Redescription of the deep-sea benthic ctenophore genus *Tjalfiella* from the North Atlantic (Class Tentaculata, Order Platyctenida, Family Tjalfiellidae)

Short Running Title: The deep-sea benthic ctenophore *Tjalfiella*

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Abstract -

Some of the most fascinating and poorly known animals on this planet are comb jellies of the phylum Ctenophora. About one-quarter of ctenophore richness is encompassed by the benthic species of the order Platyctenida, nearly all known from shallow waters. In this work, we integrate several systematic methods to elucidate an enigmatic genus, *Tjalfiella,* known previously only from deep waters near the western coastline of Greenland in the North Atlantic. For the first time, we employ microCT on museum specimens – one nearly 100 years old from the type locality of the only known species of the genus, *T. tristoma* – of extant ctenophores to visualize and compare their anatomy. With these data, we integrate in situ videography and genetic sequence data derived from newly collected deep sea specimens observed via NOAA Ship Okeanos in 2018 and 2022 at two distant localities in the North Atlantic, near North Carolina, USA, and the Azores, Portugal. The genetic data indicate that the newly collected specimens represent closely related but distinct species of *Tjalfiella*. However, neither can be named at this time because neither one could be definitively differentiated from *T. tristoma,* given that microCT and in situ imagery reveal striking morphological similarities and only variation in color and host preference. Despite the lack of new species descriptions, this work characterizes both the morphology and genetics of the benthic ctenophore genus *Tjalfiella* and specimens representing species within it, advancing our understanding of a rarely observed component of the deep-sea fauna.

Key Words - Deep sea coral associates, microCT, mitochondrial genome, ribosomal RNA, systematics

1. INTRODUCTION -

Ctenophores, also called comb jellies, include roughly 200 “accepted” marine species that are primarily planktonic and found throughout the world's oceans (Mills 2024). In general, ctenophores possess a wide variety of morphologies but typically can be recognized by the presence of swimming structures composed of fused macrocilia arranged in eight ctene rows (Parker 1905), a rotationally symmetrical body plan (Dunn et al. 2015), an apical sensory structure (statocyst), and either colloblasts (multicellular secretory organs) (Eeckhaut et al. 1997; Komai 1922; Mackie et al. 1988) or macrocilial teeth (Tamm & Tamm 1988) used to capture prey.

Taxonomic work on the phylum has been severely hindered for several reasons, including the fact that much of the diversity inhabits deep sea environments and that many species are structurally delicate, rendering them challenging to observe live and extremely difficult to preserve for later observation. Amongst the ctenophores, species in the order Platyctenida are unique in living attached to the benthos on a variety of substrates around the world's oceans. Platyctenids have dorso-ventrally compressed bodies that lack ctene rows in the adult/mature life stage while possessing them during the larval phase of their meroplanktonic life cycle (Glynn et al. 2019; Komai 1941; Mortensen 1912), which likely explains why many initial studies saw them as intermediate forms by which ctenophores gave rise to turbellarian flatworms (Kowalevsky 1880; Lang 1884; Willey 1896). Unlike flatworms and other similarly shaped benthic taxa, platyctenids possess two threadlike tentacles on opposing sides of the body, a branching gastrovascular system, and numerous papillae surrounding a central statocyst on the aboral surface (Devanesen & Varadarajan. 1942; Komai 1922). Indeed, subsequent work provided ample evidence that platyctenids are derived ctenophores, likely from a generalized cydippid-form ancestor (Komai 1922; Mortensen 1912), and a long series of phylogenetic analyses based on morphology, ribosomal genes, and RNAseq data, have concluded that Platyctenida is monophyletic and that the benthic habit is a derived character (Harbison 1985; Podar et al. 2001; Simion et al. 2015; Townsend et al. 2020; Whelan et al. 2017, Christianson et al. 2021).

From a taxonomic perspective, it is fortunate that platyctenids generally have superior preservation potential compared to most ctenophores, allowing for 49 species (Mills 2024) to be described within six genera: *Coeloplana* Kowalevsky, 1880, *Ctenoplana* Korotneff, 1886, *Lyrocteis* Komai, 1941, *Savangia* Dawydoff, 1950, *Tjalfiella* Mortensen, 1910, and *Vallicula* Rankin, 1956. However, of the known species, only three are known to live in the Atlantic Ocean, including *Vallicula multiformis* Rankin, 1956, *Coeloplana waltoni* Glynn et al. 2018, and *Tjalfiella tristoma* Mortensen, 1910, and nearly all (47 of 49) are from waters shallower than 200 meters.

[APPROXIMATE POSITION for Figure 1.]

Among the species known to live in the Atlantic Ocean, *Tjalfiella tristoma* is easily distinguished by its deep habitat and unique and well described morphology. Still, there is a lack of recent observations and representation in the literature, limiting our understanding of its geographic range and other fundamental aspects of its biology. Initially described in great detail by Dr. Theodore Mortensen (1910; 1912) living on the pennatulid Umbellula at 475–575 m on the western coastline of Greenland (Mortensen, 1912), *T. tristoma* can be distinguished from other platyctenids by eight enormous globular gonads that encircle the statocyst, numerous brood pouches that line the oral skirt, and two upright large aboral arms that lack an oral groove and house simple tentacles, i.e., tentacles without tentilla (Mortensen 1910; Fig. 1). Since its initial discovery in 1910, only the Godthaab expedition led by P.L. Kramp in 1928 confirmed the presence of *T. tristoma* on Umbellula at a locality near the original type locality and the genus *Tjalfiella* in the North Atlantic (Kramp 1942). Unfortunately, the holotype and paratypes collected by Mortensen have since deteriorated in the collections at the Natural History Museum in Denmark (L. Pavesi, pers. comm.). The only remaining definitive material of the species comes from the seven samples collected and preserved in formalin by Kramp during the Godthaab expedition.

More recently, in 2018 and 2022, NOAA Ocean Exploration, with the NOAA Ship *Okeanos Explorer* and ROV *Deep Discoverer*, observed and collected several individual platyctenids of similar forms attached to the coenenchyme of *Acanella* Gray, 1870 and *Adinisis* Lapointe & Watling, 2022 octocorals in two relatively distant localities of the North Atlantic Ocean (Fig. 2). Using the original descriptions from Mortensen (1910, 1912) and the additional collected samples from Kramp, the present study reviews and redescribes Mortensen's genus *Tjalfiella* and species *T. tristoma*. In addition, we characterize, both genetically and morphologically, the newly available material from the North Atlantic, which appears to represent two closely related species. We label the new material as *T.* aff. t*ristoma* because, at this time, we are unable to determine if either is equivalent to *T. tristoma*. The present study integrates evidence from microCT morphological scans of museum specimens, genome skimming to derive mitochondrial genomes and nuclear ribosomal loci of the ribosomal repeat (*18S*, *ITS1*, *5.8S*, *ITS2*, and *28S*) from the more recently collected specimens, and high-definition video observations. Redescribing the genus *Tjalfiella in comparison to other known benthic ctenophores* represents a necessary step for advancing the systematics of Platyctenida and, by extension, Ctenophora.

[APPROXIMATE POSITION for Figure 2.]

2. MATERIALS AND METHODS –

2.1 Collection and observation -

On July 1st, 2018, the ROV *Deep Discoverer* observed and collected (EX1806_D17_02B_A01; UNSM-IZ-1490693) three individual benthic ctenophores of similar morphology on a single octocoral belonging to the octocoral genus *Acanella* (Fig. 3A) at a depth of 1866.3m, 36°13'48.0" N 74°28'12.0" W, roughly 75 km east of the coast of North Carolina, USA (Fig. 2). Additionally, seven platyctenids with similar morphology were observed on four *Acanella* sp. on the same dive at 1840-1870 meters but were not collected (Fig. 4). The *Deep Discoverer* is part of a two-body ROV system capable of 6000m that includes the *Deep Discoverer* (3.16×1.96×2.59 meters) and the camera sled Seirios (Galvez et al., 2024). The video was recorded with an Insite Pacific "Zeus Plus" HD Video Camera, producing videos at a resolution of 1920×1080 pixels, using a frame rate of 29.97i, and the ProRes 442 @ 139 Mbps codec. Lighting was provided by Deepsea Power and Light "Sealite Sphere" LED lamps (190,000 lumens total) and two Deepsea Power and Light "LED Multi SeaLite" auxiliary lamps. Color-correcting procedures were employed when the cameras reached a stable operating temperature at depth and were held constant throughout the dive. Video Form Monitor and Vector Scope and a color chip from DSC Labs were used to obtain accurate and unbiased colors in the ROV video.

[APPROXIMATE POSITION for Figure 3.]

Additionally, on July 24th, 2022, the *Deep Discoverer* ROV (same equipment utilized as in 2018) observed a single occurrence of nine platyctenids on the coenenchyme (surface) of an octocoral in the *Adinisis* genus (Fig. 5) at a depth of 1061.43m, 42°20'22.9"N 29°08'59.3"W, approximately 300 km north of the Azores, Portugal (Fig. 2). Seven of the nine specimens were collected along with the host octocoral (EX2205_D05_04B_A03; USNM-IZ-1674065).

The specimens collected by Kramp in 1928 from 685m at Umanak Fjord (Kramp, 1942) were borrowed from the Natural History Museum in Denmark (NHMD88841). The specimens were photographed and illustrated using the microscopy techniques described below.

2.2 Fixation and Preservation -

Once collected via NOAA Ship *Okeanos Explorer*, specimens were gently separated from the coral substrate using a pipette. Of the collected specimens from 2018, one was placed in 95% ethanol for DNA extraction, a second was also preserved as a morphological voucher using 95% Ethanol (EX1806_D17_02B_A01), and the third appears to have been lost during collection. The two remaining specimens were transferred and cataloged into the Smithsonian National Museum of Natural History (NMNH) Invertebrate Collection (USMN 1490693). The body of the preserved specimen experienced minor shrinkage, wrinkling of the epidermis, and contraction. The overall structure of the sample remained intact with minimal tissue deterioration and deformation, other than the shrinkage.

Additionally, from the seven samples collected in 2022, one specimen was preserved for DNA extraction in 95% ethanol, while the remaining six specimens collected were preserved for morphological investigation in 70% ethanol (EX2205_D05_04B_A03), and later cataloged in the NMNH invertebrate collection (USNM 1674065). The preserved specimens experienced body shrinkage but to a lesser extent than the 2018 specimen because of the difference in ethanol concentration used for fixation.

[APPROXIMATE POSITION for Figure 4.]

[APPROXIMATE POSITION for Figure 5.]

2.3 DNA Extraction and Sequencing

DNA was extracted using an AutoGenPrep 965 automated DNA extraction robot (AutoGen, Holliston, MA, USA) following the manufacturer's tissue protocols. Enzymatically sheared libraries were prepared using the NEB Ultra II FS DNA library prep kit (New England Biolabs), targeting an insert size of approximately 400 bp. Our libraries were amplified using six cycles of PCR following the kit manufacturer's chemistry and thermocycler recommendations. We employed iTru y-yoke adapter stubs and iTru unique dual indices (Glynn et al. 2019) rather than NEB adapters and indices, tailoring the amount of adapter based on DNA concentrations specified in the NEB guidelines. A Qubit dsDNA HS assay (Thermo Fisher Scientific, Waltham, MA, USA) run on a High Sensitivity D1000 ScreenTape (Agilent, Santa Clara, CA, USA) was used to quantify individual library size. Sequencing (150 bp, paired-end reads) of equimolar pooled libraries was achieved using a NovaSeq 6000 (Illumina Inc., San Diego, CA, USA).

The resulting reads for each of the specimens were trimmed of adapters and poor-quality sequences using FastP (Shifu et al. 2018) and then assembled using SPAdes (Prjibelski et al. 2020). Mitofinder (Allio et al. 2020) was then used to identify the assembled mitochondrial genome and to create draft annotations of protein-coding genes. Mitochondrial genome annotations were visualized and checked for accuracy of start and stop codons, as well as translation using Geneious Prime (v. 2023; https://www.geneious.com). Every gene region was compared to available reference mitochondrial genomes in NCBI, four from RefSeq (NC_016117, NC_038065, NC_045305 and NC_045864, none of which represent platyctenes) and three available platyctene mitochondrial genomes (LN898113; *Coeloplana loyai*, LN898114; *Coeloplana yulianicorum*, and LN898115; *Vallicula multiformis*, all derived by Arafat et al. 2018). The ribosomal repeat regions were assembled using the Map to Reference function and built-in Geneious mapper with the sensitivity set to ``medium/low" and multiple iterations starting with reference sequences of *18S* derived from other ctenophores.

To conduct phylogenetic analyses, mitochondrial *cox1* and nuclear *18S* were aligned to available platyctenid sequences in GenBank using MAFFT (Katoh et al. 2013). For each dataset, the best fitting model was selected using Smart Model Selection in PhyML, using the AIC criterion (Lefort et al. 2017). Phylogenetic relationships were inferred using the Maximum Likelihood method (ML) on PhYML 3.0 software (Guindon et al. 2010), assessing node support with bootstrap indices (400 replicates) assuming the following models of nucleotide evolution: mitochondrial *cox1*: GTR+R; nuclear *18S*: GTR+I. Bootstrap values below 70 are considered not supported, following Hillis and Bull (1993). All trees were rooted with the outgroup *Lampea, following the results of Simion et al. (2015) and Townsend et al. (2020).*

2.4 Microscopy and Histology

The condition of all preserved samples was evaluated under an Amscope Trinocular Stereo Microscope (SW-2T13Z) equipped with a mounted T5i Canon camera and external lighting. Additionally, body proportions and dimensions of collected animals were measured and documented using the software ImageJ (Table 1). Due to the opaqueness of the preserved specimens, samples were prepared for a non-destructive histological analysis using microCT.

Three specimens, including one definitive specimen of *T. tristoma* from the Umanak Fjord, Greenland, one specimen of *T.* aff. *tristoma* -2018 from off the coast of North Carolina, USA, and one specimen of *T.* aff. *tristoma* -2022 from near the Azores, Portugal, were stained in 20mL centrifuge tubes with 4% Phosphotungstic acid (PTA) in 70% ethanol for 13-18 days ($\bar{x}$ - 14 days). Specimens were scanned using a GE Pheonix vitomeix M 240/180 kV Dual Tube microCT machine at the Smithsonian National Museum of Natural History Imaging Laboratory. For scanning, the specimens were propped up using spare pieces of packing styrofoam and foam sheets in a 1.5mL microcentrifuge tube filled with 70% ethanol. The microcentrifuge tube was then affixed to a small plastic rod and mounted in the holder of the machine.

Whole-body scans of each sample were conducted using the procedures used in Phillips and Goetz (2023). Scans were exported as stacked image files and later imported into the 3D reconstruction software ORS Dragonfly version 2020.1 (Dragonfly 2020). The datasets generated and analyzed in this study are available in the MorphoSource repository (*T. tristoma* - 000607650, *T.* aff. *tristoma* (2018) - 000607670, *T.* aff. *tristoma* (2022) - 000607586). Individual tissue layers were colorized in the stomodeal, tentacular, and apical planes to identify internal and external anatomical features utilizing the 3D brush feature (Fig. 6).

[APPROXIMATE POSITION for Figure 6.]

3. RESULTS AND DISCUSSION

3.1 Genetics

BioSample, SRA, and assembled nucleotides for mitochondrial genomes and ribosomal repeats were registered with National Center for Biotechnology Information (NCBI) (Table 2) under NCBI BioProject PRJNA1072111. The SRA dataset for USNM_IZ_1490693 contained 8,255,776 reads, while the two derived SRA libraries from USNM_IZ_1674065 contained 25,755,884 and 27,033,550 reads, respectively.

The derived ribosomal repeat regions containing complete *18S*, *ITS-1*, *5.8S*, *ITS-2*, and *28S* are 6100 and 6072 bp, respectively, and had average coverage of 135 and 599, respectively, the latter based only on the first of the two generated libraries. The two samples have a genetic divergence of 0.69% across the entire ribosomal repeat region (0.0% in 18S, 5.67% in *ITS2-5.8S-ITS2*, and 0.21% in *28S*).

Mitochondrial genomes derived from USNM_IZ_1490693 and USNM_IZ_1674065 were both complete and circularized, with lengths of 11397 (coverage 172X) and 11020 (coverage 389X) bp, respectively. Like those of other sampled platyctene species (Arafat et al. 2018), the assembled mitochondrial genomes are particularly AT-rich, consisting of roughly 25% A's, 60% T's, 5.5% C's, and 9% G's. As expected based on prior work characterizing mitochondrial genomes of ctenophores, the genomes are compact, with just 11 protein-coding genes, two ribosomal genes, and an absence of tRNAs. Both specimens yielded a mitochondrial gene order (*COX1-COX2-ND4-ND6-rrnL-rrnS-ND4L-ND1-CYTB-COX3-ND3-ND5-ND2*) that has yet to be observed in any other ctenophore. However, the order of four sets of genes is constant across known platyctenes (*COX1-COX2-ND4*, *rrnL-rrnS*, *ND4L-ND1*, and *CYTB-COX3-ND3-ND5*). None of these is common to all of the mitochondrial genomes in NCBI's RefSeq database of reference genomes. Whereas the genetic divergences between genes of the two *T.* aff. *tristoma* measure from roughly 2-6%, those between genes of *T.* aff *tristoma* and other platyctenes are an order of magnitude greater (Table 3).

Phylogenetic analyses (Fig. 7) of mitochondrial *cox1* and nuclear *18S* both show a close relationship between our two representatives of *Tjalfiella*, and deep divergences from the other sampled platyctenes. While all representatives of the genus *Coeloplana* form a monophyletic group, the family Coeloplanidae has conflicting results, being monophyletic (but without significant support) according to the signal in the *18S* gene and diphyletic in the *cox1*-based topology. In addition, the *cox1* topology has *Tjalfiella* branching within Platyctenida, whereas the *18S* topology suggests that *Tjalfiella* could be sister to all other platyctenes in this limited analysis. Additional sampling of molecular characters and taxa would be necessary for clarification of the phylogenetic relationships within Platyctenida.

[APPROXIMATE POSITION for Figure 7.]

3.2 MicroCT Scans

For samples stored in ethanol (>70%), PTA stains were found to impose strong tissue contrast in all sampled platyctenes (Fig. 6). The greatest example is the tentacular apparatus, which is continuously observed to have a higher contrast from surrounding tissue like the mesoglea, which forms a noisy grey mass compared to the stark whites of the aforementioned structure . Meanwhile, structures that form part of the gastrovascular system are visualized as hollowed-out negative spaces with two undescribed masses snaking through distal sections. Thin halos of highly contrasting tissue then surround these hollowed-out masses. These structures appear identical to the anatomical cross-sections illustrated by Mortensen in his description of the species. As PTA is especially useful for staining collagen and other proteinaceous layers, tissues that show high concentrations of these molecules are expected to have a higher contrast to neighboring tissues (Metscher 2009). Details for each of the scans are given in Table 1. The effects of dehydration are evident, as certain features like the gastrovascular system and mesoglea experience noticeable shrinkage, and the staining is slightly less distinct than with treatments on samples fixed for the purpose of microCT imaging (Metscher 2009).

3.3 Taxonomy

Phylum Ctenophora Eschscholtz, 1829

Class Tentaculata Eschscholtz, 1825

Order Platyctenida Bourne, 1900

Family Tjalfiellidae Komai, 1922

Grammatical gender - feminine

Type genus - *Tjalfiella* from Komai (1922): 92-93

Etymology - Formed as a stem from the genus *Tjalfiella*.

Included genus (1) - *Tjalfiella* Mortensen, 1910

Diagnosis - Platyctenida with tentacles that lack tentilla.

Description - Benthic and sessile, body laterally compressed, elongated in the transverse direction; two "chimney-like" cylindrical vertical projections originating at either end and distinctly perpendicular to the rest of the body, each housing a single tentacle without tentilla in its own sheath; pharyngeal cavity with an accessory opening on each transverse end of body at the distal end of the vertical projections; transverse canals reduced into short branching diverticula, showing no anastomoses; gonads arising on lateral walls of the above diverticula; viviparous, eggs developing into cydippid-like embryos in brood-cavities in the lateral parts of the body.

***Remarks* -** Although Mortensen included the type genus (*Tjalfiella*) in the family Ctenoplanidae due to his observation of “cydippid-like” larvae, in 1922, Komai argued that there was such a noticeable morphological difference between *Ctenoplana* and *Tjalfiella* that the creation of Tjalfiellidae was justified. Presently, five families of Platyctenida are recognized: Coeloplanidae, Ctenoplanidae, Lyroctenidae, Savangiidae, and Tjalfiellidae. The latter is differentiated from Coeloplanidae, Ctenoplanidae, and Lyroctenidae by having tentacles that lack tentilla and branching diverticula that do not form anastomoses. The monotypic and only once observed Savangiidae lack an aboral sense organ and have tentacles that lack a primary filament, consisting solely of a tuft of tentilla. The two vertical projections that originate at either end of the laterally compressed body, and which are perpendicular to it, were called “chimneys” or “chimney-tops” by Mortensen (1912), in reference to their cylindrical shape and flat tops. Similarly positioned projections from the bodies of *Lyrocteis imperatoris*, *L. flavopallidus*, and *Coeloplana meteoris* were termed arms by Komai (1941), Robilliard & Dayton (1972), and Thiel (1968), respectively. In these taxa, the projections differ from those in Tjalfiellidae by not having flat tops and possessing oral groves that run down their lateral sides. We use the more general term “arm” to describe all of these raised structures, irrespective of the shapes of their distal ends.

Genus *Tjalfiella* Mortensen, 1910

***Grammatical gender* -** feminine

***Type species* -** *Tjalfiella tristoma* from Mortensen (1910): 249-253, plates. 1-10

***Etymology* -** From the Danish Tjalfe, on which Mortensen based the name. This originated from the earlier Tjalfe expedition to Greenland on the vessel Tjalfe. “Tjalfe is a renowned figure in Northern Mythology (the companion of the good Thor on his journey to Utgård)” (Mortensen 1912: 2).

***Included species (1)* -** *Tjalfiella tristoma* Mortensen, 1910

***Description* -** Tjalfiellidae compressed in the stomodeal axis and “U” shaped; with two large aboral arms that lack oral grooves, chimney-like with flat tops, and extend perpendicularly from the main axis of the body; two tentacles (one sheathed in each respective aboral arm) that lack tentilla; statocyst sunken into a deep cavity in the center of the aboral face of the animal; hermaphroditic gonads developing into four pairs of external globular pockets centered around the statocyst on the aboral face; genital brood cavities may appear as rows of externalized pockets lining the edge of the oral skirt; meridional canals reduced to short diverticula from perradial canals; branching canals present, but rather sparse, showing no anastomoses; lack ctene rows in maturity.

Remarks - Mortensen (1912) following a relatively short original (1910) description, provides highly detailed and extensive morphological/developmental description based on microscopy and histology of many specimens and his drawings are sufficient to recognize and describe the genus when observed from a deep-sea submersible platform with high-definition imagery. No other genera have been assigned to the family Tjalfiellidae.

Species *Tjalfiella tristoma* Mortensen, 1910

Fig. 6, 8

Grammatical gender - feminine

Etymology - From the feminine noun in apposition formed from Latin *tri*, “three,” and *stoma*, “openings.” This name refers to the specimen's three-body orifices, the mouth, and one opening for each aboral arm.

Pronunciation - *Phonetic;* “Chae-l-fe-el-ah” “try-stoh-ma” *IPA;* /tˈiːd͡ʒˌælfɪˈɛlə tɹɪstˈo͡ʊmə/

Material - Seven samples of *T. tristoma* from the Natural History Museum in Denmark (NHMD88841) and references to Mortensen's original description and illustrations in his 1912 manuscript “The Danish Ingolf Expedition: *Tjalfiella tristoma* n. g., n. sp. A sessile ctenophore from Greenland”: 249-253, plates. 1-10.

Description -

Body - Benthic ctenophore, compressed in the stomodeal axis; and “U” shaped in the tentacular axis; two large highly extendable aboral arms that lack oral grooves on opposing sides of body; distal ends of arms flattened into a disk, contains two openings, a singular gastric accessory opening with serrated edges, and a single smaller tentacular opening; overall body is smooth with no warts or visible papillae, exceptions are four pairs of globular gonads on the aboral face (number of gonads vary between individuals) and two rows of brooding pouches around oral skirt.

Size - Roughly 20mm in length from the tentacular axis, 5mm in length from the stomodeal plane, and 10mm in height; height can be highly variable (6-13mm)

Coloration - Transparent; body is opaque milky white, while tentacles are yellow due to the presence of colloblasts. (These colors are reported from formalin-preserved individuals, which may have lost some color – this cannot be determined after the fact.)

Apical organ - A single sunken statocyst in the center of the aboral face, does not extend beyond the outer epidermal layer; lacks cilia used for balance and positioning; lacks a polar field and no visible anal openings; a large, thickened epithelial floor extends down to the mouth.

Ctene rows - Completely absent in the mature benthic form, while embryos possess eight equally spaced ctene rows of uniform length. See Embryo section (below) for details.

Tentacular apparatus - Two tentacular apparatus (one per aboral arm) located on the innermost facing half; contains a large spherical tentacle bulb (d - 2-3.3mm) with a single tentacle protruding up towards the distal end of the arm; lack tentilla; of an undefined thickness that gradually narrows to the distal end.

Gastrovascular system - Suboral cavity “U” shaped with three openings, a single downward-facing slit mouth, two accessory openings in the arms distal end; suboral cavity houses numerous hanging stomodeal folds that thin out towards the accessory openings; a single protrusion extends upward from the suboral cavity towards the centralized statocyst, two canals extend horizontally from protrusion into the tentacles, each horizontal canal contains four diverticula that imbed into four hermaphroditic gonads; additionally two diverticula extend between each pair of gonadal diverticula along the outer body wall, each diverticulum has numerous dichotomously branching protrusions, all protrusions lack anastomoses.

Reproductive system - Four to eight large globular gonads arranged around a statocyst (d - 3-3.5mm), number varies due to the stage of regeneration, hermaphroditic with separation of reproductive tissues;; Several large brood pouches (variable in number) arranged in 2-3 rows develop on distal ends of each sub-diverticula around the oral foot.

Embryo - Mortensen described the larvae in three stages depending on their development; these stages are described below:

Stage I - Spherical (d - 2mm), numerous small white pigment spots irregularly scattered, large furrow in the transverse plane reaches halfway down the body on oral side, lack of a developed suboral cavity; aboral side with a single statocyst and two elongations; eight rows of costae with clustering cells on aboral half of body, develop into rudimentary tentacle bulbs on tentacular axis; lack ctene rows.

Stage II - Spherical (d - 2mm), without pigmentation, large furrow with visible suboral cavity taking up half body cavity; sunken statocyst with four large elevations; variable number of ctene plates arranged in eight equidistant rows (h - 0.1mm) reaching half length of body; tentacle bulbs developed into “T” shape with simple tentacles, lacking tentilla.

Stage III - Pear-shaped (l - 5mm, d - 2mm); furrow developed into two lobes (similar to *Ctenoplana*), pressed together to form tight seal, can flatten on a surface; extends halfway down the oral side of body; suboral cavity is large and voluminous, constitutes half body cavity, fully connected to tentacle bulbs and stomodeum; costae and ctene rows sunken into deep furrows of equidistant around aboral end

Ecology - Known only residing on the surface of pennatuloid octocorals of the genus *Umbellula*, perhaps in an obligatory symbiotic relationship. Diet may include crustaceans, indicated by an unidentified, partially decomposed, 1-cm "shrimp" lying within the suboral cavity, the tail lying partly within one chimney (Mortensen 1912:10); additional ecological information unknown.

Habitat - Deeper than 400m in areas dominated by mud flats within the Umanak Fjord and Northern Baffin Bay; 71.001296N, -54.349333W

Host - *Umbellula lindahli*; occupies the upper portion of the central unbranched rachis or within the crown of polyps. Never observed independent of *Umbellula*.

[APPROXIMATE POSITION for Figure 8.]

Remarks - *Tjalfiella tristoma* is unique among the other described species of platyctenes because of the "U"shaped body with projecting arms that lack visible oral grooves, simple tentacles without tentilla, and an arrangement of large globular gonads encircling the statocyst on the aboral face of the species (Fig. 8). Species in *Coeloplana*, *Vallicula multiformis,* and *Lyrocteis imperatoris* do not possess any of these traits. Notably, *V. multiformis* and *Coeloplana* spp. lacks externalized gonads, have a flattened body when relaxed, and possess tentacles adorned with fine tentilla that exit out of the distalmost ends of a prominent oral groove. Alternatively, *L. imperatoris* can be distinguished from *T. tristoma* based on the presence of a notable oral groove and tentacles adorned with fine tentilla. Additionally, the canal structure and arrangement of gonadal tissue visually varies between *Tjalfiella*, *Coeloplana*, and *Lyrocteis*, with both *Coeloplana* and *Lyrocteis* possessing a fine network of anastomosing canals while *Tjalfiella* possesses only blind ending canals (Fig. 9). Although certain morphological characteristics used to differentiate species of platyctenes may vary during their development or regeneration, the current valid species have good support based on detailed descriptions of both mature and developmental stages.

Additionally, although Mortensen (1912) observed cilia lining the interior of the suboral cavity in the arms, we failed to detect them utilizing light microscopy and microCT analysis. Additional destructive histological analysis would be needed to verify the presence of cilia in the suboral cavity in the arms.

The geographic distribution of *T. tristoma* is described by Mortensen (1910) as being strictly in the North Atlantic Ocean on the Western coast of Greenland in the Umanak Fjord and the Northern Baffin Island beginning at about 71.001296N, -54.349333W. Additionally, Mortensen (1910) described no occurrences of *T. tristoma* south of Umanak Fjord. However, during Kramp's visit to the region through the Godthaab expedition in 1928, specimens of *T. tristoma* were observed only on *U. lindahli* within the Umanak Fjord, with no additional observations either North or South of the type locality described by Mortensen. Regardless, these sparse observations for *T. tristoma* could suggest either a tight or spotty distribution for the species. However, due to the brooding nature of *Tjalfiella*, the species may not be as widely distributed as related broadcast spawners.

[APPROXIMATE POSITION for Figure 9.]

***Species Tjalfiella* aff. *tristoma* (2018)**

Figs. 3, 4, 5, 6, 10, 11.

Material - Two specimens of *T.* aff. *tristoma* collected by the ROV *Deep Discoverer* on the NOAA *Okeanos* and stored in the Smithsonian National Museum of Natural History (EX1806_D17_02B_A01; UNSM-IZ-1490693). Additional pictures of living specimens observed during the same dive were used for morphological analyses and host availability.

Description -

Body - Benthic ctenophore, "U" shaped in the tentacular axis; two large semi-gelatinous arms (h - 3.23-5.2mm) extending perpendicular from the horizontal axis of the animal, placed on opposing sides of the body; lacking lateral oral grooves, distal ends of arms flattened into a disk with an outer margin that is capable of folding inwards, contains two openings, a singular gastric accessory opening with serrated edges, and a single smaller tentacular opening; overall body surface is smooth with no warts or visible papillae, exceptions are four pairs of globular gonads on the aboral face (number of gonads vary between individuals) and two rows of brood pouches around the oral skirt.

Size - 10mm in the tentacular plane, 5mm in the stomodeal plane, and 8-11mm in height

Coloration - Translucent with orange to yellow ochre hue, intensity varies;suboral cavity deep rust or copper in color; tentacles and tentacle bulbs are a milky white.

Statocyst – Sunken into the main body via a small pocket; exact structure is unknown.

Ctene Rows - Absent in maturity but present in observable larvae. See the embryo section below.

[APPROXIMATE POSITION for Figure 10.]

Tentacular Apparatus - Two tentacular apparatuses (one per aboral arm) located on the innermost facing half of arm; contains a large spherical tentacle bulb (d - 2mm) with a crescentic tentacle root, a single tentacle protruding up towards the distal end of the arm (d - .11mm), lacking tentilla; of an undefined thickness that gradually narrows to the distal end; coil counterclockwise when retracted into sheath; the shape and abundance of colloblasts were not documented.

***Gastrovascular System* –** Large and “U” shaped with three openings: a downward-facing slit mouth on the oral face and a single accessory opening on each aboral arm; central suboral cavity directly above the slit mouth and below the statocyst, lined with numerous thin stomodeal folds attached from the cavity's roof, folds thin out towards accessory openings; a single protrusion extends upward from the suboral cavity towards the centralized statocyst, two canals extend horizontally from protrusion into the tentacles, each horizontal canal contains four diverticula that imbed into four hermaphroditic gonads; two diverticula extend between each pair of gonadal diverticula along the outer body wall, each diverticula has numerous dichotomously branching protrusions, all protrusions lack anastomoses; development of these diverticula varies across individuals.

***Reproductive System* -** Eight large globular gonads (d - 2.2-2.44mm) protruding from aboral face around statocyst, contain both male and female reproductive tissue separated by canal diverticula; two rows of brood pouches around oral skirt (ea.1.36-1.62mm in d), house brooded embryos (see embryo section).

***Embryo* -** Spherical and cydippid-like (d - 0.8mm); eight equidistant ctene rows on aboral side stretching to half body length, number of ctene plates around six but variable; statocyst present on central aboral end with large gelatinous dome; large slit on oral end that stretches halfway up the body and opens up to suboral cavity, suboral cavity voluminous and occupies half body cavity, suboral cavity converges to stomodeum, four perradial canals diverge from stomodeum, and split into two adradial canals each which merge into meridional canal roughly a quarter way from the bottom of the ctene row at an angle of 40°; tentacle bulbs halfway up body on opposing side of tentacular plane, “T” shaped, orange/rust colored, a single tentacle without tentilla each.

***Ecology* -** Known only residing on the surface of highly ramified deep-sea octocoral of the genus Acanella, perhaps in an obligatory symbiotic relationship (it was not seen on nearby corals in other genera). The ecology of this platyctene species is otherwise unknown and requires future study.

***Habitat* -** 1866.3m, 36°13'48.0" N 74°28'12.0" W, roughly 75 km east of North Carolina, USA

***Host* -** Undescribed *Acanella* sp. but closely resembles *Acanella arbuscula*. Usually occupies the upper third of the host organism.

[APPROXIMATE POSITION for Figure 11.]

Remarks - Compared to *T. tristoma* (see description above), most morphological features have no significant difference between measured *T. tristoma* and *T.* aff. *tristoma* (2018) (Table 4). However, both *T. tristoma* and *T.* aff. *tristoma* (2018) possess slight variations in coloration and host preference. Mortensen most notably described *T. tristoma* as white to pale-yellowish, while *T. aff. tristoma* (2018) is consistently observed as bright orange with a red suboral cavity. Additionally, even in the presence of other suitable host organisms, *T. tristoma* was documented only attaching to *Umbellula lindahli,* while *T.* aff. *tristoma* (2018) was observed only attaching to *Acanella* sp. (Fig. 3). Additional sightings of *T.* aff. *tristoma* (2018) along EX1806 showed an affinity for *Acanella* sp. with no additional sighting on neighboring corals (Fig. 4). Similar situations are described between individual species of *Coeloplana,* which often have no significant anatomical differences between species. Instead, external features like coloration, papillae arrangement, and host preference allow identification. Genetically, *T.* aff. *tristoma* (2018) differs from *T.* aff. *tristoma* (2022), but not by great amounts. The often-employed mitochondrial *cox1* gene shows 3.08% divergence (Table 3), comparable to the lower end of interspecific differences among platyctenes sampled by Alamaru et al. (2017), and far exceeding any intraspecific variation among their samples.

***Species Tjalfiella* aff. *tristoma* (2022)**

Fig. 5, 6, 12

Material - Nine samples of *T.* aff. *tristoma* collected by the ROV *Deep Discoverer* on the NOAA *Okeanos* and stored in the Smithsonian National Museum of Natural History (EX2205_D05_04B_A03; USNM-IZ-1674065). Additional pictures of living specimens observed during the same dive were used for morphological analyses and host availability.

Description -

Body - Benthic ctenophore, compressed in the stomodeal plane, "U" shaped in the tentacular plane; two extendable aboral arms that lack oral grooves on opposing sides of body; distal ends of arms are flattened with a malleable outer margin that is capable of folding inwards, contains two openings, a singular gastric accessory opening, and a single smaller tentacular opening; overall body surface is smooth with no warts or visible papillae, exceptions are two to four pairs of globular gonads on the aboral face (number of gonads vary between individuals).

[APPROXIMATE POSITION for Figure 12.]

Size - 5mm in height, 7.6mm in tentacular length, 3mm in stomodeal length.

Coloration - Body yellow to goldenrod and translucent, intensity varying between individuals; tentacles white.

Statocyst – Sunken into a small pocket in body, not visible in a relaxed state.

Ctene Rows - Absent in specimens (mature and larval).

Tentacular Apparatus - Two tentacular apparatuses (one per aboral arm) within the innermost facing half of the arm; tentacle bulb spherical (d - 1.6mm), near the base of each arm; tentacle is simple, lacks tentilla, even thickness (d - 0.22-0.41mm) that gradually narrows to the distal end, ends in proximally crescentic tentacle root within tentacle bulb; coil counterclockwise when retracted into sheath; the shape and abundance of colloblasts were not documented

Gastrovascular System – Suboral cavity “U” shaped with three openings, a single downward-facing slit mouth, two accessory openings in each of the arm’s distal ends; suboral cavity houses numerous hanging stomodeal folds that thin out towards the accessory openings; a single protrusion extends upward from the suboral cavity towards the centralized statocyst, two canals extend horizontally from protrusion into the tentacles, each horizontal canal contains four diverticula that imbed into four hermaphroditic gonads; additionally two diverticula extend between each pair of gonadal diverticula along the outer body wall, each diverticulum has numerous dichotomously branching protrusions, all protrusions lack anastomoses, protrusions in distal end of arm become swollen in larger specimens.

Reproductive System - Six spherical gonads (d - 0.7-0.92mm) protruding from subepidermal pockets, four on one hemisphere, two on the adjacent hemisphere, encircle centralized statocyst on aboral face, do not extend beyond or into the aboral arms, t; male and female gonad tissue on adjacent sides of penetrating diverticula (see gastrovascular section for details); brooding pouches developing on one hemisphere of body (d - 0.46mm) as swollen extensions on the distal ends of the dichotomously branching subdiverticula.

Embryo - Not observed in collected samples

Ecology - Known only residing on the surface of deep-sea octocoral of the genus *Adinisis*, perhaps in an obligatory symbiotic relationship (it was not seen on nearby corals in the genera). The ecology of this platyctene species is otherwise unknown and requires future study.

Habitat - Azores at 42°20'22.9"N 29°08'59.3"W at a depth of 1061.43m

Host - Undescribed *Adinisis* sp. Usually occupies the upper third of the host organism.

Remarks - Similarly to *T. tristoma* and *T.* aff. tristoma (2018), *T.* aff. tristoma (2022) has almost identical internal and external morphological features with only slight variations in the size of both the brood pouches and gonads. However, these variations can be explained by the small size of *T.* aff. tristoma (2022) compared to other *Tjalfiella* specimens, likely inferring an earlier developmental stage. Additionally, *T.* aff. *tristoma* (2022) differs from the aformentioned *Tjalfiella* specimens by remaining yellow in color with a preference for *Adinisis* octocorals compared to *Umbellula lindahli* of *T. tristoma* and *Acanella* sp. of *T.* aff. *tristoma* (2018). As in remarks above, *T.* aff. *ttristoma* (2022) differs genetically from *T.* aff. *ttristoma* (2018) by an amount that is more consistent with the samples representing distinct but closely related species. Either of these putative species could be the true *T. tristoma*, but it is also possible that they represent two species distinct from *T. tristoma*.

4. Conclusion

The organisms inhabiting deep-sea environments are not particularly well known, since the percentage of the deep-sea that has been explored by biologists is minute. Nevertheless, deep-sea environments are presently threatened by the prospects for extractive industries, such as petroleum (Zhang et al. 2021) and minerals (Lusty & Murton 2018; Rabone et al. 2023). Indeed, human activities are already impacting deep environments, e.g., the Deepwater Horizon oil spill (Wiesenburg et al. 2021) and deep bottom trawl fisheries (Good et al. 2022). Where measured, biodiversity loss in impacted habitats has increased on a global scale, making the study of deep-sea biodiversity essential (Beazley et al. 2021; Claudet et al. 2021; Costello et al. 2010). The United Nations highlighted marine biodiversity in its 14th Sustainable Development Goal and seeks to “protect and restore ecosystems and biodiversity” as challenge two of this Ocean Decade. Thus, it is imperative to know the species that make up and interact within deep-sea communities. This basic information is essential for understanding species’ distributions and how organisms function as part of their respective communities.

The present study integrates several systematic methods to elucidate the enigmatic benthic ctenophore genus *Tjalfiella* and specimens probably representing additional species within it. Our study is the first to use microCT to visualize the anatomy of extant ctenophore species using museum specimens (one nearly 100 years old), demonstrating the potential for this technique to advance understanding of the morphology of, and its variation across, Platyctenida. This study also employed low-coverage whole genome sequencing to recover complete mitochondrial genomes with novel gene orders for Platyctenida and Ctenophora as a whole, as well as complete ribosomal repeat regions. All of these novel observations, including *in situ* videography, serve to characterize both the morphology and genetics of *Tjalfiella*, providing evidence that the diversity and distribution of *Tjalfiella* is greater than had previously been reported in the North Atlantic. That said, we were unable to name any new species in this study because we could not definitively differentiate either *T.* aff. *tristoma* (2018) or *T.* aff. *tristoma* (2022) from Mortensen’s *T. tristoma.* MicroCT and *in situ* imagery showed that all three samples show striking morphological similarities, with the only significant variations stemming from variations in color and host preference. This highlights one of the many difficulties in taxonomy and why it can often move more slowly than anyone would like. However, even in the absence of naming new species, this work provides morphological, ecological, and genetic data that have stabilized the meaning of the genus *Tjalfiella*, and which will be critical for comparative purposes when numerous other deep sea platyctene specimens that have been collected in recent years as part of various deep-sea explorations are characterized.

Acknowledgments -
We thank the crew of the NOAA *Okeanos* and *Deep Discoverer* ROV for the safe collection and storage of the specimens, Abigail Reft for cataloging and specimen handling, the University of Copenhagen Natural History Museum of Denmark for loaning the samples of *Tjalfiella tristoma* from Kramp's expedition to Greenland. For the morphological analysis we thank both J.J. Hill and Freya Goetz for mentoring and operating the microCT. Additionally, we would like to thank the editor and reviewers for constructive feedback and support. Finally, we acknowledge the Smithsonian NMNH Laboratories of Analytical Biology (LAB) for resources associated with the generation of genetic data, as well as the technical expertise of Stephanie Bush and Carrie Craig.

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FIGURE LEGENDS

Figure 1 - Reproduction of the original Plate 1 of Mortensen's 1912 description of *Tjalfiella tristoma*. **1.** Photograph of two specimens in their natural position on the stalk of *Umbellula lindahli*. **2.** A young specimen, without embryos, tentacular view, **3.** A larger specimen, with a single embryo, tentacular view, **4.** Specimen showing the right half in regeneration. No embryos, tentacular view. **5.** Young specimen, without embryos and possibly experiencing regeneration, tentacular view. **6.** Fully developed specimen, aboral view, **7.** Specimen having lost the right aboral arm with the tentacle and the outer pair of genital organs, tentacular view. **8.** Specimen in regeneration, tentacular view. **9.** Fully developed specimen, with numerous brood pouches, tentacular view (same specimen as in Fig. 6).

Figure 2 - Map featuring reported species distribution. *Diamond*- Type locality specimen by Mortensen (1910, 1912), *Triangle* - Sample (NHMD88841) collected by Kramp in 1928, Square- Sample collected by NOAA (UNSM-IZ-1490693) in 2018, Circle- Sample collected by NOAA (USNM-IZ-1674065) in 2022.

Figure 3 - Still images of living specimens of *Tjalfiella* aff. *tristroma* (2018)*;* **A.** *In situ* observation of four individuals on host octocoral *Acanella* sp. Scale bar - 2cm, **B.** Still image of a collected individual in a retracted state, showing the body shape and reproductive system, **C.** Closeup of the aboral side, showing the opening of the arm with a simple tentacle, **D.** Closeup of arms, showing the coiling of tentacles and the relaxed state of the animal. Scale bar B to D - 0.25cm.

Figure 4 - Additional observation by NOAA of platyctenes of similar morphology to *Tjalfiella* aff. *tristoma* (2018) on dive EX1806_D17. Arrows point to individual platyctenes in the branches of *Acanella* sp. Scale bar - 2cm.

Figure 5 - Still images of living specimens of *Tjalfiella* aff. *tristroma* (2022) with respect to host coral*;* **A.** *In situ* observation of the host coral *Adinisis* sp. Scale bar - 2cm, **B.** Close-up still image of the

collected host with several platyctenes in a retracted state. Scale bar - 0.75cm, **C.** Tentacular view of two individual platyctenes separated from host coral. Arrows point to individual platyctenes on the collected Adinisis sp. Scale bar - 0.5cm.

Figure 6 - MicroCT 3D reconstruction of three *Tjalfiella* samples: **A.** Tentacular view of *T. tristoma,* **B.** Tentacular view of *T.* aff. *tristoma* (2018), **C.** Tentacular view of *T.* aff. *tristoma* (2022), **D.** Stomodeal view of *T. tristoma*, **E.** Stomodeal view of *T.* aff. *tristoma* (2018), **F**. Stomodeal view of *T.* aff. *tristoma* (2022), **G.** Colorized internal anatomy of *T. tristoma* with low opacity body overlay, **H.** Colorized internal anatomy of *T.* aff. *tristoma* (2018) with low opacity body overlay, **I.** Colorized internal anatomy of *T.* aff. *tristoma* (2022) with low opacity body overlay, **J.** Closeup of aboral arms face with centralized opening and tentacle, **K.** Isolated view of intact larval *T.* aff. *tristoma* (2018) within the brood chamber, tentacular view, **I.** Closeup of developing brood chambers along diverticula protrusions. Blue - gastrovascular cavity (e.g., suboral cavity), Purple - Gonads, Magenta - brood pouches, Pink - tentacular apparatus, Green - canals, Teal - generalized larval anatomy. Scale bar: A-F - 3mm, G-I - 1.6mm, J-L - 0.3mm.

Figure 7 - Maximally likely (ML) phylogenetic reconstructions of Platyctenida; **A.** ML topology based on mitochondrial COI gene. ML bootstrap support indices are indicated near the corresponding nodes. **B.** ML topology based on the nuclear *18S* rDNA gene. Bootstrap support indices are indicated near the corresponding nodes, with only those exceeding 70 being shown. Representatives of *Coeloplana, Lampea, Lyrocteis*, and *Vallicula* were obtained from GenBank. Coloration is used to differentiate between genera.

Figure 8 - Illustrated Plate of *Tjalfiella tristoma* collected by Kramp: **A.** representation of specimens in their natural position on the stalk of *Umbellula lindahli*, **B.** view of flattened face of aboral arm, **C.** young specimen of *T. tristoma* with asymmetrical gonads and no brood pouches, tentacular view, **D.** mature *T. tristoma,* tentacular view, **E.** aboral schematic of arrangement of diverticula and associating canals, **F.** stomodeal view of mature *T. tristoma,* **G.** cutaway illustration featuring internalized anatomy. Tj - *Tjalfiella tristoma*, H - Host coral (*Umbellula lindahli*), a.a. - aboral arm, a.f. - aboral face, b. - brooding pouch, d.b. - diverticula that transverse along the body, d.g. - gonadal diverticula, d.p. - diverticula protrusions, d.t. - tentacular diverticula, g. - gonads, m. - mouth, o.s. - oral skirt, s.c. -suboral cavity, s. - statocyst, s.f. - stomodeal folds, s.o. - secondary opening (mouth), t. - tentacle, t.b. - tentacle bulb, t.s. - tentacle sheath, t.o. - tentacle opening.

Figure 9 - Simple schematic of the structure of the canal and reproductive system in three families of Platyctenida viewed from the aboral side; **A.** Tjalfiellae, **B.** Coeloplanidae, **C.** Lyroctenidae. c - canal system, g - gonads, s - statocyst, t - tentacular apparatus.

Figure 10 - Illustrated Plate of *Tjalfiella* aff. *tristoma* (2018) collected by NOAA: **A.** representation of specimens in their natural position on the stalk of *Acanella* sp., **B.** mature *T.* aff. *tristoma* (2018), tentacular view, **C.** Cross section of a gonad, **D.** Stomodeal view of mature *T.* aff. *tristoma* (2018), **E.** cutaway illustration featuring internalized anatomy, tentacular view. **F.** Closeup drawing showing the development of brood pouch on diverticula protrusions, **G.** view of the flattened face of aboral arm, **H.** aboral schematic of the arrangement of diverticula and associating canals; Tj - *Tjalfiella* aff. *tristoma* (2018), H - Host coral (*Acanella* sp.), a.a. - aboral arm, a.f. - aboral face, b. - brooding pouch, d.b. - diverticula that transverse along the body, d.g. - gonadal diverticula, d.p. - diverticula protrusions, d.t. - tentacular diverticula, g. - gonads, m. - mouth, o.s. - oral skirt, s.c. - suboral cavity, s. - statocyst, s.f. - stomodeal folds, s.o. - secondary opening (mouth), t. - tentacle, t.b. - tentacle bulb, t.s. - tentacle sheath, t.o. - tentacle opening.

Figure 11 - Illustrated Plate of larval *Tjalfiella* aff. *tristoma* (2018) collected by NOAA: **A.** tentacular plane, **B.** aboral view, **C.** oral view, a.c. - anal canal, c.p. - ctene plate, c.r. -ctene row, m. - mouth, p. - pharynx, s. - statocyst, t. - tentacle, t.b. - tentacle bulb.

Figure 12 - Illustrated Plate of *Tjalfiella* aff. *tristoma* (2022) collected by NOAA: **A.** representation of specimens in their natural position on the stalk of *Adinisis* sp., **B.** mature *T.* aff. *tristoma* (2022), tentacular view, **C.** Stomodeal view of mature *T.* aff. *tristoma* (2022), **D.** cutaway illustration featuring internalized anatomy, tentacular view. **E.** view of the flattened face of the aboral arm, **F.** aboral schematic of the arrangement of diverticula and associating canals, Tj - *Tjalfiella* aff. *tristoma* (2022), H - Host coral (*Adinisis* sp.), a.a. - aboral arm, a.f. - aboral face, d.b. - diverticula that transverse along the body, d.g. - gonadal diverticula, d.p. - diverticula protrusions, d.t. - tentacular diverticula, g. - gonads, m. - mouth, o.s. - oral skirt, s.c. - suboral cavity, s. - statocyst, s.f. - stomodeal folds, s.o. - secondary opening (mouth), t. - tentacle, t.b. - tentacle bulb, t.s. - tentacle sheath, t.o. - tentacle opening.

Table 1 - Summary of microCT parameters for each sample scanned in the present study.

Species name	**Catalog number**	**microCT scan ID**	**X-ray tube**	**Timing (milliseconds)**	**Number of images**	**Voltage (kV)**	**Current (uA)**	**Power (W)**	**Voxel size (µm)**
Tjalfiella tristoma	NHMD1183630	NB11	Nano-focus 180 kV	1000	2448	75	165	2.93	4
Tjalfiella aff. *tristoma* (2018)	USNM 1490693	NB04	Nano-focus 180 kV	500	2060	80	140	3.23	5.29
Tjalfiella aff. *tristoma* (2022)	USNM 1674065	NB07	Nano-focus 180 kV	333	1214	80	140	3.65	6.01

Table 2 - NCBI accession numbers for genetic data derived in this study.

Specimen Voucher	NCBI BioProject	NCBI BioSample	NCBI SRA	NCBI Nucleotide for Ribosomal Repeat	NCBI Nucleotide for Mitochondrial Genome
USNM_IZ_1490693	PRJNA1072111	SAMN39749400	SRR27906209	PP317598	PP327218
USNM_IZ_1674065	PRJNA1072111	SAMN39749539-40	SRR27906210-11	PP317599	PP331237

Table 3 - Pairwise genetic divergences across genes of the mitochondrial genome, from *Tjalfiella* aff. *tristoma* (2018)

Gene	***T.* aff. *tristoma* (2022)**	***Coeloplana loyai***	***C. yulianicorum***	***Vallicula multiformis***
COX1	3.16%	22.18%	23.88%	25.63%
COX2	3.82%	26.04%	27.26%	34.90%
ND4	2.27%	20.81%	22.07%	30.44%
ND6	1.93%	18.84%	21.26%	38.16%
rrnL	5.89%	30.31%	31.72%	37.66%
rrnS	5.59%	40.56%	41.01%	50.17%
ND4L	1.71%	21.79%	24.79%	37.18%
ND1	3.75%	27.02%	29.19%	34.12%
CYTB	2.90%	17.42%	17.60%	29.66%
COX3	4.74%	25.33%	25.59%	29.09%
ND3	4.17%	25.07%	25.66%	33.03%
ND5	3.33%	24.92%	25.83%	33.85%
ND2	2.55%	25.00%	24.73%	34.27%

Table 4 - Summary of the anatomical features for all three samples of *Tjalfiella* specimens.

Organism	Morphology	Size (mm)	Ratio to body	Remarks
Tjalfiella tristoma (1910)	Body (tent. plane)	20	1	Cylindrical in tent. plane, "U" shaped w/ tentacles, white
	Body (stom. plane)	9	0.45	Circular in stom. plane
	Arm (height)	3.5 - 8	0.17 - 0.4	Two, conical with aboral tentacle and gastric openings at vertex
	Arms width (tent. plane)	4.7	0.24	Variable depending on state of arm retraction
	Arms width (stom. plane)	3	0.15	Lacking an oral groove
	Mouth	1.3	0.07	Short and slit-like in stomodeal plane
	Suboral cavity	3.2	0.16	Long and voluminous with no documented color
	Stomodeum	-	-	-
	Tentacles(s) extended	-	-	Two, length not known but lack tentilla, yellow
	Tentacles(s) coiled	-	-	Two, length not known but lack tentilla, yellow
	Tentacle bulb	2 - 3.3	0.1 - 0.17	Two on opposing sides of apical organ, spherical
	Gonads	3 - 3.8	0.15 - 0.19	Eight, globular, on aboral face
	Brood pouches	1.7 - 2.7	0.08 - 0.13	36, Large and arranged in 2-3 rows
	Embryo(s)	2 - 4.5	0.1 - 0.22	Housed within brooding pouches, development varies
	Apical organ	0.1	0.01	Sunken with large dome
Tjalfiella aff. *tristoma* (2018)	Body (tent. plane)	10	1	Rectangular in tent. plane, "U" shaped w/ tentacles, orange
	Body (stom. plane)	4.7 - 5	.47 - 0.5	Square in stom. plane, orange
	Arm (height)	3.23 - 5.2	.32 - .52	Two, conical with aboral tentacle and gastric openings at vertex
	Arms width (tent. plane)	2.4	0.24	Variable depending on state of arm retraction
	Arms width (stom. plane)	2	0.2	Lacking a original groove
	Mouth	1.4	0.14	Short and slit-like in stomodeal plane
	Suboral cavity	3.98	0.4	Long and voluminous, red

	Stomodeum	1.13	0.11	Cylindrical and no documented color
	Tentacles(s) extended	0.17	0.01	Two, length not known but lack tentilla, white
	Tentacles(s) coiled	0.2	0.02	Two, length not known but lack tentilla, white
	Tentacle bulb	2	0.2	Two on opposing sides of the apical organ, spherical
	Gonads	2.2 - 2.44	.22 - .24	Eight, globular, on aboral face
	Brood pouches	1.36 - 1.62	.13 - .16	24, Large and arranged in 2-3 rows
	Embryo(s)	0.8	0.08	Housed within brooding pouches, development varies
	Apical organ	0.13	0.01	Sunken with large dome
Tjalfiella aff. *tristoma* (2022)	Body (tent. plane)	7.6	1	hemispherical in tent. plane, "U" shaped w/ tentacles, orange
	Body (stom. plane)	2.5	0.33	Flattened/hemispherical in the stomodeal plane, orange
	Arm (height)	2.06 - 3.42	.27 - .45	Two, conical with an aboral tentacle and gastric openings at vertex
	Arms width (tent. plane)	2.1 - 2.2	.28 - .29	Variable depending on the state of arm retraction
	Arms width (stom. plane)	1.8 - 2.1	.23 - .28	, Lacking an oral groove
	Mouth	0.8	0.11	Short and slit-like in stomodeal plane
	Suboral cavity	2.67	0.35	Long and voluminous, yellow-orange
	Stomodeum	0.29	0.04	Cylindrical and orange
	Tentacles(s) extended	0.22	0.03	Two, lack tentilla
	Tentacles(s) coiled	0.41	0.05	Two, coil and contract counterclockwise close to tentacle bulb
	Tentacle bulb	1.29	0.17	Two on opposing sides of the apical organ, spherical
	Gonads	.7 - .92	0.09 - 0.12	Six and spherical - four on one hemisphere, two on opposing
	Brood pouches	0.46	0.06	Three, small on one side of animal
	Embryo(s)	-	-	-
	Apical organ	-	-	-

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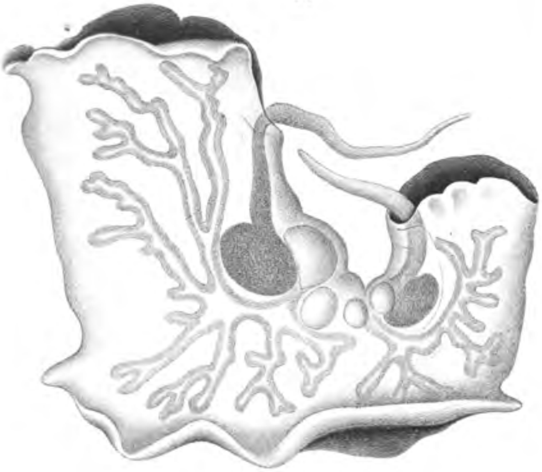

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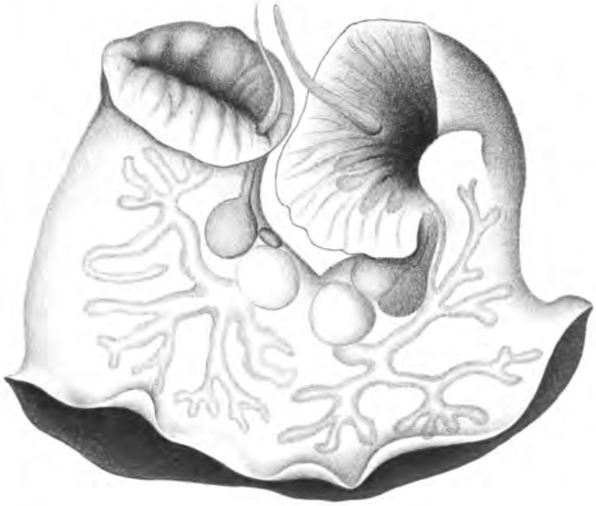

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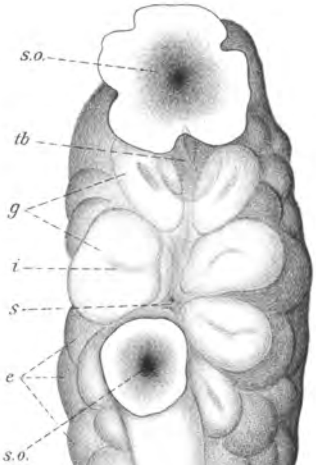

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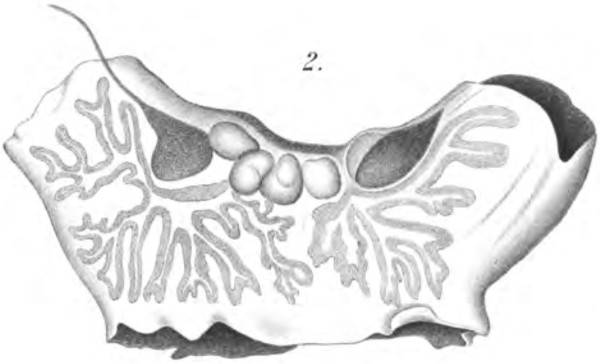

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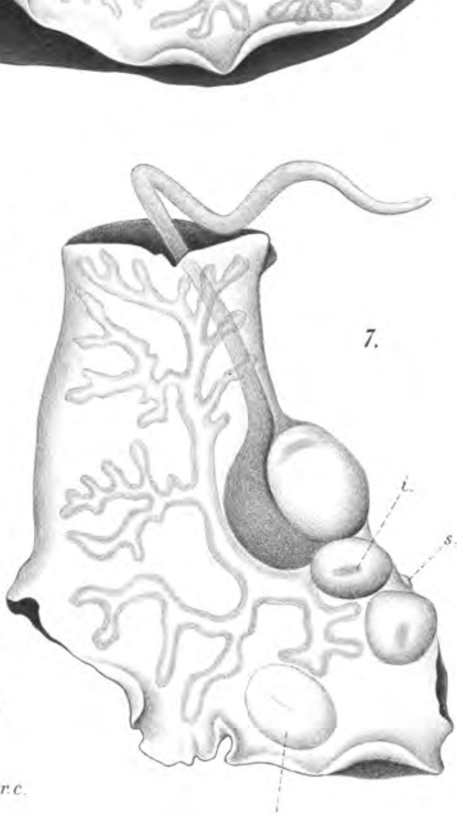

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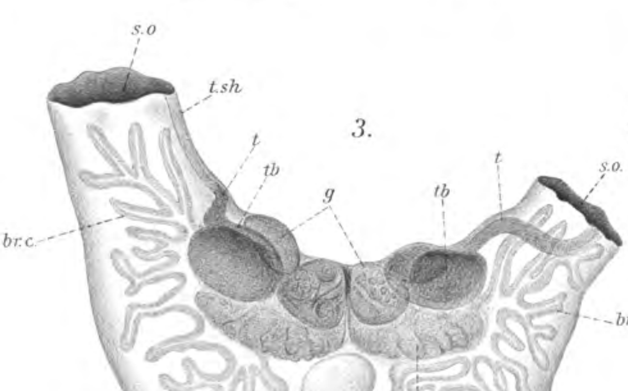

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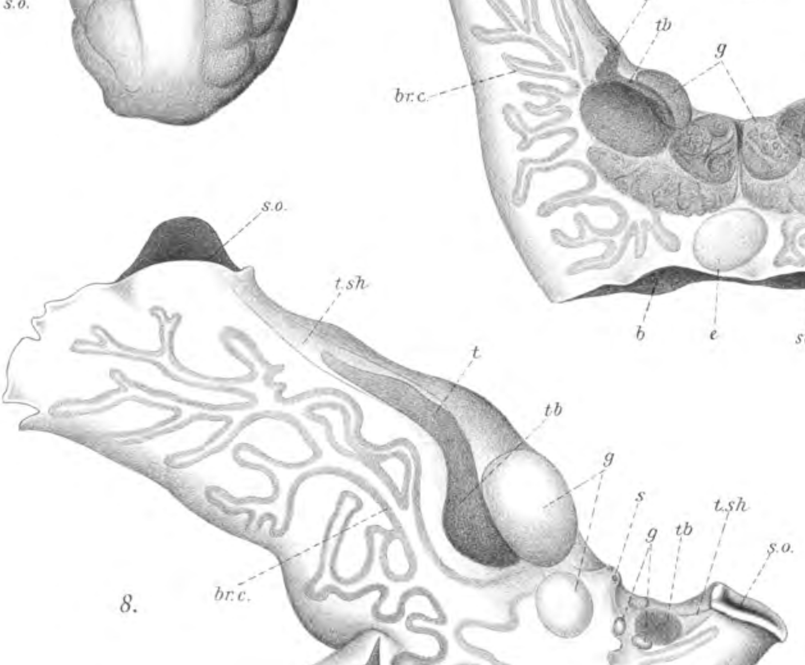

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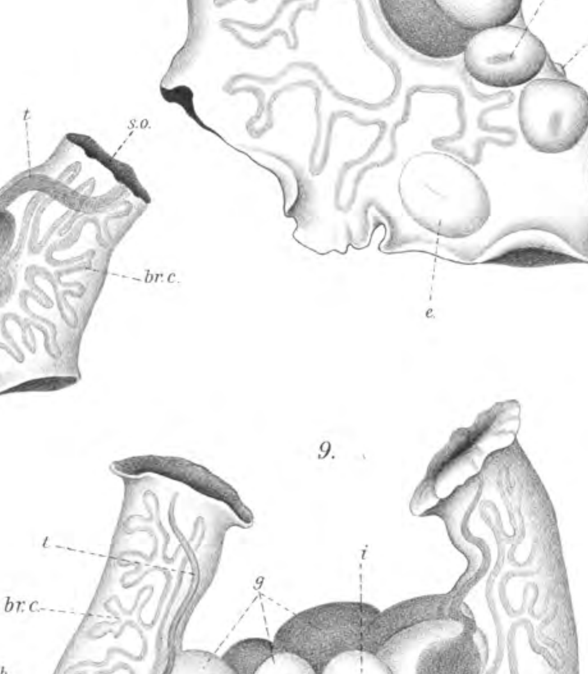

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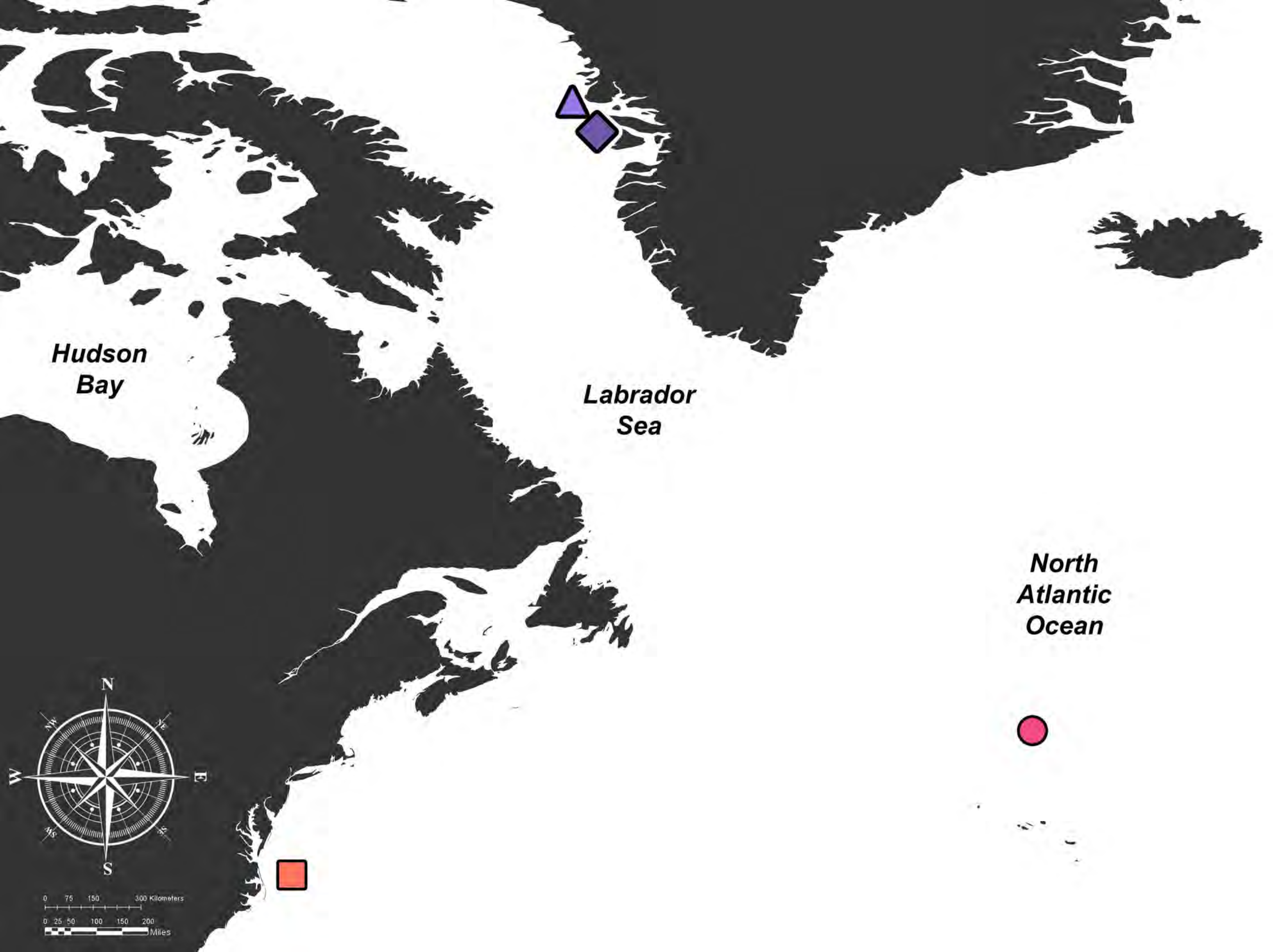
Tjalfiella tristoma Mrtsn.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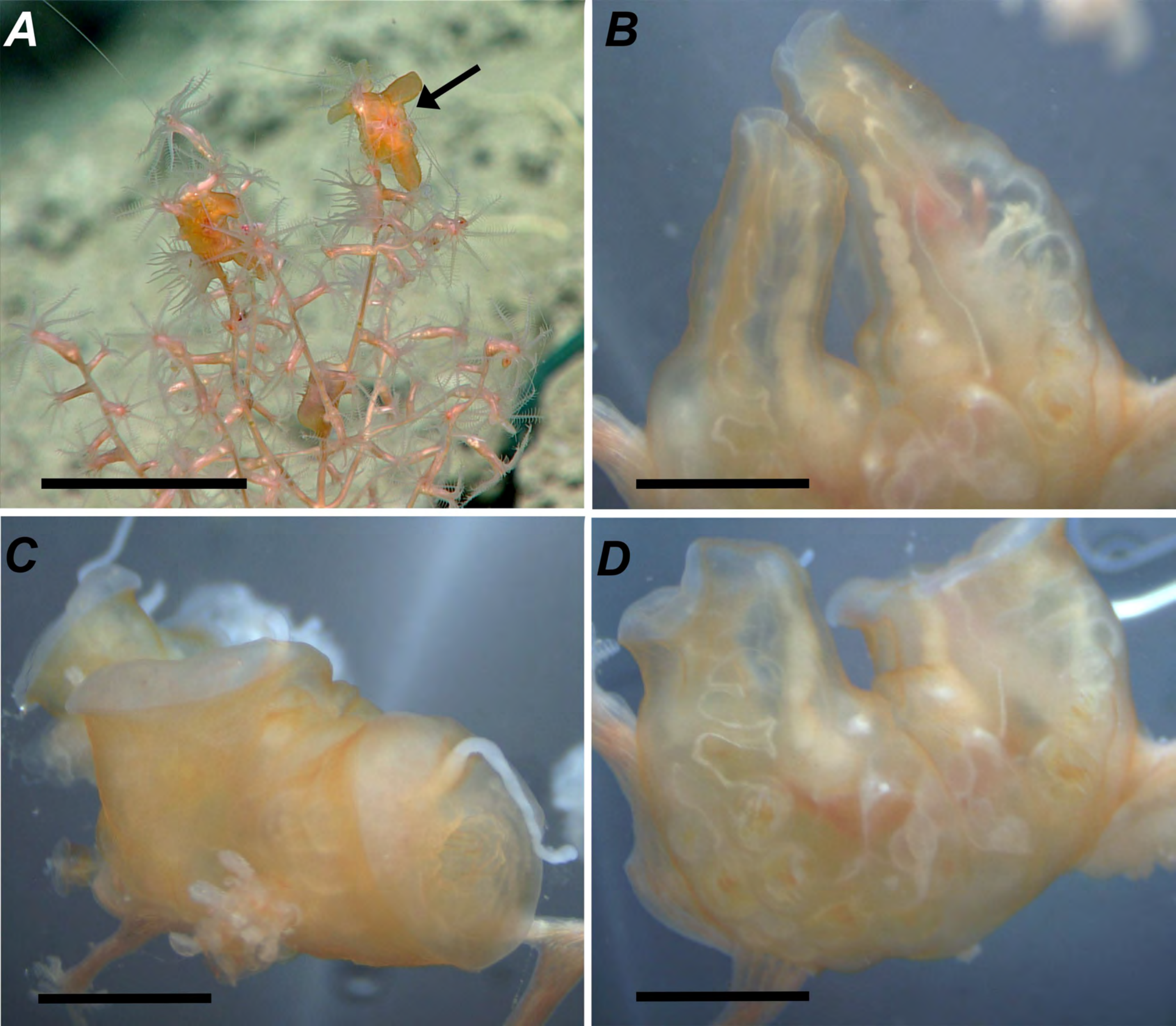
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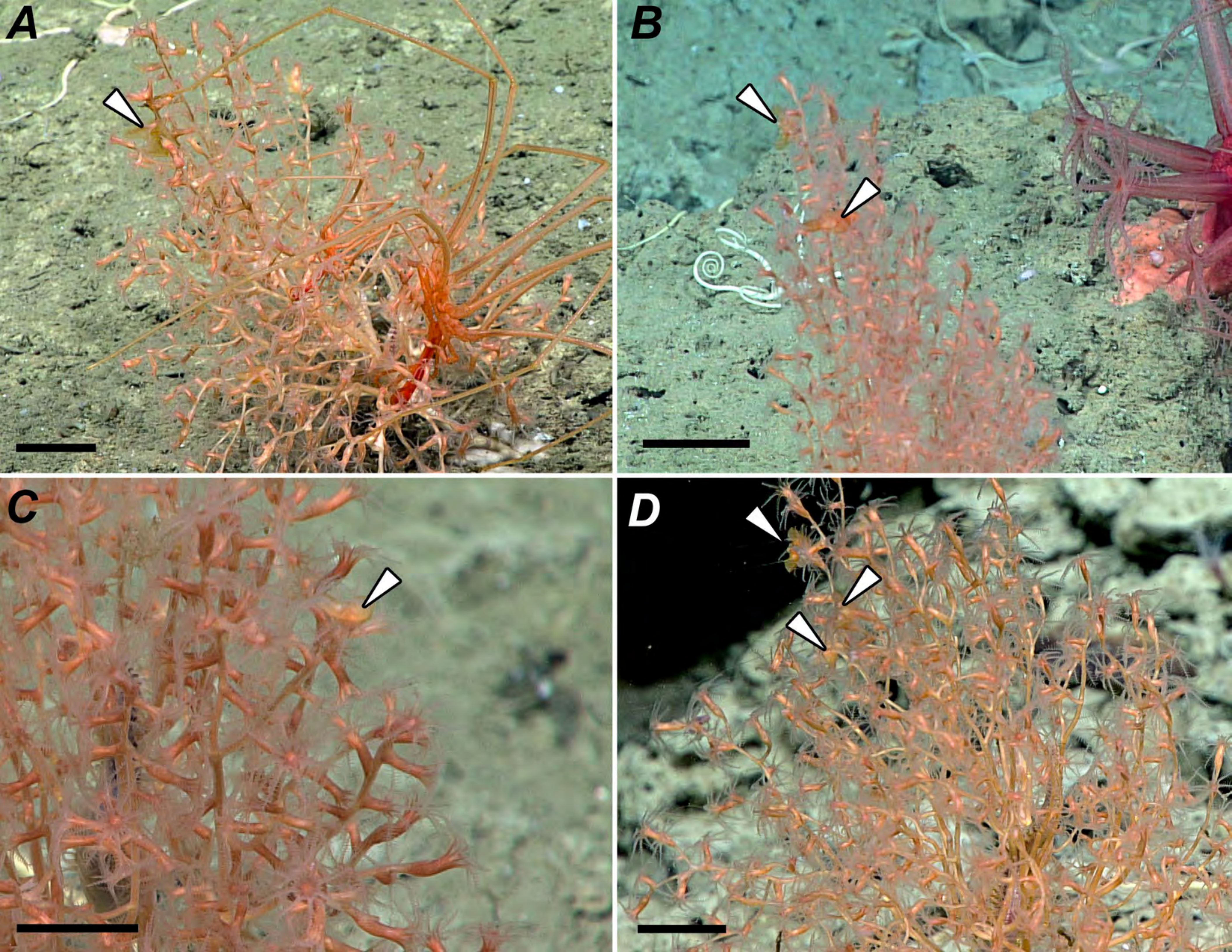
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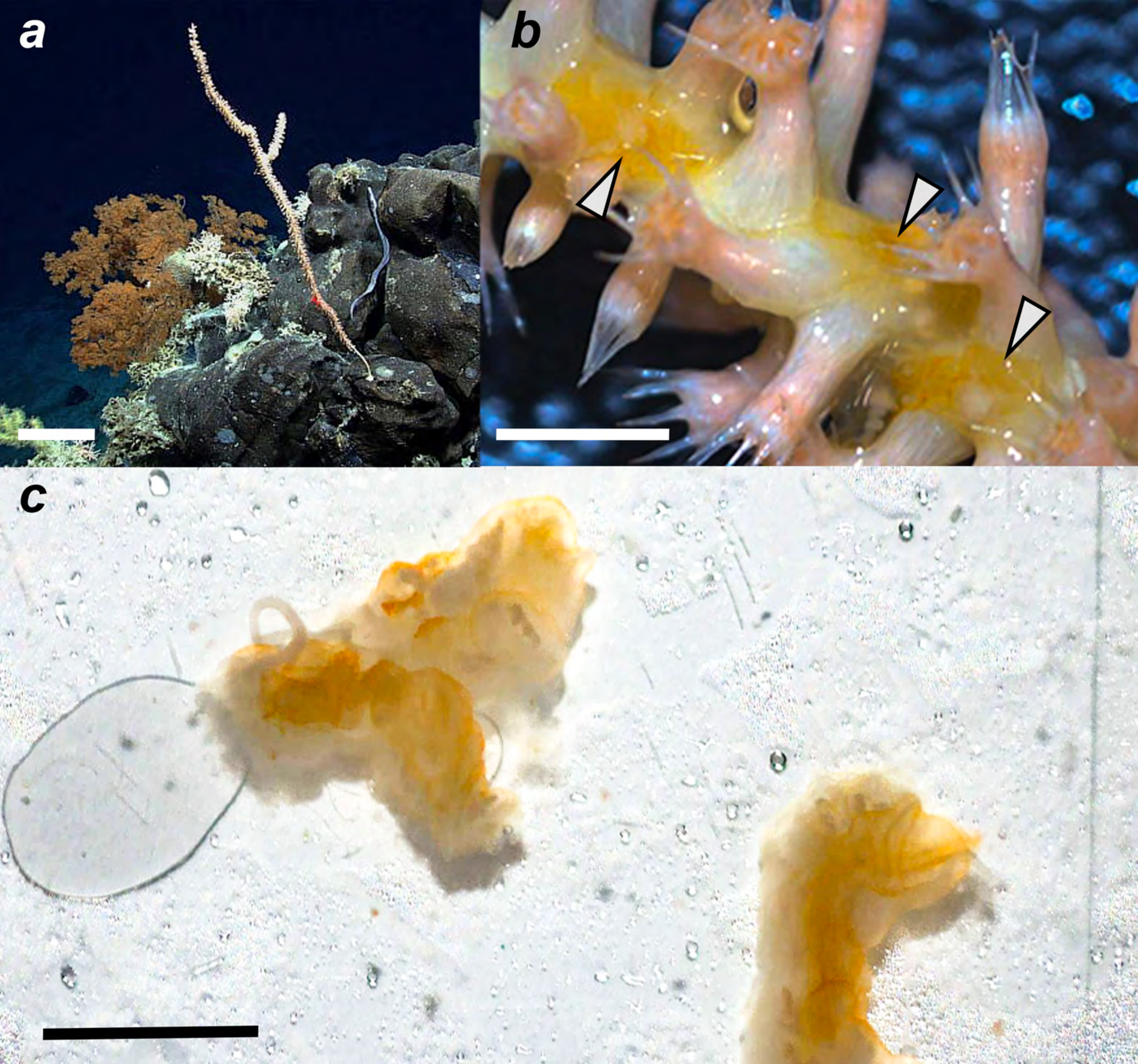
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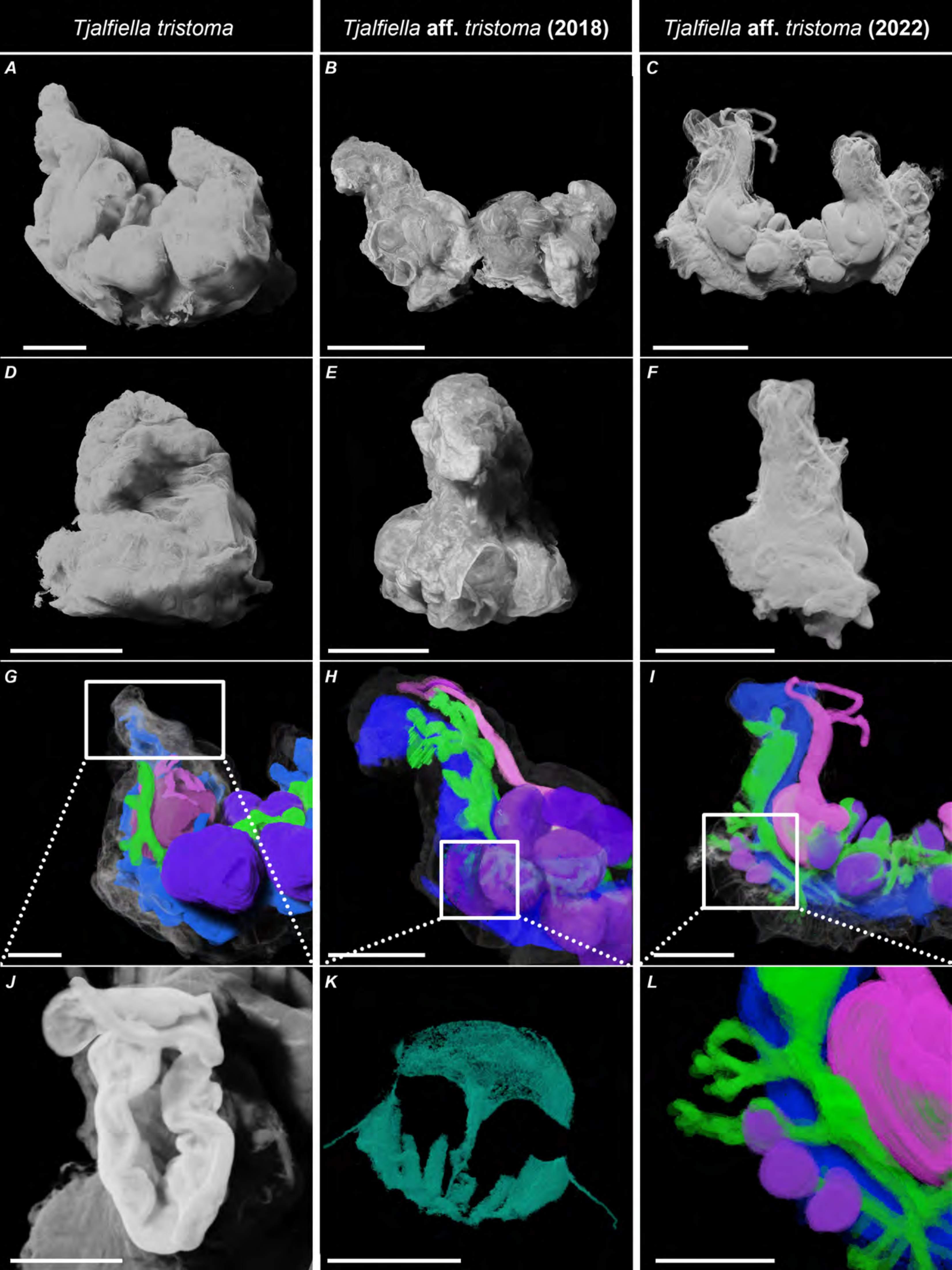

Tjalfiella tristoma
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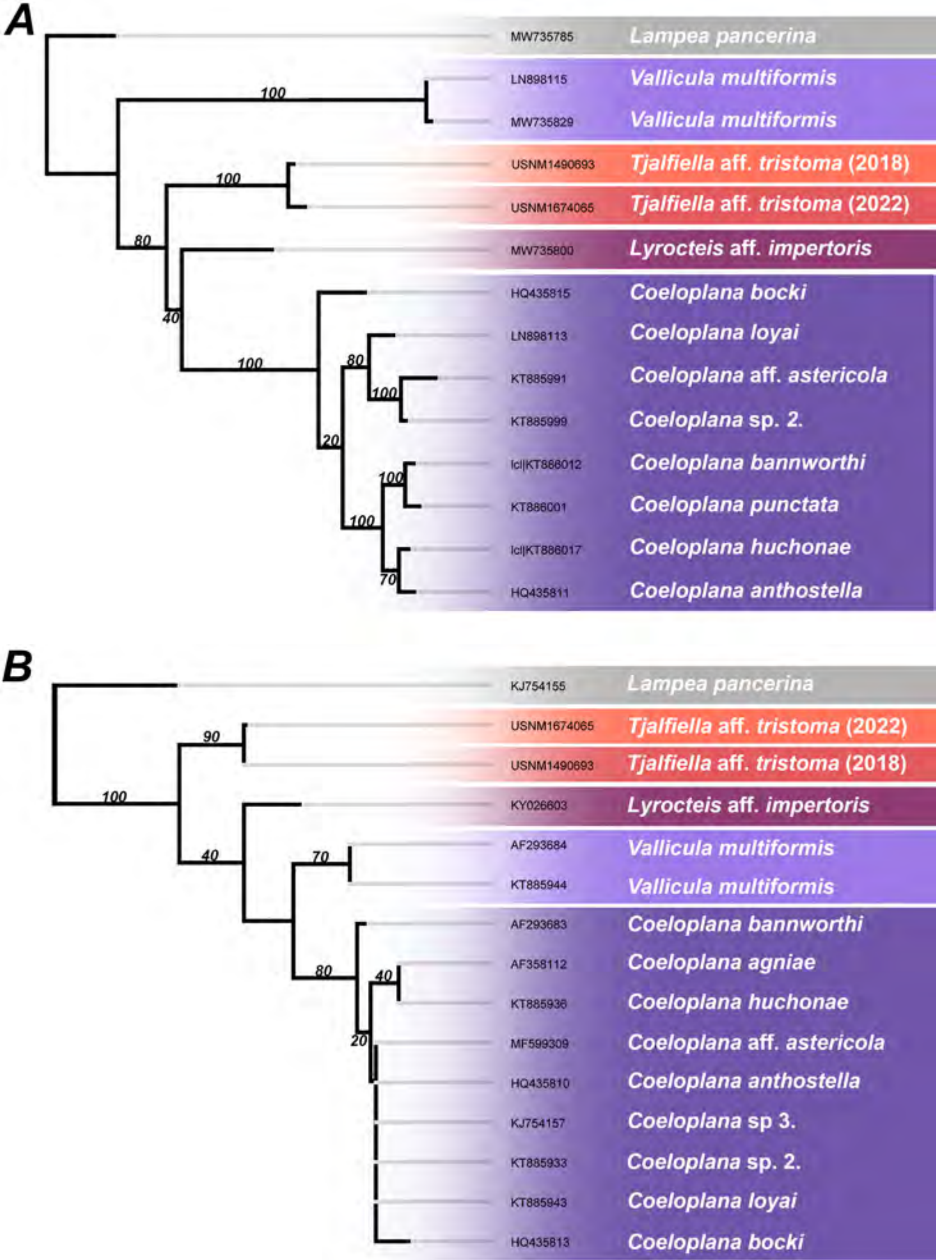
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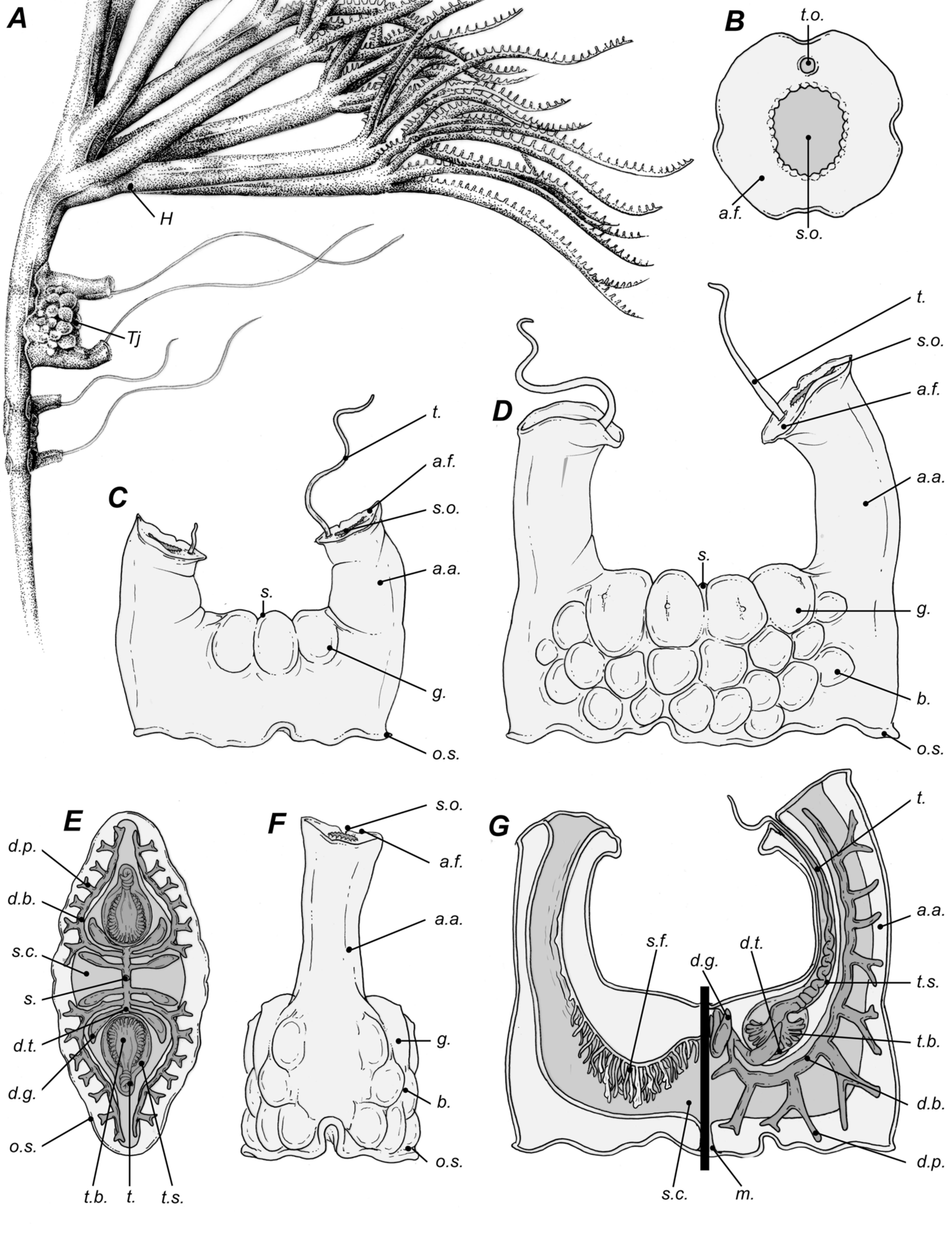
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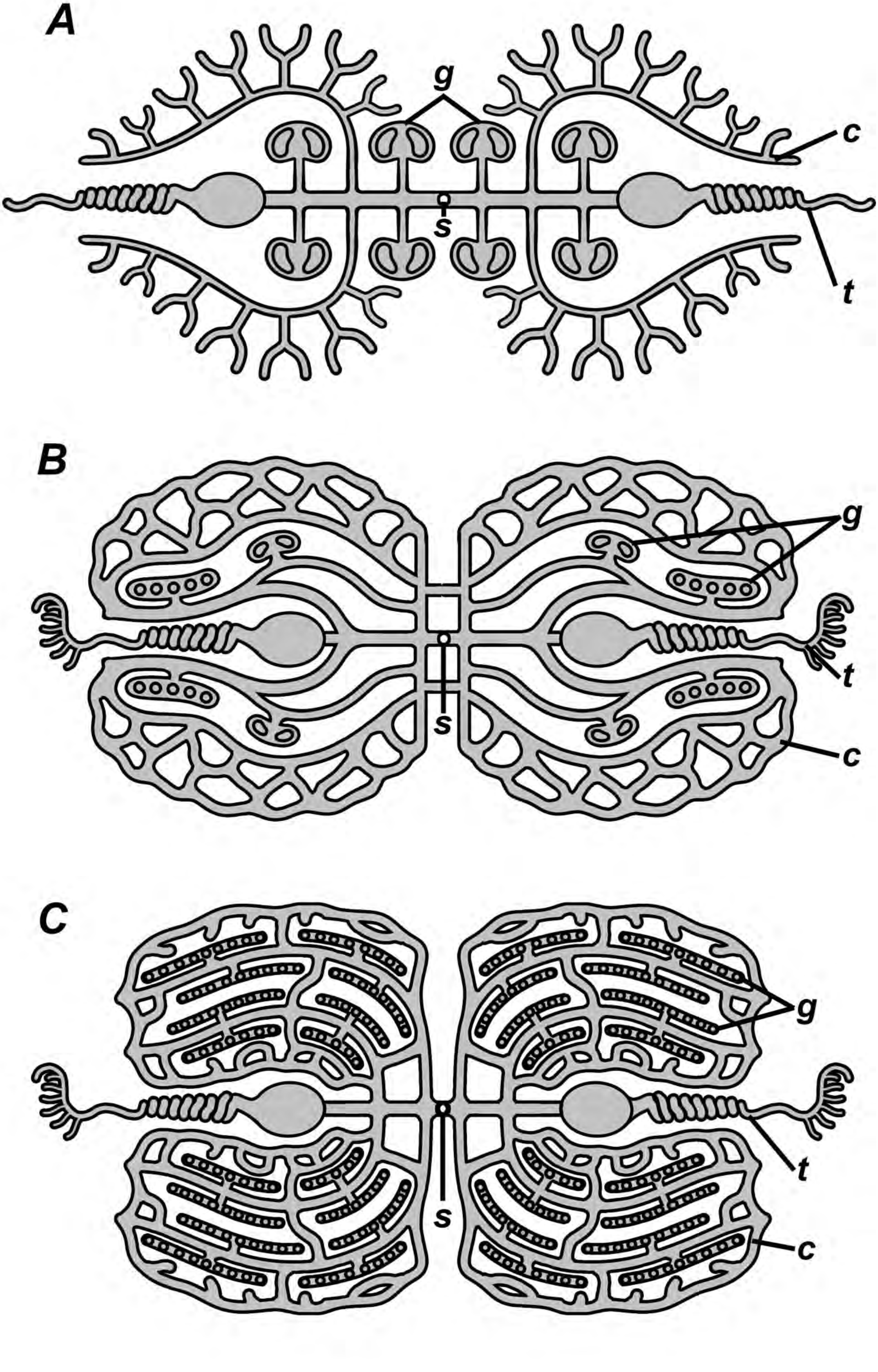

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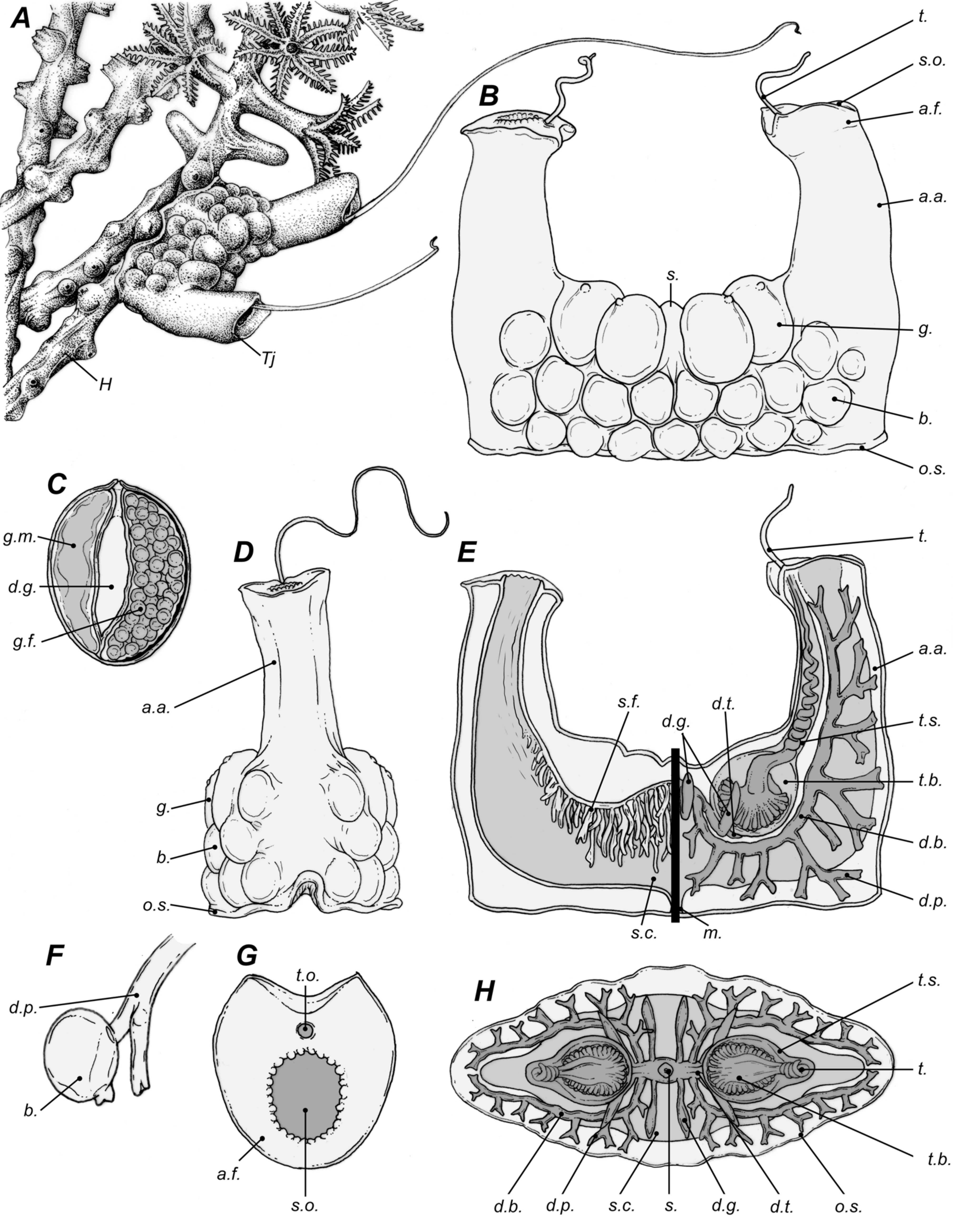
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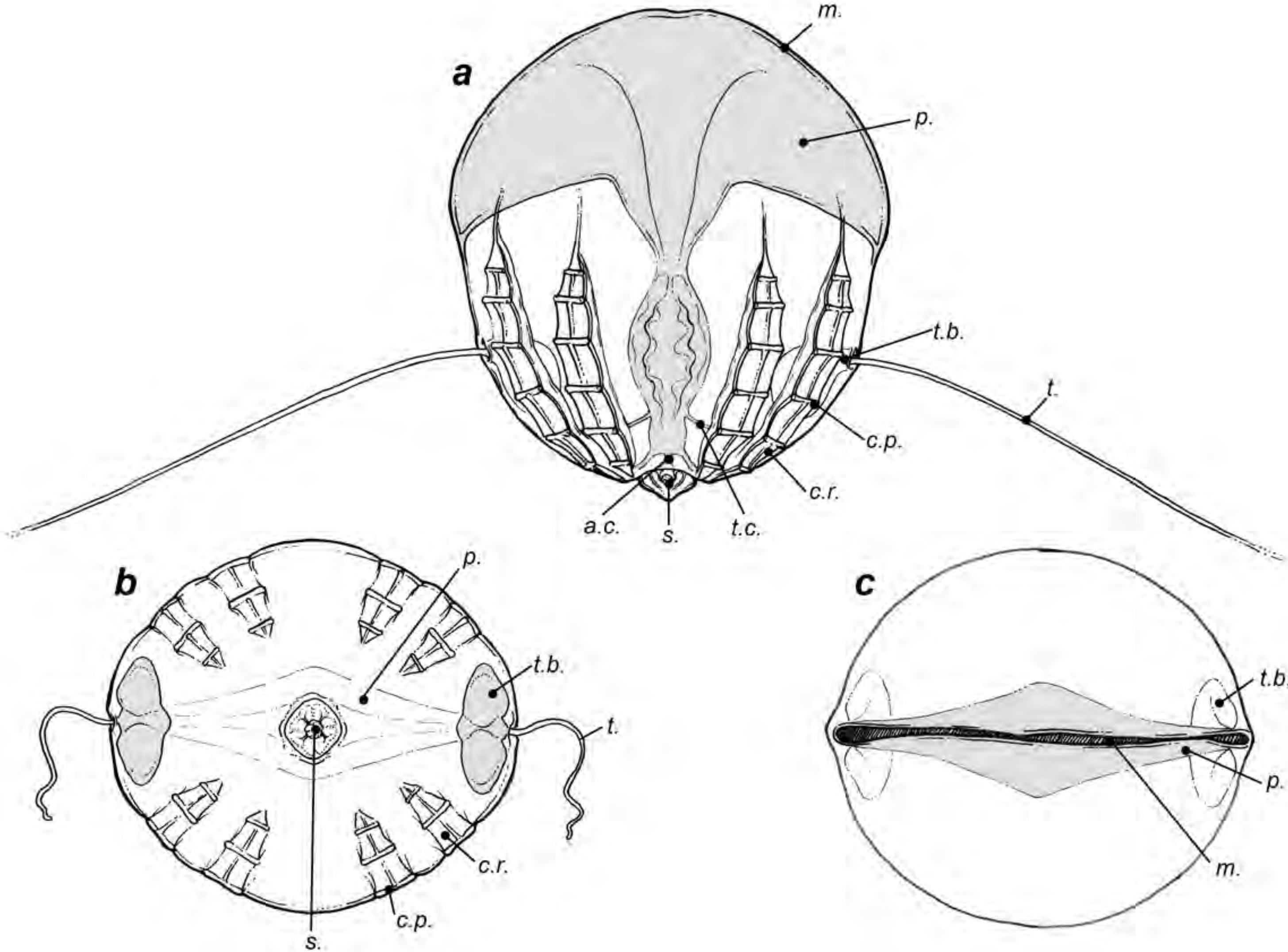
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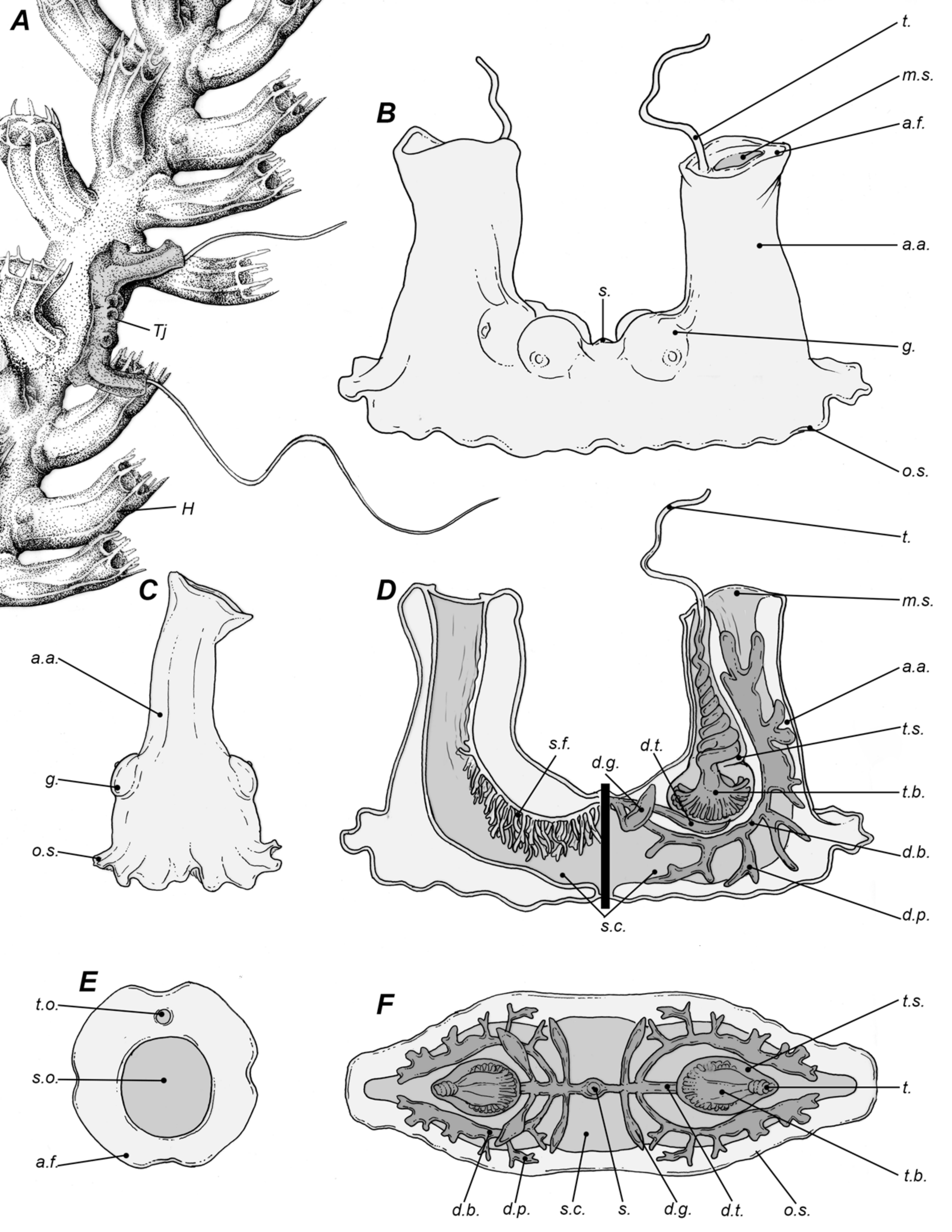

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d.t.
t.b.
d.b.
d.p.
s.c.
E
t.o.
s.o.
a.f.
F
t.s.
t.
t.b.
d.b.
d.p.
s.c.
s.
d.g.
d.t.
o.s.