Image Descriptions

In general, the images in each section are placed in order of when they were taken/produced. The order is meant to show my exploration and gradual mastery of the tools and techniques.

Macrophotography Images

1. Three Melibe leonina (hooded nudibranch) huddled up on the side of the water tank.
   This was a completely spontaneous moment that I captured on the first week using a Samsung S20 fe phone. The angle and framing of the photo were deliberate, but I didn't pay much attention to the background or lighting.
2. Garter snake hiding in the grass near the lab.
   This was one of the first photos that I took with the macro camera (on automatic mode). The image has gone through four revisions after receiving feedback throughout the course: Version 1 was unedited; version 2 sharpened the snake, blurred background elements, and received some color correction; version 3 added vignetting, as well as some additional blurring and sharpening; version 4 reduced color saturation on the blade of grass that is front and center in the image.
3. Spider wrapping up prey that had recently flown into its web.
   The photo was taken with the macro camera on manual mode. The photo demonstrates my increasing proficiency at manipulating the camera settings. Since the photo was taken on a cloudy and windy day, it was quite dark and the spider web would constantly wobble. I could only get the lighting and focus that I did by playing with the camera settings.
4. Close-up of the spider from image 3.
5. Fluorescence image of a cleared and stained sculpin.
   The stains used were alcian blue and alizarin red, which stain cartilage and ossified tissue respectively. The photo was taken with the macro camera under ultraviolet light.
6. Two tentillum (coils) and an unidentified red mass (possibly a lure) from the siphonophore Nanomia bijuga.
   The photo was taken with the Zeiss fluorescence microscope without using fluorescence lights. My goal was to test whether I could take close-up shots of the siphonophore and other small critters using the Zeiss scope. The Zeiss scope was a tool that I used only twice, but with more practice and experience using the tool, I think it might be possible to use it to capture images of small specimens with good lighting and focus.

SEM Images

7. Five SEM images of a purple sea urchin spine stitched together.
   A) This image was my first attempt at stitching together SEM images.
8. Five SEM images of a purple sea urchin spine stitched together.
   B) The same five SEM images colorized and stitched together from scratch using the photo editing skills and experience that I've obtained throughout the course.
9. Three SEM images of a leaf hopper stitched together.
   This image was my first attempt at colorizing an SEM image. (A) My first ever attempt. (B) My second attempt, which was created one week later after further familiarizing myself with Photopea's colorization tools.

Histology Images

10. Fish tissue taken at 5x magnification.
    This was my first attempt at paraffin histology. My histology slices had many areas in which the tissue fell apart, either caused by incomplete invasion of paraffin into my specimen or soaking the slices in an overly hot water bath.
11. My histology slides box.
    Histology is the technique I am the most proficient at because I've gone through the process of preparing a specimen, cutting it with the microtome, and staining it many times. Throughout the process, I've also played with variables such as how long I keep my specimens in xylene, the thinness of my slices, and staining duration.
12. H&E-stained lateral cross-section of a scallop gill taken at 10x magnification. Insets were taken at 20x magnification.
    The image demonstrates my increasing proficiency at preparing, cutting, and staining my specimens.
13. H&E-stained transverse cross-section of a scallop eye taken at 20x magnification.
    This was my second attempt. My first attempt failed because of incomplete invasion of paraffin, which caused my specimen to fall off when I cut my paraffin block. This is one of my favorite images that I've produced because I managed to preserve a lot of the tissue in a structure less than 0.5 mm in all dimensions and despite not softening up the eyeball before infiltration.

Micro-CT Images

14. Skull of a ribbed sculpin with operculum bones and gill rays highlighted in blood-red.
    My first micro-CT scan. The skull was sectioned out from my CT scan of a ribbed sculpin (12.5 mm x 2 mm x 2 mm).

Images from the Final Project

15. Melibe leonina cerata.
    PTA stains all tissue while silver nitrate stains nervous tissue. I stained one cerata with PTA and another with silver nitrate to differentiate between digestive and nervous tissue. (A) Photograph of a cerata fixed in glutaraldehyde. (B) Micro-CT scan of a PTA-stained cerata. The stained inner tissue is depicted in green. (C) Micro-CT scan of a Golgi-stained cerata. Nervous tissue is depicted in green.
16. Histology of the Melibe leonina cerata.
    Digestive tissue and nervous tissue were both identified in the same histological section.
17. Micro-CT scan of a PTA-stained Melibe leonina (hooded nudibranch).
    The stained inner tissue is depicted in green. Large organs such as the guts and penis were sectioned out. The bottom-left inset shows the autotomous plane of intact cerata. The top-right inset shows the autotomous plane after the cerata has been autotomized. The tissue seems to constrict/contract at the autotomous plane where autotomy has occurred.
18. Dirona pellucida cerata.
    Unlike in the Melibe leonina cerata, where the inner tissue stained by PTA and silver nitrate look very different, the inner tissue of the PTA-stained and silver-stained Dirona pellucida cerata look surprisingly similar. (A) Photograph of a cerata fixed in glutaraldehyde. (B) Micro-CT scan of a PTA-stained cerata. The stained inner tissue is depicted in red. (C) Micro-CT scan of Golgi-stained cerata. Nervous tissue is depicted in green.
19. Histology of the Dirona pellucida cerata.

The histological section shows nervous tissue. Surprisingly, digestive appears to be digestive tissue absent from the Dirona pellucida cerata, which may suggest that the cerata do not function as an extension of the digestive system in this species.

Final Project Summary

My goal for this project was to understand the morphological function of autotomy in nudibranchs. I began by exploring the structure and function of the cerata in nudibranchs that are capable of autotomy. I looked at two species of nudibranch: Melibe leonina and Dirona pellucida. I used micro-CT to look at the region where the cerata autotomized as well as the tissue present inside the cerata. An interesting fact about the cerata is that it functions as an extended gut in some nudibranchs, so I wanted to highlight the presence of digestive tissue inside the cerata. I stained the cerata in two different PTA (which stains all tissue) and silver nitrate (which specifically stains nervous tissue) in order to show the digestive tissue and nervous tissue separately. I used histology to identify the tissue present in the cerata. Surprisingly, digestive tissue seemed to be absent in the Dirona pellucida cerata. I also CT scanned entire nudibranchs in order to identify and observe the region at which autotomy occurs (the autotomous zone). I wanted to do a comparison of the autotomous zone before and after the cerata has been autotomized. I was able to accomplish this with the Melibe leonina. However, I was unable to show the autotomous zone in my CT scan of the Dirona pellucida because I didn't allow enough time for PTA to penetrate and stain the inside of my specimen. I also feel that my research is incomplete. If I were to continue my research, the next step would be to dissect out the autotomous zones of the intact and autotomized cerata so that I can do an up-close CT scan of the region. This would show the structure of the autotomous zone in much finer detail. Additionally, histology could also be performed on the dissected autotomous zone to try and identify the tissue types in the region. Since my areas of interest are neuroscience and stem cell research, I wonder if research into autotomy could be used to improve current methods of organ/cell growth. Although I don't know if I will continue doing research on autotomy in the future, I've learned and become proficient at using several tools and techniques throughout this apprenticeship that will serve me well in any research I do in the future and for my career in general. I've become proficient at macrophotography, taking SEM images, histology, CT-scanning, and using image editing software, as well as prepare specimens for these tools and techniques. My goal in taking this course was to learn about the many different visualization methods out there. I feel that I've accomplished my goal while additionally obtaining valuable experience working in a lab with other people.

Methods

Specimen preparation for preservation

In order to euthanize the nudibranchs, I gradually added 12g MgCl to 200mL sea water. Both species of nudibranch were very sensitive to the addition of MgCl, causing most of their cerata to fall off. I needed at least a few cerata to remain intact in order to compare the autotomous zone where cerata remain intact and where they have been autotomized. Luckily, my

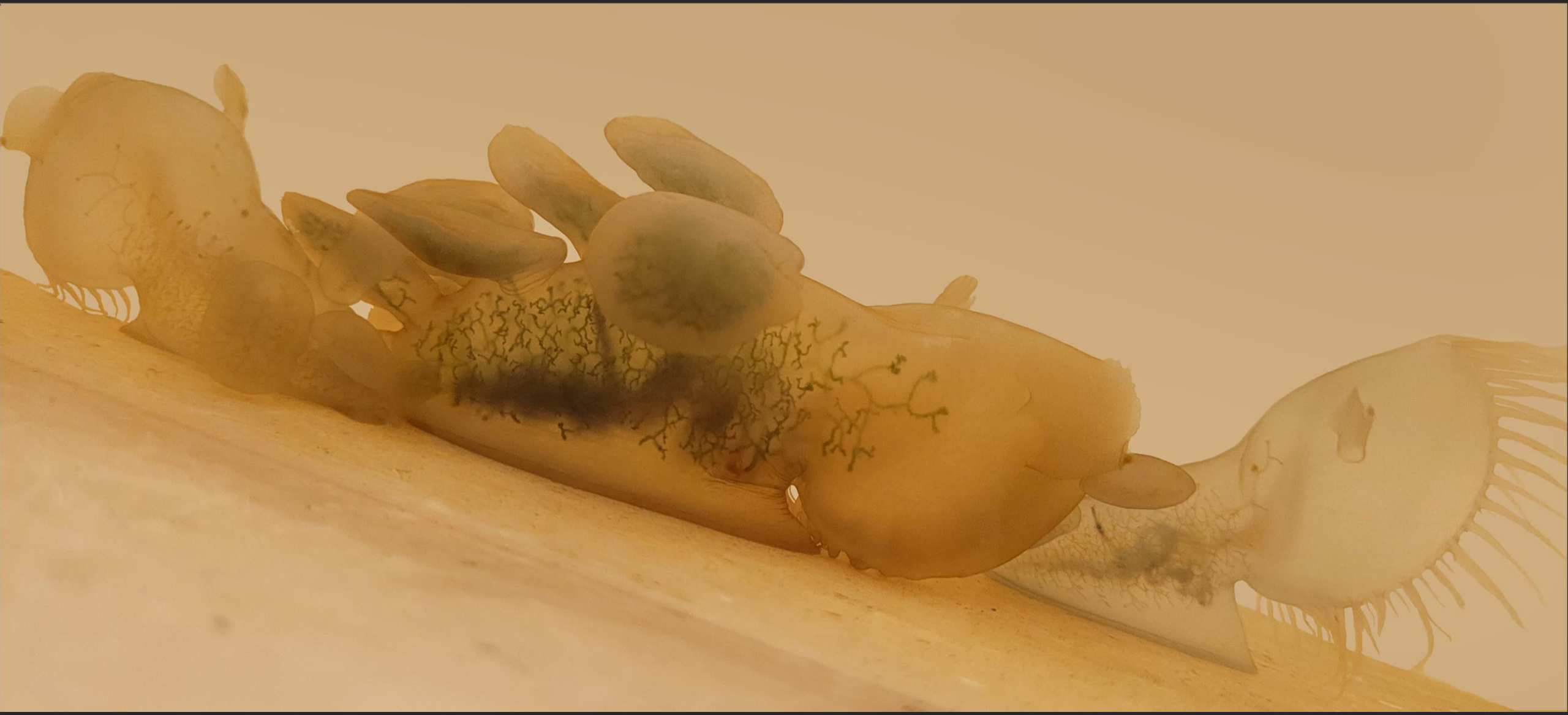
specimens had a few cerata left intact. After observing no movement for at least 5 minutes, I moved the nudibranchs to a 99% ethanol solution. I fixed the nudibranchs in 20% glutaraldehyde for at least 72 hours and the autotomized cerata in 2.5% glutaraldehyde for at least 48 hours. The nudibranchs and cerata then went through a dehydration series: I rinsed out the glutaraldehyde by putting the nudibranchs and cerata in water for 4 hours. I replaced the container with fresh water at the 2 hour mark. I then moved them to a 30% ethanol solution for 2 hours, then a 50% ethanol solution for 2 hours, then to a 70% ethanol solution where the specimens can be kept in and preserved indefinitely.

Specimen preparation for micro-CT

I stained some cerata in phosphotungstic acid (PTA) and some cerata in silver nitrate (Golgi stain). I stained the entire nudibranch specimens in PTA. I kept the nudibranchs and cerata in a 3% PTA in 70% ethanol solution for at least 72 hours. Unfortunately, 72 hours was not enough time for PTA to fully penetrate the Dirona pellucida specimen. Thus, a full scan of the Dirona pellucida was not included in my portfolio. Additionally, some of the cerata were put into silver nitrate and allowed to stain for 48 hours. When the nudibranchs are ready to be scanned, I put them into a plastic bag, placed them in an appropriately-sized tube, and used Styrofoam to suspend my specimen so that it doesn't touch the sides of the tube. For the cerata, I instead filled small centrifuge tubes with warm agar jelly, placed my specimens into the jelly, and allowed it to cool so that my specimens are suspended in the liquid. I then thoroughly wrapped the tube before putting them in the CT-scanner so that no liquid can leak out.
Micro-CT images I produced for the project can be seen in portfolio images 15-19.

Specimen preparation for paraffin histology

In order to prepare the cerata for histology, I put my specimens through another dehydration series. I moved the cerata from a 70% ethanol solution to an 80% ethanol solution for 1 hour, then to 90% ethanol for 1 hour, then to 95% ethanol for 30 minutes, then to 99% ethanol for 30 minutes, then to 100% ethanol for 15 minutes. In order to encase the cerata in a block of paraffin, xylene was used to allow paraffin to penetrate the specimen. I waited until the outer layer of the specimen became transparent and the inner layer looks like plastic before taking them out of the xylene. I then moved the cerata into smaller container of 1:1 xylene to paraffin solution for 1 hour, then into two changes 100% paraffin solution for 1.5 hours. The container was kept in a vacuum oven to prevent the paraffin from solidifying. After allowing sufficient time for the paraffin to penetrate, I moved the cerata into the paraffin block mold and filled the mold with paraffin. After the block has solidified, I used a microtome and water bath to cut the block and orient my slices onto microscope slides. After allowing time for my slides to dry, I submerged my slides in xylene to remove excess paraffin. After rehydrating my specimens in 90% ethanol and then in 70% ethanol, I then stained them with hematoxylin and eosin. After allowing my slides to dry, I then mounted my slides with cover slips and took photos with a light microscopy camera.
Histology images I produced for the project can be seen in portfolio images 16 and 19.



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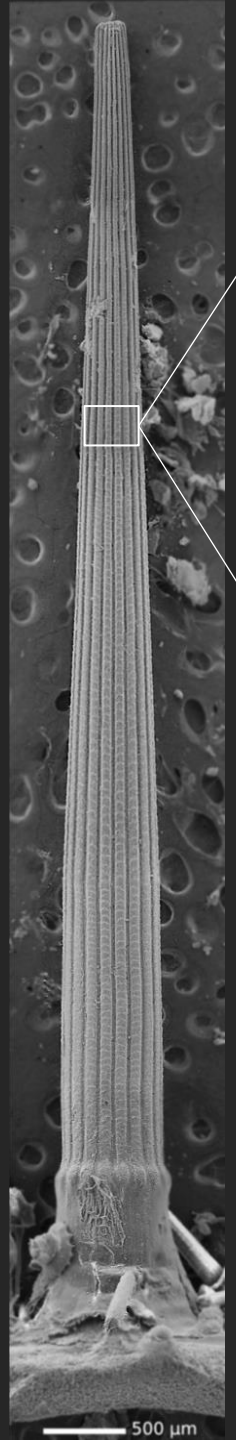
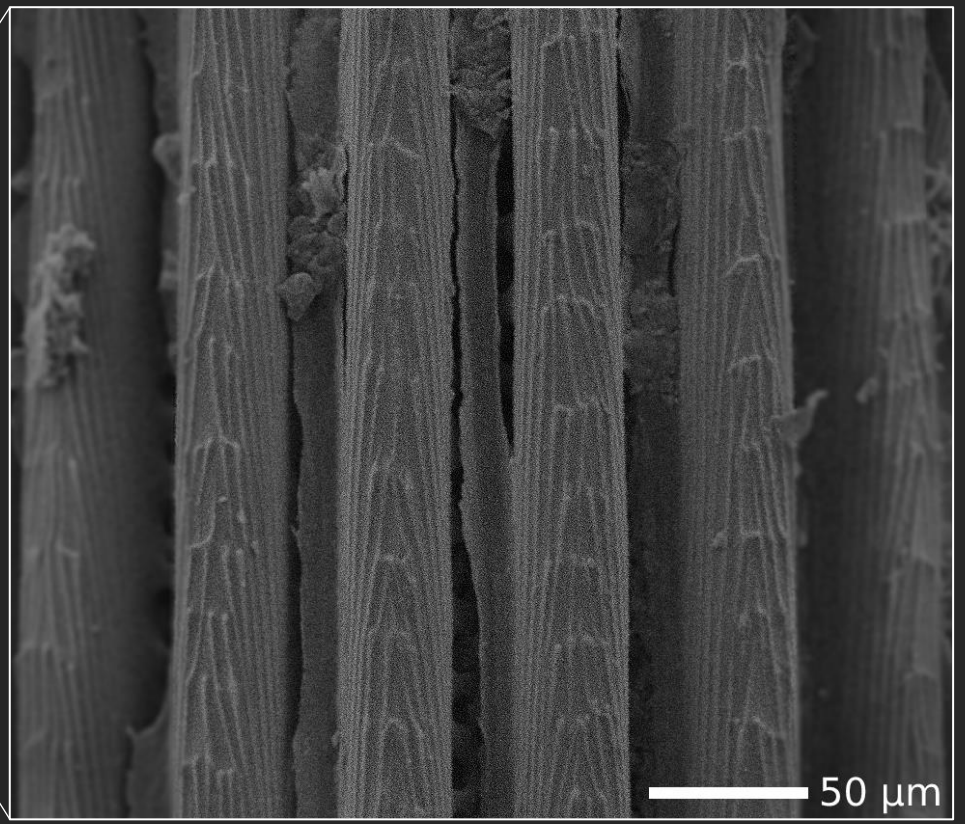
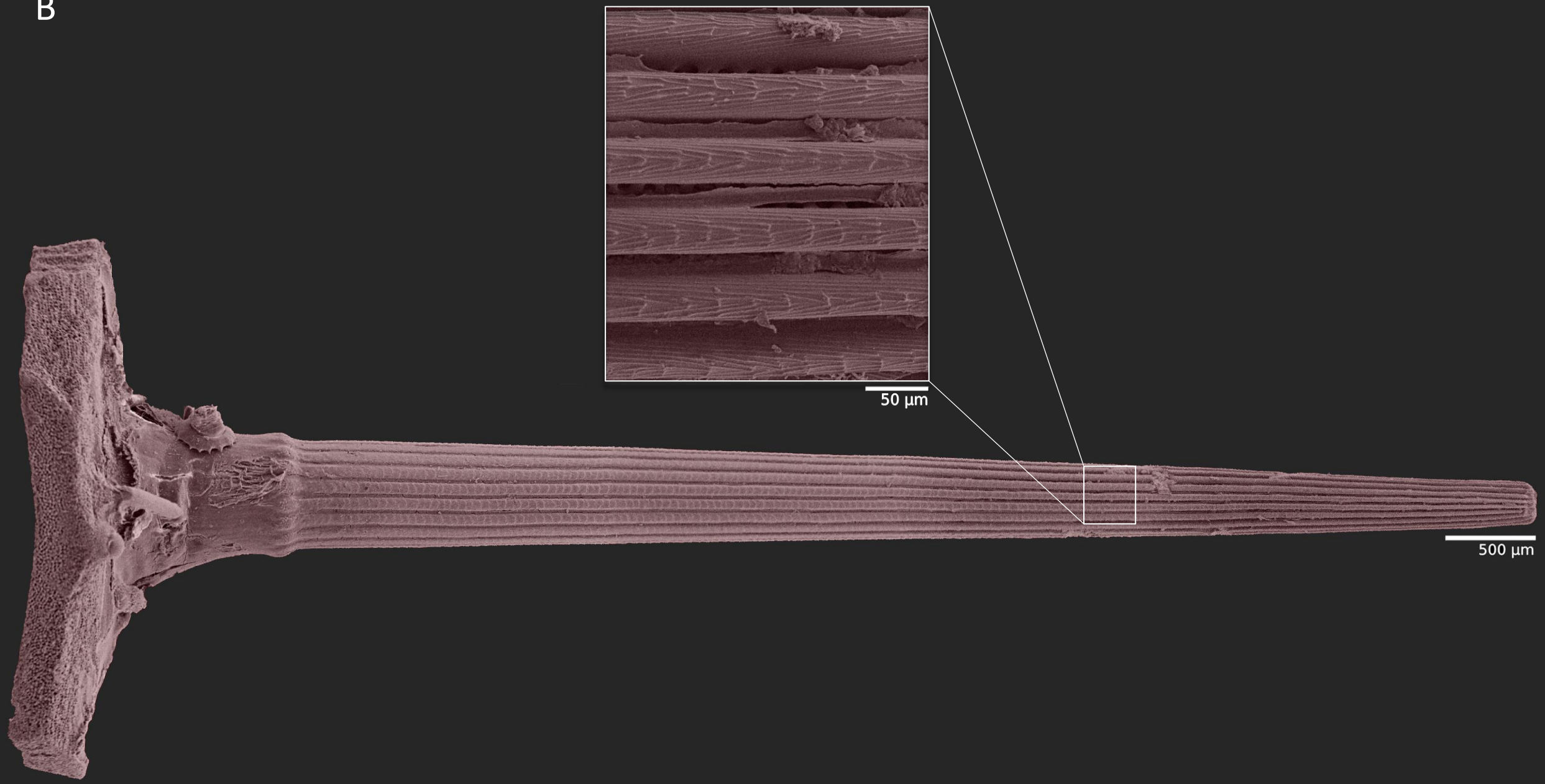
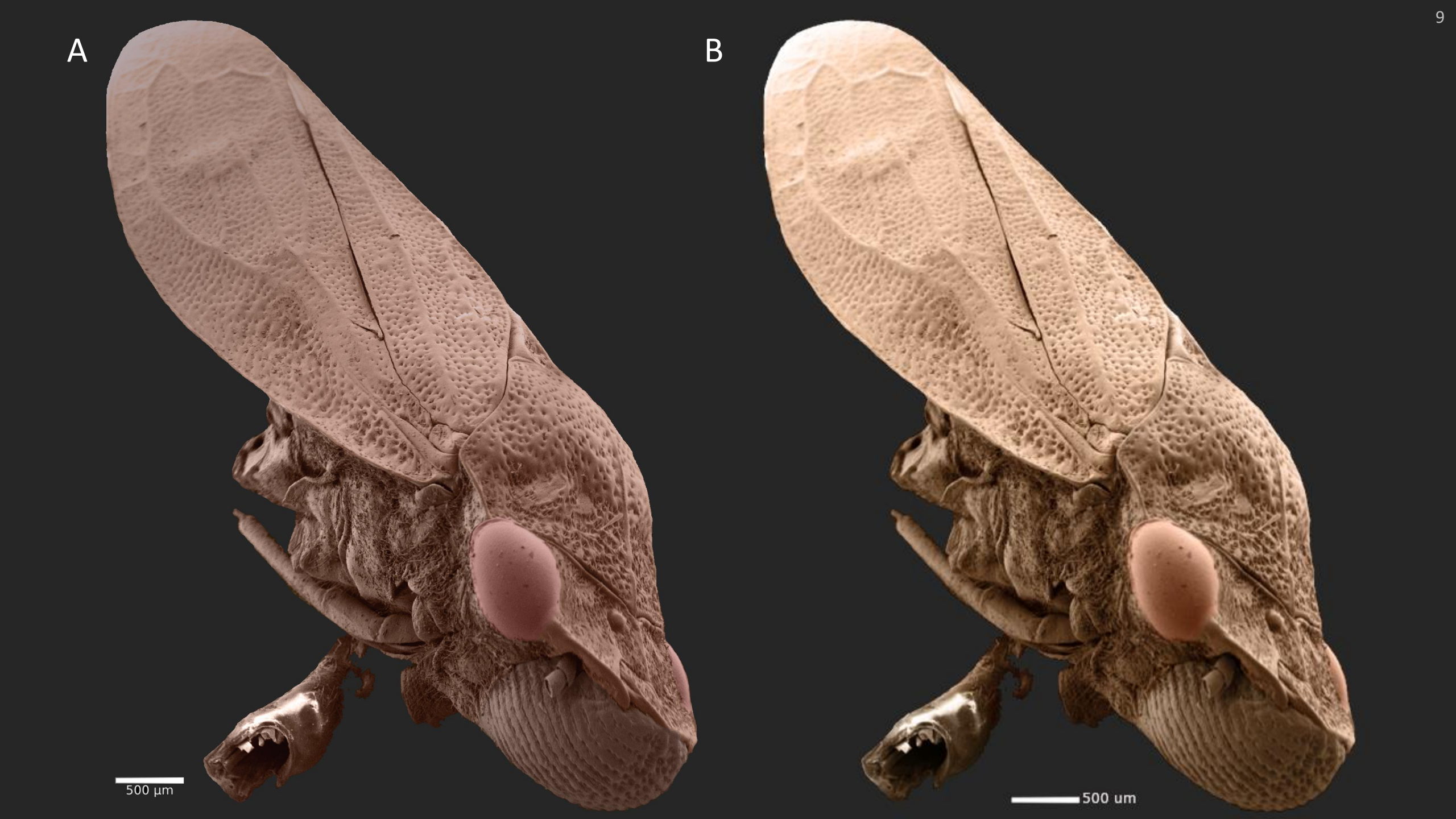
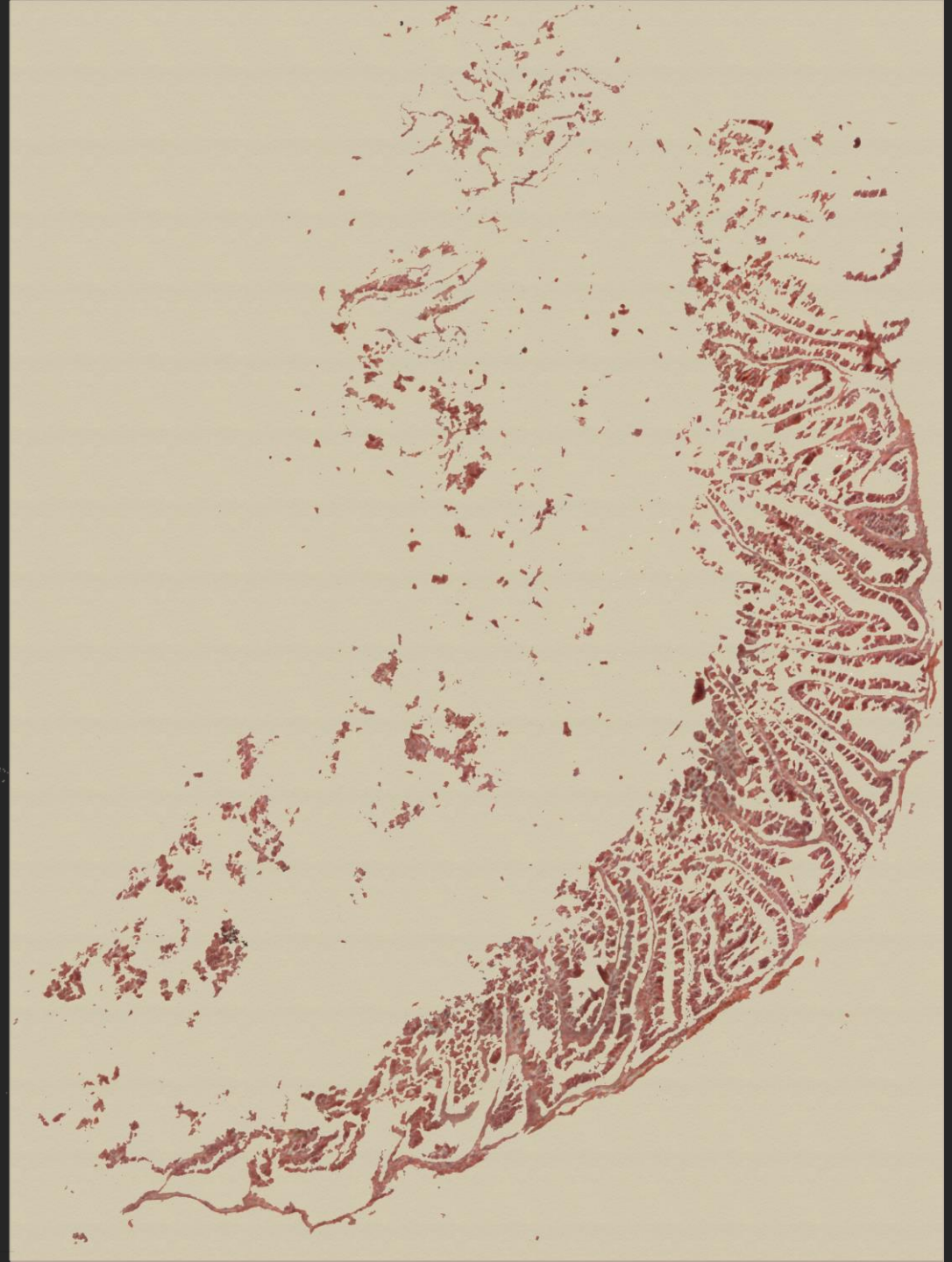


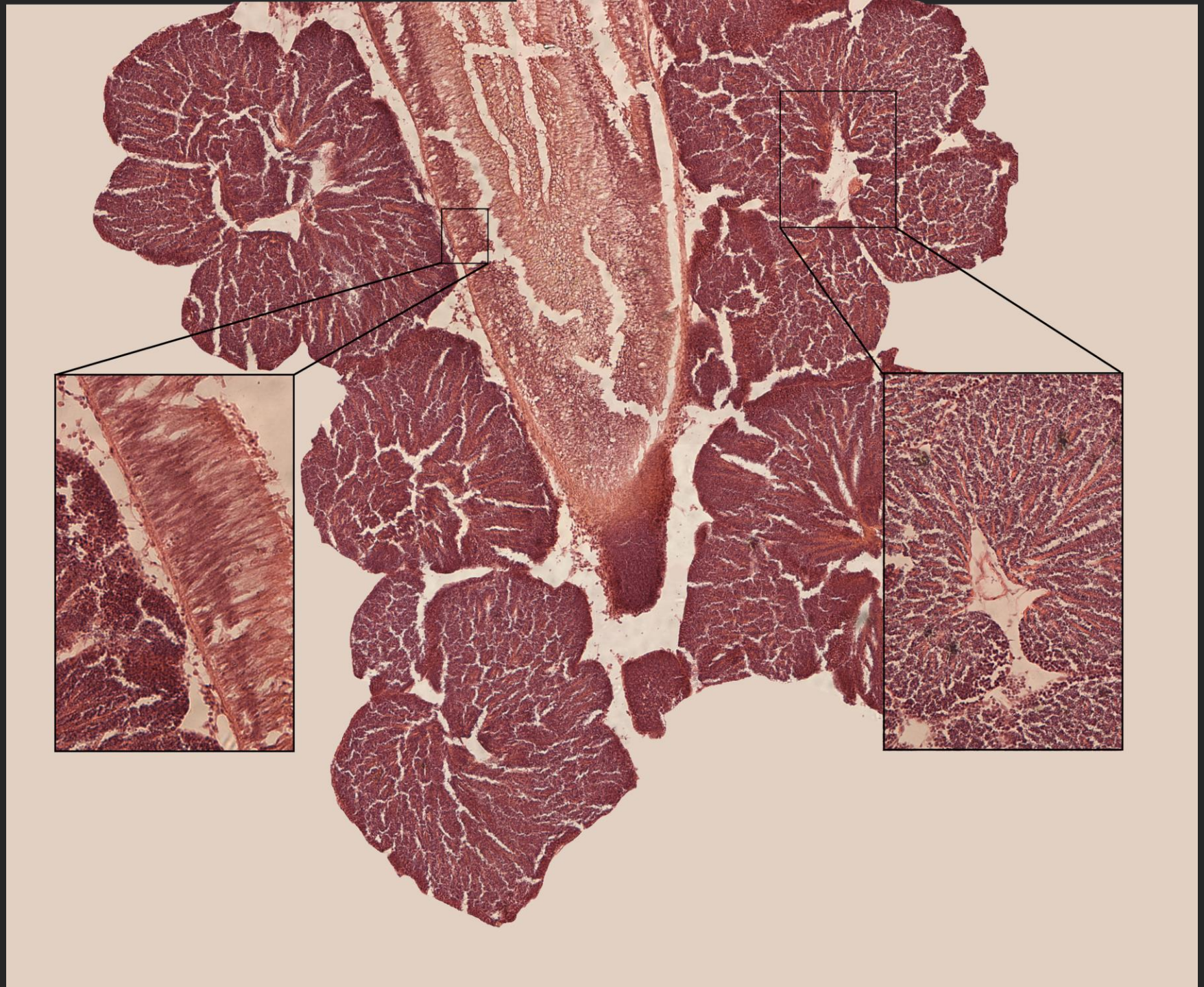


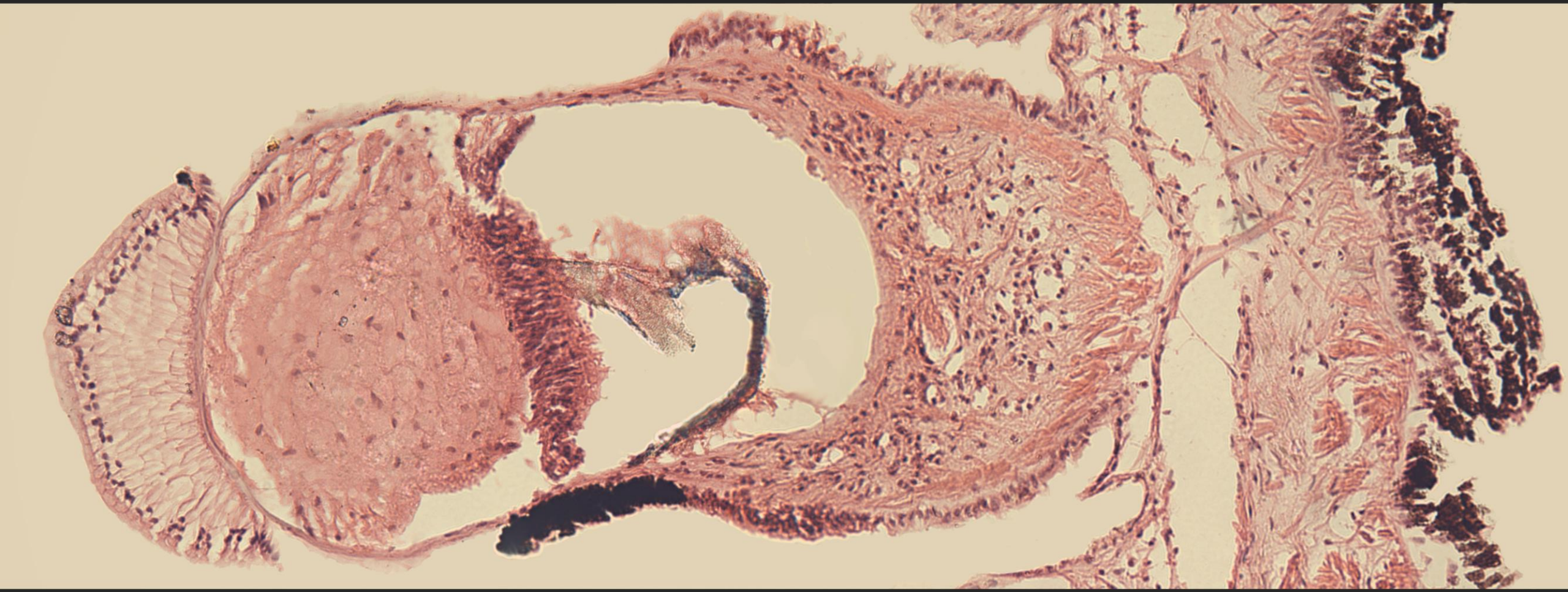
B

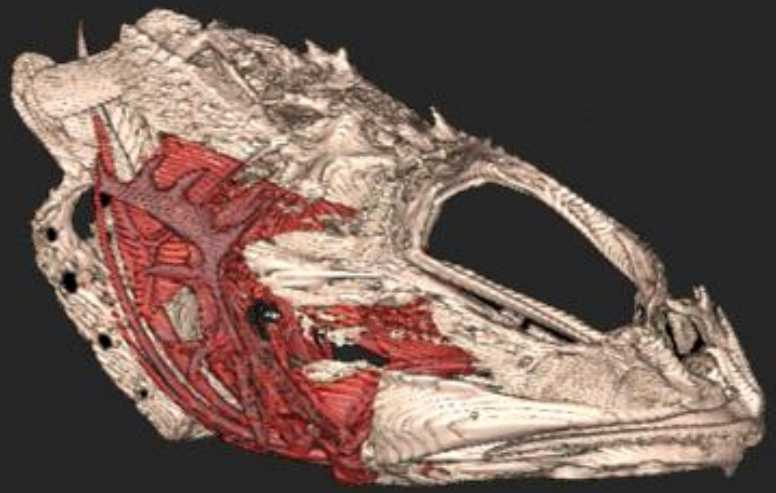



A
500 µm
B
500 um

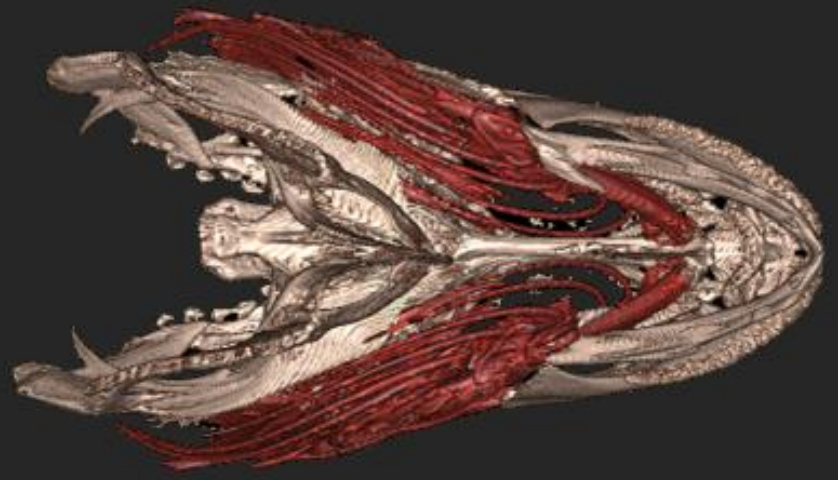






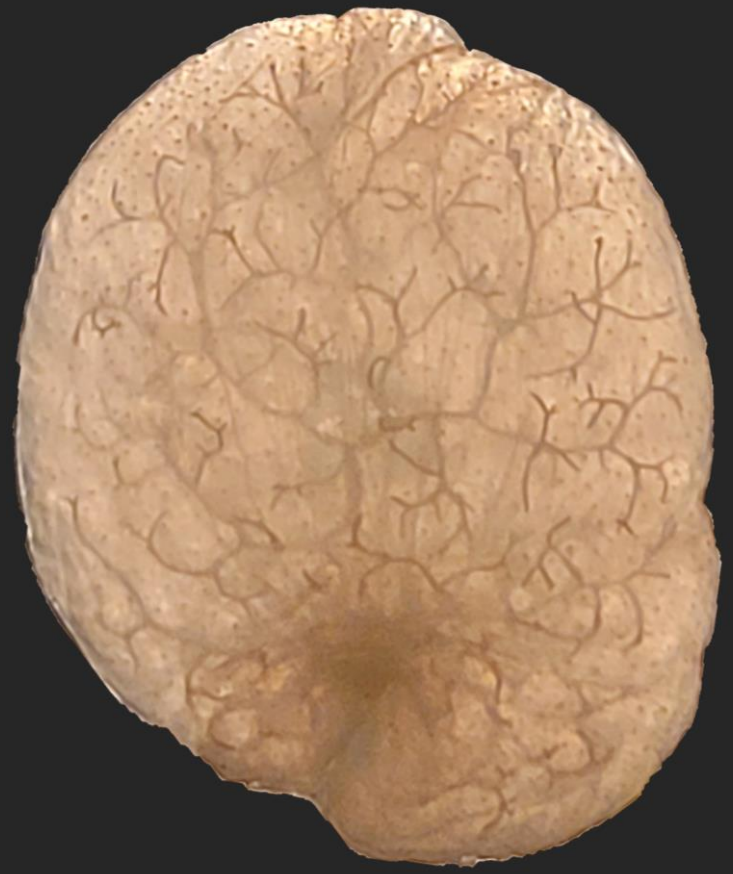


Lateral View



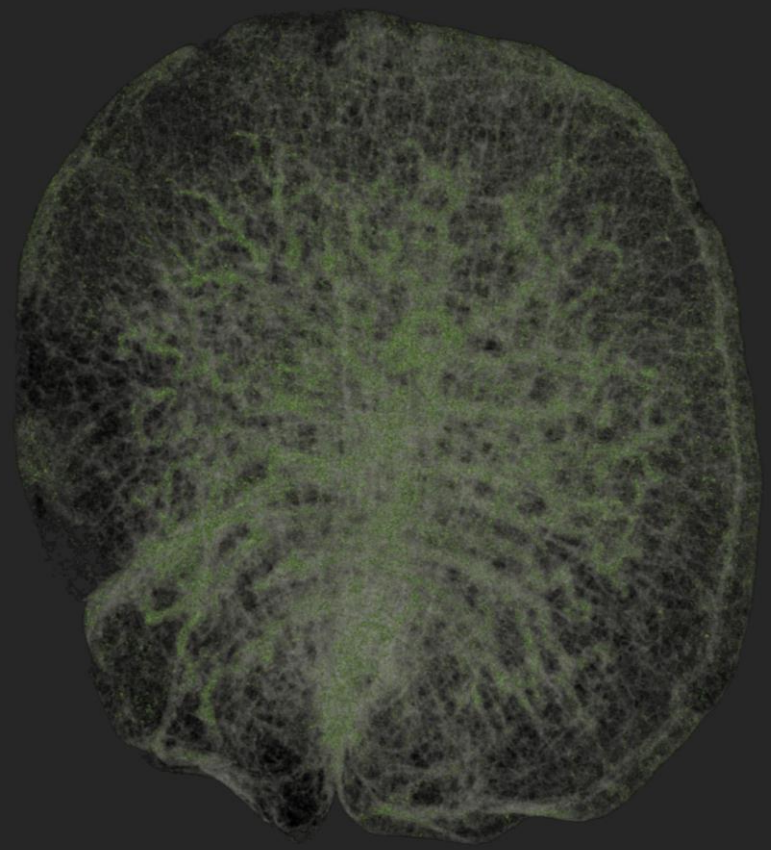
Anterolateral View

A



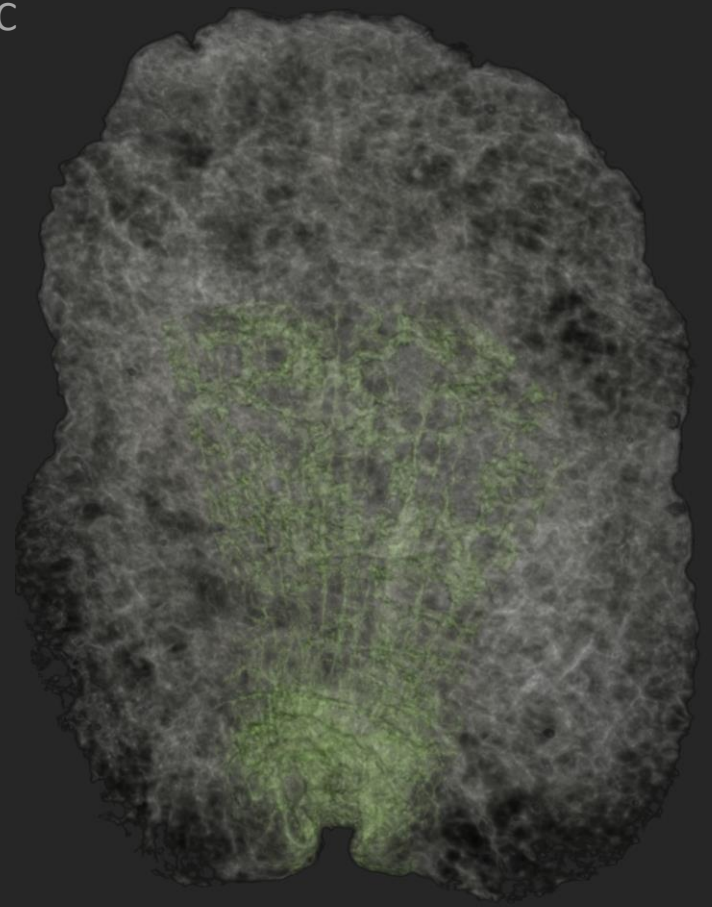
Autotomized cerata of Melibe leonina.
Fixed in glutaraldehyde.

B

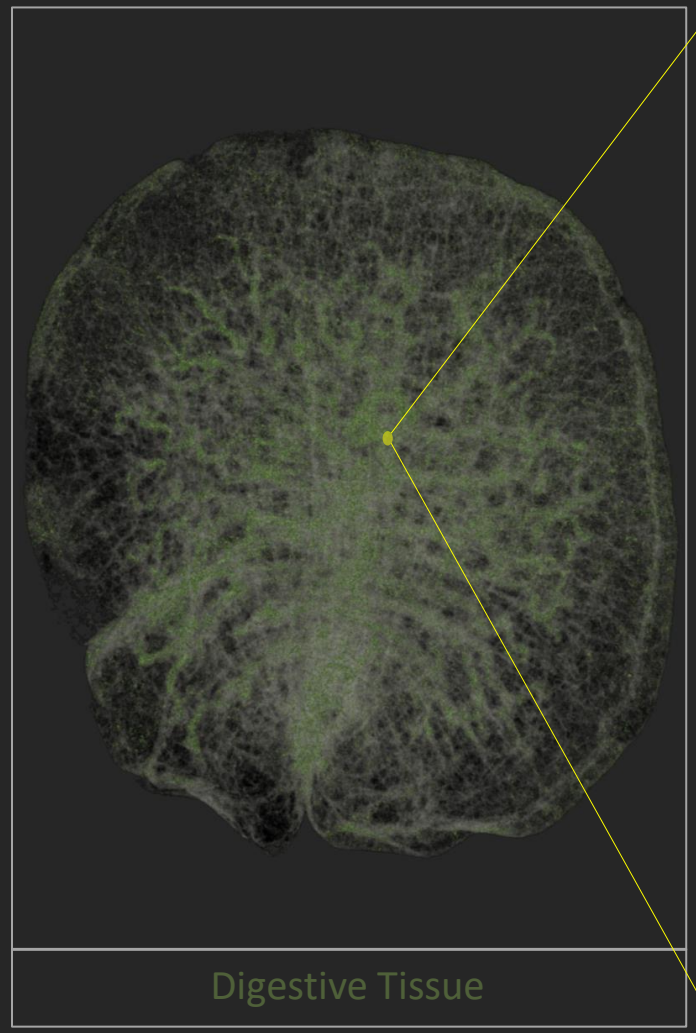


Micro-CT scan Melibe leonina cerata.
Stained in phosphotungstic acid.
Digestive tissue and nervous tissue.

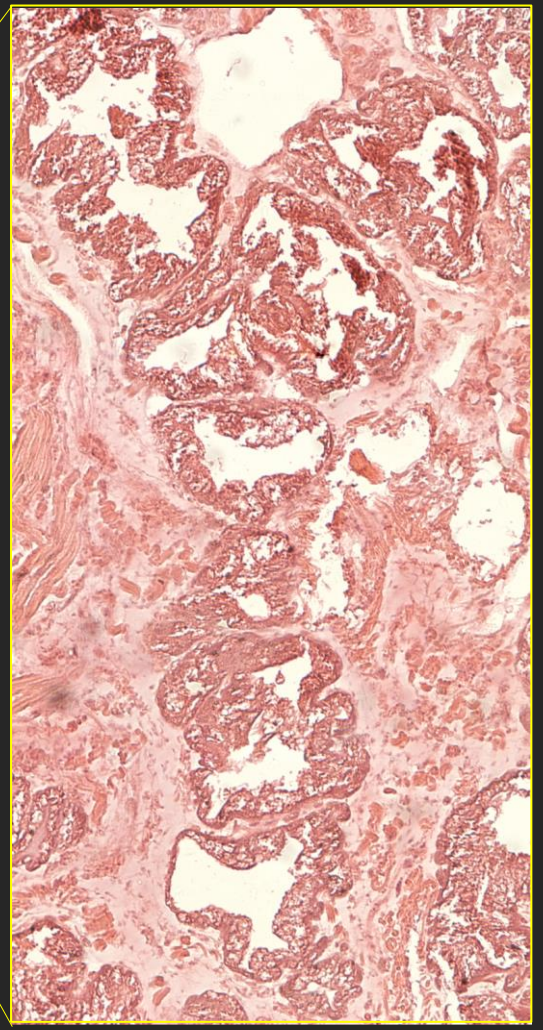
C

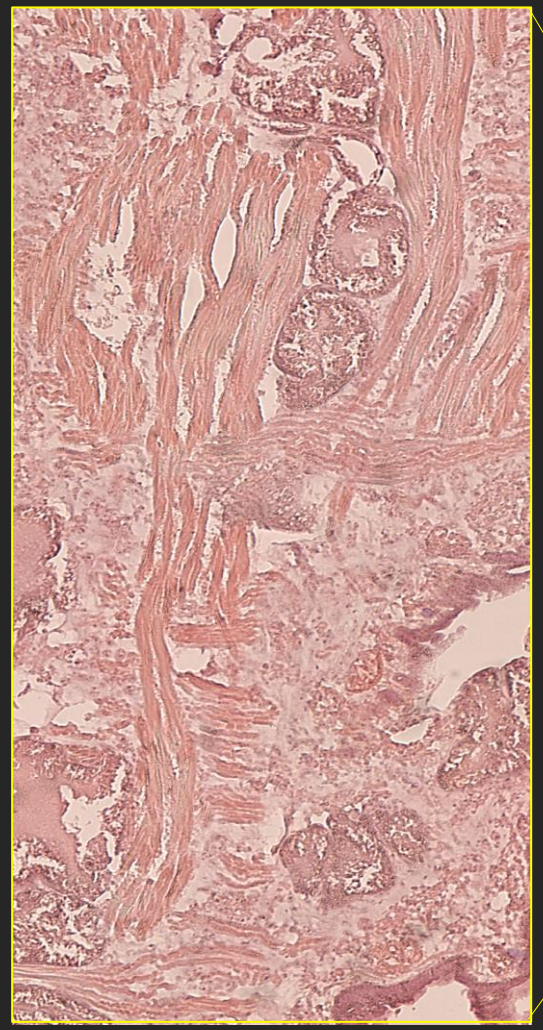


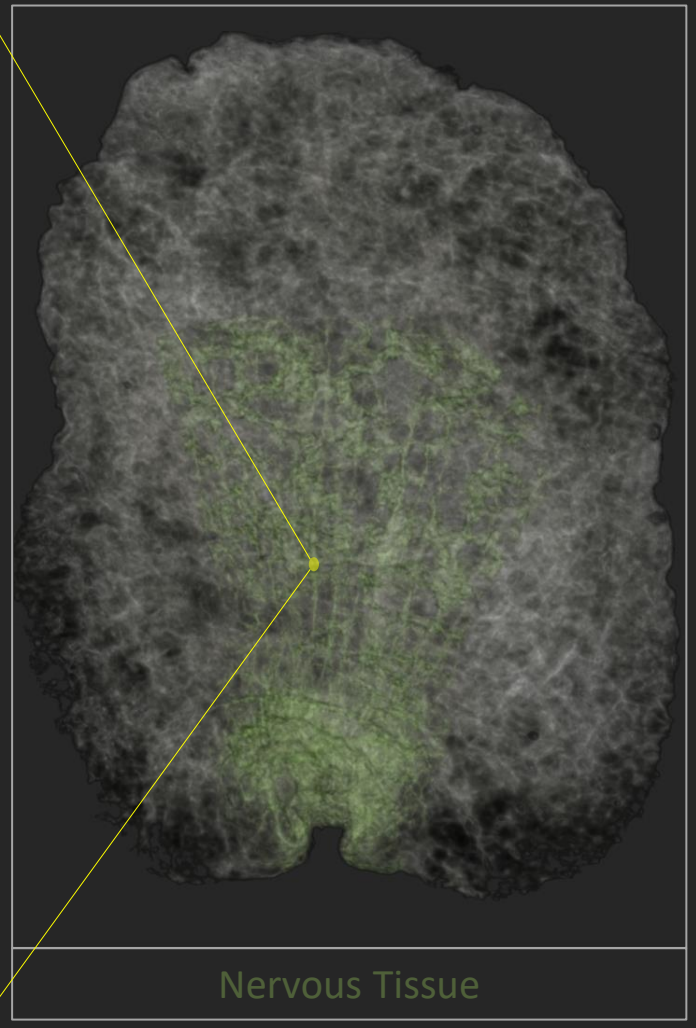
Micro-CT scan Melibe leonina cerata.
Stained in silver nitrate.
Nervous tissue only.



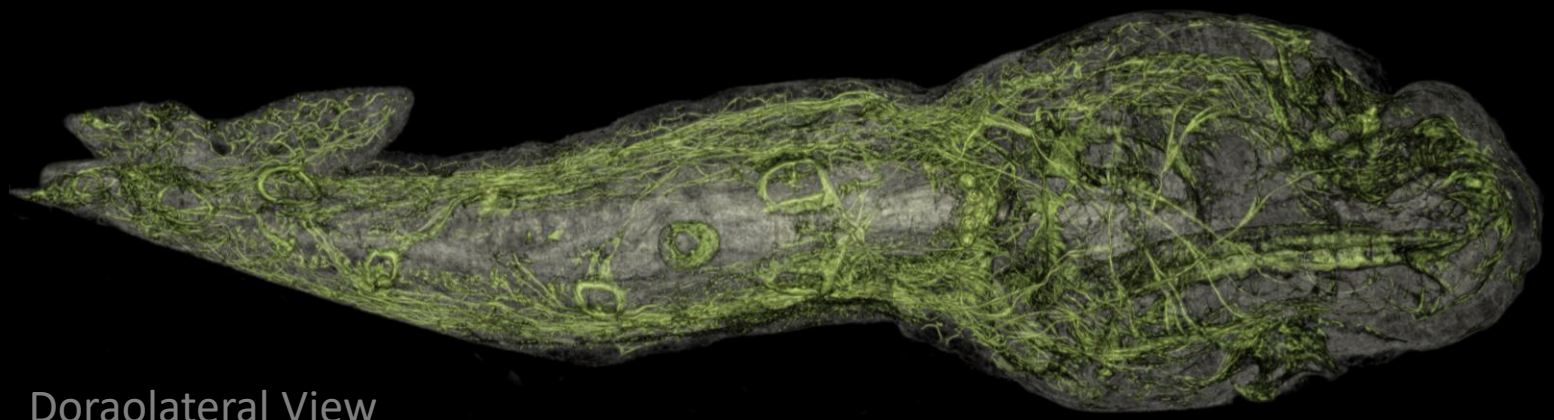
Digestive Tissue



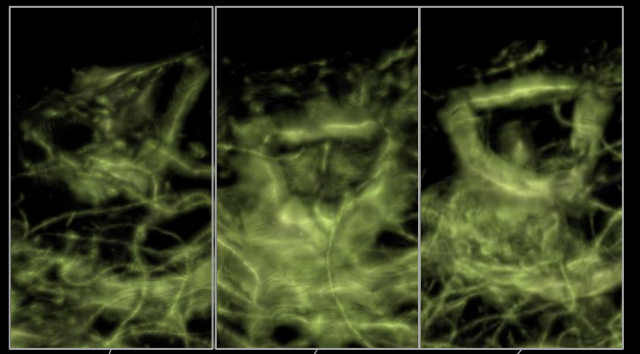


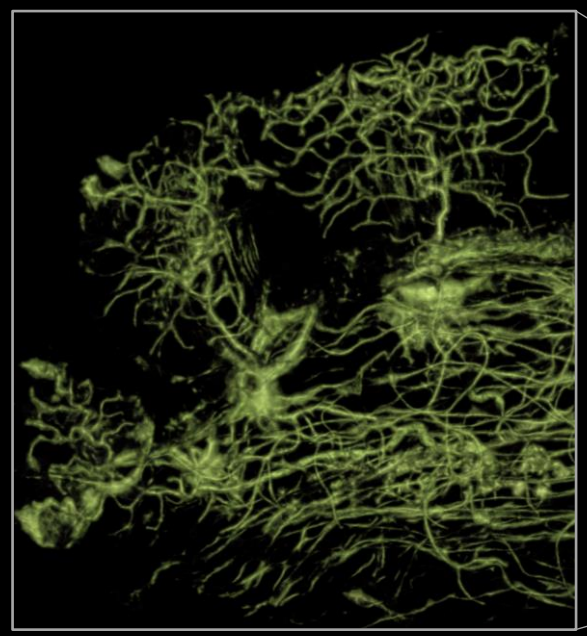


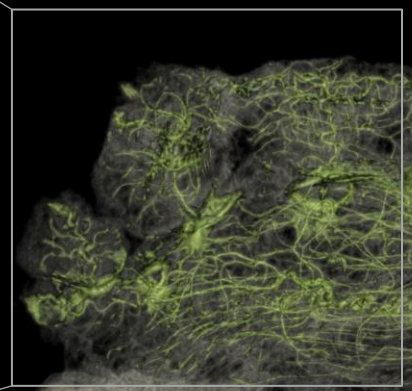
Nervous Tissue



Doraolateral View





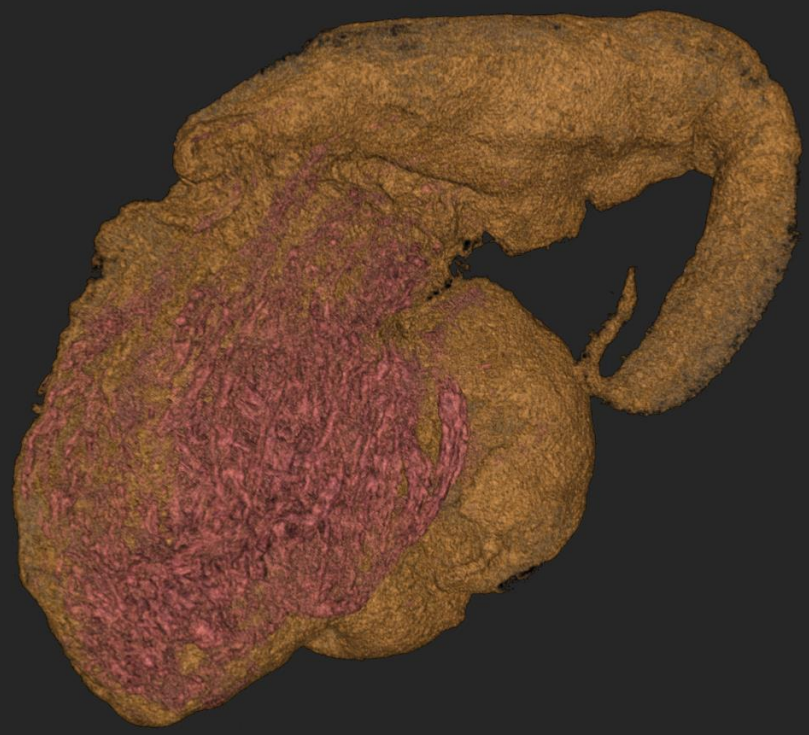


Lateral View

A

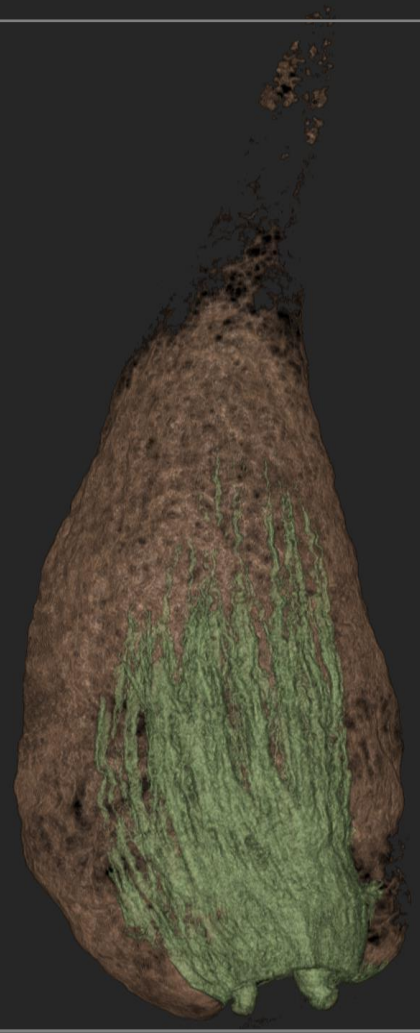
Autotomized cerata of Dirona pellucida.
Fixed in glutaraldehyde.

B

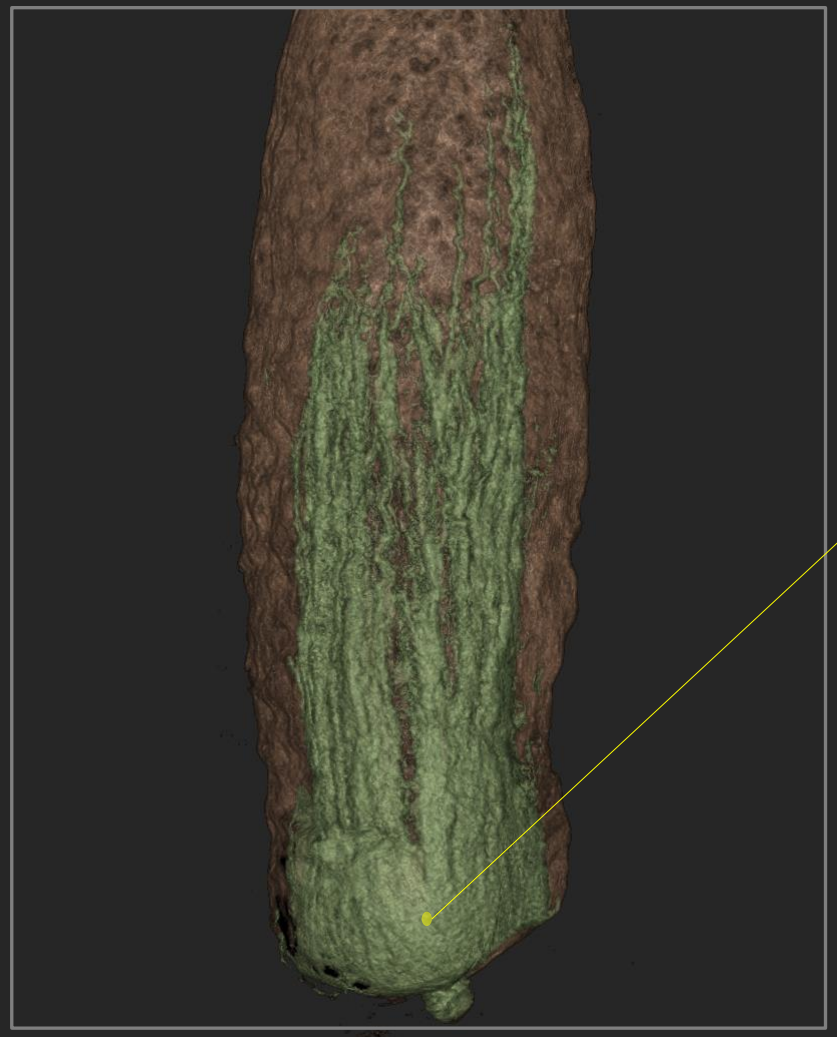


Micro-CT scan of Dirona pellucida cerata.
Stained in phosphotungstic acid.
Digestive tissue and nervous tissue.

C



Micro-CT scan of Dirona pellucida cerata.
Stained in silver nitrate.
Nervous tissue only.



Medial View