Calyx
co	Claustrum ostium
cs	Claustrum
ep	Epidermis
exb	Exumbrella
gd	Gonad
gf	Gastric filament
go	Gastric ostium
gp	Gastric radial pocket
gt	Gastrodermis
gvc	Gastrovascular cavity
in	Infundibulum
lse	Lateral septal evaginations
mc	Manubrial corner
mn	Manubrium
mr	Main radial pocket
ms	Mesoglea
mu	Longitudinal muscle
pe	Peduncle
sp	Septum
sub	Subumbrella
um	Umbrella
vg	Valve of gastric ostium

Results

Staurozoa

Stauromedusa without claustrum

Peduncle/calyx connection region (Fig. 1a, b) with four interradial gastric septa formed by thin layer of mesoglea surrounded by gastrodermis (Figs. 1c and 2a). Four infundibula (peristomial pits) associated with septa, blind ended, delimited by epidermis, deeply developed up to base of calyx, widening orally (apically), with broad apertures on subumbrella (Figs. 1c and 2a–c). Infundibula gradually larger toward oral region, delimiting gastric ostia, a narrow space between adjacent infundibula (Figs. 1d and 2d, e). Adjacent septal gastrodermis merges defining four perradial regions and dividing gastrovascular cavity (Figs. 1e and 2d). Fusion of septal gastrodermis forms basal region of manubrium and gastric radial pockets (perradial pockets), i.e., central part of gastrodermis of each septum joins forming four-sided manubrial gastrodermis while lateral parts of adjacent septa join forming gastric radial pockets. Fusion of septal

gastrodermis produces a transitory membrane with a double layer of gastrodermis and central layer of mesoglea, named valve of gastric ostium (Figs. 1e and 2d–h). Each infundibular epidermis also progressively merges orally, disrupting valve of gastric ostium (Figs. 1f, g and 2h, i). Central part of each infundibular epidermis becomes manubrial epidermis, and epidermis of adjacent infundibula forms epidermis of gastric radial pockets (i.e., epidermis of subumbrella) (Figs. 1g, h and 2j, k). Therefore, each gastric radial pocket is formed by fusion of gastrodermis and epidermis of adjacent septa, with manubrium formed by fusion of all four septa. Four gastric radial pockets laterally separated from each other by interradial septa (Figs. 1g, h and 2j, k). Manubrium internally lined by gastrodermis, externally by epidermis (Figs. 1g, h and 2i–k). Gonadal structures as serial gastrodermal evaginations, internal to gastric radial pockets (Fig. 2i–k).

Stauromedusa with claustrum

Peduncle/calyx connection region (Fig. 3a) with four interradial gastric septa formed by thin layer of mesoglea surrounded by gastrodermis (Figs. 3b, 4a, and 5a–c). Four infundibula associated with septa, blind ended, delimited by epidermis, deeply developed up to base of calyx, widening orally (apically), with broad apertures on subumbrella (Figs. 3b, 4a, and 5a–c). Lateral septal evaginations of adjacent interradial septa delimiting claustra ostia (Figs. 3c, 4b, c, and 5c–f). Union of lateral septal evaginations forming claustra, membranes delimited by central layer of mesoglea surrounded by gastrodermis, dividing gastrovascular cavity (Figs. 3d, 4d, e, and 5d–g). Four accessory radial pockets delimited (separated from main gastrovascular cavity) by claustra (Figs. 3d, 4d, e, and 5e, g). Infundibula gradually larger toward oral region, delimiting gastric ostia, a narrow space between adjacent infundibula (Figs. 3e, 4f, and 5g). Adjacent septal gastrodermis merges again, dividing gastrovascular cavity once more, forming four main (principal) radial pockets and manubrium (Figs. 3f, 4g, and 5h, i), similar to formation of gastric radial pockets in staurozoans without claustrum such as *Haliclystus tenuis* (Figs. 1 and 2). Central part of gastrodermis of each septum joins forming four-sided manubrial gastrodermis while lateral parts of adjacent septa join forming main radial pockets. Fusion of septal gastrodermis produces a transitory membrane with double layer of gastrodermis and central layer of mesoglea, named valve of gastric ostium (Figs. 3f, 4g, and 5h–j). Each infundibular epidermis progressively merges orally, disrupting valve of gastric ostium (Figs. 3g, h, 4h, and 5i–k). Central part of each infundibular epidermis becomes manubrial epidermis, and epidermis of adjacent infundibula forms epidermis of main radial pockets (i.e., epidermis of subumbrella). Therefore, each main radial pocket formed by fusion of gastrodermis and epidermis of adjacent septa, and

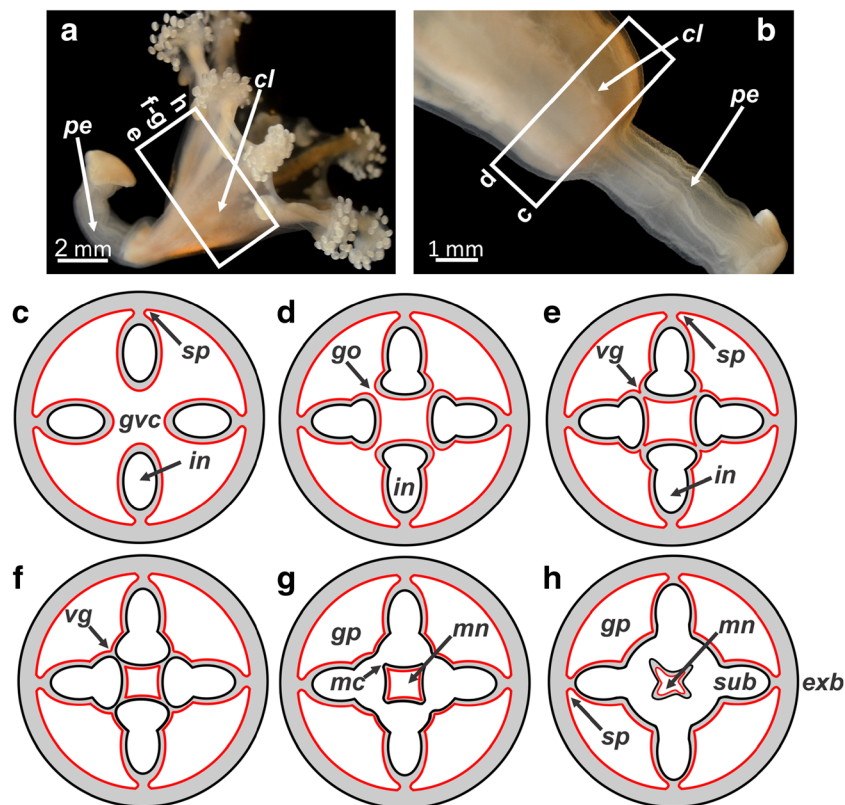


Fig. 1 General body plan of *Haliclystus tenuis* (stauromedusa stage). **a, b** Preserved specimens; **c–h** schematic representations of cross-sections through the specified regions shown in **a** and **b**. **a** Body mainly divided into oral (apical) calyx (cl) and aboral (basal) peduncle (pe); **b** detail of calyx/peduncle connection; **c** four septa (sp) with four infundibula (in), and central gastrovascular cavity (gvc); **d** progressively larger infundibula (in), and gastrodermis of adjacent septa getting closer, delimiting the

gastric ostia (go); **e** connection of gastrodermis of adjacent septa (sp), delimiting a double layer of gastrodermis with central mesoglea, the valve of gastric ostium (vg); **f, g** gradual connection of epidermis of infundibula, delimiting the four gastric pockets (gp) and base of manubrium (mn); **h** central manubrium (mn) and four gastric pockets (gp) separated by septa (sp). Legend: epidermis, black; gastrodermis, red; mesoglea, gray. See Table 2 for additional abbreviations

manubrium formed by fusion of all four septa. Eight radial pockets, four accessory radial and four main radial pockets (associated with manubrium and gonads) (Figs. 3h, 4h, and 5k). Accessory and main radial pockets unalterably separated by claustra (Figs. 3h, i, 4h, and 5k). Accessory radial pockets laterally separated from each other by interradial septa (Figs. 3h, i, and 4h). Manubrium internally lined by gastrodermis, externally by epidermis (Figs. 3i and 4h). Gonadal structures as serial gastrodermal evaginations, internal to main radial pockets (Figs. 4g, h, and 5k).

Cubozoa

Cubopolyp

Simple and undivided gastrovascular cavity, delimited by gastrodermis, from aboral (basal) to oral (apical) region (Figs. 6 and 7).

Cubomedusa

Apex of umbrella (Fig. 8a, b) with four interradial gastric septa: one thin layer of mesoglea surrounded by gastrodermis, connected to gastrodermis of gastrovascular cavity (Figs. 8c and 9a–d). Four infundibula associated with septa, blind ended, delimited by epidermis, deeply developed downwards to base of umbrella, widening orally, with broad apertures on subumbrella (Figs. 8c and 9a–c). Infundibula gradually larger toward oral region, delimiting gastric ostia, a narrow space between adjacent infundibula (Figs. 8d and 9e). Adjacent septal gastrodermis merges defining four perradial regions and dividing gastrovascular cavity (Figs. 8e and 9f), similar to formation of gastric radial pockets in staurozoan species with and without claustrum (Figs. 1, 2, 3, 4, and 5). Fusion of septal gastrodermis forms basal region of manubrium and gastric radial pockets (perradial stomach pouches), i.e., central part of gastrodermis of each septum joins forming four-sided manubrial gastrodermis while lateral parts of adjacent septa

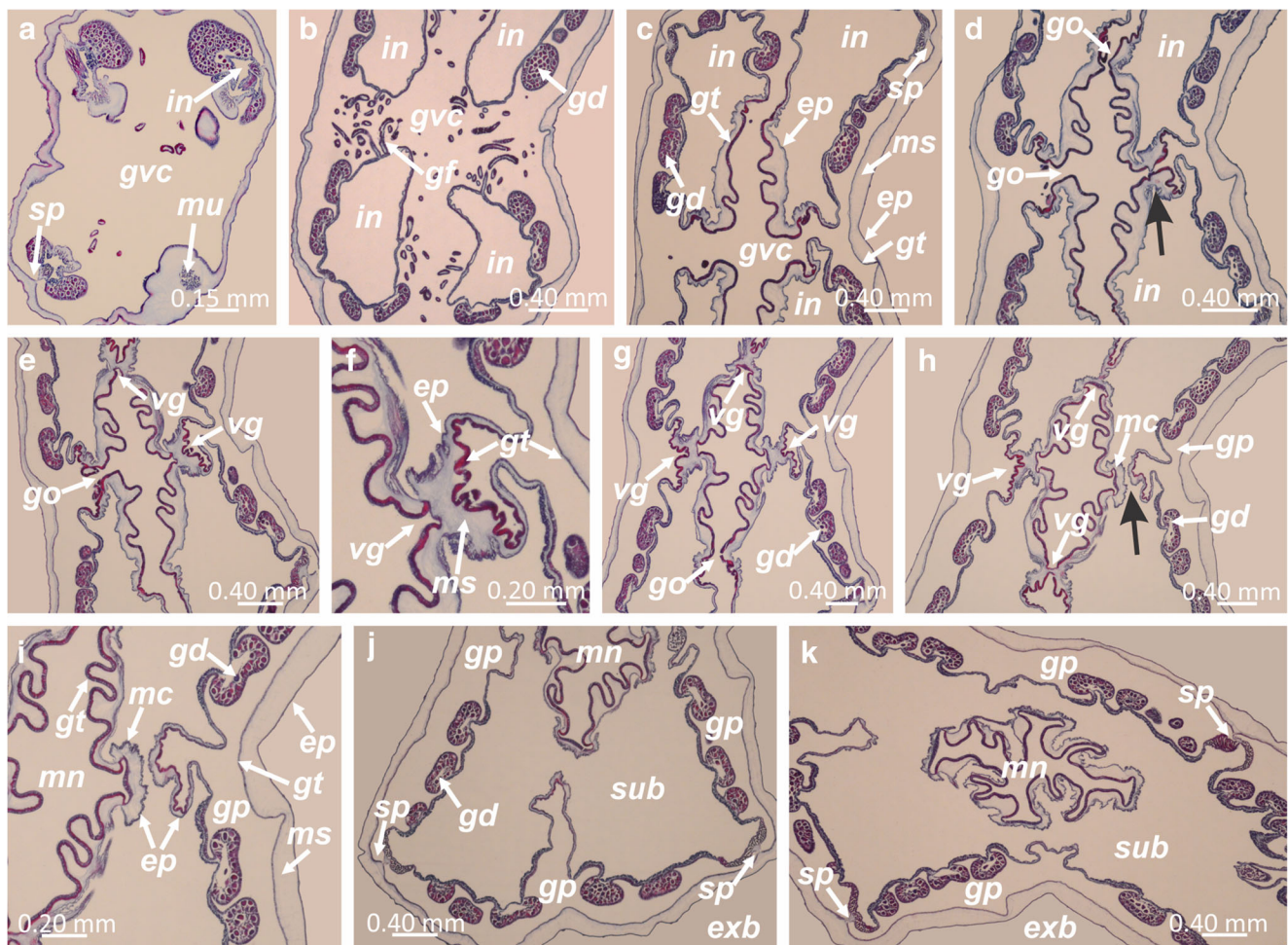


Fig. 2 Histological sections of *Halichystus tenuis* (from peduncle/calycal connection region moving upward to manubrium region in a–k) showing the gastric radial pockets and manubrium. **a** Four interradial septa (sp) and central gastrovascular cavity (gvc) at peduncle/calycal connection region; **b, c** increase of infundibula (in); **d** gastrodermis of adjacent septa start to fuse (black arrow) above the gastric ostium (go); **e** gastrodermis of adjacent septa fused, delimiting the valve of gastric ostium (vg); **f** detail of

the valve of gastric ostium (vg), a double layer of gastrodermis (gt) with central layer of mesoglea (ms); **g** delimitation of three valves of gastric ostium (vg) above gastric ostium (go); **h** epidermis of infundibula in adjacent septa fused (black arrow), delimiting central manubrium and four periradial gastric pockets (gp); **i–k** central manubrium (mn) and four periradial gastric pockets (gp) with gonads (gd). See Table 2 for additional abbreviations

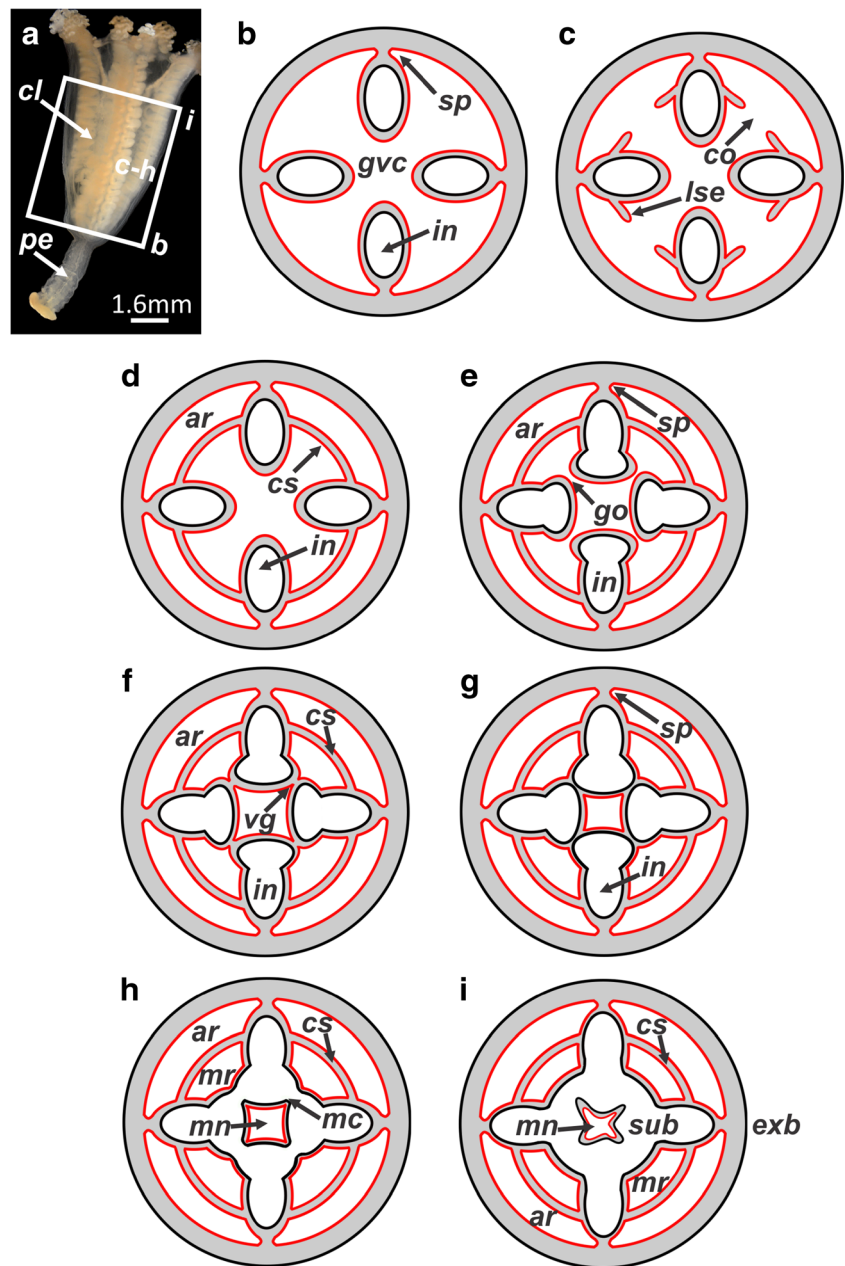
join forming gastric radial pockets (Fig. 8e). Fusion of septal gastrodermis produces a transitory membrane with double layer of gastrodermis and central layer of mesoglea, named valve of gastric ostium (Figs. 8e and 9f). Each infundibular epidermis progressively merges orally, disrupting valve of gastric ostium, but delimiting periradial suspensorium (opposite to manubrial corner), a fold of subumbrellar epidermis (Figs. 8f–h and 9g, h). Central part of each infundibular epidermis becomes manubrial epidermis, and epidermis of adjacent infundibula forms epidermis of gastric radial pockets (i.e., epidermis of subumbrella) (Figs. 8h, i and 9i). Therefore, each gastric radial pocket is formed by fusion of gastrodermis and epidermis of adjacent septa, and manubrium formed by fusion of all four septa. Four gastric radial pockets laterally separated from each other by interradial septa (Figs. 8h, i and 9i). Manubrium internally

lined by gastrodermis, externally by epidermis (Figs. 8h, i and 9i). Gonadal structures as septal gastrodermal evaginations, internal to gastric radial pockets (Fig. 9i).

Discussion

Claustra are structures delimited by the connection of lateral evaginations of adjacent interradial septa, and each claustrum is constituted by a central layer of mesoglea surrounded by gastrodermis (i.e., gastrodermis-mesoglea-gastrodermis), without communication with epidermis (Figs. 3, 4, and 5) (Gross 1900; Berrill 1963; Miranda et al. 2016b). The claustra (Gross 1900; Mayer 1910; Miranda et al. 2016b) provide an additional level of complexity to the gastrovascular system by dividing the cavity (Clark 1863; Gross 1900; Berrill 1963;

Fig. 3 General body plan of *Manania uchidai* (stauromedusa stage). **a** Preserved specimen; **b–i** schematic representations of cross-sections through the specified region in **a**. **a** Body mainly divided into oral (apical) calyx (cl) and aboral (basal) peduncle (pe); **b** four septa (sp) with four infundibula (in), and a central gastrovascular cavity (gvc); **c** lateral septal evaginations (lse), with a double layer of gastrodermis and central mesoglea, which gradually get closer, delimiting the claustra ostia (co); **d** complete connection of lateral septal evaginations, delimiting the claustra (cs) and four accessory radial pockets (ar); **e** progressively larger infundibula (in), and gastrodermis of adjacent septa getting closer, delimiting the gastric ostia (go); **f** connection of gastrodermis of adjacent septa (sp), delimiting the valve of gastric ostium (vg), a double layer of gastrodermis with central mesoglea; **g, h** gradual connection of epidermis of infundibula, delimiting the four main radial pockets (mr) and base of manubrium (mn); **i** central manubrium (mn) and eight gastric pockets, four accessory radial pockets (external) and four main radial pockets (internal), separated by claustra (cs). Legend: epidermis, black; gastrodermis, red; mesoglea, gray. See Table 2 for additional abbreviations



Collins and Daly 2005; Miranda et al. 2016a, b). They do not have a demonstrated function, but the claustra create exclusive compartments for the gonads, separating them from the gastric chambers, anchors, arms, and secondary tentacles of stauromedusae (contrasting with stauromedusae lacking claustra; see Miranda et al. 2016b), although aboral communications (claustra ostia) exist before claustra delimitation is complete (Figs. 3, 4, and 5).

Valves of gastric ostia (referred to as palatine nodes by Haeckel 1882, plate XVI, figure 6, gk) are structures at the base of the manubrium, which are also constituted by a central layer of mesoglea surrounded by gastrodermis (Figs. 1, 2, 3, 4, 5, 8, and 9) (see also Conant 1898,

which analyzed the cubomedusae *Carybdea xaymacana* Conant, 1897 and *Tripedalia cystophora* Conant, 1897). However, at the point where manubrium and gastric radial pockets (or main radial pocket in stauromedusae with claustra) are delimited, the epidermis (infundibular/subumbrellar) disrupts these structures, which never happens to claustra (Figs. 1, 2, 3, 4, 5, 8, and 9). There are also aboral communications with the main gastrovascular cavity, named gastric ostia (Figs. 1, 2, 3, 4, 5, 8, and 9).

Claustra are only present in some stauromedusae, located from the base to the margin of the calyx (Gross 1900; Mayer 1910; Miranda et al. 2016b). In contrast, the valves of gastric ostia are present in stauromedusae with and without claustra

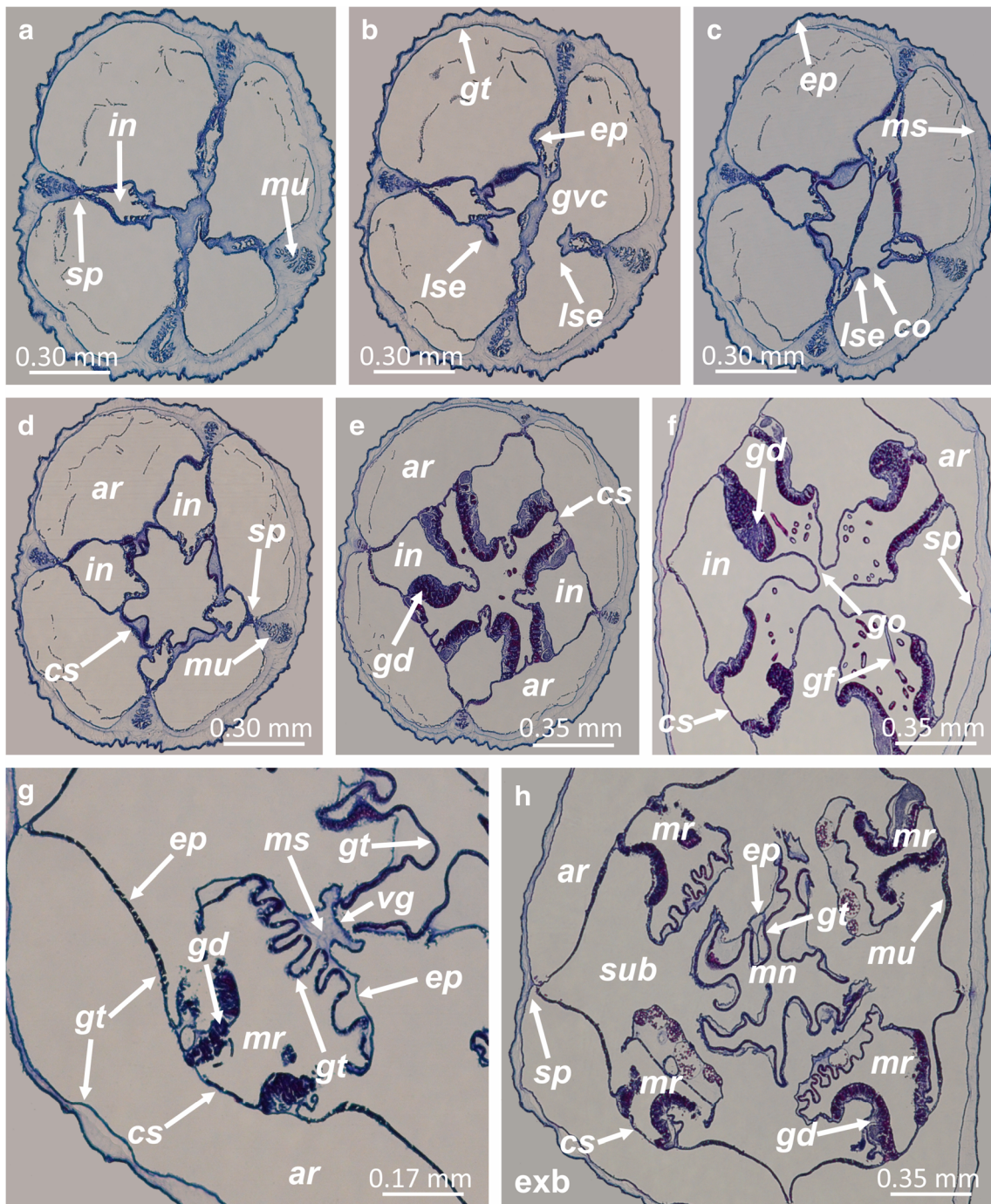


Fig. 4 Histological sections of *Manania uchidai* (from peduncle/calix connection region moving upward to manubrium region in a–h) showing accessory radial pockets, main radial pockets, and manubrium. **a** Four interradial septa (sp) at peduncle/calix connection region; **b**, **c** lateral septal evaginations (lse), with a double layer of gastrodermis and central mesoglea, which gradually get closer, delimiting the claustra ostia (co); **d**, **e** complete connection of lateral septal evaginations, delimiting the

claustra (cs) and four accessory radial pockets (ar); **f** increase of infundibula (in), delimiting the gastric ostium (go); **g** connection of gastrodermis of adjacent septa, delimiting the valve of gastric ostium (vg), a double layer of gastrodermis (gt) with central mesoglea (ms); **h** connection of epidermis of infundibula, delimiting the four main radial pockets (mr) with gonads (gd), and base of manubrium (mn). See Table 2 for additional abbreviations

(Berrill 1963; Miranda et al. 2016b) and in cubomedusae (Conant 1898), as it is involved in the delimitation of manubrium and gastric/main radial pockets (Figs. 1, 2, 3, 4, 5, 8, and 9). In stauromedusae without claustrum and in

cubomedusae, there is only one connection of septal gastrodermis at the base of the manubrium, delimiting the valves of gastric ostia (Figs. 1 e and 8e). In stauromedusae with claustra, there are two connections of septal gastrodermis, at

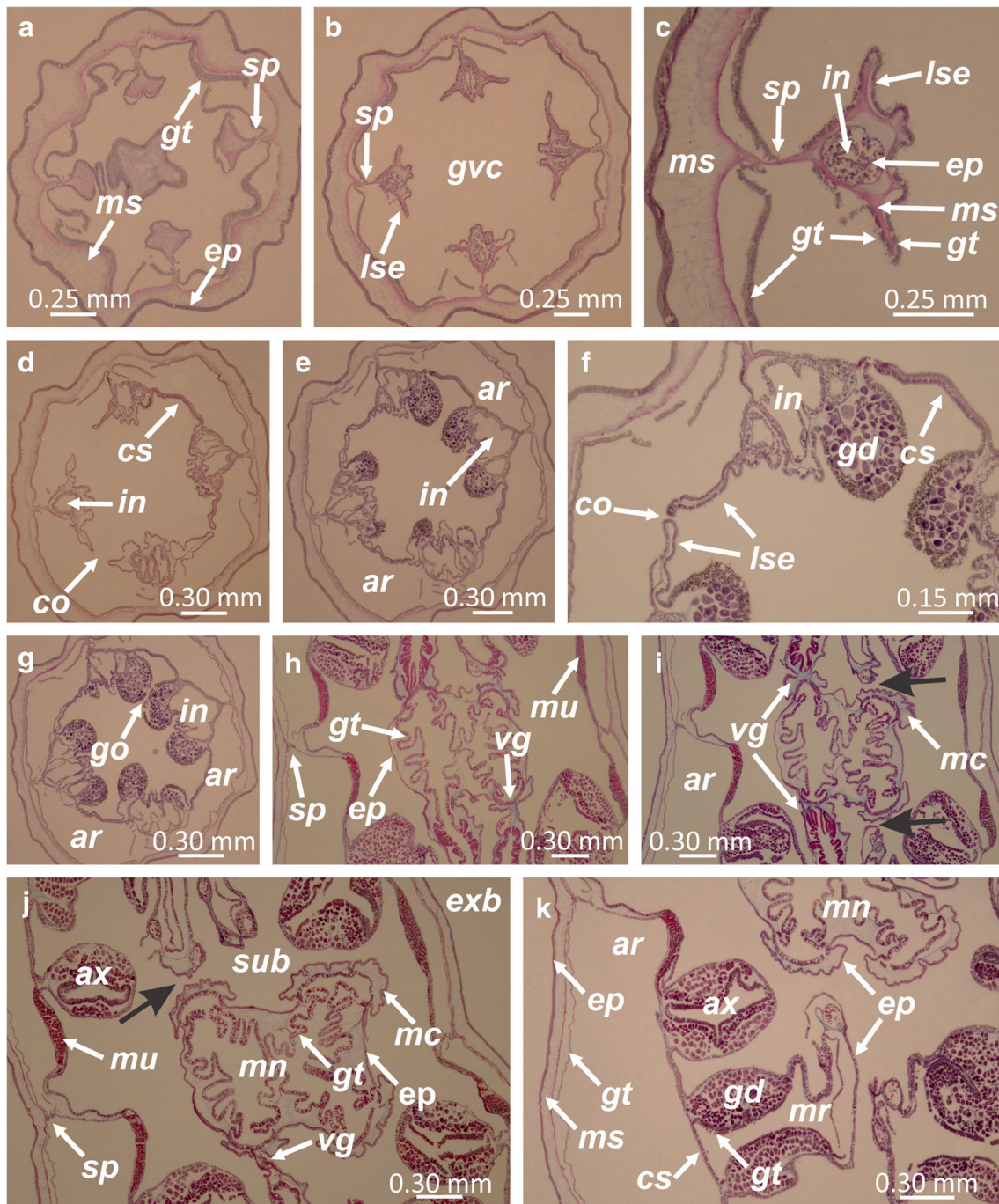


Fig. 5 Histological sections of *Craterolophus convolvulus* (from peduncle/calyx connection region moving upward to manubrium region in **a–k**) showing accessory radial pockets, main radial pockets, and manubrium. **a** Four interradial septa (sp) at peduncle/calyx connection region; **b** four septa with lateral septal evaginations (lse) and a central gastrovascular cavity (gvc); **c** detail of septum (sp) with internal infundibulum (in), and lateral septal evaginations (lse) with a double layer of gastrodermis (gt) and central mesoglea (ms); **d–f** lateral septal

evaginations gradually get closer delimiting the claustra ostia (co), and progressively the claustra (cs) and four accessory radial pockets (ar); **g** increase of infundibula (in), delimiting the gastric ostia (go); **h** connection of gastrodermis of adjacent septa, delimiting the valve of gastric ostium (vg), a double layer of gastrodermis (gt) with central mesoglea, **i–k** connection of epidermis of infundibula (black arrows), delimiting the four main radial pockets (mr) with gonads, and base of manubrium (mn). See Table 2 for additional abbreviations

the base of the calyx delimiting the claustrum (Fig. 3d) and at the base of the manubrium, equivalent to the connection in stauromedusae without claustrum and in cubomedusae, delimiting the valves of gastric ostia (Fig. 3f). Thiel (1966:

96) mentioned that “the claustrum [in Cubozoa] is formed by lateral projections of the central parts of the adjacent septa.” However, the claustrum is actually formed by lateral projections of the basal parts of the adjacent septa (Fig. 3c, d),

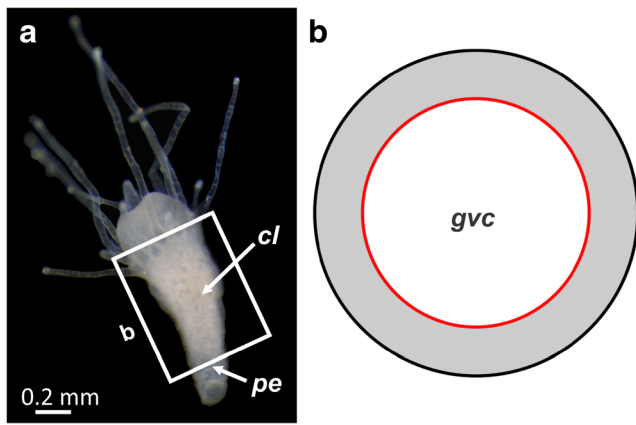


Fig. 6 General body plan of *Carybdea xaymacana* (cubopolyp stage). **a** Preserved specimen; **b** schematic representation of cross-section through the specified region in **a**. **a** Body mainly divided into oral (apical) calyx (cl) and aboral (basal) short peduncle (pe); **b** central gastrovascular cavity (gvc), without subdivisions. Legend: epidermis, black; gastrodermis, red; mesoglea, gray

whereas the valves of gastric ostia are formed by projections of the central parts of the adjacent septa (Figs. 1d, e, 3e, f, and 8d, e). Thus, cubozoans do not possess claustra.

Apparently, Krumbach was the first author to suggest the presence of claustra in Cubozoa (Krumbach 1925: 565, figure 529). Thiel used the same terminology (Thiel 1966: 79, figure 3, upper right), although he stated that Conant (1898) referred

to this structure as the “valve of gastric ostium.” According to Thiel (1966: 96) “based on the criteria of position in comparable structural systems”, the structure in Cubozoa “is homologous with the claustrum in Stauromedusae” and added that “[t]he septa are connected by a double-layered entoderm lamella, which is a real claustrum” and that the “claustrum” in Cubozoa “is very small.”

A misinterpretation in the relative position of the structures was the probable reason for Krumbach (1925) and then Thiel (1966) to suggest the presence of claustra in Cubozoa. Both authors compared the claustra of Staurozoa with the valves of gastric ostia in Cubozoa, i.e., they compared the base of the calyx in stauromedusa (aboral, Fig. 3d, e) with the base of manubrium in cubomedusa (oral, Fig. 8e, f). This assumption is clear when comparing figure 519 (*Craterolophus*) in Krumbach (1925: 539) and Fig. 2 (upper right) in Thiel (1966: 79), schematic representations of cross-sections of the base of calyx in stauromedusae showing the claustra (equivalent to our Fig. 3d, e), with figure 529 in Krumbach (1925: 565) and Fig. 3 (upper right) in Thiel (1966: 79), schematic representations of cross-sections of the base of manubrium in cubomedusae (equivalent to our Fig. 8e), supposedly showing the claustra but actually illustrating the valves of gastric ostia. Probably, that is also the reason that led Thiel (1966) to conclude that the “claustrum” in Cubozoa “is very small”, because the valve of gastric ostium is smaller than the real claustrum (Figs. 3d–f, 4d–g, and 5g–j). Both Krumbach

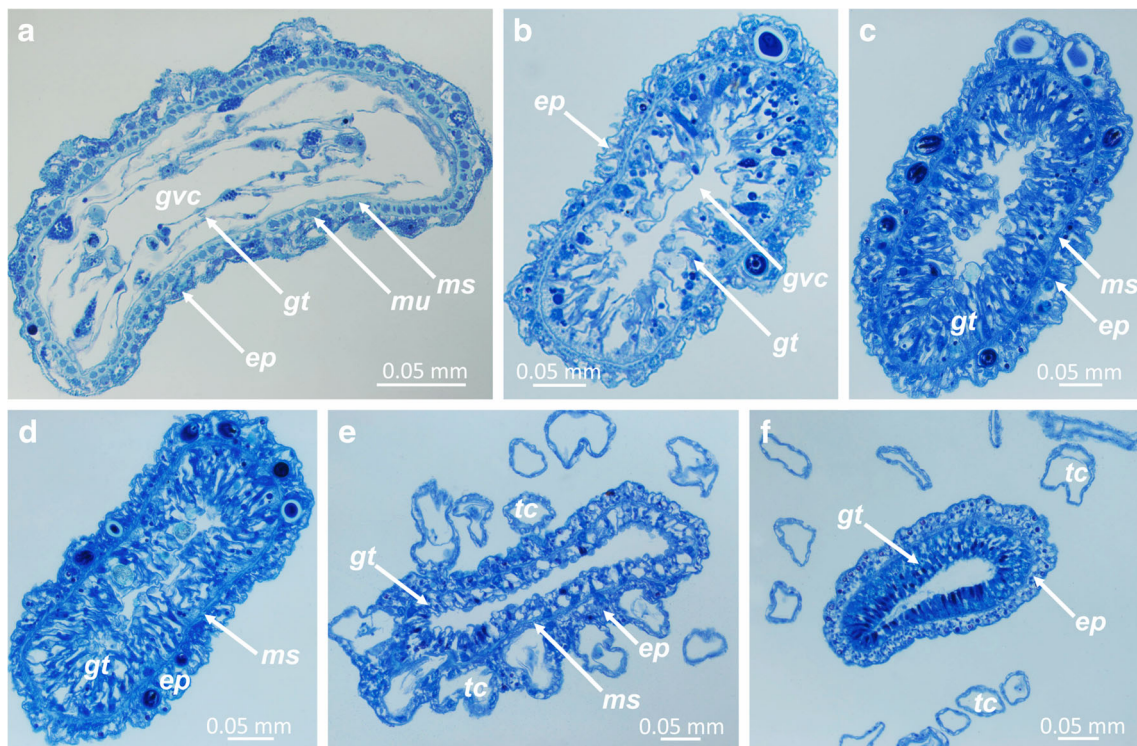


Fig. 7 Histological cross-sections of *Carybdea xaymacana* (cubopolyp stage). **a–f** Sections from aboral (basal) to oral (apical) regions; simple body, with a central gastrovascular cavity (gvc), without subdivisions. Staining: **a–f** Toluidine blue. See Table 2 for additional abbreviations

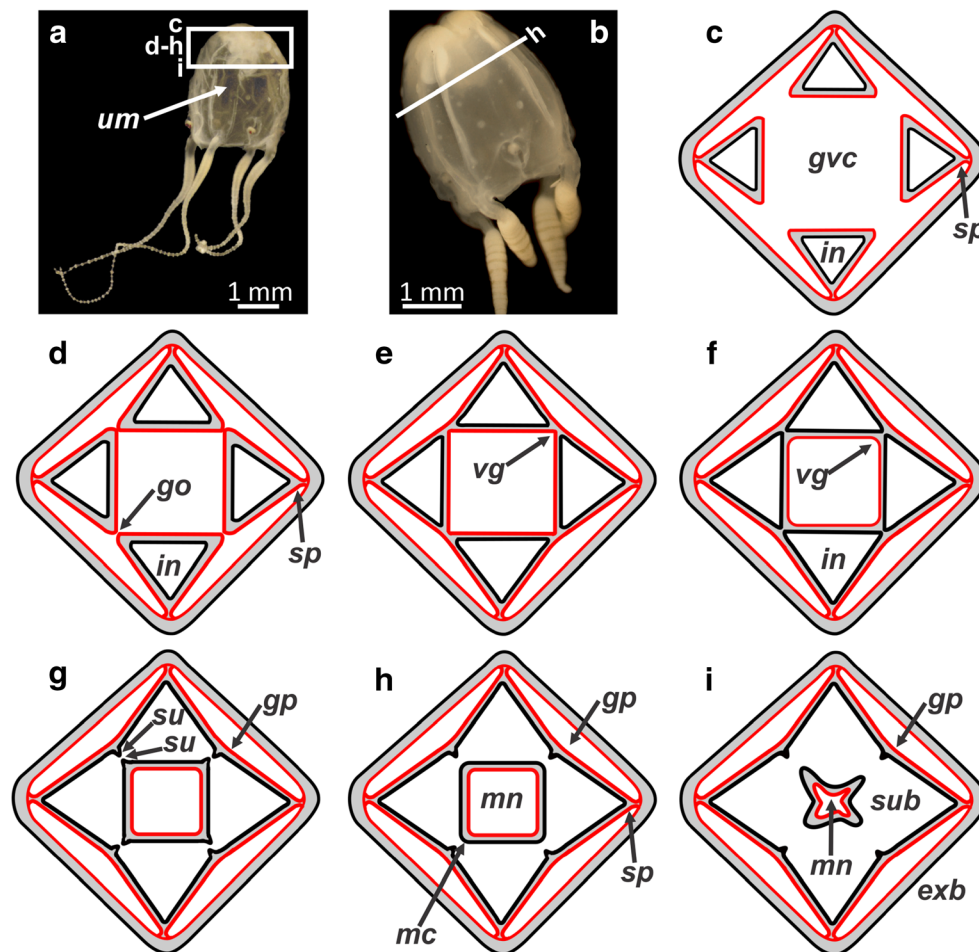


Fig. 8 General body plan of *Copula sivickisi* (cubomedusa stage). **a, b** Preserved specimens; **c–i** schematic representations of cross-sections through the specified regions in **a** and **b** (from medusa apex to oral region). **a, b** General view of cubomedusa umbrella (um); **c** four septa (sp) with four infundibula (in), and a central gastrovascular cavity (gvc); **d** progressively larger infundibula (in), and gastrodermis of adjacent septa getting closer, delimiting the gastric ostia (go); **e** connection of

gastrodermis of adjacent septa (sp), delimiting a double layer of gastrodermis with central mesoglea, the valve of gastric ostium (vg); **f–h** gradual connection of epidermis of infundibula, delimiting the suspensorium (su), four gastric pockets (gp) and base of manubrium (mn); **i** central manubrium (mn) and four gastric pockets (gp) separated by septa. Legend: epidermis, black; gastrodermis, red; mesoglea, gray. See Table 2 for additional abbreviations

(1925) and Thiel (1966) did not present any cross-section of the base of the manubrium of stauromedusae (oral, Figs. 1e and 3f), contributing to their misinterpretation. Therefore, the “claustrum” in cubomedusae (Krumbach 1925; Thiel 1966) is the valve of gastric ostium (Conant 1898), a structure at the base of the manubrium also present in stauromedusae with and without a real claustrum (Clark 1863; Haeckel 1882; Gross 1900; Uchida 1929; Uchida and Hanaoka 1933; Miranda et al. 2016b). Consequently, the claustrum, a tissue from the base to the margin of calyx, is only present in stauromedusae species of the genera *Craterolophus*, *Depastrum*, *Depastromorpha*, *Halimocyathus*, and *Manania* (Miranda et al. 2016a, b).

In stauromedusae with claustra, there are eight radial pockets, four accessory radial pockets, or exogons (Haeckel 1879; Gross 1900; Berrill 1963; Thiel 1966), directly

associated with chambers in peduncle, anchors and arms, and four main radial pockets (Miranda et al. 2016b), or mesogons (Haeckel 1879; Thiel 1966), associated with manubrium and gonads (Figs. 3, 4, and 5) (Miranda et al. 2016b). In stauromedusae without claustrum and in cubomedusae, there are only four gastric radial pockets (Figs. 1, 2, 8, and 9). These pockets occupy the same position as the accessory radial pockets (i.e., external) of stauromedusae with claustra, but they are homologous to the main radial pockets (which are internal) (Gross 1900; Berrill 1963; Miranda et al. 2016a, b). The main radial pockets of stauromedusae with claustra are considered the true gastric radial pockets, because they are delimited by the same structures (including epidermis) and also contain the gonads (Berrill 1963; Miranda et al. 2016b).

Another misconception stemming from the incorrect assertion of the presence of claustra in Cubozoa is the location of the

gonads. Thiel's evolutionary hypothesis (Thiel 1966: 110, figure 24) describes "gonads in exogon pockets" present in Cubozoa and Scyphozoa, whereas "gonads in mesogon pockets" are present in Staurozoa, and this was interpreted as a putative difference among the classes (cf. Han et al. 2013: 13). However, this separation is erroneous and, therefore, its adoption to separate taxa is incorrect as well. In stauromedusae with claustra, the gonads are indeed located in the main radial pocket (mesogon, Fig. 3h, i) (Gross 1900; Uchida and Hanaoka 1933; Berrill 1963; Thiel 1966; Miranda et al. 2016b). However, in stauromedusae without claustrum and in cubomedusae, the gonads are located in the gastric radial pocket (Figs. 1g, h and 8g–i), also called "stomach pouch" (Conant 1898; Uchida 1929; Berrill 1963; Miranda et al. 2013, 2016b), which, as discussed above, occupy the same position as the exogon (accessory radial pocket; Fig. 3h, i) in stauromedusae with claustra. Therefore, stauromedusae without claustrum have gonads in the same topological position as cubomedusae. Indeed, because the main radial pockets of stauromedusae with claustra are considered true gastric radial pockets, delimited by the same structures including epidermis (Berrill 1963; Miranda et al. 2016b), gonads are in a similar position throughout Staurozoa and Cubozoa.

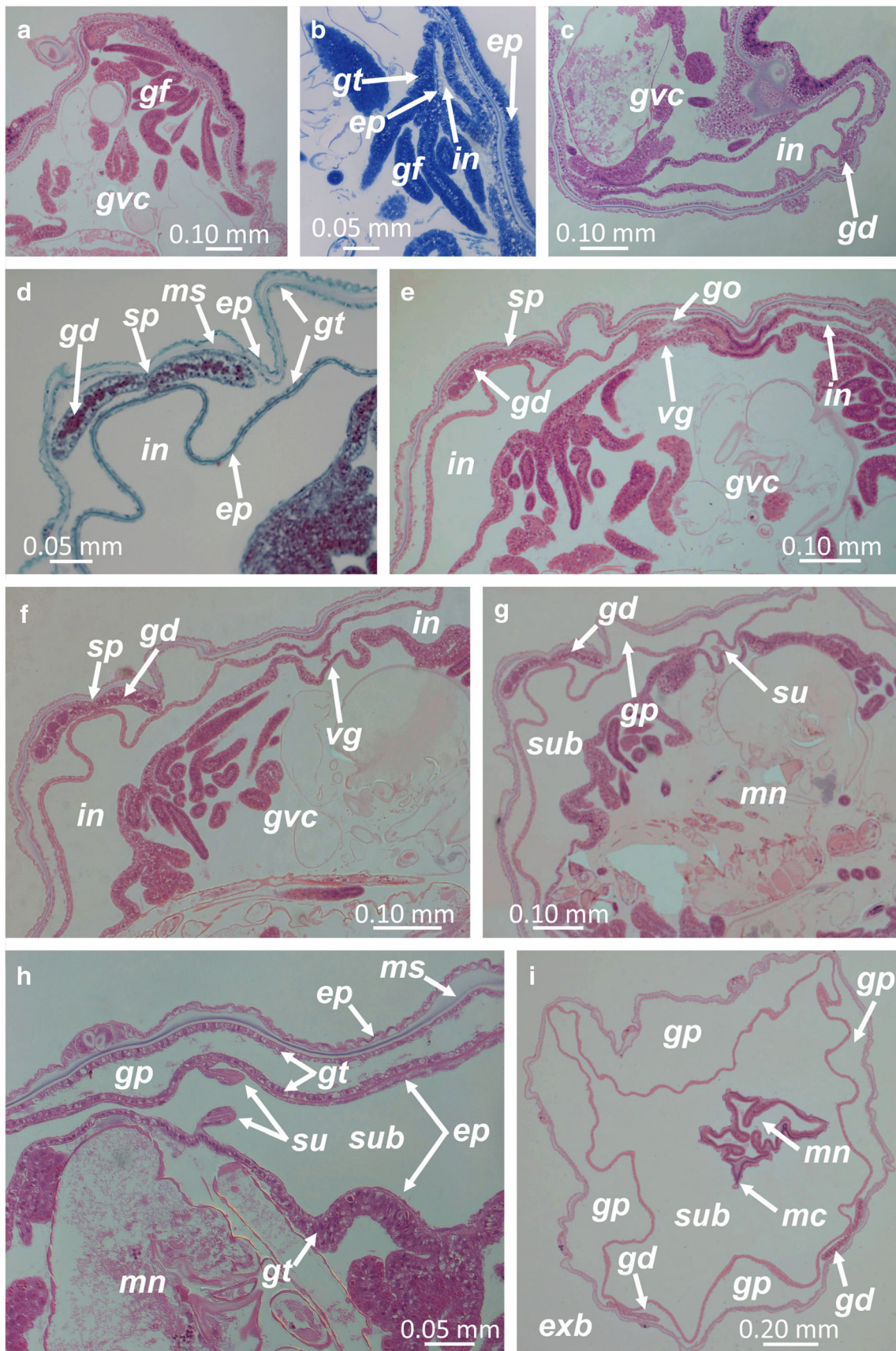
The hypothetical presence of the claustrum has played "a fundamental role" in the interpretation of early Cambrian pentamerous fossils as cubozoans (Han et al. 2013: 14). It was assumed that those fossils could not be assigned to Anthozoa, Hydrozoa, or Scyphozoa because the claustrum has never been reported in these groups (Han et al. 2013: 13). However, the claustrum is also not present in Cubozoa and, therefore, its hypothetical presence in fossils cannot be used to support a cubozoan affinity (cf. Han et al. 2016). The arrangement of gonads to distinguish Staurozoa and Cubozoa, in which the gonads in Staurozoa with claustra "develop within the mesogonial pockets rather than the exogonial pockets as in cubozoans" (Han et al. 2013: 13), is not a distinguishing character because cubozoans do not have claustra and their gonads occupy the same position as those of stauromedusae without claustrum. Han et al. (2013: 13) also stated that "living cubozoans have only two kinds of pockets separated by claustra: exogonial and mesogonial", citing Thiel (1966), but this is a misinterpretation because Thiel (1966: 96) stated that "[c]orresponding to the situation in Stauromedusae, one would expect mesogon and exogon pockets in Cubomedusae as well. This, however, is not the case [...]. While the exogon pockets, called gastric pockets, exist, there are no mesogon pockets." Finally, Han et al. (2013) proposed that "so many new gastric pockets in the current fossils are derived from further partitioning of the exogonial pockets and mesogonial pockets because new lamellae arise", an incorrect hypothesis based on a misunderstanding of the anatomy of extant cubozoans. Also, within a paleontological context, the claustrum was considered present in Cubozoa, some Staurozoa, and some fossils (e.g.,

Punctatus, *Quadrupyrigites*) (Han et al. 2016, Appendix 1, character 97, claustrum: 0—absent, 1—present; and figure 9), and the state might have been mistakenly assumed as "present" for Scyphozoa. We conclude that the affinity hypothesis of these early Cambrian pentamerous fossils, as proposed by Han et al. (2016), are refuted based on deficiencies in their morphological interpretation of extant and fossil animals, and new possibilities should be proposed.

Thiel (1966: 109) proposed that "before the origin of Cubomedusae and Stauromedusae, the claustrum developed." This hypothesis was followed in several phylogenetic studies, always citing Thiel (1966). Analyses of the small subunit of the ribosome found conflicting results based on parsimony and maximum likelihood and it was incorrectly proposed that the claustrum could favor the parsimony topology, which indicated a close relationship between Staurozoa and Cubozoa (Collins 2002). Another evolutionary hypothesis for Cnidaria based on morphology and life cycle considered that the septal shape (character 63), more specifically the state 1 (y-shaped), would be a putative synapomorphy of the clade Staurozoa, Cubozoa, and Conulatae, and that "[t]hese septa often form a claustrum" (Marques and Collins 2004, figure 1). Collins and Daly (2005: 228) hypothesized that the claustrum "was present in the ancestral medusozoan and subsequently lost independently in lineages leading to Hydrozoa and Scyphozoa", which is unlikely given the limited distribution of the claustrum with Staurozoa. Again, these hypotheses have to be refuted based on misinterpretations of the morphology of the claustrum.

In conclusion, the claustrum is a character present only in some species of Staurozoa, and the structure called "claustrum" in Cubozoa (Krumbach 1925; Thiel 1966) corresponds to the valve of gastric ostium (Conant 1898), universally present in stauromedusae, irrespective of the presence or absence of claustra (Uchida 1929; Berrill 1963; Miranda et al. 2016b). Therefore, due to the conjunction test (see de Pinna 1991), these structures cannot be considered homologous and, therefore, the presence of claustra cannot be used to support a close relationship between Staurozoa and Cubozoa (see Collins 2002; Marques and Collins 2004) nor marshaled as evidence of cubozoan affinities (see Han et al. 2013, 2016). The presence of valve of gastric ostium at the base of manubrium in other medusozoan classes remains to be assessed.

Inside Staurozoa, the claustrum has been compared in the distantly related species *Craterolophus convolvulus* and *Manania uchidai* (Naumov, 1961), with great similarity of the structures, both being lateral projections of the septa at the base of the calyx and constituted exclusively of mesoglea and gastrodermis (Miranda et al. 2016b). This was also observed in other species of *Manania* (Uchida and Hanaoka 1933; Berrill 1963) and *Depastromorpha africana* Carlgren, 1935 (monospecific genus; Carlgren 1935). There is no detailed information of claustrum anatomy for *Depastrum cyathiforme*



◀ **Fig. 9** Histological sections of *Copula sivickisi* (from the apex of umbrella moving downward to manubrium region in **a–i**) showing gastric radial pockets and manubrium. **a–c** Delimitation of interradial septum (sp) and central gastrovascular cavity (gvc) at the apex of umbrella; **d** detail of septum (sp) with infundibulum (in) and lateral gonads (gd); **e, f** gastrodermis of adjacent septa start to fuse delimiting the gastric ostium (go) and gradually the valve of gastric ostium (vg), a double layer of gastrodermis, with central layer of mesoglea; **g** epidermis of infundibula in adjacent septa fused, delimiting suspensorium, central manubrium (mn) and four perradial gastric pockets (gp) with gonads (gd); **h** detail of suspensorium (su); **i** four gastric radial pockets (gp) with gonads (gd) and central manubrium (mn). Staining: a Hematoxylin-eosin (HE); b toluidine blue; c periodic acid-Schiff + hematoxylin; d Gomori's trichrome; e–i HE. See Table 2 for additional abbreviations

(Sars, 1846) (monospecific genus; Clark 1863; Mayer 1910; but see Allman 1860, figure 4) or for *Halimocyathus platypus* Clark, 1863 (monospecific genus; see Clark 1863). Based on the working hypothesis of staurozoan relationships (Miranda et al. 2016a), we consider the claustrum to be a homoplastic character that appeared at least twice in the evolution of stalked jellyfishes, in *Craterolophus* and in the clade (*Manania*, (*Depastromorpha*, *Halicyclustus*), and it was lost in *Halicyclustus* (ACCTRAN optimization; Miranda et al. 2016a: 23, 28, figure 11). Studies on the evolutionary relationships and detailed internal anatomies of *D. cyathiforme* and *H. platypus* (species not included in the staurozoan phylogeny; Miranda et al. 2016a) are crucial for more fully understanding the evolution of this character within Staurozoa.

Acknowledgments The authors are grateful to Enio Mattos (IB-USP) and José Eduardo A. R. Marian (IB-USP) for their kind assistance with the histological procedures. We are also grateful to the editor Andreas Wanninger and to an anonymous reviewer whose comments helped improve the quality of the manuscript. This contribution involved the participation of scientists of NP-BioMar.

Funding This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) 2010/07362-7 (LSM), 2015/23695-0 (LSM), 2010/50174-7 (ACMo), 2015/21007-9 (ACMo), 2011/50242-5 (ACMa), 2013/50484-4 (ACMa); Comissão de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) PDSE 16499/12-3 (LSM), PNPd (LSM); and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) 142270/2010-5 (LSM), 301039/2013-5 (ACMo), 304961/2016-7 (ACMo), 474672/2007-7 (ACMa), 562143/2010-6 (ACMa), 305805/2013-4 (ACMa), 445444/2014-2 (ACMa).

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