



Meagre: Parasites and non-infectious diseases

Dr. Pantelis Katharios

Hellenic Centre for Marine Research



Meagre workshop, October 9, Barcelona

Outline of the presentation

Parasites

1. Sciaenacotyle panceri
2. Diplectanum sciaenae
3. Other parasites

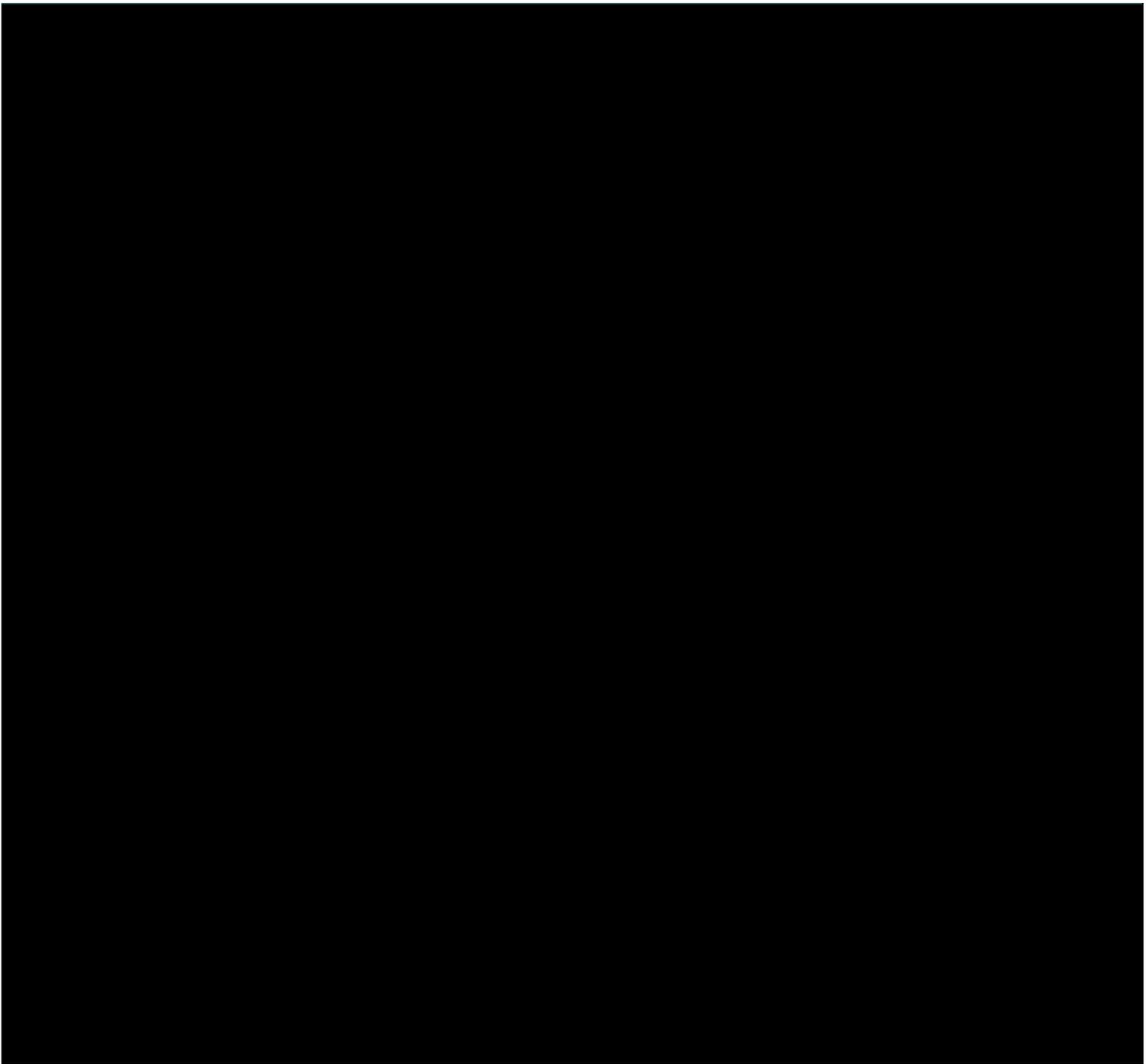
Non-infectious diseases

1. Systemic granulomatosis
2. Chronic Ulcerative Dermatopathy

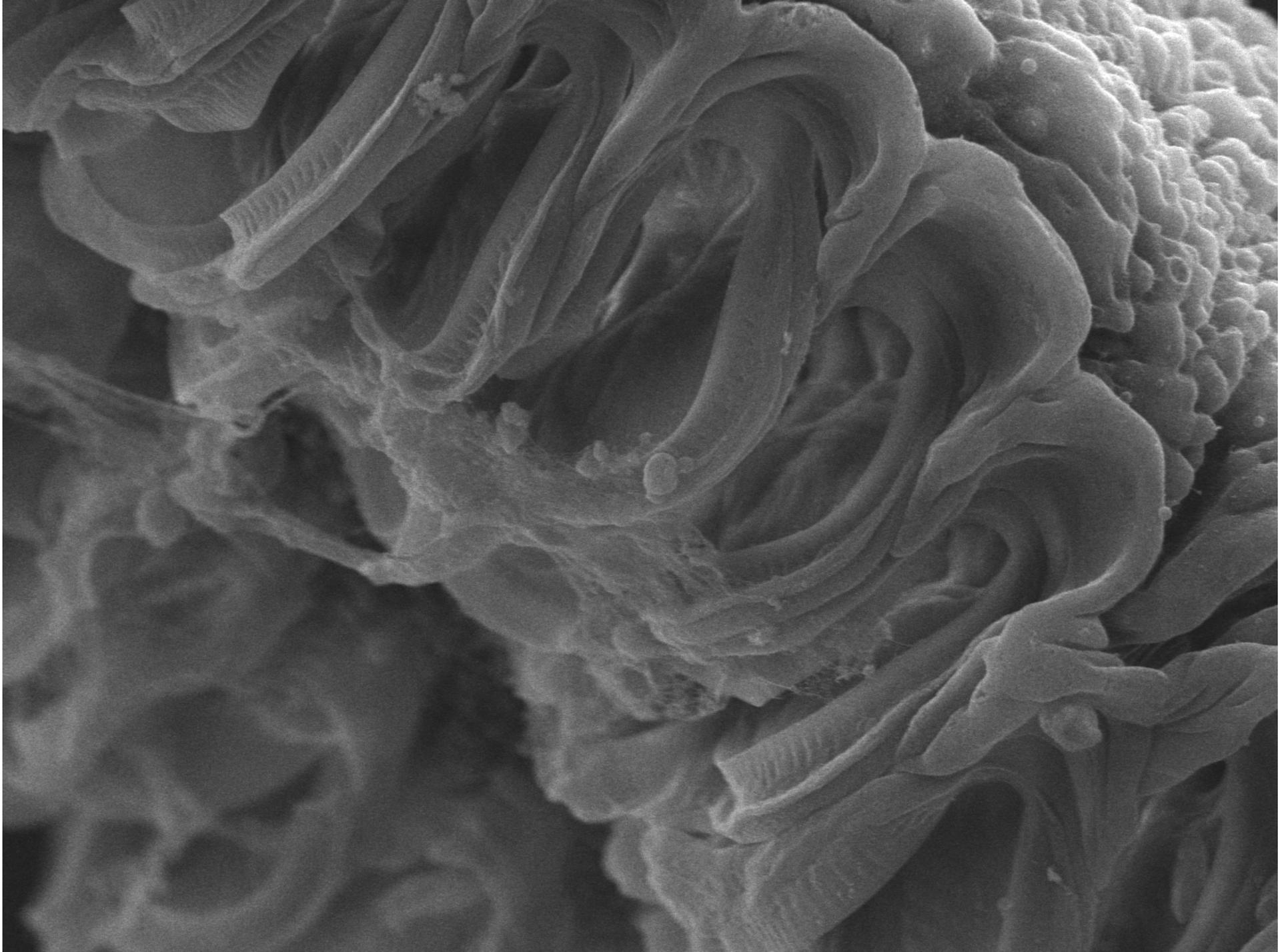
Sciaenacotyle panceri

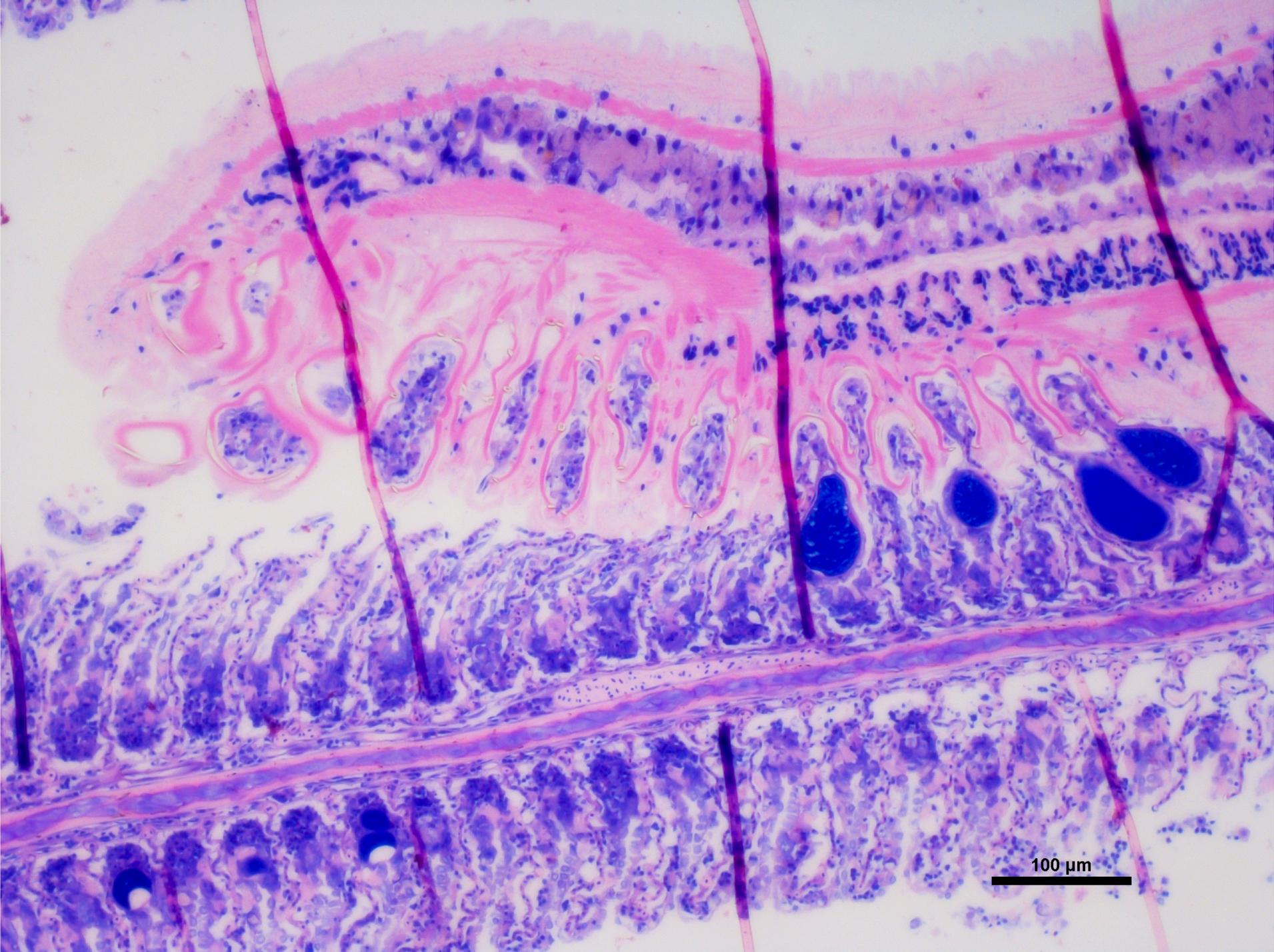
- Gill monogenean (Polyopsithocotylea)
- Highly host-specific
- Direct life-cycle
- Large size (up to 10 mm)
- Blood-feeding
- Can propagate in extremely high numbers
- Cause gill hyperplasia, anemia and eventually death
- Mortalities can be severe esp. in large fish (reaching commercial size)



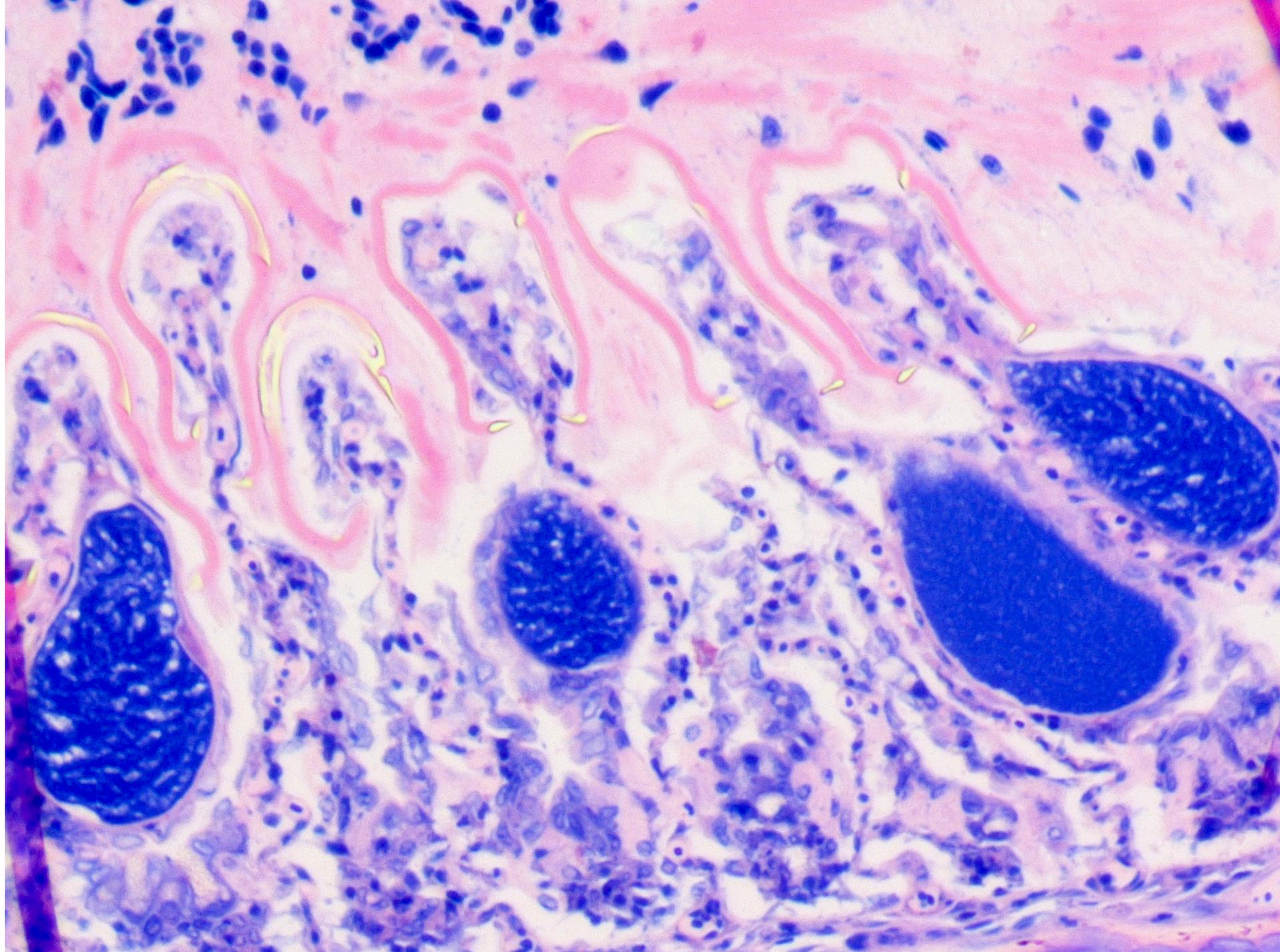


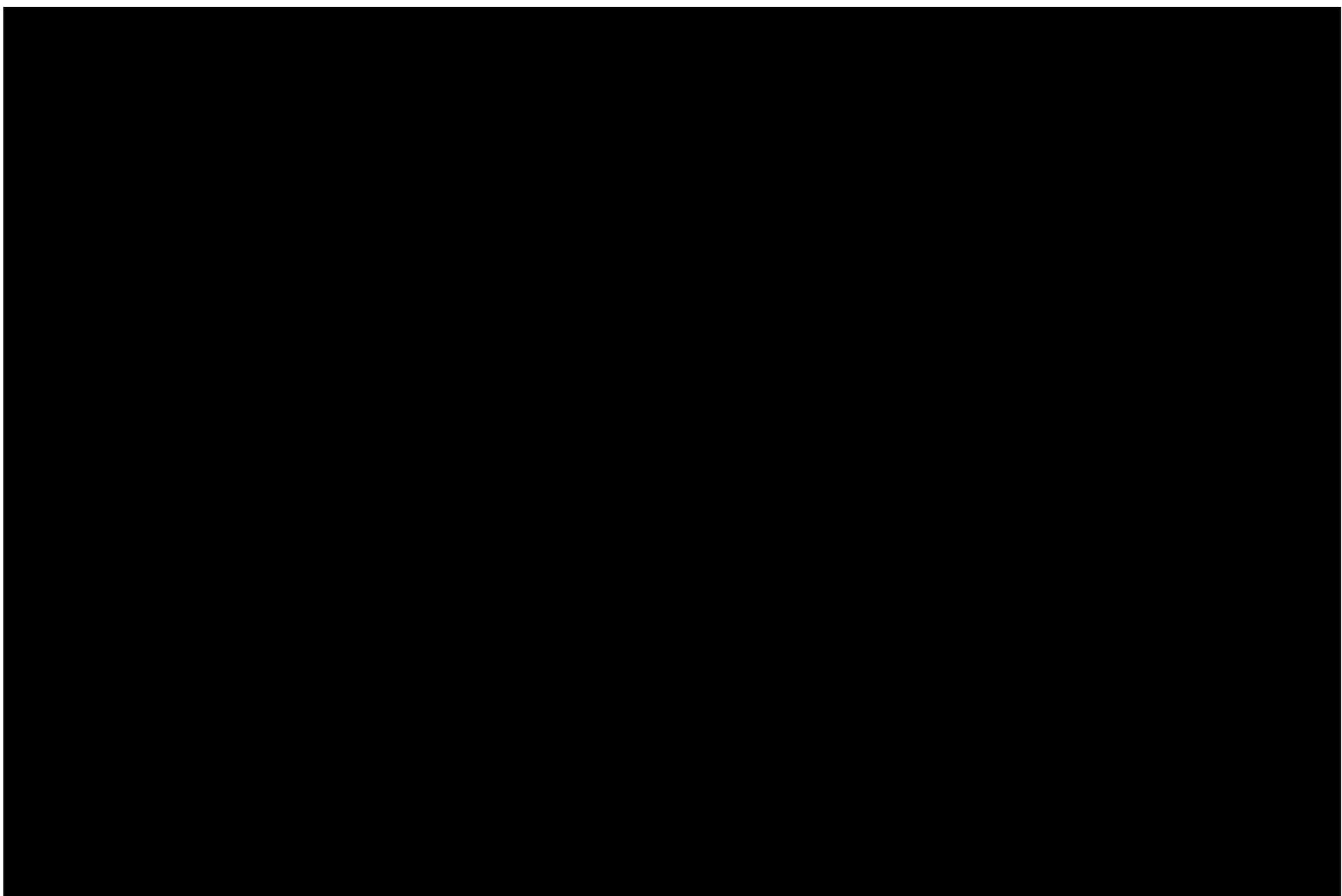




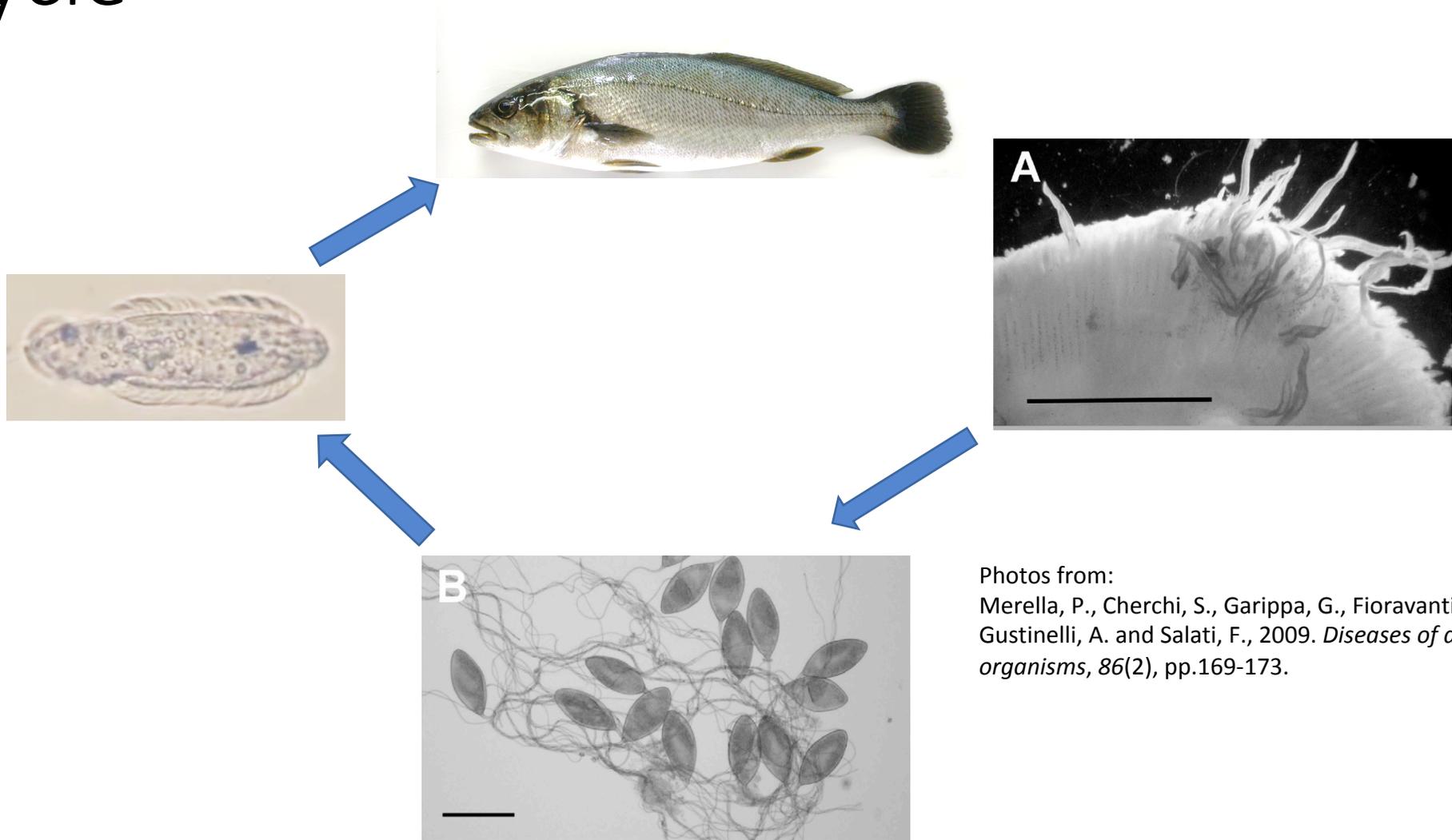


100 μ m





Life cycle



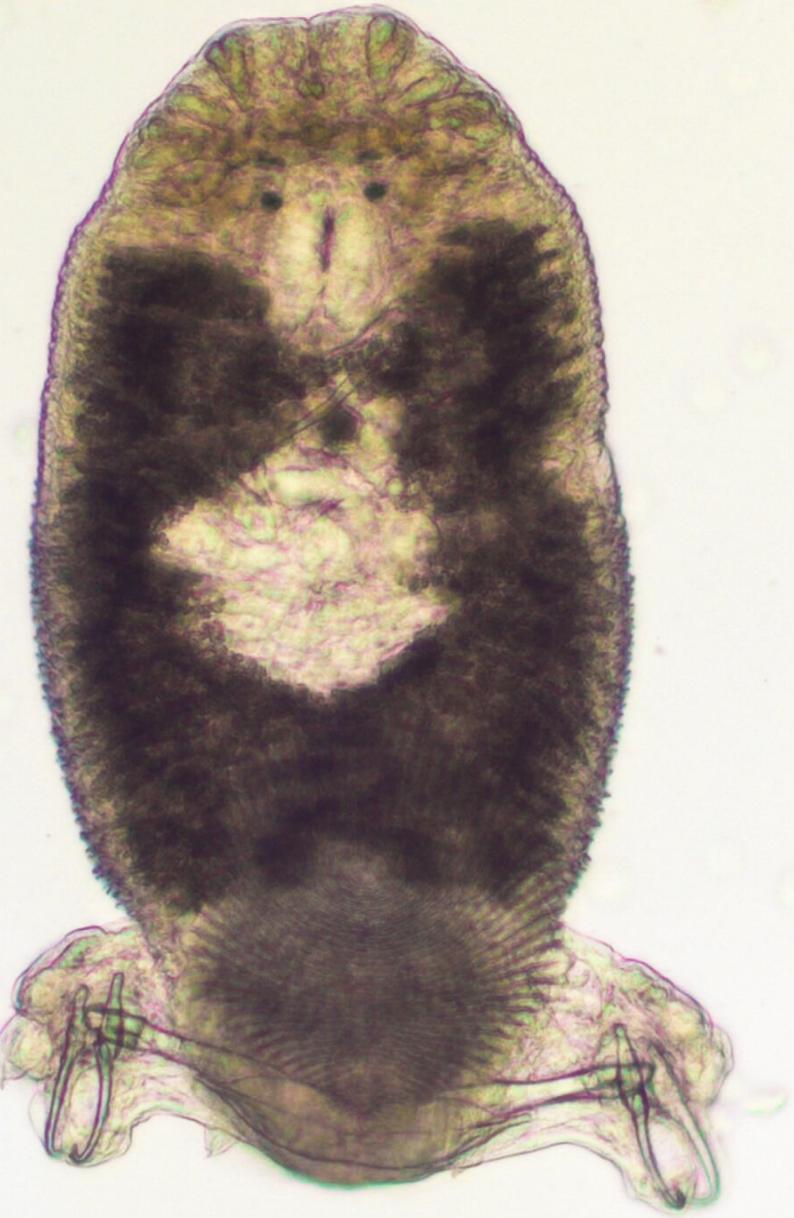
Photos from:
Merella, P., Cherchi, S., Garippa, G., Fioravanti, M.L.,
Gustinelli, A. and Salati, F., 2009. *Diseases of aquatic
organisms*, 86(2), pp.169-173.

Parasite management

- Break the life-cycle
- Adults and oncomiracidia are susceptible to treatments, eggs NO
- Two to three consecutive treatments with 15-20 days interval
- All stocks should be treated simultaneously
- Formalin treatment ineffective
- Praziquantel very effective, but not licensed
- Peroxide?
- Cinnamon as a feed additive showed promising results (IRTA)
- Monitoring

Diplectanum sciaenae

- Gill monogenean (Monoopsithocotylea)
- Host specific
- Direct life-cycle
- Mucous-feeding
- Can propagate in extremely high numbers
- Cause gill hyperplasia, secondary infections and eventually death
- Mortalities have been recorded in broodstock



200 μm



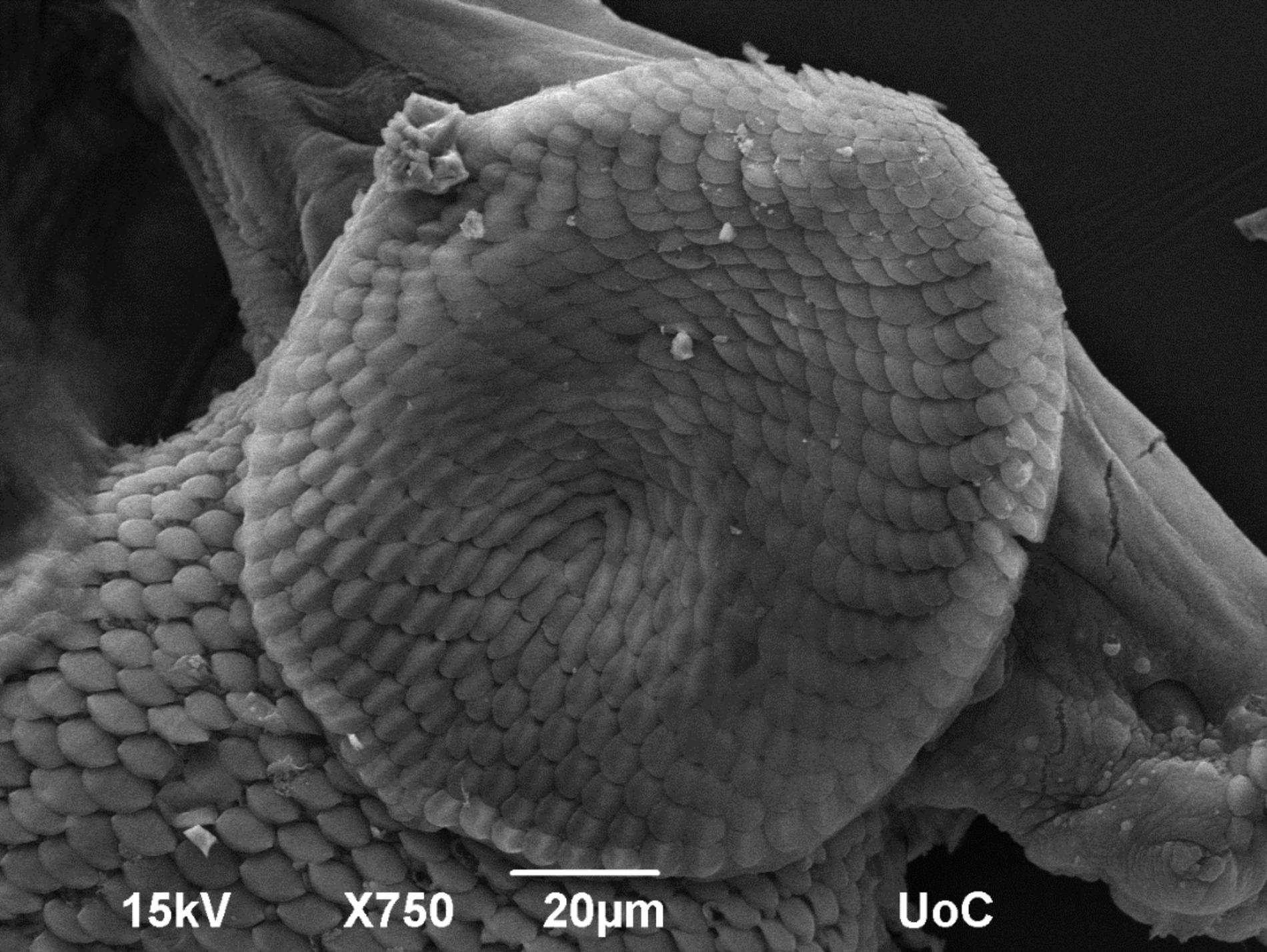
100 μm



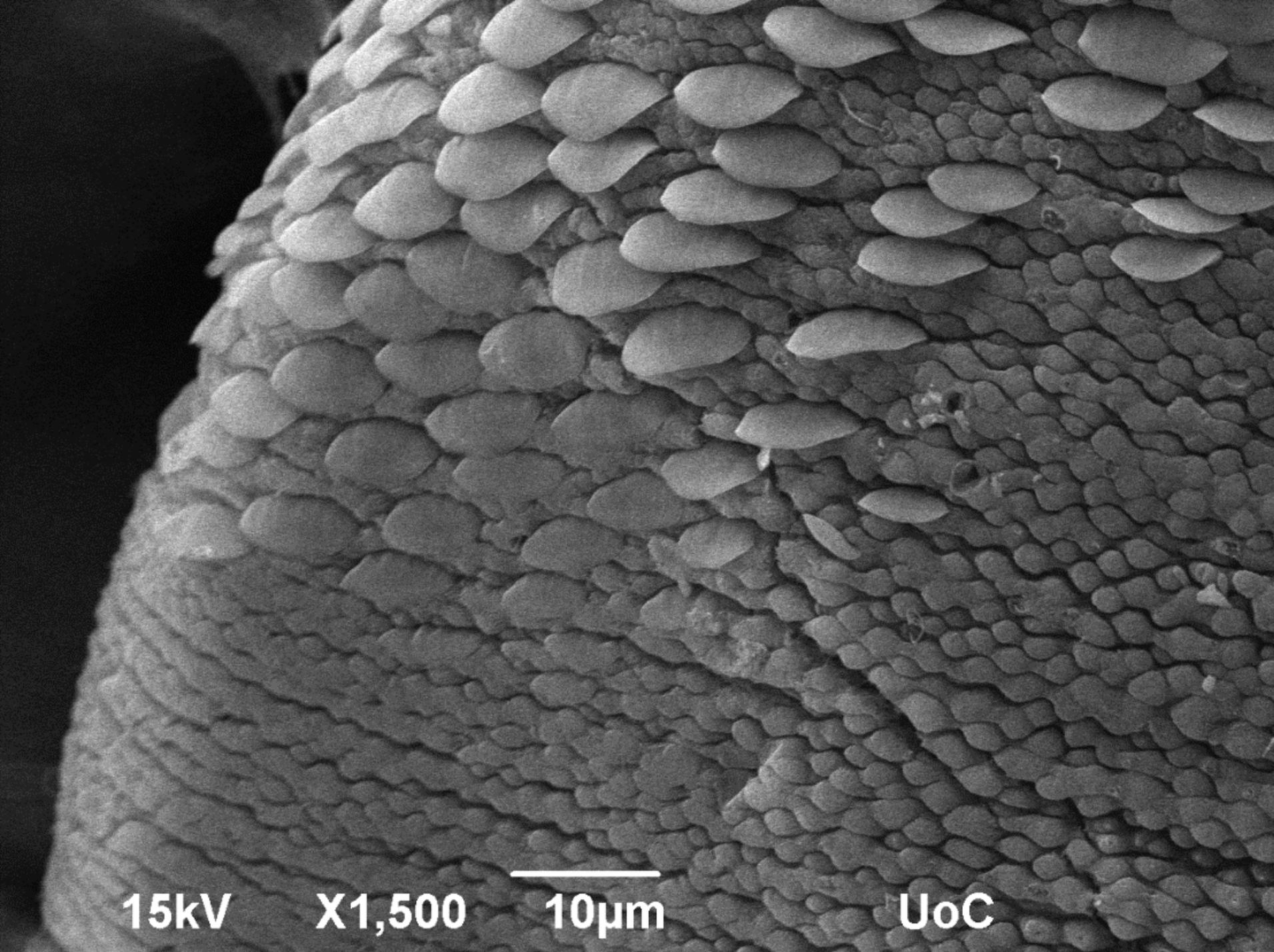
haptor

100 μ m





Squamodisc has scale-like structures.
Half of the body is covered by scales

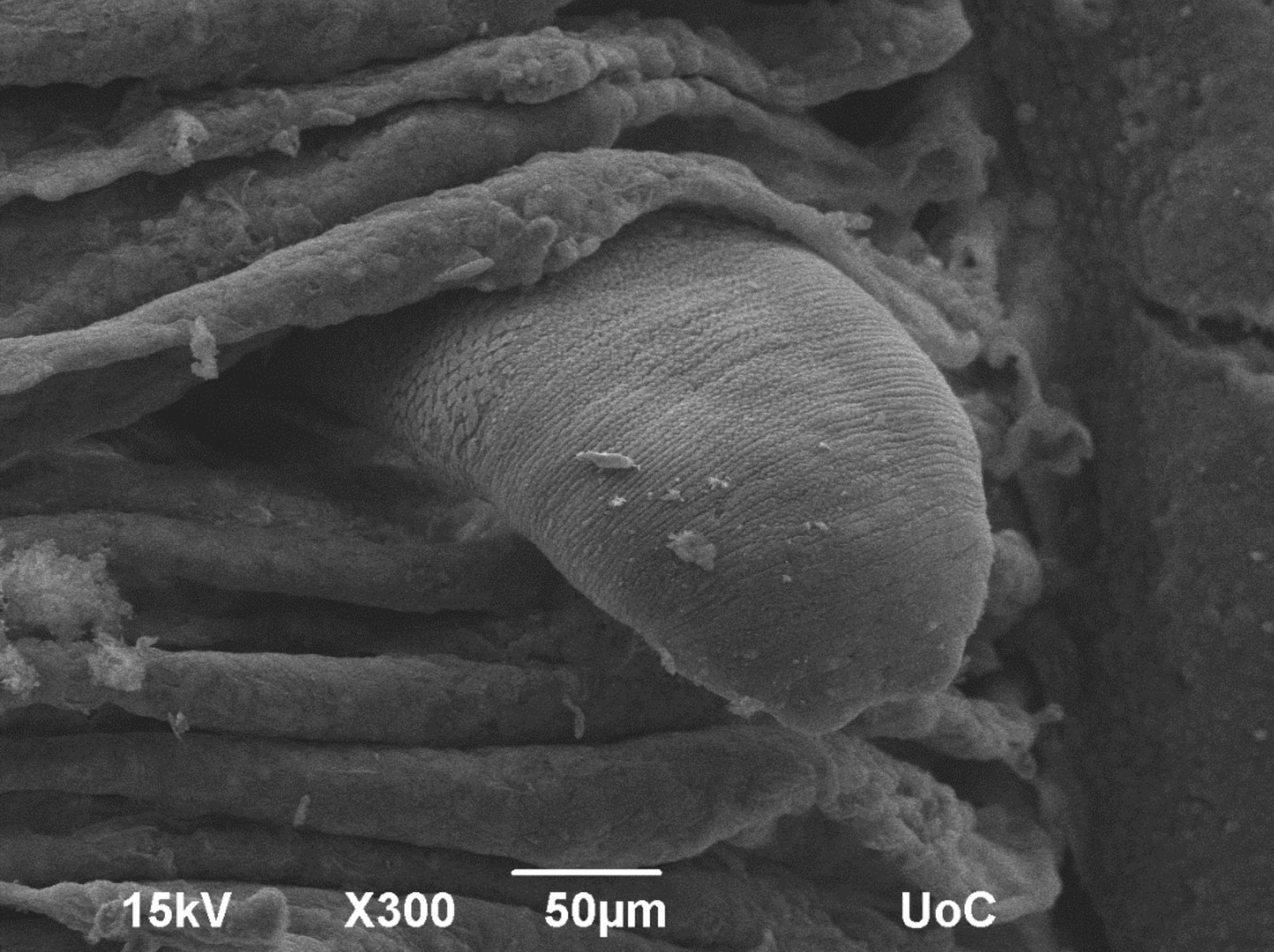


15kV

X1,500

10µm

UoC

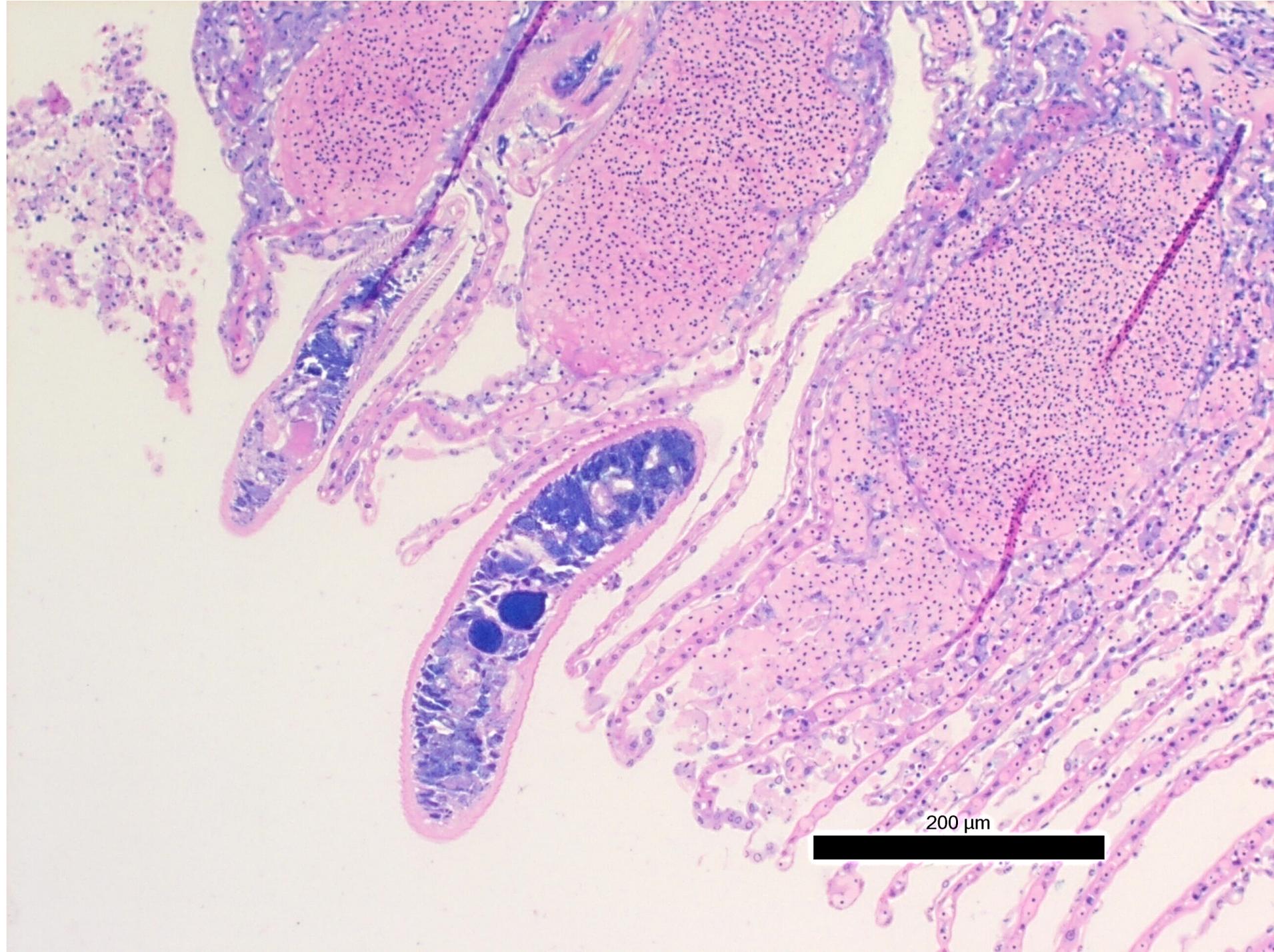


15kV

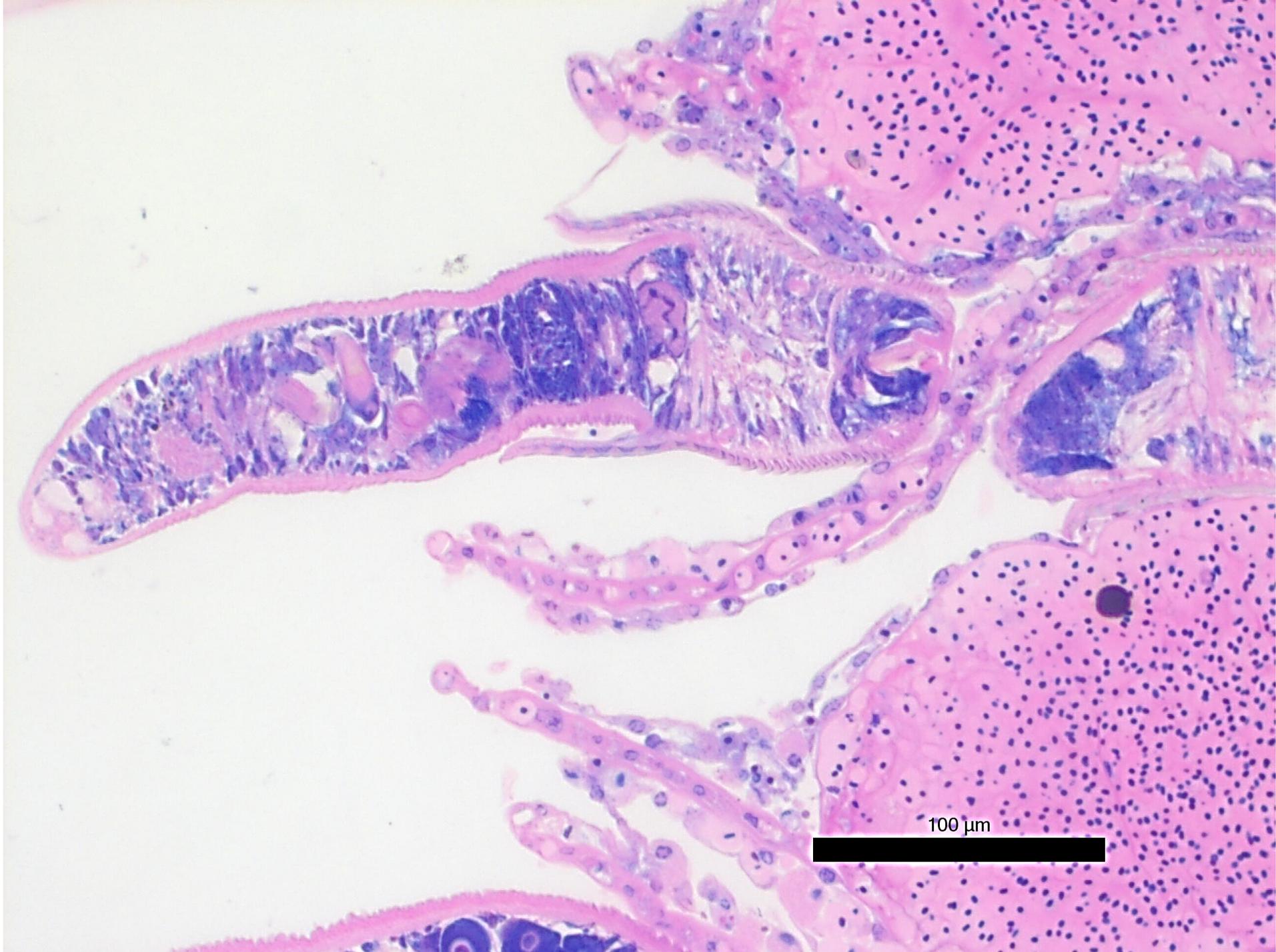
X300

50µm

UoC



200 μ m



Parasite management

- Break the life-cycle
- Adults and oncomiracidia are susceptible to treatments, eggs NO
- Two to three consecutive treatments with 15-20 days interval
- All stocks should be treated simultaneously
- Formalin treatment is effective (100 ppm for 1h)
- Monitoring of broodstock

Other parasites

- *Benedenia sciaenae* (Turkey)
- *Amyloodinium ocelatum* (Portugal)



Systemic granulomatosis

- Bottleneck for meagre production
- Disease of unknown aetiology
- Affects 100% of the population
- Severity: from very mild (undetected) to.....you will see
- Major task for WP24

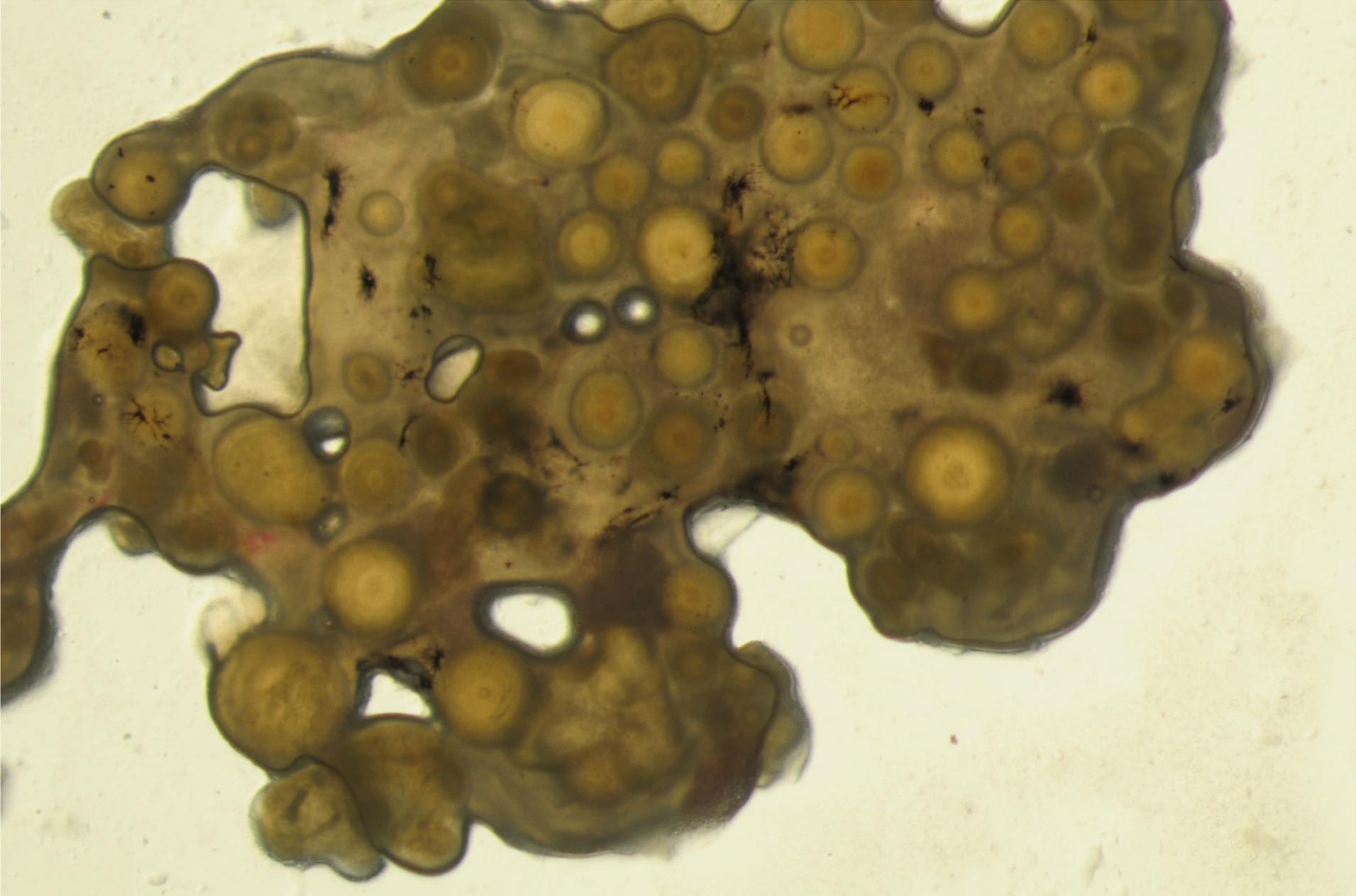


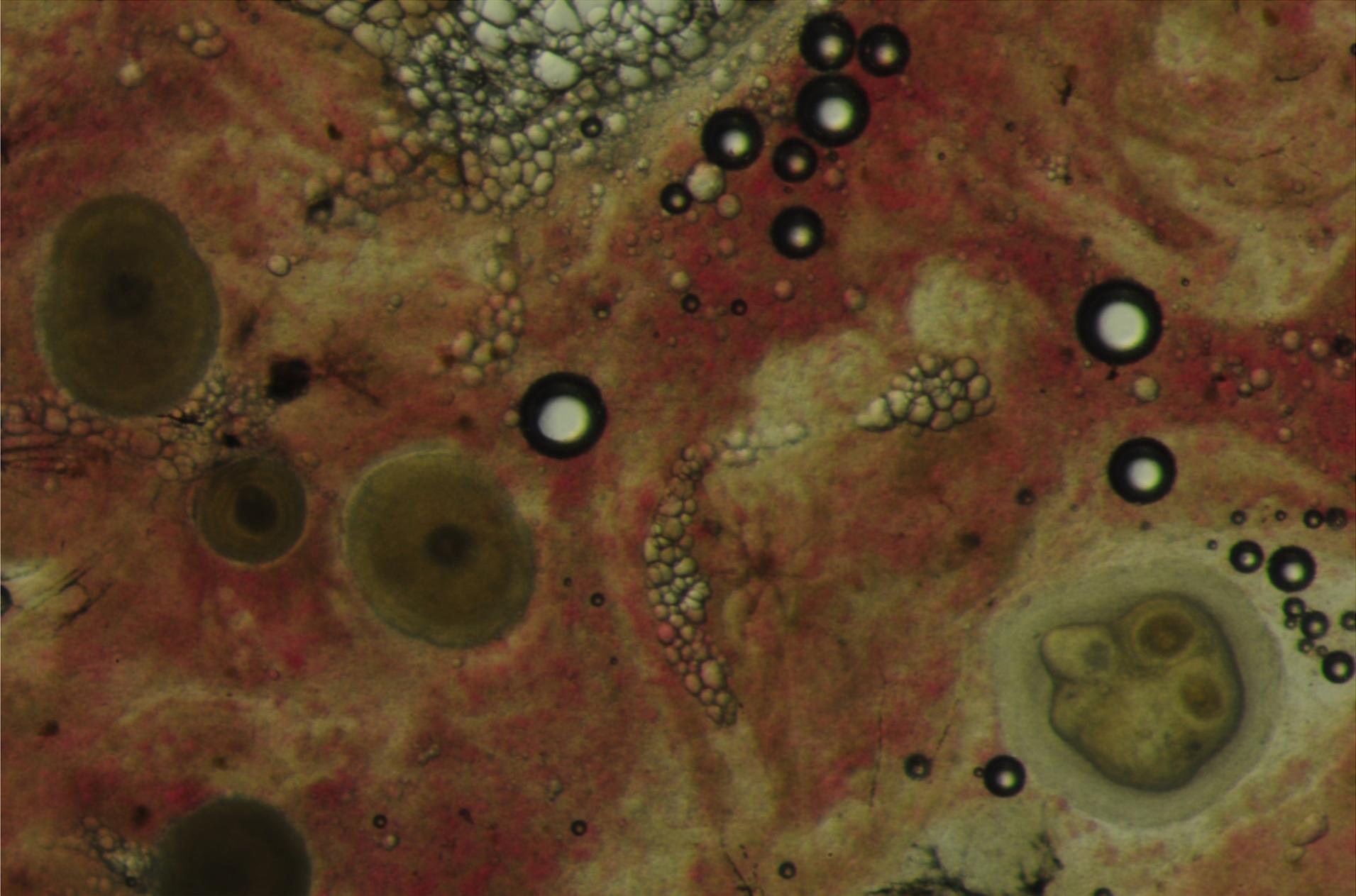


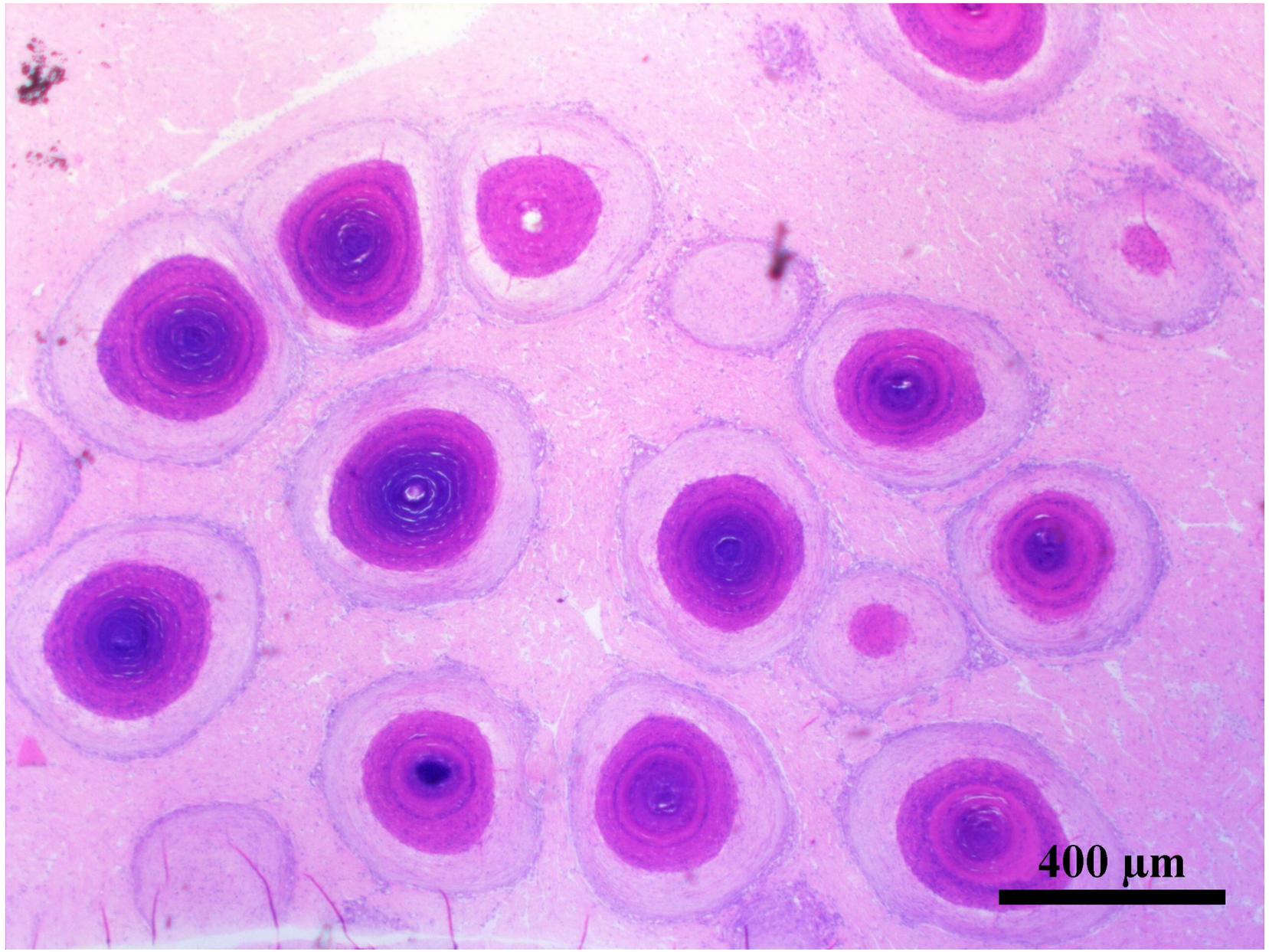


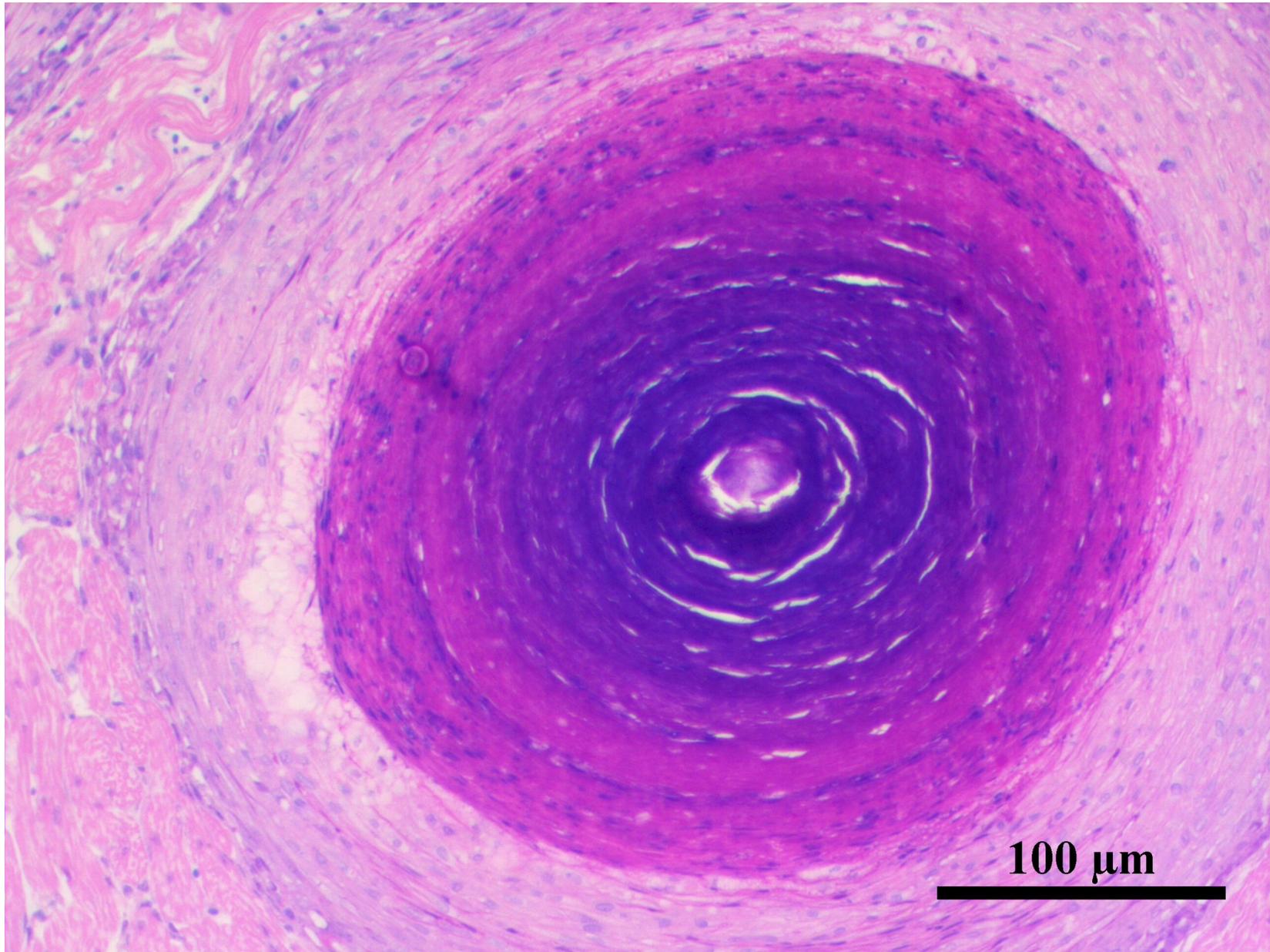




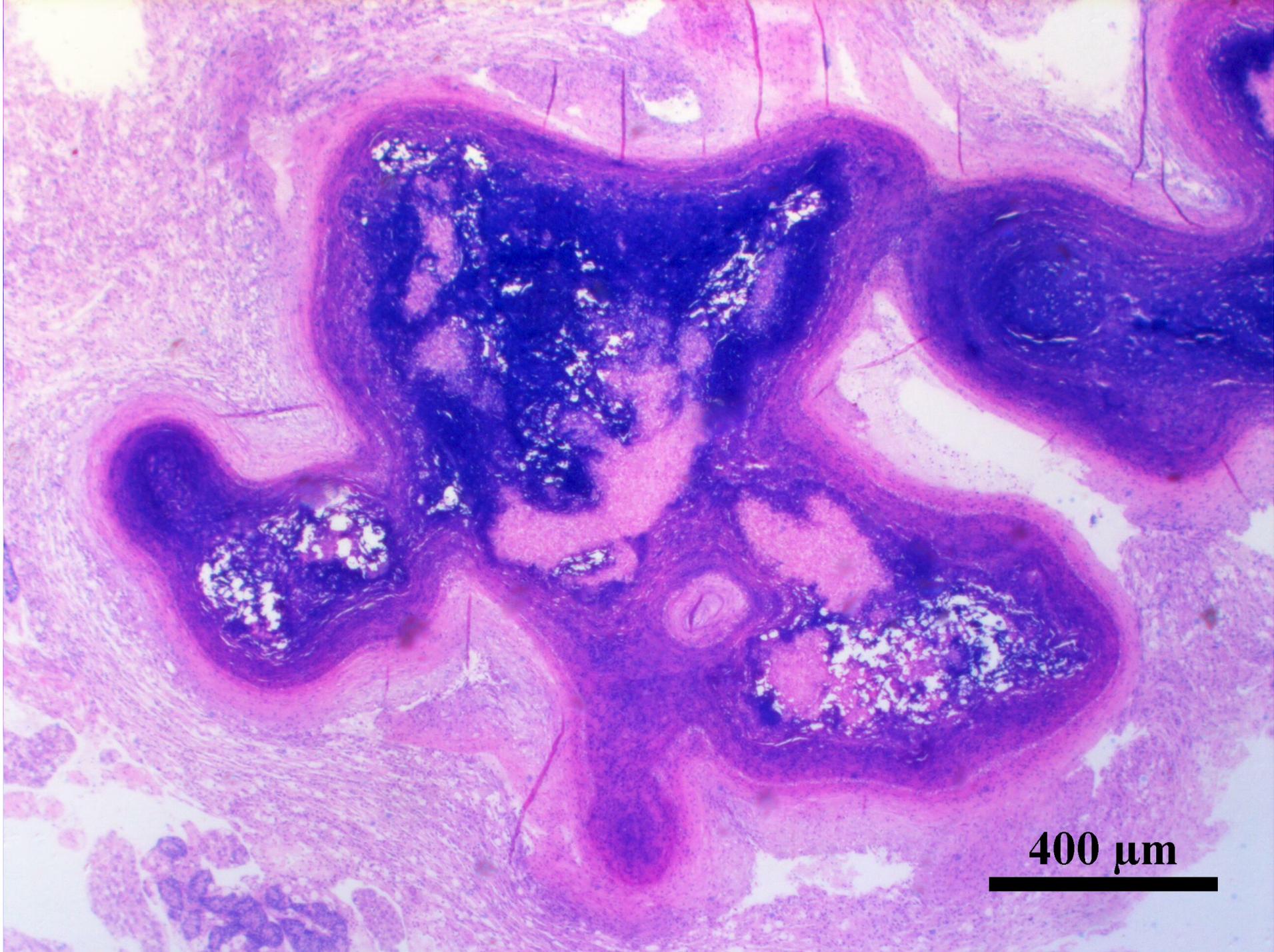




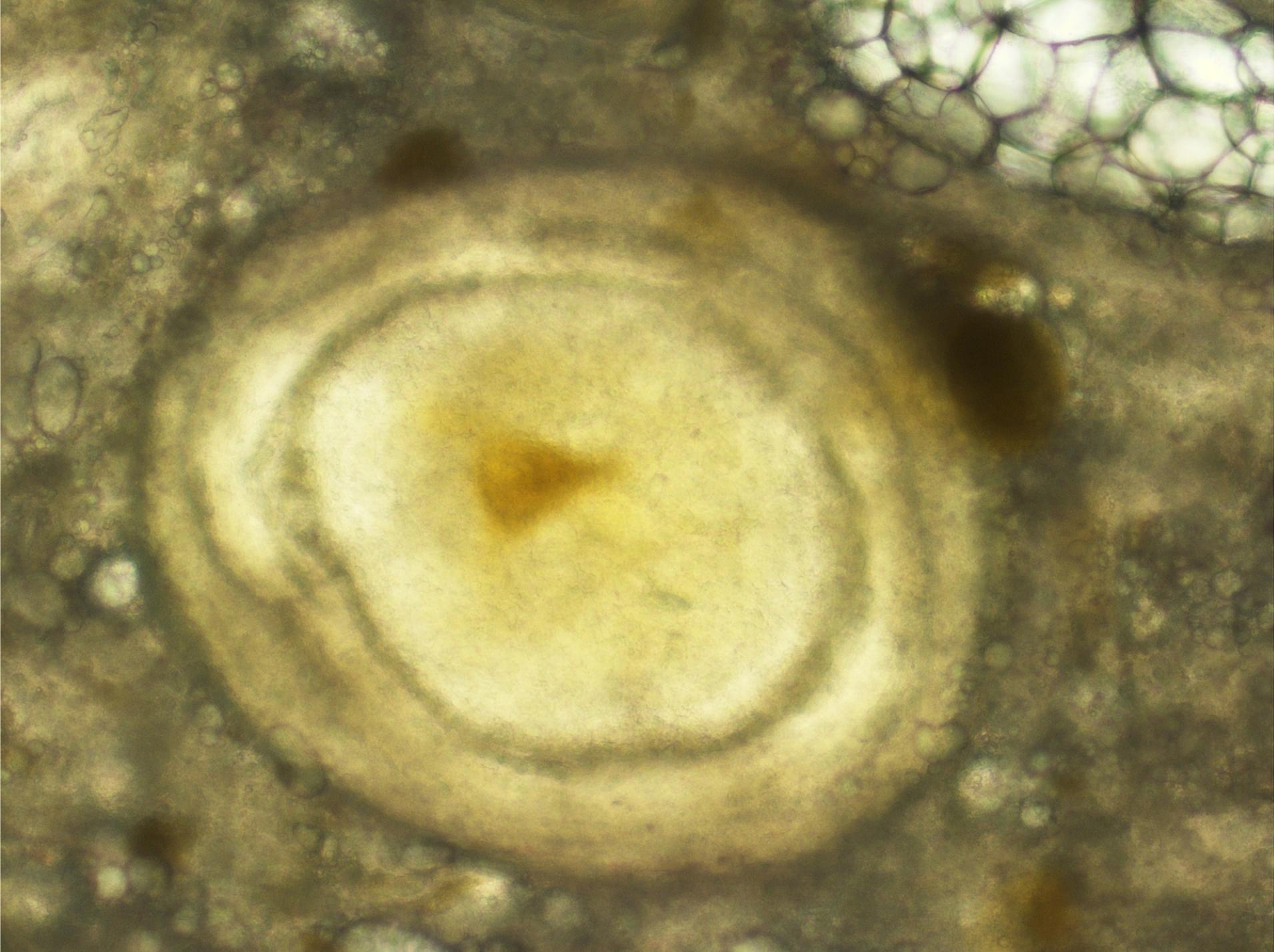


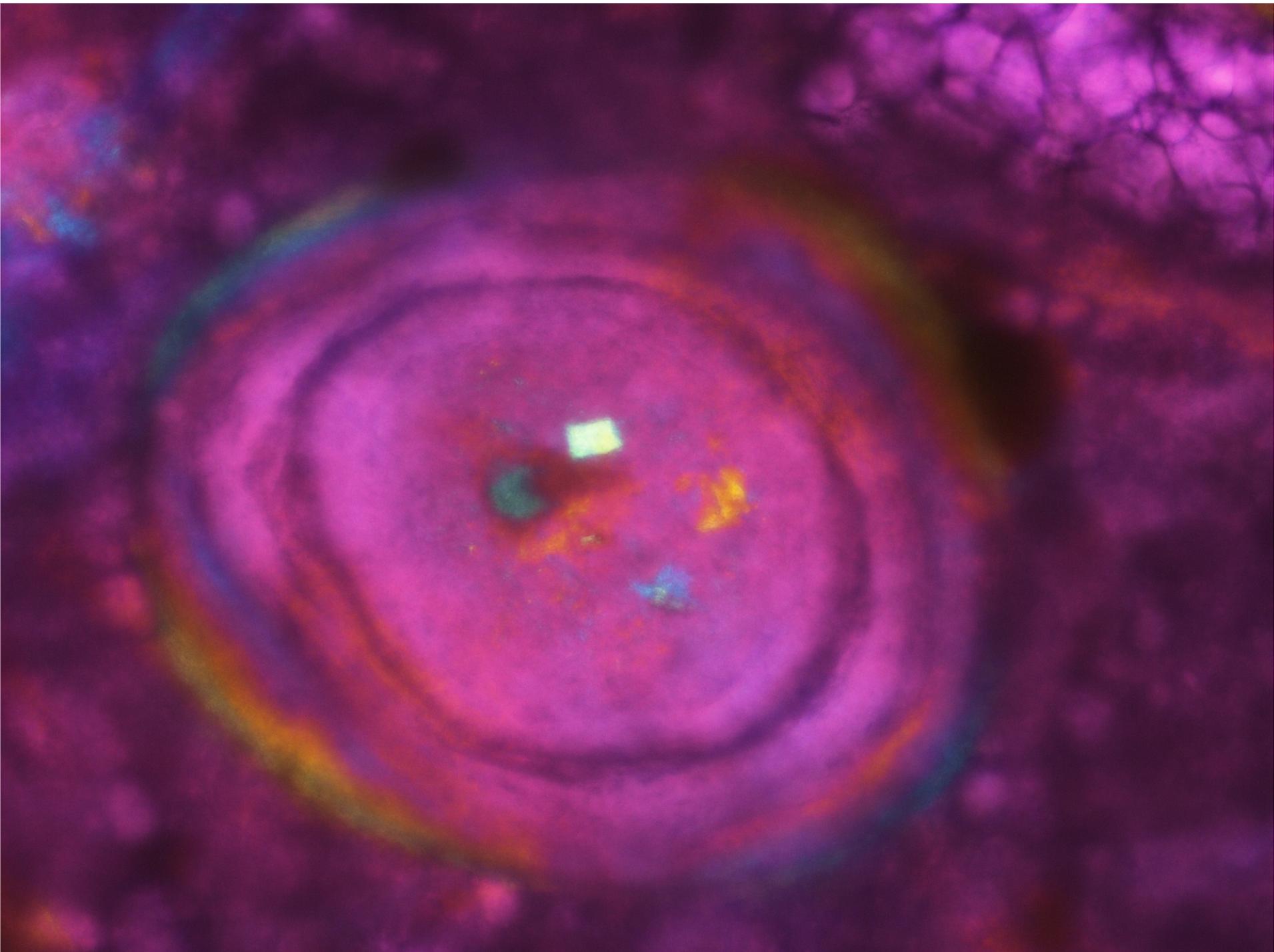


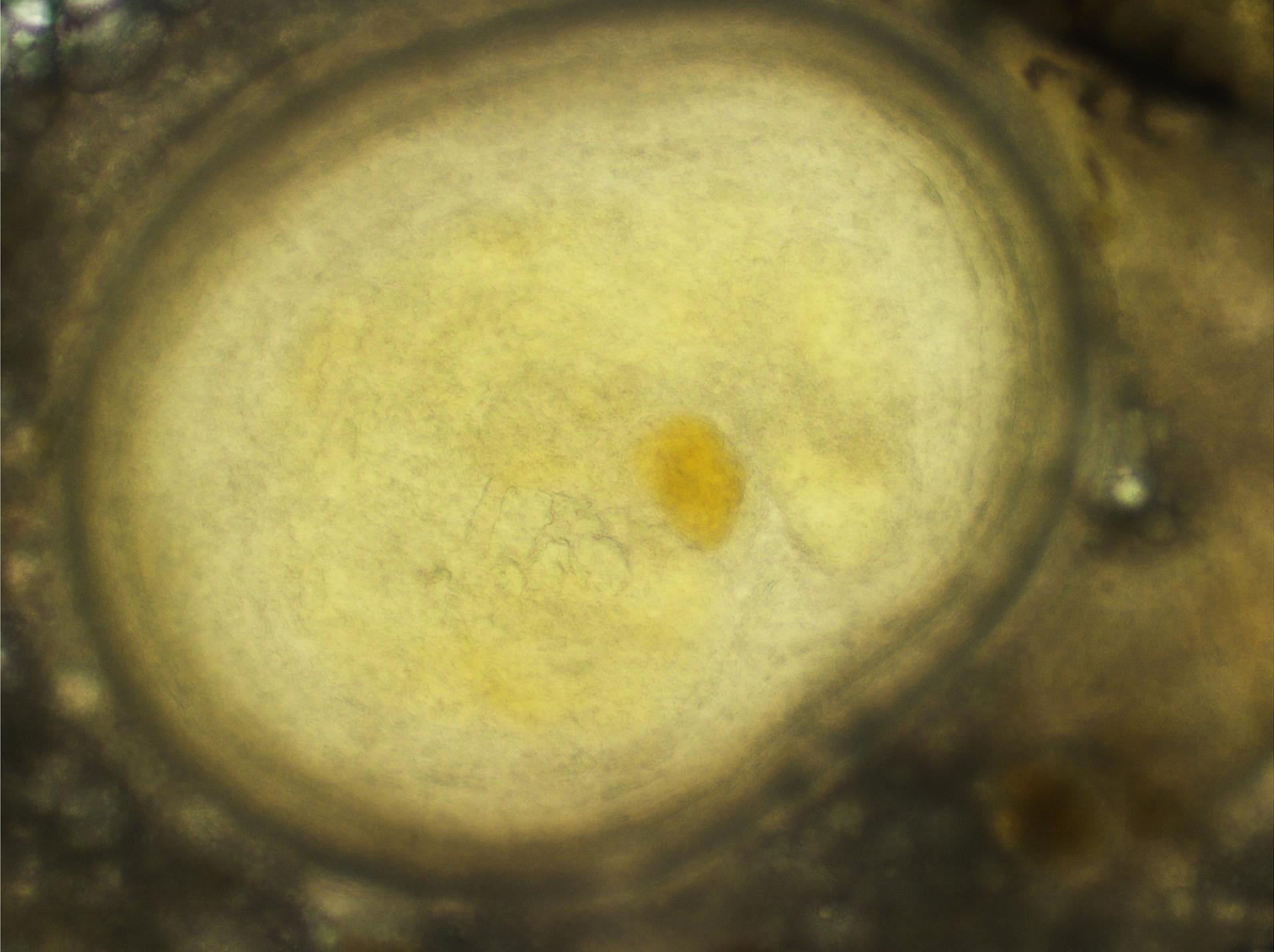
100 μm

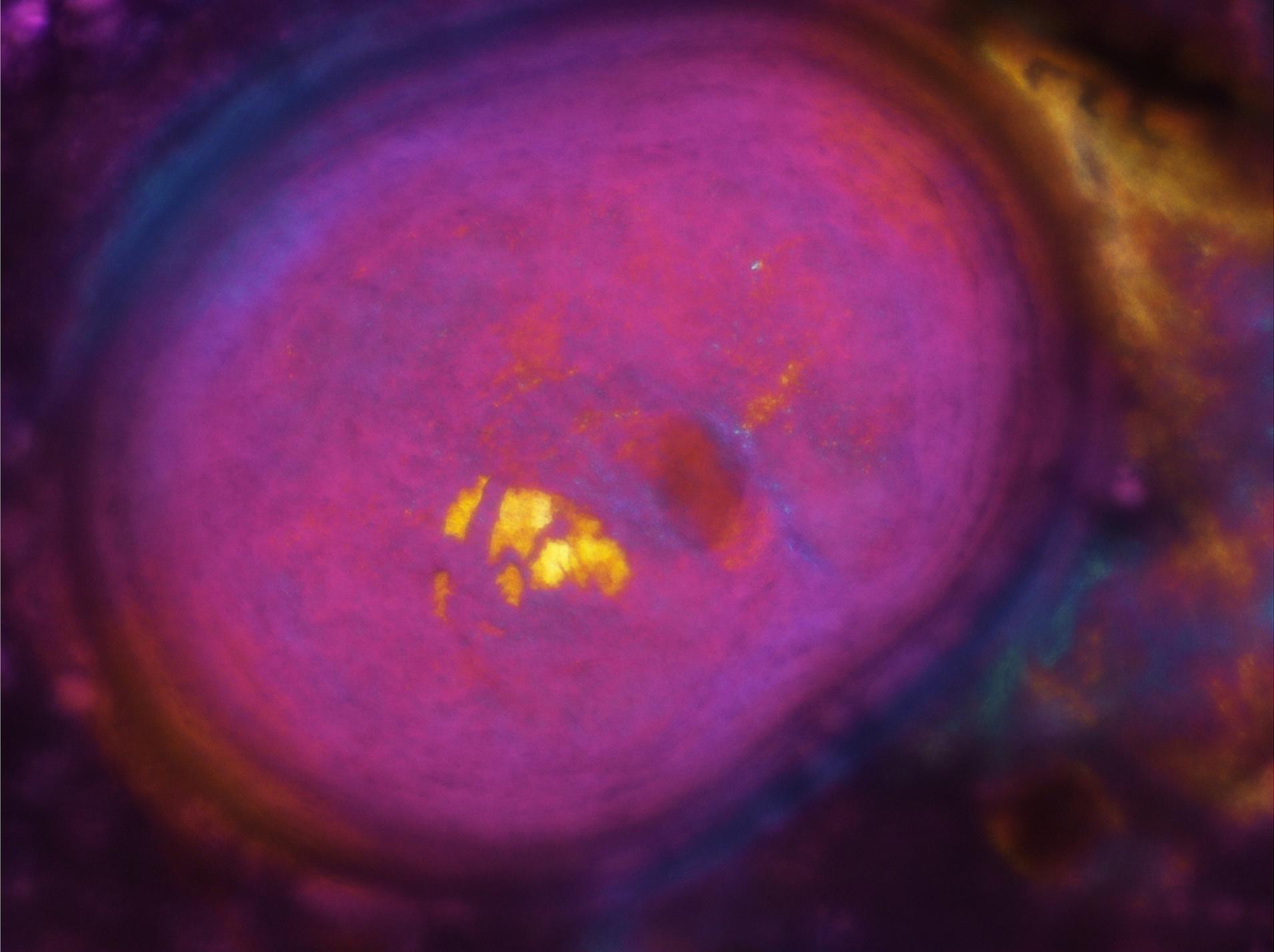


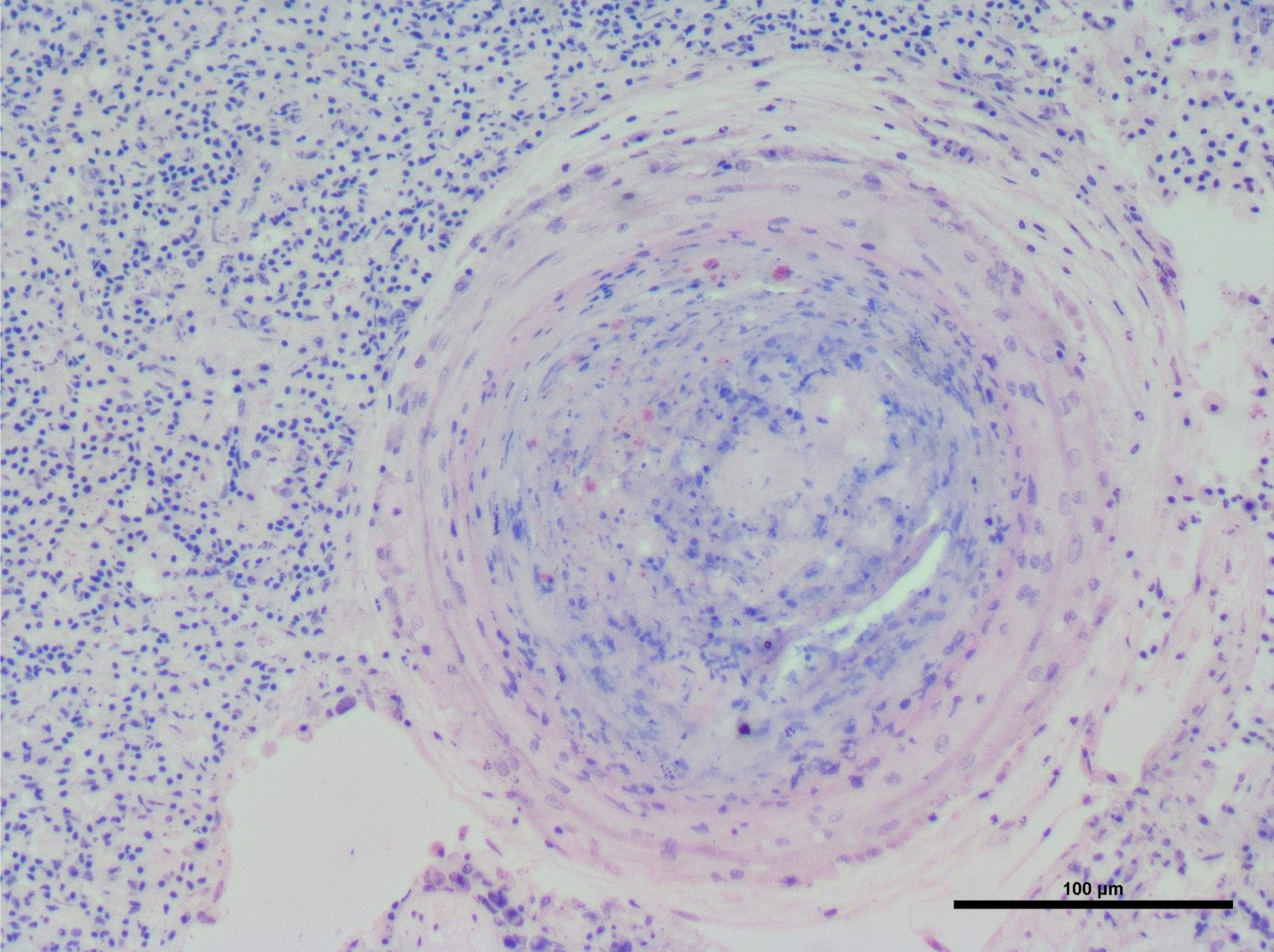
400 μm











100 μ m



Hypotheses

Nutritional (metabolic?) disease

- Visceral granulomas in sea bream-hypetyrosinaemia
- Visceral granulomas in brook trout
- Renal granulomas in turbot
- Some reports in cichlids and goldfish

Disease caused by pathogens

- Nocardia/mycobacteria
- Other granuloma-inducing pathogens (fungi, bacteria, intracellular parasites...)

Hypothesis #2

Infectious agent

Journal of Fish Diseases 2012

doi:10.1111/jfd.12015

Systemic nocardiosis in a Mediterranean population of cultured meagre, *Argyrosomus regius* Asso (Perciformes: Sciaenidae)

A Elkesh¹, K P L Kantham², A P Shinn¹, M Crumlish¹ and R H Richards¹

¹ Institute of Aquaculture, University of Stirling, Stirling, UK

² Nireus S A, Hiliadou Doridos, Focida, Greece

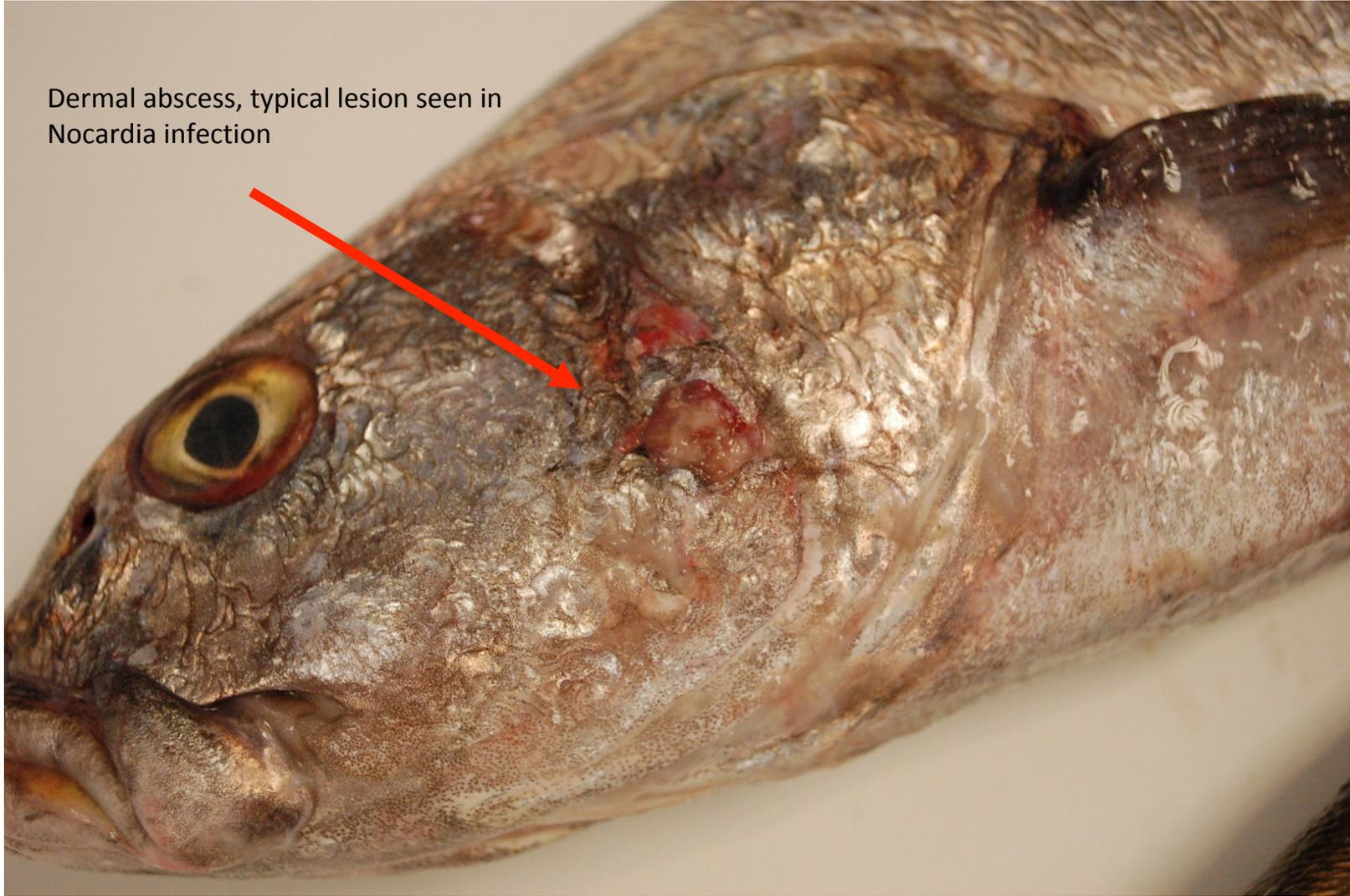


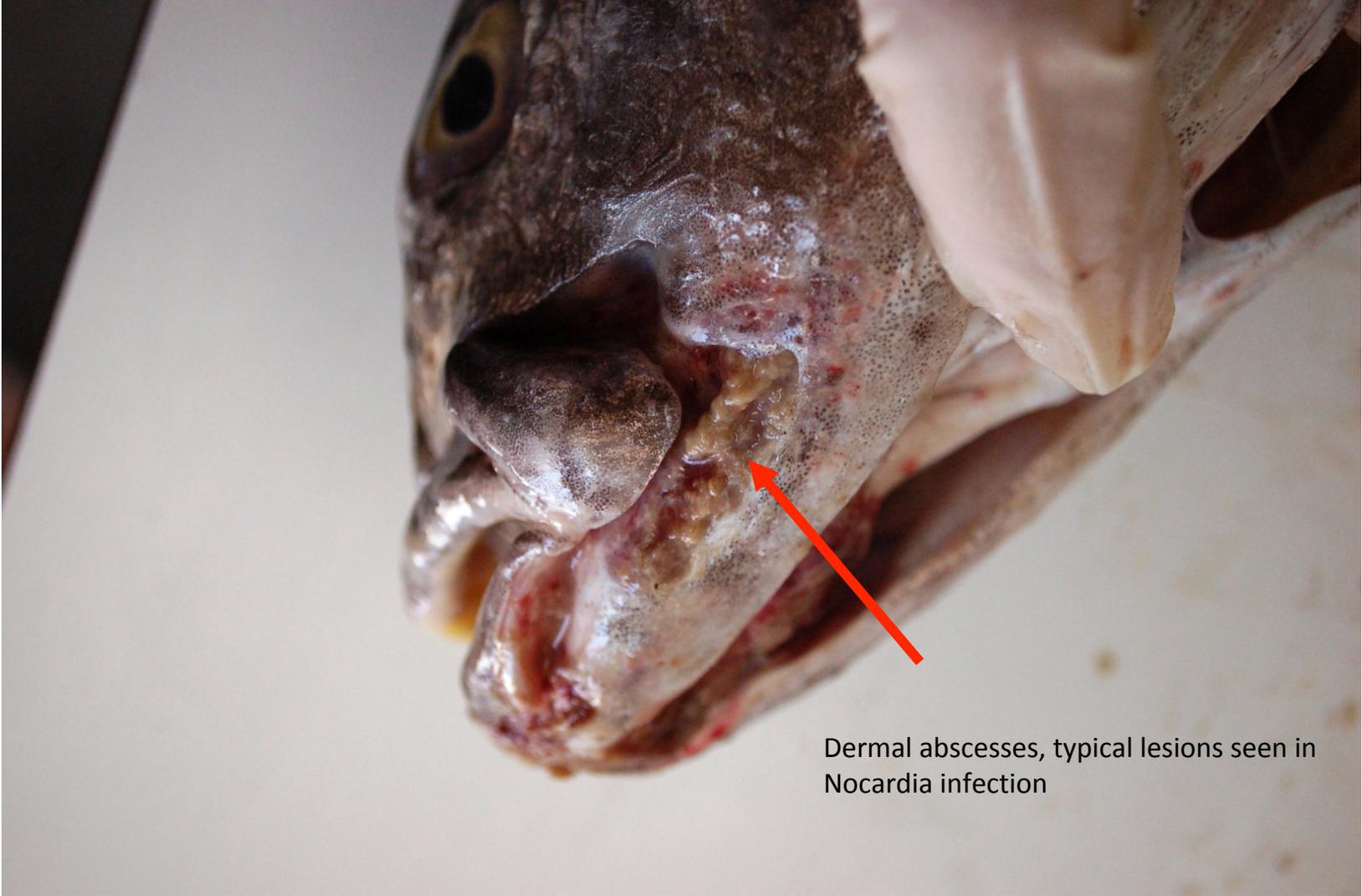
After 3 years we finally have it!!!



Severe ulceration of the skin

Dermal abscess, typical lesion seen in
Nocardia infection



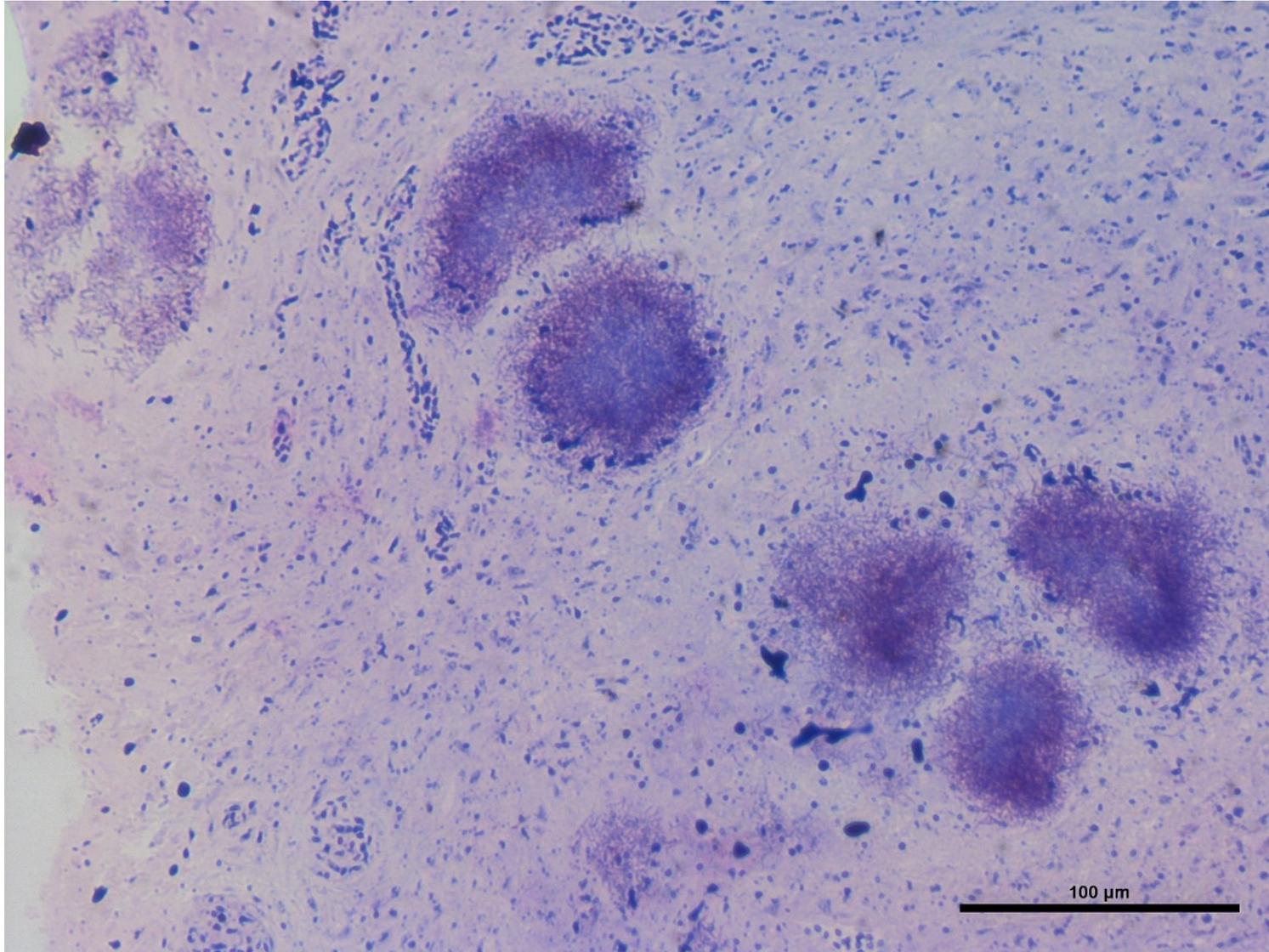


Dermal abscesses, typical lesions seen in Nocardia infection

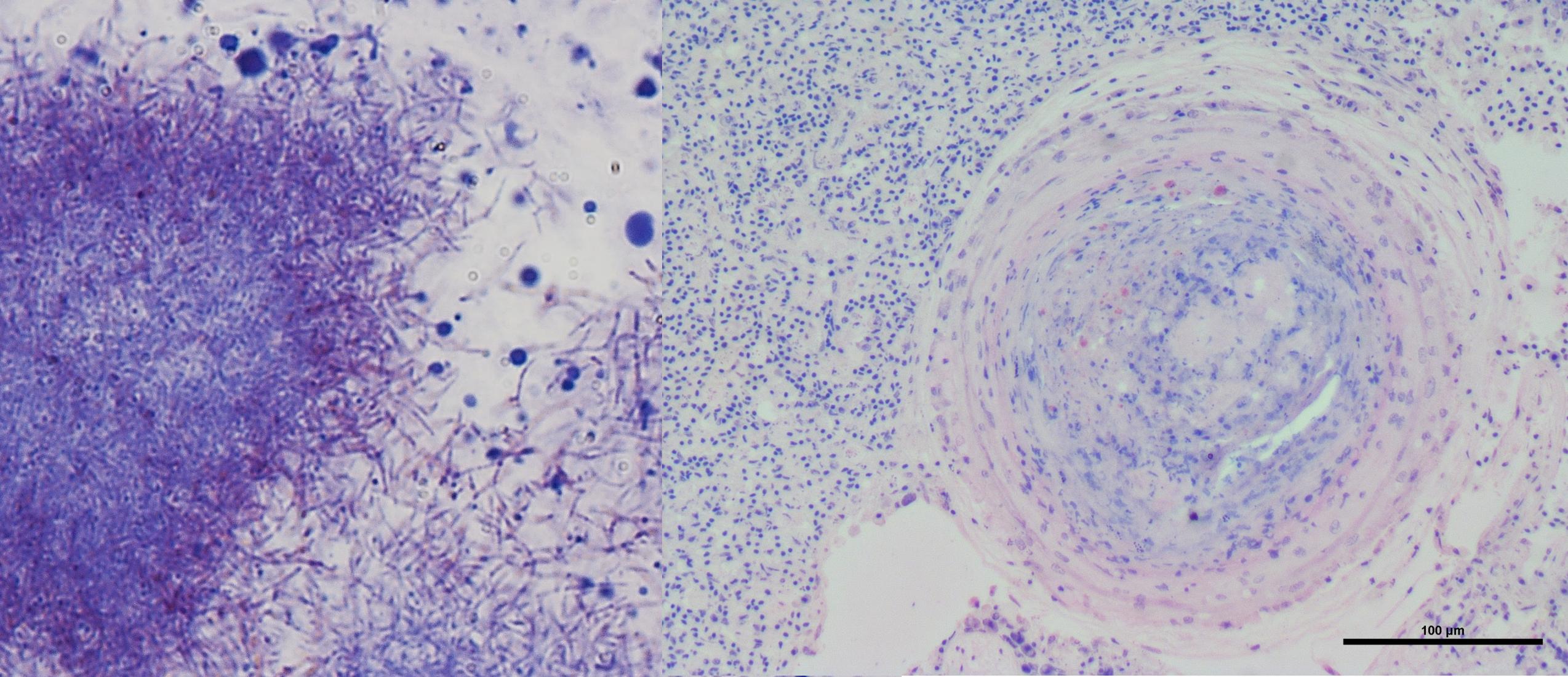
Molecular identification of the pathogen

- DNA extraction from the tissue
- PCR with primers designed for *Nocardia*
- Sequencing
- *Nocardia seriolae*

Histopathology



Section of dermal lesion showing
bacterial colonies.
Ziehl Neelsen stain



Higher magnification of the previous picture, showing acid-fast elongated and branching bacteria, consistent with the typical morphology of *Nocardia* spp.
Ziehl Neelsen stain



ELSEVIER

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep



The aetiology of systemic granulomatosis in meagre (*Argyrosomus regius*): The “*Nocardia*” hypothesis

M.I. Tsertou^{a,b,1}, M. Smyrli^{b,1}, C. Kokkari^b, E. Antonopoulou^a, P. Katharios^{b,*}

^a Laboratory of Animal Physiology, Department of Zoology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

^b Institute of Marine Biology, Biotechnology and Aquaculture, HCMR, Former American Base of Gournes, Heraklion 71003, Crete, Greece



DIVERSIFY WP24

feeding trials

HCMR

- Effect of Vitamin D
- Effect of Ca/P ratio
- Effect of plant proteins

FCPCT

- combined effect of vitamins E, C and carotenoids
- effect of Se, Mn and Zn

General conclusions

- Vitamin D₃ supplementation did not affect the development of the SG,
- High P content in the diet seems to improve the condition
- Plant protein replacement affects negatively the progression of the SG.
- High dietary content of the antioxidants vitamin E and C increased the incidence and number of fish with lower severity of SG
- The addition of Zn, Mn and Se did not ameliorate the granuloma incidence or severity.
- Nocardiosis is present in Greece, most probably in a confined geographical region; however it is not the cause of SG.

Recommendations

- A combined diet with high percentage of fishmeal (60%) and high dietary content of P (15gkg^{-1}) and antioxidants vitamins E and C.
- Since there is no data available about the reversibility of SG we recommend to start feeding with this diet when the fish weight is about 2g.

Further hypothesis for granulomatosis

- Other nutritional metabolic factors
- Genetic background (diversity of broodstock)
- Unknown and invisible pathogen



Chronic Ulcerative Dermatopathy: The disease

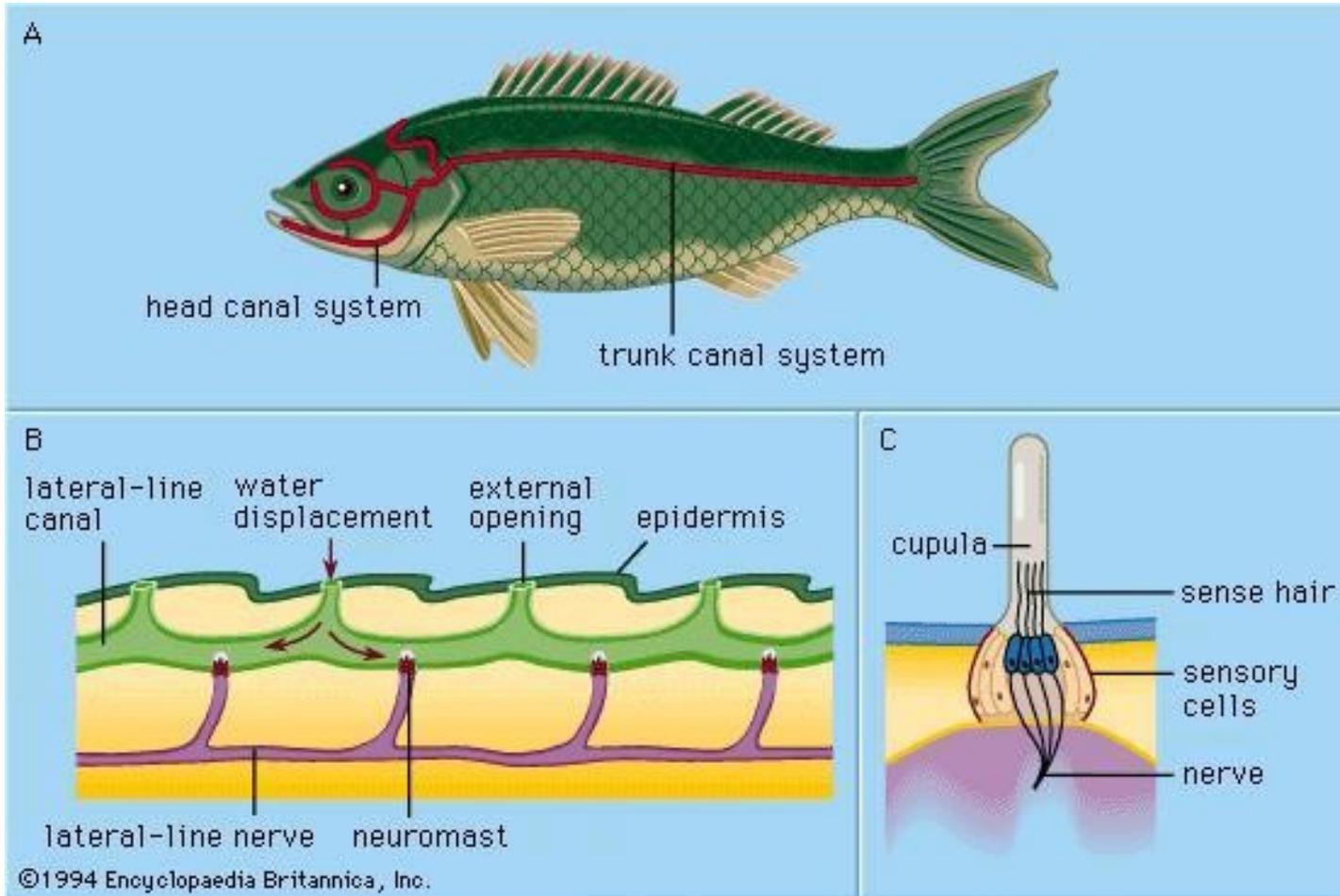


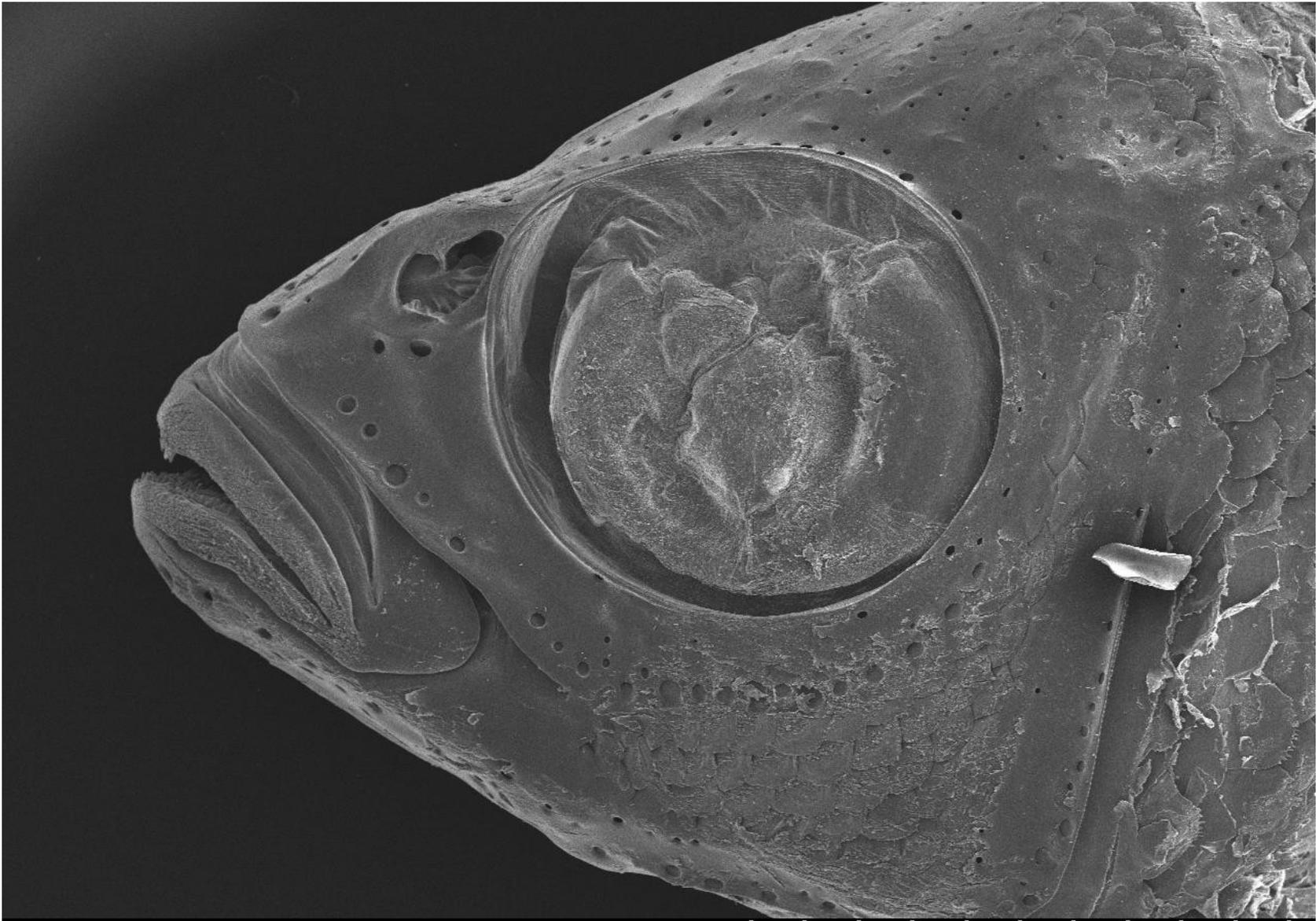
Murray cod, freshwater



Sharpsnout seabream and meagre in seawater

The lateral line organ





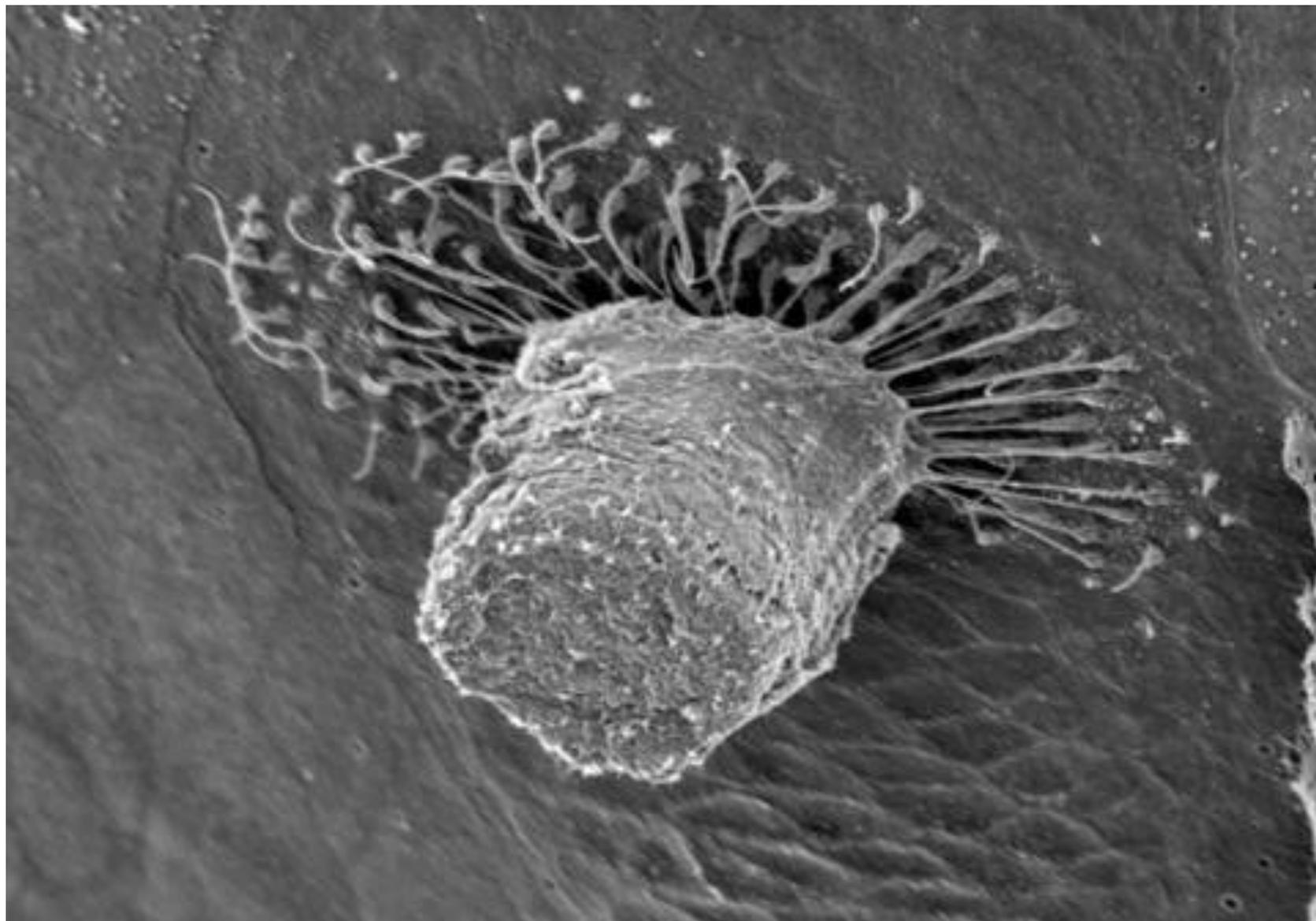
UdC 10.0kV 44.8mm x13 SE 4/17/2009

4.00mm



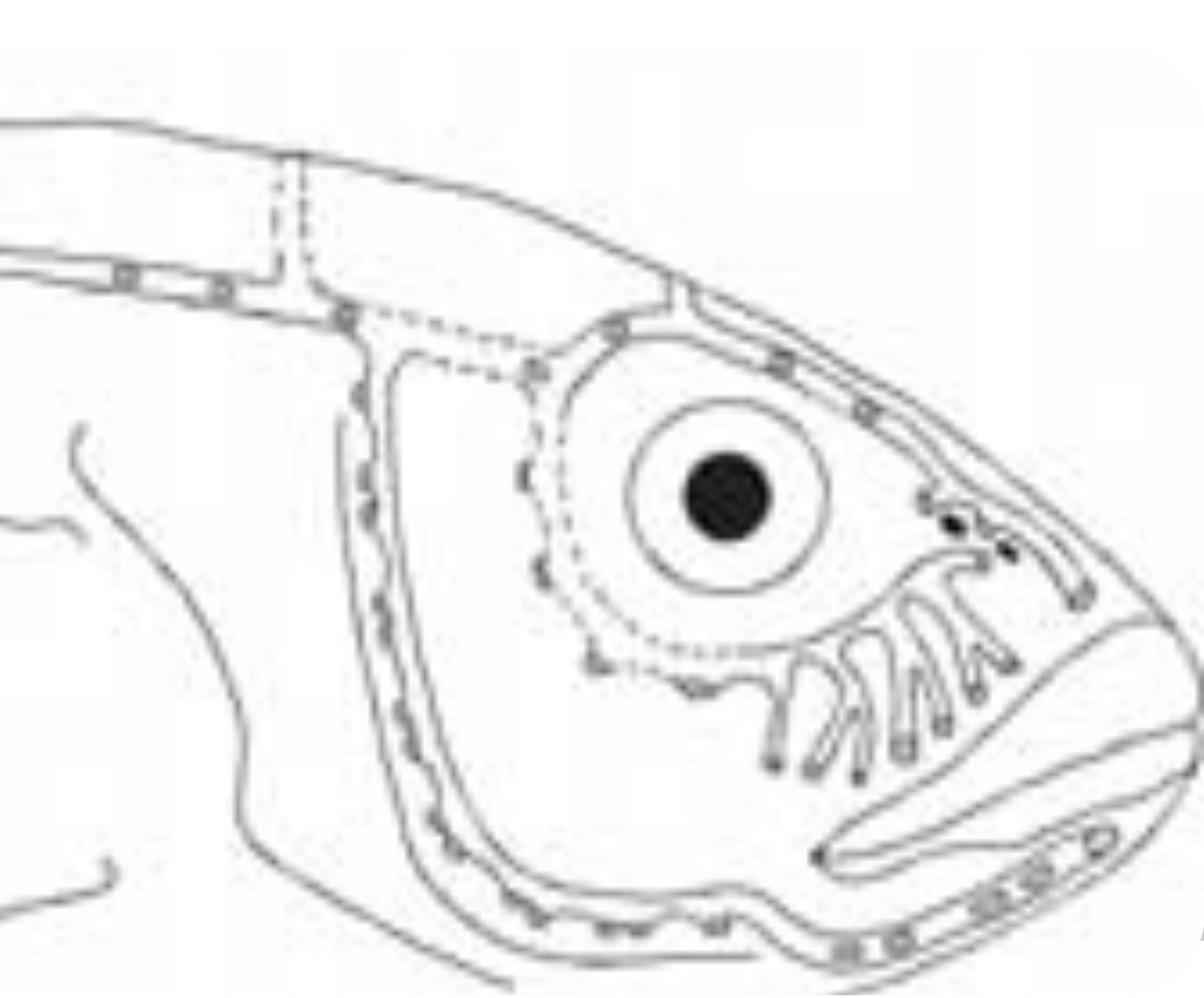
UdC 3.00kV 6.8mm x300 SE 5/12/2009

100um



UdC 3.00kV 11.7mm x1.80k SE 5/12/2009

30.0um



What do we know about the disease

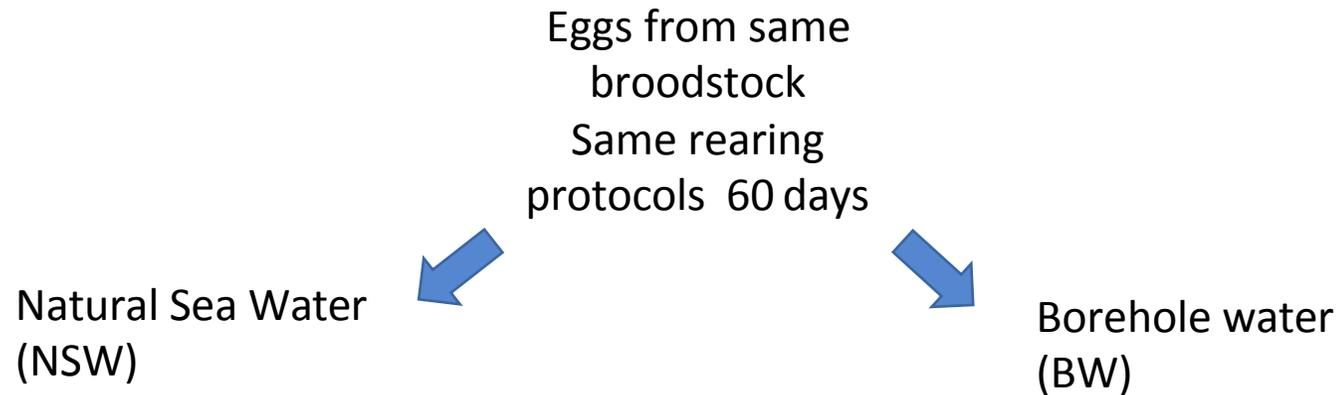
- It is non infectious
- It is directly connected to borehole (ground) water
- It affects a wide range of fish species both in fresh and seawater

- **Hypothesis**
- Increased enzymatic activity at the lateral line canals (possibly osteoclast activity)
- Katharios, P., Papadaki, M., Ternengo, S., Kantham, P.K., Zeri, C., Petraki, P.E., Divanach, P., 2011. Chronic ulcerative dermatopathy in cultured marine fishes. Comparative study in sharpsnout sea bream, *Diplodus puntazzo* (Walbaum). J. Fish Dis. 34, 459–474.

Aims of the task

- Development of the lateral line organ
- Study of Chronic Ulcerative Dermatopathy in meagre through:
 1. Histology
 2. SEM
 3. Gene expression of genes related to osteoclast/osteoblast activity (TRAP, cathK, vATP)

Experimental design (1/2)



Samplings: 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17, 19, 21, 26, 31, 36, 41, 46 and 51 dph

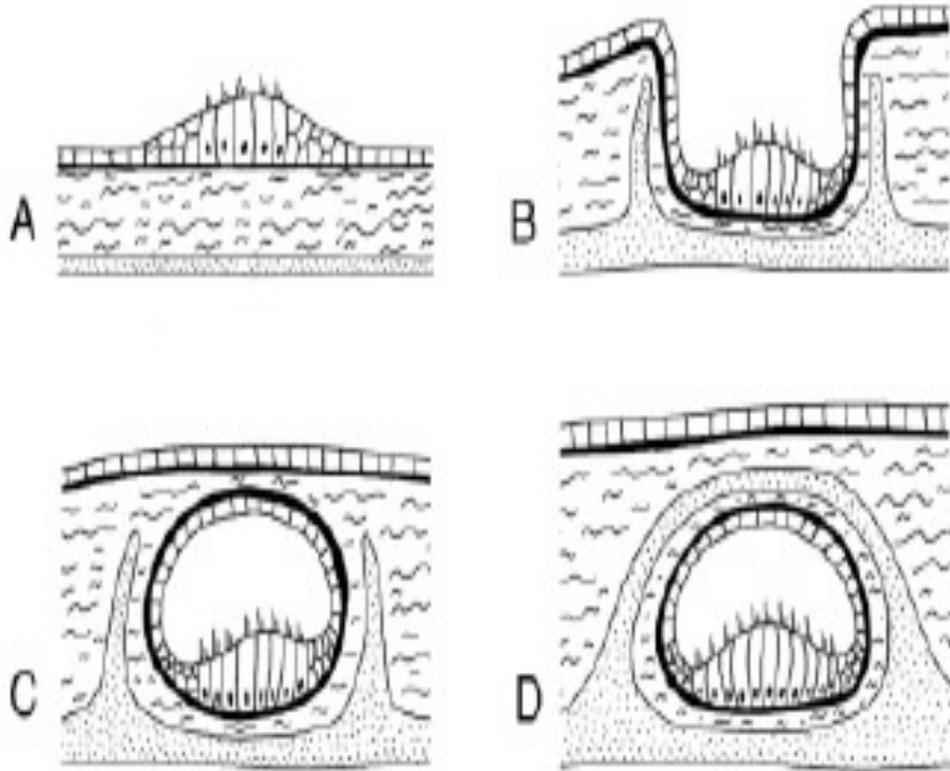
Histology, SEM, qPCR

Experimental design (2/2)



Macroscopic observations

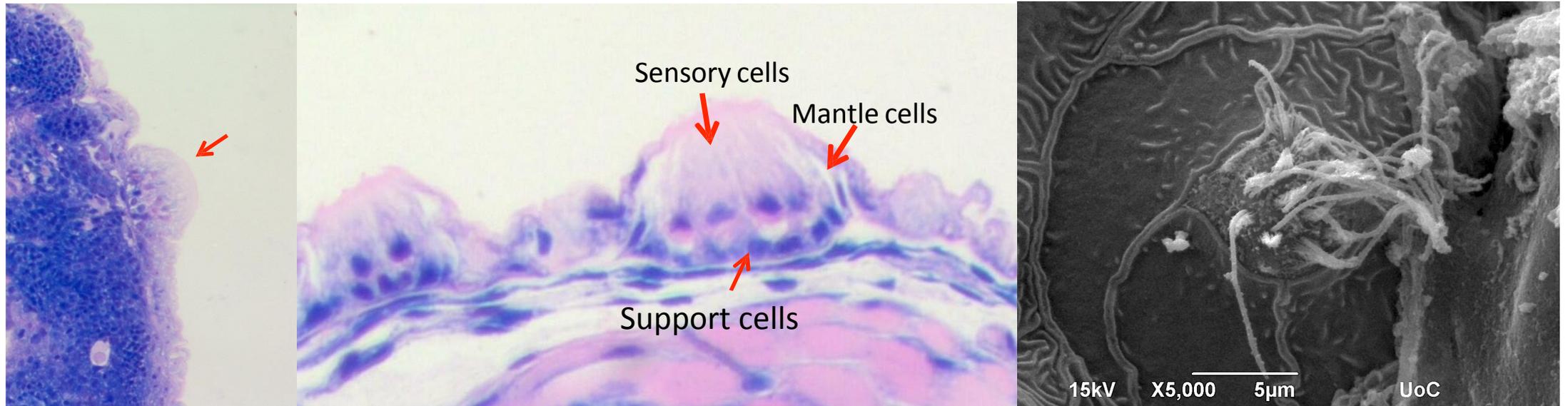
Ontogeny of the lateral line



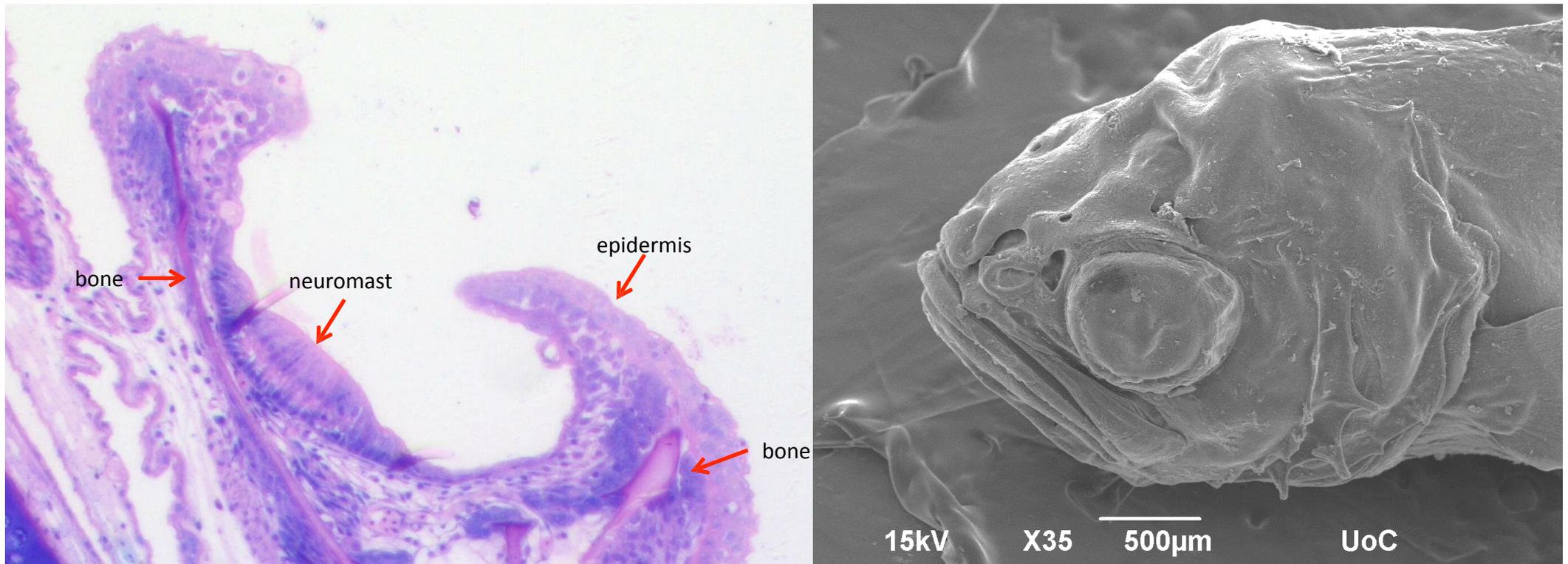
Tarby, M.L., Webb, J.F., 2003. Development of the supraorbital and mandibular lateral line canals in the cichlid, *Archocentrus nigrofasciatus*. *J. Morphol.* 255, 44–57. doi: 10.1002/jmor.10045

Ontogeny of the lateral line in meagre

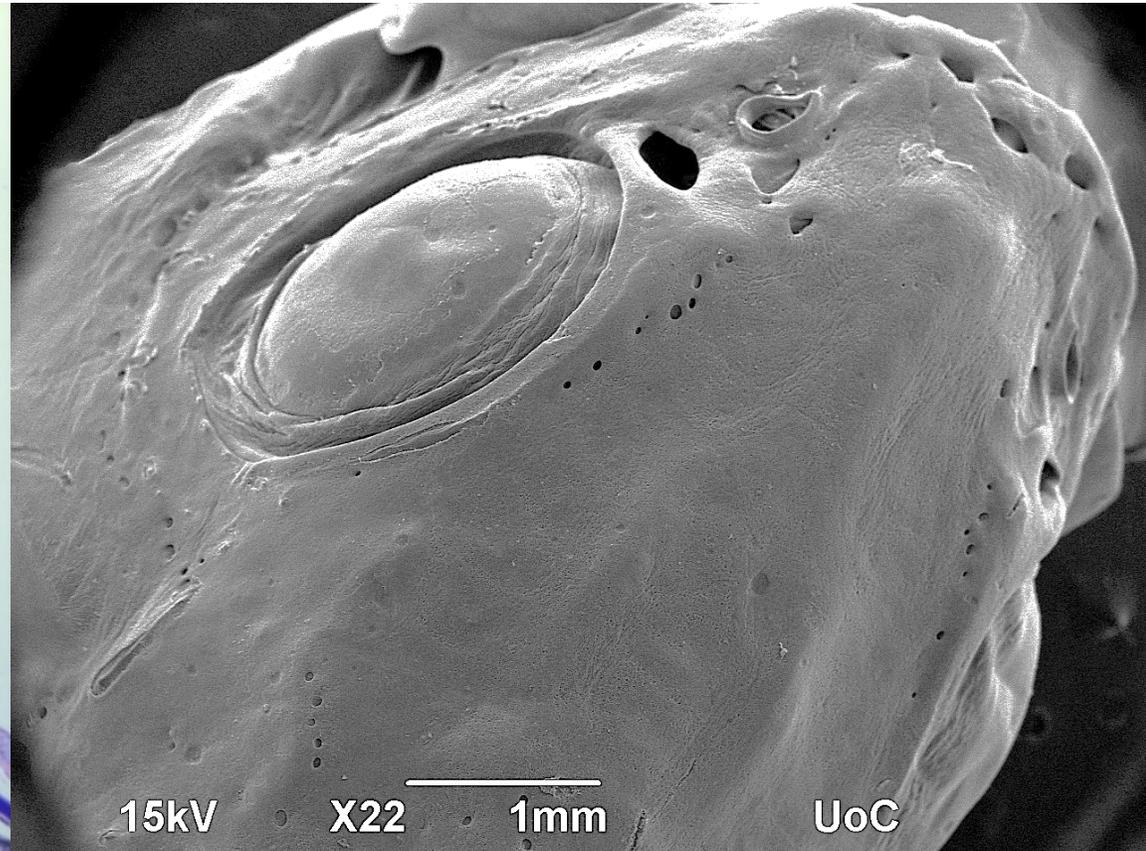
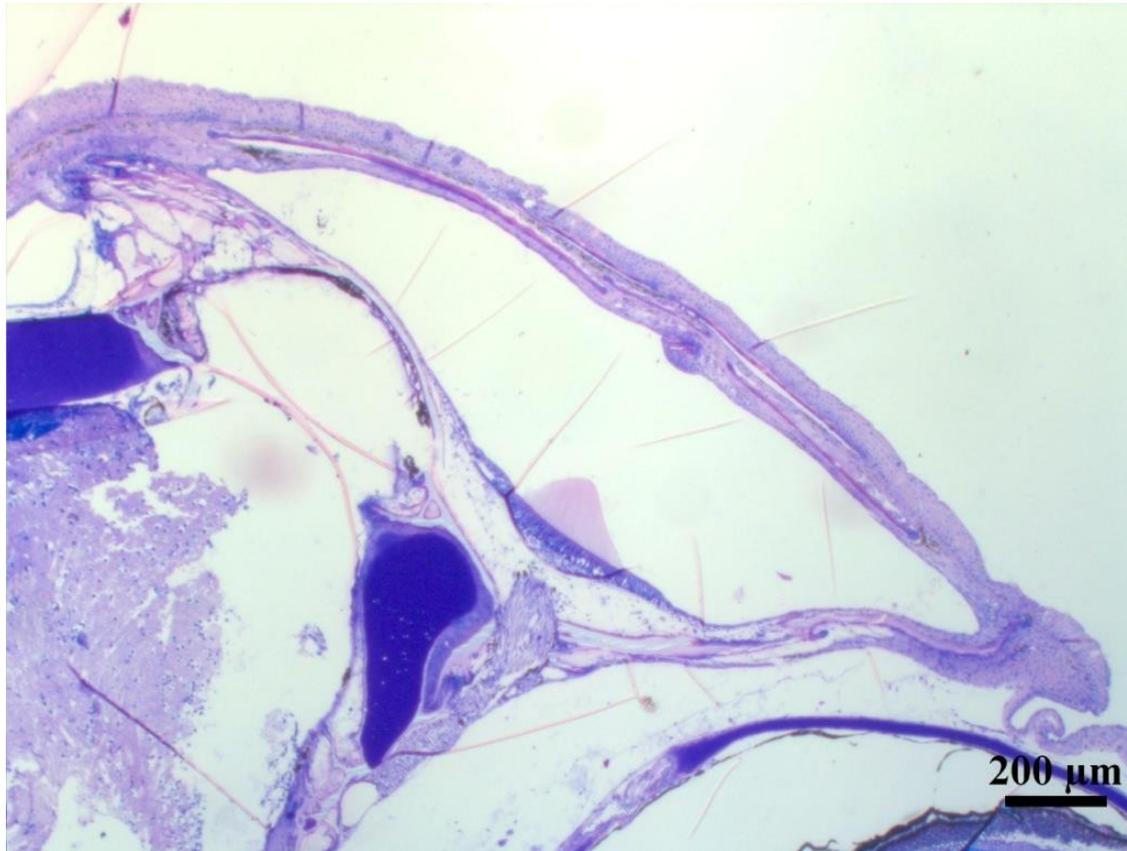
Neuromasts on the skin of meagre larvae (1-3 dph)



17-25 dph (TL: 5.7-13.7 mm) : formation of the basic lateral line canals of the head (infraorbital, supraorbital and mandibular)



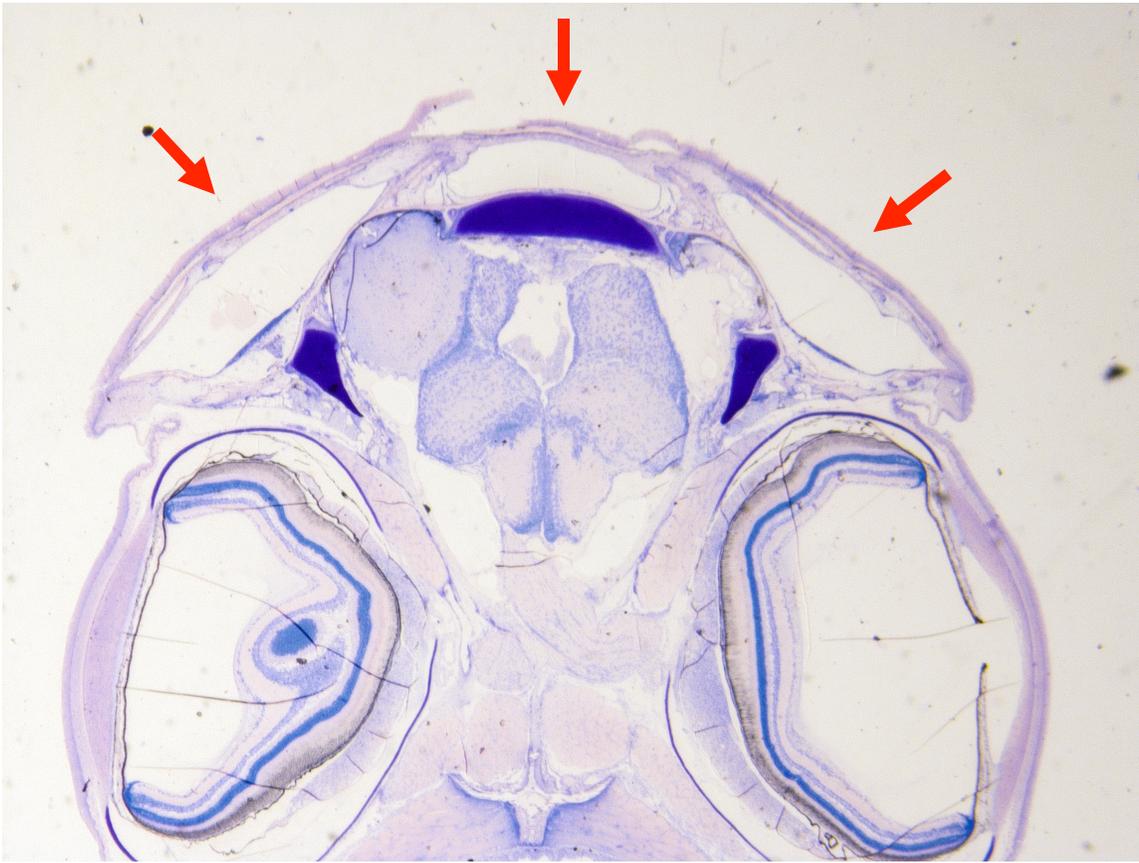
40 dph (TL: 35 mm) : completely developed lateral line



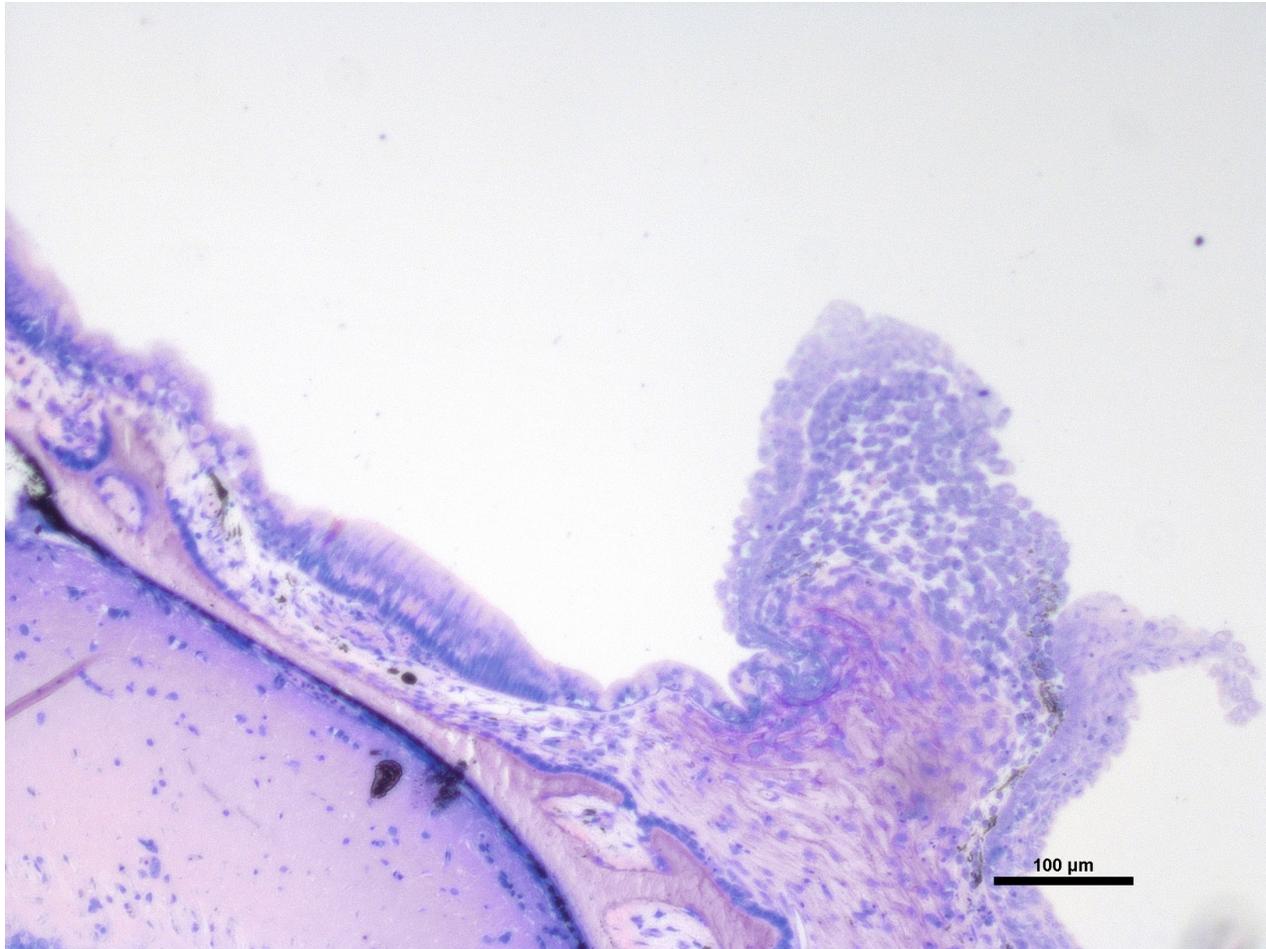
First signs of the disease (46 dph)



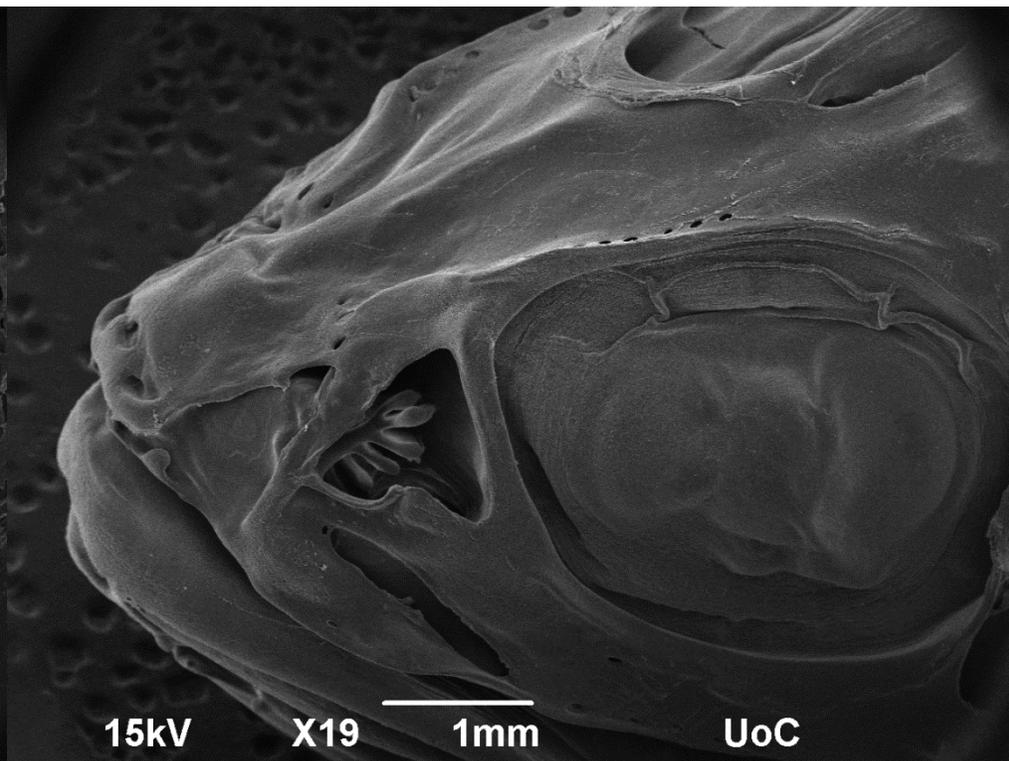
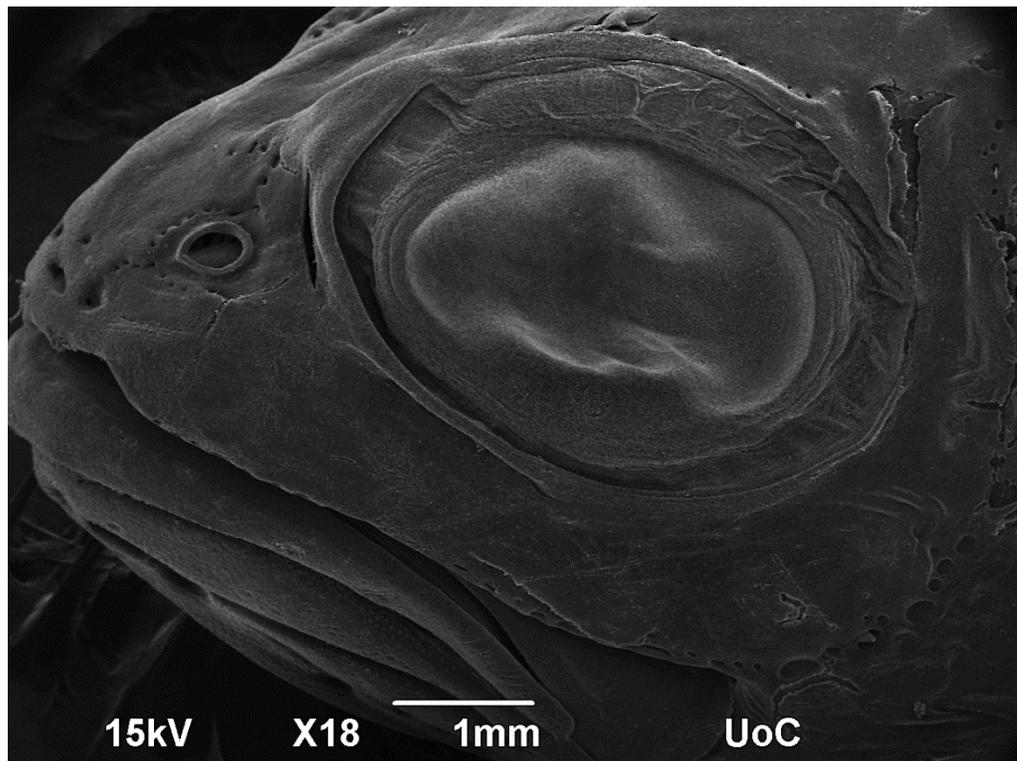
First signs of the disease (46 dph)



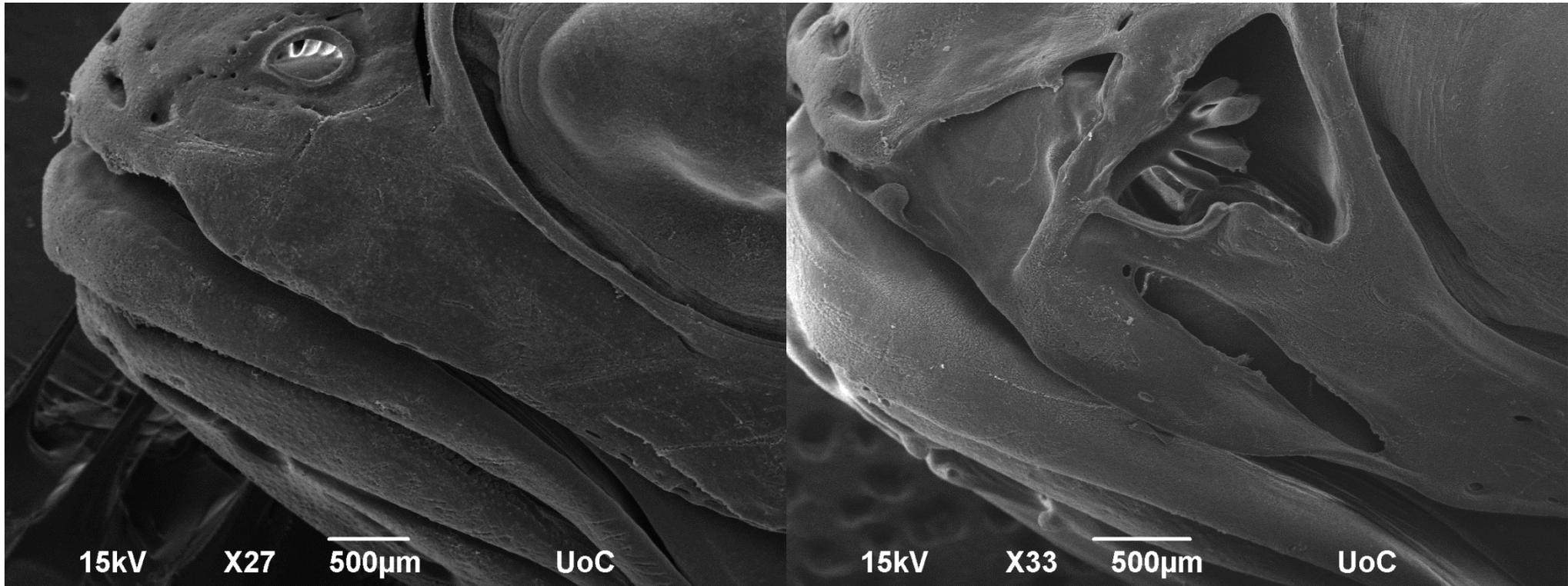
Histopathology



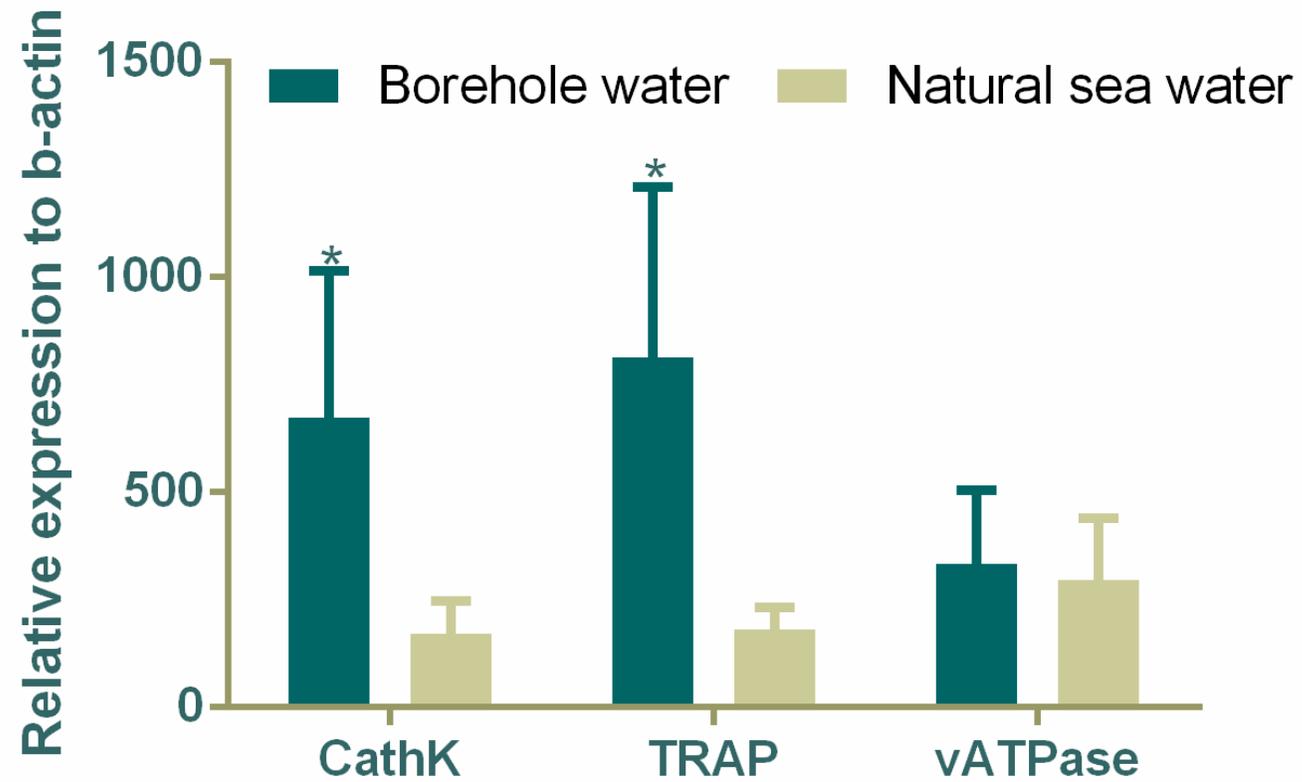
SEM



SEM



Gene expression (56dph)



Recovery



8 month fish: Borehole water

Natural seawater

Conclusions

- CUD affects meagre when grown in borehole water
- It causes severe disfigurement of the fish
- Mechanism involves osteoclastic activity
- Reversible if fish are transferred to natural seawater

- Take home message for the producers:

Do not grow fish in borehole water for more than 2 months



Acknowledgements

Marianna Tsertou (HCMR)
Maria Smyrli (HCMR)
Stavros Chatzifotis (HCMR)
Efi Cotou (HCMR)
Dida Kokkari (HCMR)
Ana Roque (IRTA)
Daniel Montero (FCPCT)
Ramon Fontanillas (Skretting)

