Emerging Gill Pathogens and their significance

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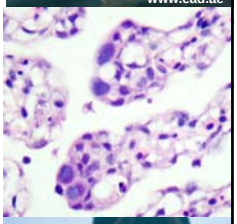
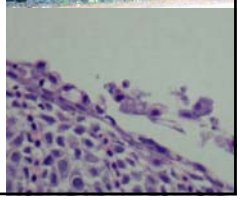
Gill Disease

- Broad term used to describe a complex condition
- Frequently multifactorial
- Combination of infectious agents and environmental challenges (e.g. phyto and zoo-plankton)
- **Very costly to the Industry!**

Costs & causes

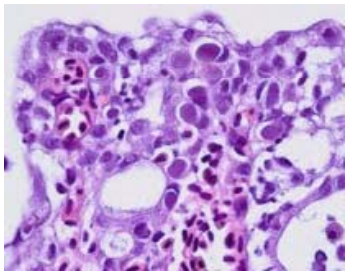
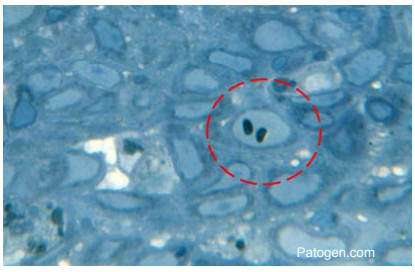
- Costs – mortality, mortality removal & disposal, growth
- Ireland - **AGD**, zoo- and phyto-plankton, epitheliocystis, other infectious causes
- Norway – "proliferative gill inflammation" (PGI) 15 to 20% marine farms (morts up to 40%), range of infectious agents involved
- Scotland – zoo- & phyto- plankton, PGI, epitheliocystis, **AGD**





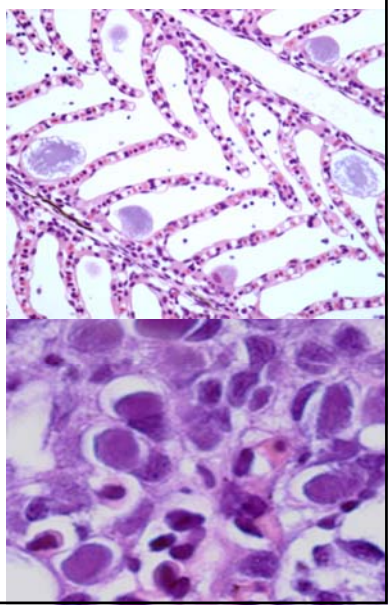
Emerging Gill Pathogens

- A novel non-chlamydial agent of epitheliocystis in Atlantic salmon
- A new microsporidean parasite of the gills

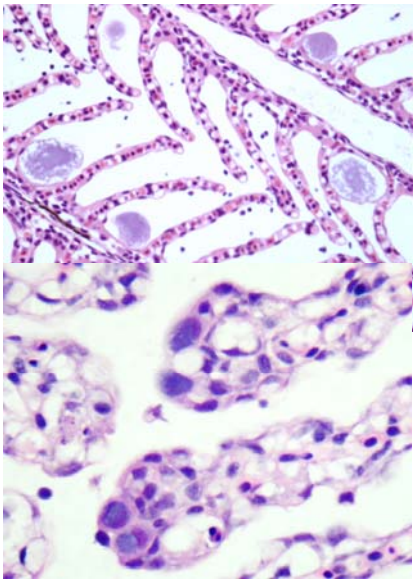
Epitheliocystis

- Infectious condition
- Characterised by intracellular cysts of bacteria
- Aetiology – Chlamydiae
- Benign Vs proliferative
- Significance?



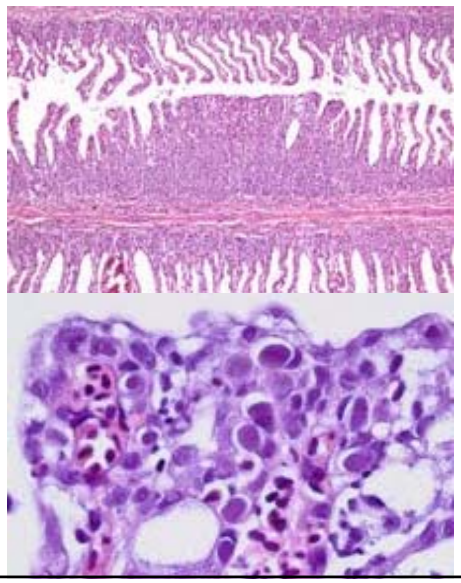
Epitheliocystis in Atlantic salmon

- '*Candidatus* Clavochlamydia salmonicola' - freshwater (Karlsen *et al.,* 2008)
- '*Candidatus* Piscichlamydia salmonis' – seawater (Draghi *et al.*, 2004)
- PGI - SW



PGI Longitudinal Study in Norway

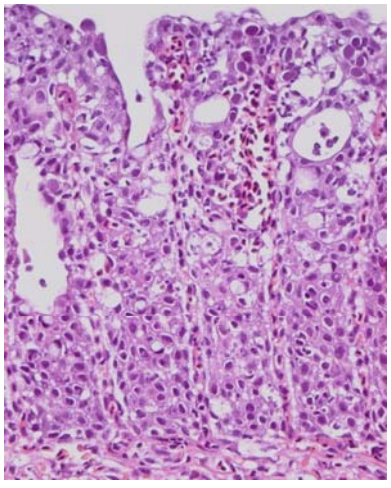
- Steinum et al. (2010)
- Proliferative gill inflammation
- Epidemiology, pathogenesis
- PGI & epitheliocystis
- Molecular detection of '*Ca*. P salmonis'



Results

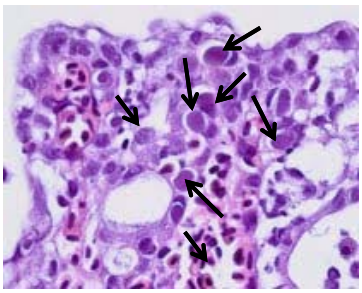
- Associations between:

- PGI & '*Ca*. P. salmonis'
- PGI & Epitheliocystis
- '*Ca*. P. salmonis' & Epitheliocystis??



Results

Ct Values
by qPCR

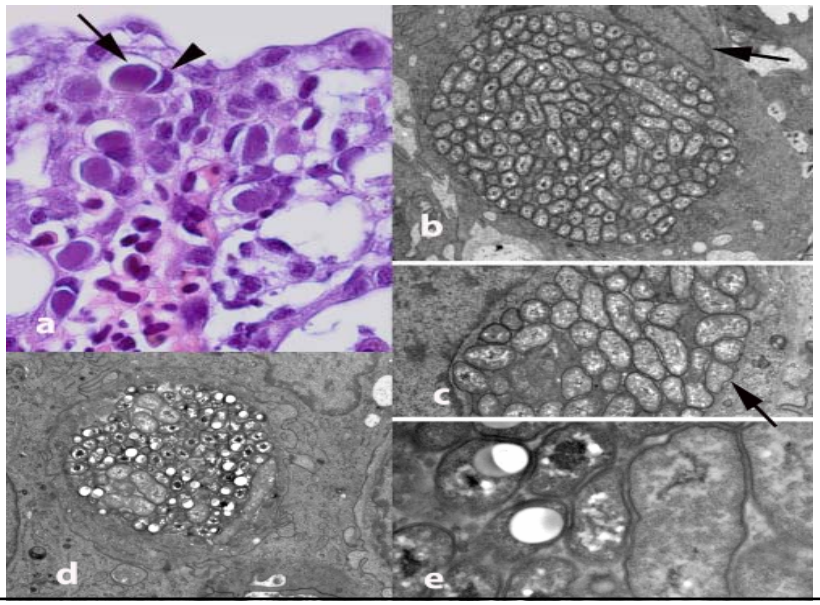


- Transmission electron microscopy
- Fluorescence *in situ* hybridization (FISH)

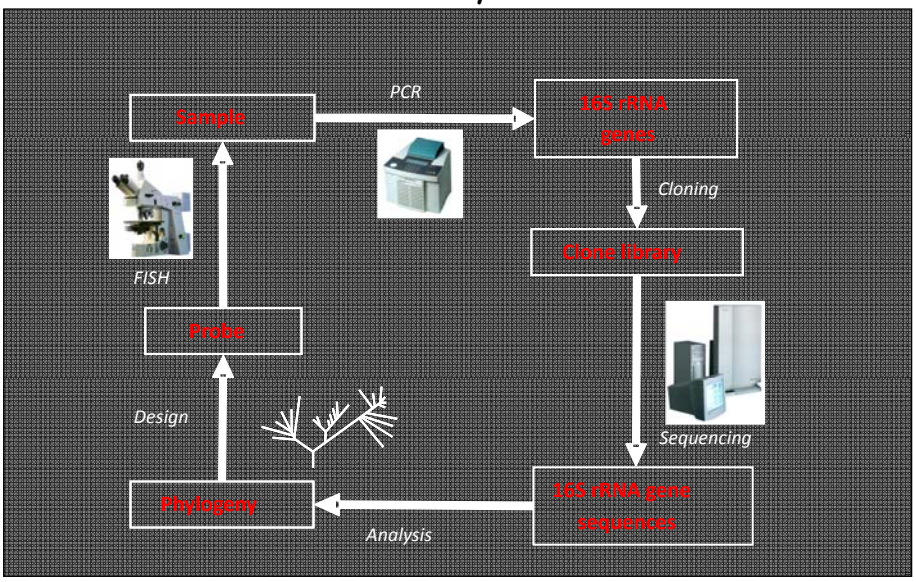
Transmission Electron Microscopy

- TEM of inclusions
- Observed forms were similar to reticulate and intermediate bodies of the chlamydial developmental cycle
- Elementary bodies were not observed

Transmission Electron Microscopy

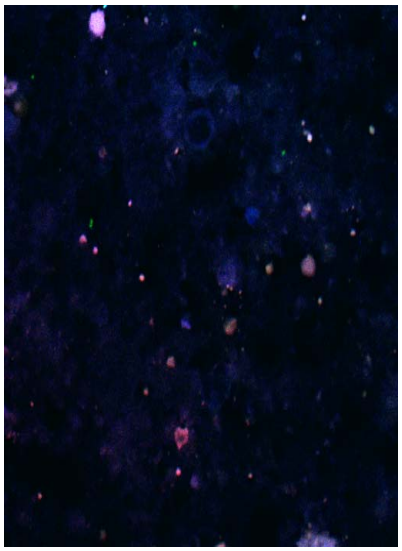


Use of FISH to identify the position of bacterial symbionts



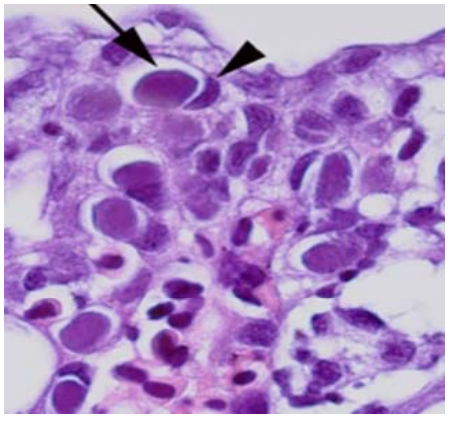
FISH for '*Ca*. P. salmonis'

- Multiple attempts on samples with ++ cysts & '*Ca*. P. salmonis'
- No signal - Why?
- Inadequate cluster for detection?
- Secondary or tertiary structure blocking the probe binding site?
- Conc. of rRNA too low?



What does this mean?

- Not '*Ca.* P. salmonis'??
- Another cyst forming bacterium?



DNA Isolation and PCR

Primer Type	Forward	Reverse
General bacterial primers	616V	1492R
Chlamydial primers	SigF2	SigR2
Primers specific for '*Ca*. P. salmonis'	Pisci183F	Pisci1353R

PCR Results

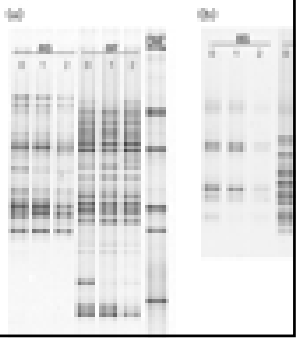
Primer Type	Quantities of Bacteria
General bacterial primers	High levels of bacteria (Average Ct. Value = 26)
Chlamydial primers	No or very little product (Ct. Value > 35)
Primers specific for '*Ca*. P. salmonis'	No or very little product (Ct. Value > 35)

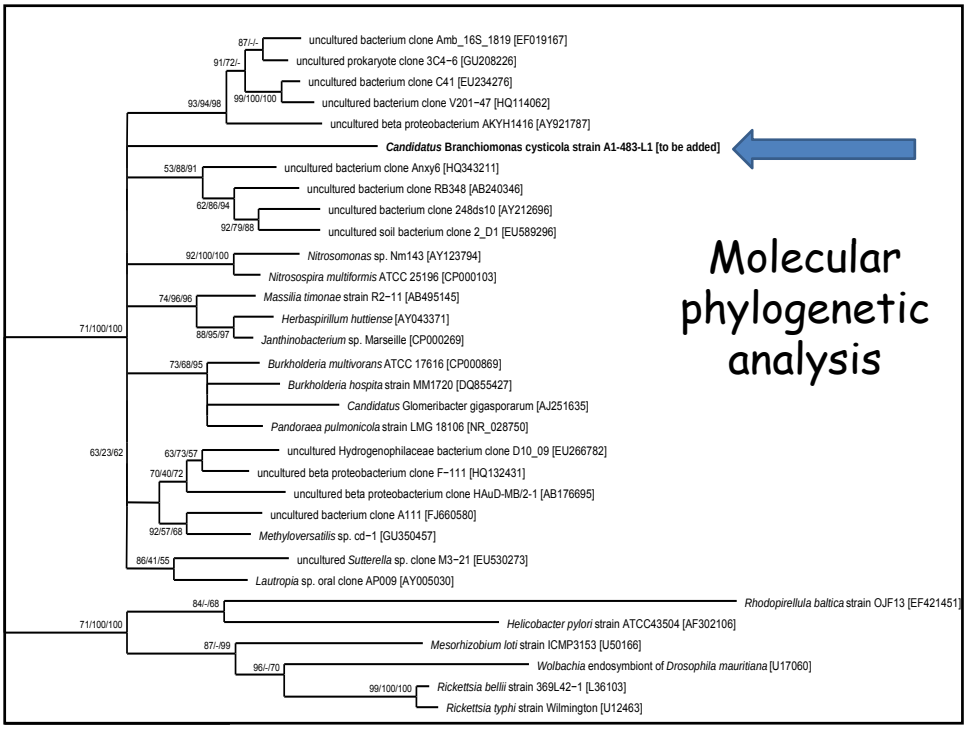
Cloning and Sequencing

- Small number of clones shared 99-100% identity with '*Ca*. P. salmonis'
- Abundant clone: 87% identity to *Janthinobacterium* and *Pandoraea*, members of the *Betaproteobacteria*
- Highly related or identical to the *Undibacterium*-like sequences

(Steinum *et al*., 2009)

DGGE Bands



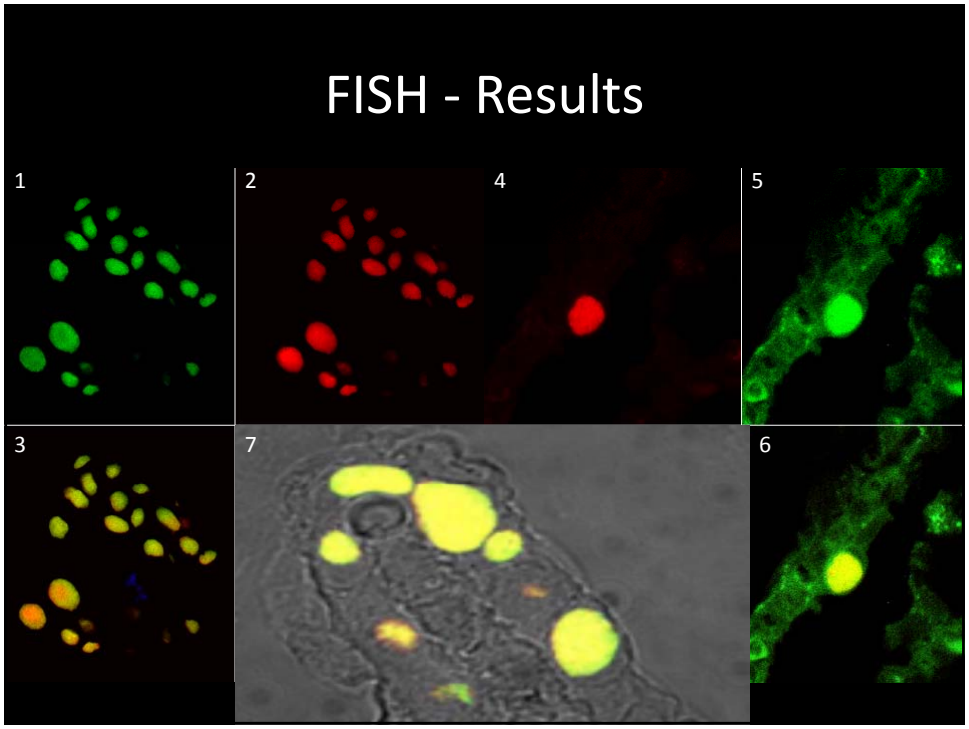


Molecular phylogenetic analysis

Fluorescence *in situ* Hybridisation

Probe Type	Probe ID	Fluorescent dye	Colour
General Bacterial Probes	EUB-MIX	Fluos	Green
Probe specific for '*Ca*. B. cysticola'	A1/A4-129	Cy3	Red

FISH - Results



Conclusions

- We have identified a novel agent responsible for causing bacterial inclusions or epitheliocysts in the gills of fish
- This bacterium is a *Betaproteobacterium* and we propose the name '*Candidatus* Branchiomonas cysticola'
- Significance? Prevalence? Geographical distribution?
- Position and role of '*Ca.* P. salmonis' ??

A Microsporidean Parasite of Atlantic salmon:

(Paranucleospora theridion / Desmozoon lepeophtherii)

A new 'phenomenon' in the Norwegian aquaculture industry

Microsporideans

- Obligate intracellular spore forming parasites
- Direct life-cycles (?)
- Can cause serious disease in cultured fish
- Clinical signs depend on organ affected
- To date not a significant disease challenge in the farming of Atlantic salmon

Paranucleospora theridion / Desmozoon lepeophtherii

- First described in sea lice by Freeman et al. (2003)
- First discovered in Atlantic salmon with gill disease in Norway in 2008 (Nylund et al. 2009)
- Also described in salmon by Freeman and Sommerville in 2009
- Present in *Salmo trutta, Oncorhynchus mykiss, L. Salmonis, C. Elongatus*

Paranucleospora theridion

- Occurs in connection with gill disease – PGI
- Also PD, HSMI and CMS
- Present in high percentage of healthy fish in low quantities
- Present in higher quantities in diseased fish
- Primary or secondary cause of the above diseases??

Life-Cycle

Horizontal Transmission??

Reproduction in salmon (Also *S. salar* and *S. Trutta*)

Reproduction in sea-lice ??(*L. salmonis* and *C. elongatus*) / Dead-end host

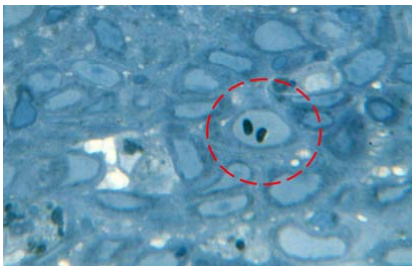
Nauplii
Copepodid

Epidemiology

- *P. theridion* - throughout Norway but highest prevalence in Southern Norway (PGI, PD)
- Highest losses from September to February
- *P. theridion* transmitted directly from sea lice to salmon (?)
- No evidence of fish-fish transmission
- Recently reported in Ireland and Scotland

Experimental Challenge

- Infection experiments with *P. theridion* have produced over 50% mortality in some groups (Are Nylund, Bergen, Unpublished results)
- No details of these challenge experiments currently available
- ???



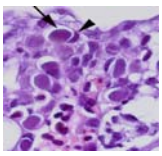
P. theridion – Significance

- *P. theridion* is present in all tissues and organs of infected salmon, not just gills
- Presence has been associated with pathology more so in the gills then other body tissues – most significant as a gill pathogen?
- Substantially higher loads in gills of fish with PGI (Steinum et al, 2010)

P. theridion – Significance

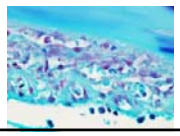
- High prevalence (80%), but not always associated with disease
- Opportunisitic microsporideans can be present in healthy humans at low levels (e.g. *Enterocytozoon bieneusi*)
- Life threatening infections in immuno-compromised individuals
- Role in disease susceptibility and development?

PGI



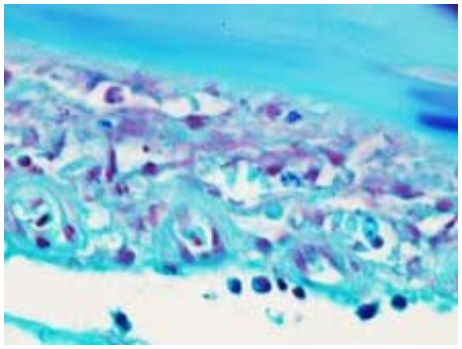
Suppression of immunity

Proliferation of *P. theridion* (reticuloendothelial system)



Treatment / Control

- Currently no available treatments
- Monitoring salmon lice and correct sea lice control may be effective against the microsporidean..
- Significance still under debate



Novel Pathogens?

- Molecular techniques have greatly enhanced our ability to find new 'pathogens'
- Care required when interpreting the significance of these novel invaders
- Primary causers of disease, or simply secondary multiplication in diseased tissues?
- Must use molecular techniques in conjunction with histopathology for meaningful interpretation

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TRINITY COLLEGE DUBLIN

Thanks for your attention!

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