

## Research Honors Thesis: Figures

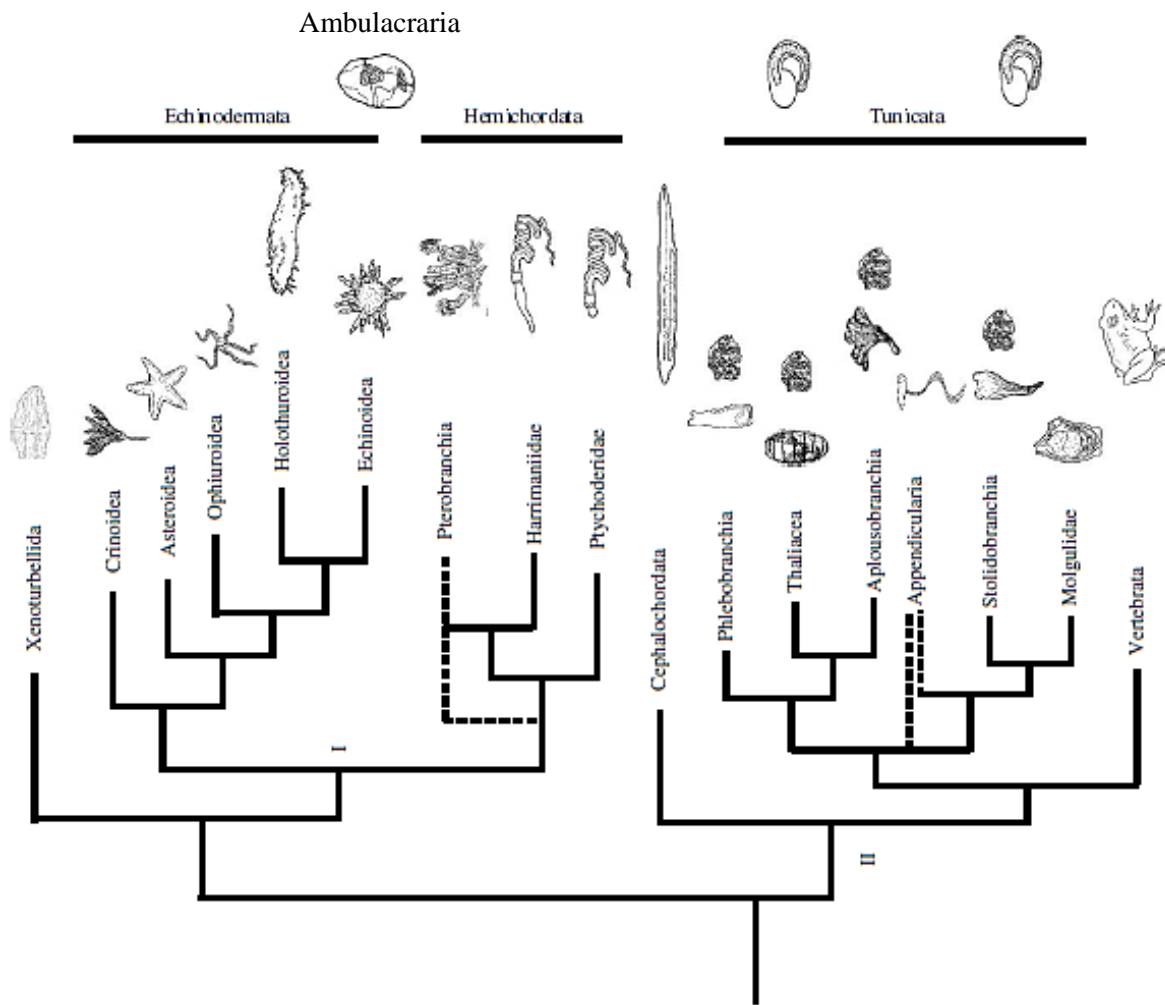


Figure 1: Phylogeny of Deuterostomia. The sister taxa Echinodermata and Hemichordata are grouped together within Ambulacraria, which is sister taxon to Chordata. Dashed lines in the hemichordate phylogeny indicate the uncertainty about the position of Pterobranchs within this group; some analyses indicate that they are basal hemichordates, others place them as a derived group within the enteropneusts that is sister to the Harrimaniidae (Enteropneusta).

Image from Swalla and Smith 2008

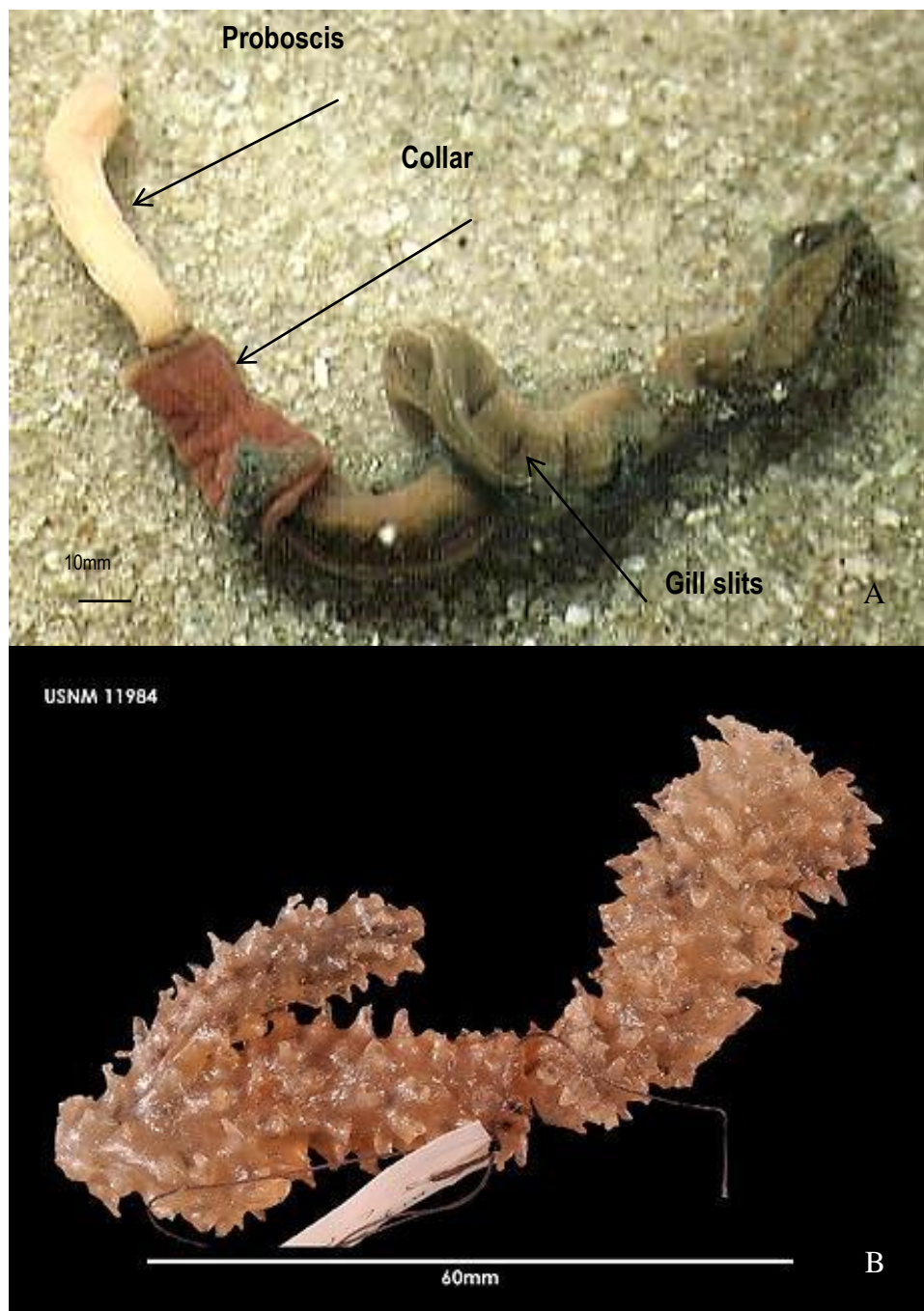


Figure 2:

A: Adult enteropneust

B: Pterobranch colony with retracted zooids

Images from:

A:<http://www.eol.org/pages/510542>

B:<http://www.theseashore.org.uk/theseashore/SpeciesPages/Additional%20Species/Acorn%20Worm.jpg.html>

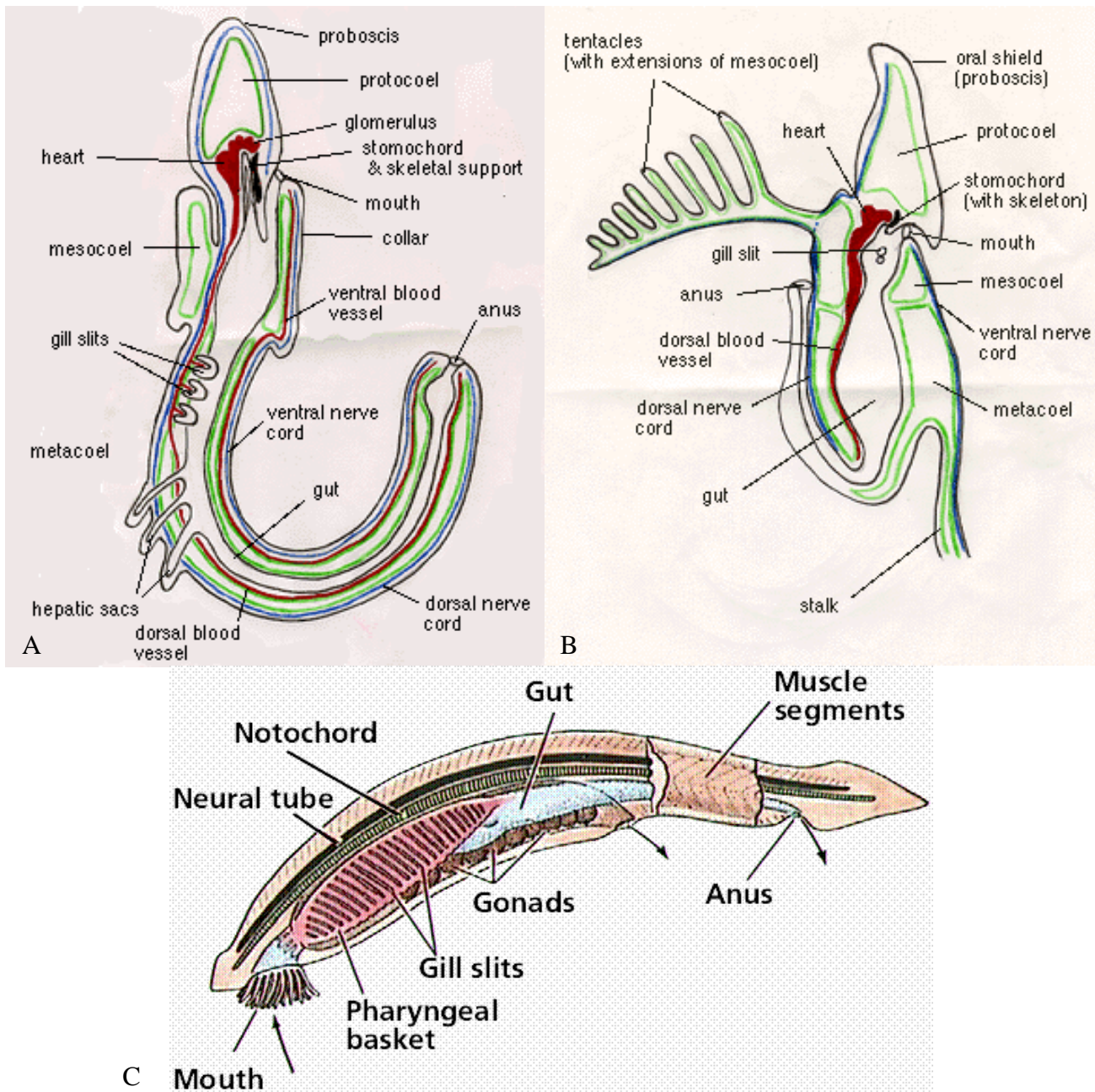


Figure 3: Comparison of the internal anatomy of enteropneusts (A), pterobranchs (B) and a chordate (Amphioxus) (C). Stomochord, collar region, glomerulus associated with the heart to form a kidney structure and three paired coelomic cavities (protocoeal, mesocoel and metacoel) can be seen in the hemichordates (A,B). The notochord, dorsal hollow nerve cord (neural tube) and pharyngeal gill slits can be seen in the chordate (C).

Images from:

A: <http://www.geol.umd.edu/~jmerck/honr219d/notes/19.html>

B: <http://www.biosku.blogfa.com/cat-10.aspx>

[www.cartage.org.lb/en/themes/sciences/zoology/biologicaldiversity/AnimalsIII/AnimalsIII.htm](http://www.cartage.org.lb/en/themes/sciences/zoology/biologicaldiversity/AnimalsIII/AnimalsIII.htm)

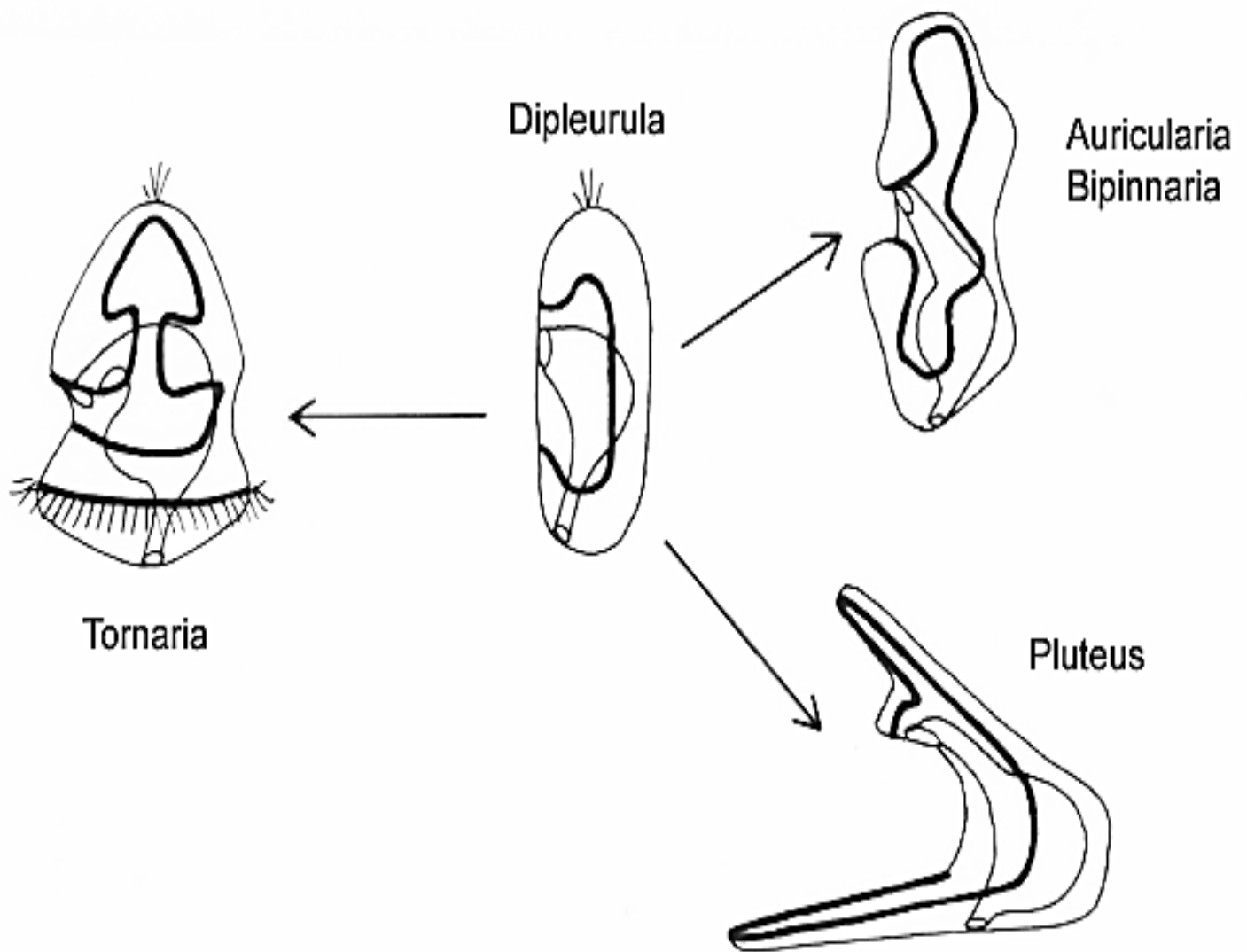


Figure 4: Dipleurula type larvae of organisms that belong to Ambulacraria. Tornaria: enteropneusts. Auricularia, bipinnaria and pluteus: echinoderms.

Source: <http://scaa.usask.ca/gallery/lacalli/tutorial/deuterostomes.php>





Figure 5

A: *Cephalodiscus nigrescens*, coenecium showing retracted zooids

B: *C. nigrescens* intact coenecium with zooids

C: *Cephalodiscus* sp. Demonstrating similar coenecium structure to *Rhabdopleura* (scale bars=1mm (a), 2mm (b,c))

Photographs by E.J. Balser

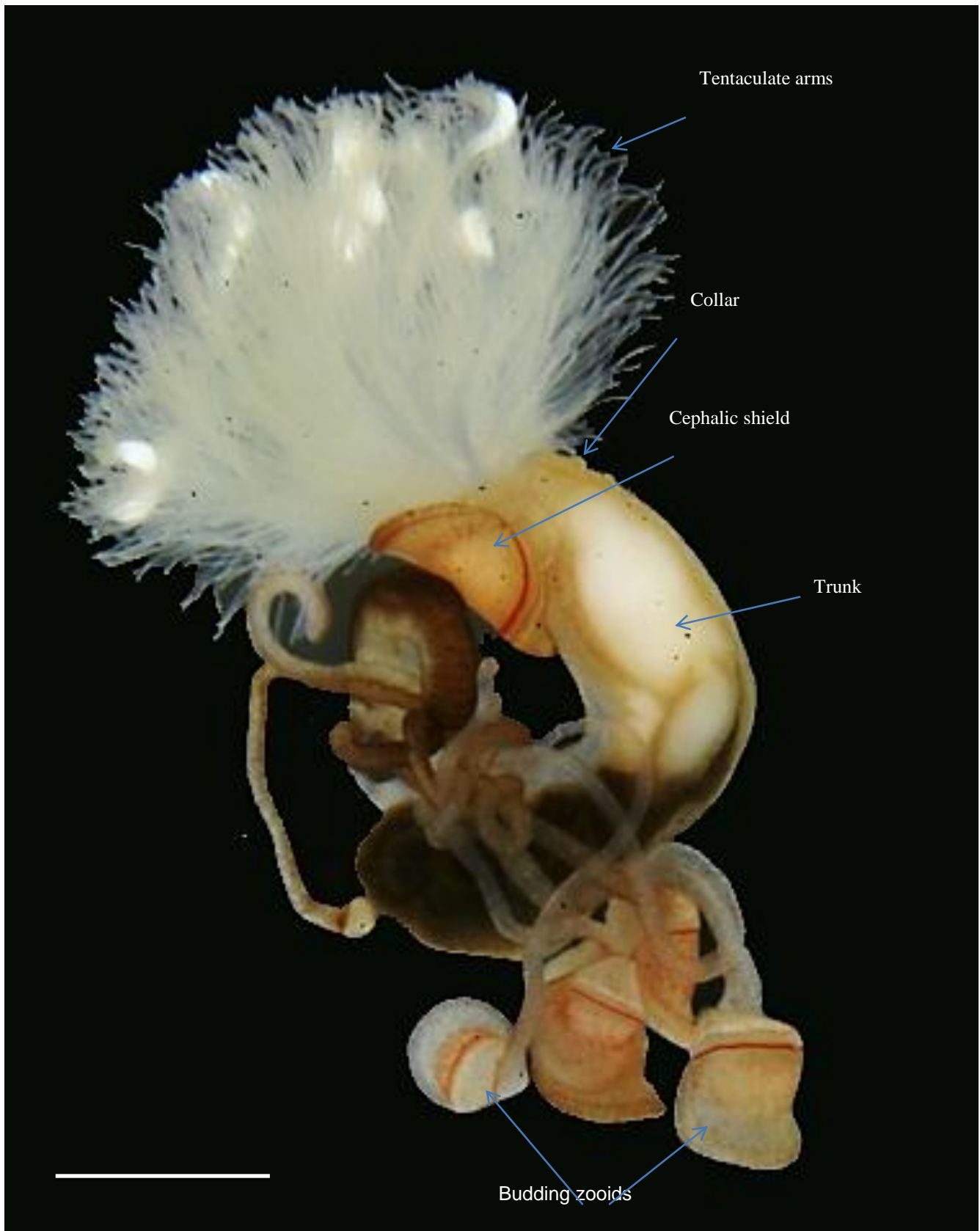


Figure 6  
Zooid of *Cephalodiscus nigrescens* removed from coenecium. Budding zooids are visible at the bottom.  
Scale bar=1mm  
Photograph by E.J. Balser

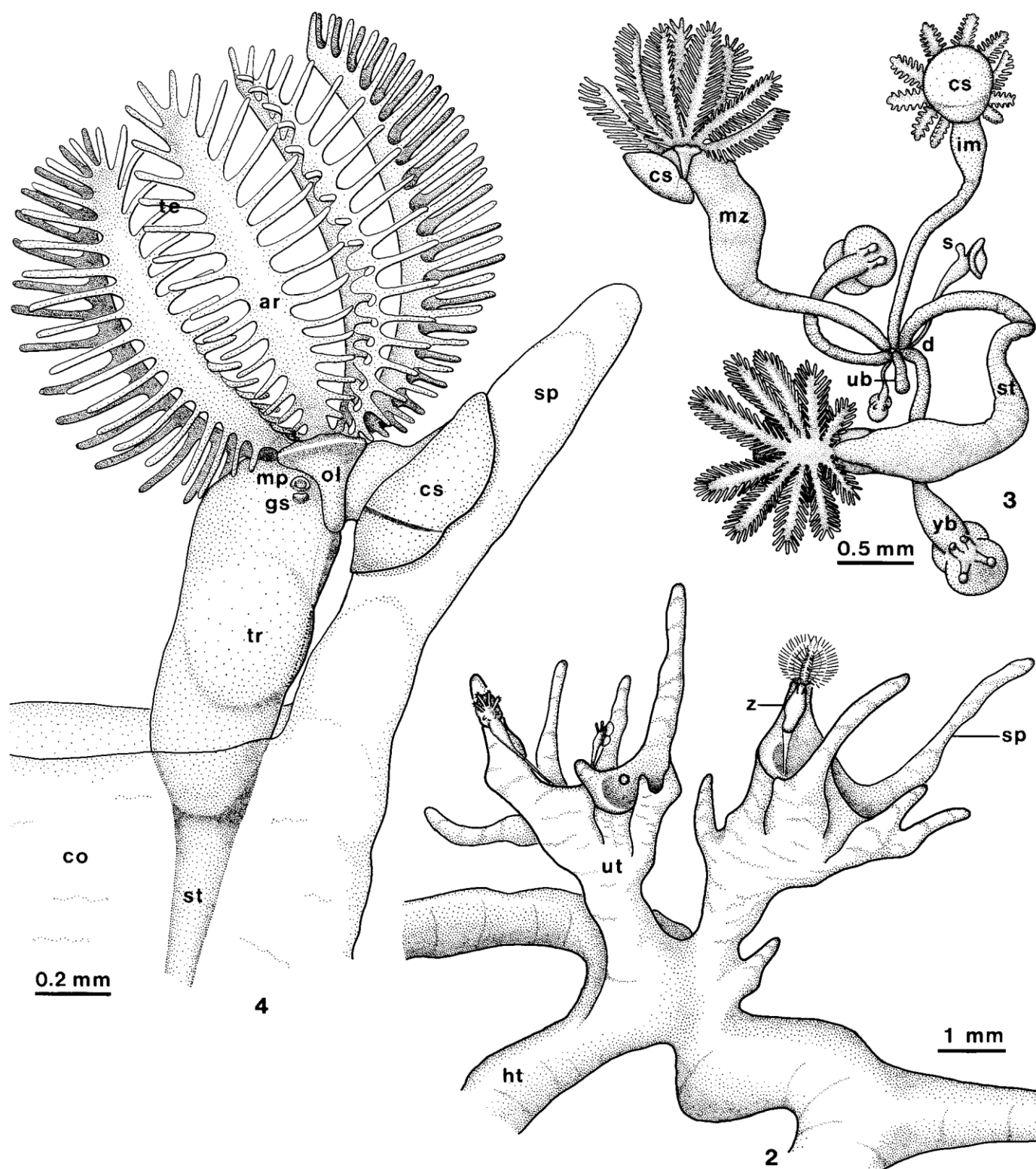


Figure 7: Drawings of coenecium and zooids of *Cephalodiscus* sp. showing attachment to a basal disc and zooid position in the coenecium during feeding.

Abbreviations: coenecium (co), zooid (z), basal disk (b), cephalic shield (cs), stalk (st), arms (ar), tentacles (te), upright tubes (ut), gill slit (gs), oral lamella (ol), young buds (yb), immature bud (ib), undifferentiated bud (ub), mesocoel pore (mp), distal ostia (o), arm tip swelling (s), horizontal tubes (ht), oral lamella (ol), stalk (st), trunk (tr)

Lester 1985



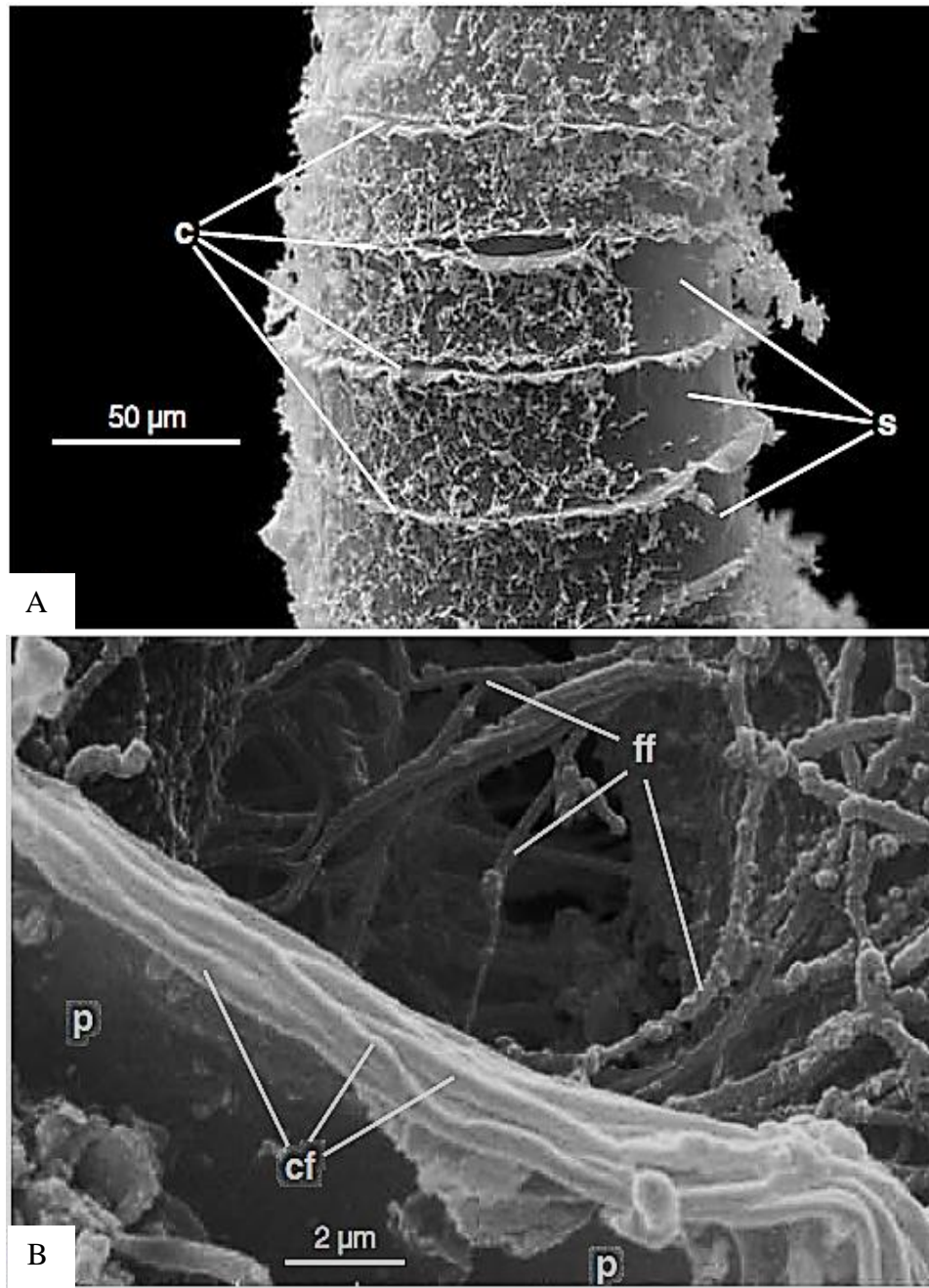


Figure 8: SEM images of coenecia of *Rhabdopleura* sp. showing fusellar and cortical fibrils.

A: cortical bands around a dwelling tube.

B: Three dimensional matrix of fusellar fibrils with an ordered bundle of cortical fibrils embedded. Fusellar fibrils are thinner than cortical fibrils.

Abbreviations: cortical fibrils (cf), fusellar fibrils (ff), fusellar collar (c), fusellar pellicle (p), outer surface of fussels (s)

Mierzejewski and Kulicki 2008



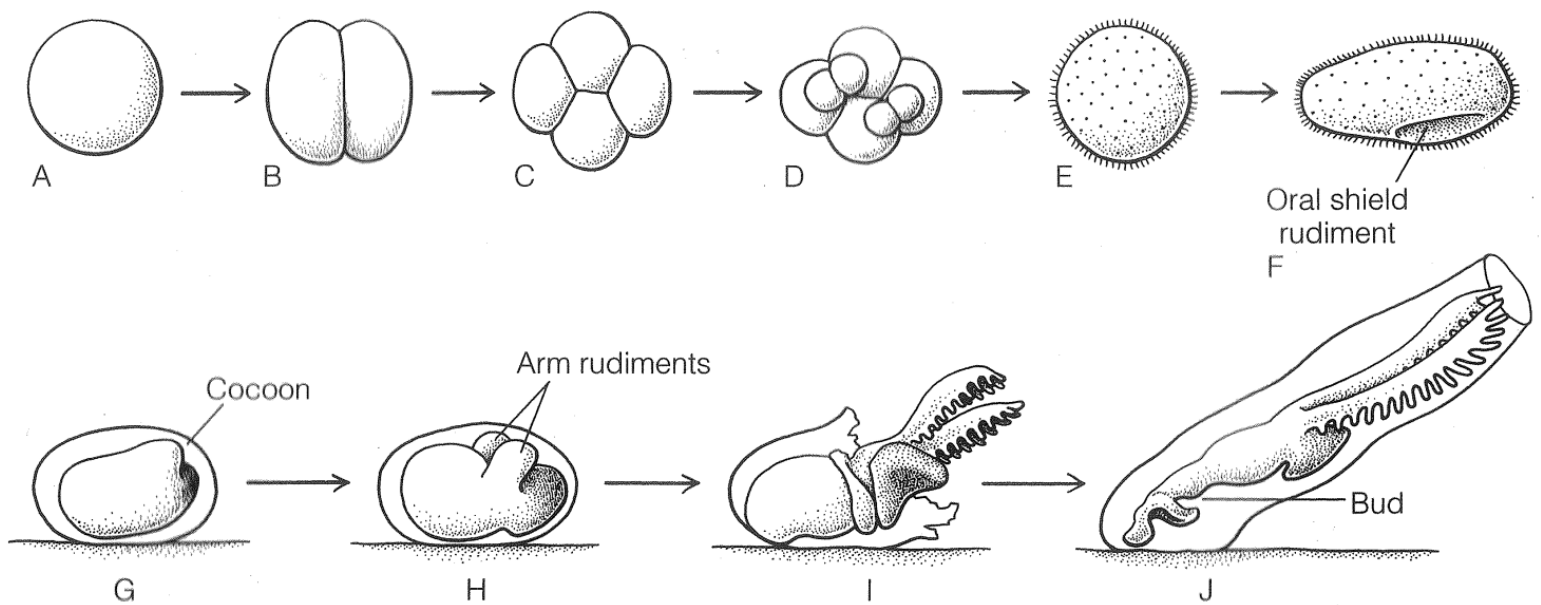


Figure 9: development of a pterobranch: A-F: formation of larva. G-E: settling of larva, secretion of cocoon, breaking out of the cocoon and development of coenecium. Zooid will begin budding to produce more zooids and will eventually form a colony.

Ruppert et al. 2004

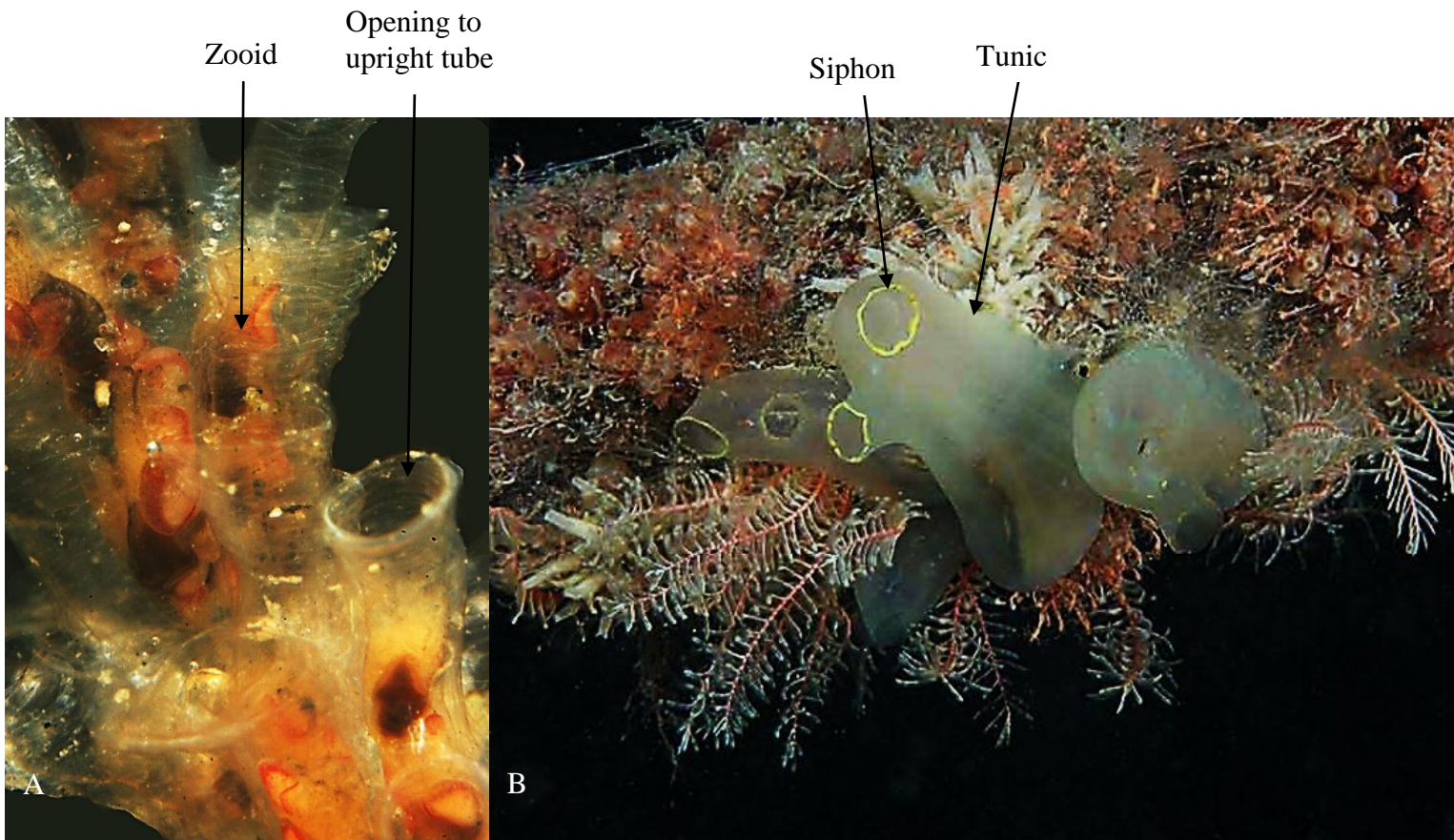


Figure 10: comparison of coenecium of *Cephalodiscus nigrescens* to the tunic of *Ciona intestinalis*. The materials composing the tunic and the coenecium display visual similarities and also have similar properties such as durability, resistance to degradation and texture.

A: Coenecium of *C. nigrescens* with retracted zooids

B: Adult specimens of *C. intestinalis*, the tunic is the outermost layer.

Source:

A: E.J. Balser

B: <http://www.marlin.ac.uk/phylumdetails.php?phylum=2341>

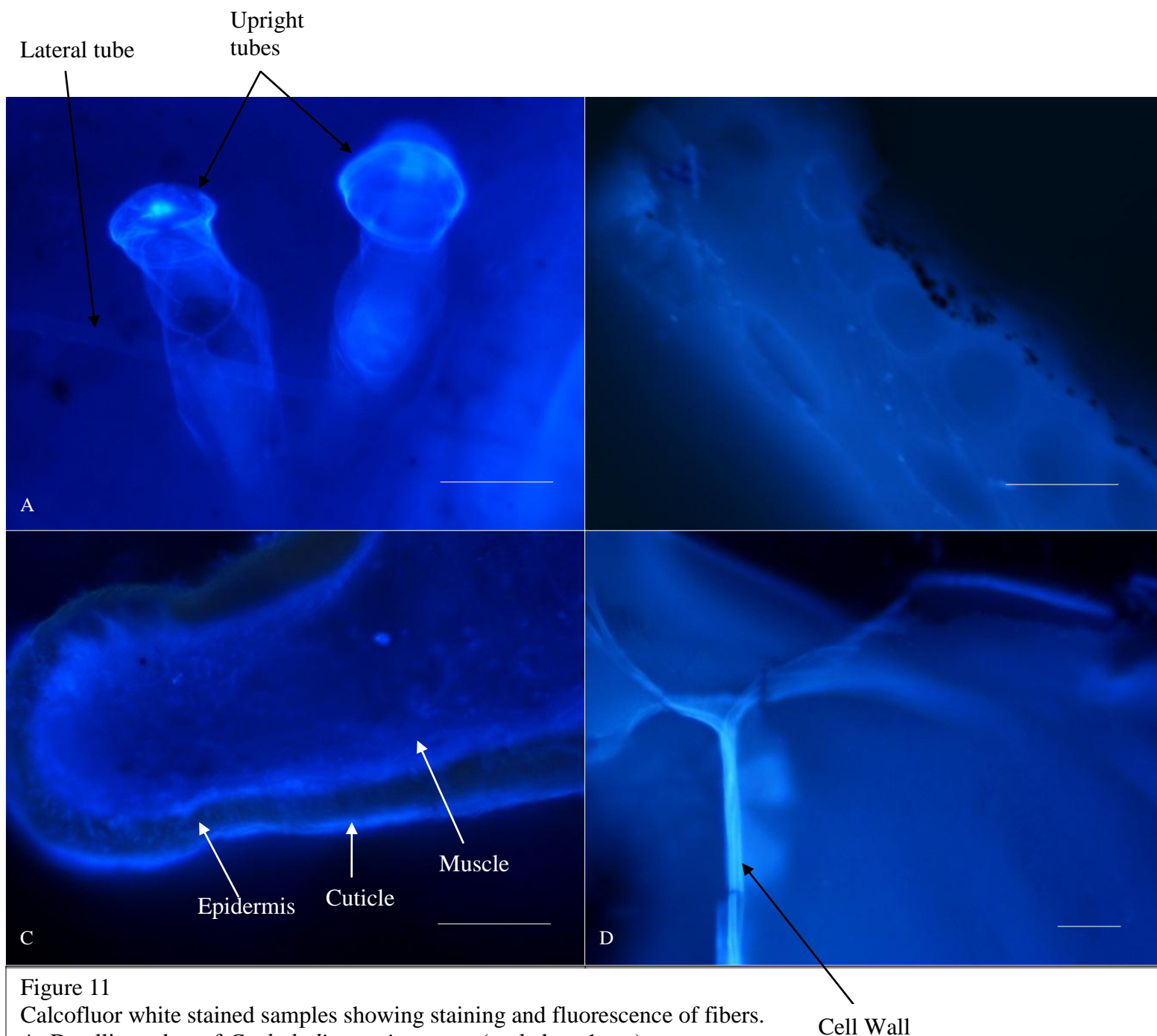


Figure 11

Calcofluor white stained samples showing staining and fluorescence of fibers.

A: Dwelling tubes of *Cephalodiscus nigrescens* (scale bar=1mm)

B: Cross section of ascidian tunic (scale bar=1mm)

C: Cross section of whole annelid (scale bar=1mm)

D: Unknown alga showing fluorescence in cell wall (scale bar=10um)

Scale bars=1mm

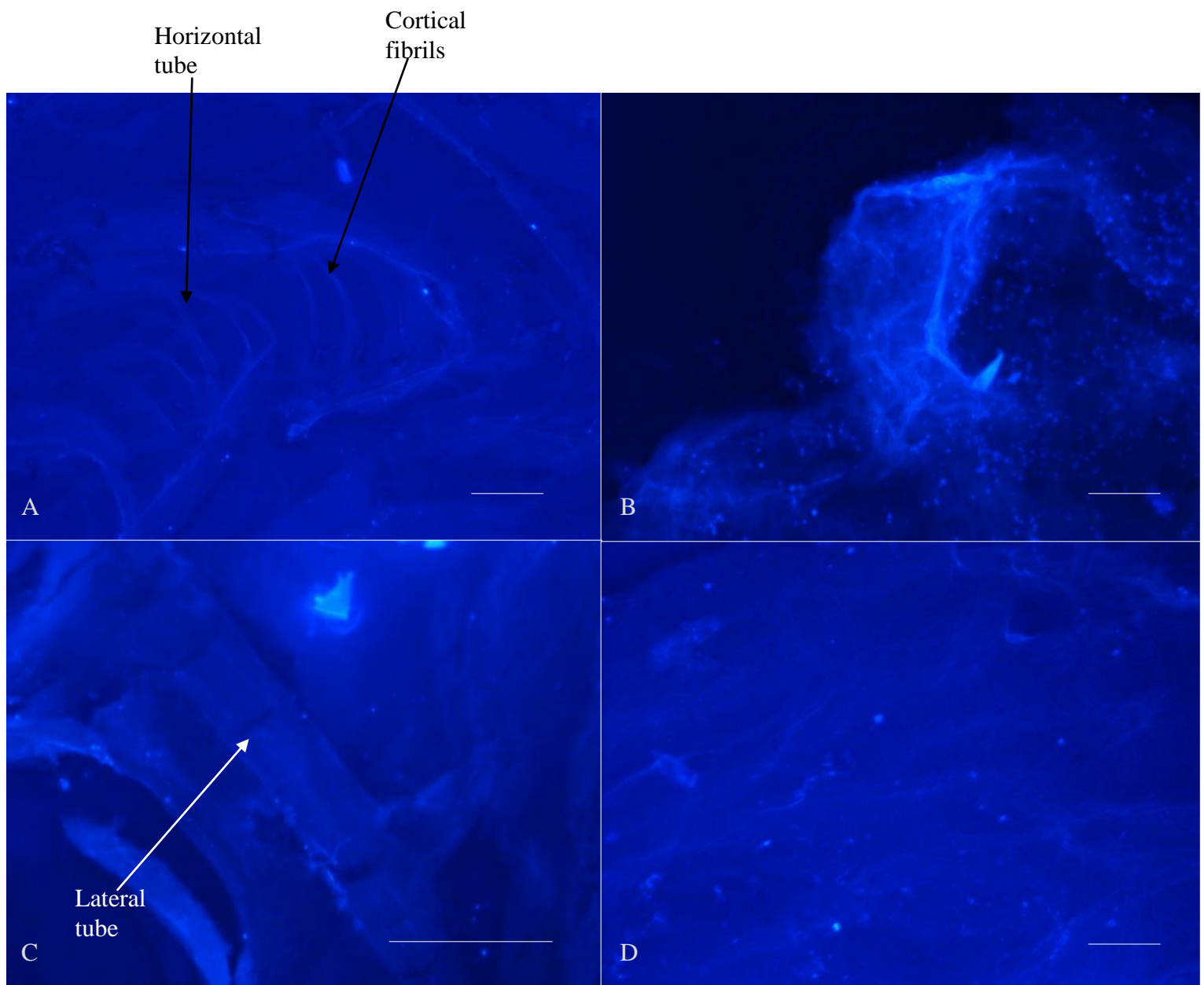


Figure 12 Sucrose impregnated cryostat sections of *Cephalodiscus nigrescens*

A: Fluorescing cortical fibrils in upright dwelling tube.

B: Fibers in coenecium.

C: Lateral dwelling tube

D: Small fibers in gelatinous matrix of coenecium.

Scale bars=0.5mm



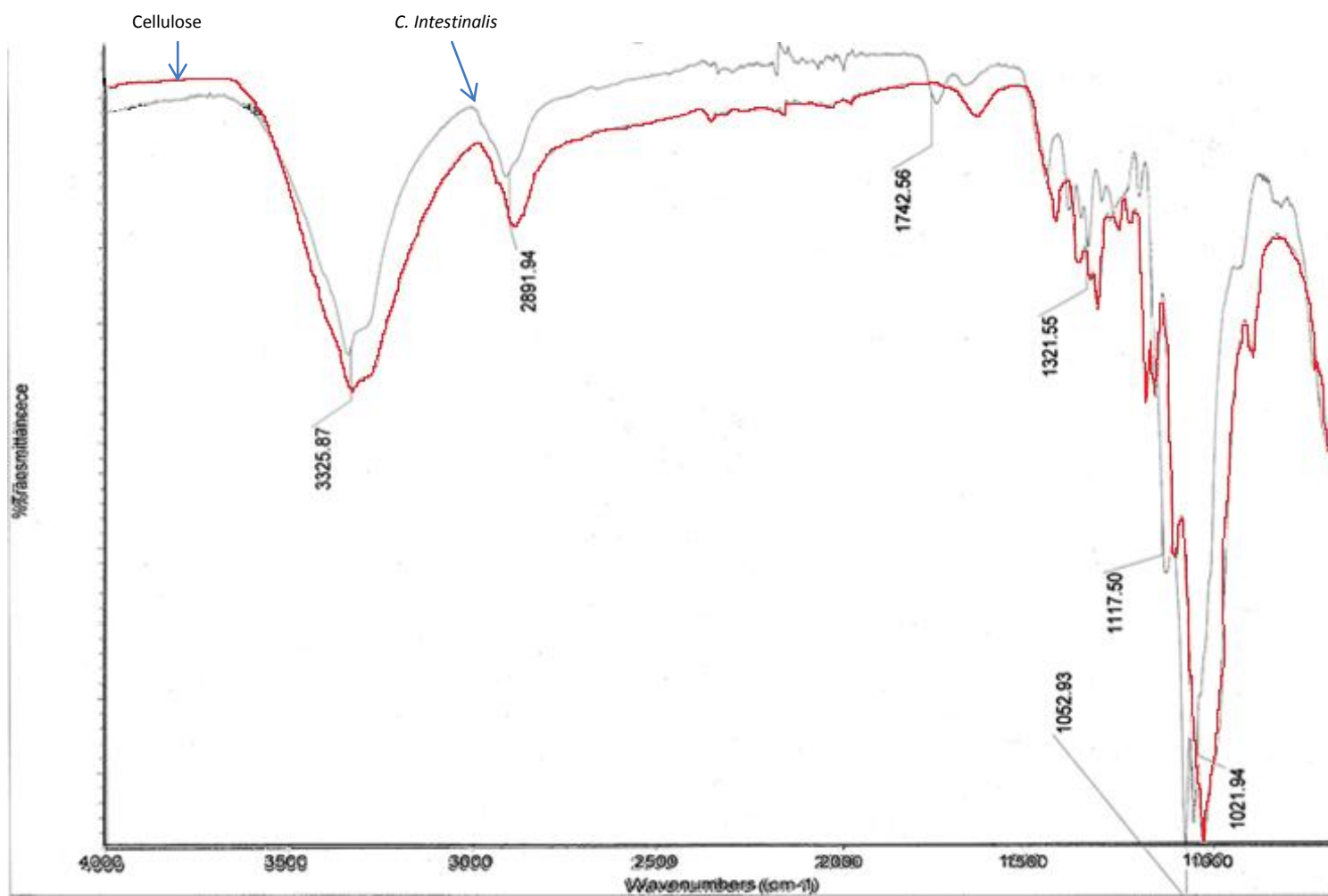


Figure 13: Overlay of IR spectra of cellulose standard (red) and *Ciona intestinalis* extract (black). Spectra confirm that the extract from *C. intestinalis* is cellulose.

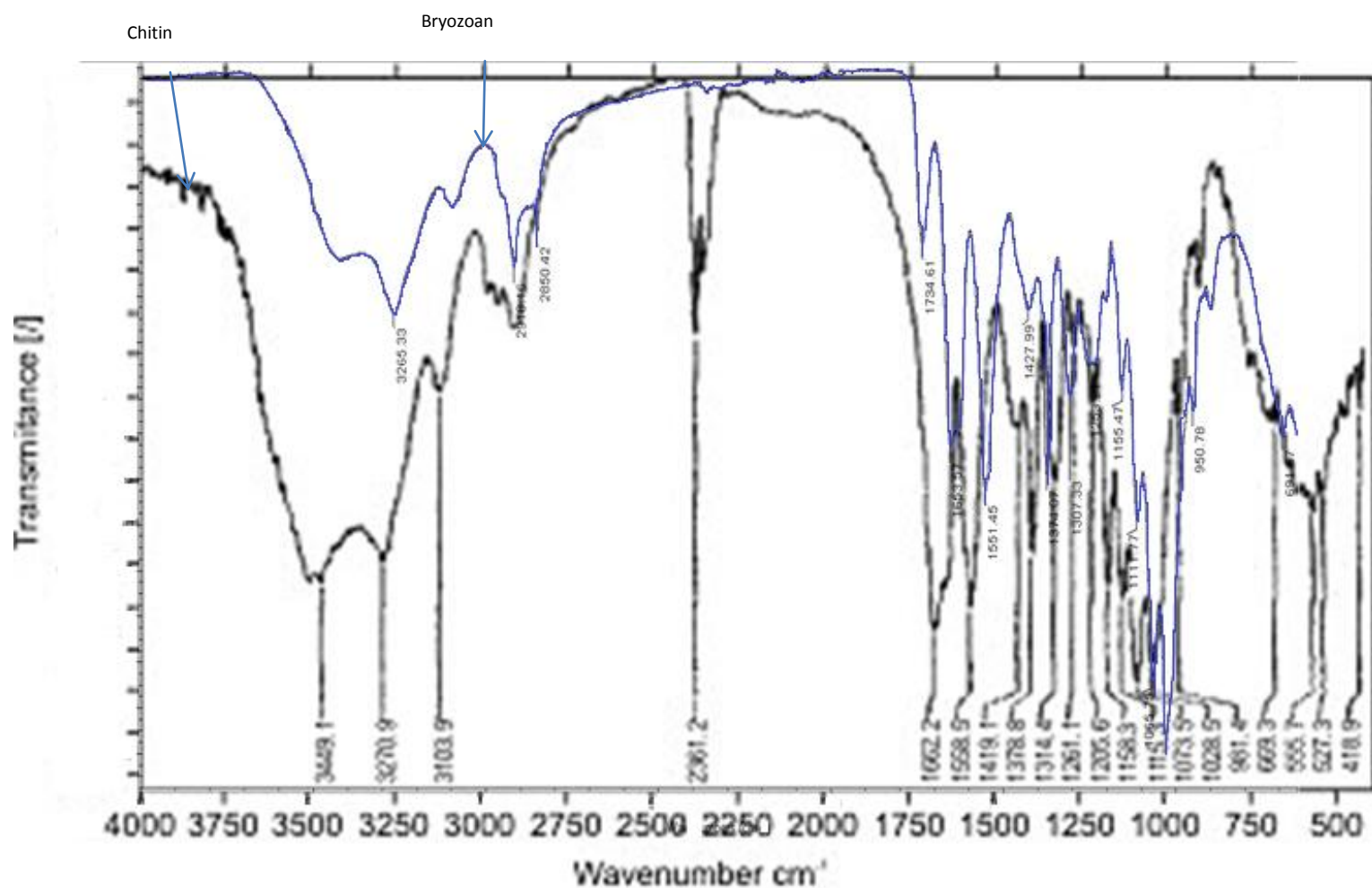


Figure 14: Overlay of IR spectra of chitin literature spectrum (black) and bryozoan extract (blue). Spectra confirm that the extract from the bryozoan is chitin.

Literature spectrum: [http://www.scielo.br/scielo.php?pid=S1517-83822004000200013&script=sci\\_arttext](http://www.scielo.br/scielo.php?pid=S1517-83822004000200013&script=sci_arttext)

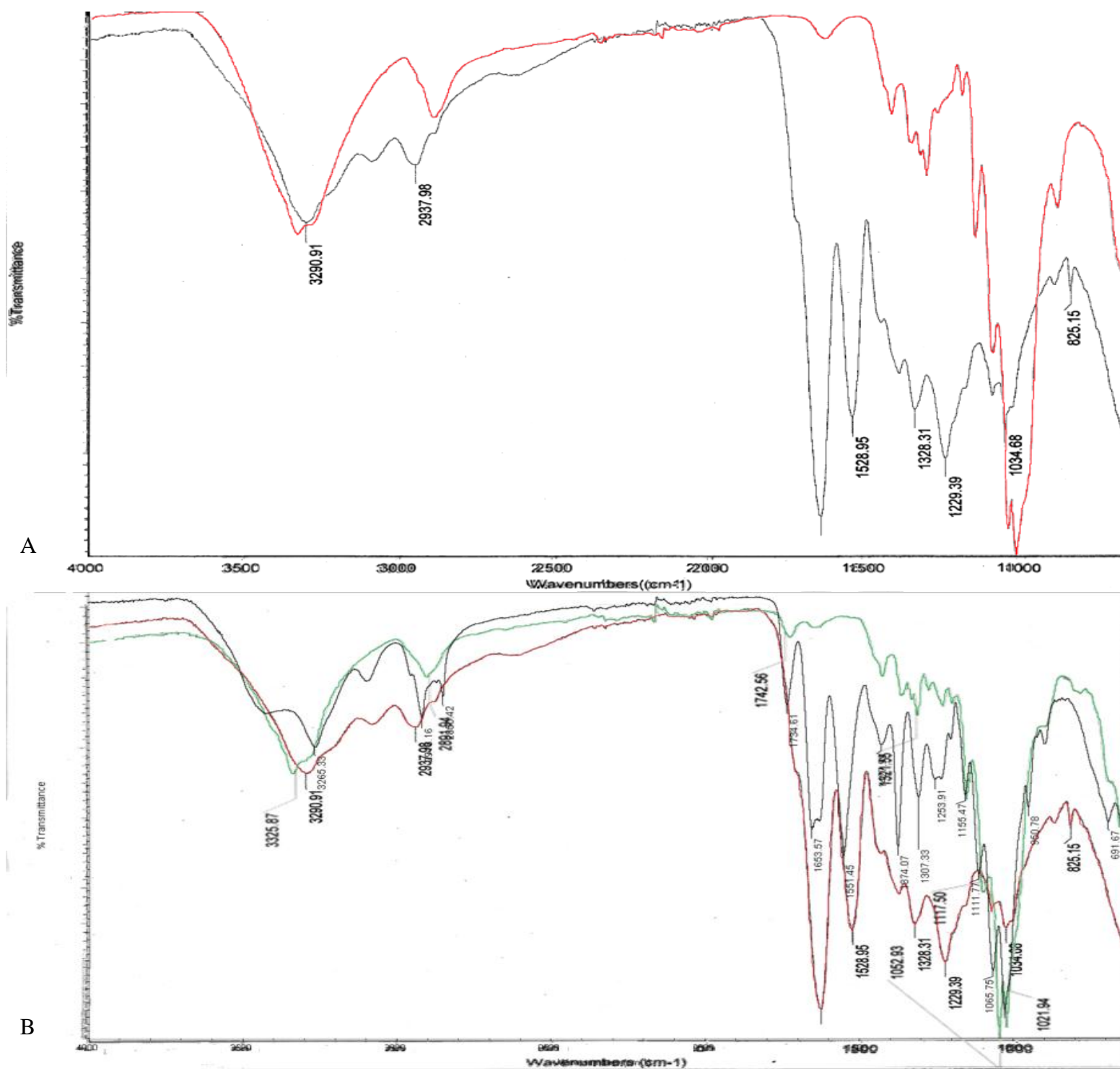


Figure 15:

A: Overlay of IR spectra of cellulose standard (red) and extract from *Cephalodiscus nigrescens* (black)

B: Overlay of IR spectra of extract from *C. nigrescens* (red), *Ciona intestinalis* (green) and a bryozoan (black).

Spectra do not match up, indicating that the structural material is neither cellulose nor chitin.



Figure 16: Stained epon sections of *C. nigrescens* shown to compare ineffectiveness of Masson's trichrome in staining epon sections.

A: Toluidine blue stained epon section of a zooid of *C. nigrescens* in its tube. Feeding arms are visible. Provided to show contrast in staining and to demonstrate the position of the zood on the section.

B: Masson's Trichrome stained specimen showing very little staining and no visible fibers. Parallel lines are knife marks, dark lines are creases in the section.

Scale bars=0.1mm



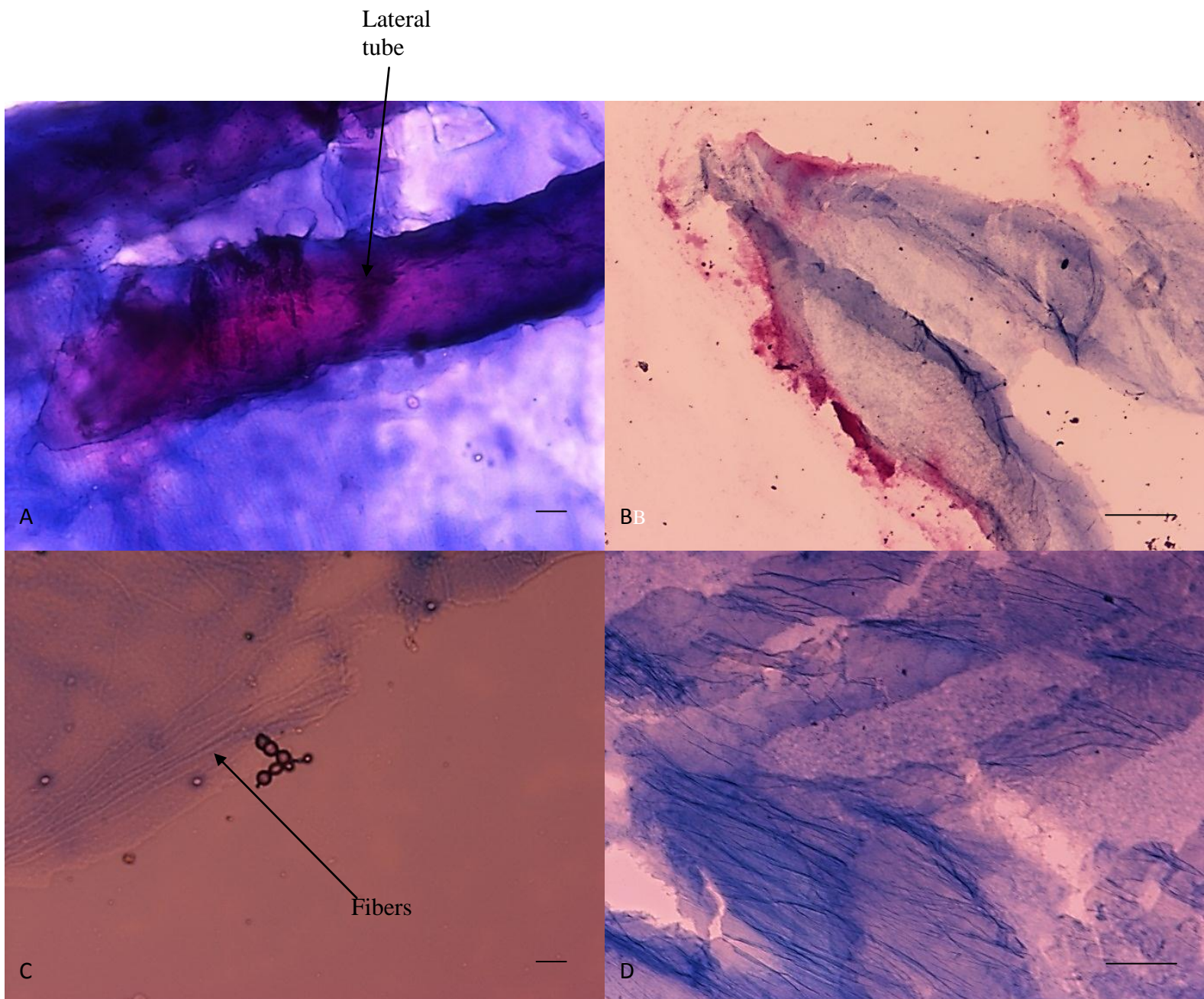


Figure 17: Frozen, sucrose embedded sections of coenecia of *C. nigrescens* stained with Masson's Trichrome.  
 A: Section with lateral dwelling tube showing trapped red stain and generalized blue staining (scale bar=0.01mm).  
 BB: Unidentified structure showing diffuse blue and red staining and loss of tissue structure (scale bar=0.1mm)  
 C: Tissue showing potential fibrils or aggregates of fibrils which are not taking up stain. Surrounding tissues show diffuse staining. (scale bar=0.01)  
 D: Tissue showing extensive folding as a result of desiccation and the sections not being fully flattened on the slide. (scale bar=0.05mm)



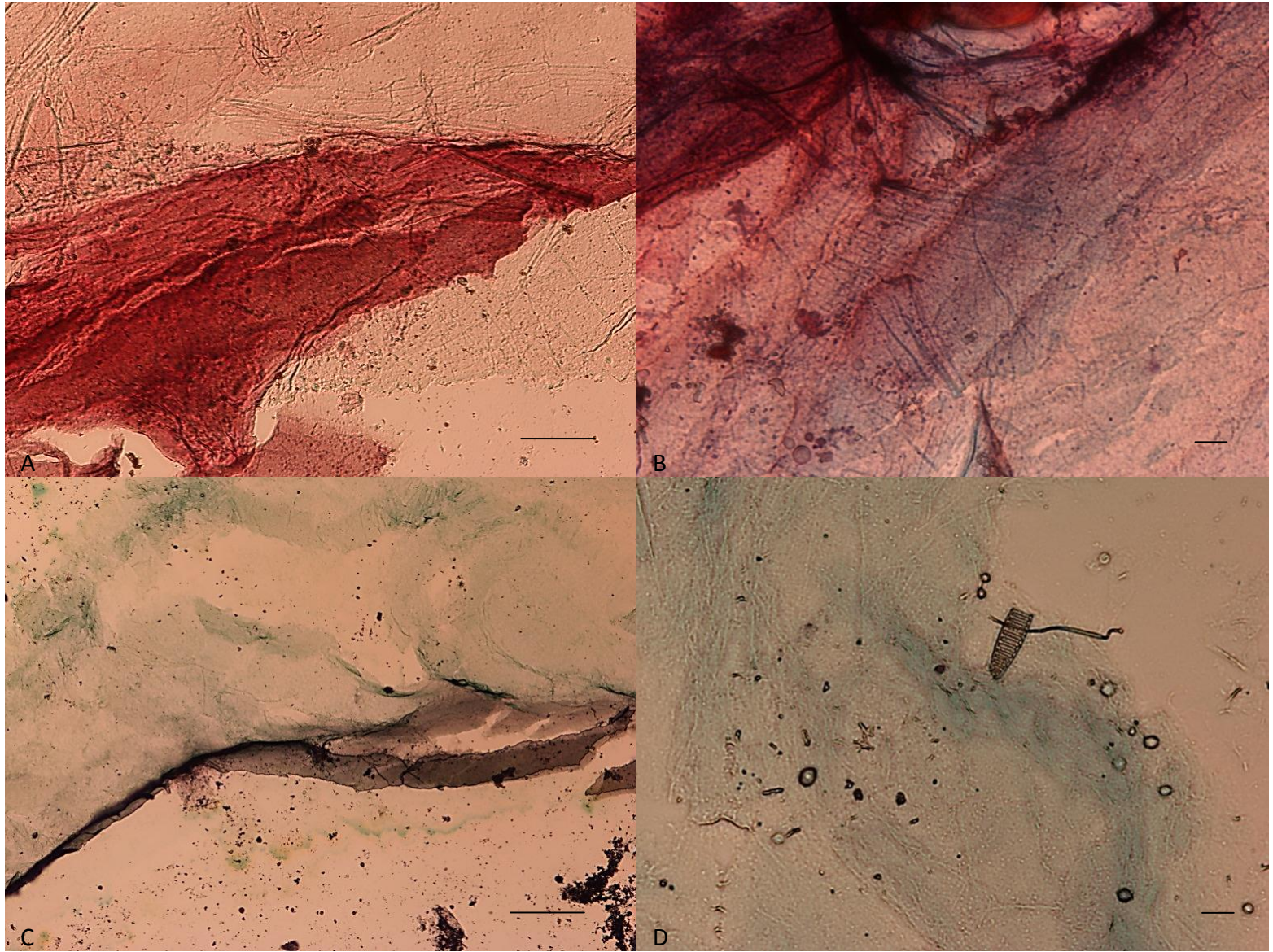


Figure 18: Frozen, sucrose embedded sections of coenecia of *C. nigrescens* stained with Hollande's Trichrome showing the inconsistency of staining and difficulty of distinguishing fibrils from creases and folds in sections.

A: Red stained section of tissue showing extensive creasing and diffuse staining that is also picked up by the slide where no tissue is present. (scale bar=0.05mm)

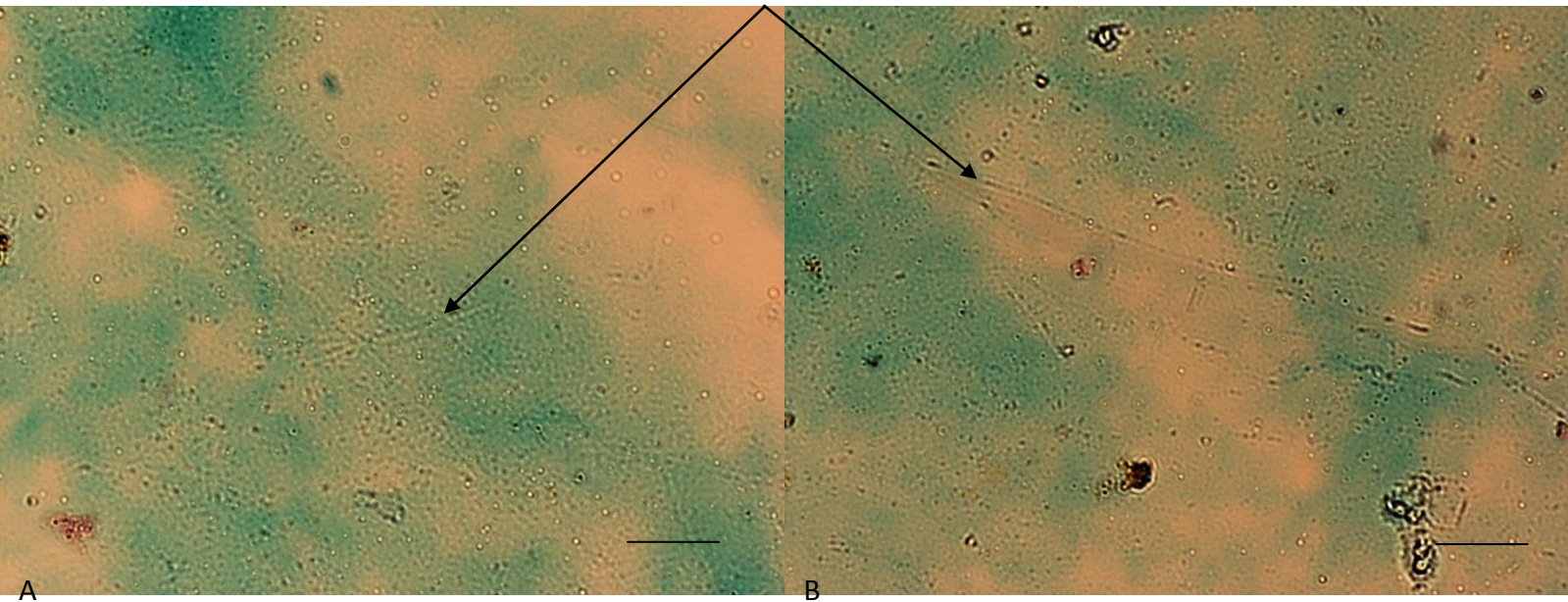
B: Section showing both red and green staining present in the same tissue sample, demonstrates the inconsistent staining. (scale bar=0.01mm)

C: Stained slide showing green stained piece of gelatin peeling from the slide. (scale bar=0.1mm)

D: Green stained section showing extensive creases and debris of unknown origin. (scale bar=0.01mm)



Fibers



A

B

Figure 19 A,B: High magnification (100X) images of frozen, sucrose embedded sections of *C. nigrescens* stained with Hollande's Trichrome. Small fibrils or bundles of fibrils are visible within indistinct, diffusely staining tissue. Fibrils are clearly not staining. (scale bar=0.01mm)