Epitheliocystis disease; results and progress

Task 25.1, D25.4

DIVERSIFY Meeting, Tenerife 2018

DR. PANTELIS KATHARIOS

INSTITUTE OF MARINE BIOLOGY, BIOTECHNOLOGY AND AQUACULTURE

HELLENIC CENTRE FOR MARINE RESEARCH

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

Inclusions in gill and skin epithelium of the fish

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

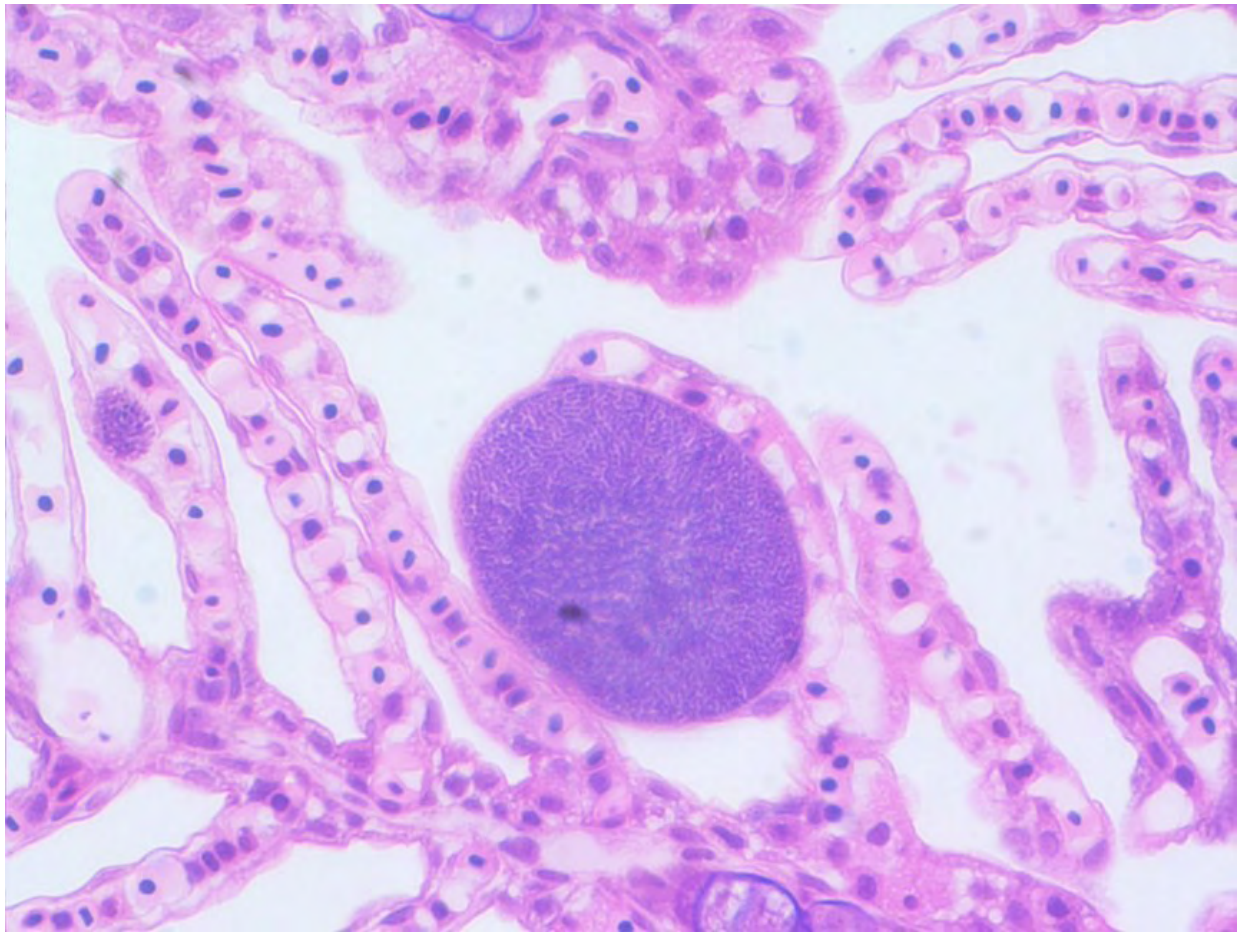
Diagnosis

Easy !

Wet mounts

Histology

PCR



Two stereotypes about epitheliocystis

It is a benign disease

It is caused by Chlamydia

Epitheliocystis and HCMR. A recurrent problem

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

Experimental investigation

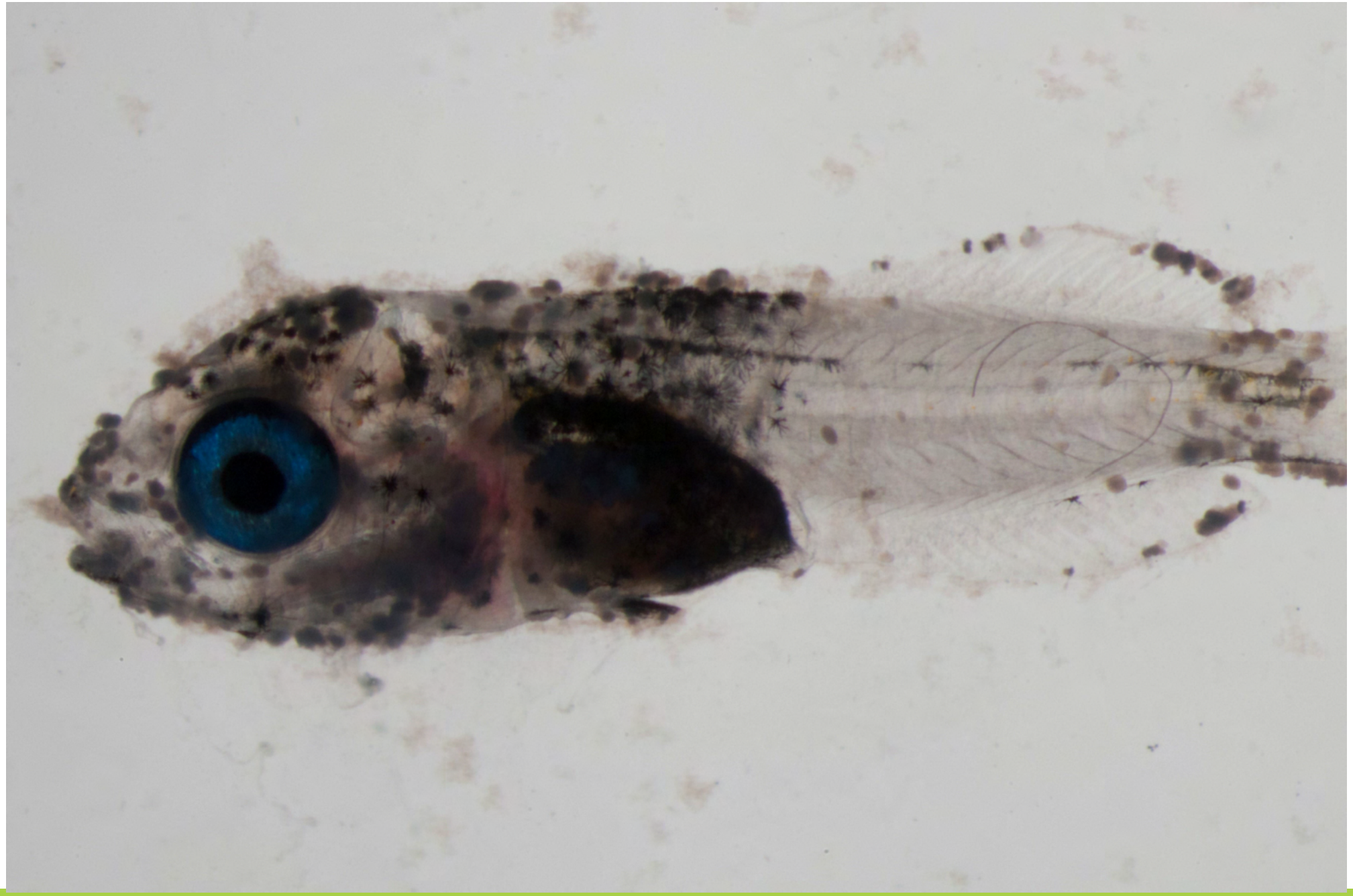
Aquaexcel Project

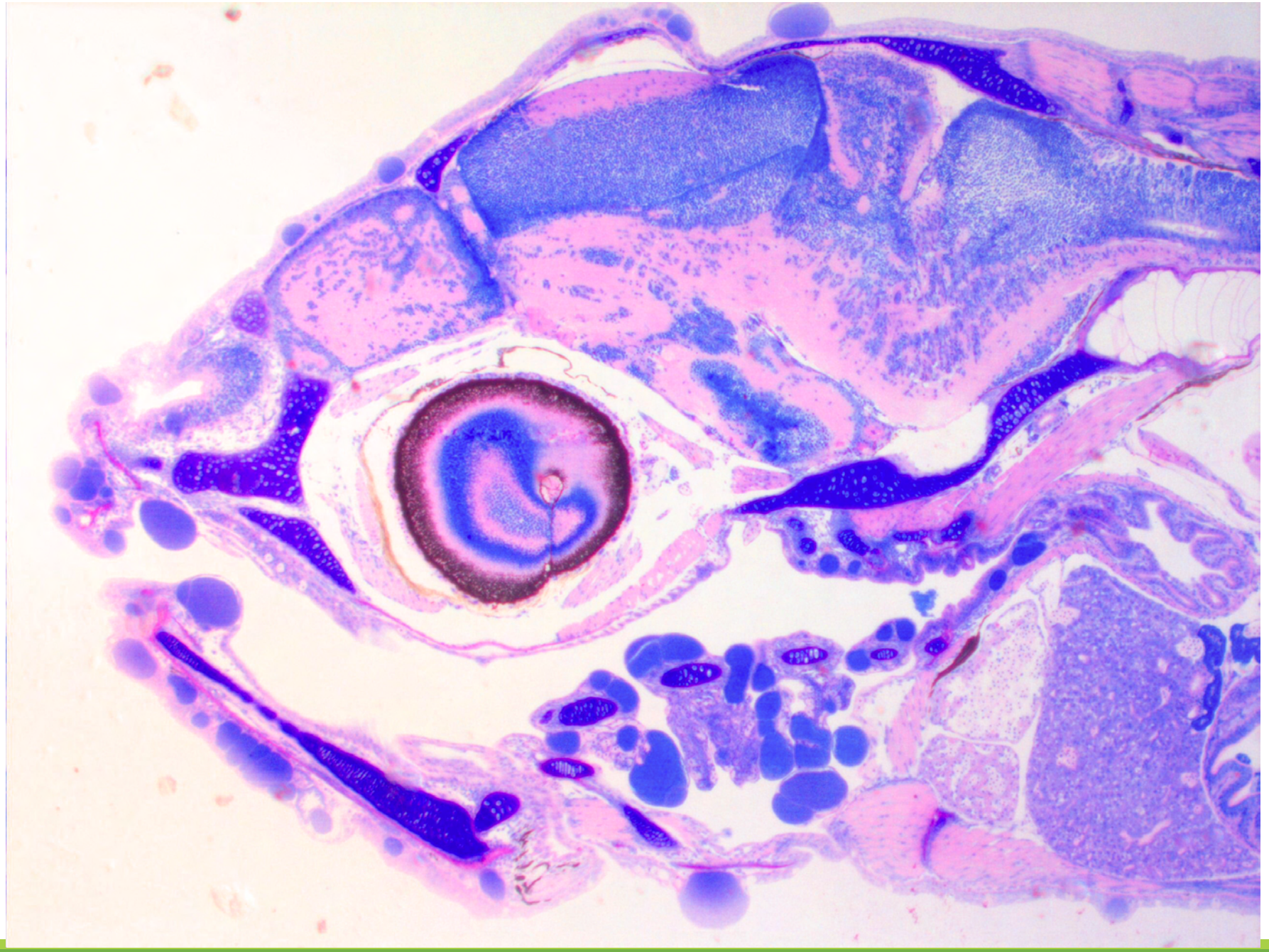
Prof. Lloyd Vaughan, Veterinary School, University of Zurich

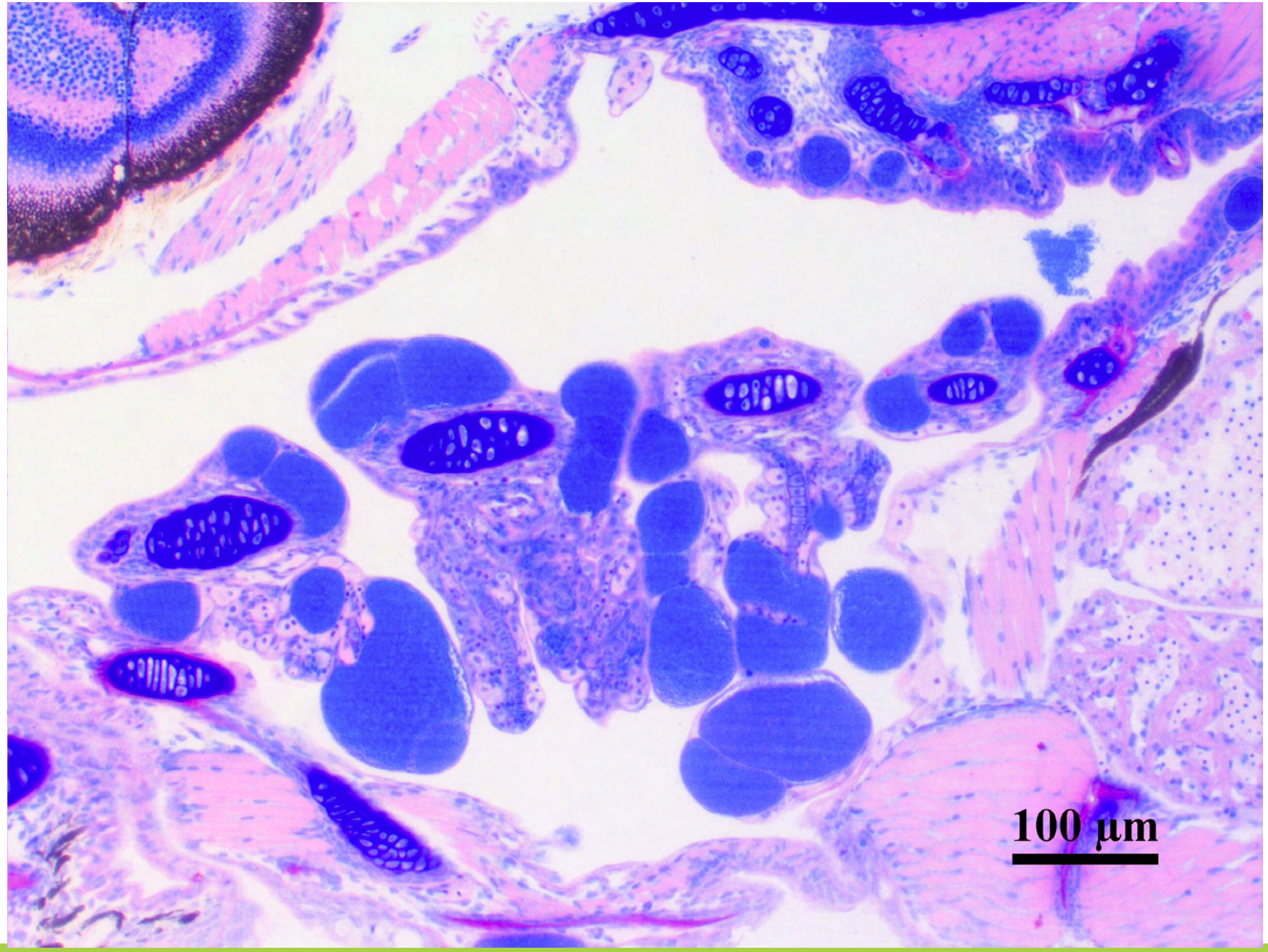
Mesocosm culture of sharpsnout seabream– Natural infection

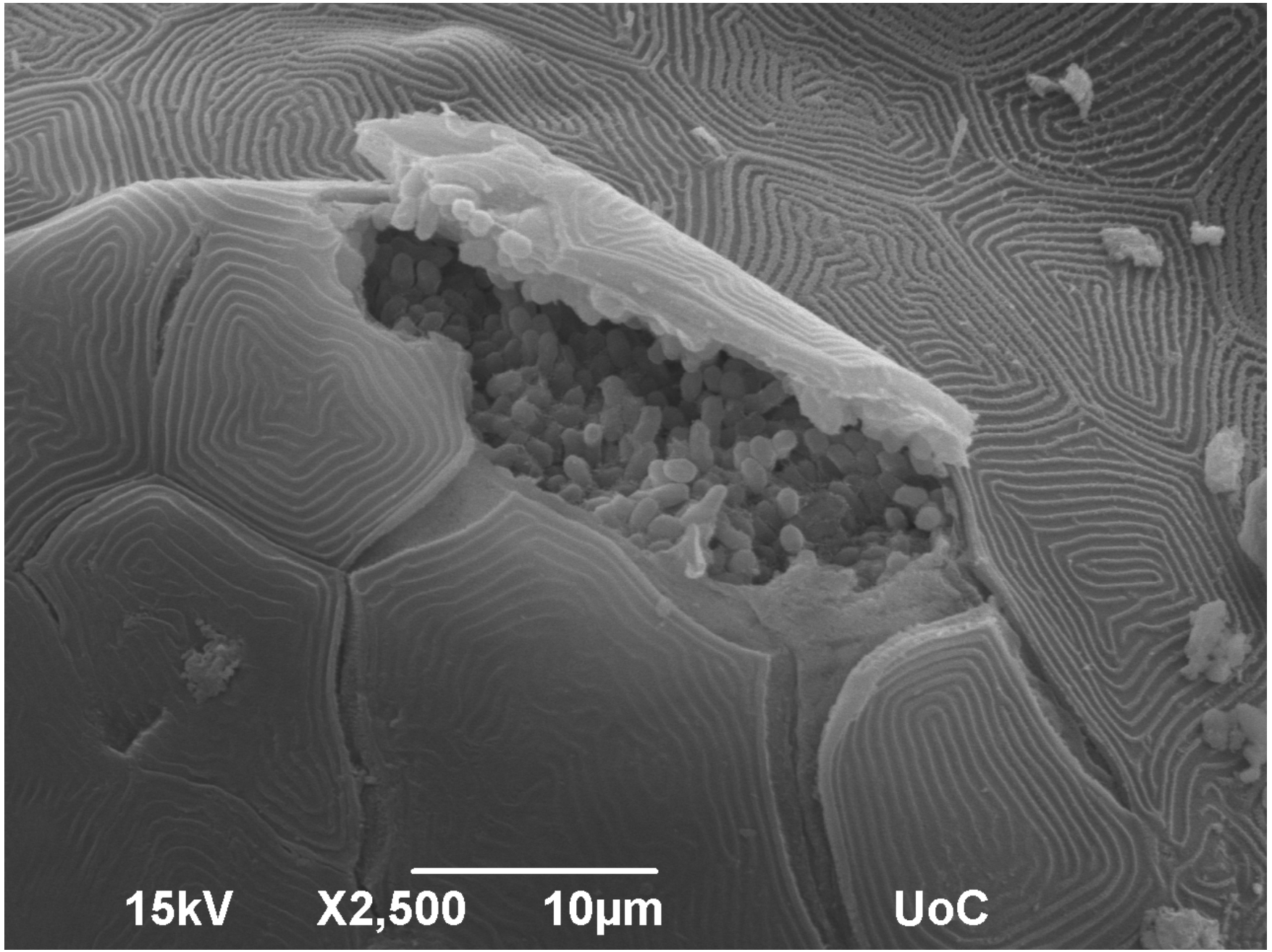
Plan B: collect samples (gilthead seabream) from Greek commercial fish farms

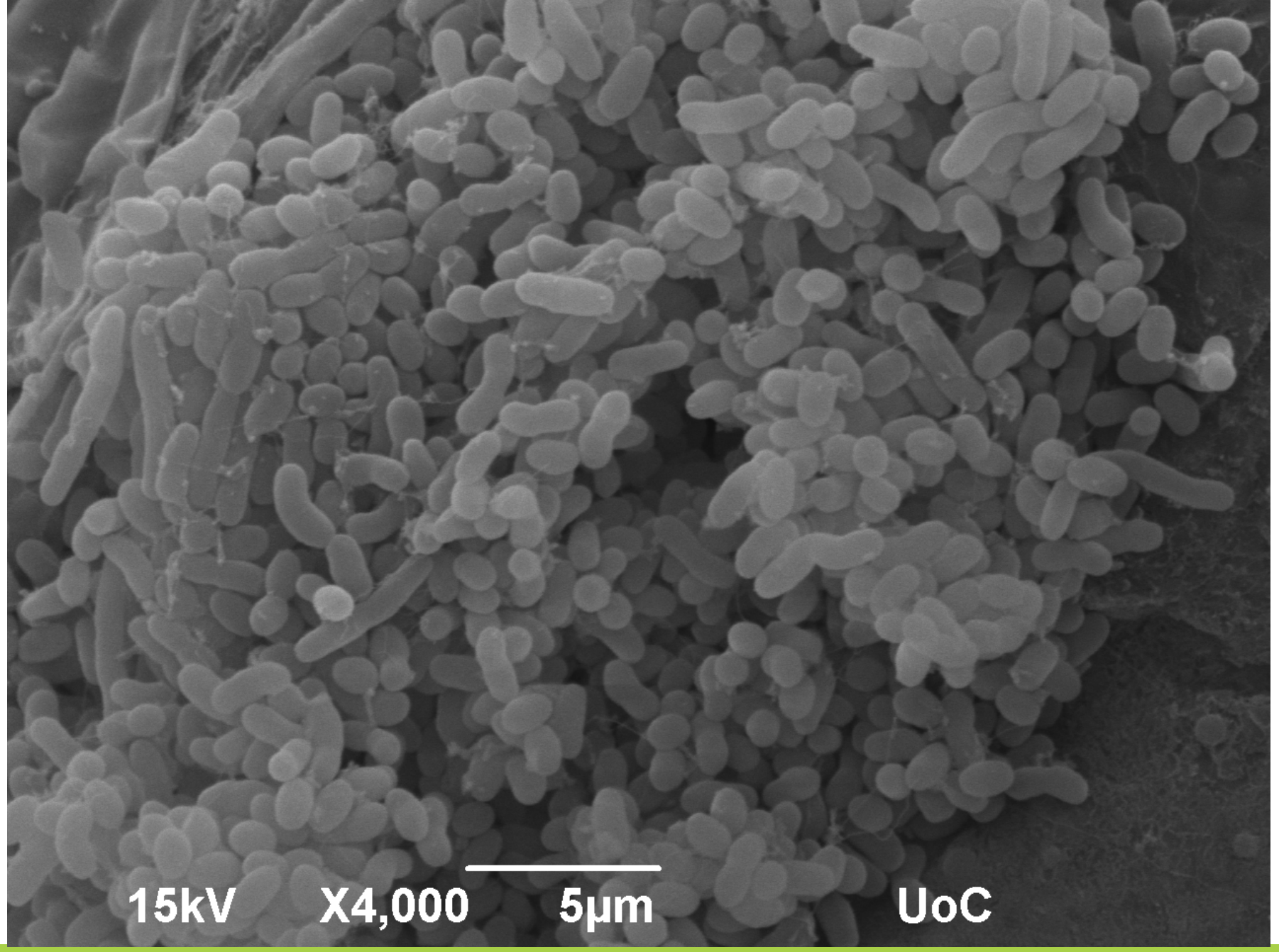
October-November 2012






100 µm


15kV
X2,500
10µm
UoC


15kV X4,000 5µm UoC

Identification pipeline

PCR for chlamydia positive

Sequencing pointed to known chlamydia pathogens

In situ for chlamydia was negative

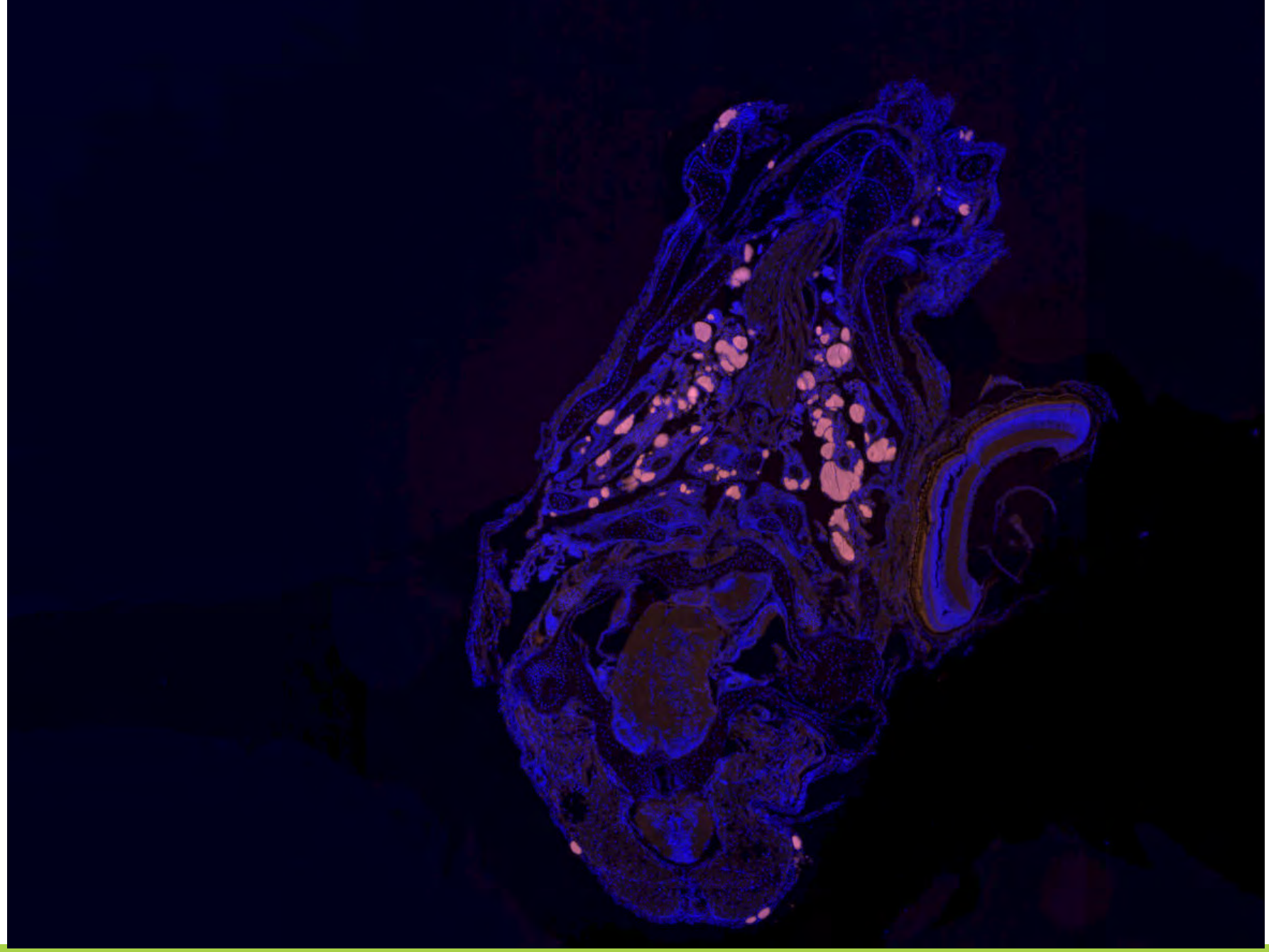
DNA extraction from single cysts

PCR 16s followed by sequencing

Endozoicomonas sp.

Construction of probes for Endozoicomonas

New in situ



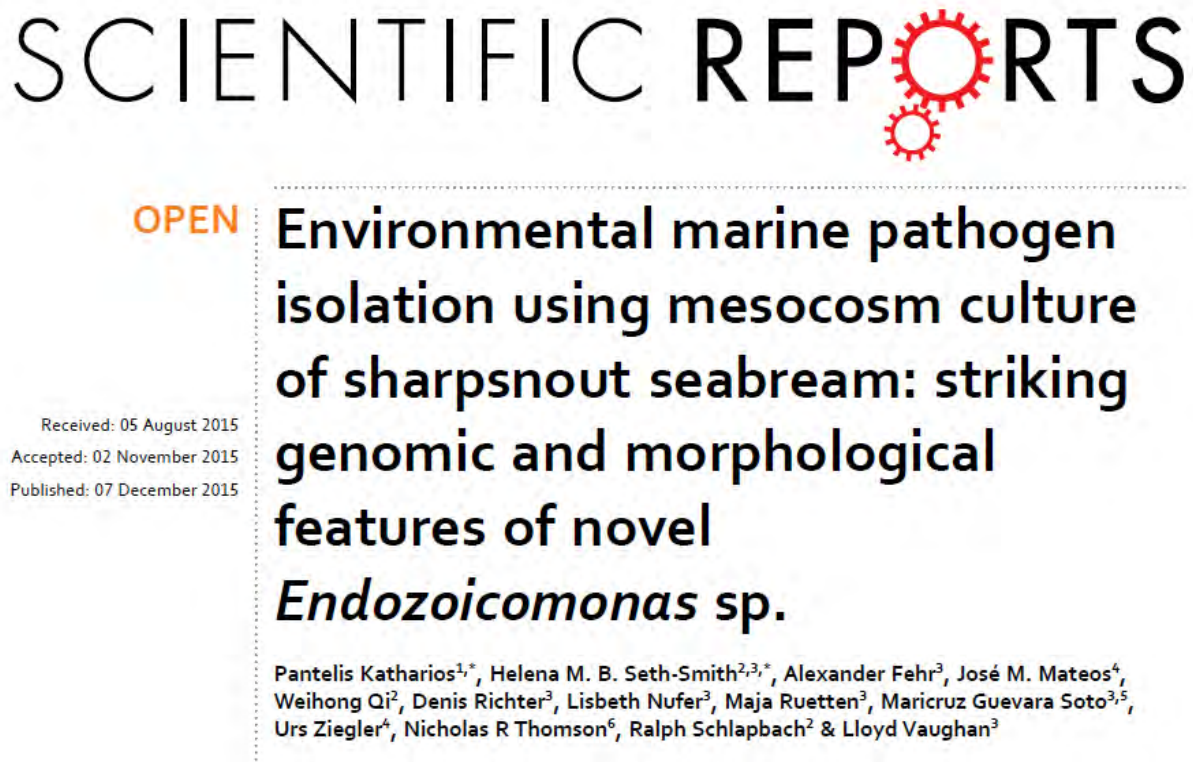
Novel pathogen

Candidatus Endozoicomonas cretensis n.sp

γ-proteobacteria

Full genome sequencing from single cysts

First genome draft published



SCIENTIFIC REPORTS

OPEN

Received: 05 August 2015
Accepted: 02 November 2015
Published: 07 December 2015

Environmental marine pathogen isolation using mesocosm culture of sharpsnout seabream: striking genomic and morphological features of novel *Endozoicomonas* sp.

Pantelis Katharios[1,*], Helena M. B. Seth-Smith[2,3,*], Alexander Fehr[3], José M. Mateos[4], Weihong Qi[2], Denis Richter[3], Lisbeth Nufer[3], Maja Ruetten[3], Maricruz Guevara Soto[3,5], Urs Ziegler[4], Nicholas R Thomson[6], Ralph Schlapbach[2] & Lloyd Vaughan[3]

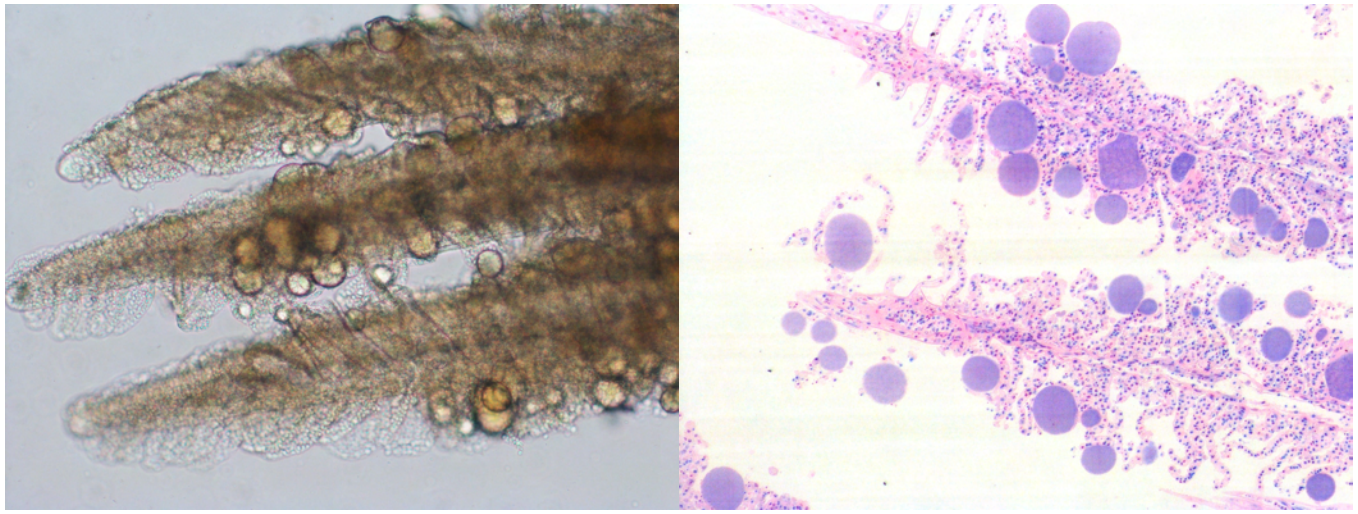
Epitheliocystis in gilthead sea bream

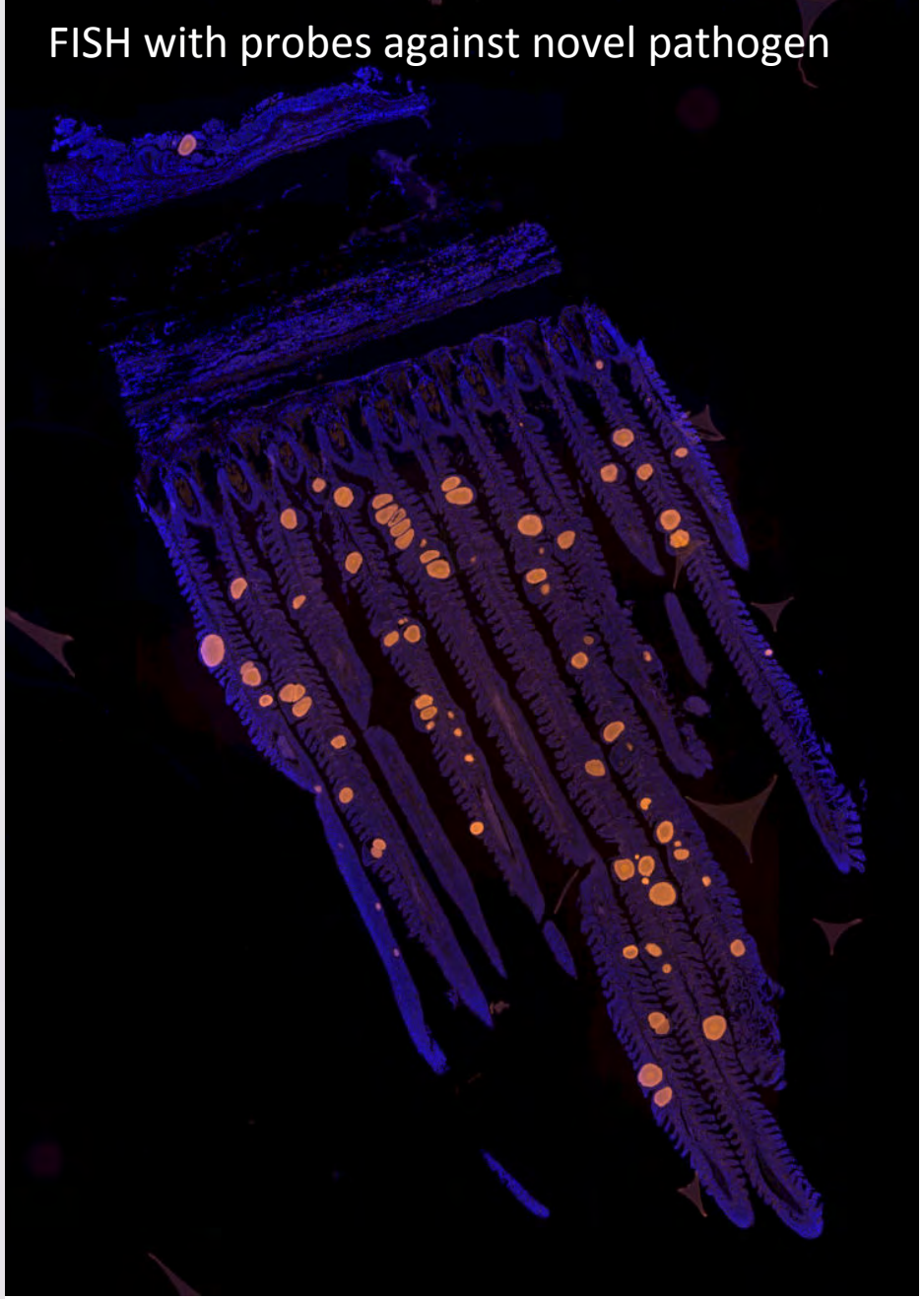
Juvenile breams (2-5g)

Similar case

PCR for chlamydia positive

In situ negative




FISH with probes against novel pathogen

Novel pathogens

Novel genus

2 new species

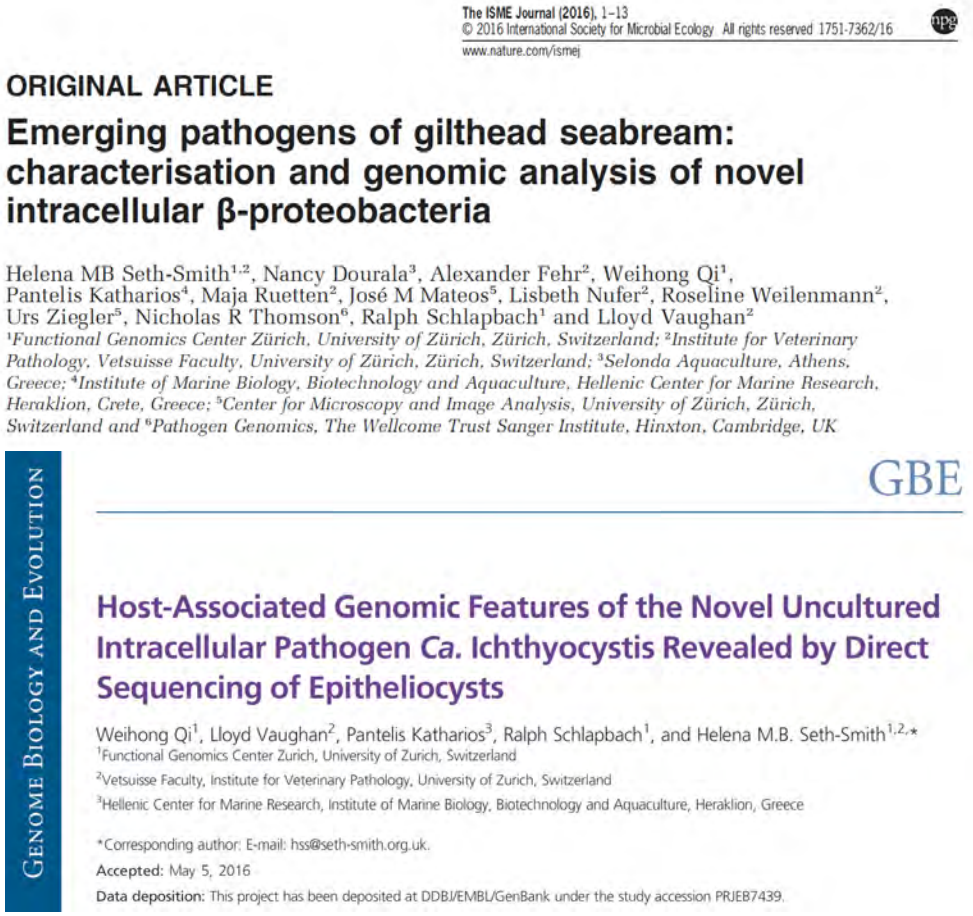
Candidatus Ichthyocystis hellenicum n.sp

Candidatus Ichthyocystis sparus n.sp

β-proteobacteria

Full genome sequencing from single cysts

First genome draft published



The ISME Journal (2016), 1–13

www.nature.com/ismej

ORIGINAL ARTICLE

Emerging pathogens of gilthead seabream: characterisation and genomic analysis of novel intracellular β-proteobacteria

Helena MB Seth-Smith[1,2], Nancy Dourala[3], Alexander Fehr[2], Weihong Qi[1], Pantelis Katharios[4], Maja Ruetten[2], José M Mateos[5], Lisbeth Nufer[2], Roseline Weilenmann[2], Urs Ziegler[5], Nicholas R Thomson[6], Ralph Schlapbach[1] and Lloyd Vaughan[2]

[1]Functional Genomics Center Zürich, University of Zürich, Zürich, Switzerland; [2]Institute for Veterinary Pathology, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland; [3]Selonda Aquaculture, Athens, Greece; [4]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, Heraklion, Crete, Greece; [5]Center for Microscopy and Image Analysis, University of Zürich, Zürich, Switzerland and [6]Pathogen Genomics, The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK

GENOME BIOLOGY AND EVOLUTION

GBE

Host-Associated Genomic Features of the Novel Uncultured Intracellular Pathogen *Ca.* Ichthyocystis Revealed by Direct Sequencing of Epitheliocysts

Weihong Qi[1], Lloyd Vaughan[2], Pantelis Katharios[3], Ralph Schlapbach[1], and Helena M.B. Seth-Smith[1,2,*]

[1]Functional Genomics Center Zurich, University of Zurich, Switzerland

[2]Vetsuisse Faculty, Institute for Veterinary Pathology, University of Zurich, Switzerland

[3]Hellenic Center for Marine Research, Institute of Marine Biology, Biotechnology and Aquaculture, Heraklion, Greece

*Corresponding author: E-mail: hss@seth-smith.org.uk.

Accepted: May 5, 2016

Data deposition: This project has been deposited at DDBJ/EMBL/GenBank under the study accession PRJEB7439.

Following the false lead

In both cases Chlamydia were co-infecting the fish

Smaller and fewer cysts were caused by fish pathogenic Chlamydia were also simultaneously present in the same tissue

Through qPCR analysis it was estimated that the chlamydia:ichthyocystis ratio was from 1:5 to 1:3000

PCR targeting chlamydia was always positive and sequencing was pointing to known pathogens

If *in situ* was not used, then the true pathogens would have been misidentified

Going after the chlamydial pathogens

Gilthead seabreams with Ichthyocystis infections

PCR Chlamydiae-specific 16s primers

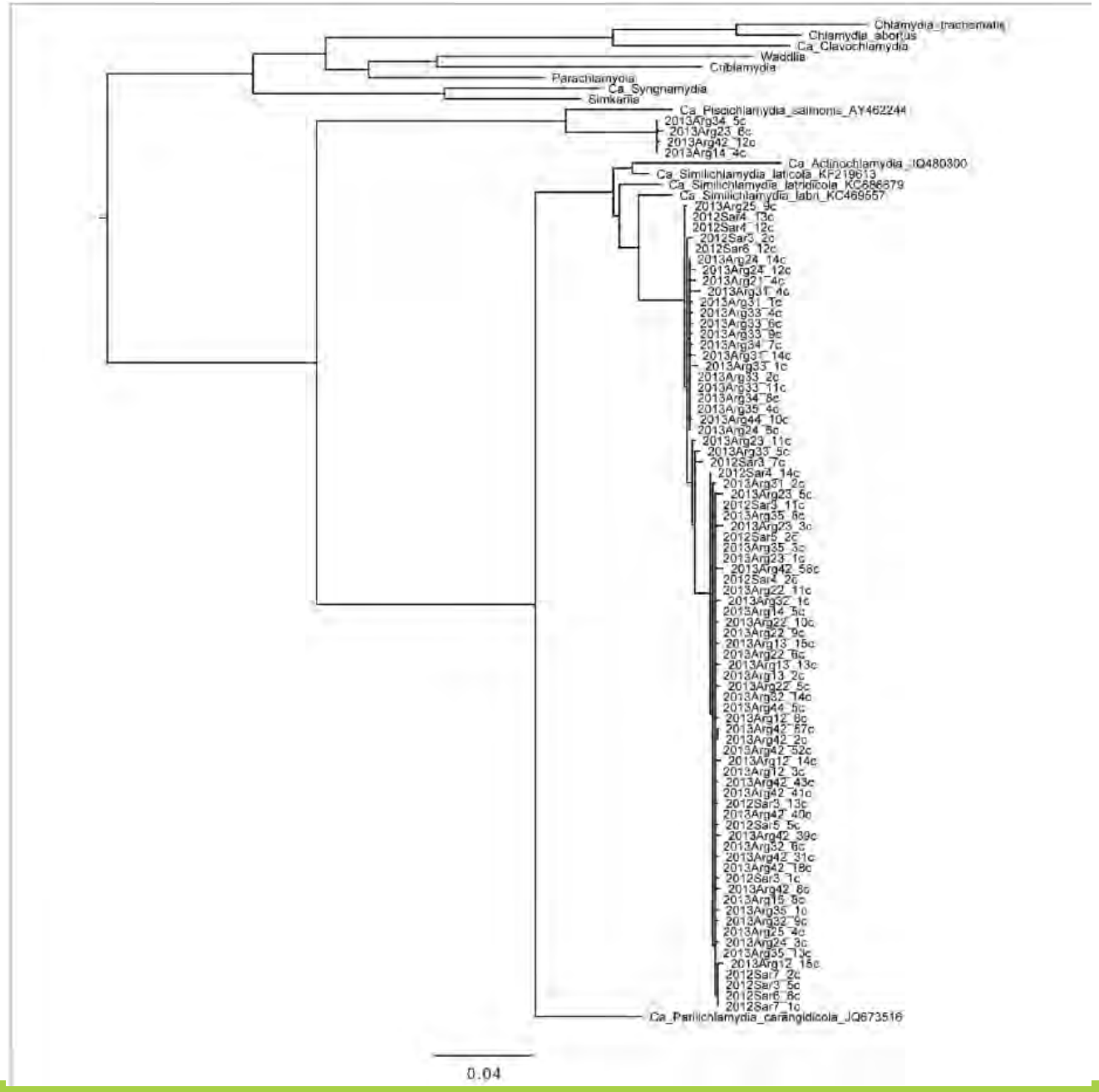
Amplicons cloned into Topo vecto pCR2.1

Blast in Genbank database

Phylogenetic analysis

Fluorescent in situ hybridization

Histology and TEM


Chlamydia_trachomatis
Chlamydia_abortus
Ca_Clavochlamydia
Waddlia
Criblamydia
Parachlamydia
Ca_Syngnamydia
Simkania
Ca_Piscichlamydia_salmonis_AY462244
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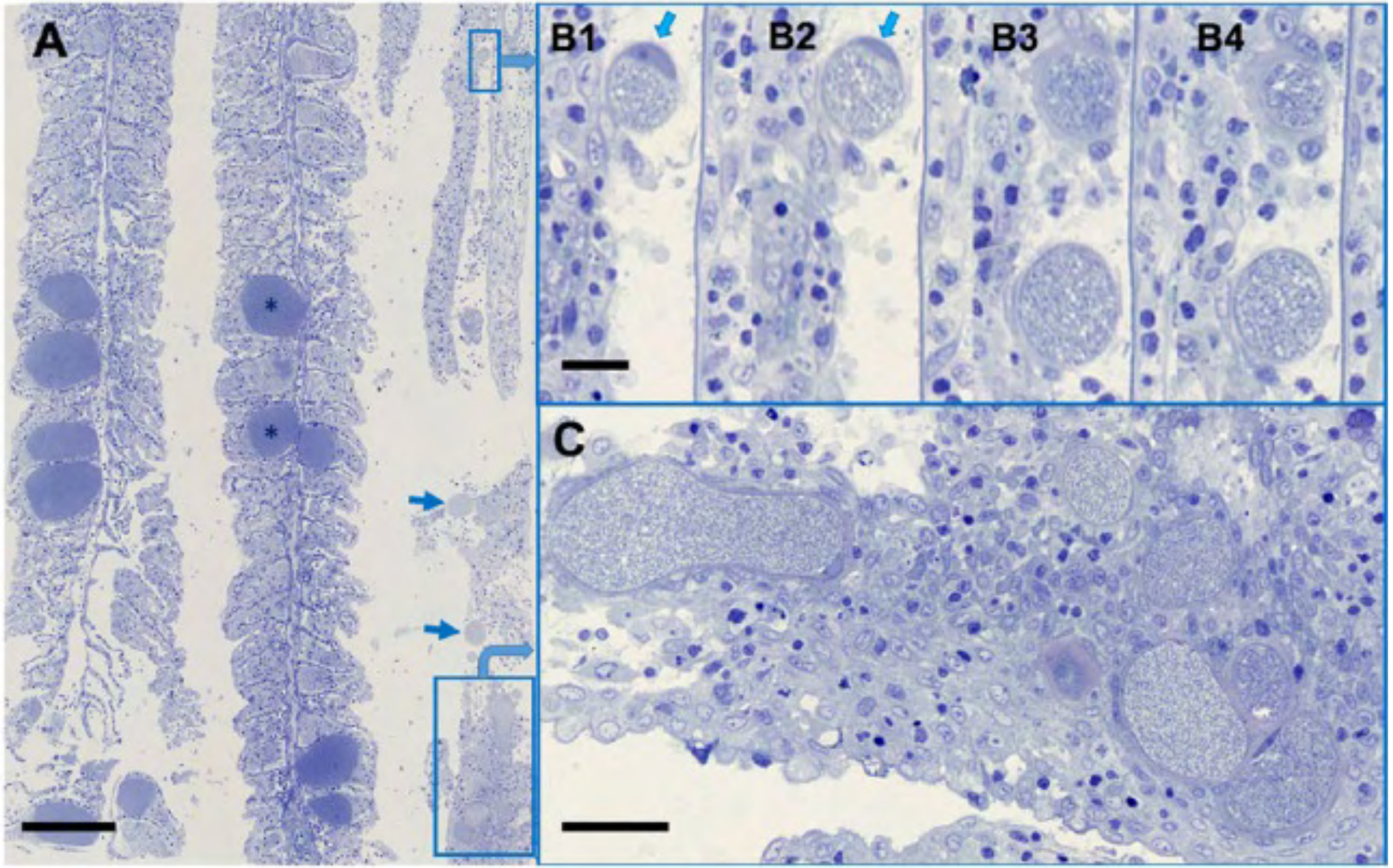
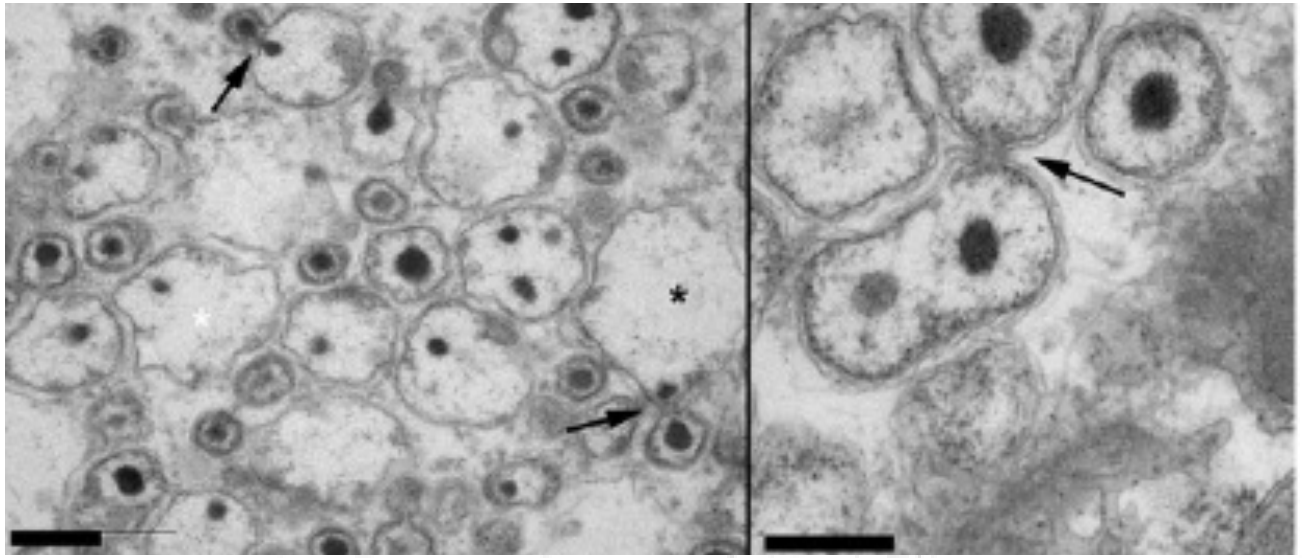
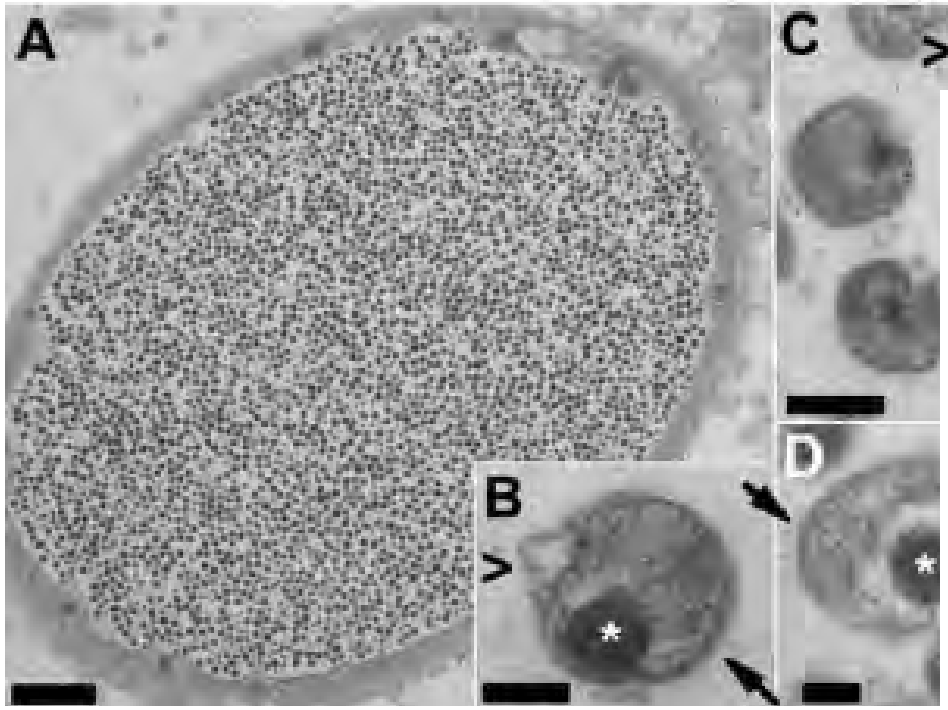

A
B1
B2
B3
B4
C





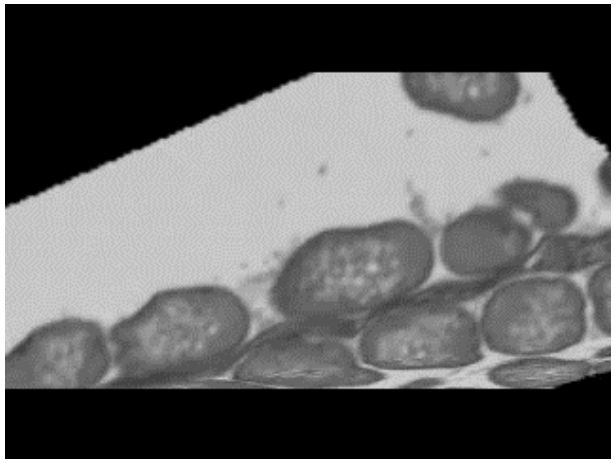
frontiers in Microbiology

ORIGINAL RESEARCH
published: 30 March 2017
doi: 10.3389/fmicb.2017.00508

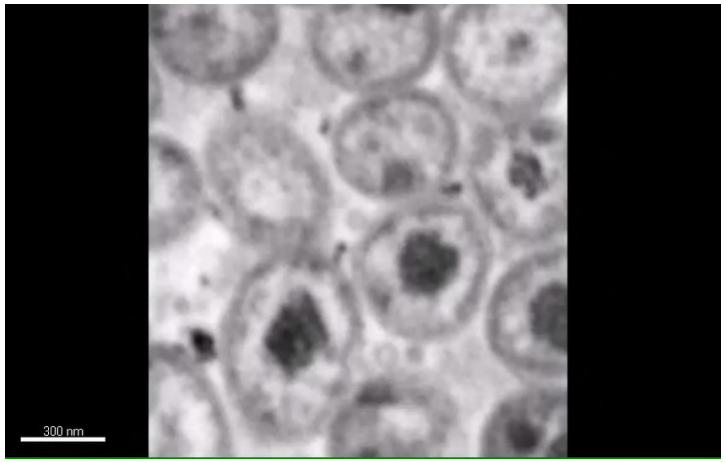
Ca. Similichlamydia in Epitheliocystis Co-infection of Gilthead Seabream Gills: Unique Morphological Features of a Deep Branching Chlamydial Family

Helena M. B. Seth-Smith[1,2†], Pantelis Katharios[3], Nancy Dourala[4], José M. Mateos[5], Alexander G. J. Fehr[1], Lisbeth Nufer[1], Maja Ruetten[1,6], Maricruz Guevara Soto[1,7] and Lloyd Vaughan[1,6]*

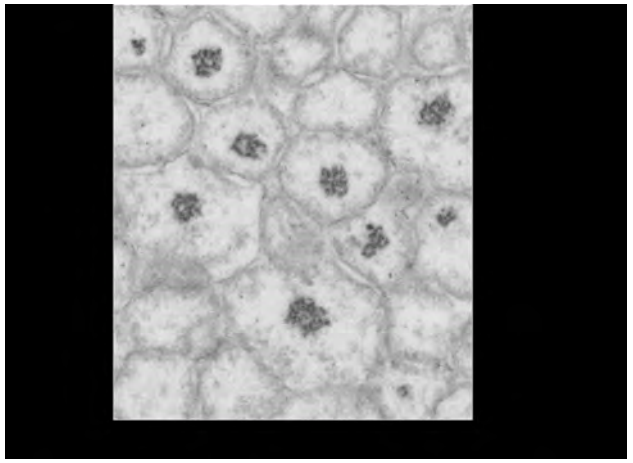
OPEN ACCESS



Ca. Endozoicomonas cretensis
γ-proteobacteria
0.8 x 2.5 µm



Ca. Ichthyocystis sp
β-proteobacteria
0.5 x 0.7 µm



Ca. Similichlamydia sp
Chlamydiae

DIVERSIFY project

Study epitheliocystis disease in greater amberjack

Mesocosm experimental setup

Isolate (?) and characterize the pathogen

Test the tools developed for early detection and diagnosis

Survey fish farms in Greece

Mesocosm trials

3 trials

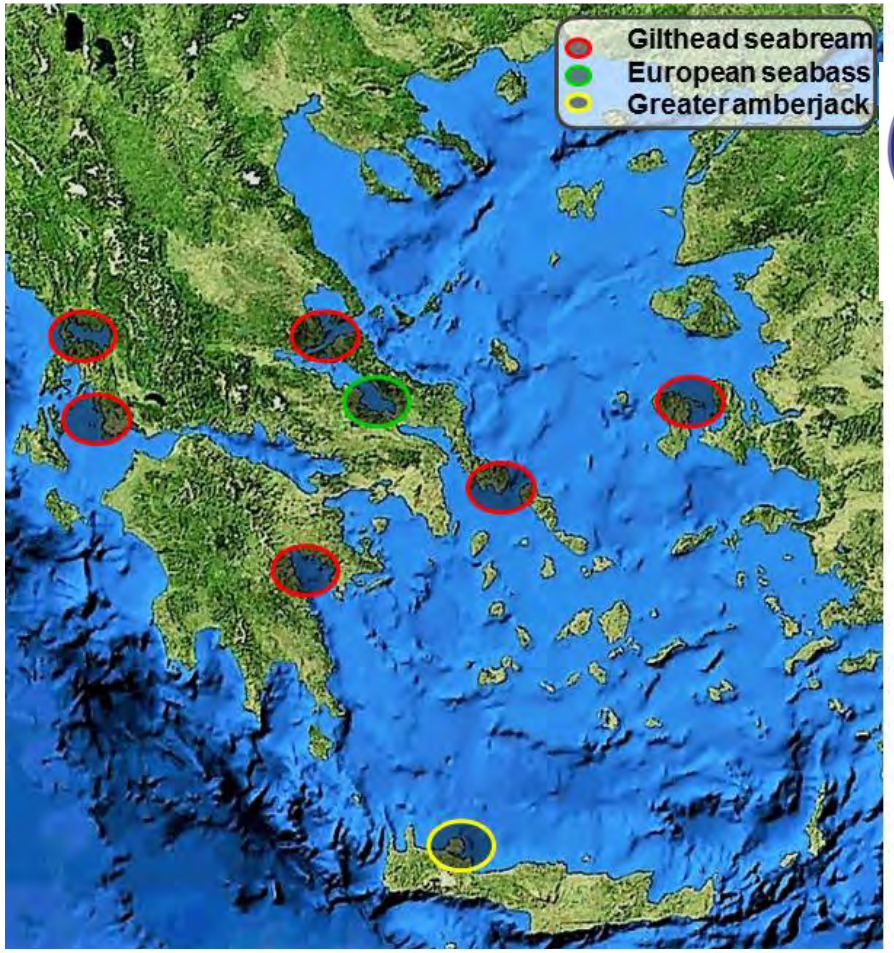
Greater amberjack, seabass, meagre

Fish did not get the infection

Screening in planktonic organisms and water

Environmental chlamydia not related to the disease

Survey in Greece



NIREUS

SELONDA

Andromeda

hcmr
ΕΛΚΕΘΕ

Gilthead seabream

- Astakos
- South Evoia
- North Evoia
- Chios
- Argolida
- Vonitsa

European seabass

- Larymna

Greater amberjack

- Souda

Identification pipeline

Amplification of 16S rRNA gene fragments with specific primers for

- *Ca.* Ichthyocystis spp.
- *Endozoicomonas* sp.
- Chlamydiae

Visualization of the results

Agarose-gel with Et-Br

Identification of pathogens

- Sequencing of the PCR products (Sanger)
- Comparison of the sequences with other sequences of NCBI GenBank (BLAST)

Phylogenetic analysis

- Alignment of sequences with ClustalW
- Phylogenetic trees were made using Tamura-Nei model with Neighbor joining analysis at 1000 bootstrap.

Results

PCR results

All (33) samples were positive for Ca. Ichthyocystis spp.

15 samples were positive for Chlamydiae

2 samples were positive for Endozoicomonas spp.

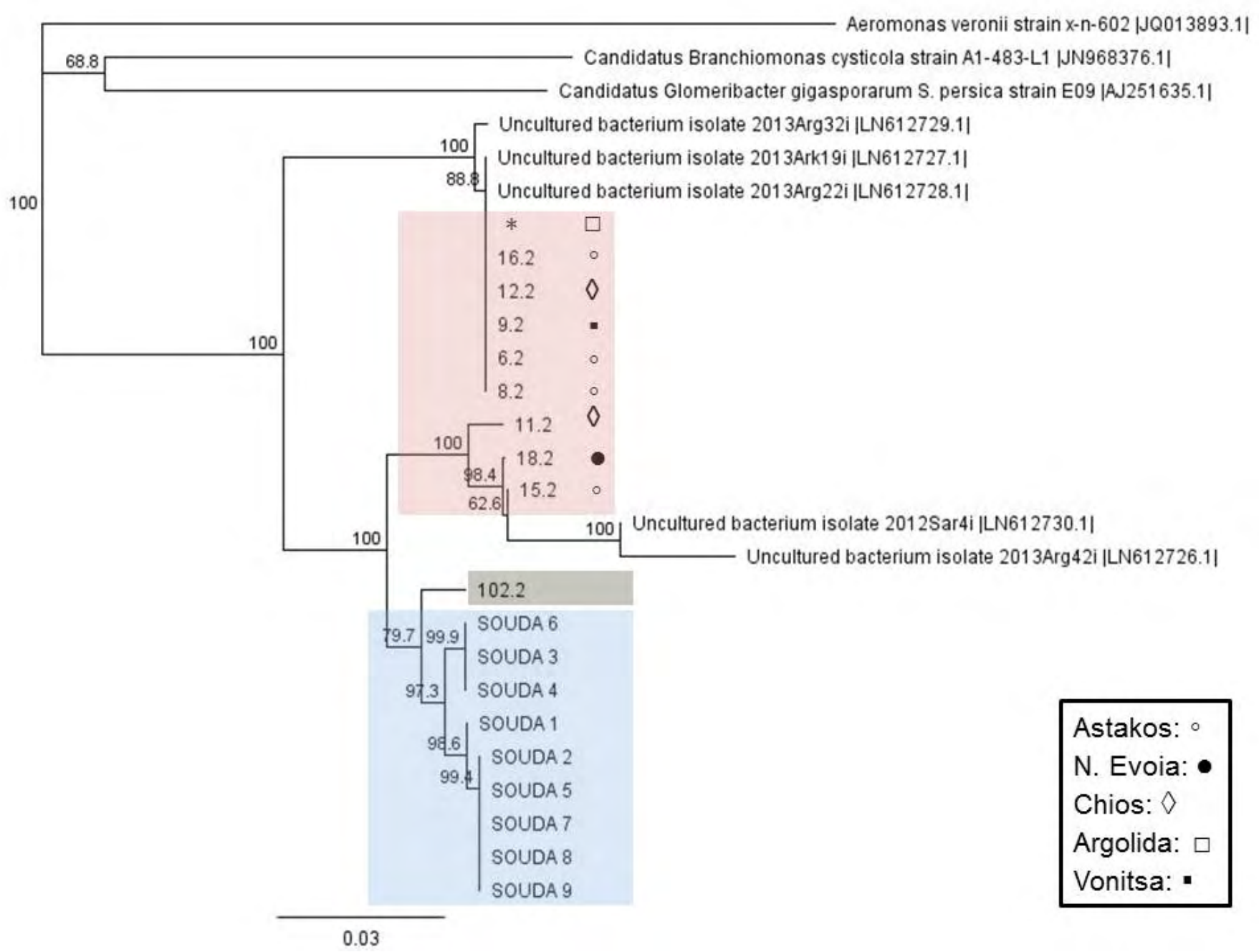
Chlamydiae

Gilthead seabream: Astakos, S. Evoia, N. Evoia, Argolida, Vonitsa

European seabass: Larymna

Endozoicomonas

Gilthead seabream: Chios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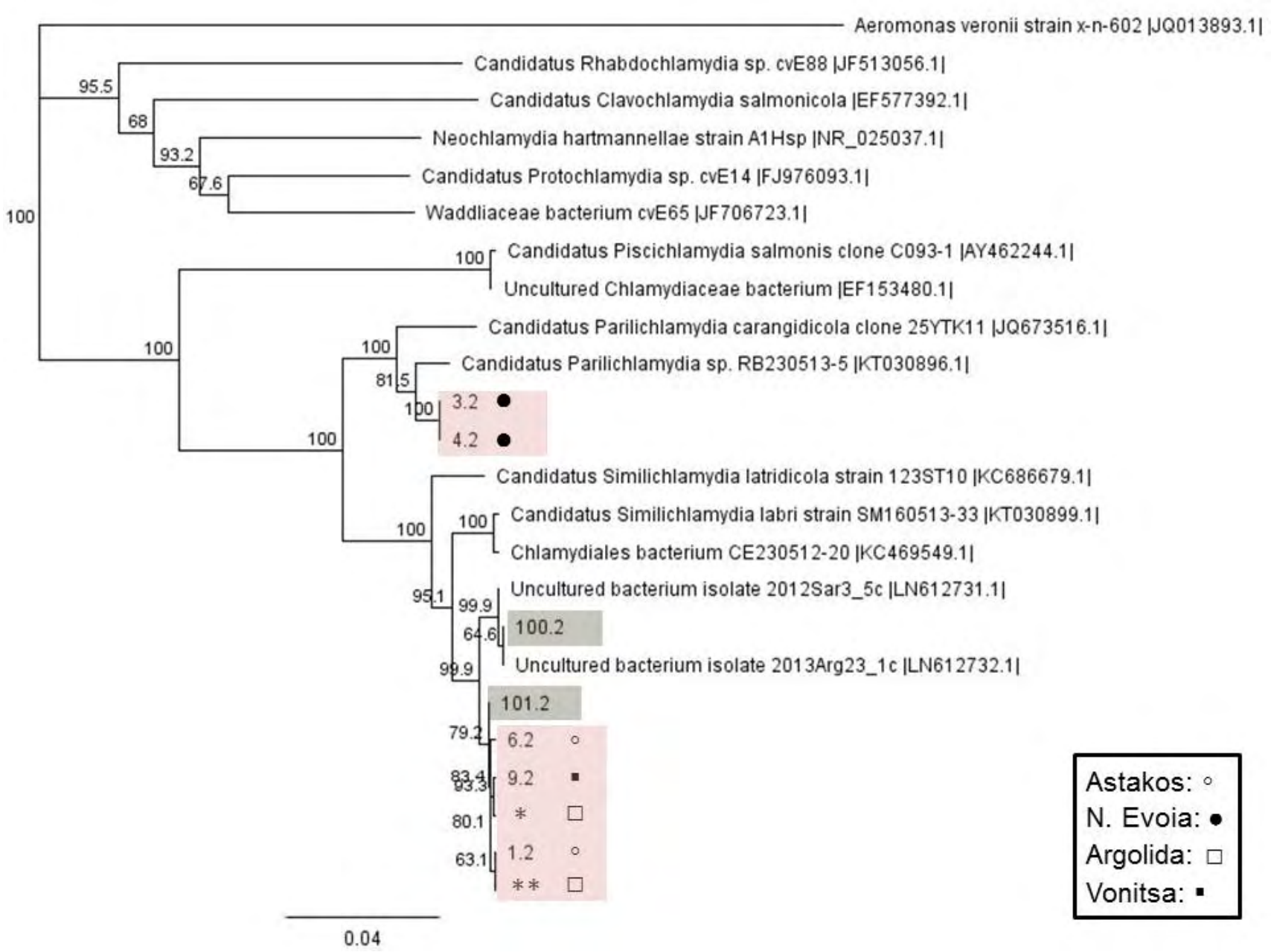
Candidatus Glomeribacter gigasporarum S. persica strain E09 |AJ251635.1|
Uncultured bacterium isolate 2013Arg32i |LN612729.1|
Uncultured bacterium isolate 2013Ark19i |LN612727.1|
Uncultured bacterium isolate 2013Arg22i |LN612728.1|
16.2
12.2
9.2
6.2
8.2
11.2
18.2
15.2
Uncultured bacterium isolate 2012Sar4i |LN612730.1|
Uncultured bacterium isolate 2013Arg42i |LN612726.1|
102.2
SOUDA 6
SOUDA 3
SOUDA 4
SOUDA 1
SOUDA 2
SOUDA 5
SOUDA 7
SOUDA 8
SOUDA 9
68.8
100
88.8
100
100
100
98.4
62.6
100
79.7
99.9
97.3
98.6
99.4
0.03
Astakos: ◦
N. Evoia: ●
Chios: ◊
Argolida: □
Vonitsa: ▪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Candidatus Rhabdochlamydia sp. cvE88 |JF513056.1|
Candidatus Clavochlamydia salmonicola |EF577392.1|
Neochlamydia hartmannellae strain A1Hsp |NR_025037.1|
Candidatus Protochlamydia sp. cvE14 |FJ976093.1|
Waddliaceae bacterium cvE65 |JF706723.1|
Candidatus Piscichlamydia salmonis clone C093-1 |AY462244.1|
Uncultured Chlamydiaceae bacterium |EF153480.1|
Candidatus Parilichlamydia carangidicola clone 25YTK11 |JQ673516.1|
Candidatus Parilichlamydia sp. RB230513-5 |KT030896.1|
3.2
4.2
Candidatus Similichlamydia latridicola strain 123ST10 |KC686679.1|
Candidatus Similichlamydia labri strain SM160513-33 |KT030899.1|
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Uncultured bacterium isolate 2013Arg23_1c |LN612732.1|
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6.2
9.2
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99.9
64.6
99.9
79.2
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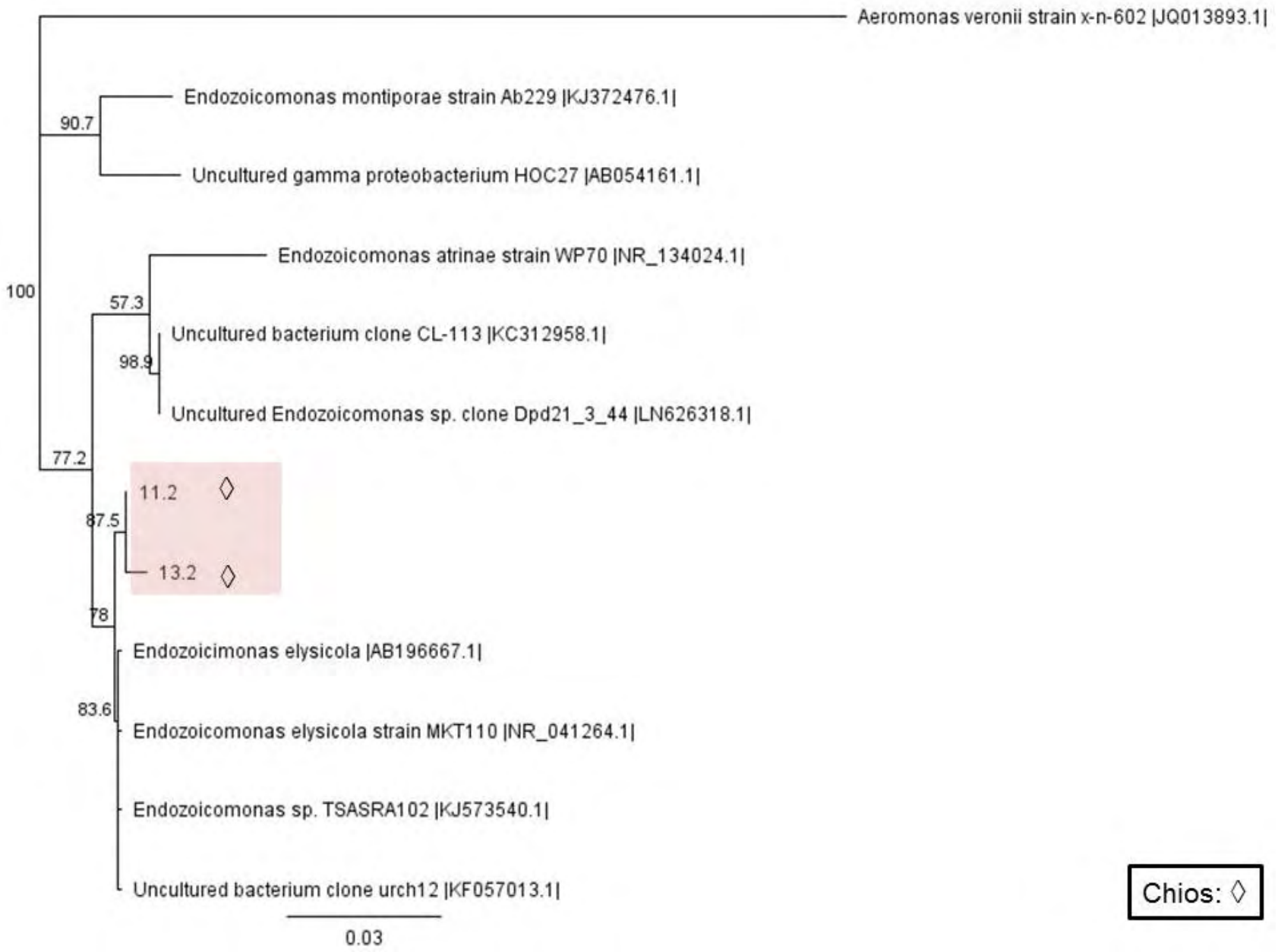
Astakos: ◦
N. Evoia: ●
Argolida: □
Vonitsa: ▪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57.3
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11.2
87.5
13.2
78
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Uncultured bacterium clone urch12 |KF057013.1|
0.03
Chios: ◊

Epitheliocystis agents in Greece

	Chlamydia	Ichthyocystis	Endozoicomonas
Taxonomic class	Chlamydiae	β-proteobacteria	γ-proteobacteria
# species found	3	2-3	1-2
# fish species infecting	3	4	2
Genome size	<1 Mb	~2.3 Mb	~5.8Mb
Life style	Obligate intracellular	Obligate intracellular	In transition

December outbreak in Souda

Sampling

2 samplings (December and January)

Fish gills (infected and uninfected), spleens

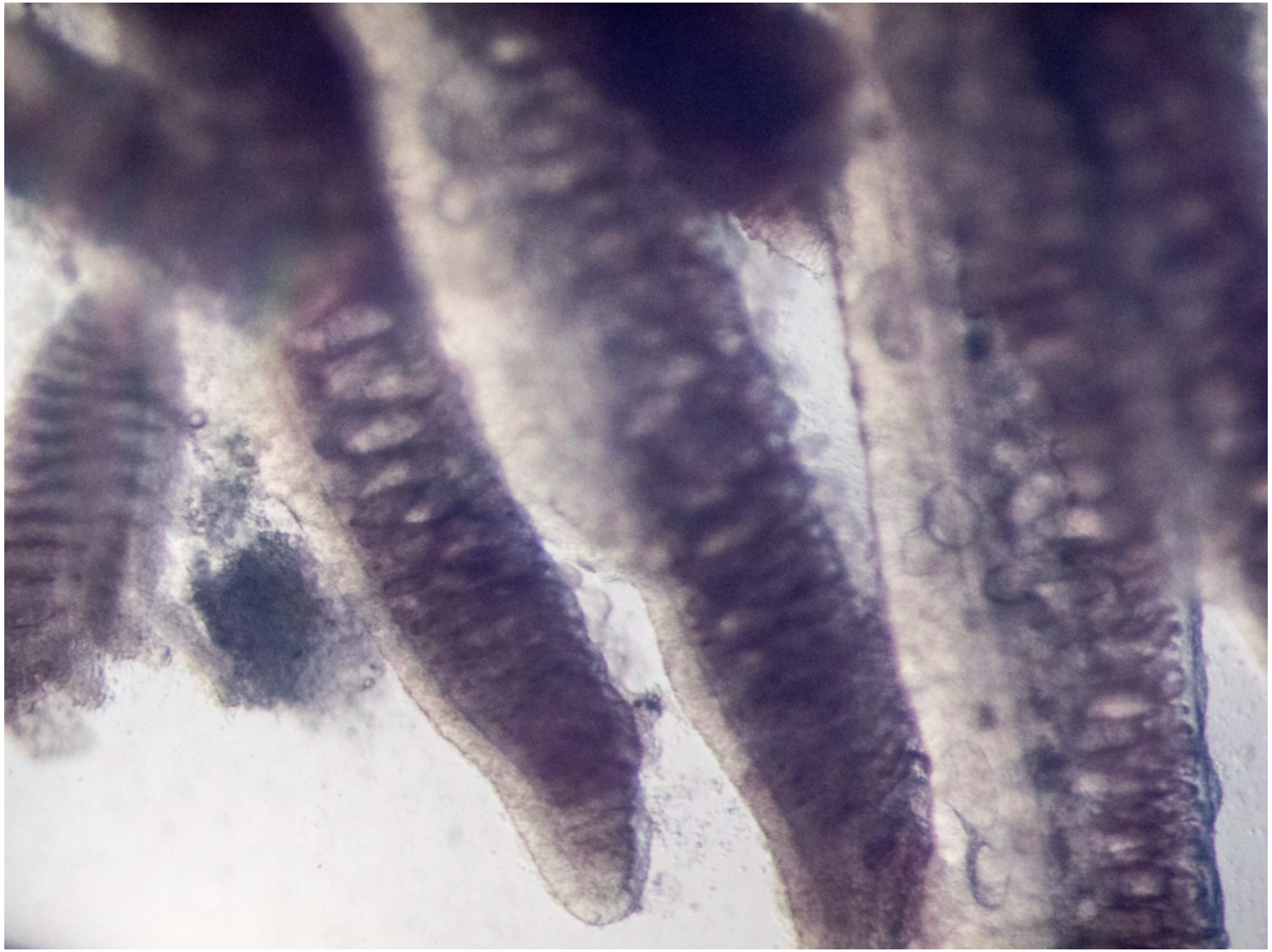
Commensal organisms (clams, oysters, mussels, anemone, ascidia, sponges)

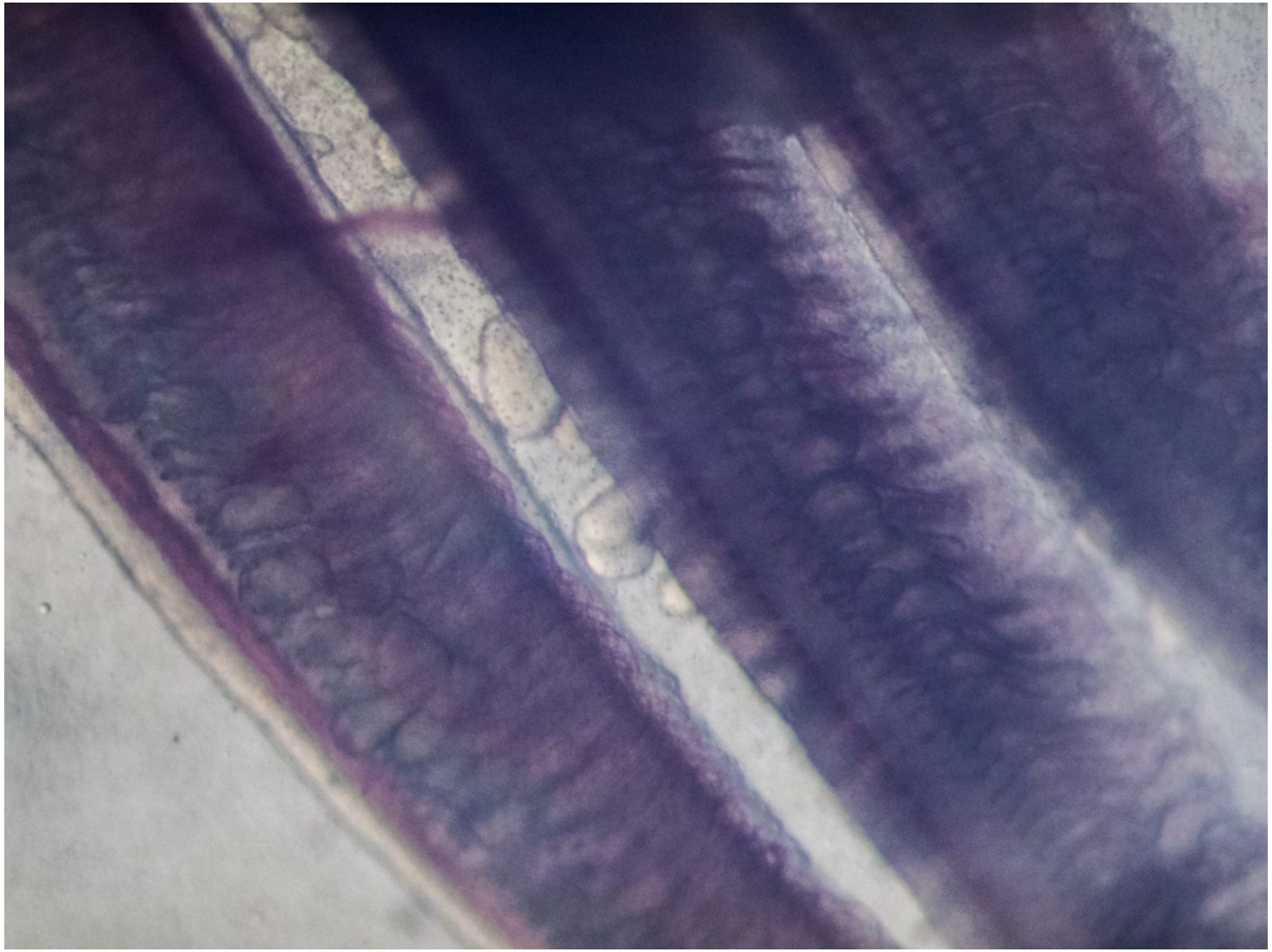
Samples for PCR, histology, TEM, genomic analysis, gene expression

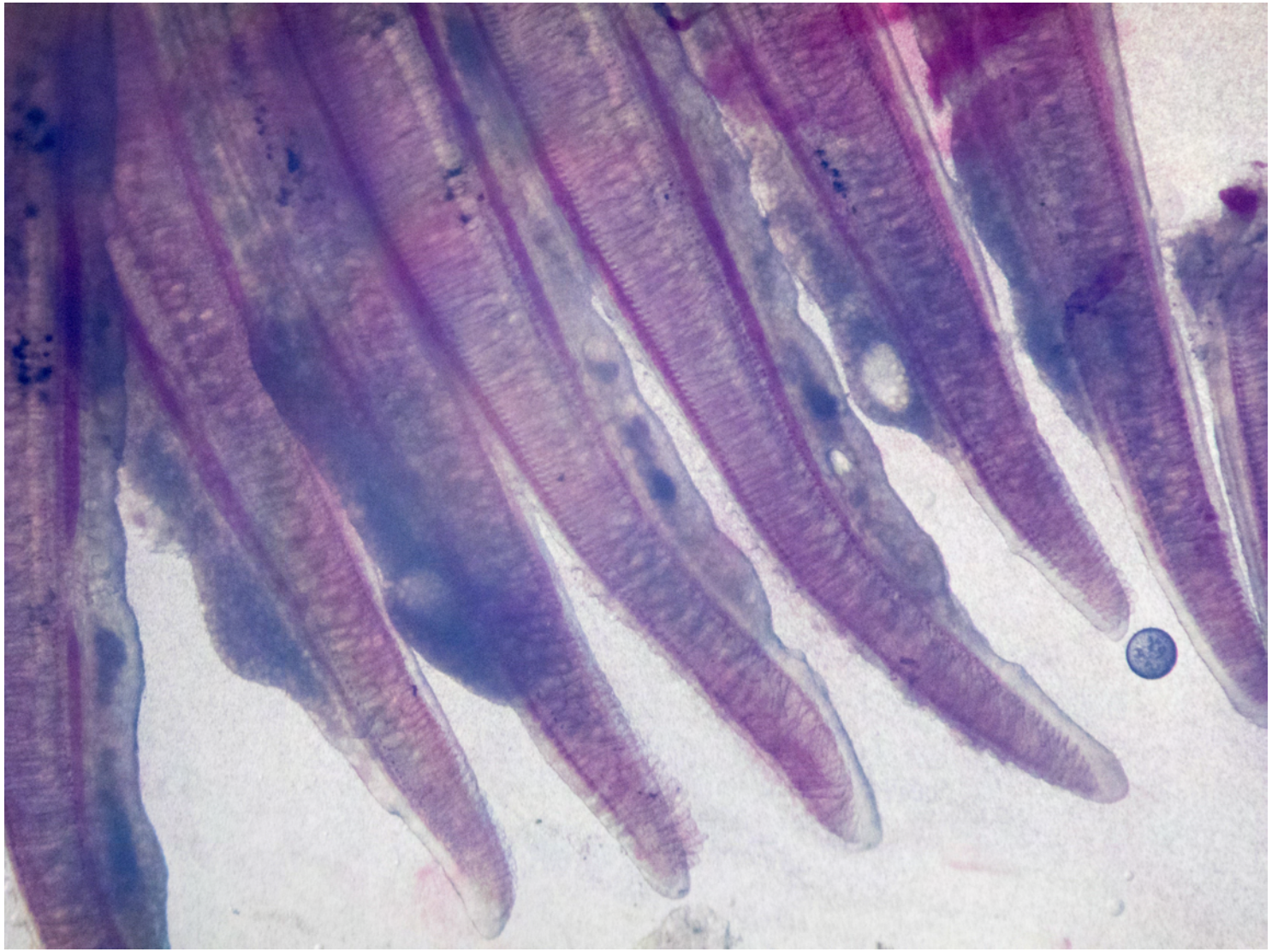
Fresh samples for infection experiments using zebrafish

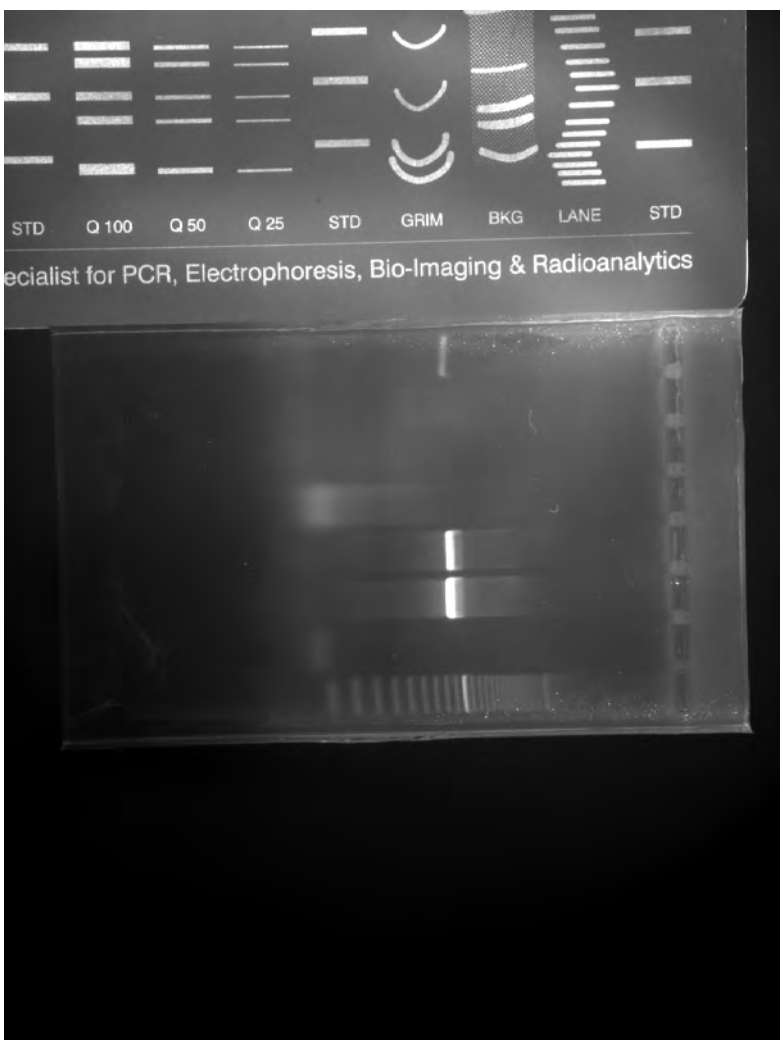
Samples sent to Chris Secombes for immune gene expression analysis

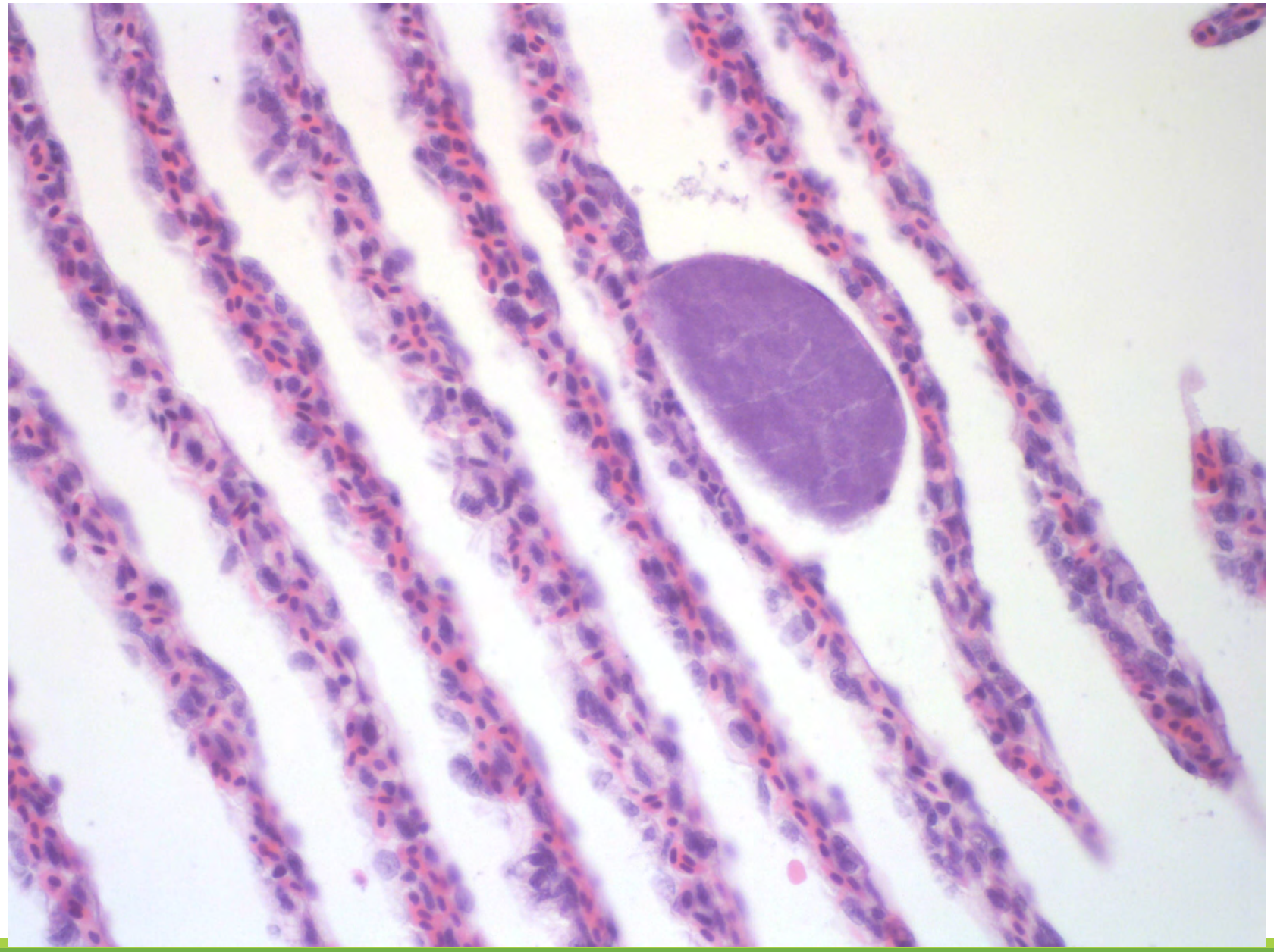
Whole Genome Sequencing

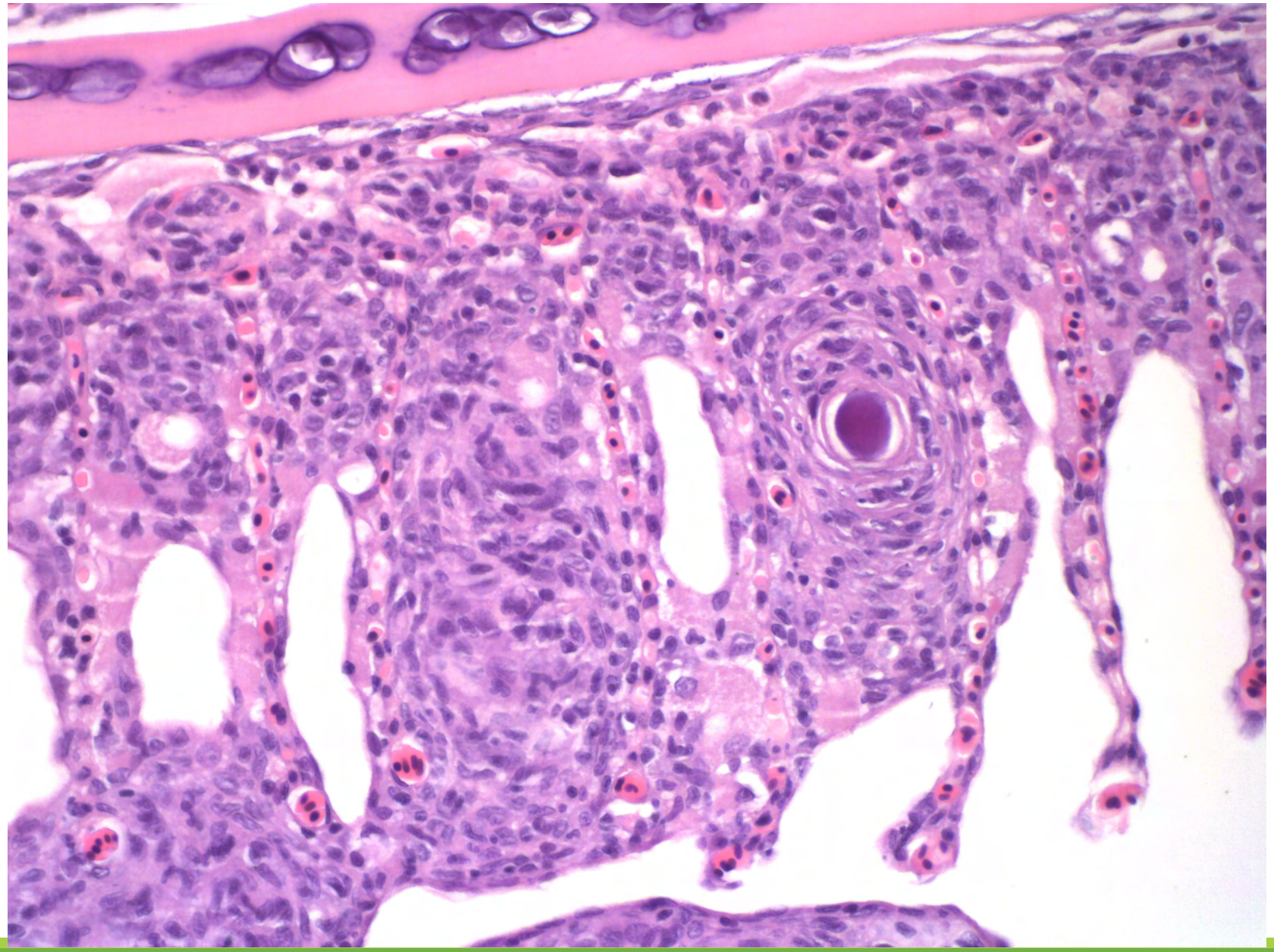


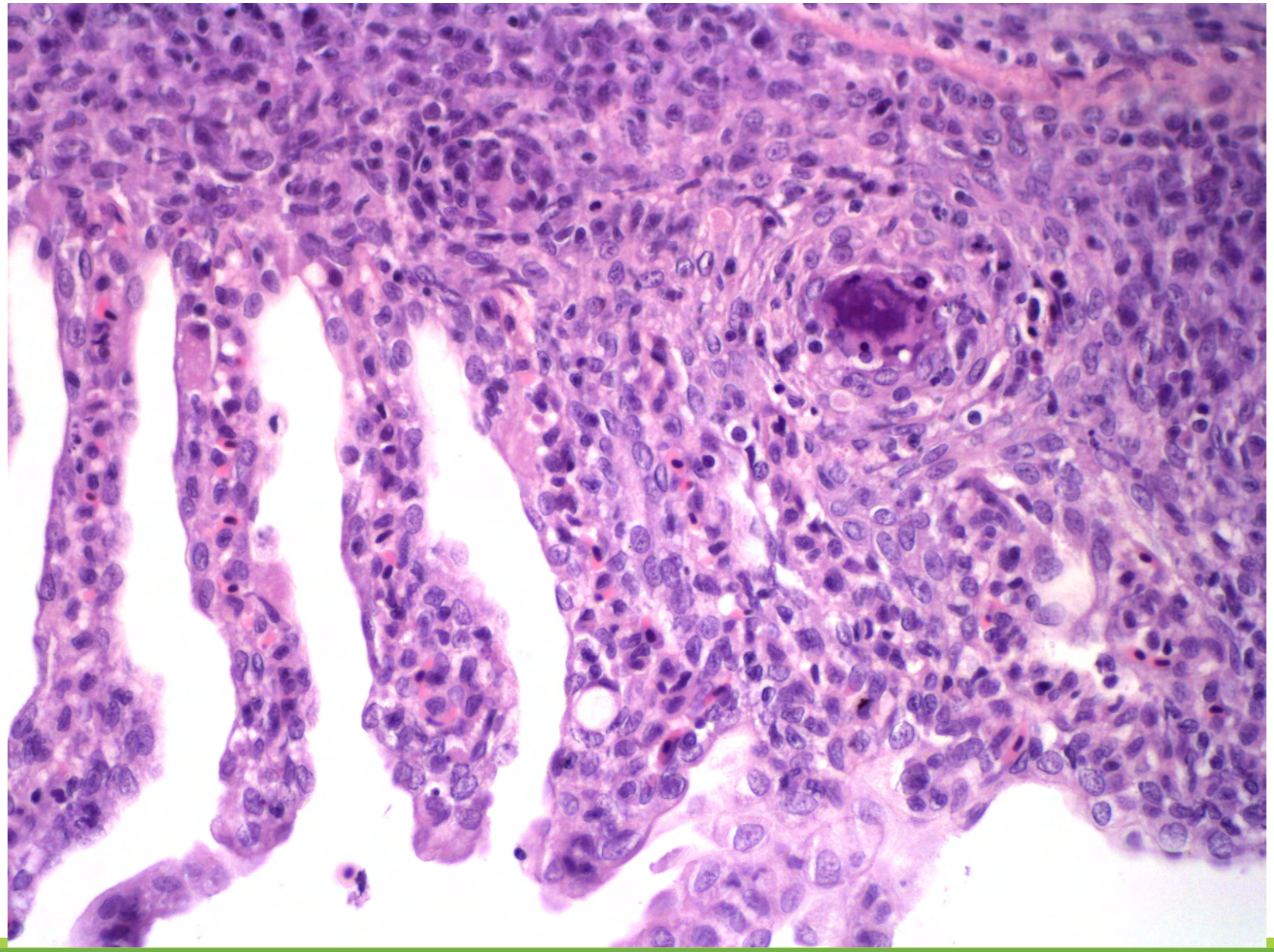


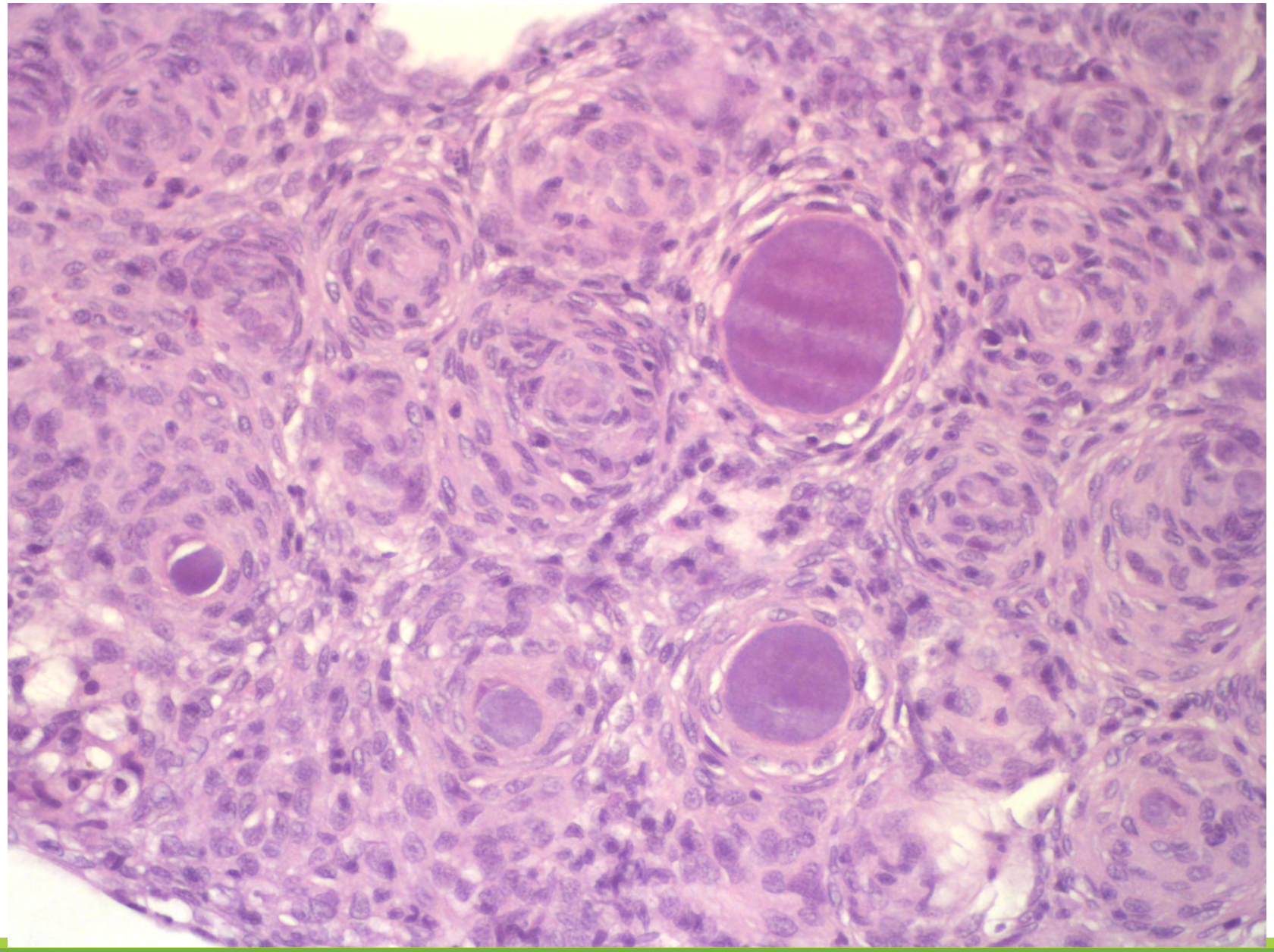



STD
Q 100
Q 50
Q 25
STD
GRIM
BKG
LANE
STD
ecialist for PCR, Electrophoresis, Bio-Imaging & Radioanalytics









Results so far

Confirmed epitheliocystis outbreak in cultured greater amberjack

Disease caused by *Ca.* Ichthyocystis sp. (possibly novel species)

Zebrafish infection unsuccessful

All commensal organisms examined are negative for the bacteria

…next steps

Whole genome sequencing

Phylogenetic analysis

Gene expression (fish for immune response, bacteria for virulence regulation

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