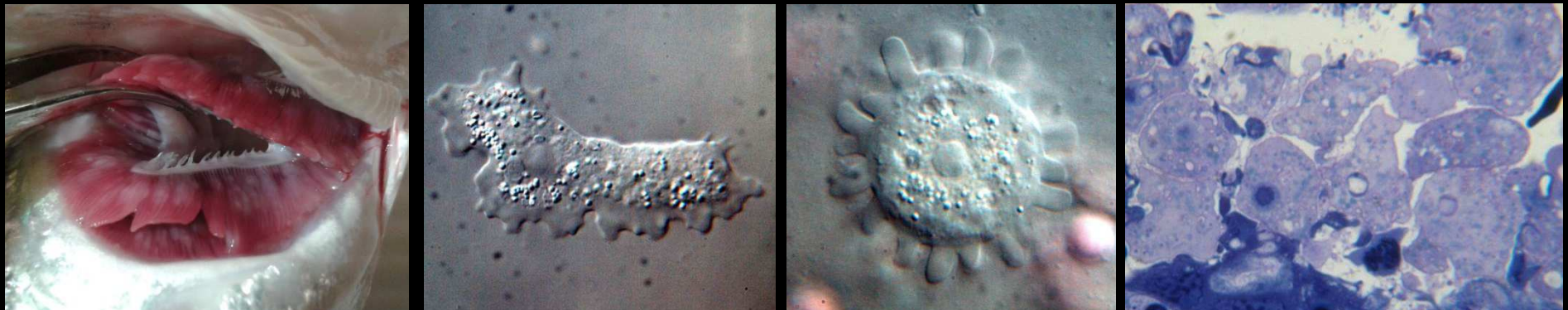


FHF-prosjekt:901053

Variasjon mellom kloner av *Paramoeba perurans*.

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Prosjektet består av følgende arbeidspakker:

Arbeidspakke I (2015 – 2016, Avsluttet):

Fenotypisk karakterisering av amøbekloner og etablering av nedfrysningsmetode.

Arbeidspakke II (2015 – 2017, Avsluttet):

Smitteforsøk og cellekulturstudier for å kartlegge vertstropisme og virulens hos utvalgte kloner.

Arbeidspakke III (2015, Avsluttet):

Fullgenomsekvensering av kloner av *P. perurans*.

Arbeidspakke IV (2016 - 2017):

Genotyping av kloner.

Arbeidspakke V (2017-2018):

Kartlegging av geografisk og historisk utbredelse, identifisering av virulensmarkører, og variasjon hos isolater fra oppdrett og villfisk.

Arbeidspakke VI (2017):

Utvikling av real time (sanntids) polymerasekjedereaksjon (RT PCR) for rask og sikker identifisering av arter (*Paramoeba* spp) og virulente kloner av *P. perurans*.

Arbeidspakke II: Tema virulens

- Et smitteforsøk gjennomført høst 2014-vår 2015
- To smitteforsøk gjennomført høst 2015
- To smitteforsøk gjennomført høst 2016
 - Testing av kloner av *P. perurans*
 - Temperatur effekter
 - Dyrkingsbetingelser (passasjer, medium (bakterier), hyppig splitting etc.)
 - Vertspopulasjoner (betydning av variasjon hos vert)
 - Spredningsevne (shedder til ko-habitant)
- **Analyse resultater:**
 - Atferdsendringer (apati, nedsatt mobilitet)
 - Dødelighet (forsøkene er i utgangspunktet laget for å unngå dødelighet)
 - Kondisjonsfaktor
 - Klinikk (gross patologi)
 - Gjellescore (0 til 5)
 - Histopatologi (gjelleforandringer)
 - Real time RT PCR (vev og vannprøver)

Gill score system for estimation of the severity of AGD

<i>Infection level</i>	<i>Gill score</i>	<i>Description</i>
Clear	0	No sign of infection and healthy red color.
Very Light	1	1 white spot, light scarring or undefined necrotic streaking
Light	2	2-3 spots/small mucus patch
Moderate	3	Established thickened mucus patch or spot groupings up to 20% of gill area
Advanced	4	Established lesions covering up to 50% of gill area
Heavy	5	Extensive lesions covering most of the gill surface

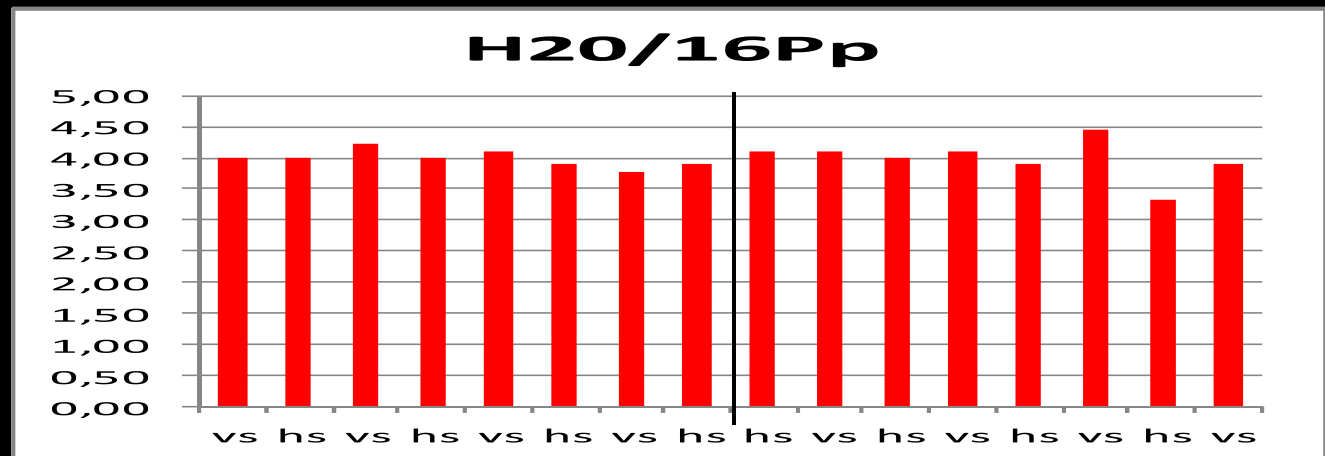
(Taylor et al., 2009)

Gjellescore

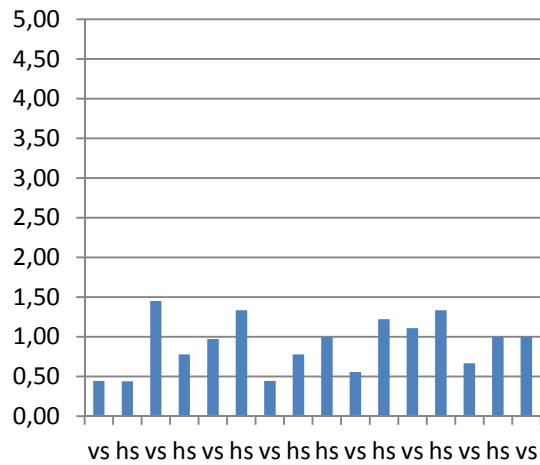
8 gjellebuer fra 10 fisk i hvert uttak

Sheddere	uttak 3	Fisk/kode	vekt	Lengde	GJELLESKORE															
					Venstre side av fisken								Høyre side av fisken							
Dato	Dag				1		2		3		4		1		2		3		4	
29.09.2016					vs	hs	vs	hs	vs	hs	vs	hs	hs	vs	hs	vs	hs	vs	hs	vs
H20/16Pp																				
29.09.2016	22dpi	K8-1	99,3	21	4	4	5	3	4	5	4	4	4	5	4	5	3	5	3	4
29.09.2016	22dpi	K8-2	80,1	18,5	4	3	3	4	4	2	3	2	4	4	4	5	4	5	4	5
29.09.2016	22dpi	K8-3	76,6	18,5	4	4	5	5	5	4	5	5	5	5	5	5	5	5	4	5
29.09.2016	22dpi	K8-4	93,2	21	5	5	5	5	5	5	5	5	4	5	5	5	3	5	3	4
29.09.2016	22dpi	K8-5	98,1	21	4	5	5	4	4	4	4	4	4	3	3	4	4	4	4	4
29.09.2016	22dpi	K8-6	77,5	19,5	4	3	3	3	3	3	3	4	4	4	3	2	4	4	3	3
29.09.2016	22dpi	K8-7	91,7	20	4	4	4	4	4	4	3	3	4	4	4	4	4	4	3	3
29.09.2016	22dpi	K8-8	87,8	21	4	4	4	3	4	4	4	4	4	3	4	3	4	4	3	3
29.09.2016	22dpi	K8-9	90	20	3	4	4	5	4	4	3	4	4	4	4	4	4	4	3	4
29.09.2016	22dpi	K8-10	88,3	20,1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4

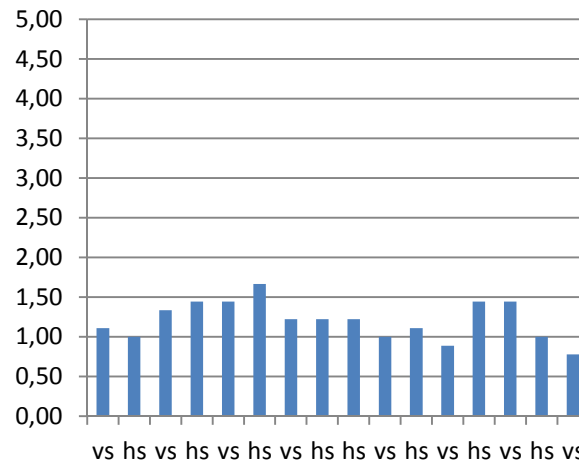
Figuren viser gj.sn. gjellescore for venstre og høyre side av alle gjellebuene (8) på begge sider.



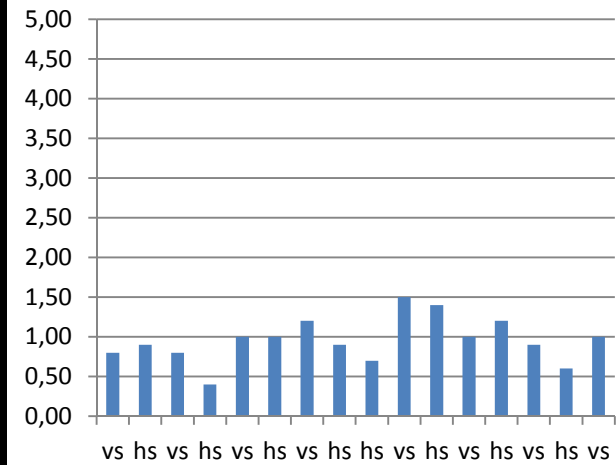
H02/13Pp



H20/16Pp

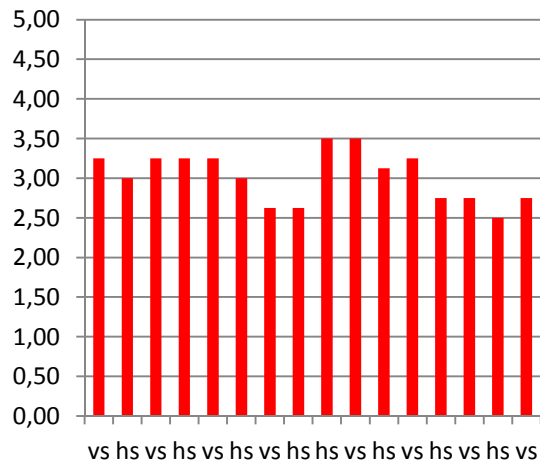


R18/15PpB



Temperatur effekt: 12 °C vs 16 °C

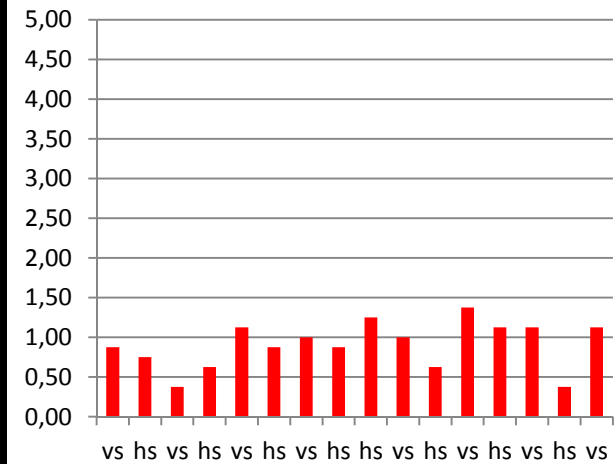
H02/13PpA



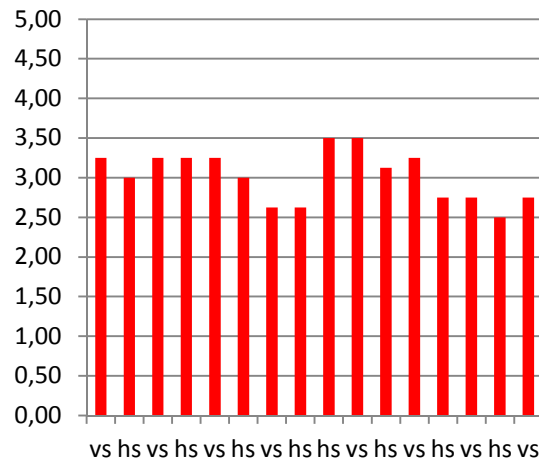
H20/16Pp



R18/15PpA

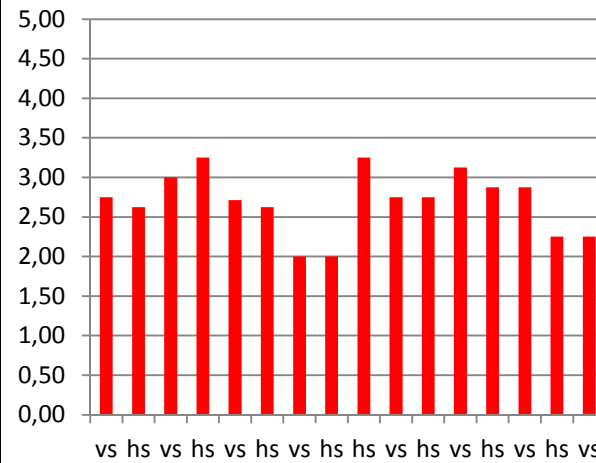


H02/13PpA



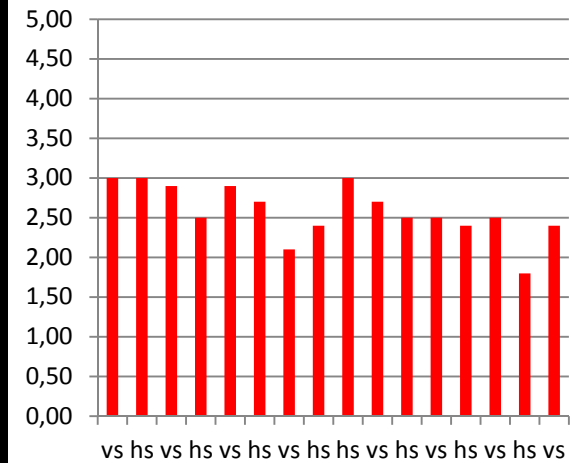
Splitting før
smitte

H02/13PpB



Vedlikeholds-
dyrking før smitte

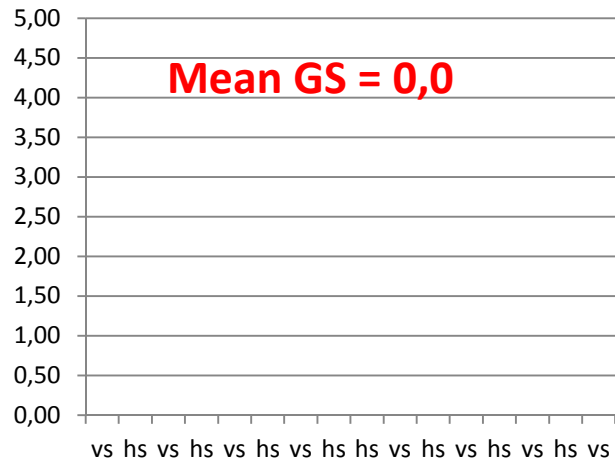
H02/13PpC



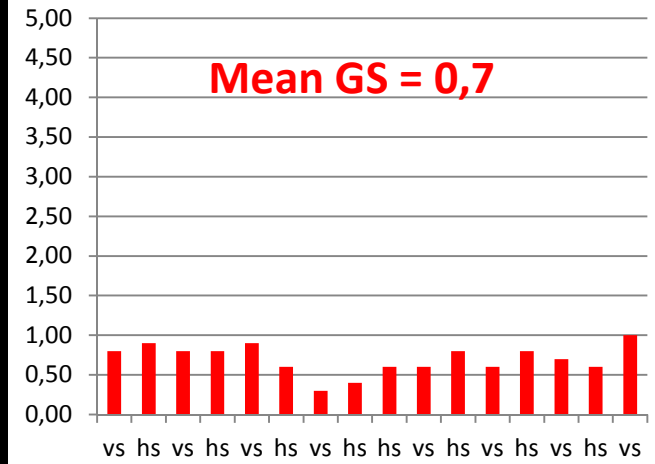
Hyppig splitting
over lengre tid før
smitte

Dyrkings-
betingelser

H03/14PpA

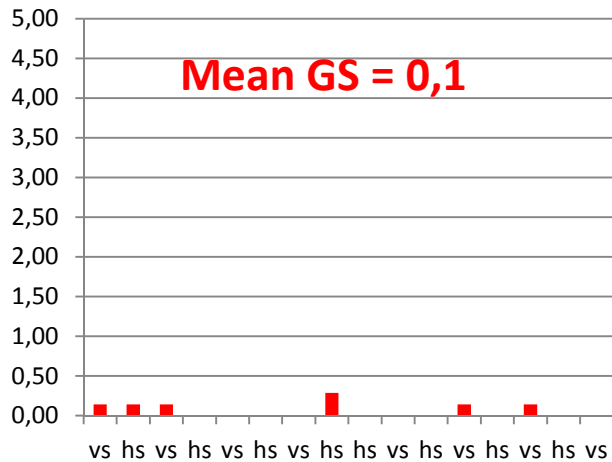


ST19/15PpA

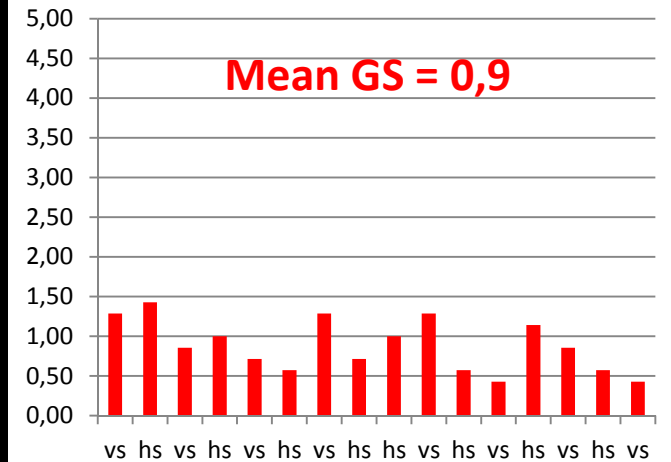


Betydning av bakterieflora i dyrkingsmedium

H03/14PpB



ST19/15PpB

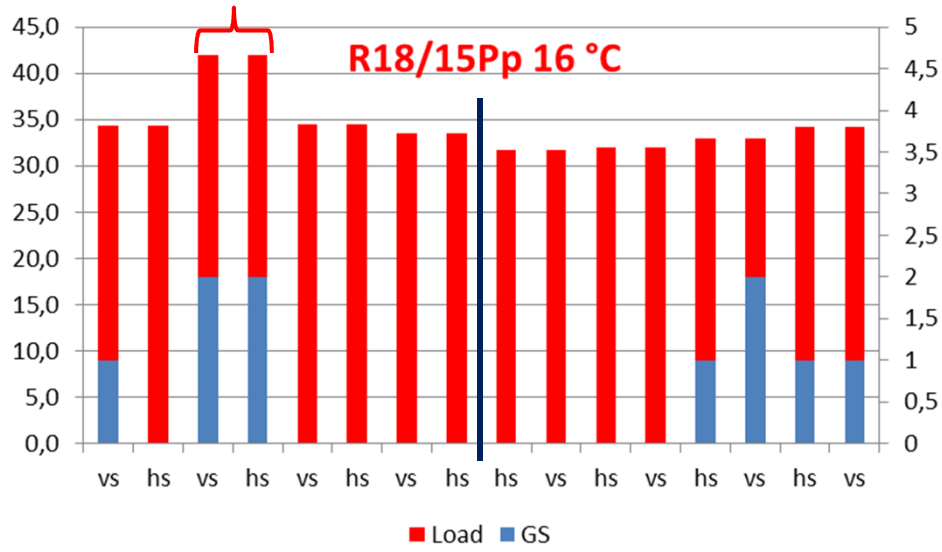




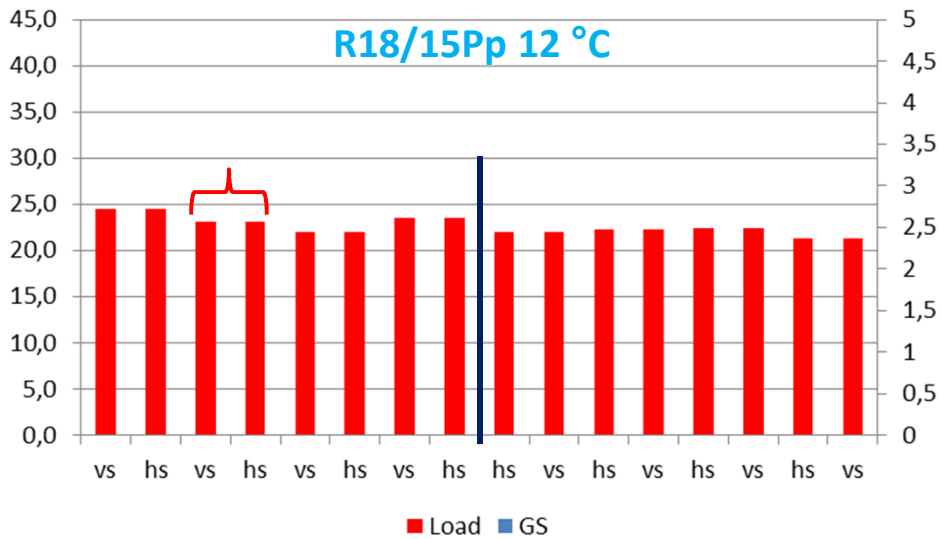
Uttak for real time RT PCR
analyser:

2 gjellebue i «knekken»

Hvor representativt er dette
uttaket for alle gjellebuene?

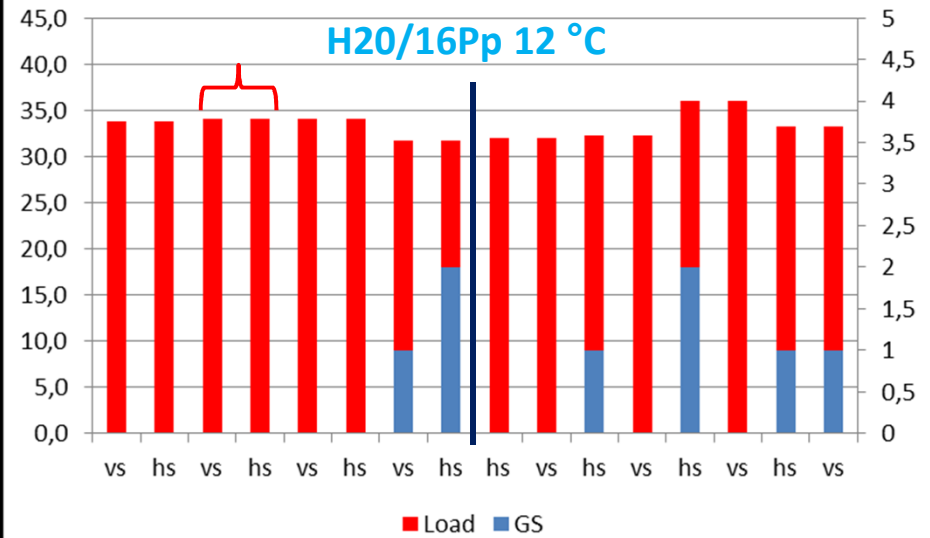


Maximum load = 45
 Minimum load = 0



Ct verdi vs Gjellescore

Load vs GS

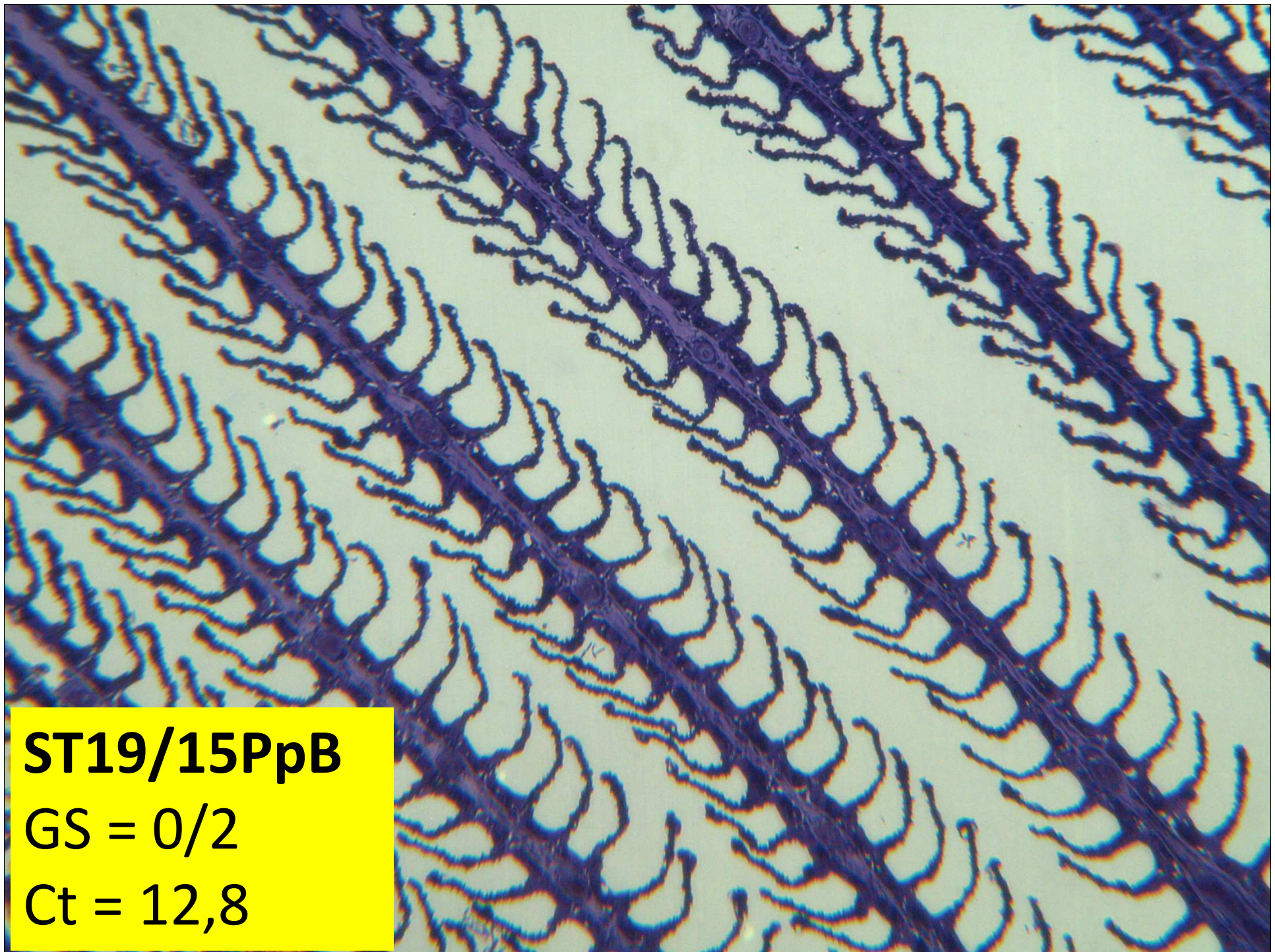


Venstre Y-akse = load
 Høyre Y-akse = GS

Patologi

Viktige virulensfaktorer hos *Acanthamoeba* spp.

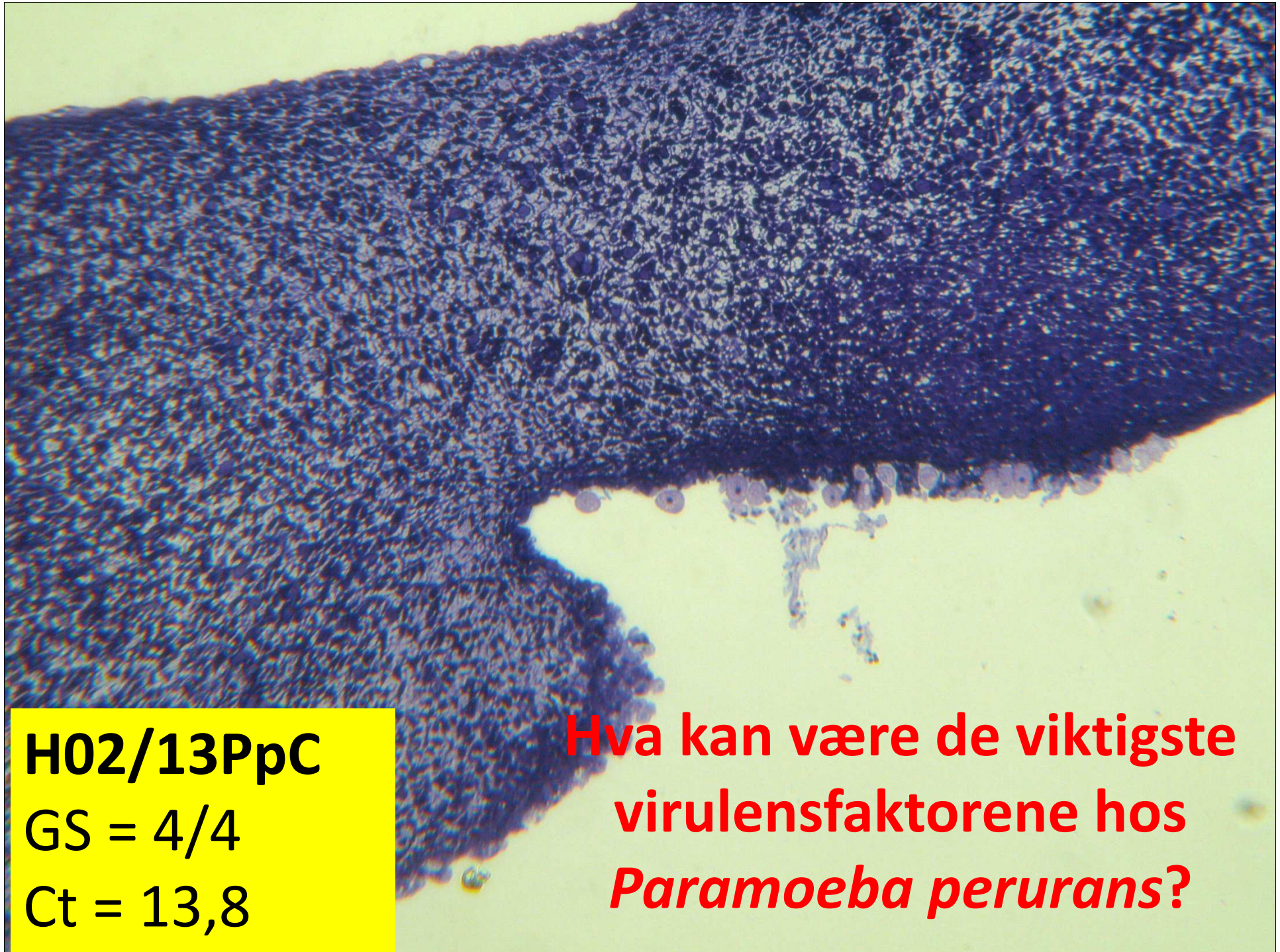
- Ødeleggelse av epitelceller:
 - Cytolyse, fagocytose, apoptose
 - Separering av celler i epitel, vakuolisering
- Årsaker til endringene
 - Mekaniske skader forårsaket av trofozoittene, og
 - Enzym aktivitet (ekstracellulære produkter, ECP)
 - Serin proteaser
 - Metalloproteaser
 - Elastaser
 - Cyctein proteaser
 - fosfolilapser
 - Cytotoksiske proteaser



ST19/15PpB

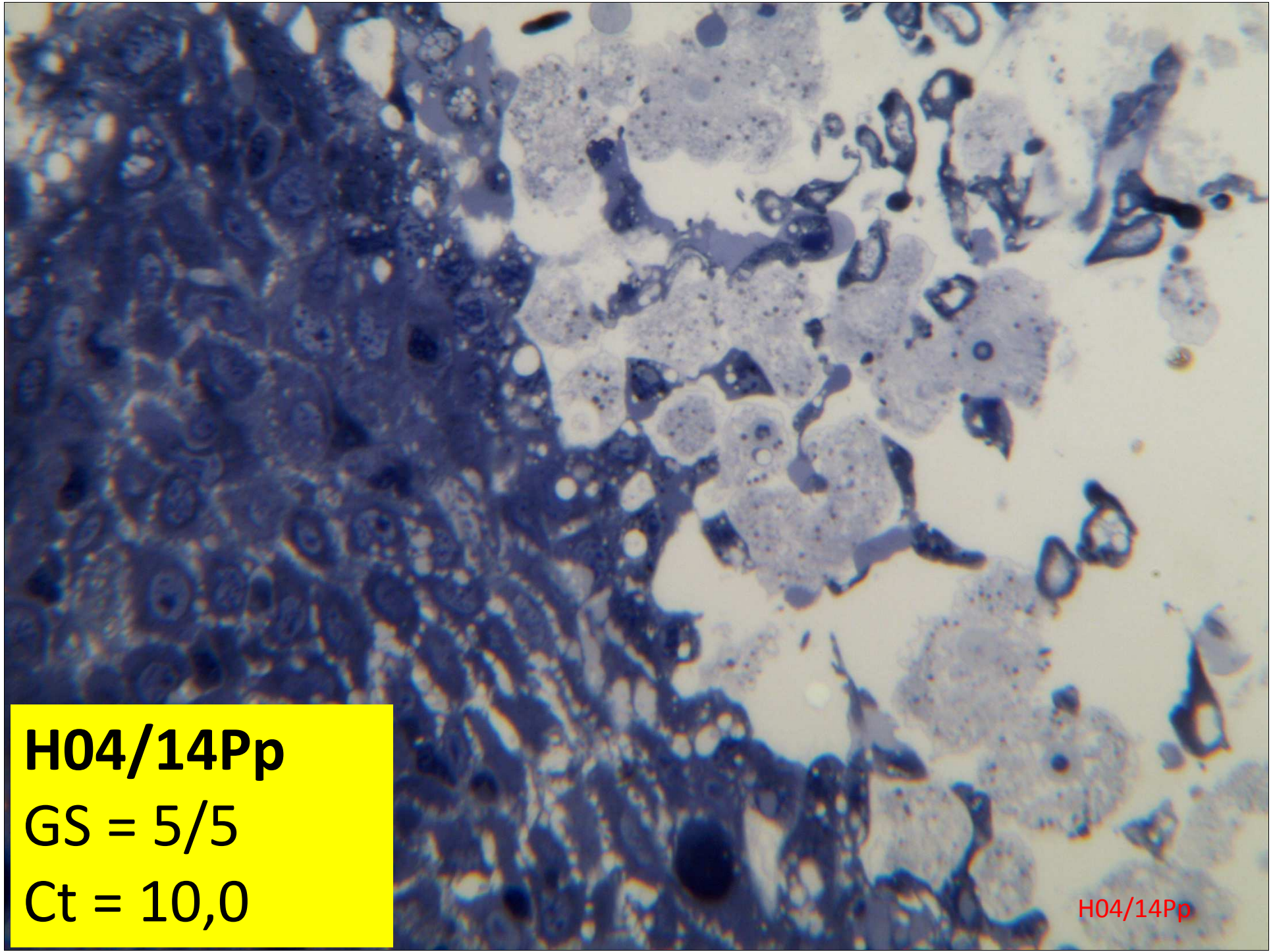
GS = 0/2

Ct = 12,8



H02/13PpC
GS = 4/4
Ct = 13,8

Hva kan være de viktigste virulensfaktorene hos *Paramoeba perurans*?



H04/14Pp
GS = 5/5
Ct = 10,0

H04/14Pp

Paramoeba perurans

- påvirkning på laksegjeller

- Enzymatisk påvirkning

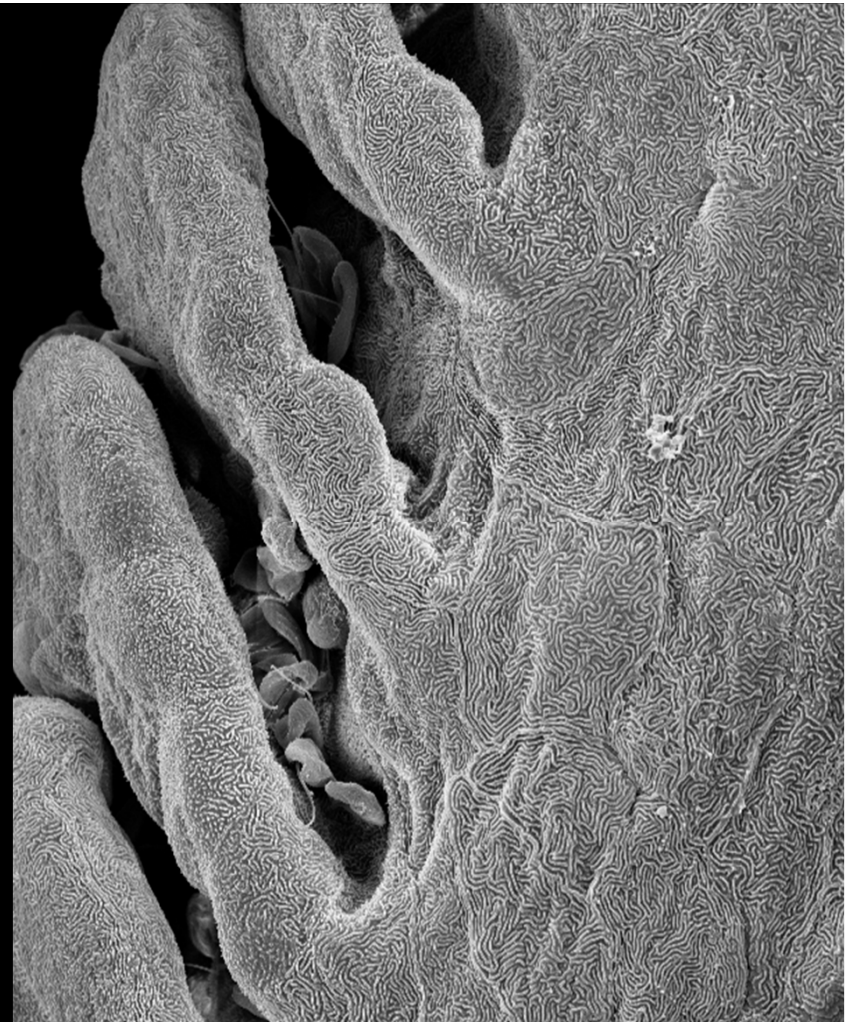
- Proteaser

- Lipaser

- Mekanisk påvirkning

- Reduserer den respiratoriske overflaten

- Ødelegger strukturen til gjelleepitelcellene



Konklusjon arbeidspakke II

- Stor variasjon i virulens
- Virulens påvirkes av:
 - Temperatur
 - Vert
 - Bakterier?
- Spredningshastighet avhengig av temperatur
- Virulensmekanismer:
 - Proteaser og lipaser (ekstracellulære enzymer)
 - Mekanisk påvirkning
- Vertsresponser:
 - Øking i antall mukusceller
 - Epitelcellehyperplasi
 - Nekroser
- Tap av respiratorisk overflate og redusert blodgjennomstrømming i gjellene

Arbeidspakke IV, 2016-2017

Oppstart høst 2016

A. Identifisering av virulens markører eller virulens gener (C. Trösse & D Pistone).

B. Identifisering av genetisk variasjon (VNTR & MLST analyser) som kan brukes i spredningsstudier og til kartlegging av geografisk variasjon (C. Trösse & D Pistone).

Gener hos *Perkinsella* sp. fra *P. perurans*

Vektlegging av
variasjon mellom
kloner
Genetisk variasjon



Name	Length	Description
H03/14Perk - 4134	2828	rRNA
perkins-like	549	R18/15Perk
Plik-B081113	392	
R18/15Per - xxx	1204	heat shock protein 90.
R18/15Perk - 10834	3006	hypothetical protein, conserved.
R18/15Perk - 12448	1519	putative alpha tubulin.
R18/15Perk - 15120	5974	RNA polymerase I second largest subunit.
R18/15Perk - 16825	2616	hypothetical protein.
R18/15Perk - 17123	1018	18S rRNA.
R18/15Perk - 17372	2186	heat-shock protein hsp70, putative.
R18/15Perk - 17959	1453	beta-tubulin (tubB) gene.
R18/15Perk - 17959x	1465	beta-tubulin (tubB) gene.
R18/15Perk - 18113	320	trans-spliced leader sequence SL.
R18/15Perk - 18279	1309	trans-spliced leader sequence SL.
R18/15Perk - 18279x	1412	trans-spliced leader sequence SL.
R18/15Perk - 18279xxxx	1970	trans-spliced leader sequence SL.
R18/15Perk - 18593	931	rRNA.
R18/15Perk - 18647	433	28S rRNA.
R18/15Perk - 18665	731	18S rRNA.
R18/15Perk - 18816	650	28S rRNA.
R18/15Perk - 19095	2084	putative heat shock 70 kDa protein, mitochondr
R18/15Perk - 19468	1478	adenosylhomocysteinase.
R18/15Perk - 19528	953	40S ribosomal protein S6 partial.
R18/15Perk - 19608	652	18S rRNA.
R18/15Perk - 20046	889	60S ribosomal protein L2 partial.
R18/15Perk - 21527	1320	eukaryotic initiation factor 4a, putative.
R18/15Perk - 21785	400	hypothetical protein.
R18/15Perk - 22250	1082	cytochrome c oxidase subunit IV.
R18/15Perk - 22300	1997	malic enzyme.
R18/15Perk - 23364	2106	putative acetyl-CoA synthetase.
R18/15Perk - 25440	695	actin I partial.
R18/15Perk - 26167	99	Leishmania ikke identifisert.
R18/15Perk - 27561	706	GTP-binding protein 1.
R18/15Perk - 2913	975	oligosaccharyl transferase subunit.
R18/15Perk - 2928	1122	aminotransferase.
R18/15Perk - 29356	301	hypothetical protein.
R18/15Perk - 3657	209	Trypanosoma ukjent.
R18/15Perk - 5717	1105	protein kinase.
R18/15Perk - 5735	5019	DNA-directed RNA polymerase II largest subunit
R18/15Perk - 62595	211	Leishmania xx.
R18/15Perk - 6262	2011	nucleolar GTP-binding protein, putative.
R18/15Perk - 6262xxx	2000	nucleolar GTP-binding protein, putative.
R18/15Perk - 6743	2725	hypothetical protein.
R18/15Perk - 7930	1062	heat shock protein 90.
R18/15Perk - 22670	1328	putative nucleolar RNA binding protein.

Name	Length	Description
H02/14Perk - 1448	5343	putative Hsc70-interacting protein, hypothetical protein, 26S proteasome regulatory complex sub
H03/14Perk - 1141	7421	eukaryotic initiation factor 4a.
H03/14Perk - 1149	9836	18S rRNA.
H03/14Perk - 1232	10993	oligosaccharyl transferase subunit*.
H03/14Perk - 1271	8068	cytochrome c oxidase subunit IV.
H03/14Perk - 1326	12224	DNA-directed RNA polymerase II largest subunit.
H03/14Perk - 13274	403	trans-spliced leader sequence SL.
H03/14Perk - 1338	6213	leucine-rich repeat protein, trans-spliced leader sequence SL.
H03/14Perk - 1392	4908	Yop effector YopM, disease resistance protein, trans-spliced leader sequence SL.
H03/14Perk - 1430	9820	DNA polymerase theta-like protein, hypothetical protein, leucine rich repeat virulence protein,
H03/14Perk - 14949	363	trans-spliced leader sequence SL.
H03/14Perk - 1553	2260	heat shock protein, mitochondrion precursor, conserved hypothetical protein,
H03/14Perk - 1674	5035	RNA polymerase II second largest subunit
H03/14Perk - 1826	443	acceptor-like protein kinase
H03/14Perk - 1844	954	ukjent
H03/14Perk - 196	6803	Acetyl-CoA synthetase.
H03/14Perk - 2008	3812	60S ribosome subunit biogenesis protein NIP7.
H03/14Perk - 2138	5174	heat shock protein 70, putative.
H03/14Perk - 2836	1960	trans-spliced leader sequence SL.
H03/14Perk - 2868	2998	leucine-rich repeat, trans-spliced leader sequence SL.
H03/14Perk - 2973	2983	beta-tubulin (tubB) gene.
H03/14Perk - 3074	1753	elongation factor 1-alpha.
H03/14Perk - 3631	2607	5S(M5) ribosomal RNA rRNA.
H03/14Perk - 4134	2828	rRNA
H03/14Perk - 481	12397	putative mitochondrial heat-shock protein hsp70.
H03/14Perk - 5003	1864	trans-spliced leader sequence SL.
H03/14Perk - 5647	850	trans-spliced leader sequence SL.
H03/14Perk - 5870	1483	hypothetical protein, conserved.
H03/14Perk - 6504	1497	trans-spliced leader sequence SL.
H03/14Perk - 653	13366	GTP-binding protein 1.
H03/14Perk - 668	14149	GTP-binding protein.
H03/14Perk - 704	18065	putative nucleolar RNA binding protein.
H03/14Perk - 7202	953	trans-spliced leader sequence SL.
H03/14Perk - 800	8078	trans-spliced leader sequence SL.
H03/14Perk - 812	8743	Leishmania ukjent.
H03/14Perk - 892	7466	40S ribosomal protein S6 partial.
H03/14Perk - 905	11313	alpha-tubulin (tubA).
H03/14Perk - 940	9021	heat shock protein 90 gene.
H03/14Perk - 9783	514	aminotransferase.

Ingen genetisk variasjon påvist

H03/14Perk

Andre årsaker til virulens variasjon

- Betydning av symbionter
 - Perkinsela sp.
 - Andre symbionter (bakterier, virus, sopp???)
- Betydning av vert (og variasjon hos vertsart)
- Betydning av bakterier i dyrkingsmediene
 - Tilsette bakterier fra kloner med høyvirulente *P. perurans* til kloner som er lav-virulente (testes i smitteforsøk høsten 2016)

Virulensmarkører

- Fylogenetiske analyser
- MLST analyser
- VNTR analyser



Amoebic gill disease (AGD) in Atlantic salmon (*Salmo salar*) farmed in Chile

Patricio A. Bustos ^{a,1}, Neil D. Young ^{b,c,1}, Marco A. Rozas ^{a,1}, Harry M. Bohle ^a, Ricardo S. Ildefonso ^a, Richard N. Morrison ^{b,d}, Barbara F. Nowak ^{b,*}

2.5. PCR product amplification and sequencing

Universal oligonucleotide primers complementary to conserved regions of the eukaryotic 18S rRNA gene were used to PCR amplify and sequence the entire *N. perurans* 18S rRNA gene from genomic DNA isolated from AGD-affected Atlantic salmon gill tissues collected in this study. Briefly, genomic DNA was extracted from gill tissues using a

2011

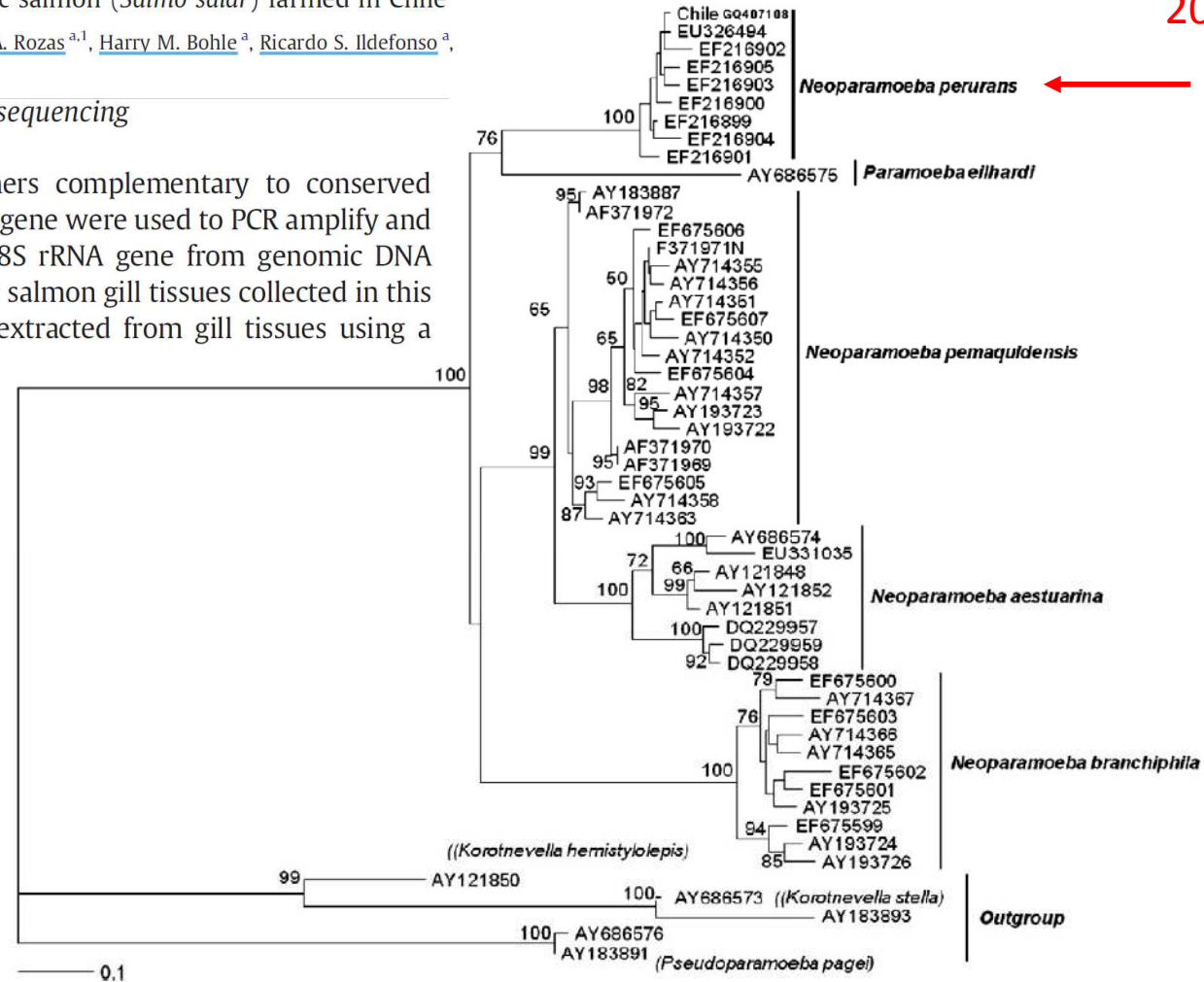
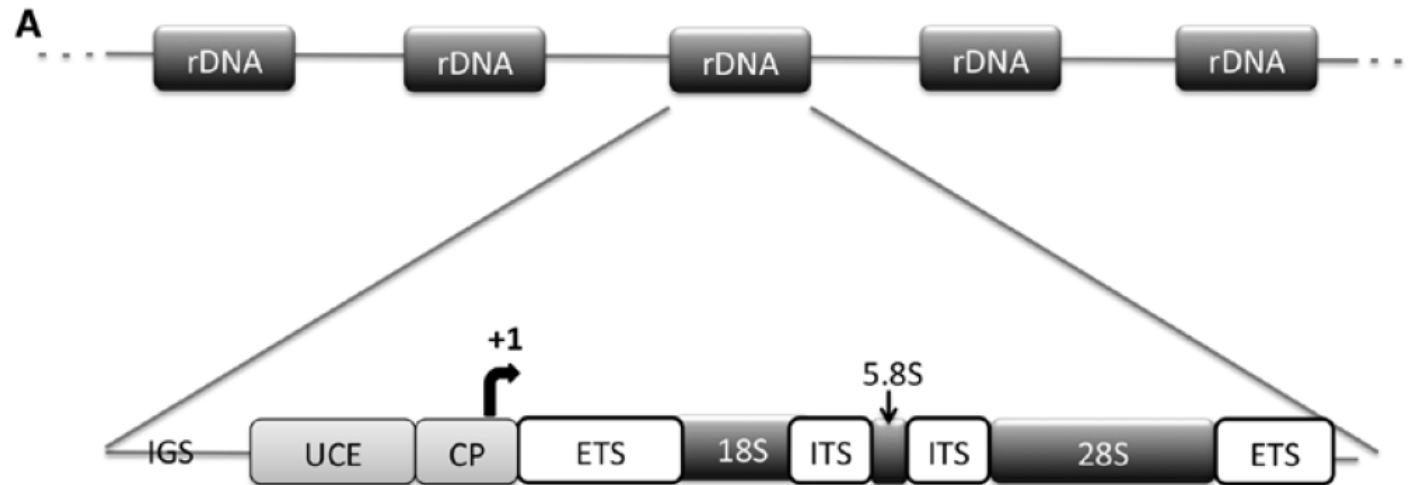


Fig. 5. Tree from the phylogenetic analysis of *Neoparamoeba* 18S rRNA gene sequences and position of *N. perurans* from Chile with related to marine amoebae. *Pseudoparamoeba pagei*, *Korotnevella hemistylepis* and *K. stella* were used as the outgroup. All sequences are presented with their GenBank accession numbers. The topology was inferred using maximum likelihood analysis incorporating the GTR + G + I model and values at nodes represent bootstrap support by fast stepwise addition analysis with 100 replicates and re-sampling of all characters using the PAUP software. All branches are to scale and the bar represents 0.1 substitutions per site.

rDNA organisering i genomet

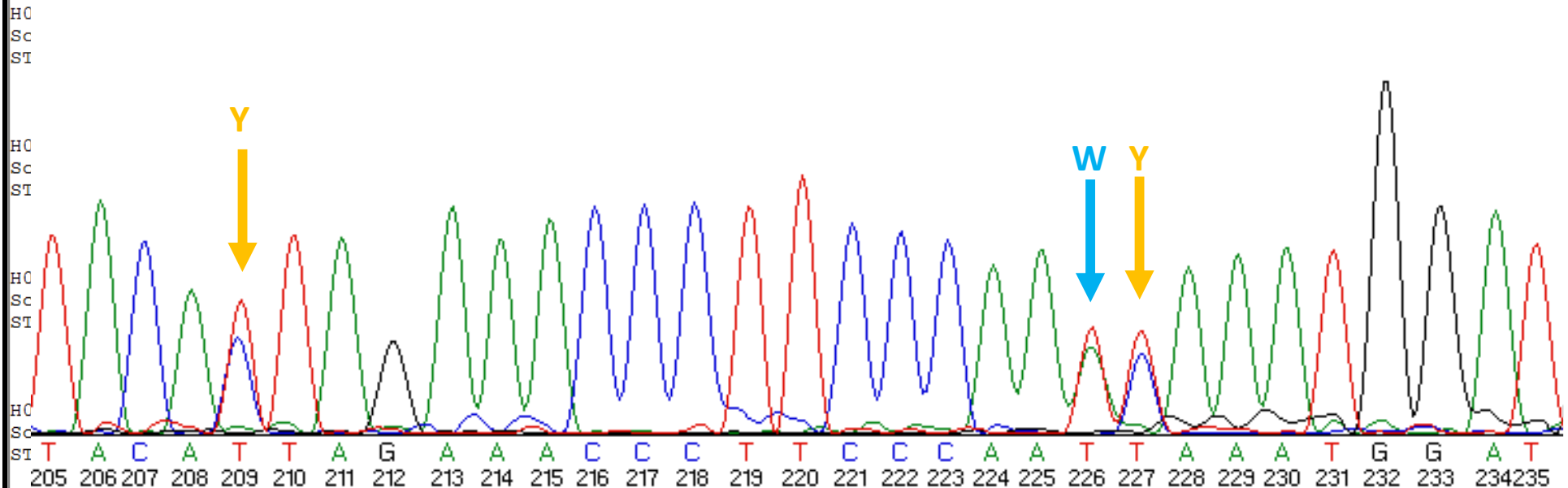


18S rDNA

```

                *      20      *      40      *      60      *      80      *      100      *
H03/14Pp : TTCGTATCAGTGTAGCGCGCGTGCAGCCAGAACATCTAAAGGGCATCACAGACCTGTTATTGCCTCAGACTTCCCTTGGTTAAACACCAAGTGTCCCTCTAAGAAGTTAT
Scotland : TTCGTATCAGTGTAGCGCGCGTGCAGCCAGAACATCTAAAGGGCATCACAGACCTGTTATTGCCTCAGACTTCCCTTGGTTAAACACCAAGTGTCCCTCTAAGAAGTTAT
ST19/15Pp : TTCGTATCAGTGTAGCGCGCGTGCAGCCAGAACATCTAAAGGGCATCACAGACCTGTTATTGCCTCAGACTTCCCTTGGTTAAACACCAAGTGTCCCTCTAAGAAGTTAT
                TTCGTATCAGTGTAGCGCGCGTGCAGCCAGAACATCTAAAGGGCATCACAGACCTGTTATTGCCTCAGACTTCCCTTGGTTAAACACCAAGTGTCCCTCTAAGAAGTTAT

                120      *      140      *      160      *      180      *      200      *      220
H03/14Pp : TAAC-R-AATTTGATAAATCAAAT-Y-CTAACAAATAAAAAACAAGTTTTTCTAATCAATCTCAGAGTCAAACCTCCAAGCTTACA-Y-TAGAAACCCCTTCCCAA-WY-AAAT
Scotland : TAAC-R-AATTTGATAAATCAAAT-Y-CTAACAAATAAAAAACAAGTTTTTCTAATCAATCTCAGAGTCAAACCTCCAAGCTTACA-Y-TAGAAACCCCTTCCCAA-WY-AAAT
ST19/15Pp : TAAC-R-AATTTGATAAATCAAAT-Y-CTAACAAATAAAAAACAAGTTTTTCTAATCAATCTCAGAGTCAAACCTCCAAGCTTACA-Y-TAGAAACCCCTTCCCAA-WY-AAAT
                TAAC R AATTTGATAAATCAAAT Y CTAACAAATAAAAAACAAGTTTTTCTAATCAATCTCAGAGTCAAACCTCCAAGCTTACA Y TAGAAACCCCTTCCCAA WY AAAT
    
```



```

                *      680      *      700      *      720      *      740      *      760      *
H03/14Pp : AATGCTTTCGAGAAGTTCGTCTTTCATAAATCCAAGAATTCACCTCTGACAATGAAATACGAATGCCCCCAACTGTCCCTTTTAAATCATTACACTTCATCTCTCTC : 7
Scotland : AATGCTTTCGAGAAGTTCGTCTTTCATAAATCCAAGAATTCACCTCTGACAATGAAATACGAATGCCCCCAACTGTCCCTTTTAAATCATTACACTTCATCTCTCTC : 7
ST19/15Pp : AATGCTTTCGAGAAGTTCGTCTTTCATAAATCCAAGAATTCACCTCTGACAATGAAATACGAATGCCCCCAACTGTCCCTTTTAAATCATTACACTTCATCTCTCTC : 7
                AATGCTTTCGAGAAGTTCGTCTTTCATAAATCCAAGAATTCACCTCTGACAATGAAATACGAATGCCCCCAACTGTCCCTTTTAAATCATTACACTTCATCTCTCTC
    
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18S - SSU



Variable numbers of tandem repeats - VNTR hos *P. perurans*

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CAGAATATCA GAATATGAGAATATGA GAATATCAGAATATCA GAATCTGAGAAGATCA GAATATCAGAATATCA GAATATGAGAATCTCAGAAT
CAGAATATCAGAATATGAGAATATGAGAATATCAGAATATCAGAATCTGAGAAGATCAGAATATCAGAATATCAGAATATGAGAATCTCAGAAT

      100          *          120          *          140          *          160          *          180
AT--GAGAATATCA--GAATATCA--GAATATCA--GAATATCA--GAATATCA--GAATATCA--GAATATCA--GAATATCA--
AT  GAGAATATCA  GAATATCA  GAATATCA  GAATATCA  GAATATCA  GAATATCA  GAATATCA  GAATATCA

*          200          *          220          *          240          *          260          *          280
-GAAGAGGAGAATGTGAGAATATGAGAATATGAAAAATGAAAATATGAGACTATCCGAATATCAGAATATCA CAATAGCA GAATATCAGAATA
GAAGAGGAGAATGTGAGAATATGAGAATATGAAAAATGAAAATATGAGACTATCCGAATATCAGAATATCACAATAGCAGAATATCAGAATA
  
```

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      *          1100          *          1120          *
ACAAACCAGTTCCCTGTGAAAAAGGGGGAAGGGAAAGAAACAAAAGAAATACC
ACAAACCAGTTCCCTGTGAAAAAGGGGGAAGGGAAAGAAACAAAAGAAATACC

      1140          *          1160          *          1180
TAGAAGAGGAA AAA--GAGGAA--GAGGAA A--GAGGAA--GAGGAA
TAGAAGAGGAAAAA  GAGGAA  GAGGAAA  GAGGAA  GAGGAA

*          1200          *          1220          *          1240
GAGGAA--GAGGAA--GAGGAA--GAGGAA--GAGGAA--GAGGAA
GAGGAA  GAGGAA  GAGGAA  GAGGAA  GAGGAA  GAGGAA

*          1260          *          1280          *
GAGGAA--GAGGAA--GAGGAA--GAGGAA--GAGGAA--GAGAAAGAG
GAGGAA  GAGGAA  GAGGAA  GAGGAA  GAGGAA  GAGAAAGAG

      1300          *          1320          *          1340          *
AAAGAGAAACGAAAGAGAAAGGCCATTAATTCATAACGCCATTATATGATAA
AAAGAGAAACGAAAGAGAAAGGCCATTAATTCATAACGCCATTATATGATAA

      1360          *          1380          *          1400
AAGGAGGAAAAGAGAAAGAGAAAGAGAAAAGAGAAACAGAAAGAGAAAT
AAGGAGGAAAAGAGAAAGAGAAAGAGAAAAGAGAAACAGAAAGAGAAAT

*          1420          *          1440          *          14
AGAAAGAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAA
AGAAAGAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAA

      60          *          1480          *          1500
AGAAAA-----: 1362
AGAAAA
  
```

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      480          *          500          *          520          *
CCCCAGTGGGGAATTGAAAACAATGGTCCAGTTTCAGGTAAGTGAATCTGAGCT
CCCCAGTGGGGAATTGAAAACAATGGTCCAGTTTCAGGTAAGTGAATCTGAGCT

      540          *          560          *          580
TCTTGAACGAATNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAGAGAAAGAGATAAAGAAG
TCTTGAACGAATNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAGAGAAAGAGATAAAGAAG

*          600          *          620          *
GAGCTAGAGACAAAGATGAAAGAGCTAGAGAT--AGAGAA--AGAGAA--
GAGCTAGAGACAAAGATGAAAGAGCTAGAGAT  AGAGAA  AGAGAA

      640          *          660          *          680
AGAGAA--AGAGAA--AGAGAA--AGAGAA--AGAGAA--AGAGAA--
AGAGAA  AGAGAA  AGAGAA  AGAGAA  AGAGAA  AGAGAA

*          700          *          720          *          740
-AGAGATAGAAAAAGAAAAAGAGAAACAGAAAGAGAAAGAGAAAAAGAAACTCAGGTAC
AGAGATAGAAAAAGAAAAAGAGAAACAGAAAGAGAAAAAGAAACTCAGGTAC

*          760          *          780          *
TGAAGAGGGGGAAGAGAAATATGAGAATATGAGAATTTGAGTAATTTGATTGAA
TGAAGAGGGGGAAGAGAAATATGAGAATATGAGAATTTGAGTAATTTGATTGAA

      800          *          820          *          840
AATTGGCTGCAAAAAGCTCAGATTTGGCAACTGAAAATGGAACAAAATCCCCAG
AATTGGCTGCAAAAAGCTCAGATTTGGCAACTGAAAATGGAACAAAATCCCCAG

*          860          *          880          *          900
TGGGGAATTGAAAACACTGGTCCAGTTTCAGGTAAGTGAATATGAGCTTCTTGG
TGGGGAATTGAAAACACTGGTCCAGTTTCAGGTAAGTGAATATGAGCTTCTTGG
  
```


Konklusjon

Virulens markører

NB:

Ingen genetiske virulensmarkører er påvist

Arbeidspakke V, 2017

Oppstart 2017

- Kartlegging av geografisk utbredelse/spredning
 - Dario Pistone & Mats Kindt
- Kartlegging av historisk utbredelse
 - Dario Pistone & Mats Kindt
- Sikker påvisning
 - 2006, 2012 - 2017

Molekylær epizootiologi

FHF-prosjekt:901053

