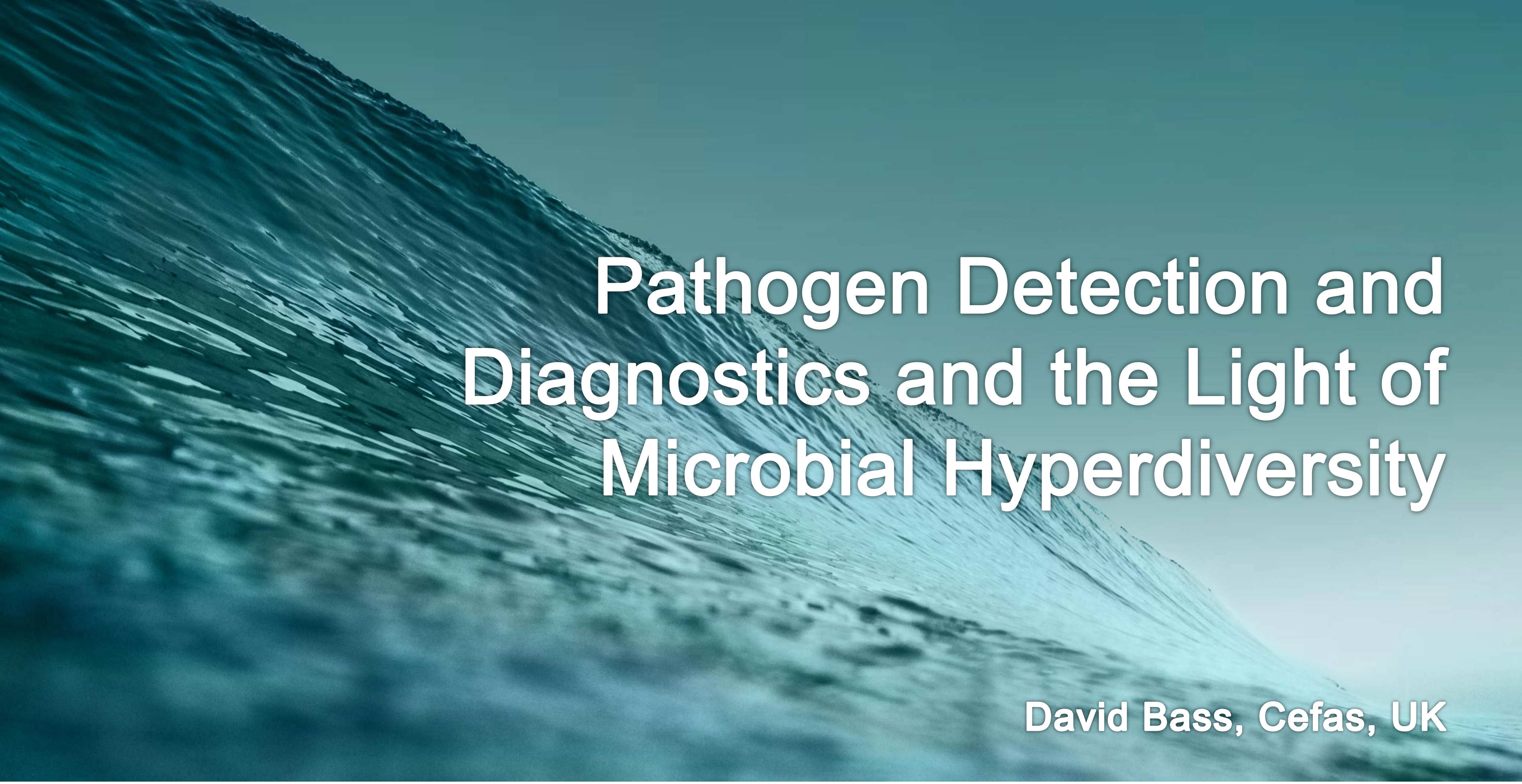


Pathogen Detection and Diagnostics and the Light of Microbial Hyperdiversity

David Bass, Cefas, UK

Cefas

VIVALDI

Preventing and mitigating farmed bivalve diseases

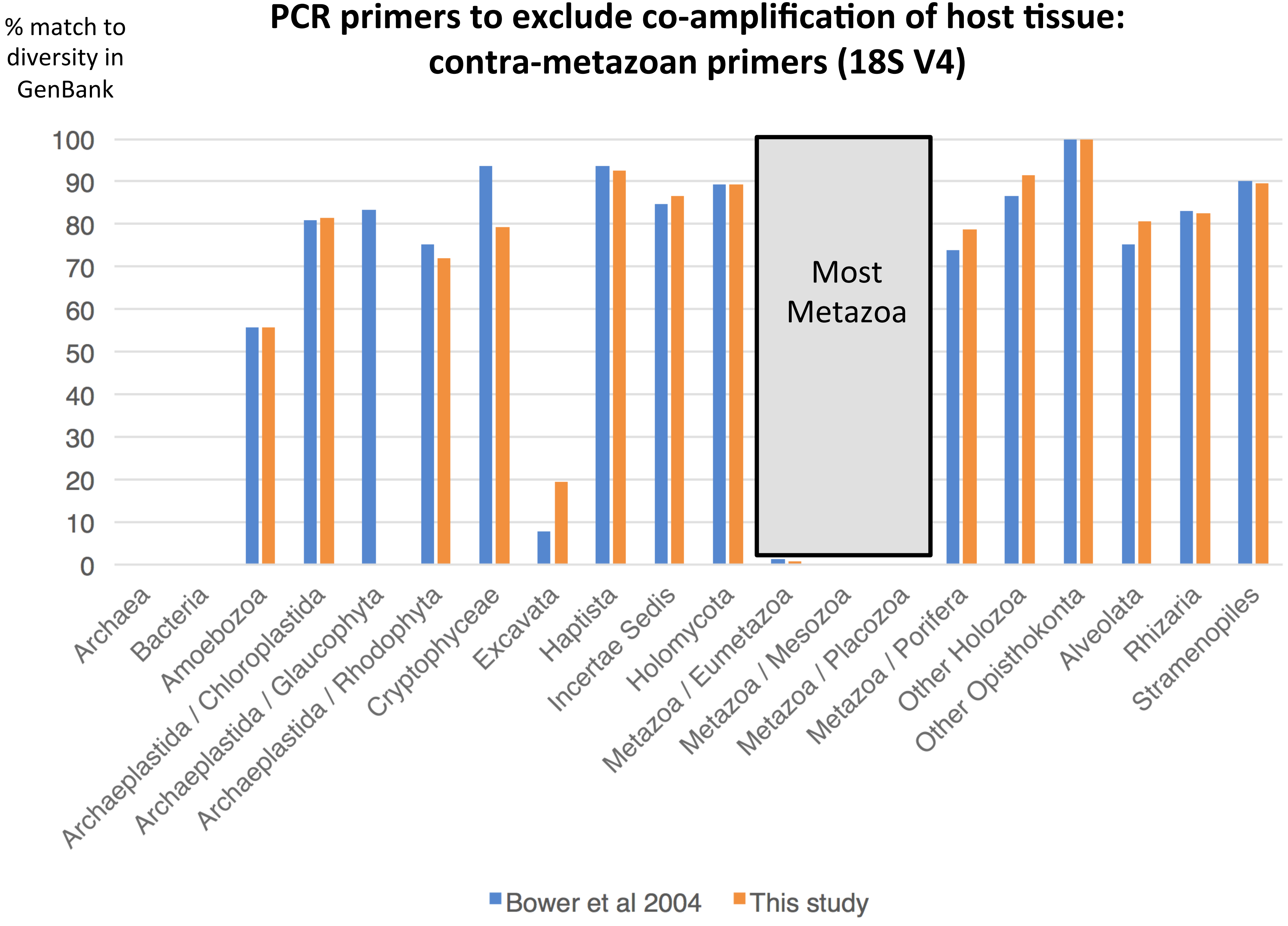
Cellular/molecular/microscopy protocols for pathogen detection, isolation, and diagnostics

- Investigating diversity of host-associated microbes
- PCR primers to exclude co-amplification of host tissue
- Long-range PCR to generate long amplicons for improved systematics, phylogenetic inference, and development of diagnostic assays
- Metagenomic approaches to pathogen detection and diversity assessment
- Differential detection of pathogens from DNA and RNA starting material: is RNA a more reliable indicator of infections?
- Laser Dissection Microscopy (LDM) and molecular characterisation of parasites

Cellular/molecular/microscopy protocols for pathogen detection, isolation, and diagnostics

- **Investigating diversity of host-associated microbes**
- **PCR primers to exclude co-amplification of host tissue**
- **Long-range PCR to generate long amplicons for improved systematics, phylogenetic inference, and development of diagnostic assays**
- Metagenomic approaches to pathogen detection and diversity assessment
- Differential detection of pathogens from DNA and RNA starting material: is RNA a more reliable indicator of infections?
- Laser Dissection Microscopy (LDM) and molecular characterisation of parasites

PCR primers to exclude co-amplification of host tissue: contra-metazoan primers (18S V4)



PCR primers to exclude co-amplification of host tissue: host-blocking primers (18S V9)

Shore crab (*Carcinus maenas*) infected with i) *Hematodinium;* ii) a microsporidian

V9 primer region, amplified with 1389F and 1510R, including blocking primer to shore crab (& other crustaceans)

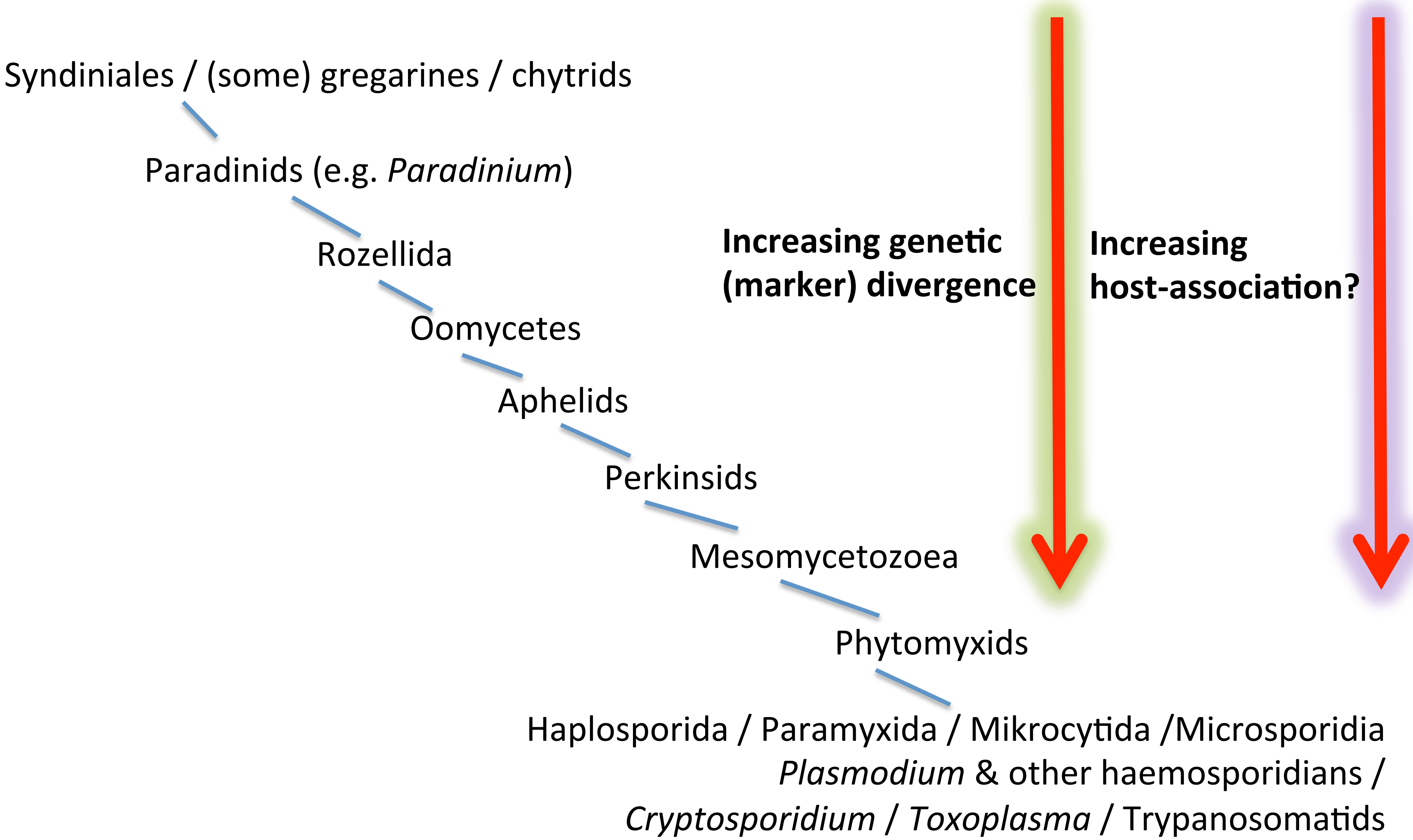
	NO BLOCK	0.25 µm BLOCK (1	1 µm BLOCK (13	NO BLOCK	0.25 µm BLOCK (83	1 µm BLOCK (83
HOST	14305	6519	742	15145	14816	163
BACTERIA	879	13557	14825	424	16041	19215
HEMATODINIUM	2280	3324	448	0	2	2
CILIATES	173	1403	1460	56	1128	936
FUNGI	2	0	5	1	58	1
STRAMENOPILES	13	59	23	7	109	25
PLATYHELMINTHES	45	18	0	5	3	0
CERCOZOA	0	0	0	0	2	0
AMOEBOZOANS	7	11	2	0	1	2
DINOFLAGELLATES	3	20	0	0	0	0
EXCAVATA	0	2	0	0	0	13

Hematodinium microsporidian

0.25 µm block revealed significantly more non-host diversity than no block
Doesn’t surmount problem of amplifying v. divergent sequences (e.g. microsporidian)

PCR primers to exclude co-amplification of host tissue: group-specific primers (18S)

Occurrence of host-associated lineages in broadly-targeted eukaryotic eDNA datasets



Syndiniales / (some) gregarines / chytrids

Paradinids (e.g. *Paradinium*)

Rozellida

Oomycetes

Aphelids

Perkinsids

Mesomycetozoea

Phytomyxids

Haplosporida / Paramyxida / Mikrocytida /Microsporidia
Plasmodium & other haemosporidians /
Cryptosporidium / *Toxoplasma* / Trypanosomatids

Increasing genetic (marker) divergence

Increasing host-association?

Group-specific PCR detection of Haplosporidia

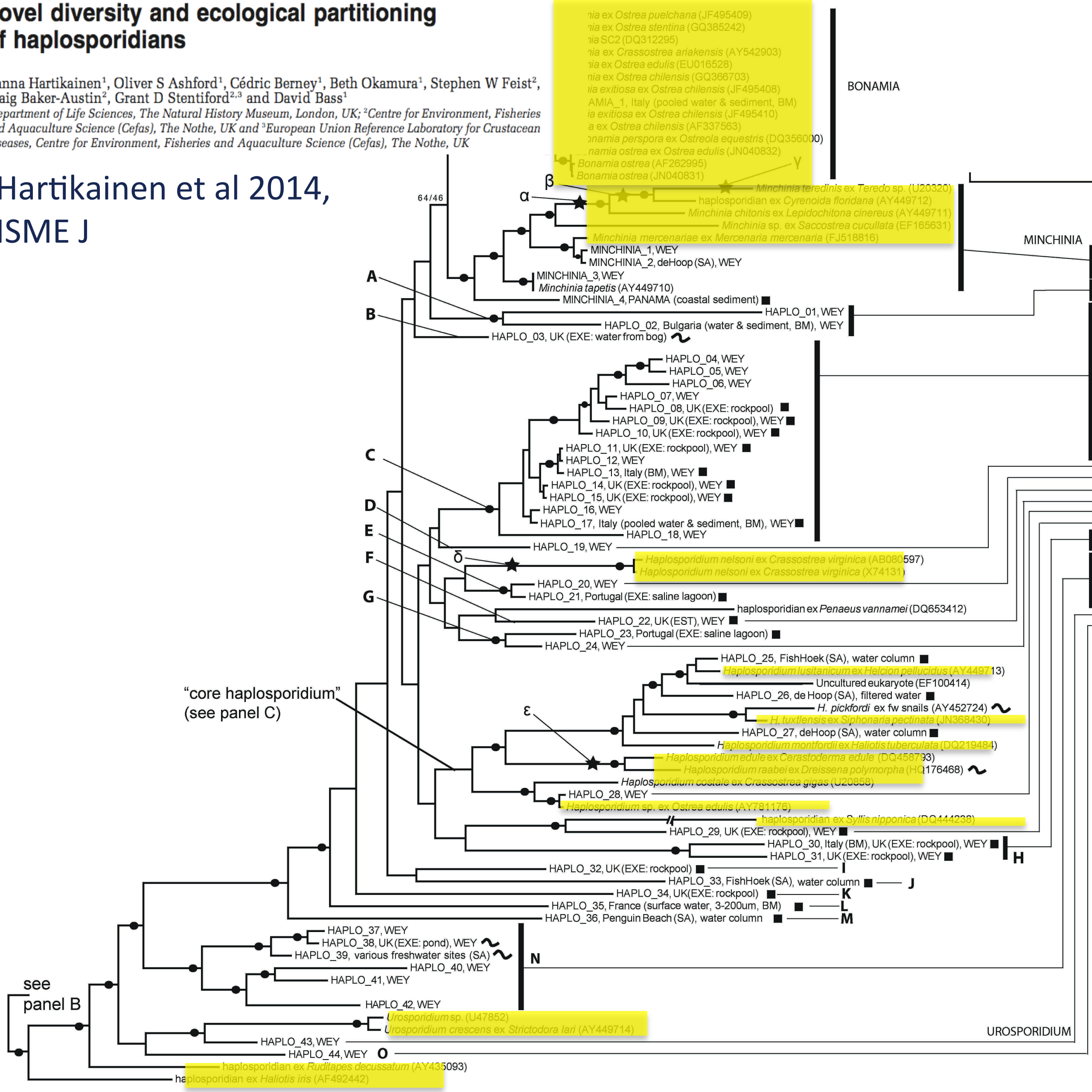
The ISME Journal (2013), 1–10
© 2013 International Society for Microbial Ecology All rights reserved 1751-7362/13
www.nature.com/ismej

ORIGINAL ARTICLE

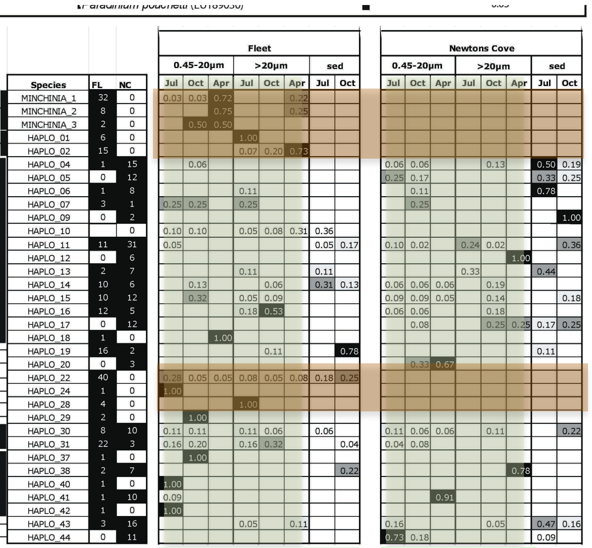
Lineage-specific molecular probing reveals novel diversity and ecological partitioning of haplosporidians

Hanna Hartikainen[1], Oliver S Ashford[1], Cédric Berney[1], Beth Okamura[1], Stephen W Feist[2], Craig Baker-Austin[2], Grant D Stentiford[2,3] and David Bass[1]
[1]*Department of Life Sciences, The Natural History Museum, London, UK;* [2]*Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, UK and* [3]*European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, UK*

Hartikainen et al 2014, ISME J



Ecological distribution (time & space)



			Fleet								Newtons Cove							
			0.45-20µm			>20µm			sed		0.45-20µm			>20µm			sed	
Species	FL	NC	Jul	Oct	Apr	Jul	Oct	Apr	Jul	Oct	Jul	Oct	Apr	Jul	Oct	Apr	Jul	Oct
MINCHINIA_1	32	0	0.03	0.03	0.72			0.22										
MINCHINIA_2	8	0			0.75			0.25										
MINCHINIA_3	2	0		0.50	0.50													
HAPLO_01	6	0				1.00												
HAPLO_02	15	0				0.07	0.20	0.73										
HAPLO_04	1	15		0.06							0.06	0.06			0.13		0.50	0.19
HAPLO_05	0	12									0.25	0.17					0.33	0.25
HAPLO_06	1	8				0.11						0.11					0.78	
HAPLO_07	3	1	0.25	0.25		0.25						0.25						
HAPLO_09	0	2																1.00
HAPLO_10		0	0.10	0.10		0.05	0.08	0.31	0.36									
HAPLO_11	11	31	0.05						0.05	0.17	0.10	0.02		0.24	0.02			0.36
HAPLO_12	0	6														1.00		
HAPLO_13	2	7				0.11			0.11					0.33			0.44	
HAPLO_14	10	6		0.13			0.06		0.31	0.13	0.06	0.06	0.06		0.19			
HAPLO_15	10	12		0.32		0.05	0.09				0.09	0.09	0.05		0.14			0.18
HAPLO_16	12	5				0.18	0.53				0.06	0.06			0.18			
HAPLO_17	0	12										0.08			0.25	0.25	0.17	0.25
HAPLO_18	1	0			1.00													
HAPLO_19	16	2					0.11			0.78							0.11	
HAPLO_20	0	3										0.33	0.67					
HAPLO_22	40	0	0.28	0.05	0.05	0.08	0.05	0.08	0.18	0.25								
HAPLO_24	1	0	1.00															
HAPLO_28	4	0				1.00												
HAPLO_29	2	0		1.00														
HAPLO_30	8	10	0.11	0.11		0.11	0.06		0.06		0.11	0.06	0.06		0.11			0.22
HAPLO_31	22	3	0.16	0.20		0.16	0.32			0.04	0.04	0.08						
HAPLO_37	1	0		1.00														
HAPLO_38	2	7								0.22						0.78		
HAPLO_40	1	0	1.00															
HAPLO_41	1	10	0.09										0.91					
HAPLO_42	1	0	1.00															
HAPLO_43	3	16				0.05		0.11			0.16				0.05		0.47	0.16
HAPLO_44	0	11									0.73	0.18					0.09	

Brackish lagoon

Rocky shore

Freshwater clades
Soil clades
More diverse hosts

Where

Vol. 133: 57–68, 2019
https://doi.org/10.3354/dao03326
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org
Published online February 21
OPEN ACCESS

Detection and characterisation of haplosporidian parasites of the blue mussel *Mytilus edulis*, including description of the novel parasite *Minchinia mytili* n. sp.

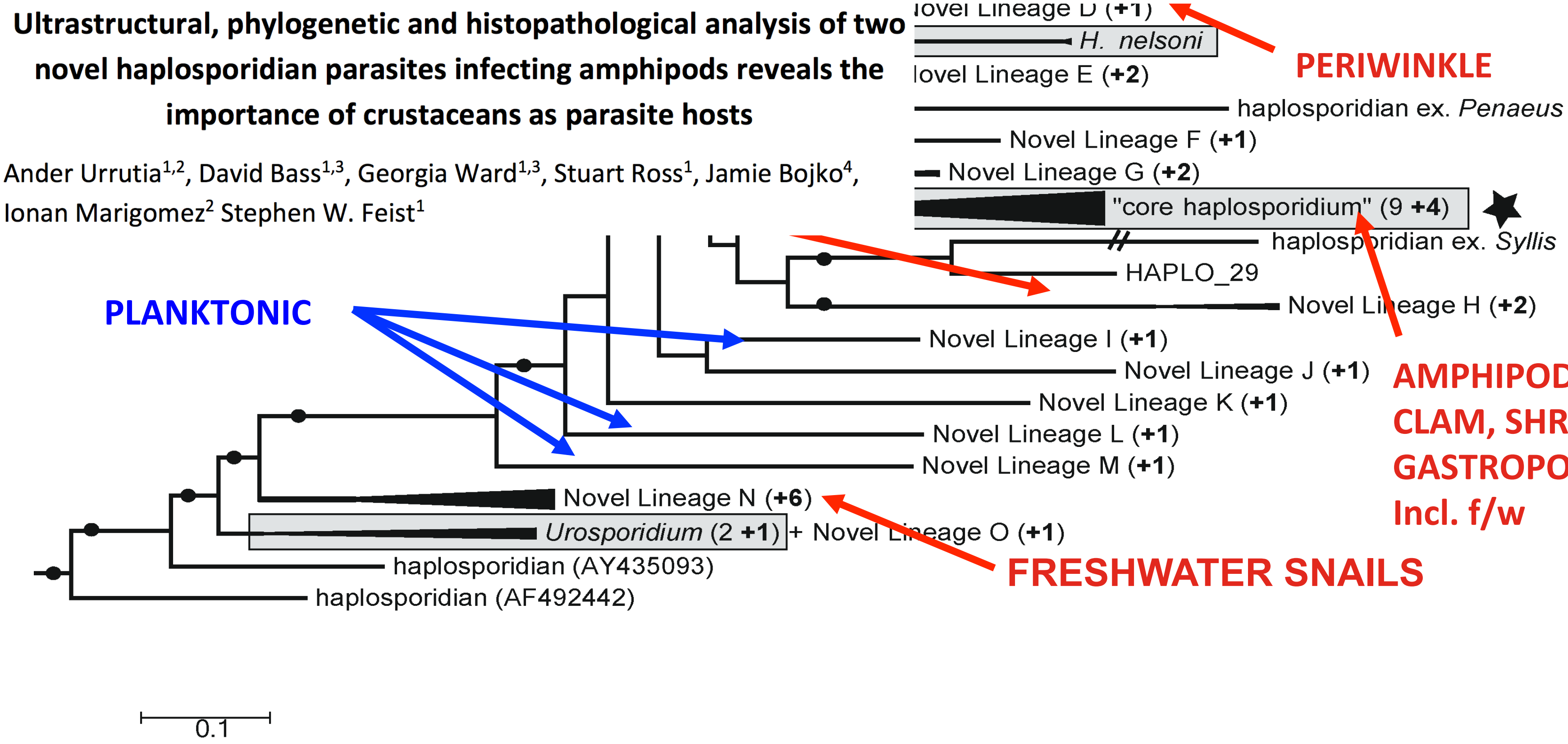
Georgia M. Ward[1,2,3,*], Stephen W. Feist[2], Patricia Noguera[4], Mar Marcos-López[4,5], Stuart Ross[2], Matthew Green[2], Ander Urrutia[2], David Bass[1,2]

MANY SMALL
f/w INVERTS
& PLANKTONIC

AMPHIPODS

Ultrastructural, phylogenetic and histopathological analysis of two novel haplosporidian parasites infecting amphipods reveals the importance of crustaceans as parasite hosts

Ander Urrutia[1,2], David Bass[1,3], Georgia Ward[1,3], Stuart Ross[1], Jamie Bojko[4], Ionan Marigomez[2] Stephen W. Feist[1]



PERIWINKLE

PLANKTONIC

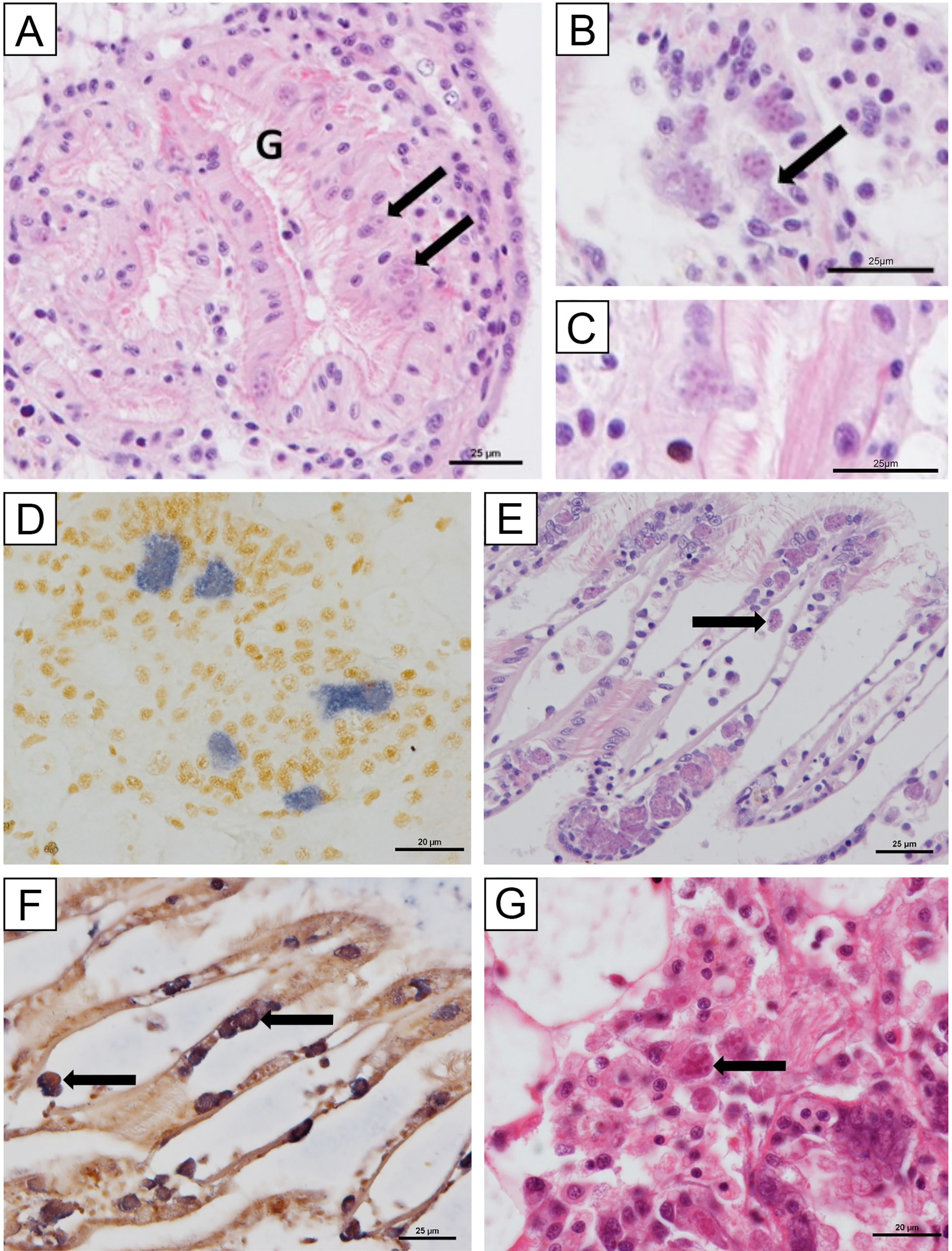
AMPHIPODS,
CLAM, SHRIMP
GASTROPODS,
Incl. f/w

FRESHWATER SNAILS

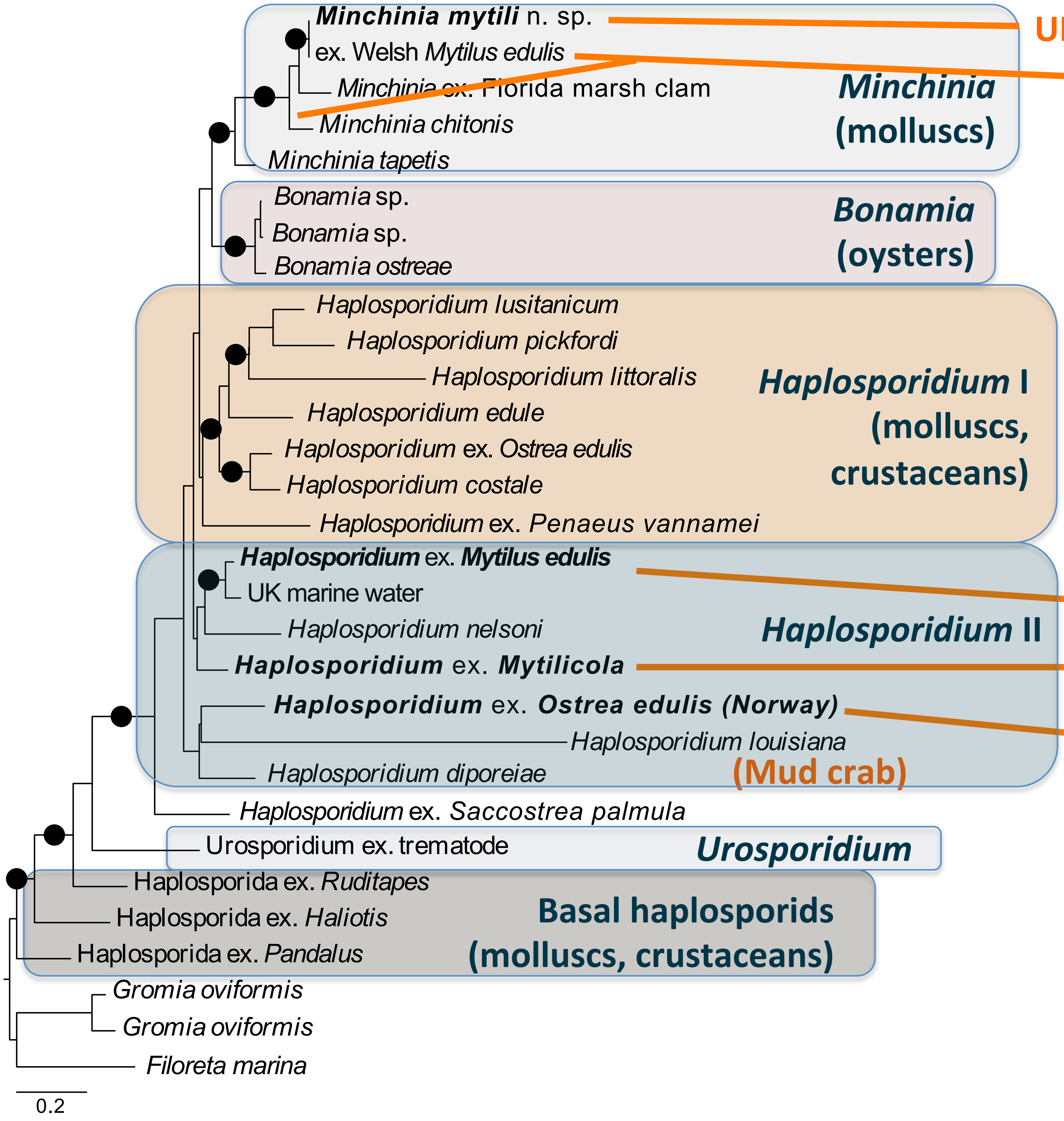
Ward et al, in prep.

Minchinia mytili in _Mytilus edulis_

Ward et al 2019



Order **Haplosporida**



UK mussels ~ 30% prevalence

Cockles, mussels, oyster spat

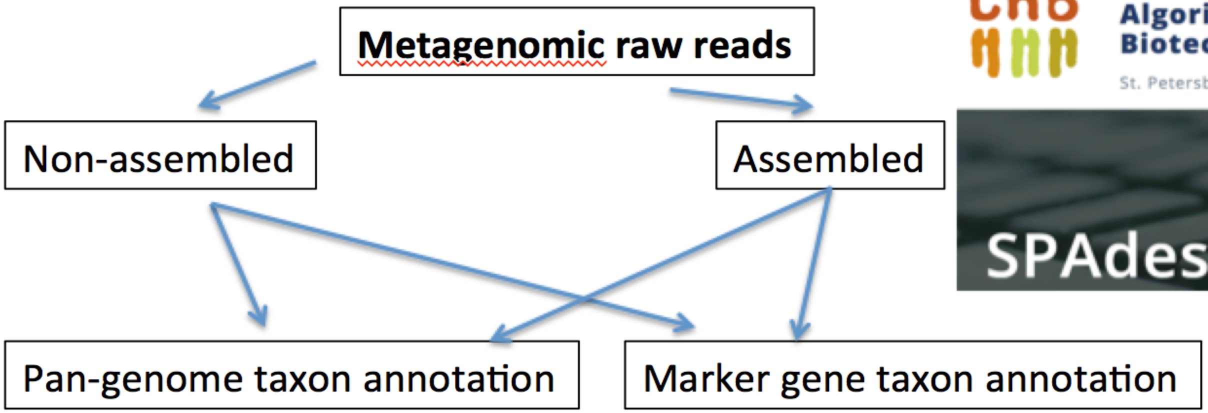
UK mussels

Hyperparasite (copepod)

Novel oyster parasite (Norway)

(Mud crab)

Metagenomic sequencing and data mining for diversity studies and pathogen ID



Metagenomic raw reads

Non-assembled

Assembled

Pan-genome taxon annotation

Marker gene taxon annotation

CAB Center for Algorithmic Biotechnology
St. Petersburg State University

SPAdes

Home Web Server Source

KAIJU

Fast and sensitive taxonomic classification for metagenomics

Centrifuge
Classifier for metagenomic sequences

OK for bacteria & viruses;
not eukaryotes

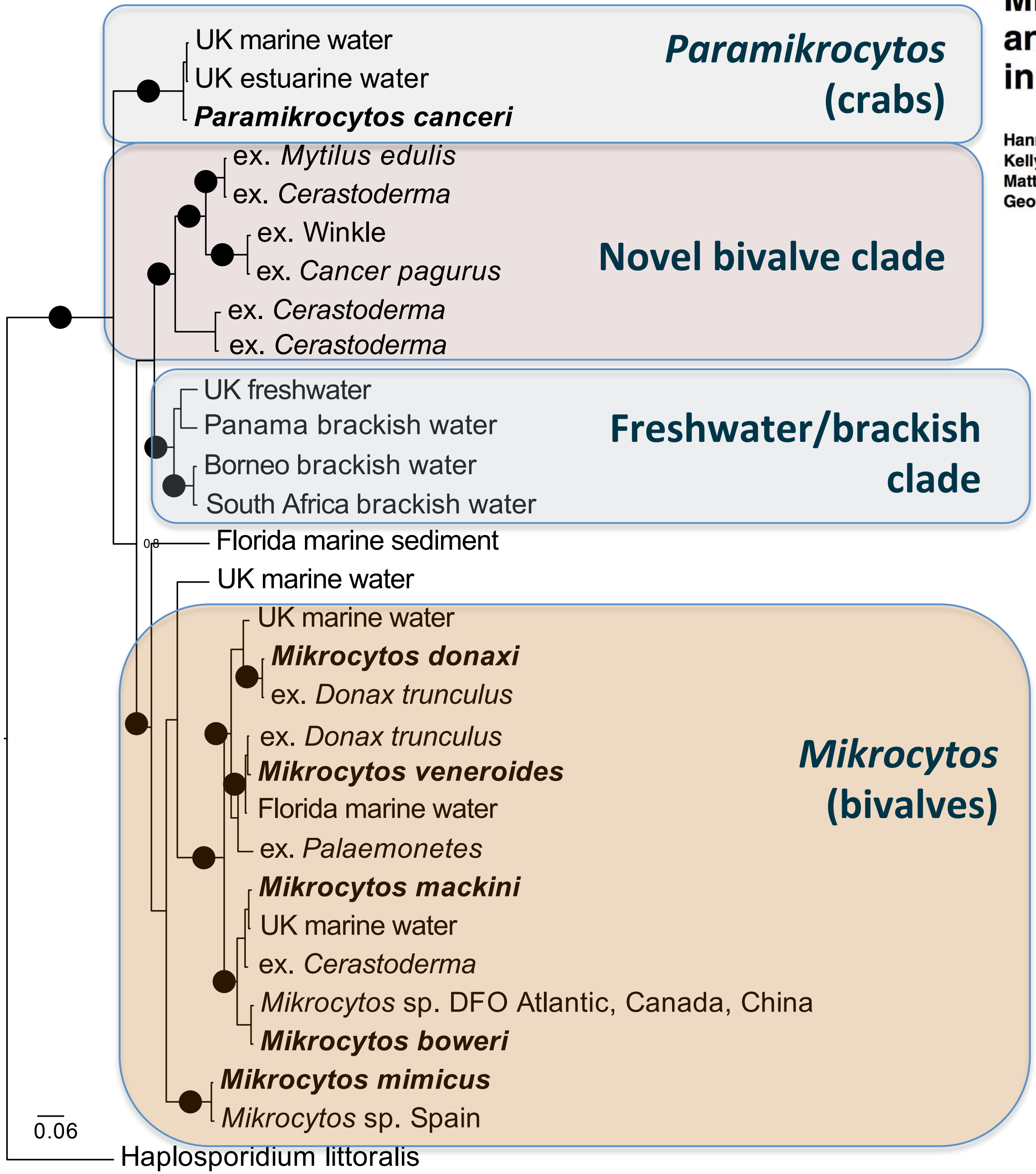
Metaxa2

PR2 Protist Ribosomal Reference Database

EUKREF

OK for eukaryotes &bacteria;
Not viruses

Doherty, van Aerle et al, in prep



UK marine water
UK estuarine water
Paramikrocytos canceri
***Paramikrocytos* (crabs)**

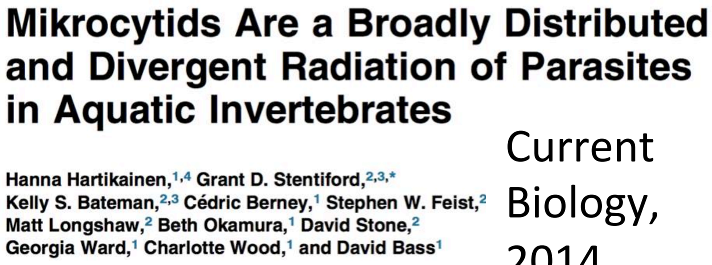
ex. *Mytilus edulis*
ex. *Cerastoderma*
ex. Winkle
ex. *Cancer pagurus*
ex. *Cerastoderma*
ex. *Cerastoderma*
Novel bivalve clade

UK freshwater
Panama brackish water
Borneo brackish water
South Africa brackish water
Freshwater/brackish clade

0.8
Florida marine sediment
UK marine water

UK marine water
Mikrocytos donaxi
ex. *Donax trunculus*
ex. *Donax trunculus*
Mikrocytos veneroides
Florida marine water
ex. *Palaemonetes*
Mikrocytos mackini
UK marine water
ex. *Cerastoderma*
Mikrocytos sp. DFO Atlantic, Canada, China
Mikrocytos boweri
Mikrocytos mimicus
Mikrocytos sp. Spain
***Mikrocytos* (bivalves)**

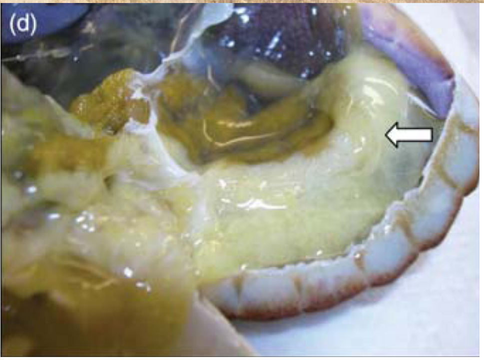
0.06
Haplosporidium littoralis

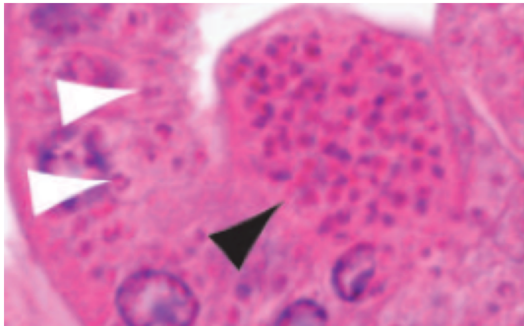


Mikrocytids Are a Broadly Distributed and Divergent Radiation of Parasites in Aquatic Invertebrates

Hanna Hartikainen,[1,4] Grant D. Stentiford,[2,3,*] Kelly S. Bateman,[2,3] Cédric Berney,[1] Stephen W. Feist,[2] Matt Longshaw,[2] Beth Okamura,[1] David Stone,[2] Georgia Ward,[1] Charlotte Wood,[1] and David Bass[1]

Current Biology, 2014





Challenges for modern molecular diagnostics

Previously unknown hyper-diversity of pathogen lineages

Many diagnostic primer sets produce small amplicons (<500 bp)

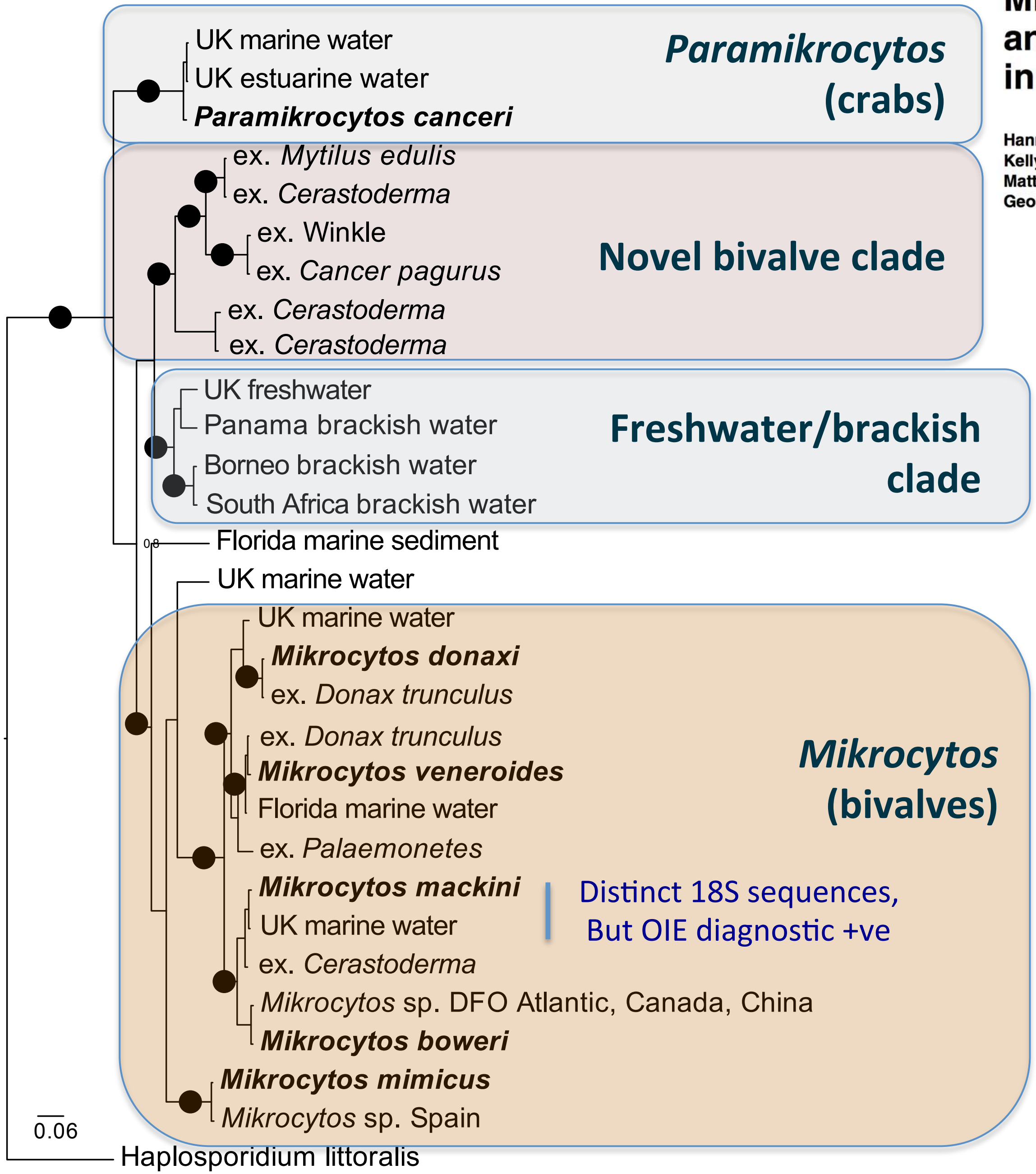
These ‘diagnostic’ gene regions are unable to distinguish between closely related lineages – therefore danger of false positives

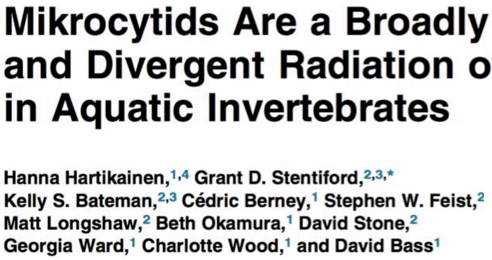
Phylogenetic interpretations complicated by low signal to background noise ratios

Missing fragments when Sanger sequencing ‘walk-through’ methods are used.

Generating and assembling rRNA arrays from metagenomic sequencing is high-cost and can be prone to errors

Therefore single read/single molecular sequencing approaches are needed

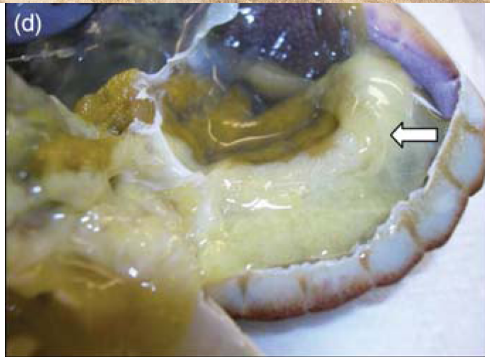


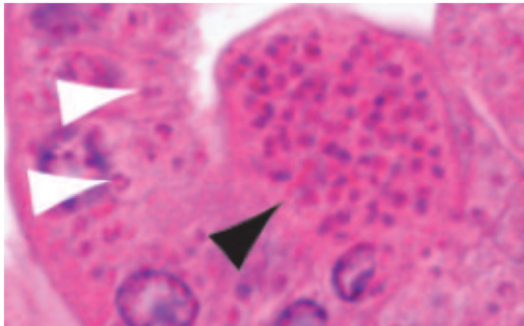


Mikrocytids Are a Broadly Distributed and Divergent Radiation of Parasites in Aquatic Invertebrates

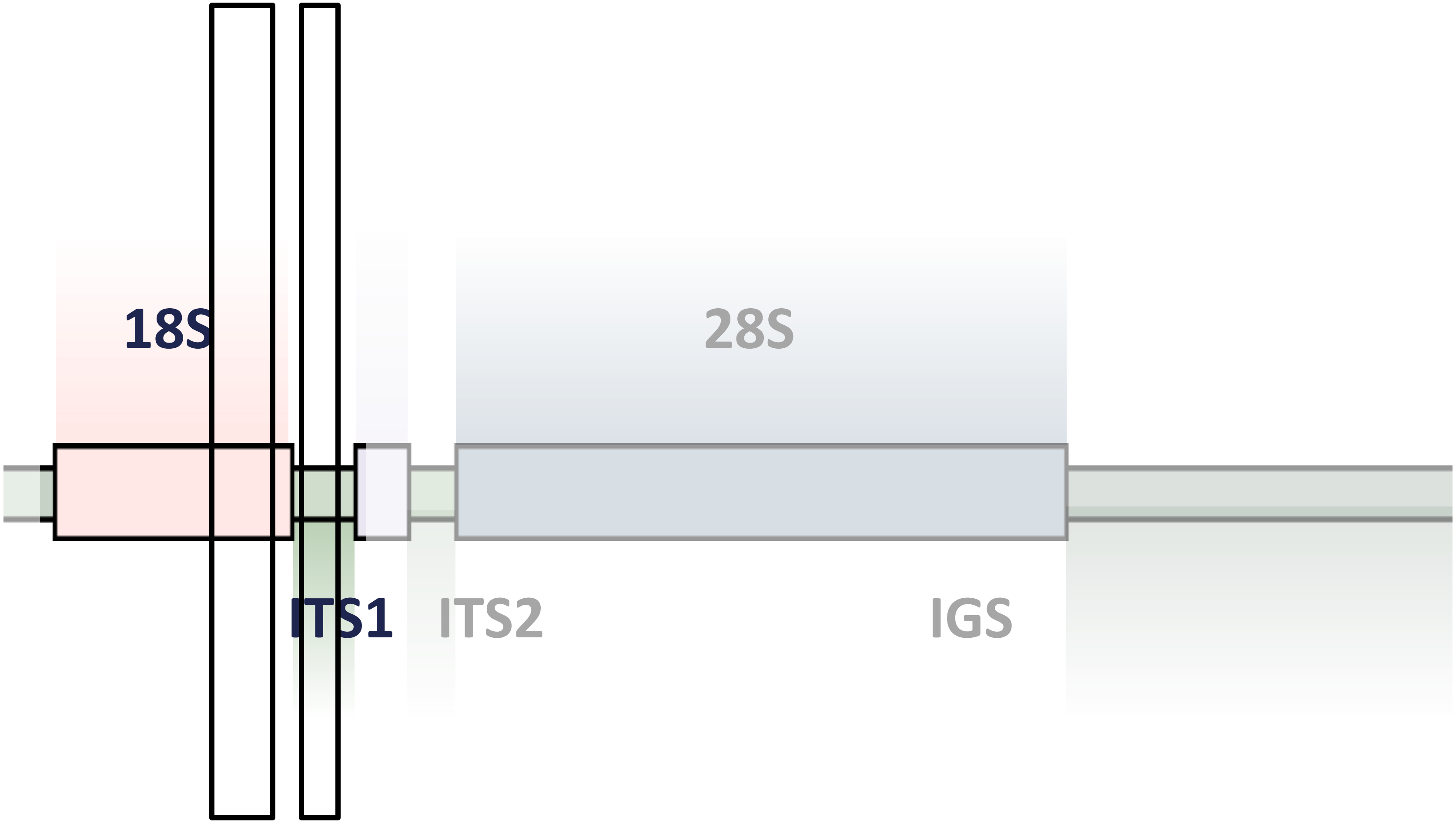
Hanna Hartikainen,[1,4] Grant D. Stentiford,[2,3,*] Kelly S. Bateman,[2,3] Cédric Berney,[1] Stephen W. Feist,[2] Matt Longshaw,[2] Beth Okamura,[1] David Stone,[2] Georgia Ward,[1] Charlotte Wood,[1] and David Bass[1]

Current Biology, 2014

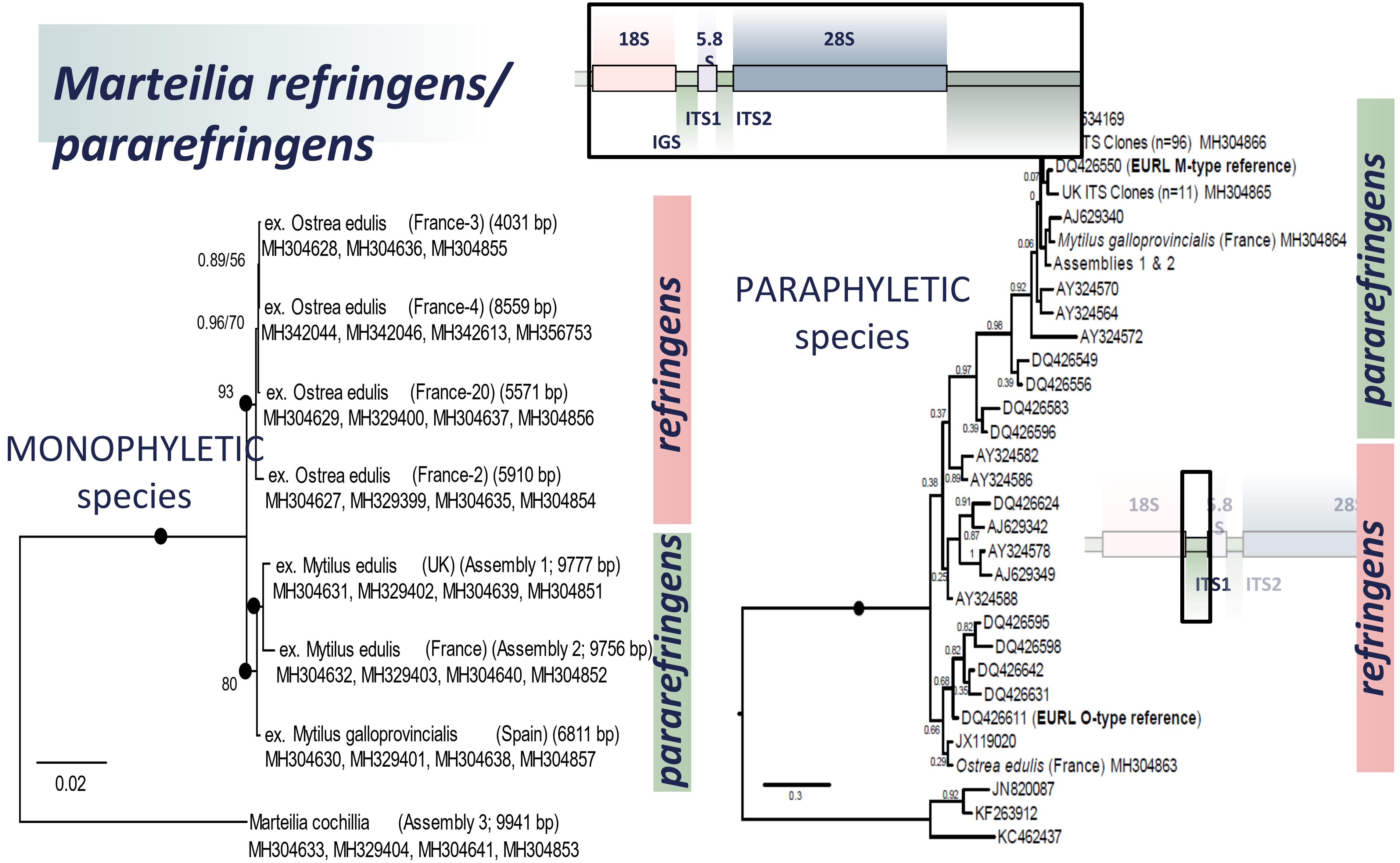




Short 'diagnostic' gene regions need to be revised / are insufficient



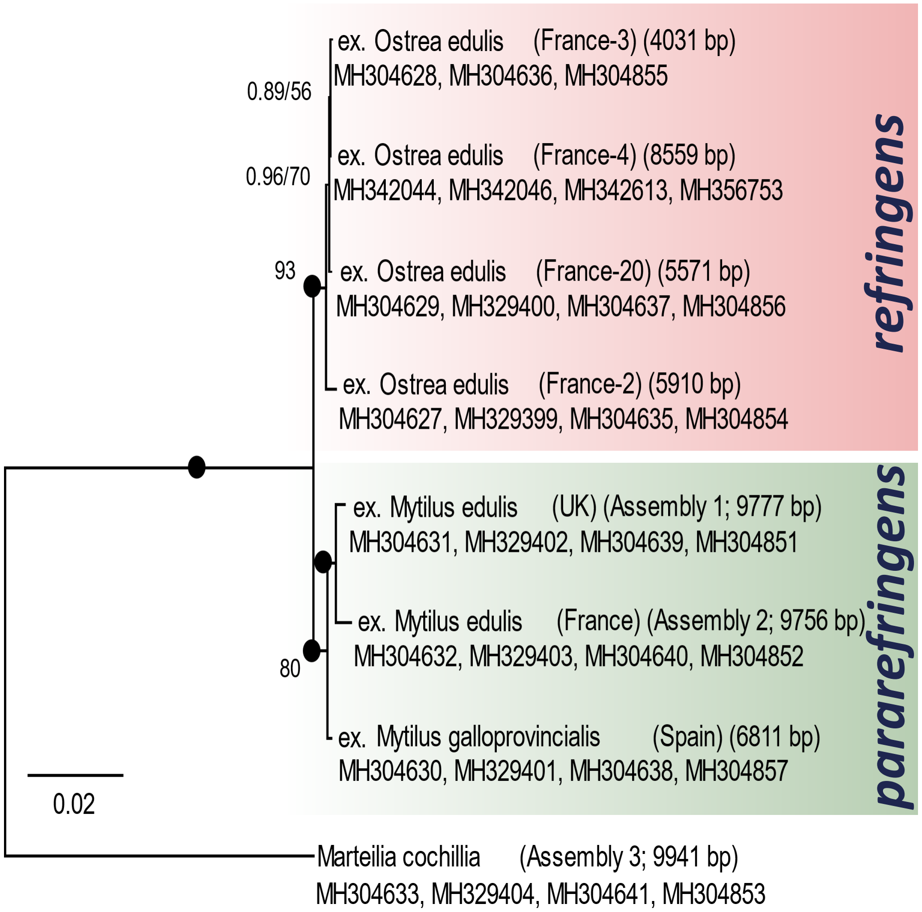
Marteilia refringens/ pararefringens



MONOPHYLETIC species

PARAPHYLETIC species

Marteilia refringens/ pararefringens



ex. Ostrea edulis (France-3) (4031 bp)
MH304628, MH304636, MH304855

ex. Ostrea edulis (France-4) (8559 bp)
MH342044, MH342046, MH342613, MH356753

ex. Ostrea edulis (France-20) (5571 bp)
MH304629, MH329400, MH304637, MH304856

ex. Ostrea edulis (France-2) (5910 bp)
MH304627, MH329399, MH304635, MH304854

ex. Mytilus edulis (UK) (Assembly 1; 9777 bp)
MH304631, MH329402, MH304639, MH304851

ex. Mytilus edulis (France) (Assembly 2; 9756 bp)
MH304632, MH329403, MH304640, MH304852

ex. Mytilus galloprovincialis (Spain) (6811 bp)
MH304630, MH329401, MH304638, MH304857

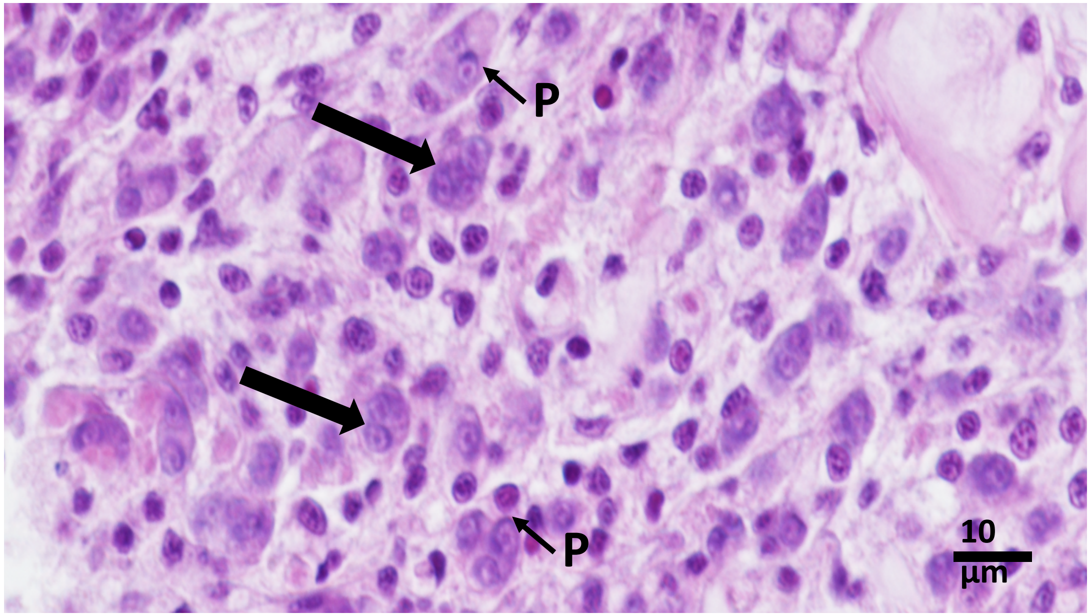
Marteilia cochillia (Assembly 3; 9941 bp)
MH304633, MH329404, MH304641, MH304853

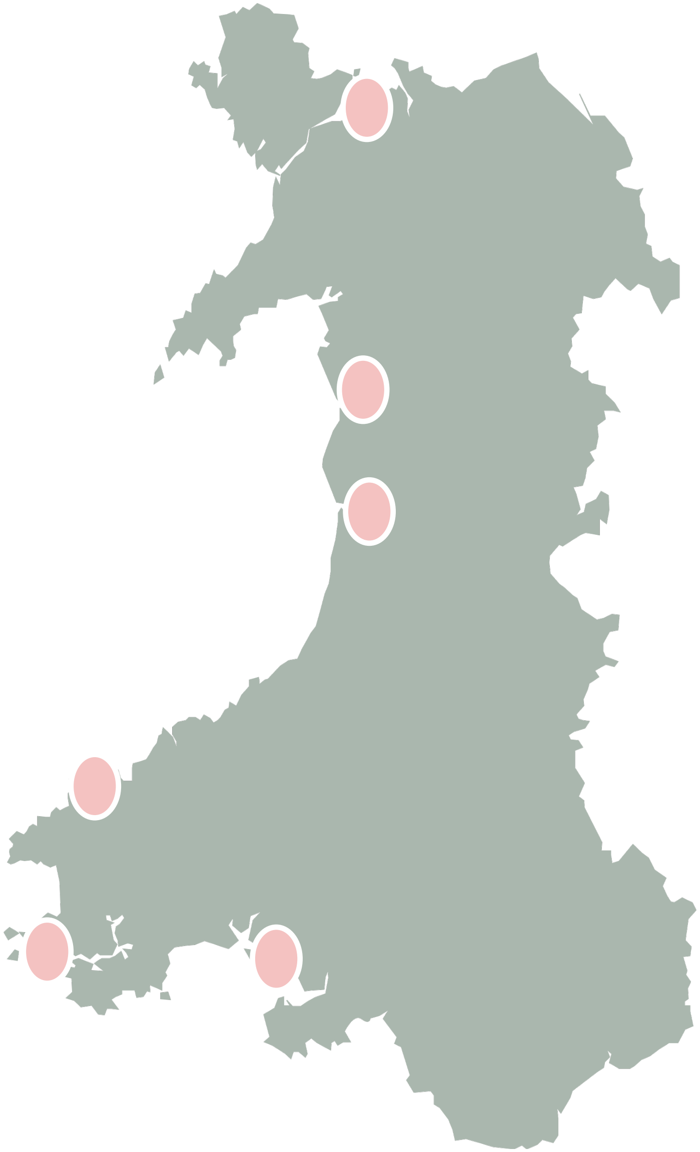
Marteilia cochillia

Diagnostic primers indicated that *M. cochillia* was infecting cockles at several sites in Wales, UK

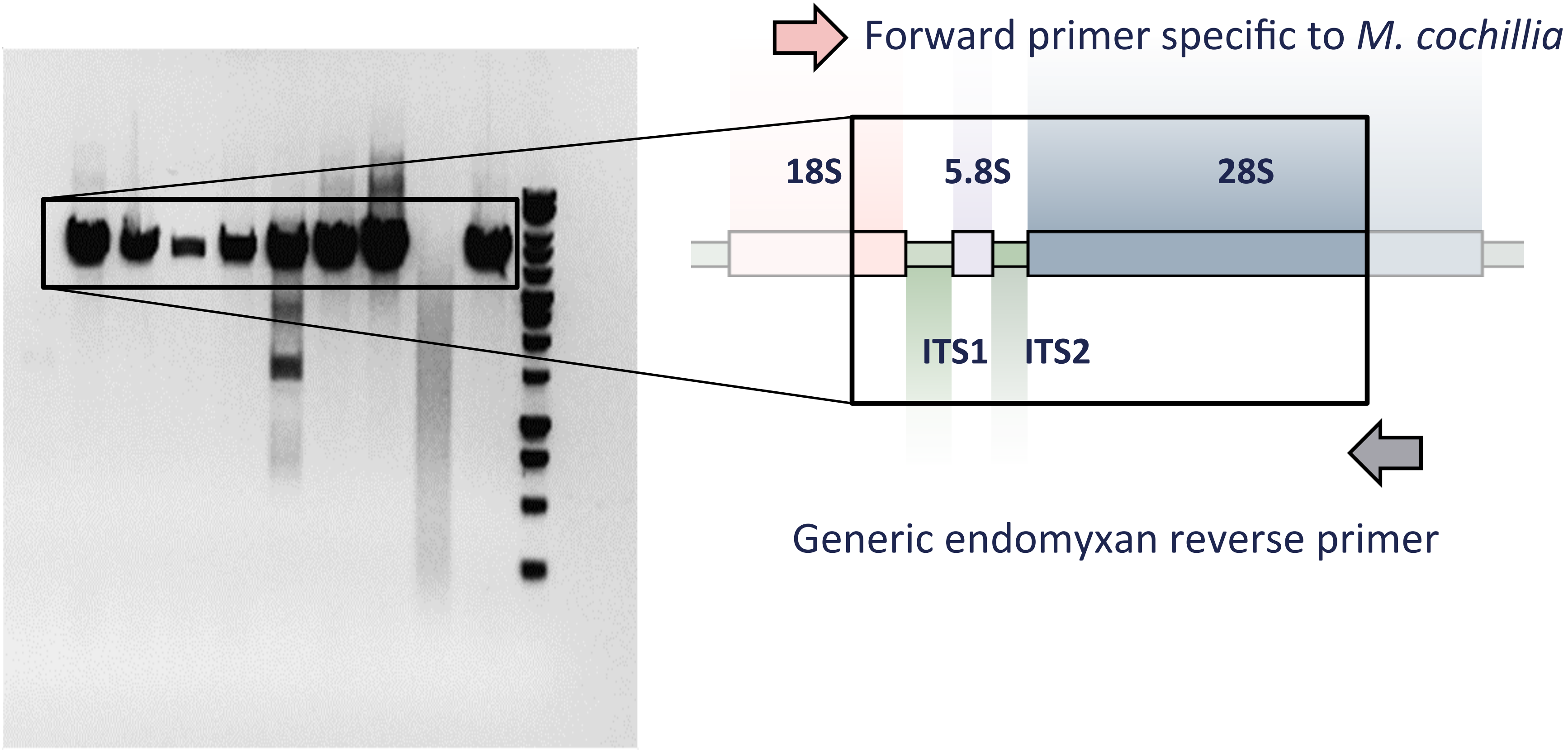
However, histopathology showed differences between infections in Wales and Spain

Are these parasites the same?





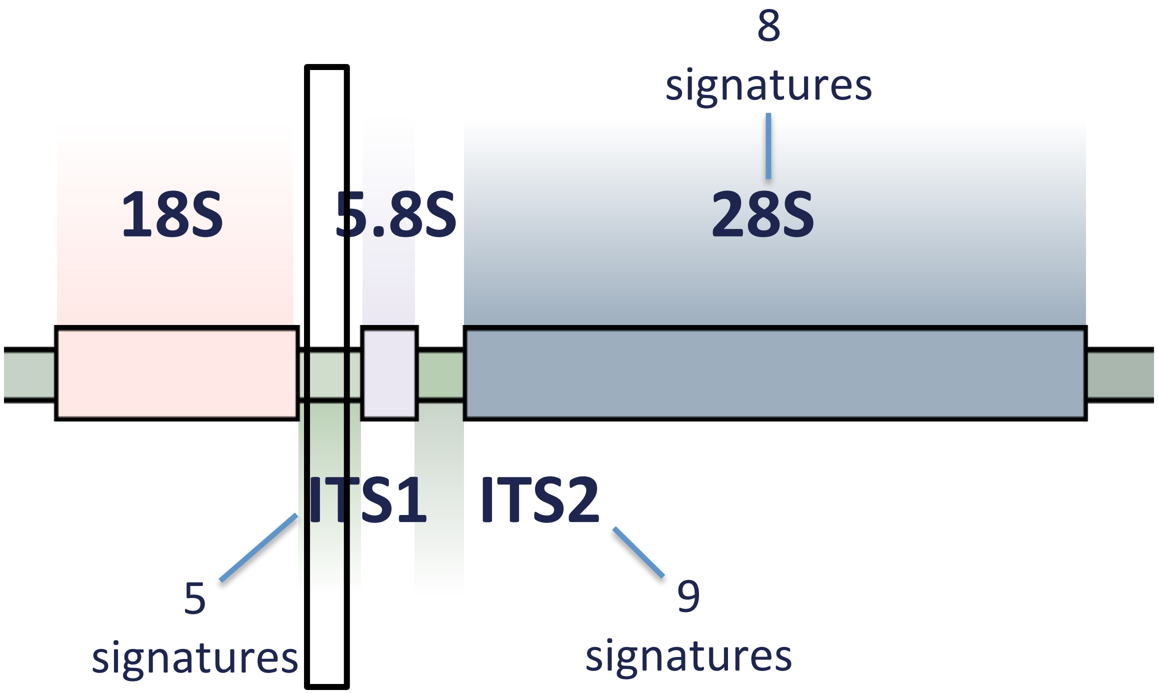
Marteilia cochillia



Forward primer specific to *M. cochillia*

Generic endomyxan reverse primer

Marteilia 'cochillia': Spain vs wales



8 signatures

18S

5.8S

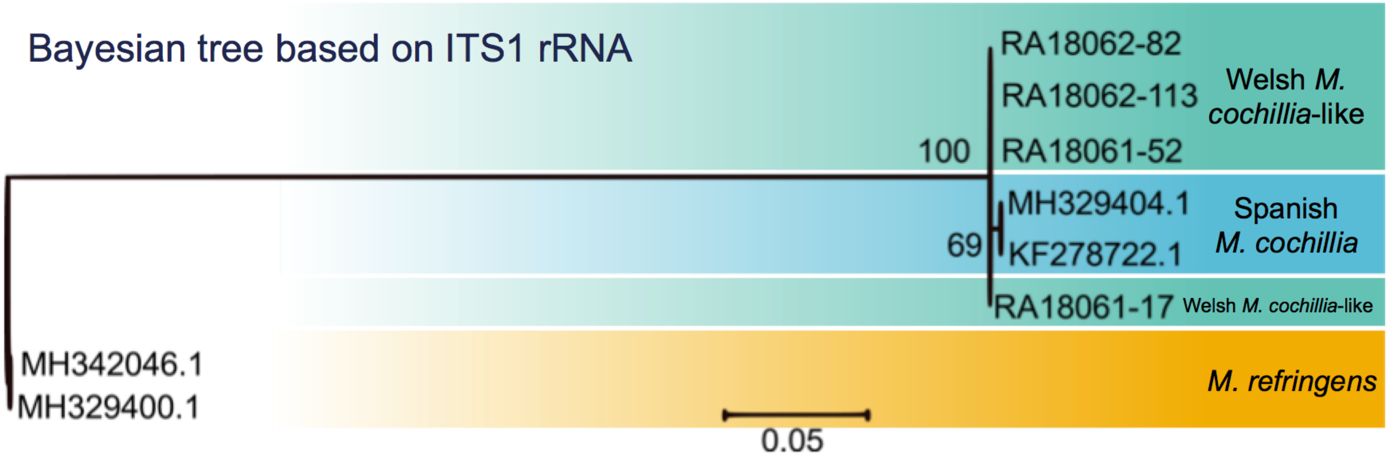
28S

ITS1

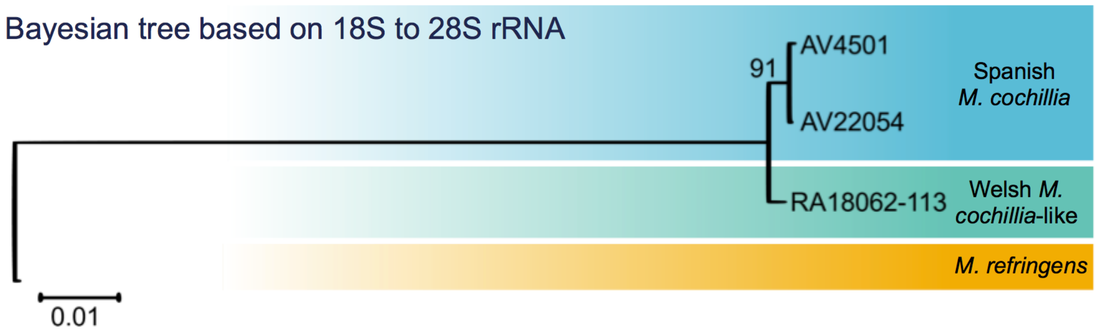
ITS2

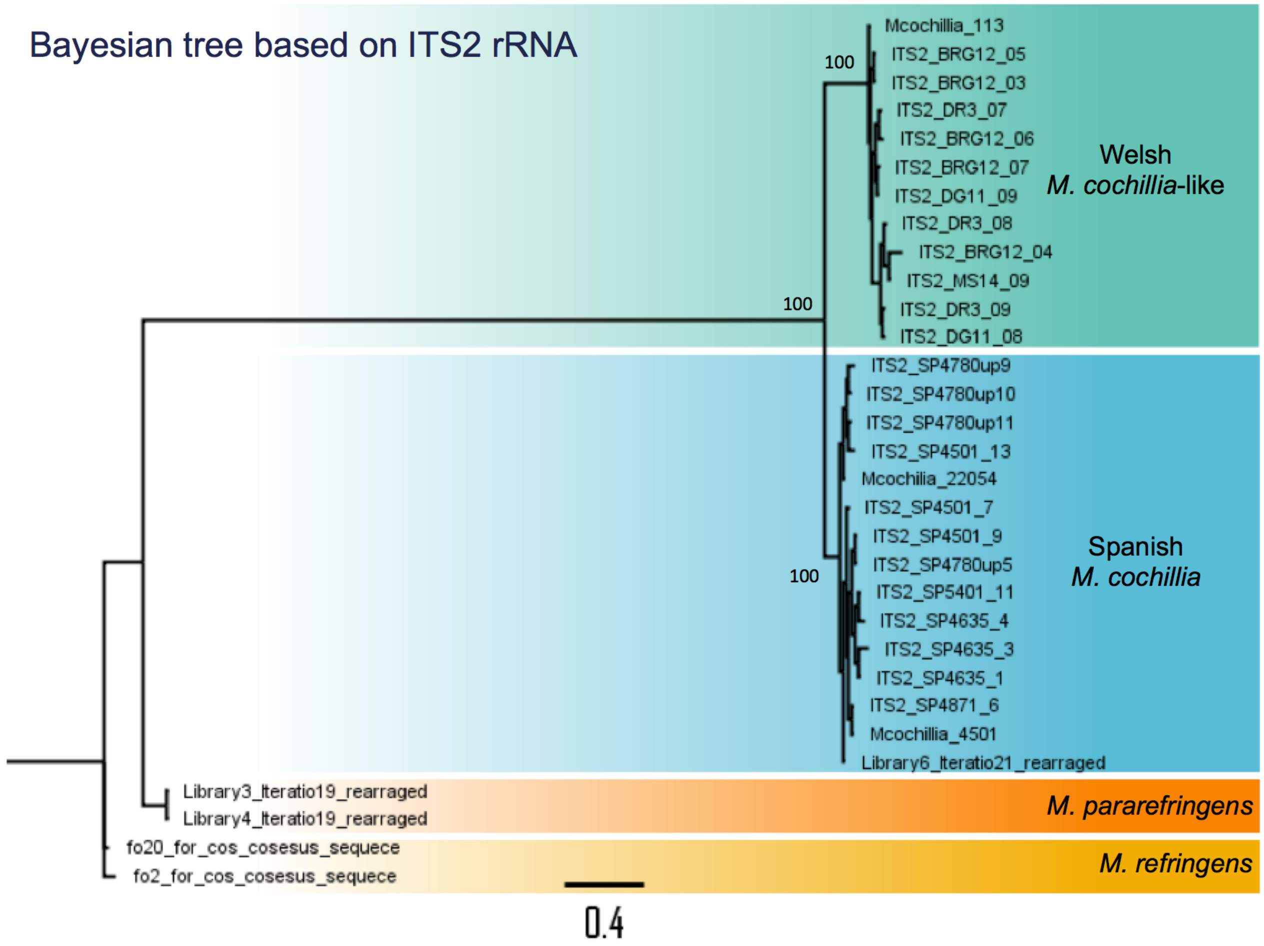
5 signatures

9 signatures

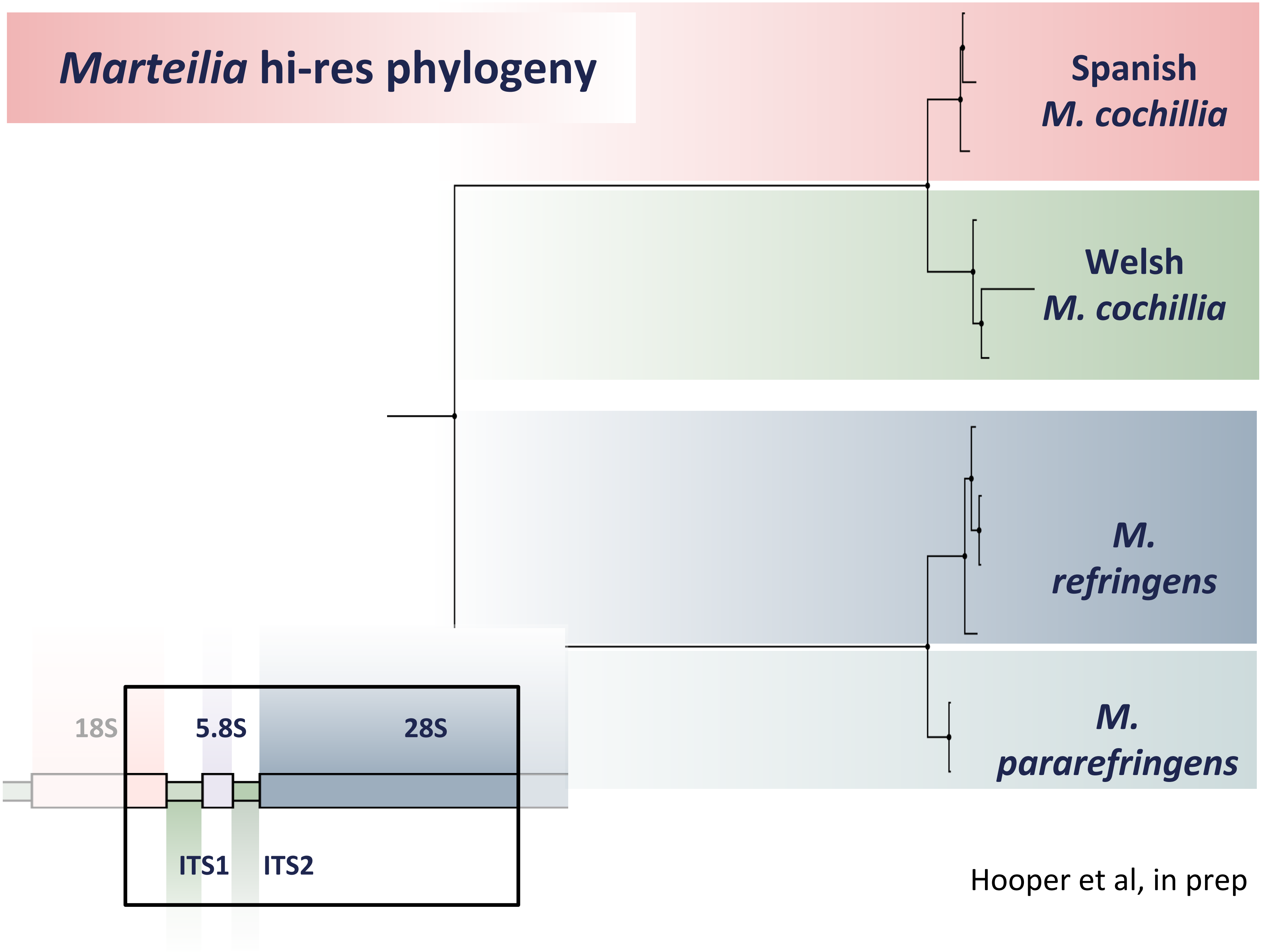


Bayesian tree based on ITS1 rRNA


Bayesian tree based on 18S to 28S rRNA
91
AV4501
AV22054
Spanish *M. cochillia*
RA18062-113
Welsh *M. cochillia*-like
M. refringens
0.01


Bayesian tree based on ITS2 rRNA
Mcochillia_113
ITS2_BRG12_05
ITS2_BRG12_03
ITS2_DR3_07
ITS2_BRG12_06
ITS2_BRG12_07
ITS2_DG11_09
ITS2_DR3_08
ITS2_BRG12_04
ITS2_MS14_09
ITS2_DR3_09
ITS2_DG11_08
100
100
Welsh *M. cochillia*-like
ITS2_SP4780up9
ITS2_SP4780up10
ITS2_SP4780up11
ITS2_SP4501_13
Mcochilia_22054
ITS2_SP4501_7
ITS2_SP4501_9
ITS2_SP4780up5
ITS2_SP5401_11
ITS2_SP4635_4
ITS2_SP4635_3
ITS2_SP4635_1
ITS2_SP4871_6
Mcochillia_4501
Library6_Iteratio21_rearraged
100
Spanish *M. cochillia*
Library3_Iteratio19_rearraged
Library4_Iteratio19_rearraged
M. pararefringens
fo20_for_cos_cosesus_sequece
fo2_for_cos_cosesus_sequece
M. refringens
0.4

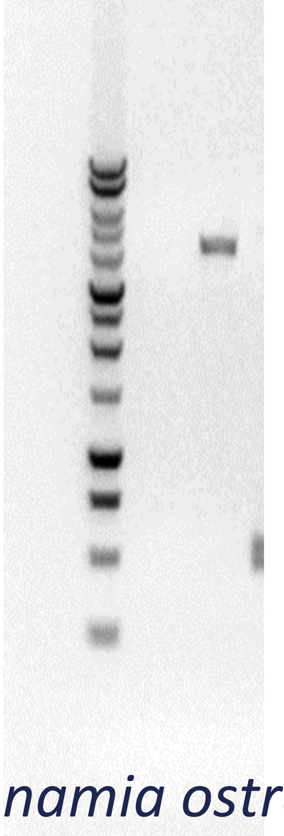
Marteilia hi-res phylogeny



Hooper et al, in prep

Future Work

Sequence more lineages, create reference datasets, improve diagnostics



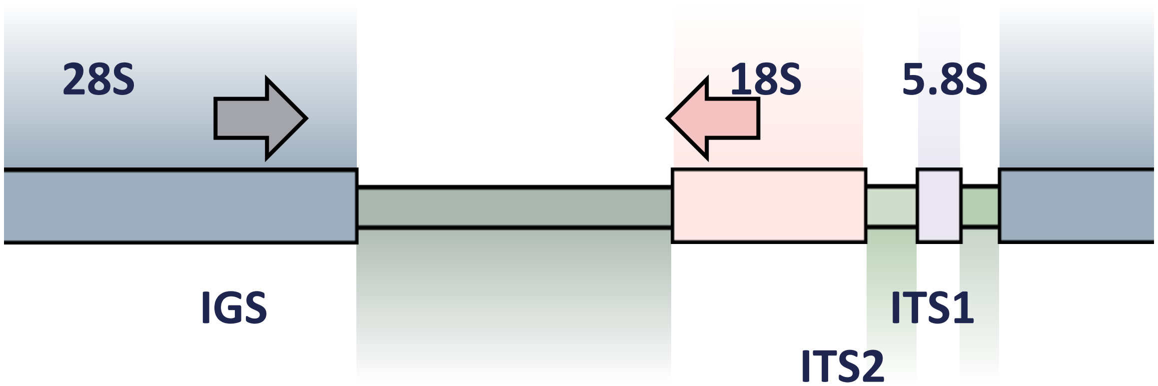
Bonamia ostreae

Haplosporidium spp

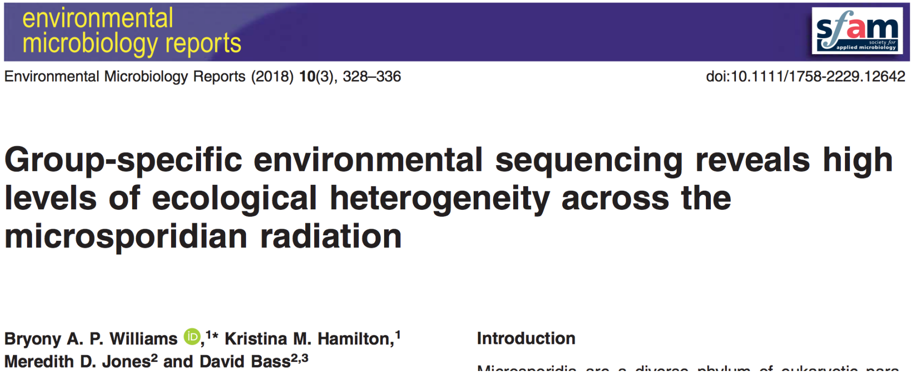
~ 3.5 kb

PacBio sequencing of amplicons

Sequencing of the IGS?



Group-specific studies of parasitic groups: discovery of novel parasites, lifecycle and ecological insight



environmental microbiology reports

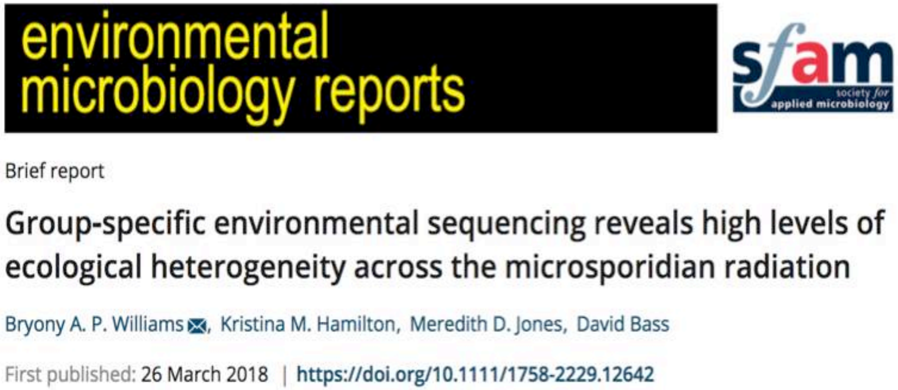
Environmental Microbiology Reports (2018) **10**(3), 328–336 doi:10.1111/1758-2229.12642

Group-specific environmental sequencing reveals high levels of ecological heterogeneity across the microsporidian radiation

Bryony A. P. Williams ,[1]* Kristina M. Hamilton,[1] Meredith D. Jones[2] and David Bass[2,3]

Introduction

Microsporidia are a diverse phylum of eukaryotic para



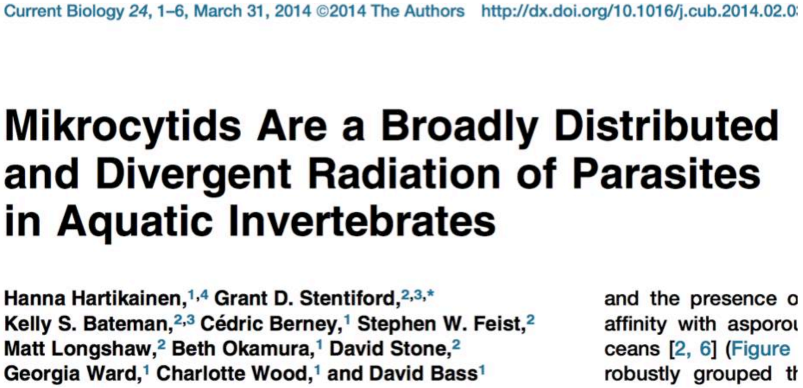
environmental microbiology reports

Brief report

Group-specific environmental sequencing reveals high levels of ecological heterogeneity across the microsporidian radiation

Bryony A. P. Williams, Kristina M. Hamilton, Meredith D. Jones, David Bass

First published: 26 March 2018 | **https://doi.org/10.1111/1758-2229.12642**

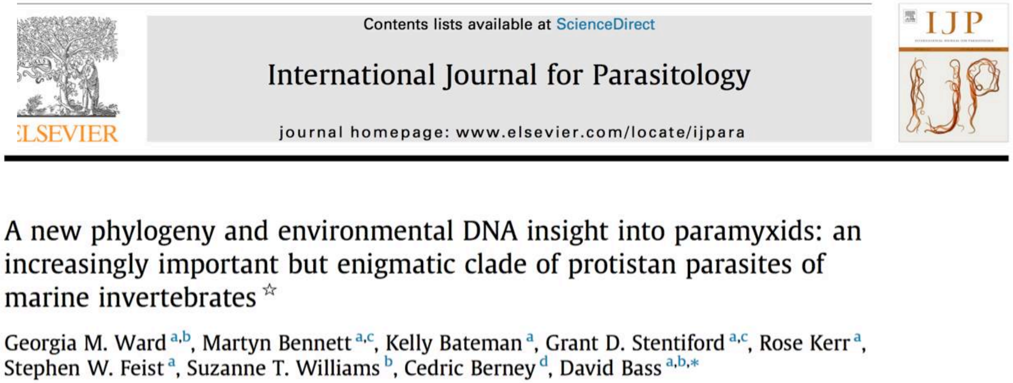


Current Biology *24*, 1–6, March 31, 2014 ©2014 The Authors http://dx.doi.org/10.1016/j.cub.2014.02.0

Mikrocytids Are a Broadly Distributed and Divergent Radiation of Parasites in Aquatic Invertebrates

Hanna Hartikainen,[1,4] Grant D. Stentiford,[2,3,*] Kelly S. Bateman,[2,3] Cédric Berney,[1] Stephen W. Feist,[2] Matt Longshaw,[2] Beth Okamura,[1] David Stone,[2] Georgia Ward,[1] Charlotte Wood,[1] and David Bass[1]

and the presence o
affinity with asporo
ceans [2, 6] (Figure
robustly grouped tl



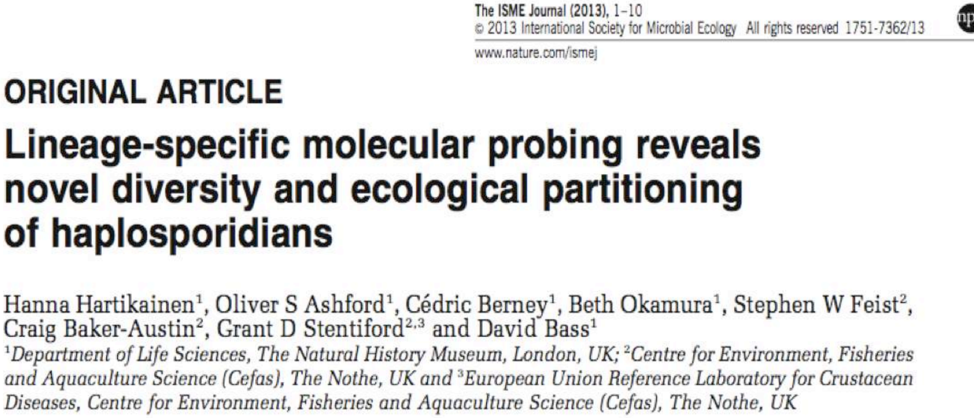
Contents lists available at ScienceDirect

International Journal for Parasitology

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

A new phylogeny and environmental DNA insight into paramyxids: an increasingly important but enigmatic clade of protistan parasites of marine invertebrates ☆

Georgia M. Ward[a,b], Martyn Bennett[a,c], Kelly Bateman[a], Grant D. Stentiford[a,c], Rose Kerr[a], Stephen W. Feist[a], Suzanne T. Williams[b], Cedric Berney[d], David Bass[a,b,*]



The ISME Journal (2013), 1–10
© 2013 International Society for Microbial Ecology All rights reserved 1751-7362/13
www.nature.com/ismej

ORIGINAL ARTICLE

Lineage-specific molecular probing reveals novel diversity and ecological partitioning of haplosporidians

Hanna Hartikainen[1], Oliver S Ashford[1], Cédric Berney[1], Beth Okamura[1], Stephen W Feist[2], Craig Baker-Austin[2], Grant D Stentiford[2,3] and David Bass[1]

[1]Department of Life Sciences, The Natural History Museum, London, UK; [2]Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, UK and [3]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, UK

The Journal of Eukaryotic Microbiology

Published by the International Society of Protistologists

Journal of Eukaryotic Microbiology ISSN 1066-5234

ORIGINAL ARTICLE

Environmental Sequencing Fills the Gap Between Parasitic Haplosporidians and Free-living Giant Amoebae

Georgia M. Ward[a,b,c], Sigrid Neuhauser[d], René Groben[e,1], Stefan Ciaghi[d], Cédric Berney[f], Sarah Romac[f] & David Bass[a,b]

Pathobiome concept

Trends in Ecology & Evolution

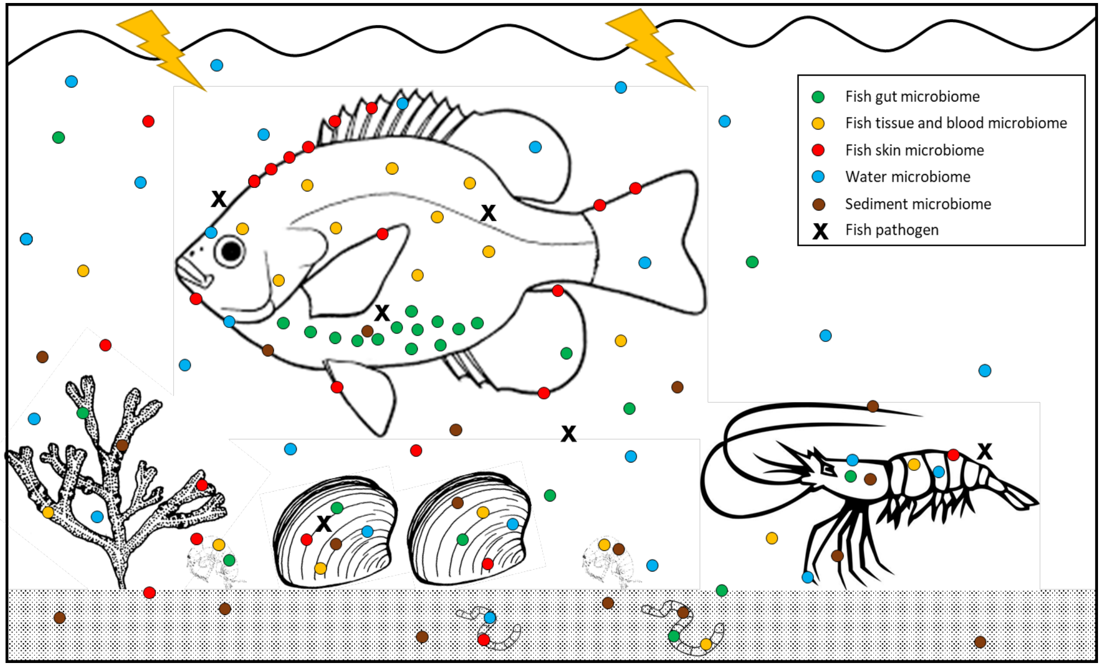
CellPress REVIEWS

Review

The Pathobiome in Animal and Plant Diseases

David Bass,[1,2,3,@,*] Grant D. Stentiford,[1,2,@] Han-Ching Wang,[4,5,@] Britt Koskella,[6,@] and Charles R. Tyler[2,7]

Trends in Ecology and Evolution, 2019



Pathobiome:

set of host-associated organisms (prokaryotes, eukaryotes, and viruses) associated with reduced health status, as a result of interactions between symbionts, host, and environment.