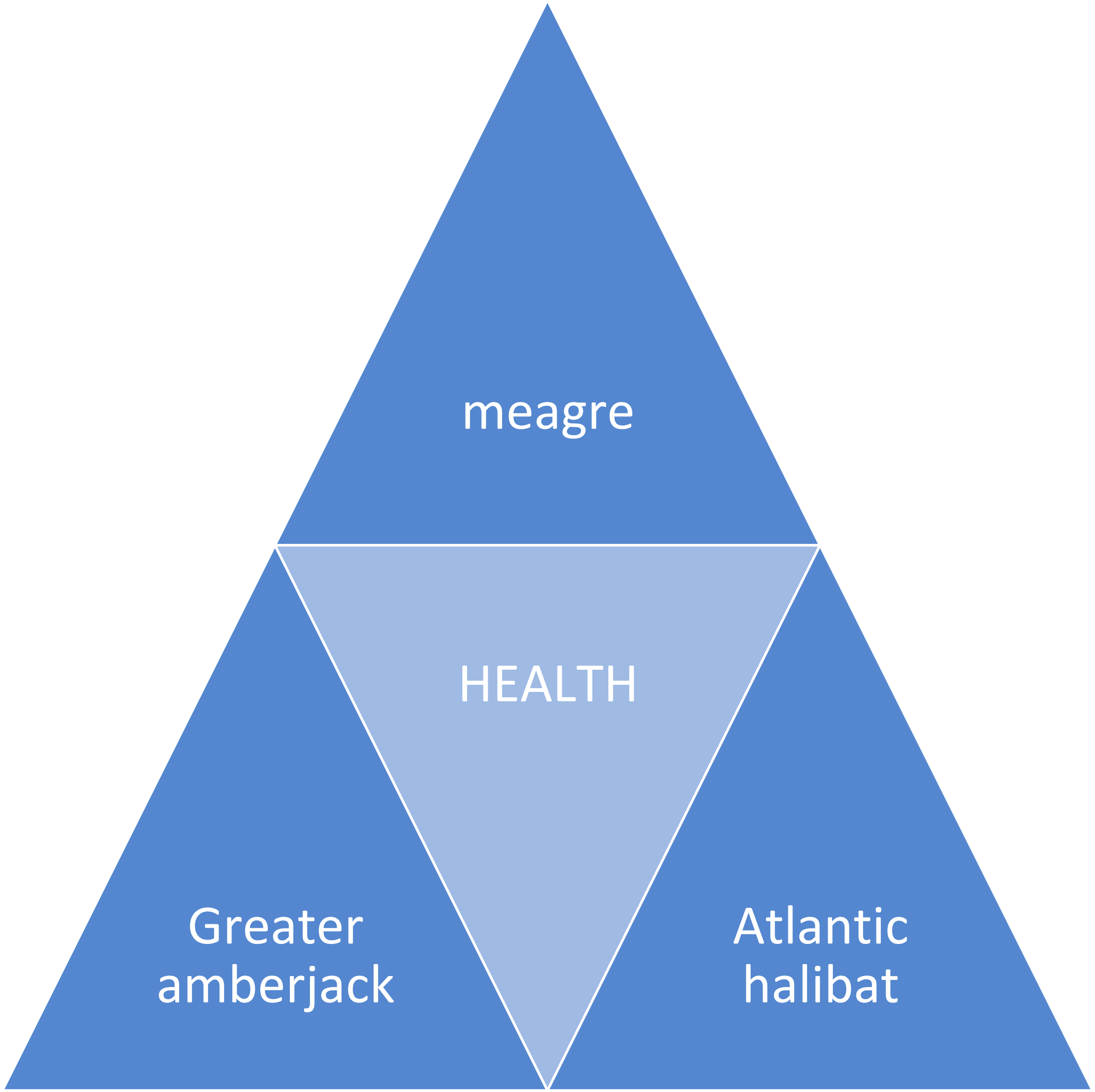
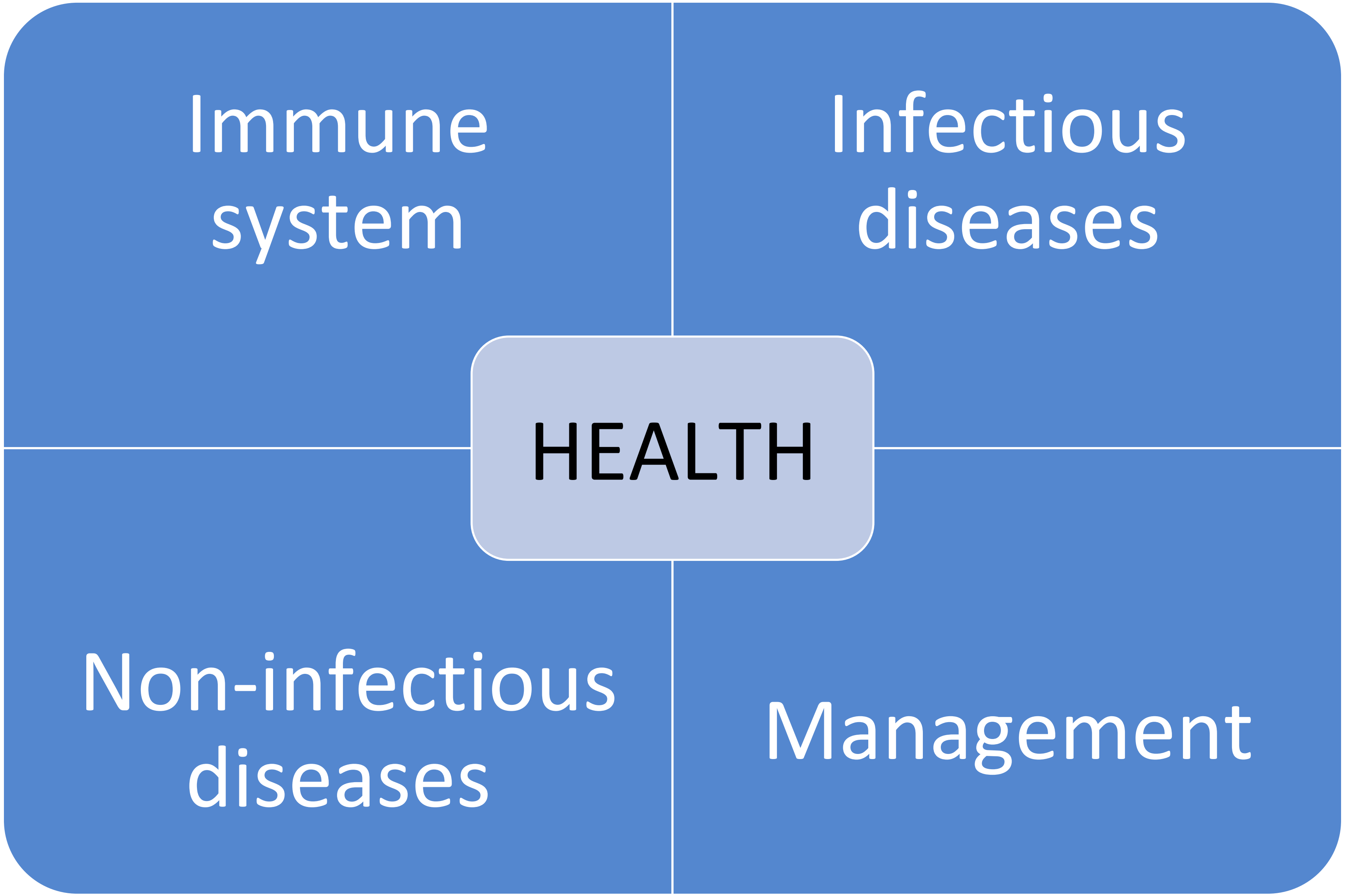
Fish Health Tasks in the DIVERSIFY project

Dr. Pantelis Katharios

Hellenic Centre for Marine Research

Workshop, November 22, Brussels


meagre
HEALTH
Greater amberjack
Atlantic halibat


Immune system
Infectious diseases
HEALTH
Non-infectious diseases
Management

Immune system

- Characterization of immune related genes for meagre and amberjack
- Vaccine development (Halibut and meage)

Bacterial diseases

- Nocardiosis (meagre)
- Epitheliocystis (amberjack)
- Vibriosis and other bacteria

Parasites

- Life cycles
- Antiparasitic treatments
- Management

Non-infectious diseases

- Systemic granulomatosis
- Chronic Ulcerative Dermatopathy

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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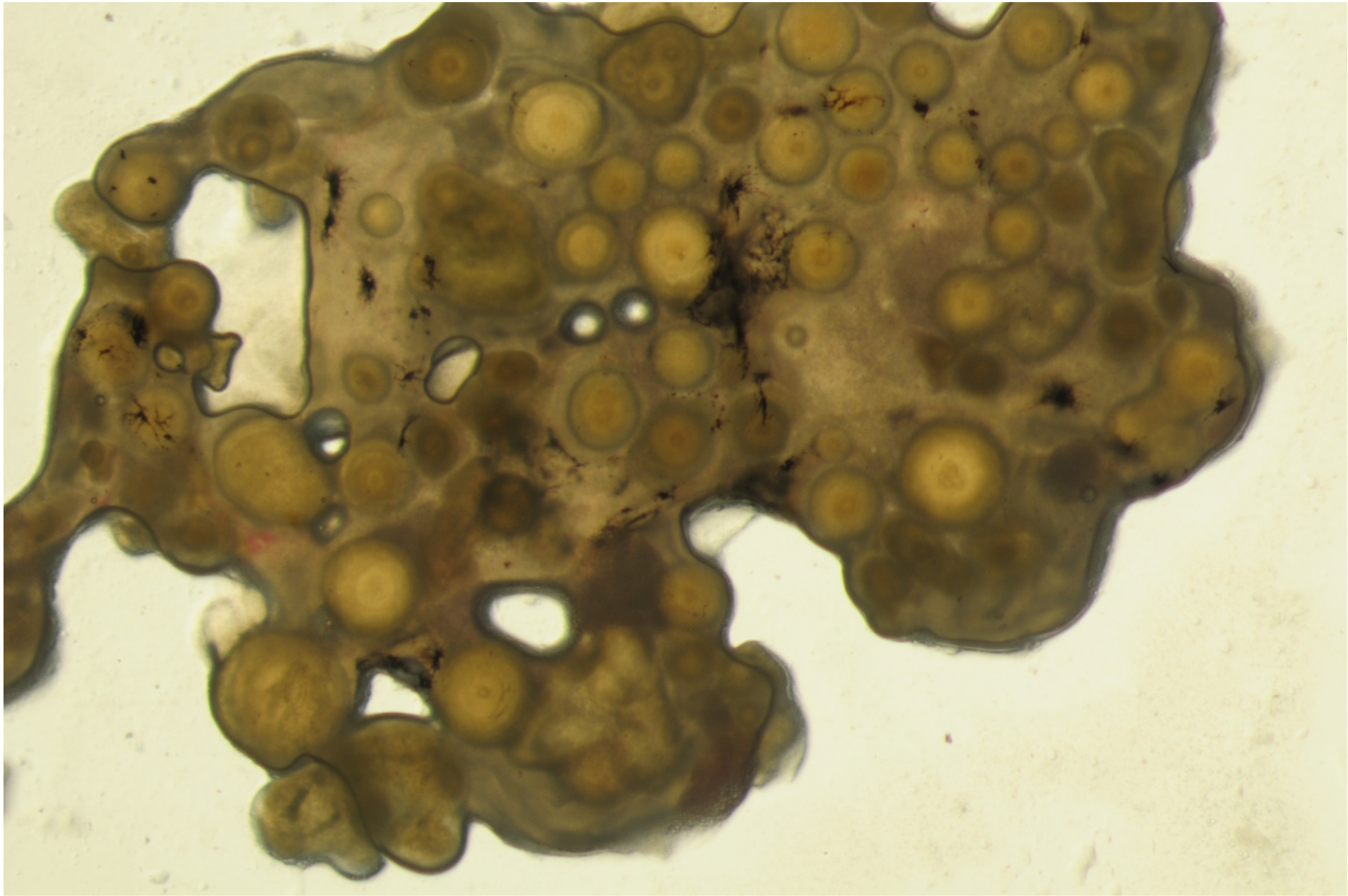

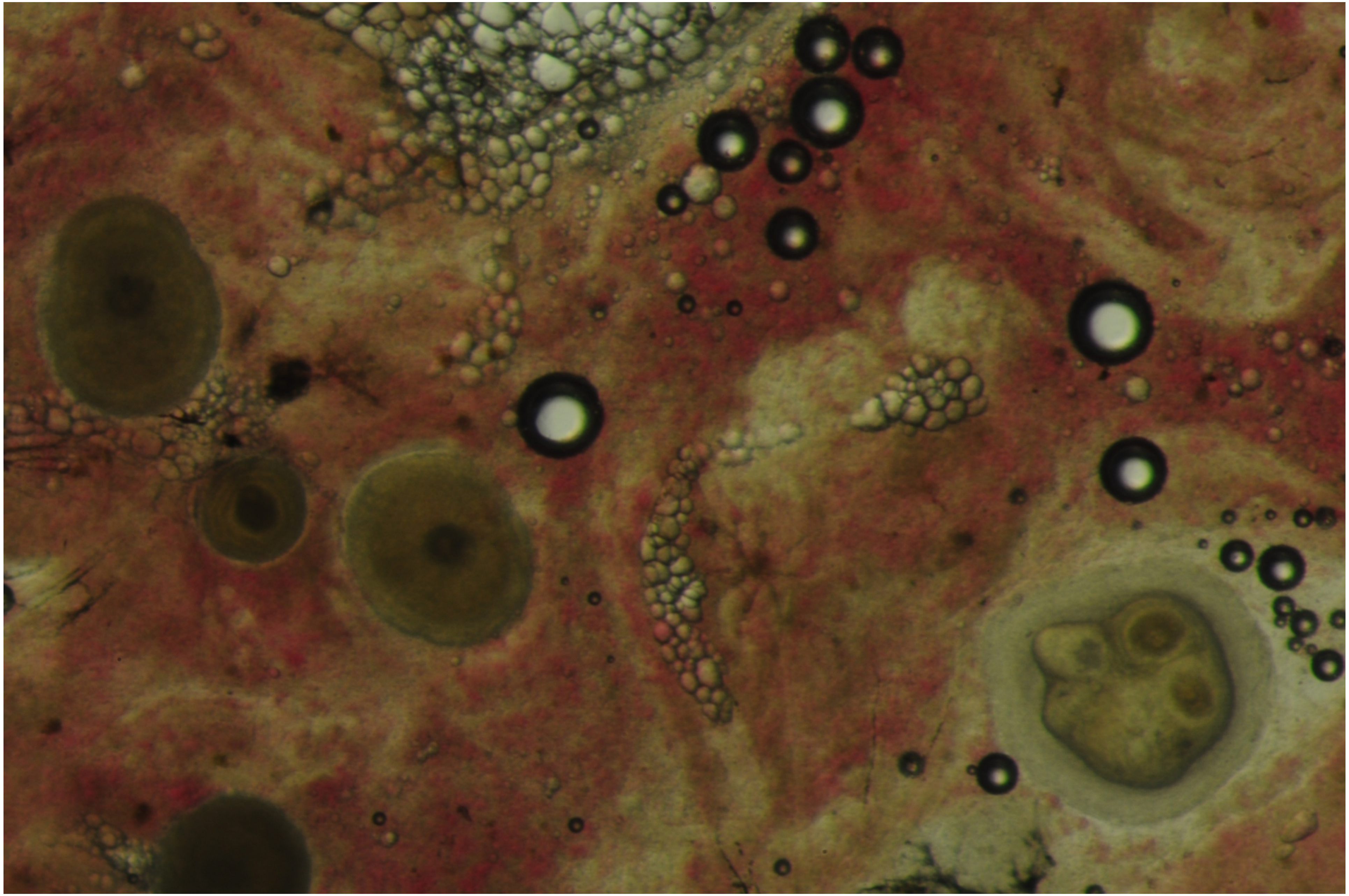

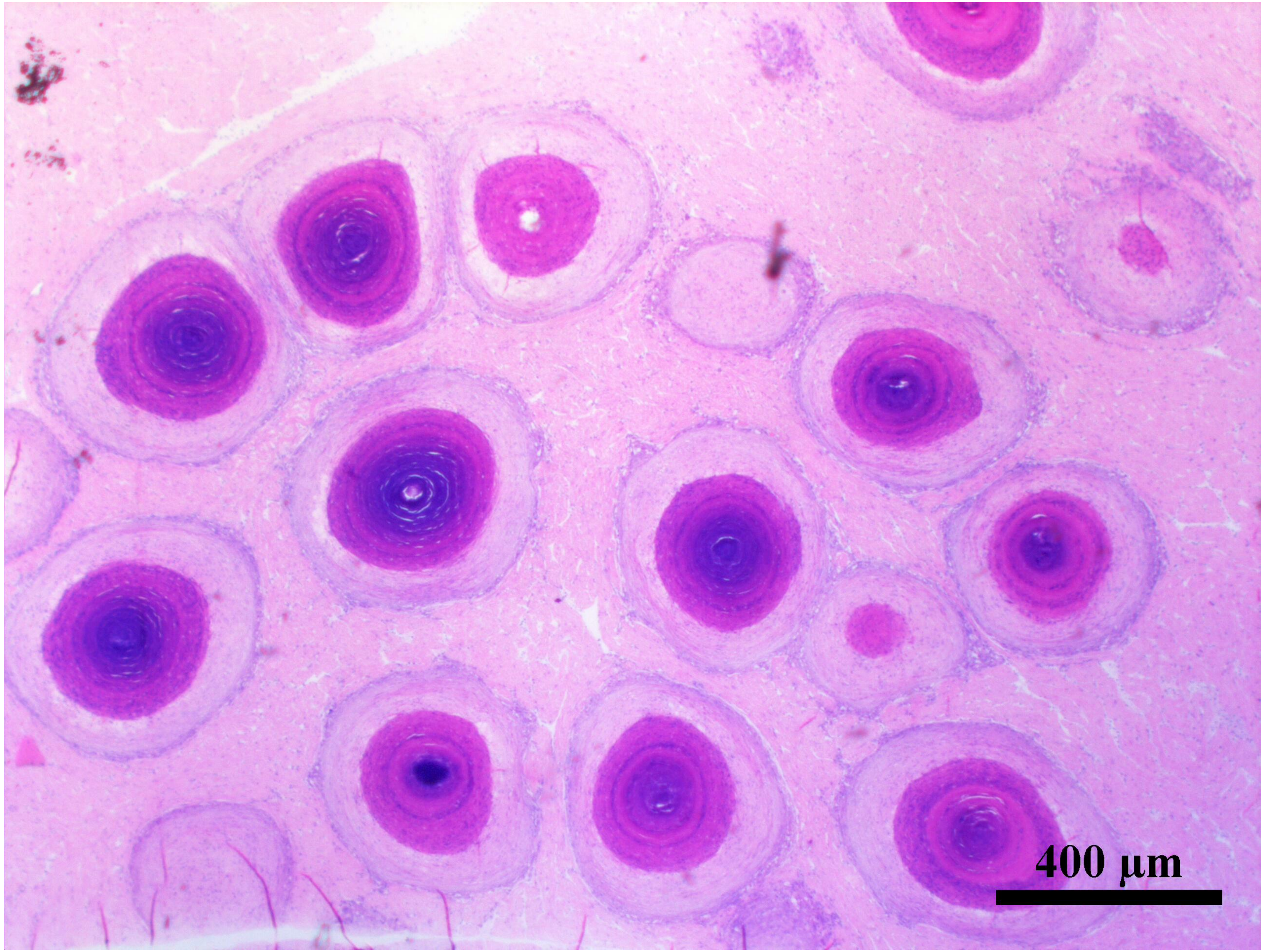
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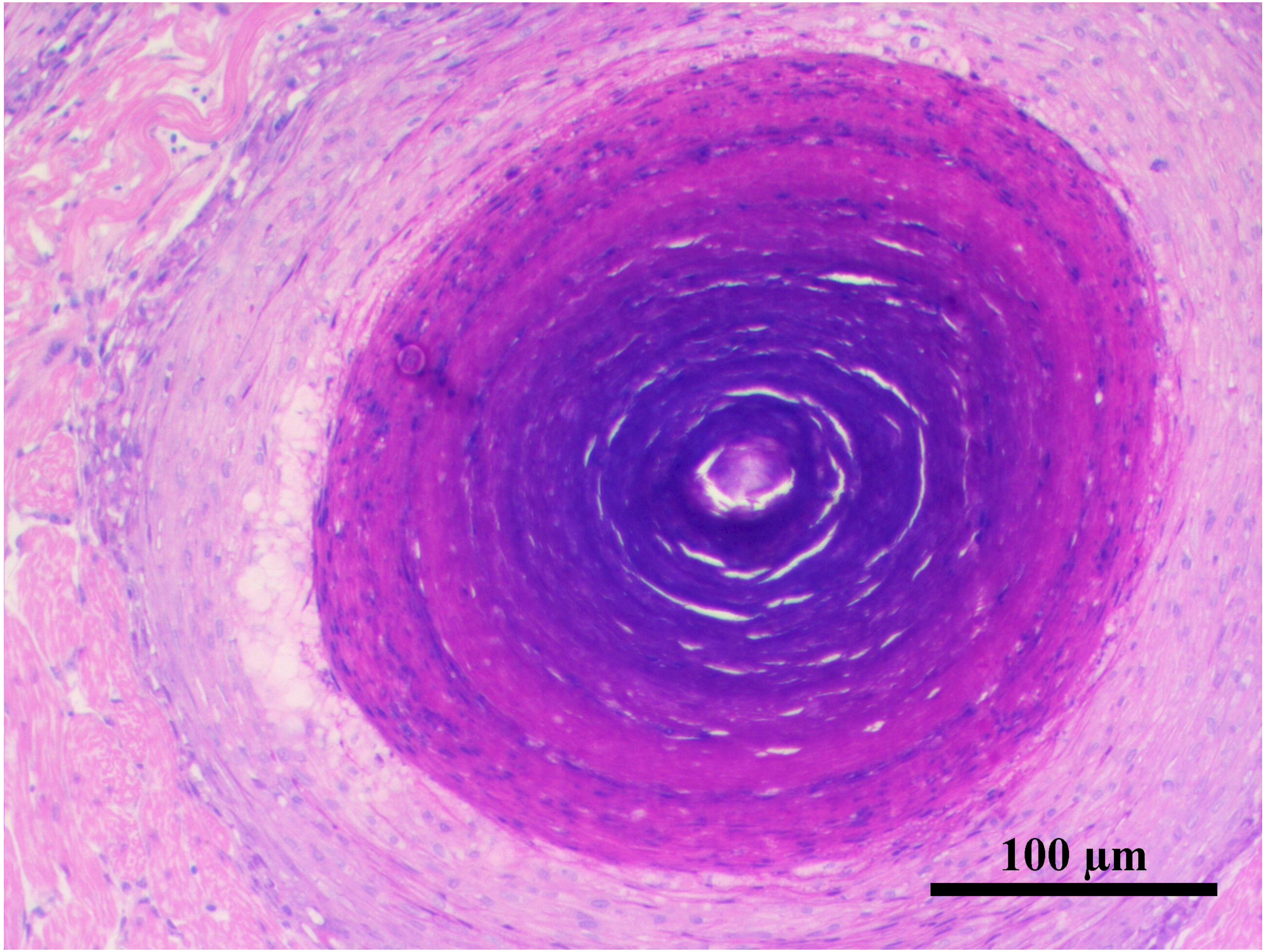
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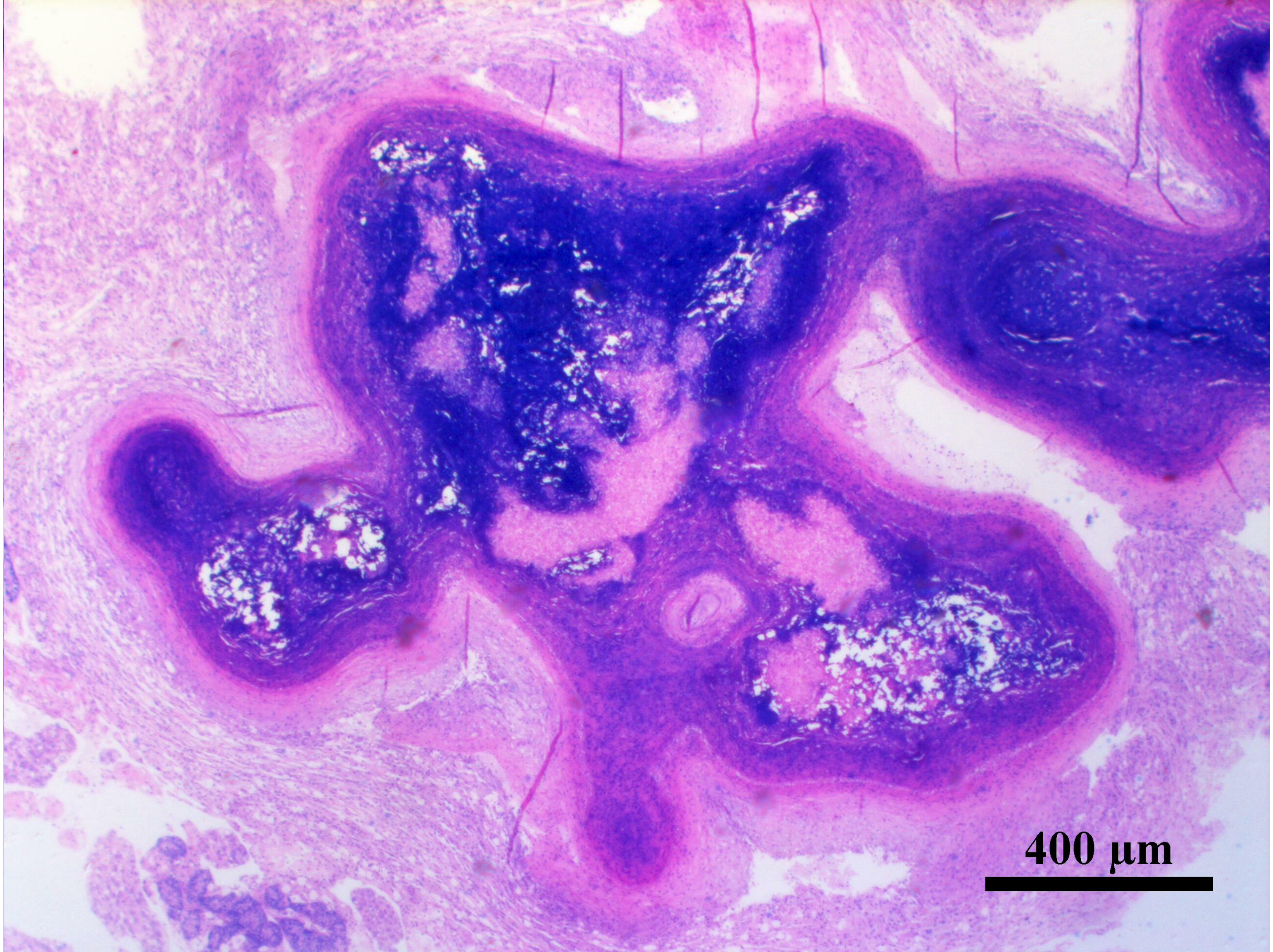
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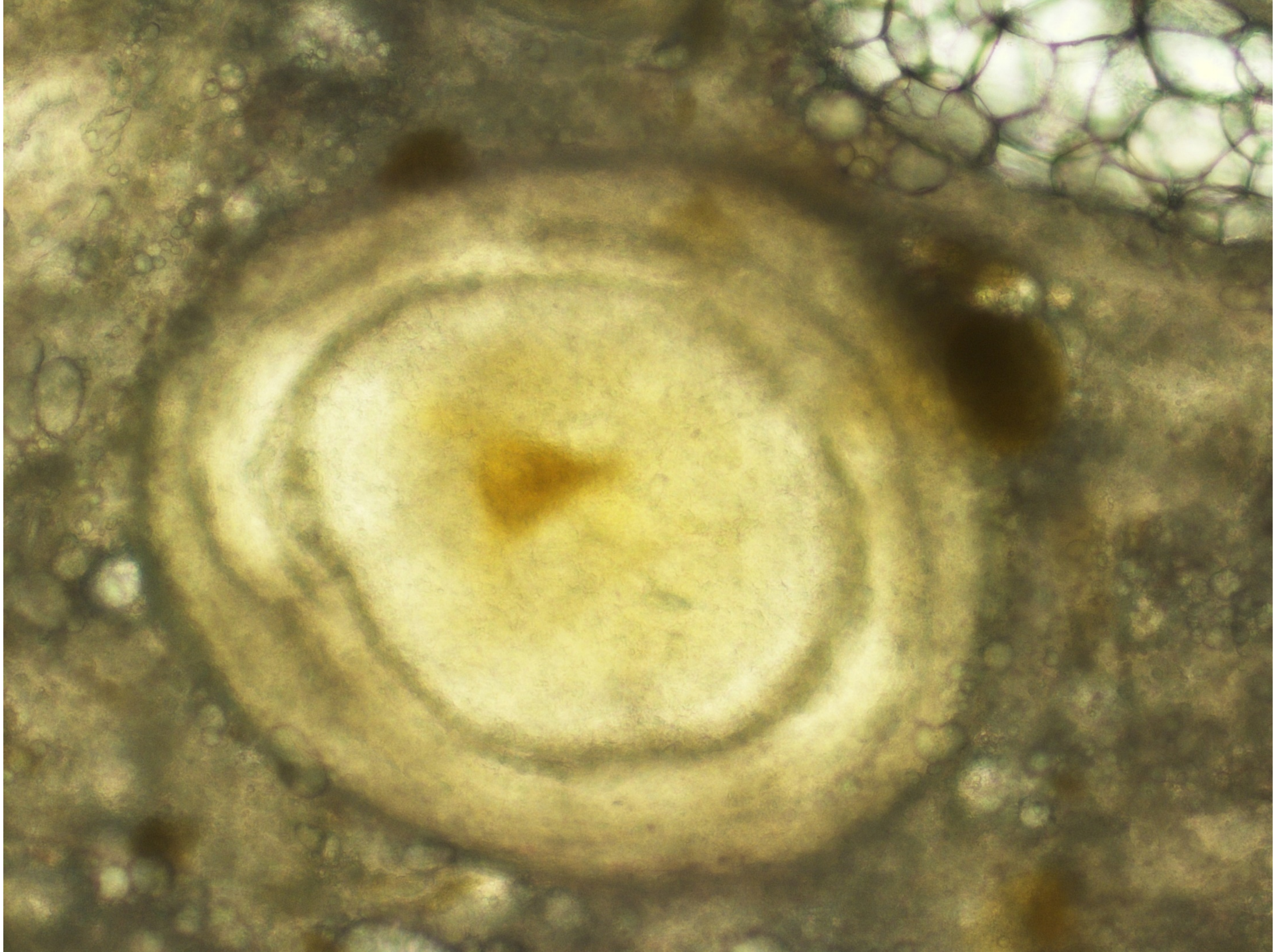
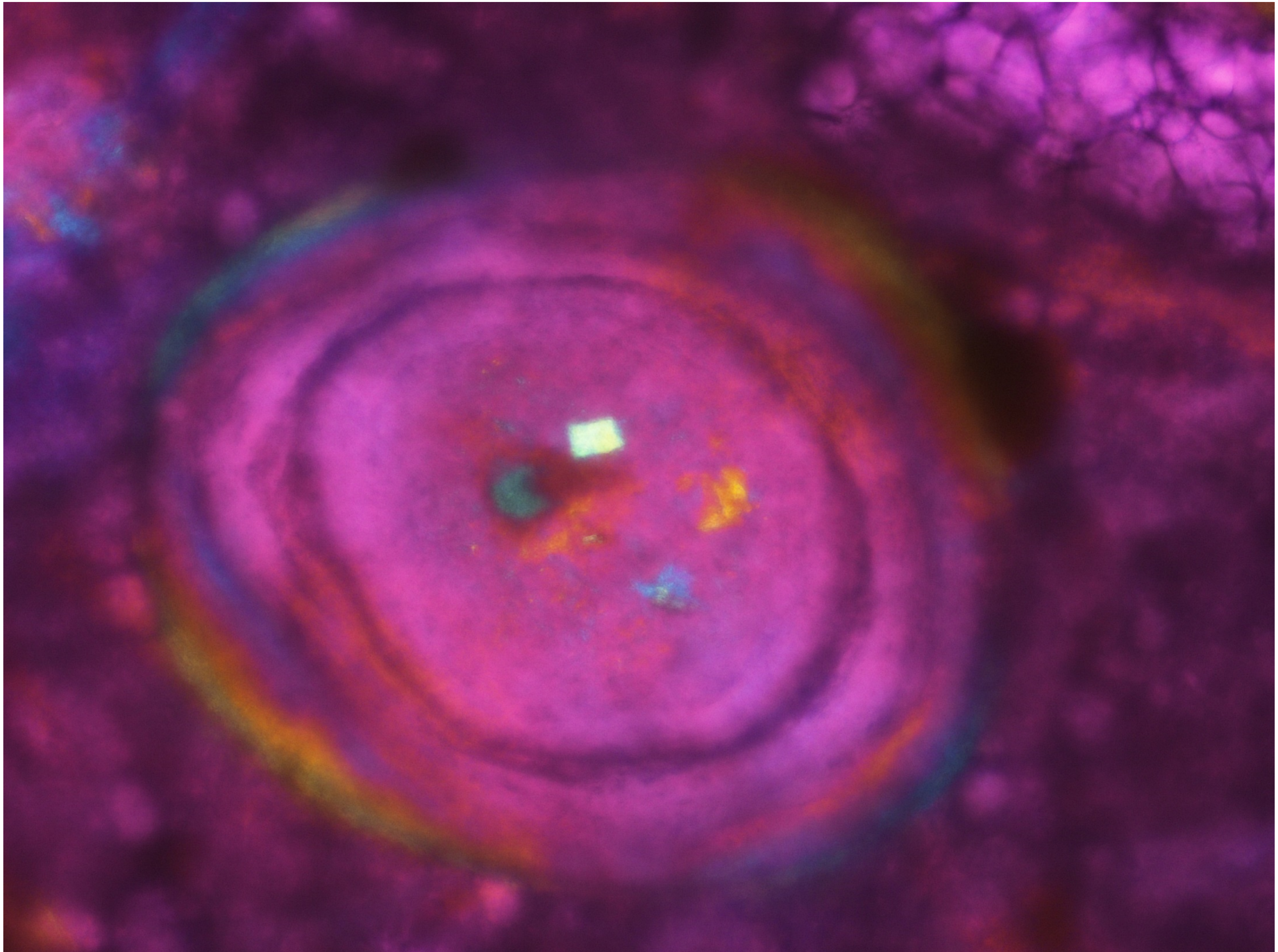




Hypotheses

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…)

Aquaculture Reports 12 (2018) 5–11

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

Ramon Fontanillas

SKRETTING
a Nutreco company

General conclusions

- Vitamin D_3 supplementation did not result in improvement of SG
- High P content in the diet improved the condition
- Replacement with plant protein worsens 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

Recommendations

A combined diet with high percentage of fishmeal (60%) and high dietary content of P (15gkg^{-1}) and antioxidants vitamins E and C.

Further hypothesis for granulomatosis

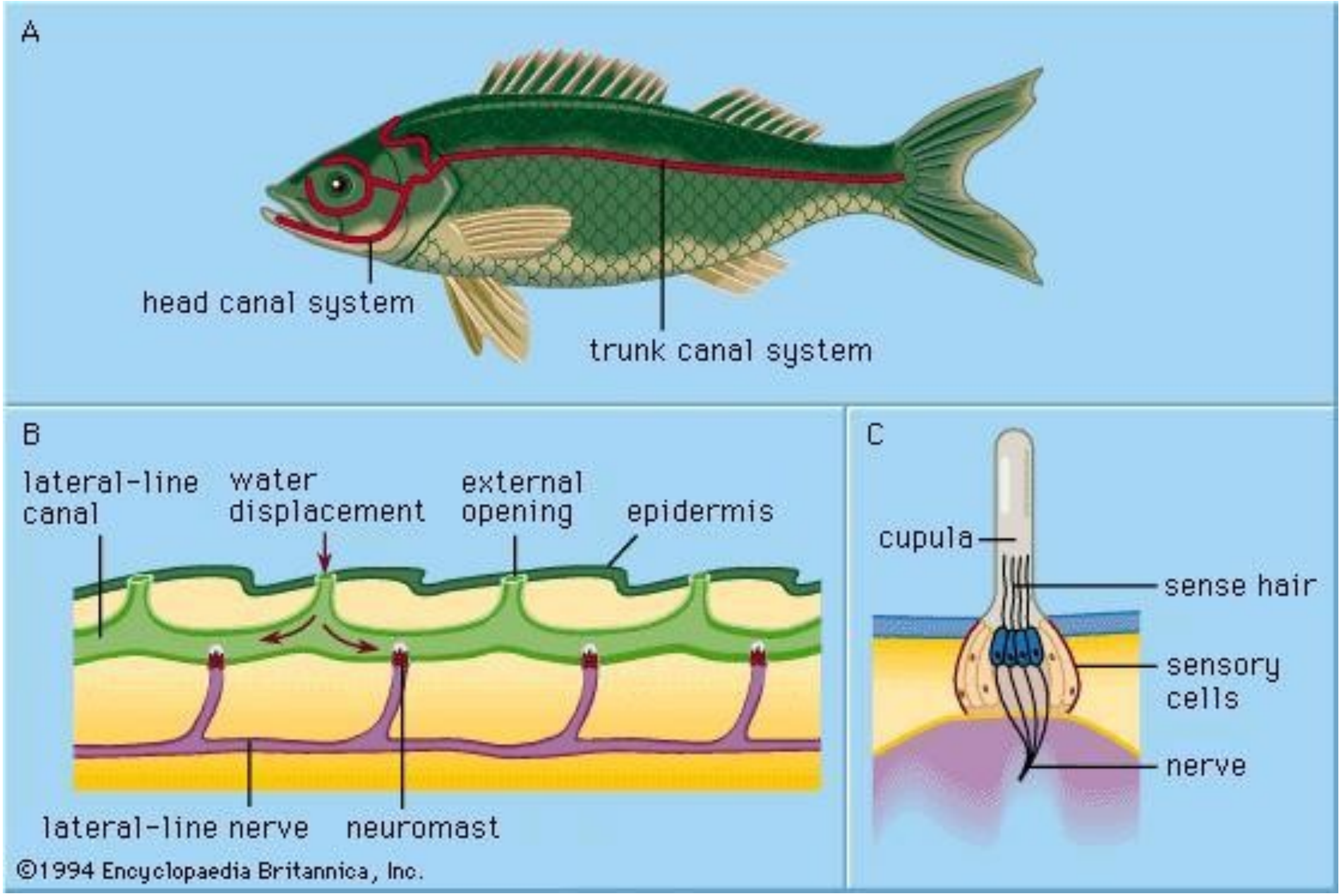
- Other nutritional metabolic factors
- Genetic background (diversity of broodstock)
- Unknown and invisible pathogen

Chronic Ulcerative Dermatopathy: The disease

Murray cod, freshwater

Sharpsnout seabream and meagre in seawater

The lateral line organ

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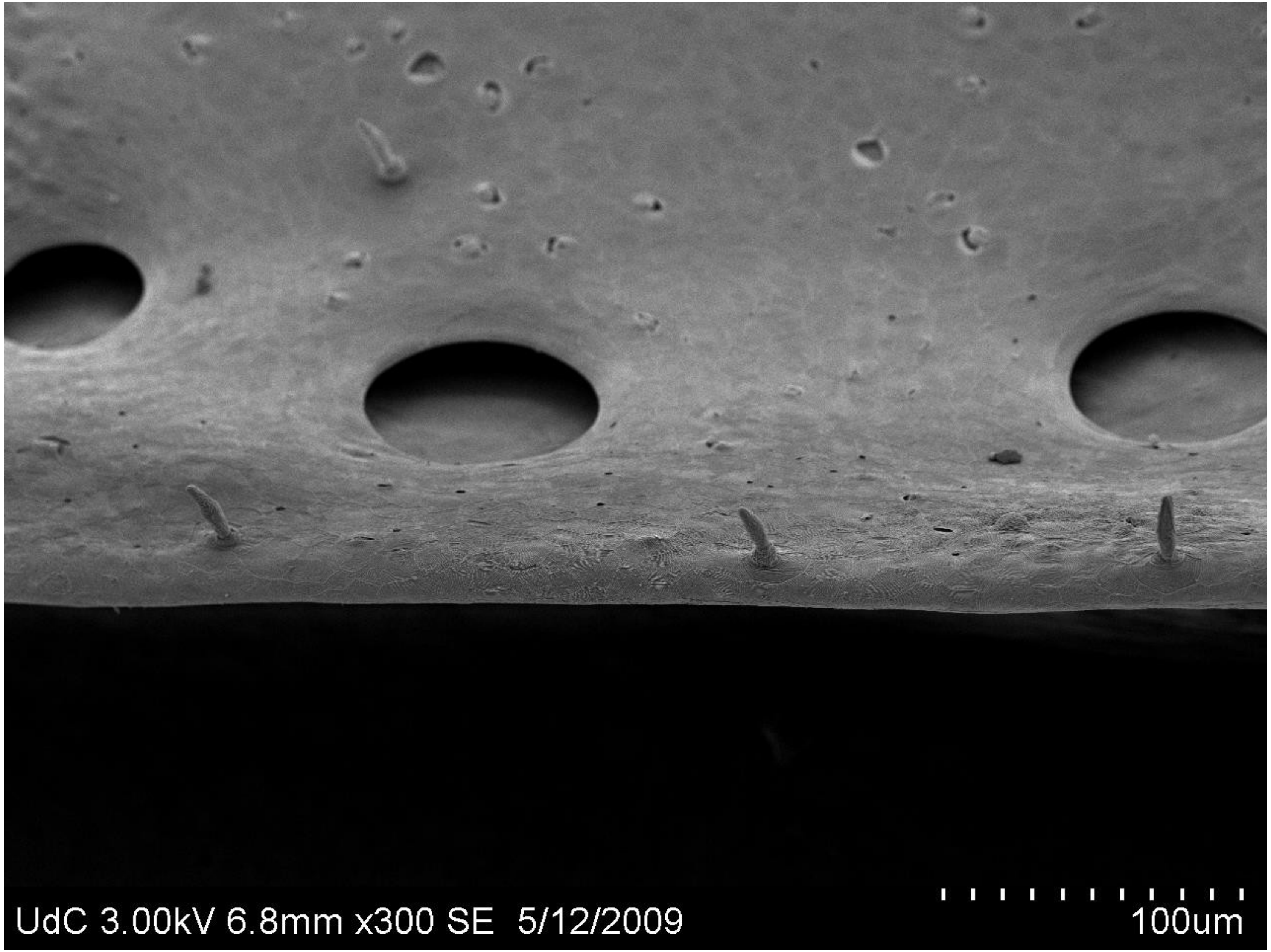
4.00mm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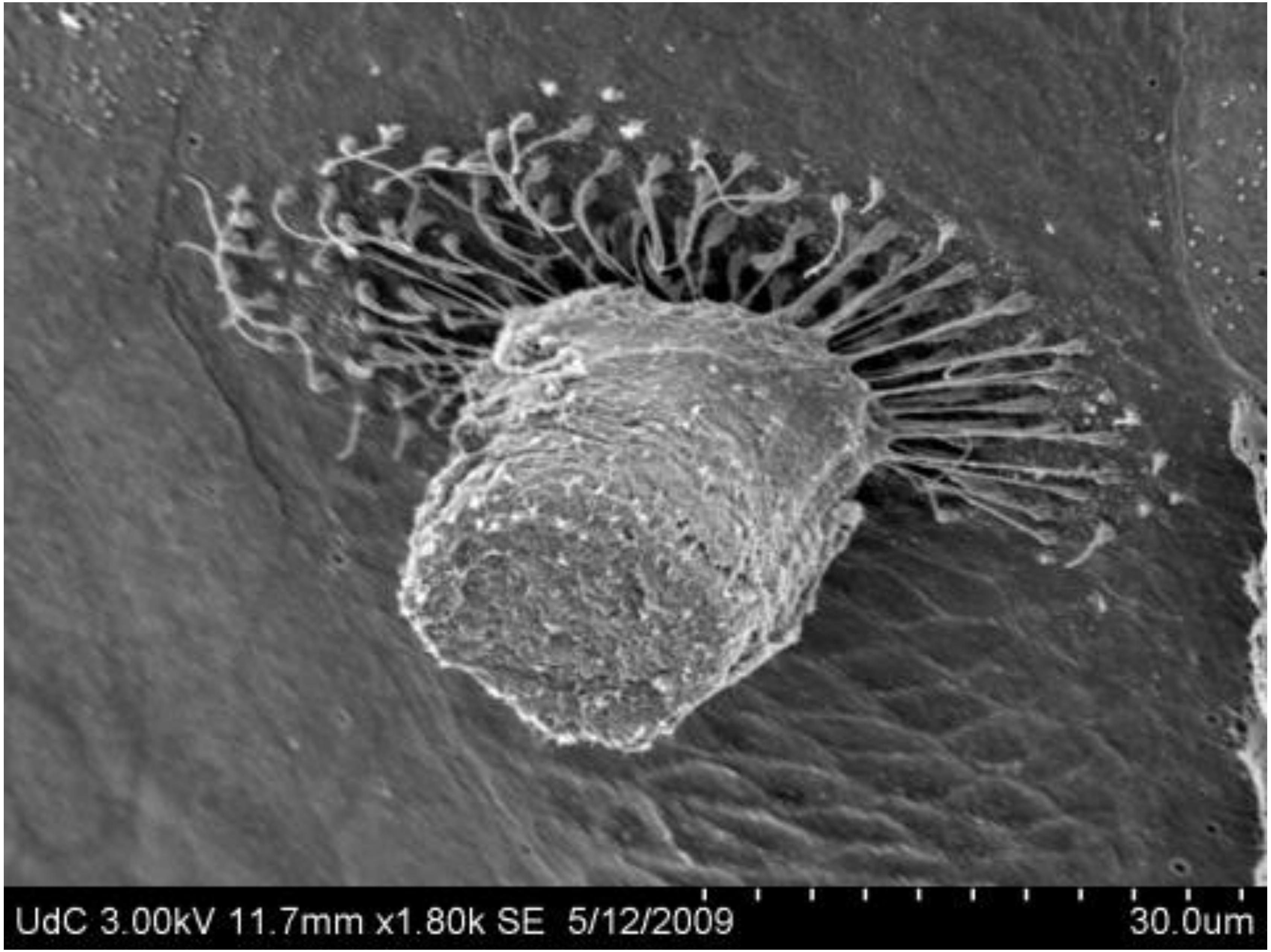
100um

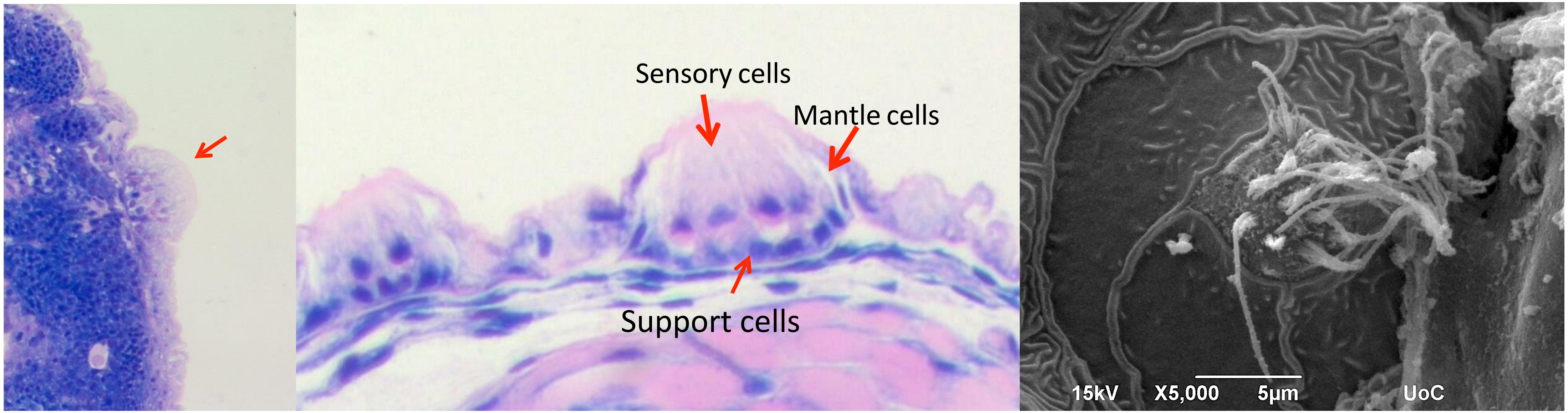

UdC 3.00kV 11.7mm x1.80k SE 5/12/2009
30.0um

Chronic Ulcerative Dermatopathy

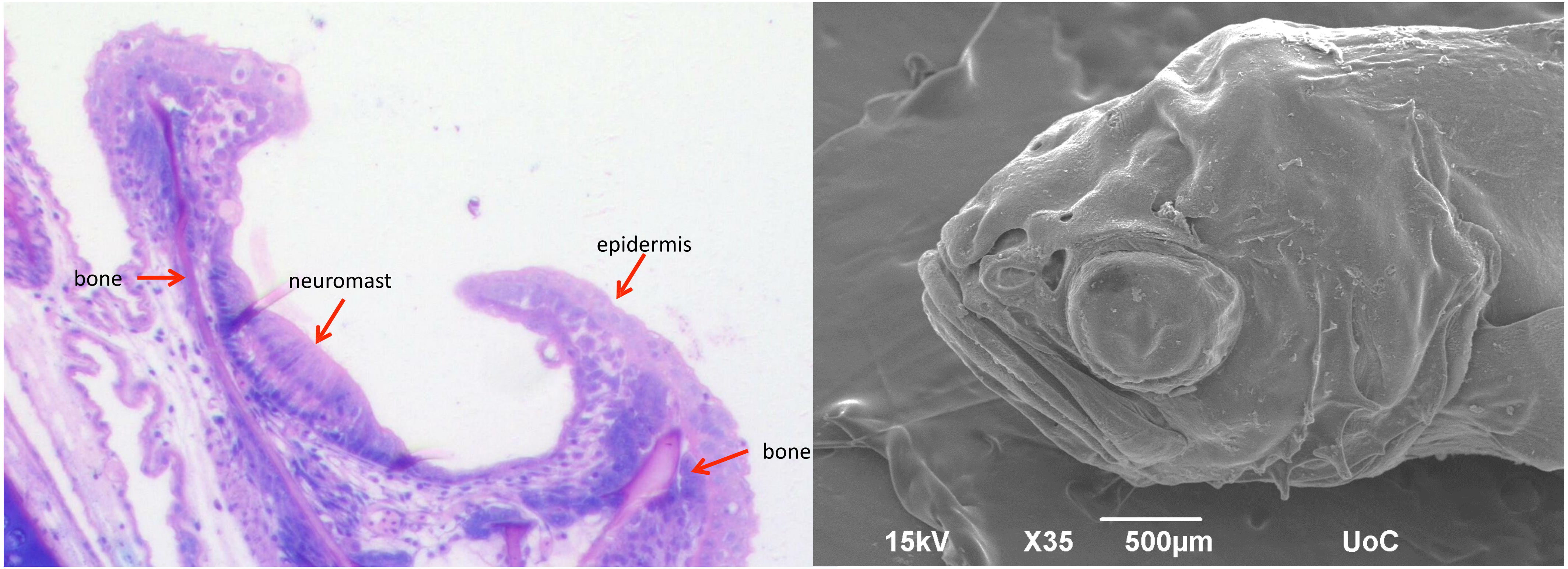
- Directly linked with the use of borehole water
- Unknown aetiology
- Implication of osteoclast/osteoblasts

Ontogeny of the lateral line in meagre

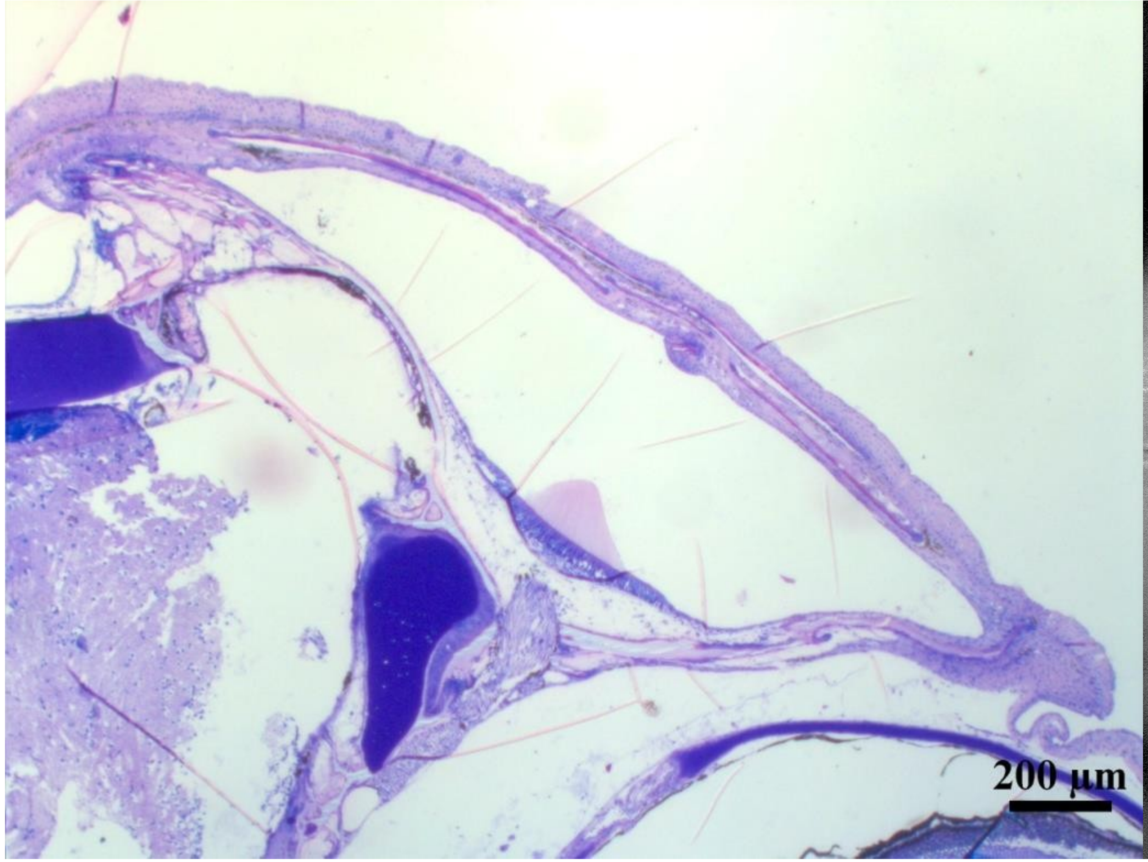
Neuromasts on the skin of meagre larvae (1-3 dph)

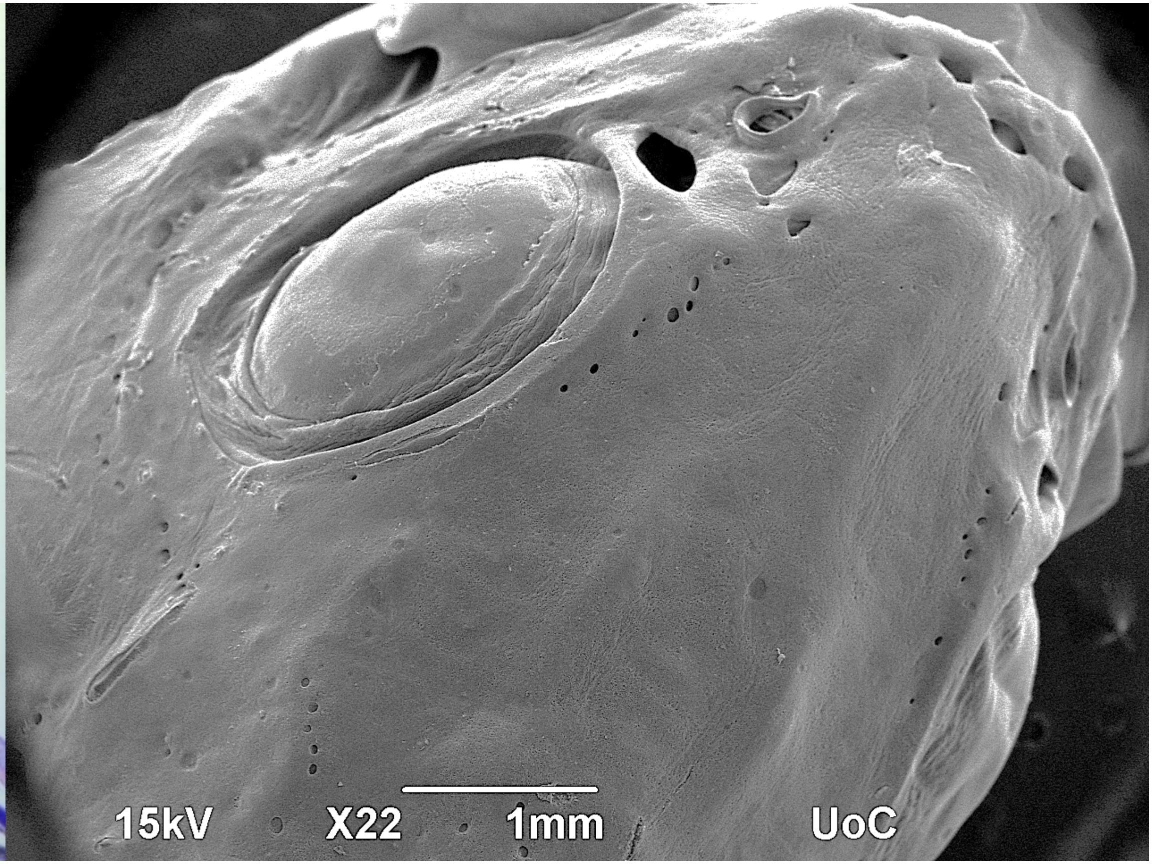


17-25 dph (TL: 5.7-13.7 mm) : formation of the basic lateral line canals of the head (infraorbital, supraorbital and mandibular)



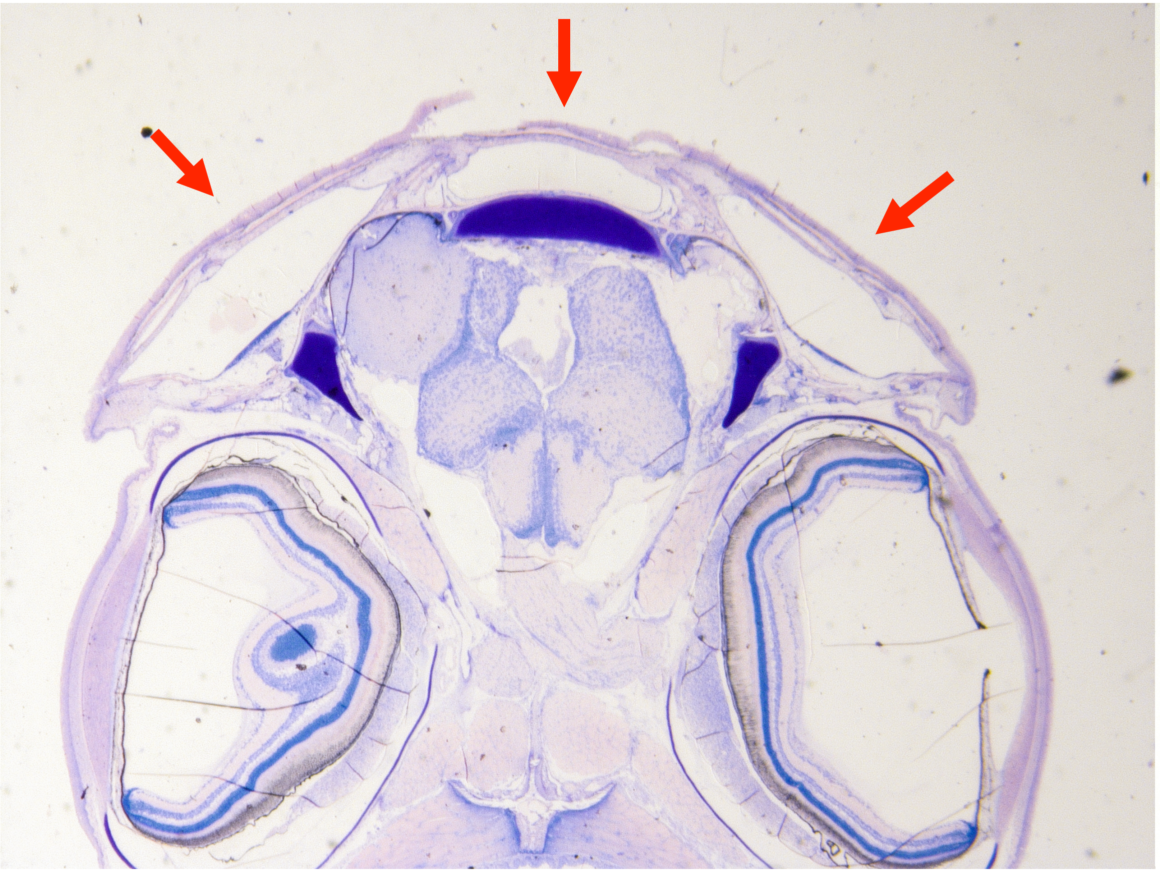
40 dph (TL: 35 mm) : completely developed lateral line



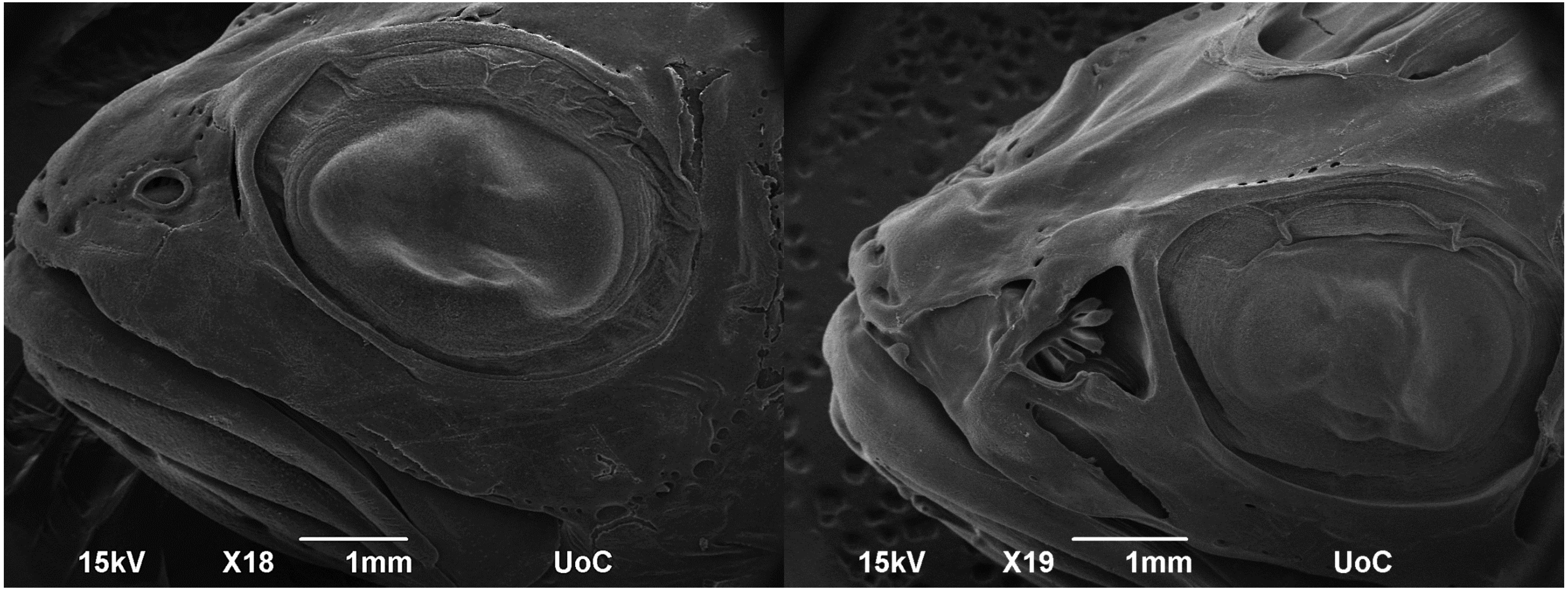


First signs of the disease (46 dph)

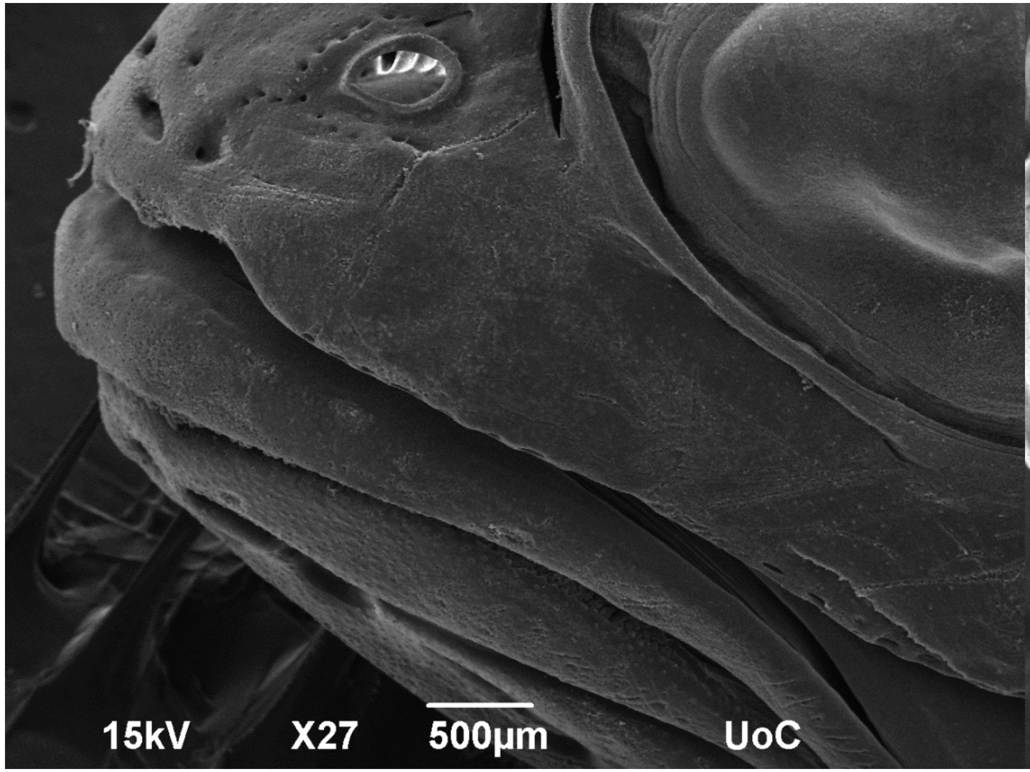
First signs of the disease (46 dph)

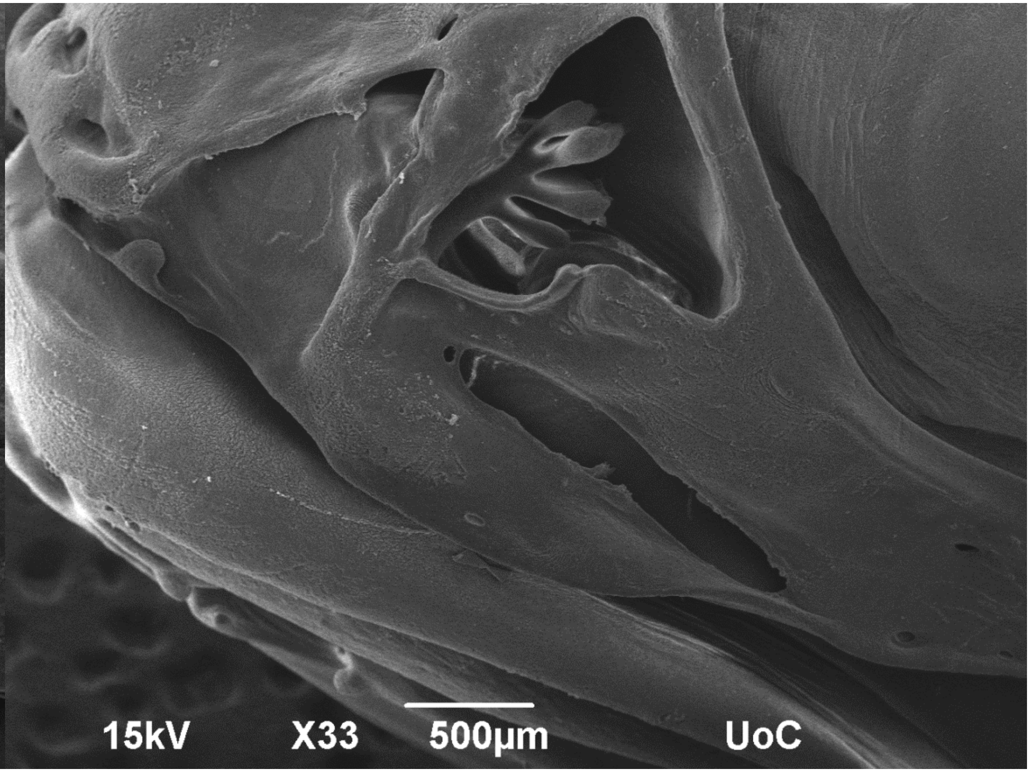


SEM

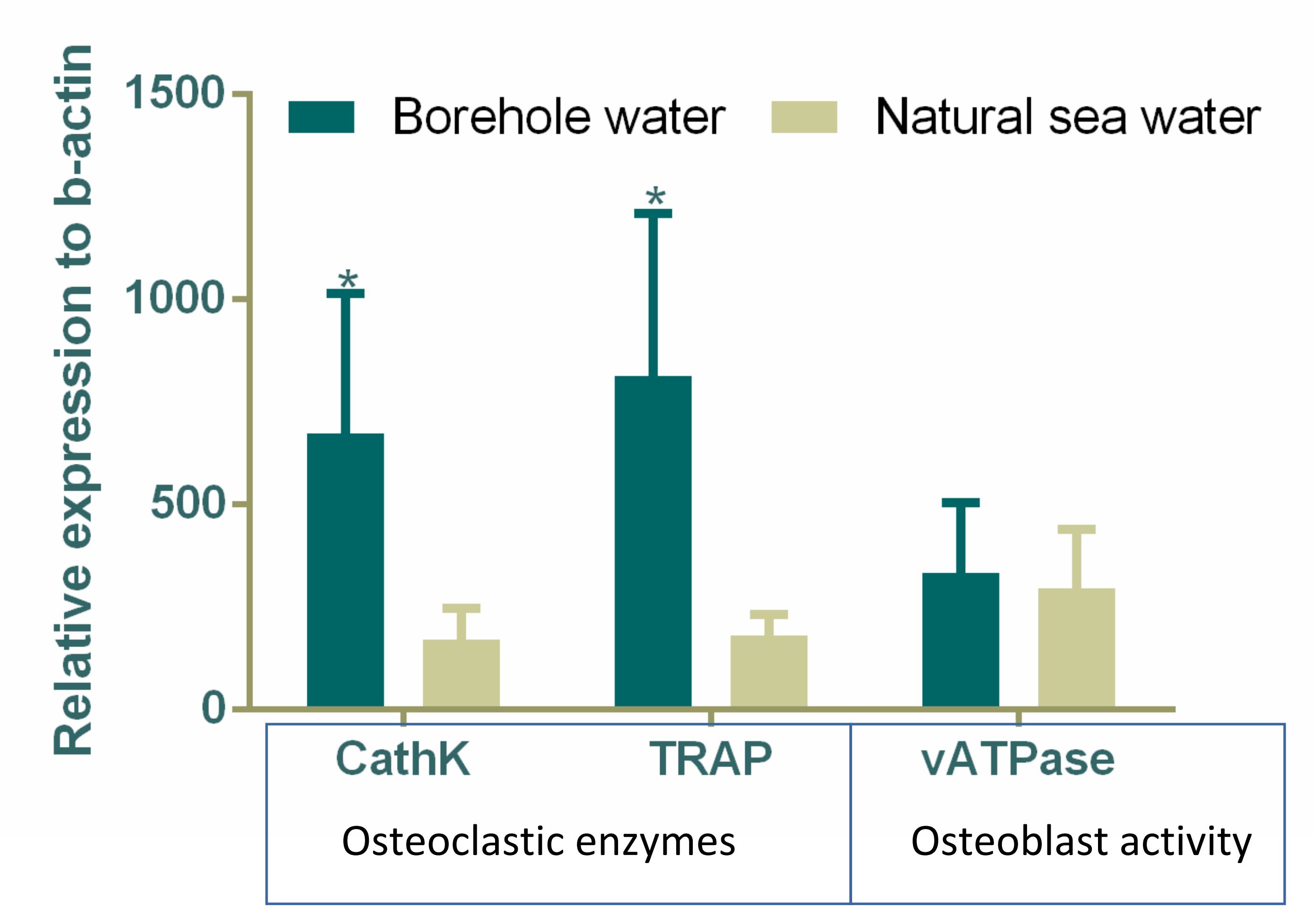


SEM





Gene expression (56dph)



Recovery

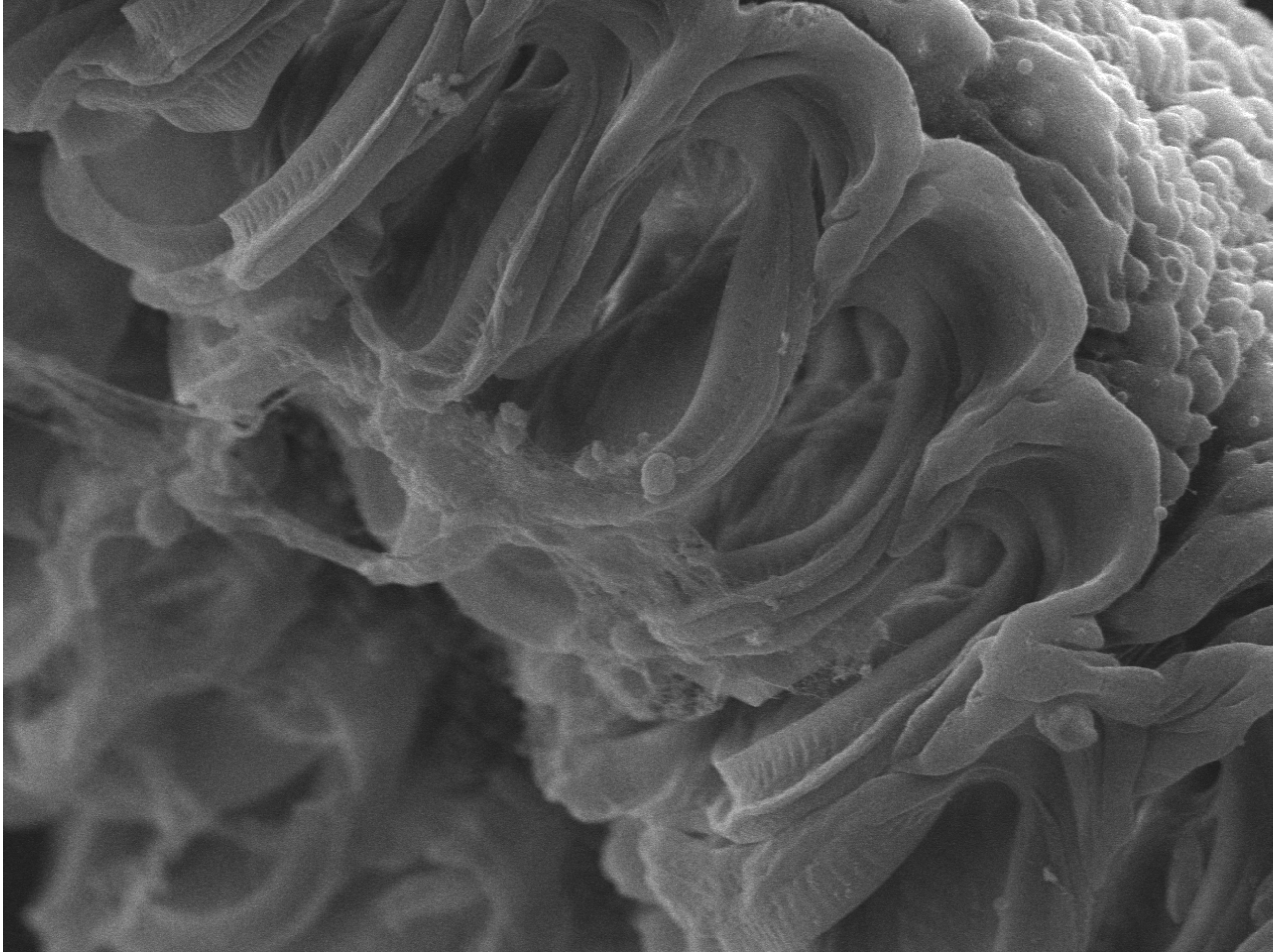
8 month fish: Borehole water

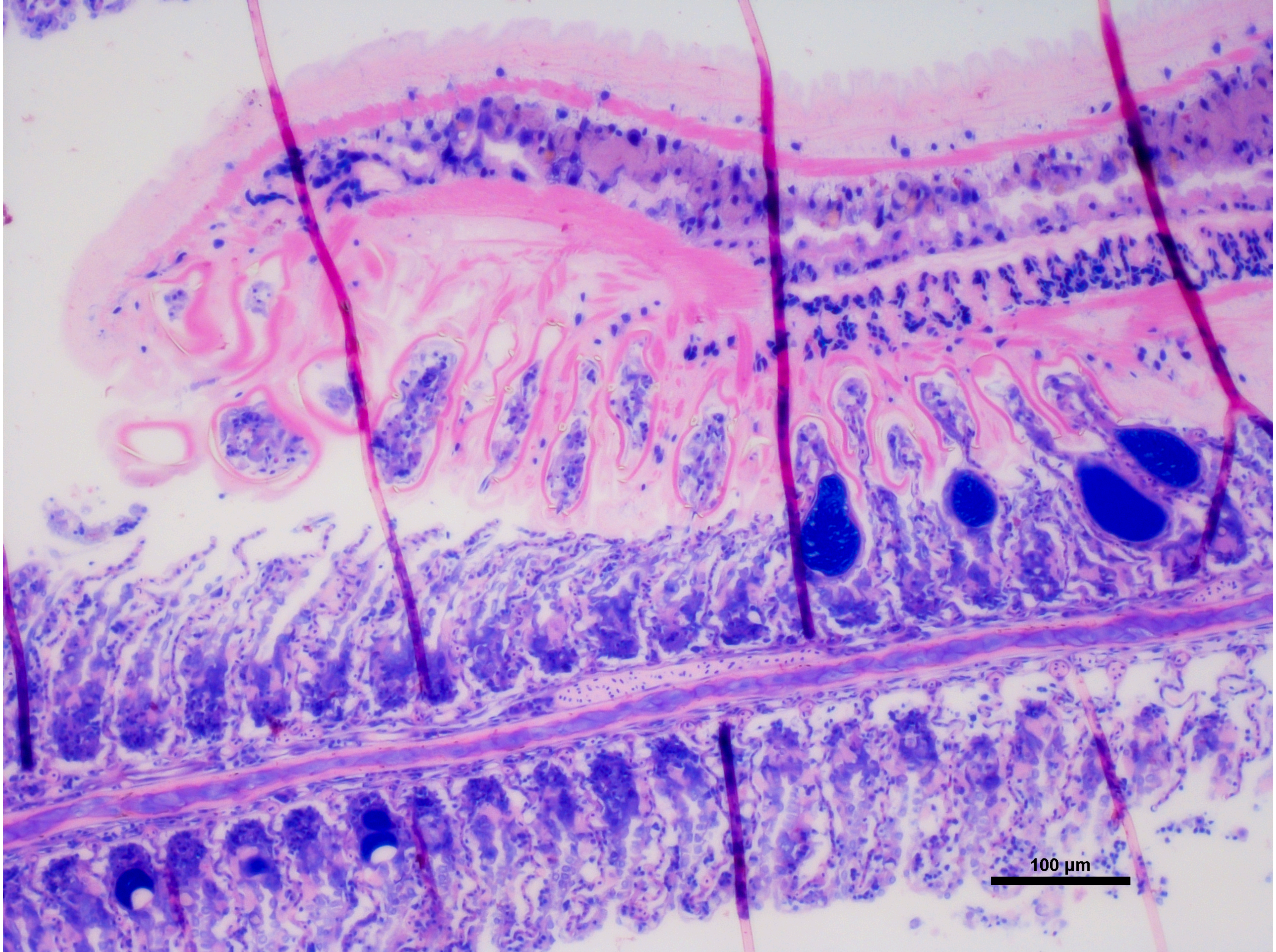
Natural seawater

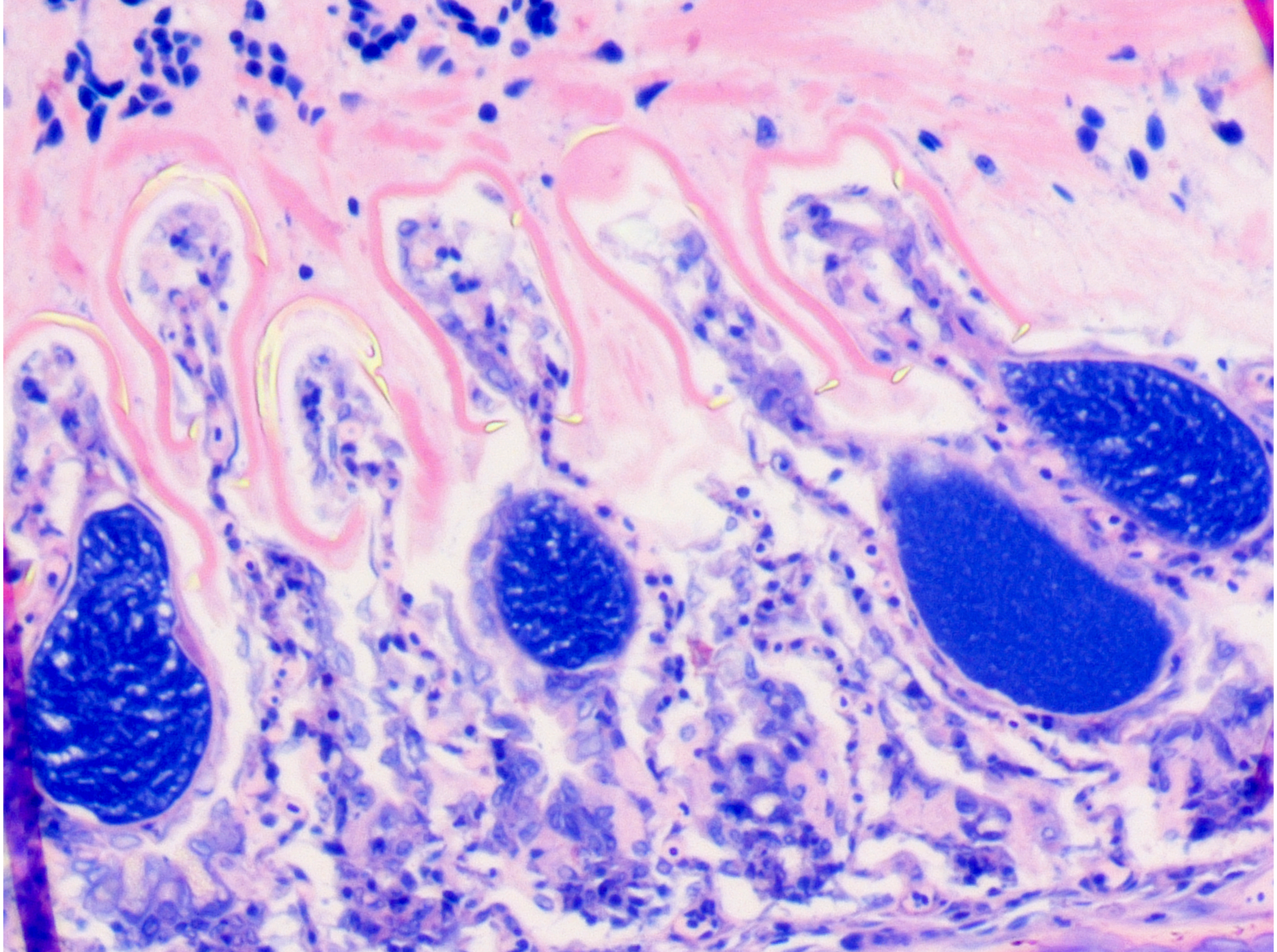
Sciaenacotyle panceri

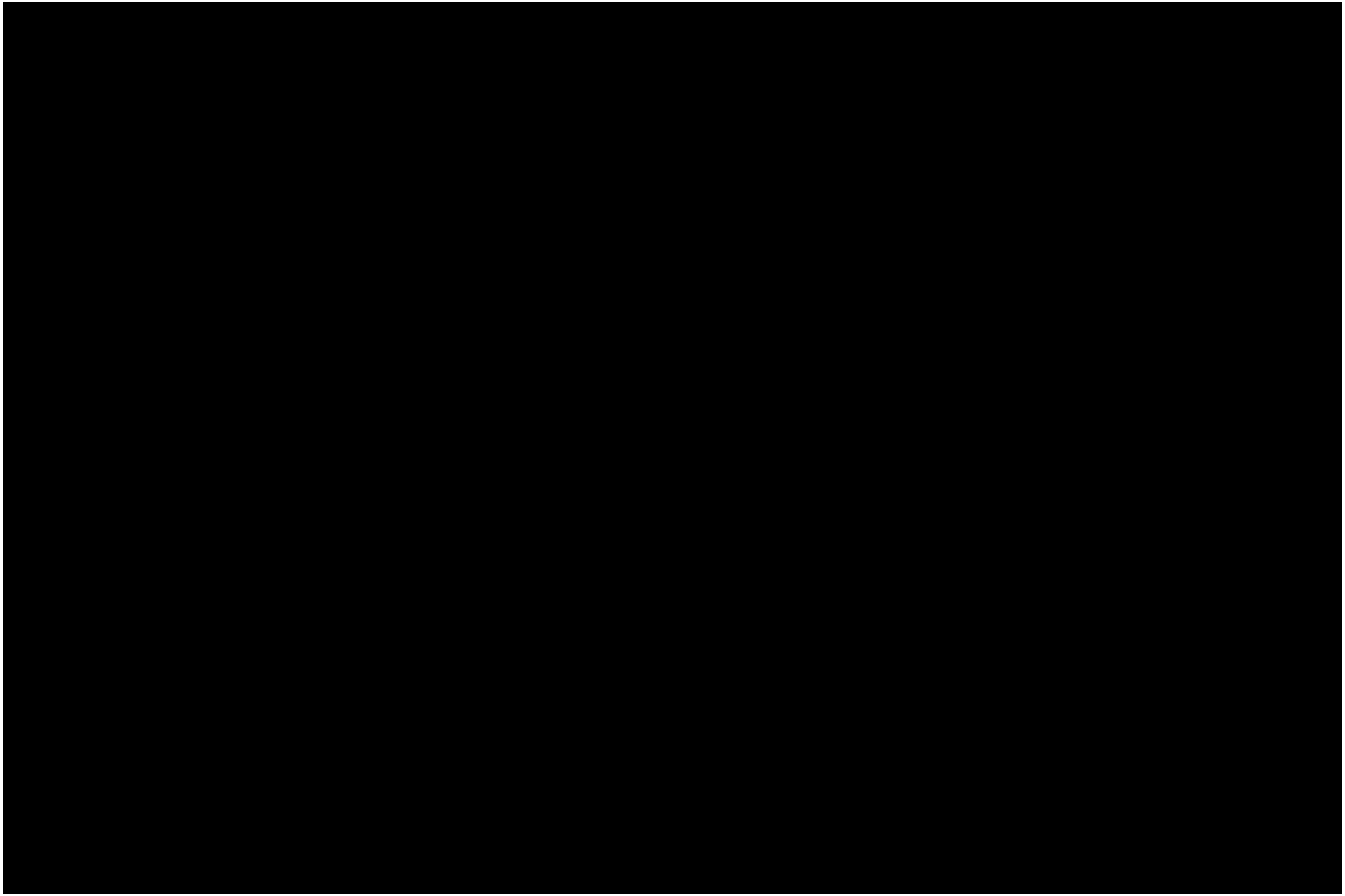
- Gill monogenean (Polyopsithocotylea)
- Highly host-specific
- Direct life-cycle
- Large size (up to 10 mm)
- Blood-feeding
- Can propagate in extremely high numbers
- Cause gill hyperplasia, anemia and eventually death
- Mortalities can be severe esp. in large fish (reaching commercial size)

[redacted]

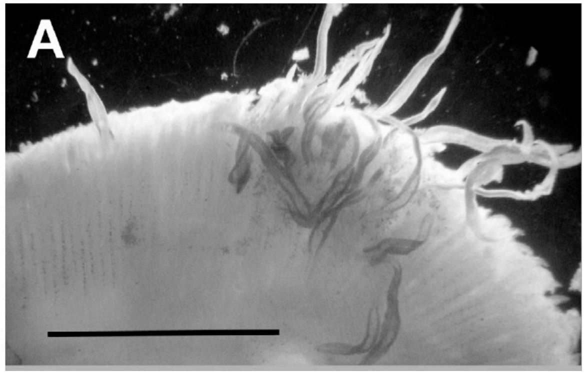


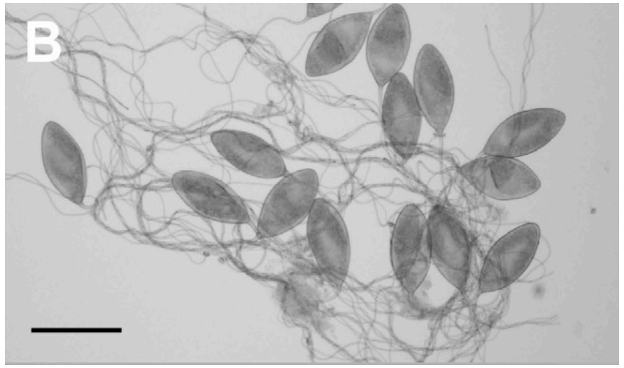

100 µm





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

Epitheliocystis

Infectious disease affecting a wide range of wild and cultured fish

Global distribution

First observed in 1920

Described and named in 1969

Caused by intracellular pathogens (Chlamydia?)

Inclusions in gill and skin epithelium of the fish

Despite efforts, no epitheliocystis-related agent has been isolated in culture until today

Epitheliocystis and HCMR

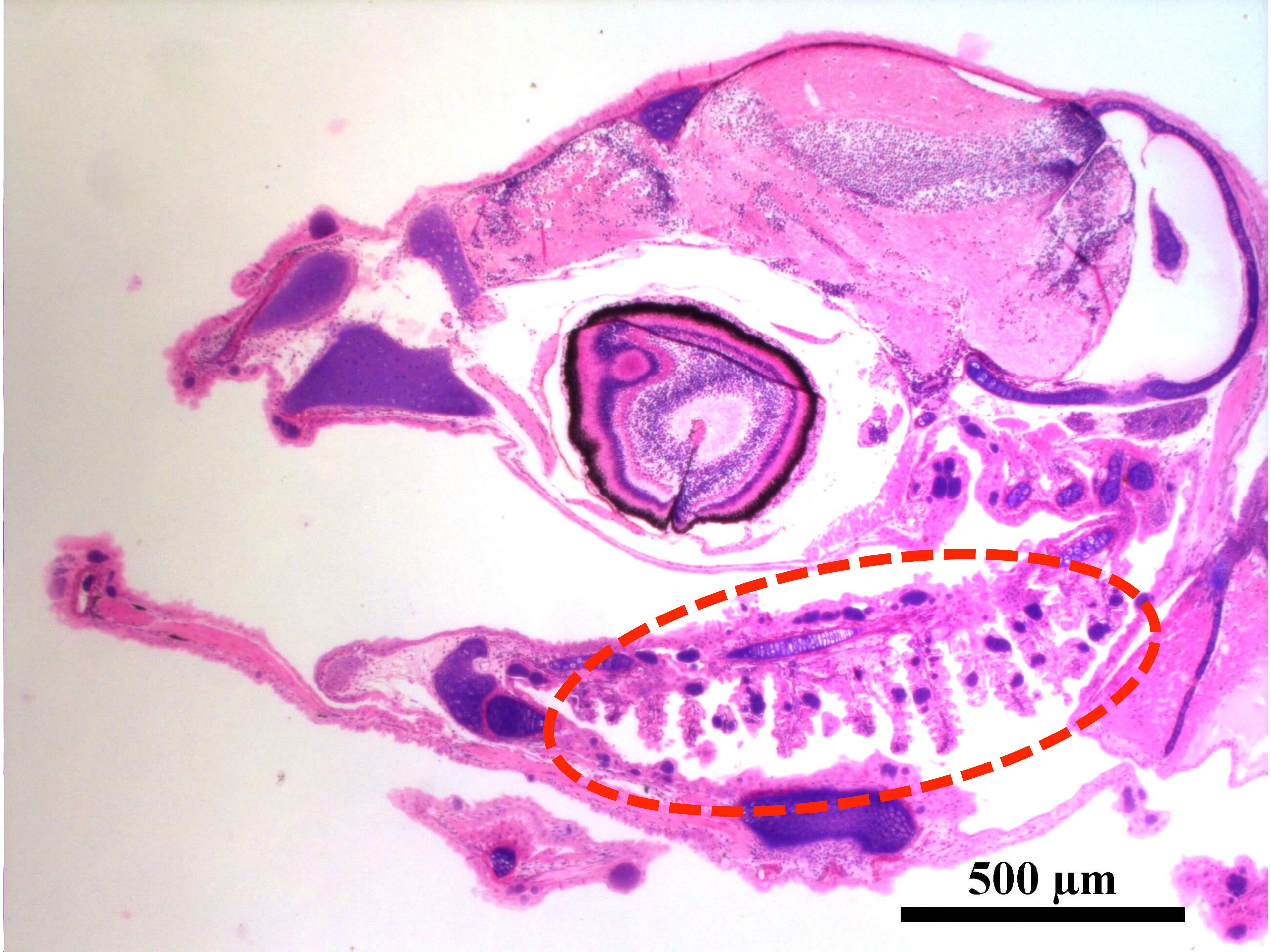
100% mortality in greater amberjack (*Seriola dumerili*) larvae

80% mortality in common dentex (*Dentex dentex*) larvae

>50% mortality in sharpsnout seabream (*Diplodus puntazzo*)

In some cases 100% mortality overnight

hcmr
ΕΛΚΕΘΕ


500 µm

Aquaculture, 90 (1990) 197–207
Elsevier Science Publishers B.V., Amsterdam

Epitheliocystis disease in the cultured amberjack, *Seriola dumerili* Risso (Carangidae)

S. Crespo, A. Grau and F. Padrós
Laboratori de Biologia, Facultat de Veterinària, U.A.B., Bellaterra, Barcelona, Spain

(Accepted 12 March 1990)

Aquaculture, 95 (1991) 1–6
Elsevier Science Publishers B.V., Amsterdam

Epitheliocystis in the wild and cultured amberjack, *Seriola dumerili* Risso: ultrastructural observations

A. Grau and S. Crespo
Laboratori de Biologia, Facultat de Veterinària, U.A.B., Bellaterra, Barcelona, Spain

(Accepted 2 October 1990)

JOURNAL OF THE WORLD AQUACULTURE SOCIETY

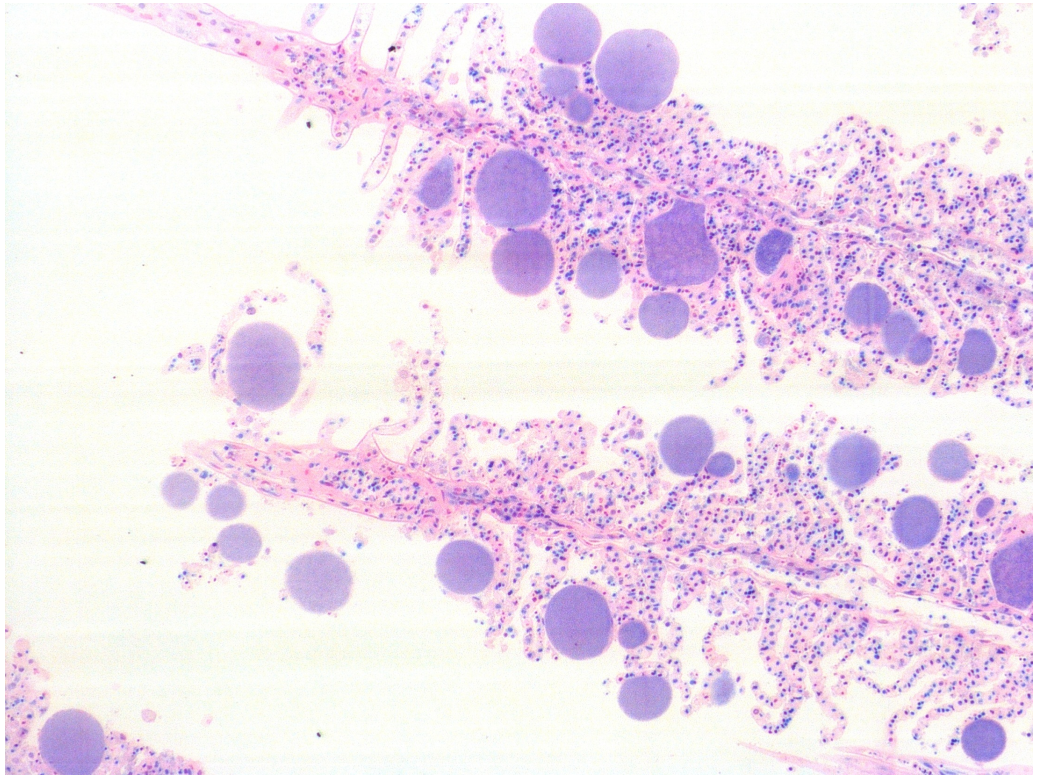
Vol. 27, No. 2
June, 1996

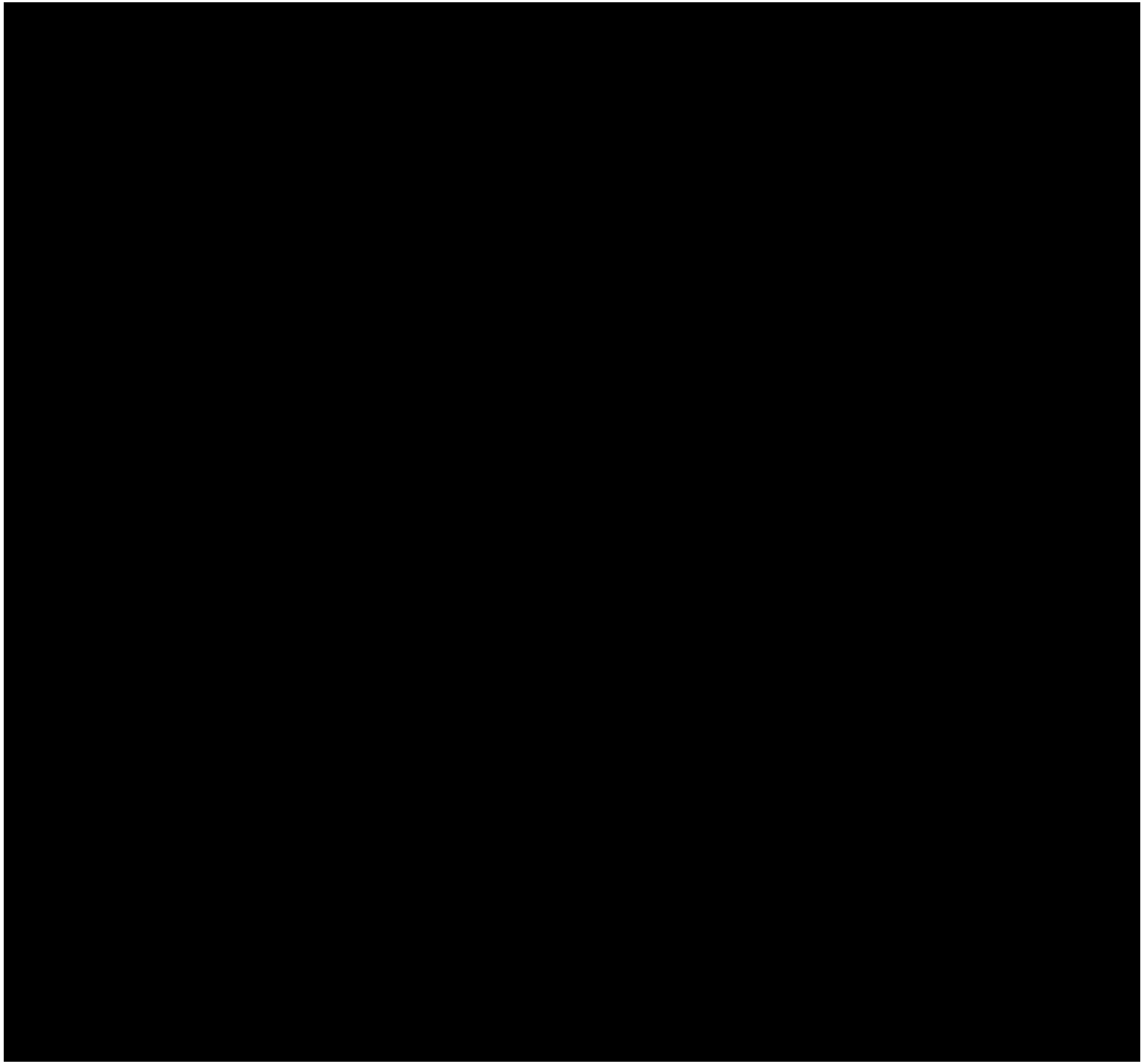
Epitheliocystis Disease in Cultured Yellowtail *Seriola mazatlana* in Ecuador

Arietta Venizelos[1]
AIC—Aquaculture International Consulting, 228 Seaview Drive, Key Biscayne, Florida 33149 USA

Daniel D. Benetti
Rosenstiel School of Marine and Atmospheric Science, Division of Marine Biology and Fisheries, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149 USA

Epitheliocystis





hcmr
ΕΛΚΕΘΕ

Novel findings regarding epitheliocystis

Caused by various unrelated bacteria

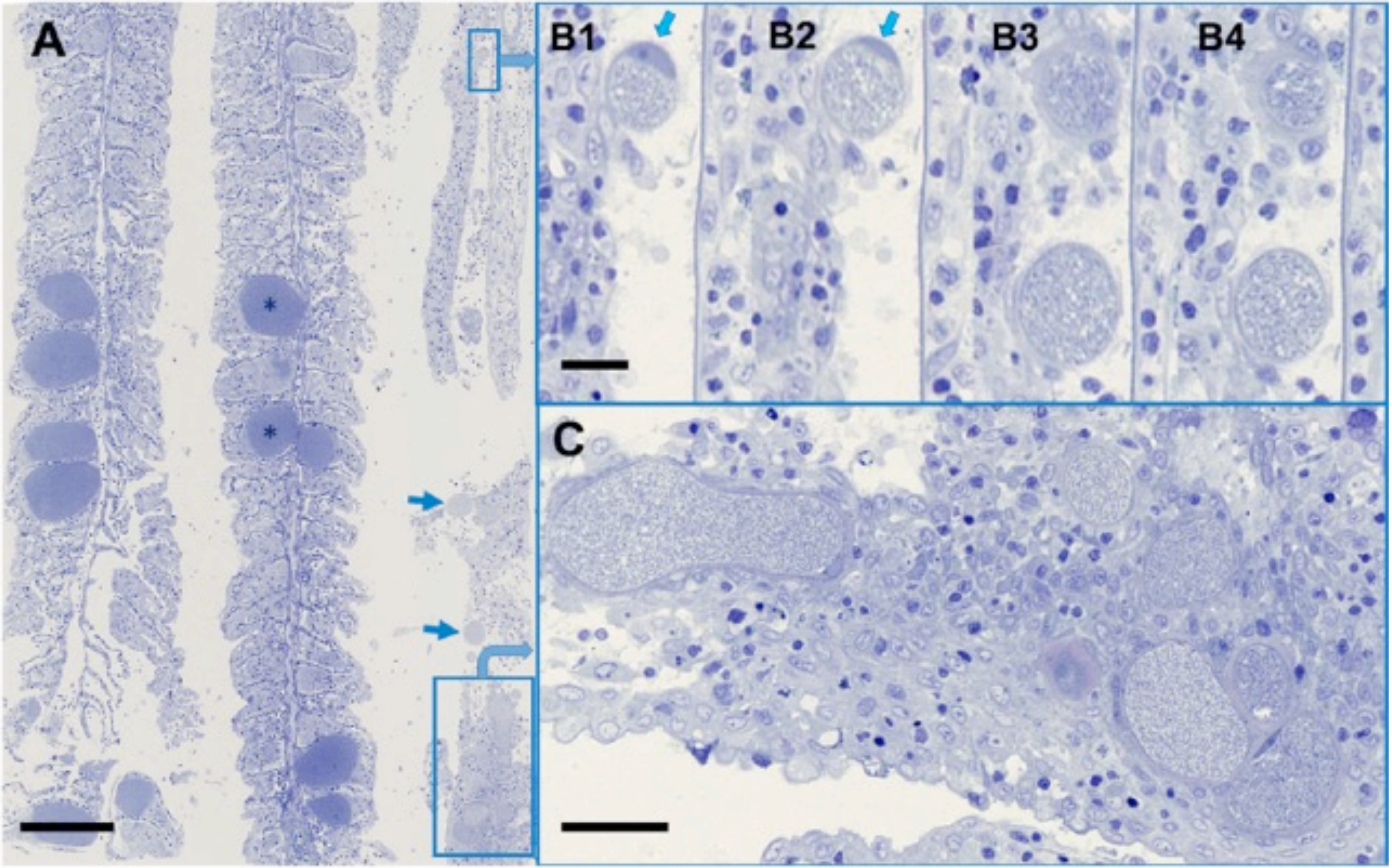
Ca. Endozoicomonas cretensis

Ca. Ichthyocystis sparus

Ca. Ichthyocystis hellenicum

…and various Chlamydia

In most of the times more than one species co-infect the same fish (same filament)



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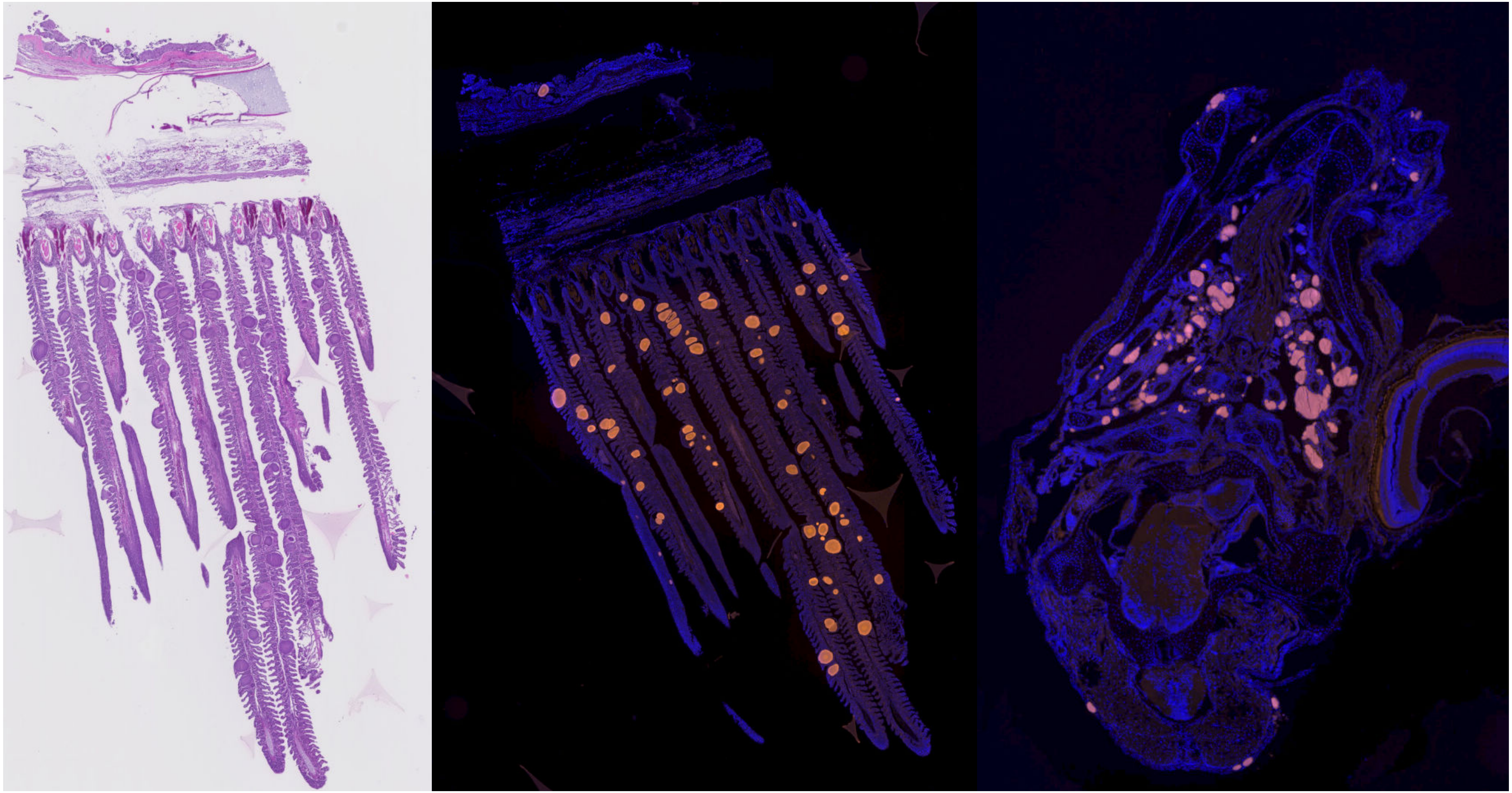
ΕΛΚΕΘΕ

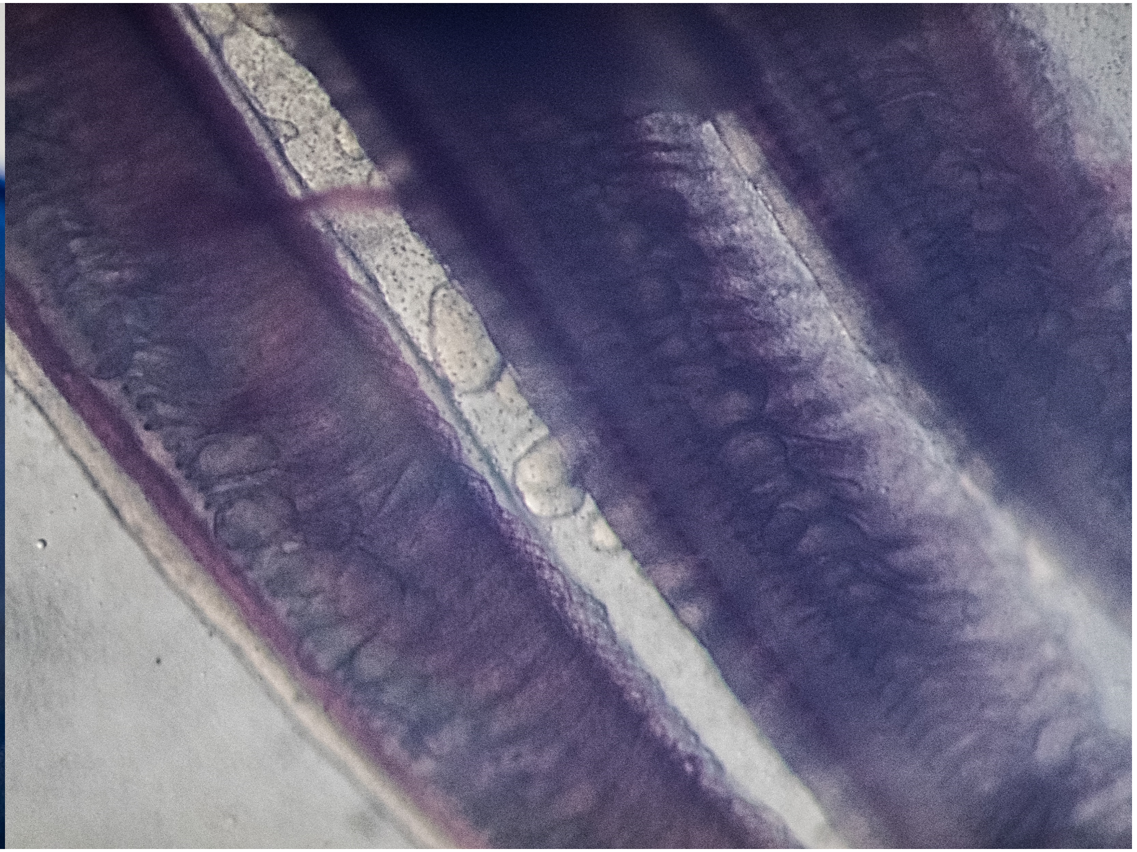


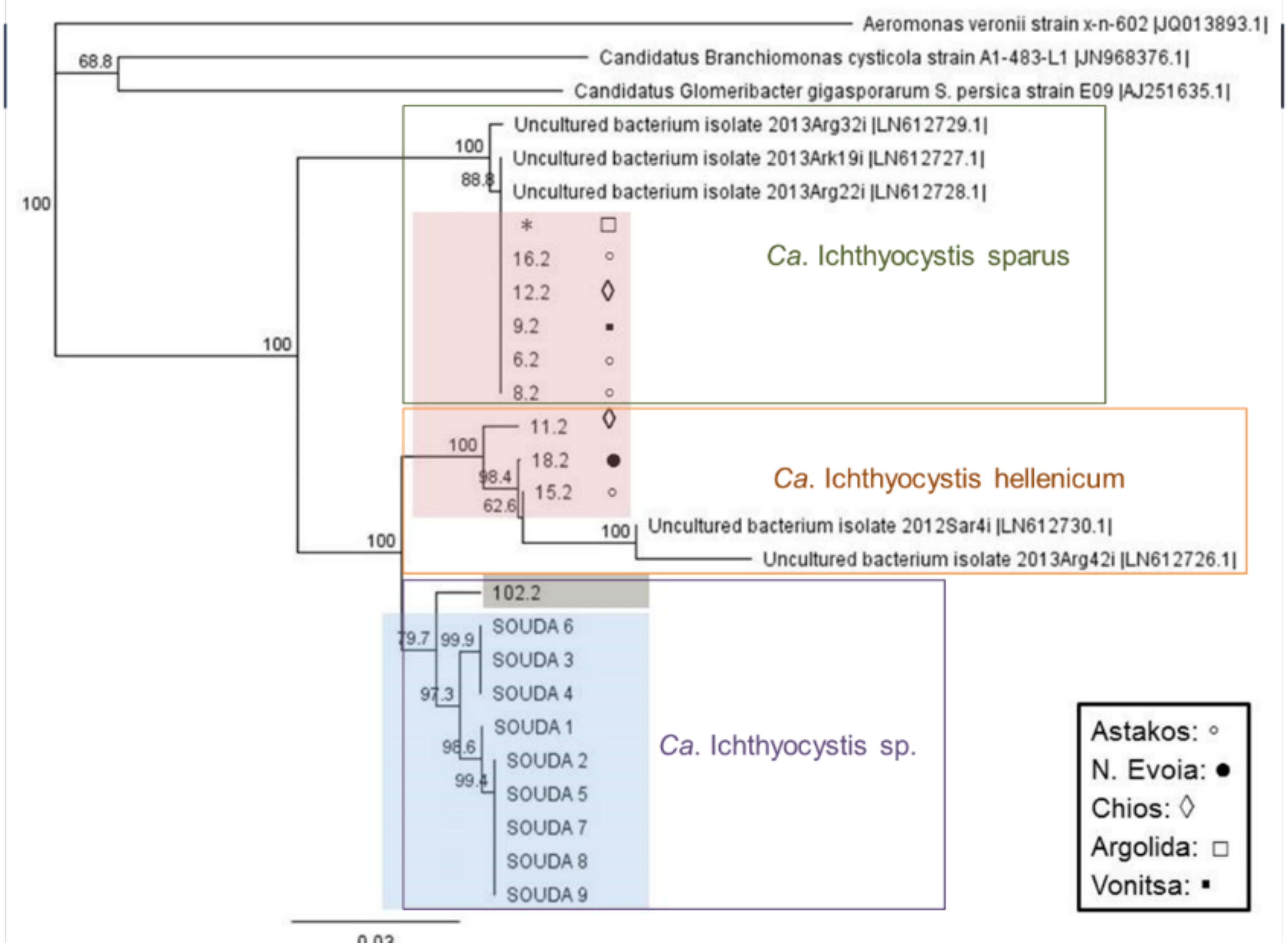
Toolbox for proper diagnosis

- PCR
- qPCR
- histology
- Fluorescence In Situ Hybridization (FISH)
- Electron Microscopy
- µCT

Prof. Lloyd Vaughan
University of Zurich
Functional Genomic Centre
HCMR

Epitheliocystis in greater amberjack in Greece is caused by a novel Ichthyocystis species





Aeromonas veronii strain x-n-602 |JQ013893.1|
Candidatus Branchiomonas cysticola strain A1-483-L1 |JN968376.1|
Candidatus Glomeribacter gigasporarum S. persica strain E09 |AJ251635.1|
Uncultured bacterium isolate 2013Arg32i |LN612729.1|
Uncultured bacterium isolate 2013Ark19i |LN612727.1|
Uncultured bacterium isolate 2013Arg22i |LN612728.1|
68.8
100
88.8
100
100
16.2
12.2
9.2
6.2
8.2
Ca. Ichthyocystis sparus
11.2
18.2
15.2
100
98.4
62.6
100
100
Ca. Ichthyocystis hellenicum
Uncultured bacterium isolate 2012Sar4i |LN612730.1|
Uncultured bacterium isolate 2013Arg42i |LN612726.1|
102.2
SOUDA 6
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SOUDA 5
SOUDA 7
SOUDA 8
SOUDA 9
79.7
99.9
97.3
98.6
99.4
Ca. Ichthyocystis sp.
Astakos:
N. Evoia:
Chios:
Argolida:
Vonitsa:
0.03

hcmr
ΕΛΚΕΘΕ

Epitheliocystis in greater amberjack

At early larval stages can be lethal

Transition from hatchery to sea-cages (first months)

Mortality is generally low and lesions resolve without treatment quite fast

However, epitheliocystis does not come alone !!!

Mortalities can be high when gills are also infected by *Zeuxapta seriolae*

Zeuxapta seriolae

Monogenean parasite

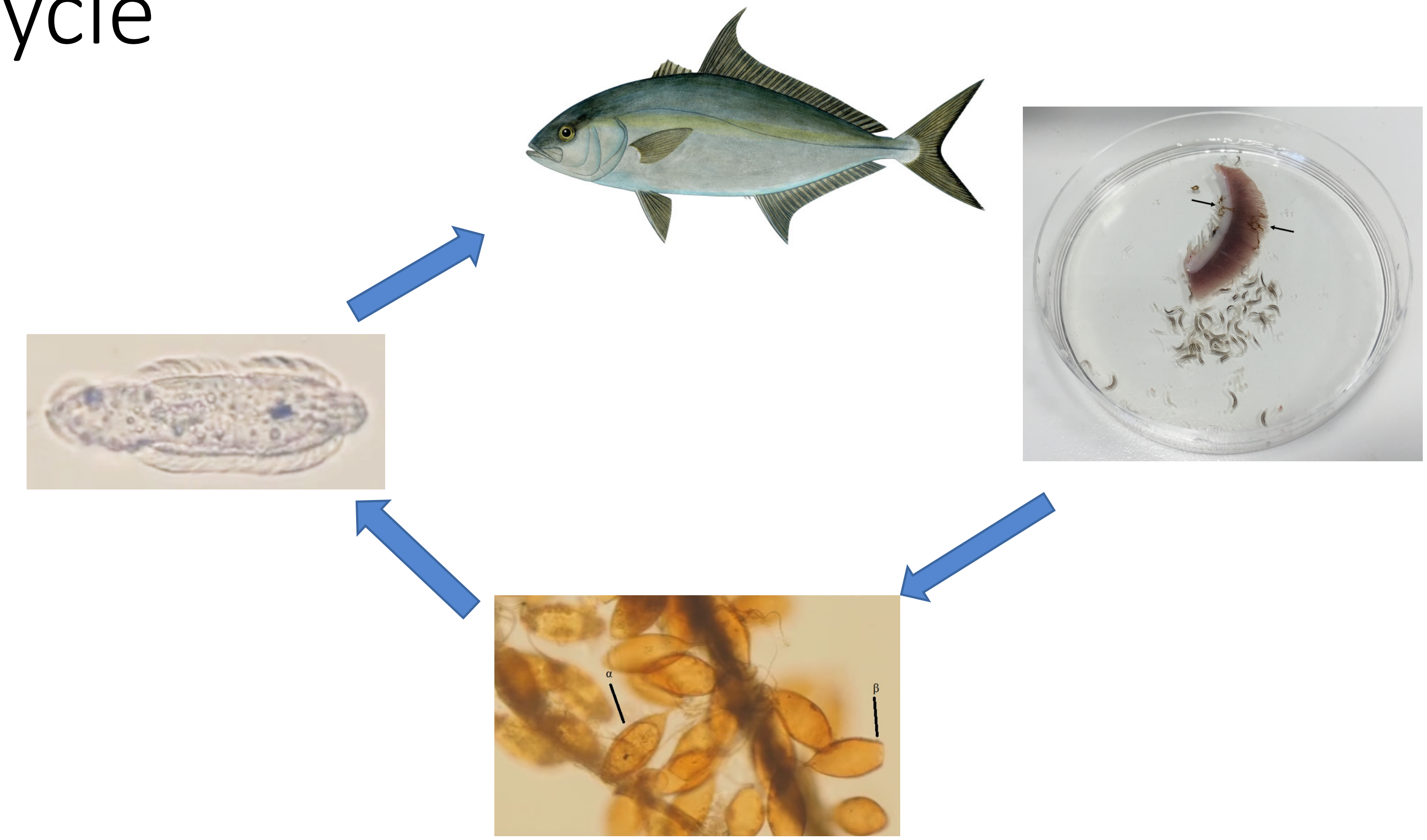
Infects the gills

Causes anemia

High mortalities (~100%)

The most important pathogen of greater amberjack

Life cycle



The monogenean *Zeuxapta seriolae* parasite of greater amberjack, *Seriola dumerili*

Dr. Pantelis Katharios
Institute of Marine Biology, Biotechnology and Aquaculture
Hellenic Centre for Marine Research

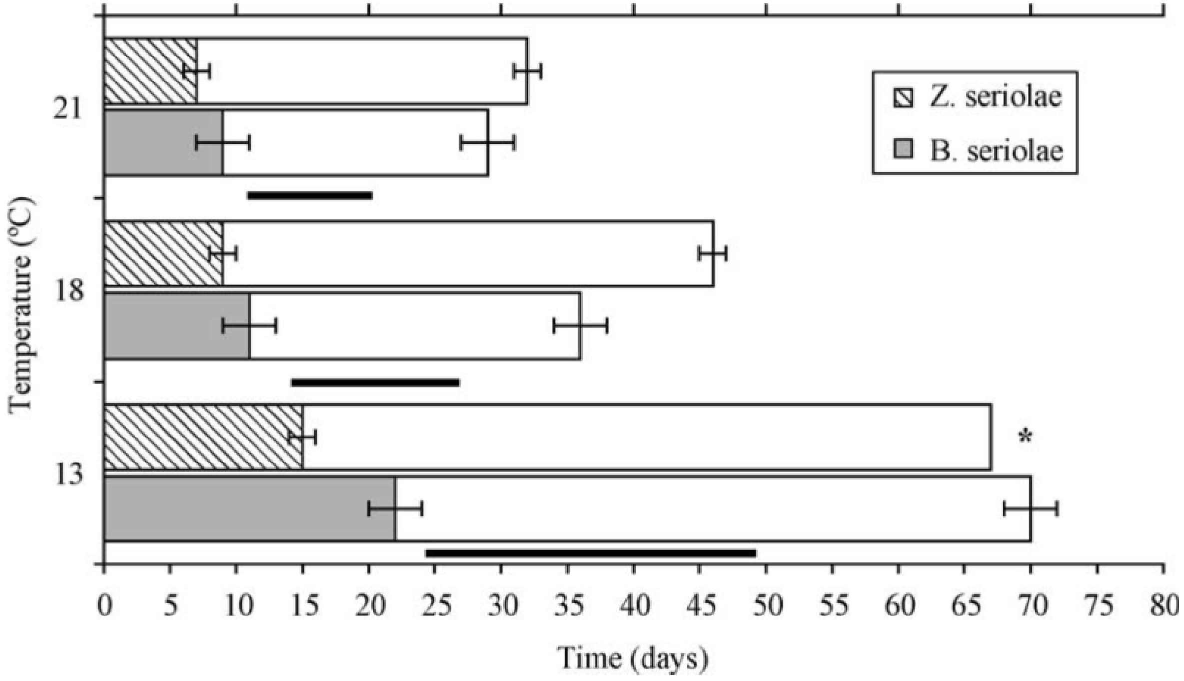
Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal for Parasitology 35 (2005) 315–327
www.parasitology-online.com

Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*

L.A. Tubbs[a,*], C.W. Poortenaar[b,1], M.A. Sewell[a], B.K. Diggles[b,2]

[a]School of Biological Sciences, University of Auckland, Private Bag 92019, P.O. Box 109 695, Auckland, New Zealand
[b]National Institute of Water and Atmospheric Research (NIWA), P.O. Box 109 695, Auckland, New Zealand

Received 30 August 2004; received in revised form 12 November 2004; accepted 15 November 2004



Generation times

Egg hatching times:

13°C: 21d

18°C: 10d

21°C: 7d

From hatching to maturity:

13°C: 45d

18°C: 36d

21°C: 21d

Aquaculture 299 (2010) 2–7

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The influence of different water temperatures on *Neobenedenia girellae* (Monogenea) infection, parasite growth, egg production and emerging second generation on amberjack *Seriola dumerili* (Carangidae) and the histopathological effect of this parasite on fish skin

Noritaka Hirazawa [a,*], Ryoko Takano [b], Hiroko Hagiwara [b], Mitsuyo Noguchi [b], Minoru Narita [b]

[a] Central Research Laboratories, Nippon Suisan Kaisha, Ltd., 559-6 Kitano-machi, Hachioji, Tokyo 192-0906, Japan
[b] Advanced Technology Development Center, Kyoritsu Seiyaku Corporation, 2-9-22 Takamihara, Tsukuba, Ibaragi 300-1252, Japan

Table 1
Neobenedenia girellae infection and parasite growth on amberjack *Seriola dumerili* at 13 days after exposure to oncomiracidia at different water temperatures.

	Seawater temperature					
	20 °C		25 °C		30 °C	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Number of mature parasites						
Pre fish	124.4 ± 30.6[a]	123.2 ± 9.0[a]	118.2 ± 40.0[a]	128.8 ± 23.4[a]	83.6 ± 16.6[ab]	34.0 ± 47.8[b]
Per cm^2 per fish surface	0.44 ± 0.09[a]	0.40 ± 0.05[a]	0.43 ± 0.12[a]	0.41 ± 0.07[a]	0.28 ± 0.04[ab]	0.12 ± 0.16[b]
Mature parasite body length (mm)	2.53 ± 0.21[a]	2.52 ± 0.23[a]	4.43 ± 0.21[b]	4.51 ± 0.30[b]	4.94 ± 0.22[c]	4.81 ± 0.29[c]
First laid eggs-recorded days after exposure	13	13	8	8	6	6
Number of laid eggs per net	0.3 ± 0.5[a]	2.3 ± 3.9[a]	1,369,000 ± 1,036,000[b]	4,903,000 ± 2,183,000[bc]	5,550,000 ± 4,725,000[bc]	9,698,000 ± 5,491,000[c]
Number of larval parasites						
Per fish	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.8 ± 1.8[a]	4.0 ± 1.9[a]	4223.9 ± 2026.3[b]	2980.5 ± 1017.0b
Per cm^2 per fish surface	0.000 ± 0.000[a]	0.000 ± 0.000[a]	0.002 ± 0.005[a]	0.012 ± 0.005[a]	14.618 ± 8.704[b]	10.175 ± 3.458[b]
Larval parasite body length (mm)	—	—	0.28 ± 0.03[ab]	0.24 ± 0.04[b]	0.47 ± 0.16 [ac]	0.54 ± 0.15[c]

Values are mean and standard deviations.
Values with different superscripts are significantly different at $P<0.05$.

Treatment

Formalin ineffective even at very high doses

Hydrogen peroxide extremely effective

75 - 100 ppm for 20-30 min

Toxic to fish at higher temperature

It kills adults and juvenile parasites but not eggs

It needs a second (and third) treatment

Praziquantel in feed (and as a bath)

Recurrent infections throughout the year

Will greater amberjack become the Med salmon?

Integrated Pest Management

- Salmon aquaculture in Canada and Norway
- Sea lice
- Sustainability
- Use of chemicals
- Protection of the environment and the consumer
- Protection of the wild stocks

Developing an Integrated Pest Management approach for marine finfish aquaculture activities in B.C.

Aquaculture Management Advisory Committee

March 15 2016

- Multifactorial approach to pest management;
- Series of evaluations, decisions and controls;
- Take advantage of all pest management control options;
- Strategies to achieve long-terms solutions.

- Prevention
- Monitoring
- Threshold for action
- Medicinal and non-medicinal controls

Prevention

- Prevention is fundamental to IPM – reduces the likelihood and severity of sea lice infestations.
- Location of sites – sources of infection and water quality.
- Year class separation – probably the most effective husbandry technique; slowing down acquisition of sea lice.
- Fallowing of sites – reduce or eliminate self-sustaining lice popn's.
- Husbandry – minimize stress, stocking densities, nutrition, hygiene, regular removal of mortalities, predator control.
- Innovative technologies – cleaner fish, vaccines, immunostimulants.

Monitoring of pest issue

- Decisions about when to conduct treatment should be based on a program of monitoring lice numbers.
- Sampling programs (frequency and sample size) should be conducted continuously following transfer to sea water.
- Selection of appropriate treatment should be based on sea lice population dynamics.
- Monitoring is necessary to ensure interventions are carried out at the correct time with appropriate product.
- Monitoring also allows the site operator to build up a picture of the dynamics of sea lice populations and make predictions around optimal management/treatment approaches.

Threshold for action

- Treatment triggers should be low enough to protect the salmon and reduce risks associated with the transfer of sea lice from farmed to wild fish.
- Too low a trigger can lead to unnecessary therapeutant use, which can be difficult, costly and environmentally unsound.
- Current regulatory approach in Pacific Region requires:
  - March 1 to June 30; if the sea lice abundance exceeds 3 motile lice (Lep spp.) per fish then implement a plan to reduce absolute sea lice inventory within 15 days.
  - July 1 to February 28; if the sea lice abundance exceeds 3 motile lice (Lep spp.) per fish then provide a plan to address exceedance to Department within 30 days.

Integrated Pest Management

- Collective decision
- Regulatory authorities
- Veterinary authorities
- Research centers
- The most important future direction for sustainability


Thank you very much