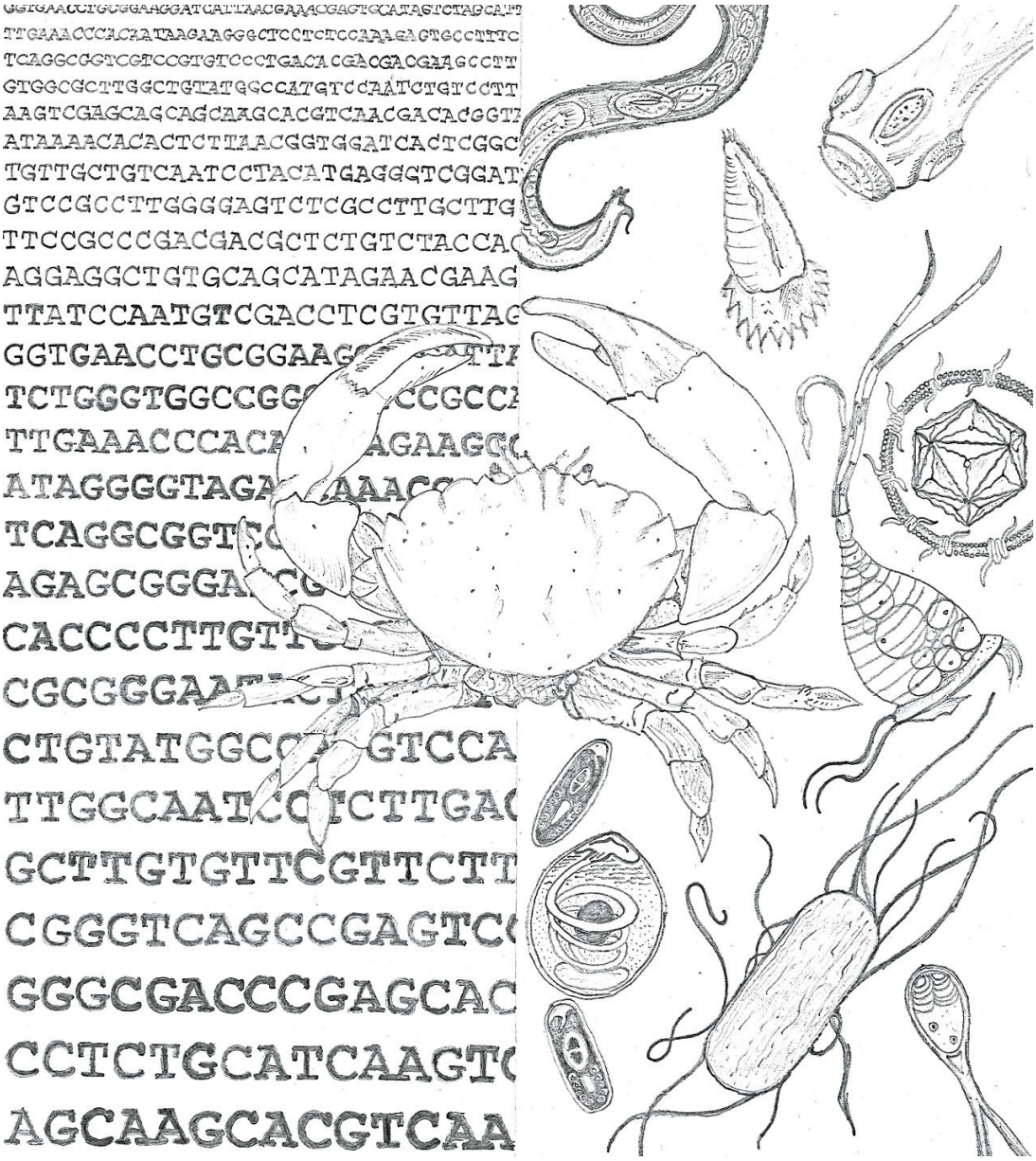
I

PARASITES OF INVASIVE CRUSTACEA: RISKS AND OPPORTUNITIES FOR CONTROL

Jamie Bojko



Submitted in accordance with the requirements for the degree of

Doctor of Philosophy

University of Leeds

Faculty of Biological Sciences

Submission date: June 2017

II

DECLARATION AND AUTHOR CONTRIBUTIONS

The candidate confirms that the work submitted is his own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Author contributions to publications by chapter:

CHAPTER 4

Publication reference: Bojko, J., Clark, F., Bass, D., Dunn, A. M., Stewart-Clark, S., Stebbing, P. D., & Stentiford, G. D. (2017). *Parahepatospora carcini* n. gen., n. sp., a parasite of invasive *Carcinus maenas* with intermediate features of sporogony between the *Enterocytozoon* clade and other microsporidia. Journal of invertebrate pathology, 143, 124-134.

J. Bojko (candidate): Experimental design, animal collection, histology, TEM, molecular diagnostics, phylogenetics, diagram design and writing.

F. Clark: Collection of *C. maenas* from Canadian coastline.

D. Bass: Phylogenetic analysis of the parasite.

A. M. Dunn: Supervisor (contributor to experimental design and text).

S. Stewart-Clark: Collection of *C. maenas* from Canadian coastline.

P. D. Stebbing: Supervisor (contributor to experimental design and text).

G. D. Stentiford: Supervisor (contributor to experimental design and text).

CHAPTER 5

Publication reference: Bojko, J., Dunn, A. M., Stebbing, P. D., Ross, S. H., Kerr, R. C., & Stentiford, G. D. (2015). *Cucumispora ornata* n. sp. (Fungi: Microsporidia) infecting invasive ‘demon shrimp’ (*Dikerogammarus haemobaphes*) in the United Kingdom. Journal of invertebrate pathology, 128, 22-30.

J. Bojko (candidate): Experimental design, animal collection, histology, TEM, molecular diagnostics, phylogenetics, diagram design and writing.

A. M. Dunn: Supervisor (contributor to experimental design and text).

P. D. Stebbing: Supervisor (contributor to experimental design and text).

S. H. Ross: Help with TEM.

R. C. Kerr: Advice for phylogenetic analysis.

G. D. Stentiford: Supervisor (contributor to experimental design and text).

CHAPTER 6

Publication reference: Bojko, J., Bacela-Spychalska, K., Stebbing, P. D., Dunn, A. M., Grabowski, M., Rachalewski, M., & Stentiford, G. D. (2017). Parasites, pathogens and commensals in the “low-impact” non-native amphipod host *Gammarus roeselii*. Parasites and Vectors, 10(193), 1-15.

J. Bojko (candidate): Experimental design, animal collection, histology, TEM, molecular diagnostics, phylogenetics, diagram design and writing.

K. Bacela-Spychalska: Co-supervisor for an eCOST STSM grant.

P. D. Stebbing: Supervisor (contributor to experimental design and text).

A. M. Dunn: Supervisor (contributor to experimental design and text).

M. Grabowski: Phylogenetics advice and help with animal collection.

M. Rachalewski: Help with animal collection.

G. D. Stentiford: Supervisor (contributor to experimental design and text).

ACKNOWLEDGMENTS

Firstly I would like to thank each of my supervisors: Alison Dunn; Grant Stentiford; and Paul Stebbing; for their time, help, and dedication to making me a better scientist. We have now been working together for 7 years (2011-2017), including my industrial placement and PhD studies, and I hope to continue to work with these fantastic scientists as I progress through my career. Many a parasite is yet to be discovered, I am sure!

A huge thanks to all of my newly acquired colleagues and friends who have helped guide me through my PhD studies and provided me with unending enthusiasm for the subject area. To Åsa Johannesen for helping me to visit the Faroe Islands, catch *C. maenas* and watch birds. To The Clarks (Sarah, Fraser and Rory), Brad Elliot and Stephanie Hall for making me feel at home in Canada; not to mention the many hours of collecting, dissecting, and squid and lobster fishing! To Karolina Bacela-Spychalska, Michał Grabowski, Michał Rachalewski and Piotr Gadawski for their help, enthusiasm, fun and fantastic shrimp merchandise provided during my visit to Poland. To David Bass (NHM/Cefas) for training me in phylogenetics, to Ronny van Aerle (Cefas) for training me in bioinformatics and Chris Hassall for much needed statistical advice (great job guys!).

I doubt I would have held onto my sanity without my friends and family! I would like to thank my Mum (Sonia Mellor), Grandma (Rita Mellor), Granddad (Barry Mellor), Sister (Jodie Bojko) and Brother (Danny Bojko) for making me laugh, annoying me, but always being there to help me and listen to my many boring problems. To my partner, Martin Rogers (PP) for all those lifts to and from University, both literally and metaphorically, and for his death-defying triumph of dealing with me for the past 4+ years (I think it's time for a holiday 🐶).

To the entire 'Dunn Lab' (past and present) and to my Leeds drinking buddies: James Rouse; Jack Goode; James Cooper; and John Grahame (to name a few) for all those extremely important, couldn't-be-missed meetings at the pub to discuss life, love, snails and everything in between. Similarly, to my Cefas/Weymouth buddies: Owen Morgan (password protected), Michelle Pond, Matt Green, Rose Kerr, John Bignell, Kayleigh Taylor, Stuart Ross, Georgina Rimmer, Tim Bean and to everyone at Cefas who didn't mind wasting their life talking about amphipods over a pint.

I couldn't forget Georgia Ward who was the pear-fect conference companion… Many conference-antics to follow I hope!

Funding acknowledgements:

I would like to thank the Natural Environment Research Council (NERC) for funding the majority of this PhD (award #: 1368300), and additionally the funding acquired by Alison M. Dunn from NERC (Grant: NE/G015201/1), which also contributed to my studies.

Thanks to the Centre for environment, fisheries and aquaculture sciences (Cefas) (CASE Partners) for all their funding contributions, particularly contract DP227X to Grant Stentiford and myself. Special thanks to Ioanna Katsidaki and Lisa Sivyer, who provided extra funding from Cefas seedcorn for pathogen discovery, to contract DP227X. Also to the Crustacean EURL (Grant Stentiford) who contributed payment to my work and travel. Thanks to David Bass for providing funding to conduct a metagenomics screen of invasive amphipods.

Thanks to the Polish National Science Centre (grant No. 2011/03/D/NZ8/03012) who provided funding to Karolina Bacela-Spychalska, which contributed to Amphipod collection in Poland. Additionally to TD1209AlienChallenge (eCOST) who provided a short term scientific mission grant to allow me to visit Poland.

Final thanks to Katrin Linse at the British Antarctic Survey who provided funding to allow me to continue PhD study whilst delving into the interesting world of Antarctic and deep sea microsporidia.

Acknowledgements by Chapter:

Chapter 2: Thanks to Fraser Clark, Sarah Stewart-Clark and Åsa Johannesen for helping me to visit Canada and the Faroe Islands, and for their help collecting and dissecting shore crabs. Thanks to Brad Elliot and Stephanie Hall for their help in dissecting. To Kelly Bateman for raw 2010 UK *C. maenas* histology data and HLV TEM images. To Stuart Ross for ultrathin sectioning for TEM. To Chris Hassall for providing statistical advice.

Chapter 3: Thanks to Karolina Bacela-Spychalska, Michał Grabowski, and Michał Rachalewski for their help in identifying and collecting amphipods in Poland. To Stuart Ross for ultrathin sectioning for TEM.

Chapter 4: Thanks to Fraser Clark and Sarah Stewart-Clark for help collecting and dissecting the crabs. To David Bass for helping me to construct the phylogenetic tree. To Stuart Ross for ultrathin sectioning for TEM.

Chapter 5: Thanks to Rose Kerr for looking over my phylogenetic tree and to Stuart Ross for ultrathin sectioning for TEM.

Chapter 6: Thanks to Karolina Bacela-Spychalska, Michał Grabowski and Michał Rachalewski for help in collecting and identifying amphipod specimens and for help with the phylogenetic analysis. To Stuart Ross for ultrathin sectioning for TEM.

Chapter 7: Thanks to Karolina Bacela-Spychalska, Michał Grabowski and Michał Rachalewski for help in collecting and identifying amphipod specimens. To Tim Bean who trained me to use the Illumina MiSeq and to Ronny van Aerle for training me to use the bioinformatics software. To Stuart Ross for ultrathin sectioning for TEM.

Chapter 8: Thanks to David Bass for finding the money for me to put some shrimp through the MiSeq. To Rose Kerr who also trained me to use the Illumina MiSeq and to Ronny van Aerle for training me to use the bioinformatics software.

Chapter 9: Thanks to Ben Pile, Ben Cargill and Alice Deacon for their help in collecting behavioural and survival data. To Chris Hassall for providing statistical advice. To Stuart Ross for ultrathin sectioning for TEM.

ABSTRACT

Invasive species are one of the foremost damaging environmental problems for biodiversity and conservation, and can affect human health and man-made structures. They pose a great challenge for pest management, with little known about their control and few available success stories. Many crustacean species are invasive and can affect both biodiversity and aquaculture. Controlling invasive Crustacea is a complex and arduous process, but success could lead to increased environmental protection and conservation. Invasive Crustacea also comprise a significant pathway for the introduction of invasive pathogens. If these invaders carry pathogens, parasites or commensals to a new site they may threaten native species. Alternatively, pathogens can control their invasive host and could be utilised in a targeted biological control effort as a biocontrol agent.

Looking specifically at one species of invasive brachyuran crab (*Carcinus maenas*) collected from the UK, Faroes Islands and Atlantic Canada, and several species of invasive amphipod from the UK and Poland, I explore which groups of microorganisms are carried alongside invasions, and if any could be used as biocontrol agents or whether they pose a threat to native wildlife.

This thesis involves wide-scale screening of *Carcinus maenas* and several amphipod species, identifying a range of metazoans, fungi, protozoa, bacteria and viruses; many new to science. Taxonomic descriptions are provided for previously unknown taxa: *Parahepatospora carcini*; *Cucumispora ornata*; *Cucumispora roeselii*; and *Aquarickettsiella crustaci*. The application of metagenomics to pathogen invasion ecology is also explored, determining that it can be used as an early screening system to detect rare and/or asymptomatic microbial associations. Finally, I used experimental systems to assess the impact of pathogens carried by *Dikerogammarus haemobaphes* upon both itself and alternate host species (*Dikerogammarus villosus* and *Gammarus pulex*), identifying that *C. ornata* can infect native species and decrease their chance of survival.

Overall this thesis describes a research process following through three main steps: i) invasive pathogen detection, ii) taxonomic identification, and iii) host range and pathological risk assessment and impact. Screening invasive and non-native hosts for pathogens is recommended for invasive species entering the UK, to provide a fast and informed risk assessment process for hazardous hitchhiking microbes.

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ABBREVIATIONS

16S: 16S Ribosomal Gene/Protein

18S: 18S Ribosomal Gene/Protein

23S: 23S Ribosomal Gene/Protein

28S: 28S Ribosomal Gene/Protein

5.8S: 5.8S Ribosomal Gene/Protein

5S: 5S Ribosomal Gene/Protein

AquaNIS: Aquatic Alien Species Database

Bt Toxin: *Bacillus thuringiensis* Toxin

CmBV: *Carcinus maenas* Bacilliform Virus

DhbfIV: *Dikerogammarus haemobaphes* bi-facies-like Virus

DhBV: *Dikerogammarus haemobaphes* Bacilliform Virus

DNA: Deoxyribose Nucleic Acid

DvBV: *Dikerogammarus villosus* Bacilliform Virus

EASIN: European Alien Species Information Network

eDNA: Environmental DNA

RNA: Ribose Nucleic Acid

RNAi: RNA interference

SEM: Scanning Electron Microscopy

SMT: Sterile Male Technique

snRNA: Small Nuclear RNA

SSU: Small-Sub Unit

TEM: Transmission Electron Microscopy

WSSV: White Spot Syndrome Virus

GISD: Global Invasive Species Database

GLM: Generalised Linear Model

GMO: Genetically Modified Organism

GrBV: *Gammarus roeselii* Bacilliform Virus

GvBV: *Gammarus varsoviensis* Bacilliform Virus

H&E: Haematoxylin and Eosin

IAI: Invasive Aquatic Invertebrate

IAS: Invasive Alien Species

IMS: Industrial Methylated Spirit

INNS: Invasive Non-Native Species

IPM: Integrated Pest Management

mRNA: Messenger RNA

NNS: Non-Native Species

PrBV: *Pontogammarus robustoides* Bacilliform Virus

rDNA: Ribosomal DNA

RLO: Rickettsia-Like Organism

CHAPTER 1

Introduction: Invasive crustaceans and their pathogens

1.1. Outline

Biological invasions can lead to changes in host-parasite relationships (Dunn and Hatcher, 2015). Carrying, losing, or gaining pathogenic and parasitic hitchhikers can alter the invasive potential of non-native species (Torchin et al. 2003; Vilcinskas, 2015) and can drive changes in the invaded community (Dunn and Hatcher, 2015). The pathogens carried by invasive species have the potential to infect and cause harm to native wildlife (Roy et al. 2016), but alternatively can have the potential to control the invasive population through biological control (Messing and Wright, 2006).

In this chapter I review the literature on invasive crustaceans to identify invasive pathogens (pathogens carried by invasive species) that could cause wildlife disease, and/or biological agents that could be utilised in integrated pest management to control their host. Herein I use the terms: pathogen (infective viral, bacterial or unicellular agent that reduces survival and host health); parasite (infective eukaryotic agent that reduces host health and may induce mortality); commensal (epibiont or ectobiont that does not increase or decrease host health); and mutualist (a symbiont that increases host health via a given mechanism), which all come under the primary term ‘symbiont’. Firstly I explore our current knowledge of the hitchhikers carried by invasive and non-native crustaceans and the legislation surrounding the discovery, control and risk assessment of these symbionts. Secondly, I explore the range of control options currently tried and tested for crustaceans, focussing primarily on the potential for biological control. I then introduce the study systems used throughout this thesis and explore the available pathogen-discovery techniques. Finally I lay out the study areas covered in each chapter. Broadly, this thesis follows a three part process, exploring firstly the broad-scale

screening of invasive Crustacea, secondly the taxonomic description of those pathogens, parasites and commensals identified, and ending with the experimental assessment of whether those pathogens act as biological control agents for the invasive host, or whether they pose a greater threat as invasive pathogens.

1.2. Invasive Crustacea and their hidden entourage of parasites, pathogens and commensal hitchhikers

1.2.1. Invasive aquatic invertebrates and their parasites

Invasive species success has increased due to human activity (Hulme, 2009). In recent decades, biologists surveying invasions have come to realise the importance of combating invasive alien species (IAS) and their pathogens, which constitute a major threat to natural biodiversity (Dunn and Hatcher, 2015; Hulme et al. 2015). IAS can affect both the environmental integrity and ecosystem services (Pyšek and Richardson, 2010), and the associated cost of repair can be significant, with high costs (>$1bn USD) associated with maintaining and re-constructing invaded areas (e.g. economic impact of invasive species in the USA: Pimental et al. 2005).

The success of an invader can depend on an array of “invasive” characteristics, for example, increased competitive capability (Human and Gordon, 1996); beneficial morphological features (e.g. size) (Roy et al. 2002); and behaviour (competitive, predatory, etc.) (Sol et al. 2002). Other factors can also be involved with an invasion dynamic; one being the presence or absence of parasites and pathogens.

In some cases, invaders lose their parasites and pathogens along their invasion pathway (via ‘enemy release’), increasing their fitness and competitive capability (Colautti et al. 2004). Alternatively, parasites and pathogens can infect susceptible native species and persist in novel locations and invasive and native populations (spill-over and spill-back) (Kelly et al. 2009). Transporting pathogens along an invasion route can result in the infection of susceptible native species and thus remove competition (e.g. parasite mediated competition: Prenter et al. 2004) or the parasite could provide the invader with a benefit, increasing its invasive success (e.g. *Fibrillanosema crangonictidae* and the invasion success of *Crangonyx* sp.: Hatcher et al. 1999; Slothouber-Galbreath et al. 2004). In some cases, when an invasive propagule (sub-set of invasive individuals) maintains an infection that is detrimental to the invasive host, it may result in the control of that invasive population and lower the impact of the invader via biological control (Hajek and Delalibera, 2010).

The invasive aquatic invertebrates (IAIs) comprise a group of invaders that include all freshwater, marine and semi-aquatic invertebrate species that have been termed invasive across the globe by online databases. These databases provide data on invaders, including: their country of origin; invasion site(s); invasion pathway(s); and their relative impact rating (Luque et al. 2014), avoiding the need to trawl scientific literature (Ricciardi et al. 2000). Compiling data in an accessible fashion can help predict future invasions (Roy et al. 2014b), aid control and eradication programmes, support policy development, aid citizen science, and identify species that deserve greater research attention based on their environmental and health-based impacts (Will et al. 2015). The future of invasive species databases will benefit from the creation of INVASIVESNET; an online, and all-encompassing, database that will coalesce pre-existing databases and information into one accessible place (Lucy et al. 2016).

Using three of the available invasive species databases [Global Invasive Species Database (GISD), the European Alien Species Information Network (EASIN) and the Aquatic Alien Species Database (AquaNIS)] a list of IAIs has been compiled and includes 1054 species (Appendix Table 1.1). GISD comprises the main global database for invasive species; detailing their distribution across the globe (Appendix Table 1.2; Fig.1.1a-b). EASIN and AquaNIS are European focussed and catalogue invaders located in, and threatening, the countries of the EU. The IAIs highlighted using this method is dominated by crustaceans, molluscs and annelids (Fig. 1.2). Interestingly, few IAIs were universally highlighted on all three databases (n=22/1054) and each database provided differing numbers of IAIs (GISD=63, EASIN=896, AquaNIS=282). This suggests there is a lack of communication between databases and the development of one main database, as discussed previously, will greatly benefit the field of invasion biology (Ricciardi et al. 2000; Faulkner et al. 2014; Luque et al. 2014; Roy et al. 2014a; Will et al. 2015; Lucy et al. 2016).

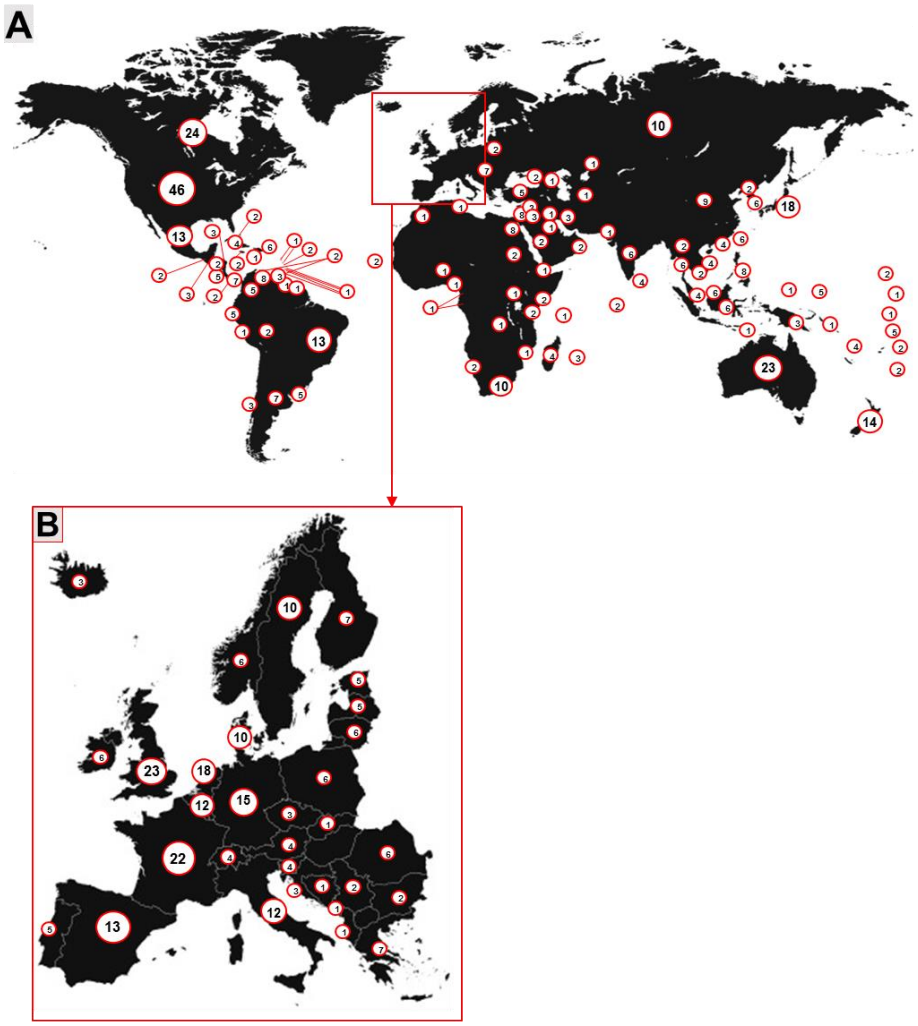


Figure 1.1: European and global numbers of IAIs listed on the Global Invasive Species Database. Countries without a number do not have IAIs as a listed priority.

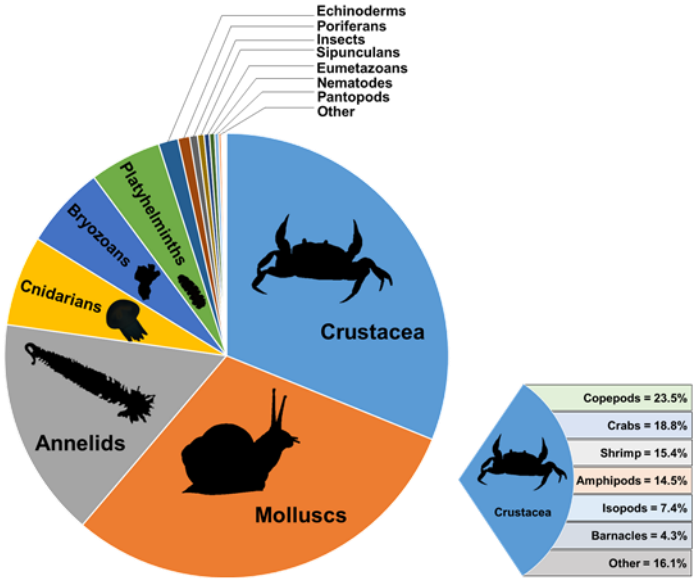


Figure 1.2: A breakdown of the taxonomic position of the 1054 IAIs obtained from three invasive species databases (GISD; EASIN; AquaNIS), focussing primarily on the Crustacea. The invasive Crustacea break down into seven groups: copepods (Copepoda); Crabs (Brachyura); Shrimp (Pleocyemata); amphipods (Amphipoda); isopods (Isopoda); Barnacles (Cirripedia); and other.

Of the 1054 IAIs catalogued by the various databases, 324 are crustaceans. Invasive Crustacea form the most numerous group within the IAIs and have been shown to impact upon biodiversity (MacNeil et al. 2013), ecosystem services and species diversity (MacNeil et al. 2013) and the environment (Dittel and Epifanio, 2009). By far, the damage to biodiversity is the most well understood consequence of crustacean invasion, with some key examples including the global European shore crab (*Carcinus maenas*) invasion (Darling et al. 2008), and the killer shrimp (*Dikerogammarus villosus*) invasion of the UK (MacNeil et al. 2013). Preservation of biodiversity is crucial to maintain the health of ecosystems and their services, whereby invasions are considered one of the most devastating processes to hinder conservation (McGeoch et al. 2016).

Based on their relative risk and impact, some crustacean species have been the focus of intense research activity for various reasons, where others are little researched. *Carcinus maenas*, for example, is utilised as a model organism for genetic/developmental studies (e.g. Verbruggen et al. 2015), ecotoxicology studies (e.g. Rodrigues and Pardal, 2014), parasitology studies (e.g. Stentiford and Feist, 2005), behavioural studies (Sneddon et al. 2000), and much more. Other invasive crustacean species such as the marine Brachyuran, *Actumnus globulus*, have received little attention aside from detection at invasion sites (Galil et al. 2008). This difference in research effort is reflected in the disease profiling of many invasive crustaceans. Diseases of invasive organisms (invasive pathogens/wildlife pathogens) are becoming recognised as an area of investigation for invasion biologists as we begin to recognise the threat posed to human and animal welfare (Roy et al. 2016).

1.2.2. Invasive crustaceans and their invasive pathogens

It has been highlighted that parasites in invasive species are heavily understudied (Roy et al. 2016). A clear understanding of the parasites and pathogens carried by IAIs is imperative to effectively assess the risk of invasive pathogens to native biodiversity, humans and livestock. Additionally, further knowledge of these pathogens allows for a true assessment of potential biological control agents. Here, invasive Crustacea are utilised as an example study-group to explore what is currently known about the pathogen profiles of an invasive group of organisms. This data are based on a review of the literature, and provides an insight into where the knowledge gaps are in invasive crustacean pathobiology.

The 324 invasive Crustacea highlighted from the 1054 IAIs (Appendix Table 1.1) split into seven broad groups: Copepods; Crabs; Shrimp; Amphipods; Isopods; Barnacles;

and Others (Fig. 1.2). Of these crustacean species 31.5% (102/324) have one or more documented associations with pathogenic, parasitic, commensal, or symbiotic organisms (Appendix Table 1.3). Adversely this indicates that 68.5% (222/324) of invasive Crustacea have no known parasitic or pathogenic associations – possibly reflecting a lack of research effort in some species.

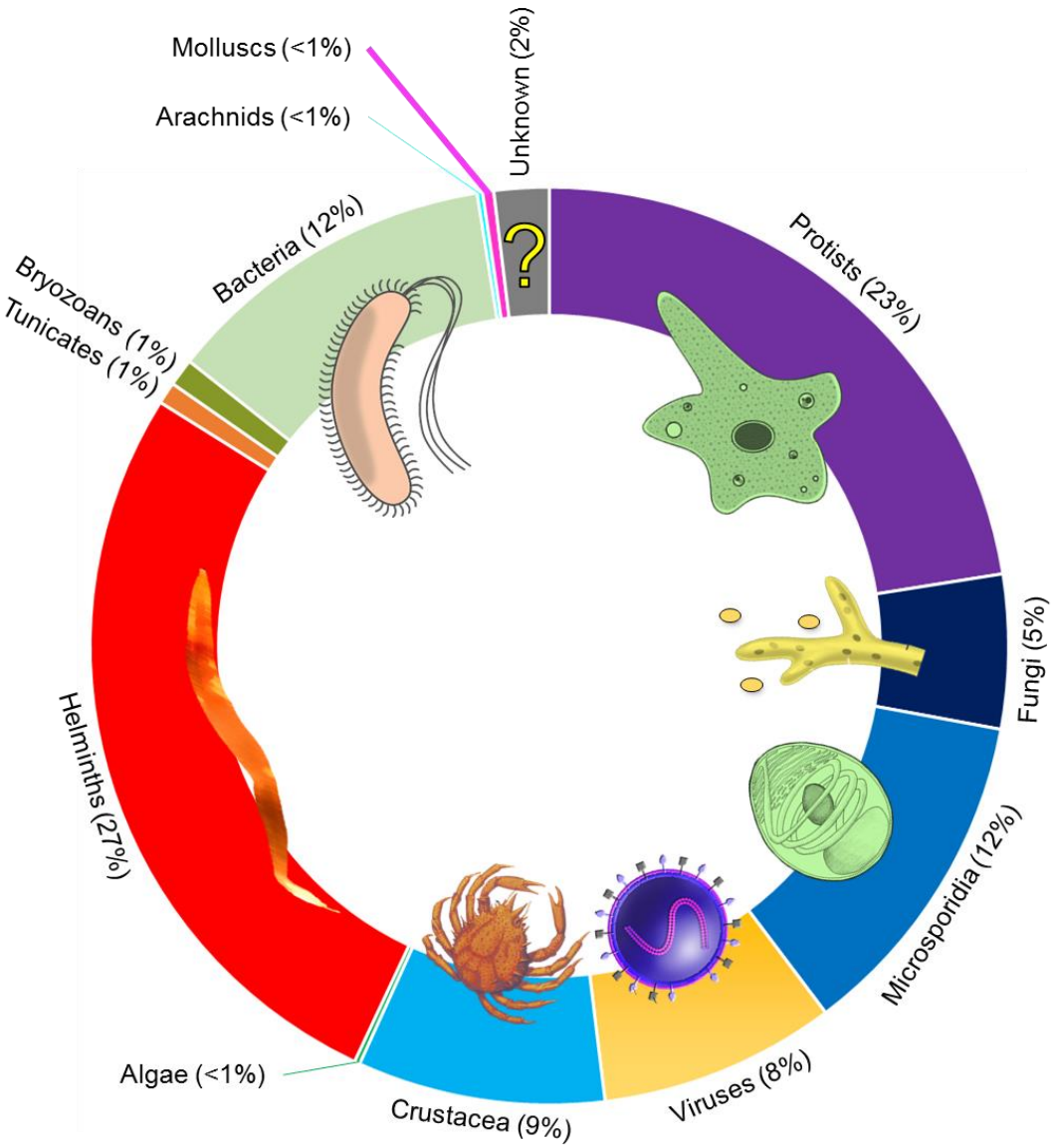


Figure 1.3: The relative number of different taxonomic groups found to associate with invasive crustaceans (n=324) from their native and invasive territories. Each broad grouping (microsporidia, viruses, etc.) are equipped with a percentage relative to the other taxa observed across the invasive crustaceans. In this case the 'Helminth' group refers to worm or worm-like parasites, such as nematodes, acanthocephala and trematodes.

Cumulatively, the invasive crustaceans have been associated with at least 391 symbionts that are taxonomically identified to genus level or higher (Appendix Table 1.3).

Ignoring the need for full taxonomic description, this number increases to at least 529 individual hitchhikers that infect, or are carried by, the invasive crustaceans (Appendix Table 1.3) (Fig. 1.3). In total, 670 associations have been made between the invasive crustacean hosts and a pathogen, parasite, commensal or mutualist.

Some invaders are difficult to attribute a clear total number of hitchhikers because they have been involved with large scale metagenomics and eDNA (environmental DNA) studies that detect a large diversity of microbial presence, such as the biofilm analysis of the American lobster, *Homarus americanus* (Meres et al. 2012). A certain level of scepticism must be taken in cases such as these due the possibility of environmental contamination or improper categorisation of gene sequence data (Chistoserdova, 2014). Despite this, metagenomics studies are at the forefront of rapidly assessing the microbiome of organisms, and applications of this technique would greatly increase our knowledge of the hidden organisms hitchhiking upon or within invasive Crustacea.

The most common invasive crustaceans are copepods (23.5% of invasive crustaceans), however this group plays host to only 39 known symbionts (Appendix Table 1.3). The group with the largest number of symbionts is the crabs (18.8% of invasive crustaceans), which are host to 240 symbionts. Shrimp and amphipods are also relatively well researched with 132 and 93 associations documented respectively. The isopods and barnacles have fewer associations, with only 32 and 5 symbionts documented respectively. Lobsters, despite only 6 being recognised as invasive species, have been well researched and have been found with 35 associations, which increases to 205 associations when large scale DNA studies are taken into account. Certain species have been the focus of many parasitological studies, such as the European shore crab, *C. maenas*, which has ~72 documented parasites, pathogens and commensals, many with full taxonomic descriptions (Appendix Table 1.3).

Some of the most devastating pathogens for wildlife and aquaculture are associated with Crustacea and several of these are linked to invasive counterparts, which have the potential to transmit them to novel locations where they could find susceptible hosts. *Aphanomyces astaci* is one of the greatest risks for endangered crayfish conservation and can be transmitted by several invasive crayfish species, within which the pathogen is asymptomatic (Alderman, 1990; Kozubíková and Petrusek, 2009). White Spot Syndrome Virus (WSSV) constitutes the worst disease to hit crustacean aquaculture; holding both a high host range and low host survival rate, and is known to infect 7.4% of invasive crustaceans (Stentiford et al. 2012; Stentiford et al. 2017; Appendix Table 1.3). Other pathogens, such as *Vibrio cholerae*, constitute a human health risk and is carried

by several invasive crustaceans, particularly invasive copepods (Daszak et al. 2000; Appendix Table 1.3).

Invasive groups such as the barnacles, isopods and copepods are little researched in comparison to some of the larger invaders such as crabs, shrimp and lobsters, however they still hold the ability of carrying invasive pathogens. *Carcinus maenas* is host to a conservative 72 organisms that could act as hitchhikers and travel to novel locations. *Homarus americanus* has 29 potential hitchhikers, however this increases to 199 if you include the large number of bacterial species identified through DNA sequence studies (Meres et al. 2012). If we assume that each invasive crustacean has the potential to carry a similar number of hitchhikers as those currently known for *C. maenas* to novel invasion sites, the 324 invasive crustaceans listed by invasive species databases may have the potential to carry 23,328 taxonomically different symbionts. This estimation touches upon how little we know about invasive pathogen diversity, and how much of a drawback this is to current research efforts to understand the risk associated with invasive pathogens (Roy et al. 2016). Based on available literature, we know of 670 observations of 529 supposedly different parasites, pathogens, commensals or symbionts (this could be the same species or different) across the invasive Crustacea, which accounts for only 2.9% of the above estimate. All of these hitchhikers would not necessarily have a negative impact at an invasion site, however an understanding of this diversity requires further research to recognise these species taxonomically and to assess their risk to native wildlife, aquaculture and human health, or their possible benefit for biologically controlling an invasive host.

1.3. Policy and the invasive pathogen

Human and livestock disease control, biosecurity and prevention is monitored by a range of different regulatory bodies like the World Health Organisation (WHO) and the World Organisation for Animal Health (OIE), which provide lists of diseases that must be reported if diagnosed (Stentiford et al. 2014). For invaders that are strongly associated with human disease, WHO often provide detailed responses such as the global vector control response (www.who.int/malaria/areas/vector_control/Draft-WHO-GVCR-2017-2030.pdf?ua=1) and develop control strategies for the eradication of disease vectors; some are invasive species (Mendis et al. 2009).

The OIE provides a similar function but for animal diseases of aquatic and terrestrial livestock involved in trade, and has the main aim to increase food security (Stentiford et al. 2014). One example includes the Aquatic animal health regulations (EU directive:

200688) for England and Wales, which outlines basic responses to wildlife disease outbreaks (such as Chitrid fungus, crayfish plague, or white spot syndrome virus) (associated with high wildlife mortality), which can be associated with invasive species. In conservation, few regulatory bodies are involved with the prevention and control of diseases that impact upon wildlife, and no regulatory body currently exists to solely serve this purpose (Dunn and Hatcher, 2015; Roy et al. 2016). Some invasive pathogens have begun to be listed alongside invasive hosts on invasive species databases (e.g. GISD lists the oomycete pathogen *A. astaci* (crayfish plague) in addition to the host, *P. leniusculus*); constituting a step forward for recognition of invasive pathogens as discrete IAS candidates, irrespective of the host that carries them.

The policy involved with invasive species is gaining a foothold, however it remains fragmented in places, particularly where invasive pathogens are concerned (Dunn and Hatcher, 2015; Roy et al. 2016). Agencies in the UK like the Department for Environment, Food and Rural Affairs (Defra) have priorities in the field of invasion biology, but often this is from the perspective of an invasive host, not the invasive pathogen. Research institutes such as the Centre for environment, fisheries and aquaculture sciences (Cefas) have taken to identifying the pathogens of aquatic invasive species (Stentiford et al. 2011; Bojko et al. 2013; Chapter 5). Early screening for newly identified invasive populations would be a crucial step forward to better understand the risk posed by invasive and non-native species and their pathogens (Chapter 6).

1.4. Control and management of aquatic crustaceans

Across the globe, food production and conservation efforts are hindered by pest species and disease causing agents. In agriculture and aquaculture, many species damage crops and livestock through consumption (Oliveira et al. 2014), competition (Gallandt and Weiner, 2007), or by vectoring disease (Lambin et al. 2010). This in turn affects the local and global economy through reduction in yield (Savary et al. 2012), health costs and loss of biodiversity (Roy et al. 2014).

Many industrial and domestic activities can be impacted by crustacean pests. Crop production and horticulture in terrestrial environments are hindered by terrestrial crustacean consumers (Gratwick, 1992; Martínez et al. 2014); some aquaculture industries produce lower yields because of pest crustaceans (Nicotri, 1977; Dumbauld et al. 2006); households can be invaded and compromised by pest and parasite infestations; and water purification and irrigation services can suffer from their colonisation (Bichai et al. 2008). In aquatic environments specifically, several pests thrive

by taking advantage of aquatic crops, livestock and harvestable food items. Examples include the parasitic salmon louse (*Lepeophtheirus salmonis*) that elicits disease in farmed and wild species of fish (Tully and Nolan, 2002); and the burrowing shrimp (*Neotrypaea californiensis* and *Upogebia pugettensis*) that impact heavily on oyster aquaculture (Dumbauld et al. 2006). Controlling these industrial and disease-causing pests is imperative to protect aquaculture industries world-wide.

Crustacea are additionally hazardous to wild environments as invasive species (Lovell et al. 2006). Invasive Crustacea can cause damage when their populations become established, grow and compete with native species: impacting upon the environment, ecosystems, and biodiversity (Hänfling et al. 2011). This in turn can have social and economic impacts as ecosystem services are compromised (Stebbing et al. 2015). Species that become invasive tend to possess certain 'characteristics' that increase their capability to become a substantial issue in novel environments (Kolar and Lodge, 2001). Each successful invader poses different threats to native ecology and imposes unique circumstances that must be considered before applying control (Allendorf and Lundquist, 2003). Such unique circumstances include: habitat choice; niche occupation; genetics; and behaviour – each of which can be exploited to increase the chance of successful control (Hänfling et al. 2011). Invasions can have varied impacts upon the economy and may require costly mitigation measures for their control and to maintain affected environments (Lovell et al. 2006). The invasive European shore crab (*Carcinus maenas*) constitutes a high-profile global invader, and aquaculture pest, that has been found to heavily impact invaded sites through decreasing biodiversity and predating on aquaculture species (Smith et al. 1955; Walton et al. 2002). Several invasive crustaceans have been observed to cause indirect damage to biodiversity by transporting pathogens that subsequently infect native species (Roy et al. 2016); one example is the non-native demon shrimp (*Dikerogammarus haemobaphes*) transporting microsporidian pathogens to the UK (Chapter 5).

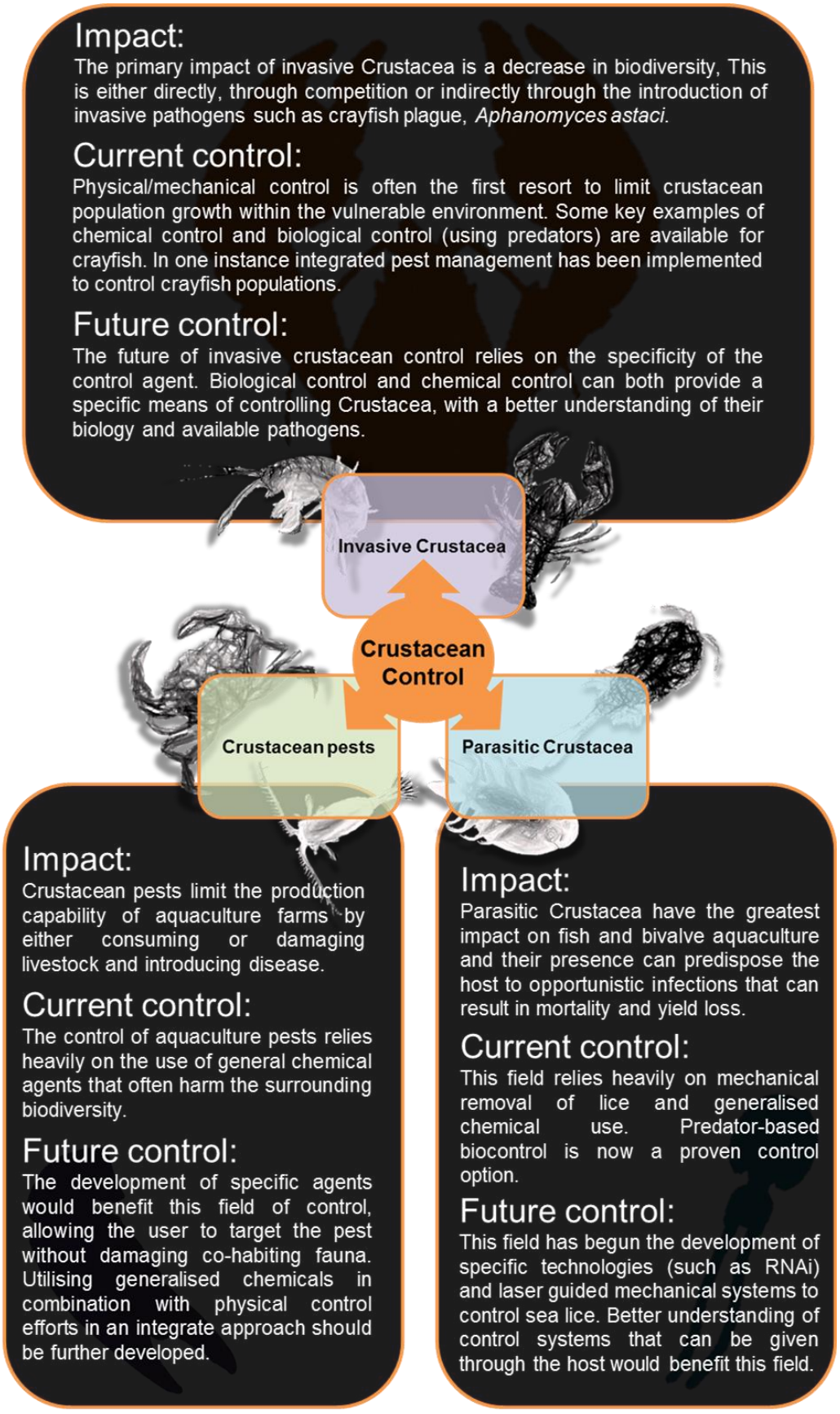


Figure 1.4: The impact, current control efforts and future potential for control outlined for the three crustacean pest groups.

Preventing the introduction of non-native crustaceans, and controlling established invaders, provides a difficult task. The applications of management measures, either to control invasive species already established or to prevent their introduction and spread, is a complex and difficult process; with management required to deal with a variety of invasive organisms, and their pathogens, travelling via multiple pathways and invading a wide array of environments (Dunn and Hatcher, 2015). Invasive species management requires input from ecologists, social scientists, resource managers, and economists (Simberloff et al. 2013), to develop and implement the control and eradication of invasive species, which is often complicated and open to scrutiny from many perspectives.

The concept of control in these scenarios provides an interesting and highly policy-relevant research effort (Fig. 1.4). As novel technologies, discoveries, and further understanding of biological mechanisms come about, the potential for crustacean control becomes more feasible and will begin to overtake the current dependence on chemical and physical control methods (Burridge et al. 2010). This next section looks at where current science has advanced in the field of controlling and managing aquatic Crustacea, specifically: industrial crustacean pests; disease-causing crustacean pests; and invasive crustacean pests. Current methods of control are discussed in addition to how new technologies and recent findings might benefit this field in the future.

1.4.1. Controlling aquatic crustacean pests

Aquaculture and wild fisheries provide a range of species, including: plants and algae; amphibians; fish; cnidarians; echinoderms; crustaceans; molluscs; and rotifers. The organisms harvested from these methods serve several purposes, usually as a food source (for human or animal consumption) but some provide an alternate purpose, such as farming coral(s) for conservation efforts (Delbeek, 2001), growing algae for gas (H_2, O_2) production (Melis and Happe, 2001), or breeding species for sale as ornamental animals (Andrews, 1990). Each can suffer from various crustacean pests.

In aquaculture, a wide range of crustacean pests are known to lower yield through consumption/predation of farmed species or wild harvest produce; many affecting aquatic crops (such as the herbivorous isopod: *Paridotea reticulata*) or sessile molluscs (such as burrowing shrimp) (Nicotri, 1977; Dumbauld et al. 2006). Many aquaculture efforts must pay a large amount to preserve their industry from pests by buying control agents and implementing biosecurity (Pillay and Kutty, 2005).

Copepods are common pests that impact upon rotifer aquaculture (Lubzens, 1987) and have recently been recorded to impact Chinese mitten crab (*Eirocheir sinensis*)

aquaculture (Zhao et al. 2012). The control of these pests is often approached from a biosecurity perspective, via the use of copepod-free water to prevent the problem arising, however some generalised chemical biocides have been tested for the removal of copepods in-situ (Zhao et al. 2012). “Pests-cleaner”, (active constituent: avermectin) and beta-cypermethrin are reported by Zhao et al (2012) to have crustacicidal properties, but “pests-cleaner” was identified as the better treatment of the two for crab aquaculture despite both avermectin and beta-cypermethrin affecting crab zoea growth (Zhao et al. 2012).

The seaweed and algal growth industry suffers from crustacean pests such as the isopod, *Idotea baltica* and the amphipod, *Ampithoe valida* (Nicotri, 1977; Smit et al. 2003). At high densities, these pests lowered algal growth by grazing (Nicotri, 1977). Another isopod pest, *Paridotea reticulata*, acts as a macro-algal grazer at high density and affects the growth of cultured *Gracilaria gracilis*. It is noted that this species can be beneficial in low numbers but high density populations result in *P. reticulata* becoming a significant pest (Smit et al. 2003). Attempts to control this pest have been made in-situ (Smit et al. 2003). Treatment was a simple process of submersion in freshwater for a 3 hour period, resulting in the *P. reticulata* being removed and the algal stock unharmed (Smit et al. 2003).

Burrowing shrimp (*Neotrypaea californiensis* and *Upogebia pugettensis*) have been shown to affect cultured and wild populations of sea grass as well as farmed oysters, resulting in a bid to develop a control regimen (Dumbauld et al. 2006). Carbaryl, a biocide used for over 40 years in the American oyster aquaculture industry, has been shown to be affective at high concentration (96% pest mortality) at reducing the numbers of burrowing shrimp but due to non-target effects on the native fauna, new methods are required to reduce environmental impact (Dumbauld et al. 2006). This resulting system consisted of a “decision tree” based on a variety of factors (bed type, ecology, etc.) that aided in the development and implementation of an integrated control process, including the use of carbaryl alongside particular physical control methods (Dumbauld et al. 2006).

1.4.2. Controlling disease-causing, parasitic Crustacea

The majority of biosecurity and control effort appears to be focussed on parasitic Crustacea, such as fish lice (Copepoda), which heavily impact piscine aquaculture (Costello, 2009). Control of fish lice is highly diverse and reaches into new technologies to forward the field of pest control.

Several crustacean species have specialised to become parasites. The most well-known examples include: ectoparasitic fish lice (Copepoda) (Johnson et al. 2004; Costello, 2006); copepods that dwell within the gut of farmed molluscs (Rayyan et al. 2004); parasitic isopods, such as *Cymothoa sp.*, which infest wild and aquaculture fish species (Costa et al. 2010); and parasitic crabs (*Pinnotheres* sp.) that live inside mussels and oysters (Trottier et al. 2012).

The highest impacting parasitic crustaceans are, by far, the fish lice. Fish lice are ectoparasitic copepods that puncture the flesh of fish, opening wounds that predispose fish to secondary infections and indirectly cause mortality (Johnson et al. 2004). This group of parasites also provide the widest range of examples for control; where research has not only focussed on chemical and physical control methods but has utilised genomic, transcriptomic and proteomic technologies to further understand weaknesses to exploit (Yasuike et al. 2012; Christie, 2014; Sutherland et al. 2014).

No fewer than 11 different chemicals have been adapted for the control/eradication of fish lice [Teflubenzuron, Ivermectin, Emamectin benzoate (SLICE®), Azamethiphos (Salmosan®), Cypermethrin (Excis®), Dichlorvos (Calicide®), Hydrogen Peroxide, Pyrethroids (Neguvon®)], which can be provided within feed or as a bath solution (Jensen et al. 2015; Jansen et al. 2016). The application of chemicals has positive results but can affect the environment and the flesh of the fish, making them less marketable (Haya et al. 2005). In many cases the use of these biocides has resulted in resistance to treatment, meaning one form of treatment usually becomes redundant after a given period, requiring constant development of new products (Aaen et al. 2015).

Physical control of sea lice involves monitoring to catch early infections, considering parasite transmission dynamics, and manual labour to remove and control infection levels. Farms benefit by reducing their chances of infection by understanding where best to place the farm in the catchment. When farms are located outside the eddy currents, where lice pool, the risk of infection is lowered (Amundrud and Murray, 2009). Lice can be manually removed from fish without subjecting them to harmful chemicals or risking biocontrol, but this is a costly method due to human labour and is often insufficient (Costello, 1993). Temperature and freshwater has also been applied to control the lice without harming the fish or environment, with varied success (Costello, 1993).

Biological control of salmon lice (*Lepeophtheirus salmonis*) uses two main fish species (wrasse: Labridae, and lump-fish: *Cyclopterus sp.*) that act as lice-predators and readily remove lice from infected stock (Groner et al. 2013). It is now becoming apparent that some of the fish used as biocontrol agents may have heritable behaviours that can be bred into the fish to increase the quality of the control (Imsland et al. 2014; Imsland et al.

2016). The application of hyper-parasites may have a role in the future of controlling sea lice; examples such as mortality-inducing microsporidians (*Paranucleospora theridion*) may provide useful alternatives to chemical treatments (Økland, 2012). Sea lice are one of the only crustaceans that have reached environmental trialling of biocontrol agents [e.g. wrasse act as cleaner fish in the Scottish salmon industry (Murray, 2015)].

Some control techniques bring salmon lice control to the cutting edge of the field. RNA interference is a method of silencing genes *in vivo* through the use of dsRNA tailored to the mRNA of an expressed gene (Katoch et al. 2013). This method is often used in cellular and developmental biology as a research tool, however, it can be repurposed to silence genes crucial for survival on a cellular or organismal level to control pests (Katoch et al. 2013). For salmon lice, the ecdysone receptor gene has been characterised as a potential target for RNAi trials in the future (Sandlund et al. 2015).

Some control methods for sea lice have become almost futuristic, such as the adaptation of laser technology with re-purposed facial recognition software, which detects lice on the skin of the fish and zaps lice with a laser as fish pass through specialised structures, limiting the need for human intervention and the associated costs (http://optics.org/news/5/5/52: “Laser technique combats sea parasites”).

1.4.3. Controlling invasive crustaceans

Invasive crustaceans are one of the most abundant groups of aquatic invaders and examples of their harmful effects to native species, ecosystems and habitats are numerous (Karatayev et al. 2009). Their impact on the economy is also a major concern as they diminish key ecosystem services (Hänfling et al. 2011). In recent years the killer shrimp (*Dikerogammarus villosus*) has been observed to rapidly replace native species across Europe (Dick and Platvoet, 2000). Chinese mitten crabs (*Eriocheir sinensis*) have been identified as highly damaging organisms to the structural integrity of the banks of the River Thames in London (Clark et al. 1998). Invasive burrowing isopods have polluted waters with microplastics due to their boring activity in polystyrene floats under ship docks (Davidson, 2012). European shore crabs (*Carcinus maenas*) have been identified as global invaders that affect biodiversity and aquaculture on a planet-wide scale (Walton et al. 2002). Finally, signal crayfish (*Pacifastacus leniusculus*) (as well as many other invasive crayfish species) have been identified as a vector and introductory pathway for one of the worst aquatic wildlife diseases, crayfish plague (*Aphamomyces astaci*), which has caused white clawed crayfish (*Austropotamobius pallipes*) to become endangered across Europe (Svoboda et al. 2017). In addition, signal crayfish, as with

other invasive crayfish species, are ecosystem engineers and can significantly alter the ecosystem they invade.

Attempts to control invasive Crustacea or implement successful eradications remain a rarity (Lafferty et al. 1996; Hänfling et al. 2011). Of the few examples available, the control methods that have been explored for invasive Crustacea include: autocidal; physical/mechanical; chemical; and biological control (Goddard et al. 2005; Hänfling et al. 2011; Gherardi et al. 2011; Stebbing et al. 2014).

The introduction and spread of invaders can be difficult to predict, making the targeted application of control and management methods difficult. The application of computational modelling to predict invasion routes can be a considerable aid in the most effective deployment of resources. For example, modelling the movement of Chinese mitten crabs (*E. sinensis*) is aiding in the development of control programmes (Herborg et al. 2007). Likewise, computational modelling can be used to forecast where organisms, such as the killer and demon shrimp are able to invade (Gallardo et al. 2012), or in the identification of hotspots of introduction and spread, allowing for the development of targeted monitoring (Tidbury et al. 2016). Population modelling can also allow for the testing of the effects of long term management programmes without the need for resource intensive field trials (Stebbing et al. 2012), in addition to aiding in the development of control programmes.

1.4.3.1. Autocidal control of invasive Crustacea

Autocidal control is a generic term, including intra-species competition between fertile and infertile males, often referred to as the Sterile Male Technique (SMT), to lower the breeding success of a pest population, in addition to the use of pheromones as control agents (Gherardi et al. 2011; Stebbing et al. 2014). In its original form SMT was applied to terrestrial insect pests and involves irradiation of males to promote infertility/sterility, these are then released en masse into wild populations of the target species, where the infertile/sterile males compete with normal males for females. Sterilisation can also be achieved through removal of sex organs or genetic engineering (Alphey, 2014; Stebbing et al. 2014; Blum et al. 2015). The technique is species specific and inversely density dependent. As the fertile male population decreases, the rate of control increases as an increasing portion of the female population is mated by released sterile males. SMT has been used successfully used to control and in some cases eliminate several insect pest populations (Alphey, 2014), for example the screw worm (*Cochliomyia hominivorax*) was successfully eliminated from North America starting in the 1950s (Knipling, 1960). The technique has been used successfully against a number of other pest species such as

Mediterranean fruit fly (*Ceratitis capitate*), melon fly (*Bactrocera cucurbitae*), pink bollworm (*Pectinophora gossypiella*), codling moth (*Cydia pomonella*) and tsetse fly (*Glossina austenii*) (Wyss 2000; Hendrichs et al. 2005; Klassen and Curtis 2005).

The application of SMT to invasive crayfish populations has been examined via both laboratory and field testing. Methods developed and partially tested include X-ray treatment and removal of gonopods, each providing promising results (Aquiloni et al. 2009a; Gherardi et al. 2011; Stebbing et al. 2014). Successes in this field provide a foundation for the application of this technique for other crustacean invaders and, due to the limited environmental threat, it provides a seemingly risk-free approach for control and eradication. However, the mass rearing of invasive Crustacea may be difficult to justify financially and may be viewed as unacceptable. In addition, the technology to breed only male animals would need to be developed. It is therefore likely that the application of SMT to invasive Crustacea will be limited by the ability to physically remove animals from a water system, treat the males and then return them to the water.

Semio-chemicals in the form of pheromones have been used in the control and management of insect pest populations (specifically lepidopteran and coleopteran) for some time (Kirsch, 1988). Pheromone based control is normally applied either as: i) mating disruptor, whereby pheromone plumes are released to confuse males in their search for a mate, limiting reproduction, ii) ‘attract and kill’ traps where the pheromone is used to lure males or females into the trap, removing them from the population or, iii) mass trapping large numbers of animals for removal from the population (El-Sayed et al. 2006).

Despite being extensively used in terrestrial environments, there has been little progress in the application of semio-chemicals in the control of aquatic invasive crustacean species. Some work using putative sex pheromones of invasive crayfish has been conducted (Stebbing et al. 2003; Aquiloni et al. 2009b) with promising results, revealing that males only need olfaction to identify a mate, where females require olfaction and visual ques to identify a mate, but no finalised control method has yet been developed. A sex pheromone, specifically a nucleotide pheromone, of the invasive European shore crab (*Carcinus maenas*) has also been identified (Hardege et al. 2011), and again no application to control has yet been developed.

Semio-chemicals present a species specific and environmentally friendly means of controlling invasive species. Despite some obstacles that need over-coming, such as reliable means of controlled release of the pheromone into the environment, there are a number of promising examples of where this technique could be applied successfully.

1.4.3.2. Physical/Mechanical control of invasive Crustacea

A more common form of invasive crustacean control is the application of physical or mechanical control. Mechanical control is based on the removal of animals from a population, usually in the form of trapping the target species, followed by euthanasia. These methods tend to be labour intensive and time consuming, needing to be applied over multiple years, which can sometimes limit their implementation as effective control measures (Gherardi et al. 2011; Hänfling et al. 2011; Stebbing et al. 2014).

Trapping invasive crustaceans has rarely been proven to be effective, but is commonly used for many species (Hänfling et al. 2011). There is evidence to suggest that limited success may be a result of insufficient effort being applied and for too short a period (Stebbing et al. 2014), further highlighting trapping as a method that is too resource dependant for extensive management programmes. In some cases, advanced trapping has been designed to increase its efficacy by including the use of specific baits (pheromones, prey) or lures (social lures, light, shelter) and designing the trap with the invader in mind to avoid trapping native species and further specifying the technique (Stebbing et al. 2003; Stebbing et al. 2014).

In some cases, physical removal can be easily achieved, especially where the target species has specific habitat preferences, for example, the aquatic isopod *Sphaeroma quoianum* that is invasive in the USA; where control in this instance has been achieved by placing artificial rotting wood habitats into water systems, allowing colonisation, then removing to lower the population (Davidson et al. 2008).

Many invaders, such as the American signal crayfish, have become invasive through escape from aquaculture farms (Goddard and Hogger, 1986) and are still prized as a food source, and are now trapped extensively within their invaded range for human consumption. Other invaders share a similar story, such as the Chinese mitten crab, where suggestions have been made to sell this species back to China from trapped populations in its invasion range, as a delicacy (Clark et al. 2009). Invaders that provide this added benefit can end up being distributed further due to their associated price tag, however licencing, such as that seen in the UK (Environment Agency), acts as an important restriction used to avoid future invasive propagules and track where novel invasions could be occurring through sale or husbandry of the invader (Hänfling et al. 2011). Although public movement can often increase the distribution of invaders (Anderson et al. 2014) their involvement in “citizen science” through engagement and education is becoming a benefit for invader control: identification of invasion sites for new and existing invaders is an example (Crall et al. 2010; Hänfling et al. 2011; Tidbury et al. 2016). In some cases, invaders can be inedible, such as metal-contaminated

Procambarus clarkii, which can accumulate heavy metals toxic to humans: in cases such as this, control can be more difficult as people may be less keen to become involved (Gherardi et al. 2011).

Approaches such as electro-fishing to control crayfish (Gherardi et al. 2011; Stebbing et al. 2014) and "electro-screens" to prevent the migration of *E. sinensis* (Gollasch, 2006) may provide an easier, more efficient and cheaper method of control.

Mechanical removal of organisms from fomites (materials likely to carry infection/organisms) is often one of the first defences to invasion (i.e. biosecurity), initially through the decontamination of vessels that may be transporting invaders. The bay barnacle, *Amphibalanus improvisus*, provides a good example where temperature, anti-fouling paints, oxygen deficient hulls, chlorine treatment and mechanical removal are combined to help prevent invasion (Hänfling et al. 2011). *Chelicorophium curvispinum*, an invasive amphipod from the Ponto-Caspian, provides a second example where heating (40.8˚C) and filtration of ballast and sludge cause 90% mortality and heavily reduces the likelihood of invasion (Rigby and Taylor, 2001; Horan and Lupi, 2005; Hänfling et al. 2011). Heat treatments have also been examined for a number of other aquatic invasive species, including plants (Anderson et al. 2015), and are now being recommended as a biosecurity measure by the Environment Agency in the UK.

Where invasions have reached unmanageable levels, large scale efforts such as entire drainage of ponds and lakes, or the construction of barriers, have been attempted to remove or prevent the movement of invaders, such as crayfish (Johnsen et al. 2008). In the laboratory, such processes followed by substratum drying have been trialled with some success, such as the control of Ponto-Caspian invaders (Poznańska et al. 2013). The efficiency of methods like this is questionable and has been shown in the past to be ineffective (Johnsen et al. 2008).

1.4.3.3. Chemical control of invasive Crustacea

Chemical biocides are commonplace in aquaculture and agriculture, and in all cases an assessment of their impact toward non-target species is considered before their application as a pesticide or herbicide (Ruegg et al. 2007). However, despite rigorous testing it is difficult to be certain that biocides will not negatively affect the environment and surrounding wildlife. Chemical run-off into rivers and streams, and the effect of chemicals on non-target species within agricultural/aquacultural land, remain a concerning problem for their continued, and in some cases excessive, use (Bunzel et al. 2015). Recent studies have highlighted the risk of non-target neonicotinoids which are

meant to control invasive and pest insect species (insecticidal), but also effect bee populations, identifying their wide ranging impacts upon invertebrates and, to a greater extent, ecosystem health (Robinson et al. 2017). This study highlights the importance of understanding non-target chemical effects on surrounding wildlife. The application of general biocides to areas of high biodiversity to control invasive species may be a particular problem due to greater risk of non-target species interacting with the biocide (Green et al. 2005).. . In wild habitats biodiversity can be higher, relative to farmed environments, meaning that non-specific chemical biocides have a greater chance of impacting a greater variety of species as well as the target, and are more likely to impact upon the ecology (Green et al. 2005).

Chemicals have been used in the past to control invasive crustacean populations that also effect wild, aquatic, environments. Saline treatment is commonly used as a preventative for invasion, evacuating invasive freshwater crustaceans in ship ballast water (Ellis and MacIssac, 2009). The process of increasing lake or river salinity would cause large amounts of ecological damage as many species are highly sensitive to saline conditions, limiting applications of this technique (Haddaway et al. 2015).

A variety of biocides have been applied to control invasive Crustacea in the past: Organophosphates, Organochlorines, Pyrethroids, Rotenone, and Surfactants are all examples however most lack the specificity required to avoid harm to native/co-habiting species (Hänfling et al. 2011). Most appear to result in bioaccumulation and biomagnification in the food chain, which have ripple effects across an ecosystem (Hänfling et al. 2011). The trialling of natural pyrethrum (i.e. Pyblast) has been applied to the North Esk catchment in Scotland to control the signal crayfish population (Peay et al. 2006), showing some success, with no crayfish being found in the following summer but some found at the pre-treated site. It is important when chemicals like this have been applied to monitor the biodiversity and invader in the area to avoid ecosystem breakdown and assess the efficacy of the biocide to prevent resistant strains of the target species from arising (Peay et al. 2006; Hänfling et al. 2011). The same chemical biocide has also been trialled in the laboratory to control red swamp crayfish (*P. clarkii*) in Italy and was found to induce mortality in crayfish but not a co-habiting native crustacean, *Daphnia magna* (Cecchinelli et al. 2012). Given recent developments of chemicals with more specific modes of action for the agriculture industry, there are likely to be candidates suitable for the control of invasive Crustacea that have reduced environmental damage (Stebbing et al. 2014).

Microbe toxins such as Bt-toxin (derived from *Bacillus thuringiensis*) have been suggested (Hänfling et al. 2011) but none are designed to target crustacean species.

1.4.3.4. Biological control of invasive Crustacea

Biological control (biocontrol) utilises organisms to control a pest population through the augmentation, introduction or conservation of a biocontrol agent, which can naturally predate, compete with, or parasitize the target pest. Often, biocontrol agents are suggested for the control of certain invasive Crustacea, but reaching the level of laboratory and field trialling is rare. The effectiveness of biocontrol in aquatic environments is often debated as a high-risk control strategy, however identifying novel agents for crustacean control are researched (Atalah et al. 2015). In principle, biocontrol is a more 'natural' approach to the control of pests, particularly due to growing concerns surrounding over-reliance on non-specific chemicals and the development of resistance. In addition, the cost of development and production of some chemicals may be prohibitively expensive (Stebbing et al. 2014).

The predatory impacts of native fish on invasive Crustacea has been tested for the Asian shore crab (*Hemigrapsus sanguineus*) and could lead to a conservation of fish predators to promote control (Heinonen and Auster, 2012). Several studies have also examined the impact of fish predation, both environmentally and experimentally, on crayfish populations and many suggest that fish predators can be used to reduce the size of crayfish populations (e.g. Westman, 1991). Eels (*Anguilla anguilla*), burbot (*Lota lota*), perch (*Perca fluviatilis*), pike (*Esox lucius*), chub (*Squalius cephalus*), trout (*Salmo trutta* and *Oncorhynchus mykiss*), tench (*Tinca tinca*) and carp (*Cyprinus carpio*) are all recognised predators of crayfish (Stebbing et al. 2014). Aquiloni et al. (2010) found that eel gape size limited the maximum size of the animals predated on; while eels could enter into burrows, which other fish species could not. Eels may have been the main contributor to the decline in crayfish populations in a study by Frutiger and Müller (2002). The declining eel stocks in many European rivers may inadvertently aid in the expansion of signal crayfish. This is illustrated by a study where the removal of fish from a lake in Finland resulted in a dramatic increase in the crayfish population, further highlighting the natural control that the fish were having on the crayfish (Westman 1991). Predatory fish (eel, perch, burbot, pike) have been introduced in Italy to control the *P. clarkii* population and have been found to target only juveniles, benefiting control (Aquiloni et al. 2010). Some resistance has already been noticed, where the introduction of these fish has resulted in a behavioural change of the invader, making it hide more and evade predation (Aquiloni et al. 2010). The presence of predatory fish may, therefore, reduce growth and rate of sexual maturity in crayfish, while altering behaviour, for example increased utilisation of shelter (Blake and Hart 1995).

Although the introduction of predators does apply some level of control to invasive populations, there are potential issues. The effectiveness of biocontrol using predators is proportionate to the population density of the target species, meaning that relative effectiveness will decline over time. Introduced biocontrol organisms may predate on nontarget species, a particular issue once the target population has been reduced. In addition, the introduced predators may impact on the environment (e.g. carp causing turbidity), and may migrate away from the area of control if used in open systems.

Pathogens, such as: nematodes; parasites; fungi; microsporidia; bacteria; and viruses, may be utilised to control invasive crustacean populations (Ovcharenko et al. 2010; Stentiford et al. 2011; Cordaux et al. 2012; Chapter 5). Although pathogen based biocontrol methods are viewed as a high-risk control strategy (Thomas and Willis, 1998), pathogens are commonly used in agriculture to control insect pests with great success, and the application has links and lessons for invasive crustacean control (Hajek et al. 2007). To date there do not appear to be any examples of successful commercial-scale control of aquatic crustaceans. Even engineered forms of Crayfish plague have been suggested in the past as a crayfish control agent (Hänfling et al. 2011). In some cases, laboratory trials for the biocontrol of Crustacea have been undertaken: the best available example for this involves *C. maenas* and its Sacculinid parasite (*Sacculina carcini*) (Goddard et al. 2005). *Sacculina carcini* both castrates and parasitizes the invasive host, allowing a combination of pathogen-based-biocontrol with the added benefits of autocidal control. A drawback however is the lack of host specificity of *S. carcini*: a common draw-back of many biocontrol agents (Goddard et al. 2005).

Despite the possible benefits of applying pathogenic biocontrol agents to control Crustacean pests, it is important to learn from past mistakes and the history of application of pathogenic biocontrol agents to agricultural land. Generally, non-target effects of biocontrol agents should be avoided, and some studies have identified that non-target hosts can acquire the pathogen (Kasson et al. 2015), and that the pathogen can persist in the environment and result in unwanted affects to the environment (Bruck, 2005). Firstly, non-target host infection is usually tested at the preliminary stage and is outlined well by Kasson et al (2015), who describe biocontrol specificity testing of a pathogenic fungus (*Verticillium nonalfalfae*) to control an invasive tree (*Ailanthus altissima*). They identify that some non-target species can become infected by the potential biocontrol agent. Entomopathogenic fungi have been found to survive outside their host and persist in the environment, interacting with the rhizospehere and affecting microbial diversity in the environment (Bruck, 2005). Persistence could benefit the control of insect pests, however a decrease in microbial biodiversity may affect soil nutrition, structure and affect

plant growth (Bruck, 2005). In some cases such control agents have been found to evolve in the environment and may evolve to infect non-target species and have previously undetermined consequences (Wright and Bennett, 2017). Such mechanisms are important to consider if choosing to apply a biocontrol agent to a novel area, such as an aquatic environment to control and invasive crustacean species.

1.4.4. Integrated pest management for invasive Crustacea

Integrated pest management (IPM) has been shown to have high success rates in a variety of fields (Wey and Emden, 2000). Acknowledging that there is very rarely a silver bullet, the remaining option is to examine how the integration of a variety of demonstrated control methods act together towards the management of the target species (Stebbing et al. 2014). One well documented example exists in the control of the invasive crayfish *Orconectes rusticus* (Hein et al. 2006; Hansen et al. 2013). This system started with mechanical removal of crayfish between 2001-2005 and legislative restriction on the harvest of fish predators in the area (a form of conservation-based biocontrol). This resulted in a decline in trap-caught crayfish by 95% and the native community also showed some recovery. Similarly in Switzerland, extensive trapping in addition to the introduction of predatory fish (eel and pike) significantly reduced the size of a population of red swamp crayfish by a factor of 10 over 3 years (Hefti and Stucki 2006). Work is currently being conducted examining the potential application of male sterilisation of signal crayfish as part of a trapping programme, where females and subordinate males are removed (Stebbing et al. 2014).

A potential reason for the lack of long-term, multi-disciplinary approaches to invader control may be as a result of costs. The development of robust population models allowing for the effectiveness of combinations of management methods to be tested over long time periods could be a viable means by which management strategies can be refined prior to field trials. Knowledge of a species' life history and population dynamics are essential in the development of such models (Stebbing et al. 2014).

1.4.5. Lessons to be learnt from past attempts at invasive crustacean control and biosecurity

When control fails it is often not reported, however when biosecurity fails the evidence is visible through the presence of new invasive populations. An example of this is the recent invasion of the killer and demon shrimp in the UK (MacNeil et al. 2010), where little biosecurity was originally present to prevent these species entering the UK. Further

threat from future invaders, such as *Pontogammarus robustoides*, requires a step-up in biosecurity to prevent invasion. Using this same example, 6 years on from initial invasion, the killer shrimp has not had any application of control; but has undergone screening to assess the possibility of biocontrol (Bojko et al. 2013) and reviews of potential means of control have been conducted (Stebbing et al. 2013). The presence of this species has however sparked a stream of research into biosecurity techniques and legislation to prevent further movement of the invader and increase the monitoring of aquatic areas (Anderson et al. 2014; Anderson et al. 2015).

On occasion, invasive species can become a benefit for the economy, whilst still damaging the environment and its inhabitants. This often comes in the form of edible or ornamental species such as: the signal crayfish (*P. leniusculus*); the red king crab (*Paralithodes camtschaticus*); the Kuruma prawn (*Marsupaneus japonicus*); the swimming crab (*Portunus pelagicus*) (DAISIE, 2009) and the American lobster (*Homarus americanus*) (Stebbing et al. 2012). Invasion from commodity species such as these slows the response of legislation and control processes as a possible economic benefit is considered through harvesting these invaders, despite conservation impacts (Hänfling et al. 2011). Issues can arise from making invaders a commodity in non-native areas; including increased dispersal as a bi-product of trade (Hulme, 2009). Methods of avoiding issues like this have been suggested in the past such as the use of native species as ornamentals instead of invasive species (Ewel et al. 1999).

1.4.6. The future of crustacean control in industry and wild environments

Crustacean control efforts rely heavily on predefined techniques and agents pioneered by other fields of science, such as the use of generalised chemical and physical control methods developed by the field of insect control. Crustacean control research can learn a great deal from the insect control sector and, despite the similarities between crustacean and insect biology, a clear understanding of crustacean biology, behaviour and genetics is integral to successfully apply control.

To bring crustacean control up to speed with current technologies this section explores which technologies may aid the field, how knowledge of new processes may bring about new ways of controlling Crustacea, and finally a suggestion as to where the future of crustacean control should be focussed.

1.4.6.1. Bt toxin is not alone

Recently, shrimp mortalities across Asia raised great concern for the industry as large amounts of shrimp died from an unknown pathogen. This outbreak was found to be caused by a strain of *Vibrio paraheamolyticus* carrying a plasmid [OIE recognised disease: acute hepatopancreatic necrosis disease (AHPND)] that contained two protein coding genes: Photorhabdus insect-related A (PirA) and Photorhabdus insect-related B (PirB) (Han et al. 2015). These genes produce proteins that interact and result in a toxic effect to the gut system of susceptible hosts, displaying a similar pathology to that observed by Bt toxin and susceptible insects (Bravo et al. 2007).

Full understanding of this mechanism could lead to a specific form of crustacean control, parallel to that used in the control of agriculturally important insect pests. This could involve the application of a bacterial agent or purified protein. Discovery of novel pathogens that contain similar genes to the PirA/PirB complex could be used directly to control a target host. Similar screening efforts have been conducted to discover novel Bt-like toxins for insect control (Mani et al. 2015). The potential is present for re-adaptation of the currently identified PirA/PirB toxin genes through amino acid substitution at the genetic level, as seen for Bt toxin (Chandra et al. 1999). Development/discovery of such agents could control some of the world's worst invaders such as the mitten crab, signal crayfish and killer shrimp.

1.4.6.2. Knocking out crustaceans with RNA interference

A relatively recent discovery is the biochemical mechanism of RNAi, which is used by the cell to naturally prevent viral infection (Fire et al. 1998). This mechanism can now be exploited by researchers to knock out genes in an attempt to understand their function by developing sequence-specific dsRNAs complementary to mRNA sequences transcribed by the host (Crustacea examples: Kato et al. 2011; Hirono et al. 2011; Nagaraju et al. 2011; Pamuru et al. 2012). Activation of the RNAi pathway involves several protein complexes and results in the breakdown of mRNA and a lack of protein translation (Tijsterman et al. 2004). This method has been considered for the control of parasitic sea lice (Katoch et al. 2013); however, its theoretical applications are highly diverse and include the development of specific dsRNA biocides for a huge number of pests.

By targeting housekeeping genes required for continued cellular function, one could induce apoptosis in entire tissues and cause mortality though organ failure (Baum et al. 2007). For insects, several genes have been targeted in the past (such as: V-ATPase,

Ecdysone receptor gene) many synonymous in Crustacea (Baum et al. 2007; Katoch et al. 2013).

A benefit for this method of control is the level of specificity. RNA biocides can be developed to target a gene with a unique sequence, meaning that specific species can be targeted as long as enough genetic variation is present (Baum et al. 2007). This would allow implementation of a control regimen in the wild, where non-target species would be wholly unaffected even if they consume the dsRNA biocide - depending on their relative genetic variation to the target. A further benefit is the mechanism of up-take in arthropods: dsRNA can enter the gut epithelia through the SID-1 membrane-protein complex (Feinberg and Hunter, 2003) meaning the target arthropod pest need only consume the biocide.

Drawbacks to this technique provide serious problems for the implementation of RNAi-based control. The first is the relative instability of RNA. RNA, even as dsRNA, is easily degraded in the environment and can be broken down by RNase enzymes. This makes delivery of this biocide an important process to consider and requires in-depth analysis of the current possibilities of biocide delivery. Despite the issue of delivery, the RNA biocide must also reach the target host, which can provide complications to its function but could be remedied by providing the biocide in a prey/food item (Huvenne and Smagghe, 2010). RNA biocides must be ingested to function so knowledge of the food eaten by the target species must be well understood. The RNA provided is only capable of knocking down one gene, due to specificity, and so this must be chosen well and could be inhibited by mutation in certain genes (Huvenne and Smagghe, 2010).

1.4.6.3. Delivery of control agents

Before an effective biocide is developed it is important to consider how it will reach the target pest. This process can be difficult, taking into account that the biocide must be present in an attractive form (such as a food source) to bring the pest into contact. Sufficient quantities of the biocide must be present to induce mortality. Finally, the biocide must be stable enough to remain in the environment long enough to make contact with the pest.

An attractant can come in the following forms: specific food sources; light lures; species specific pheromones (Stebbing et al. 2003); and attractive chemical smells [rotting flesh (Putrescine)]. Use of specific attractants and trap design can make generalised chemical control agents more specific, resulting in the chemical reaching the target pest preferentially (Stebbing et al. 2003).

Pioneers in this field have focussed upon isolating and synthesising sex pheromones and kairomones from target Crustacea (Rittschof and Cohen, 2004; Hardege, 2011). The synthesis of pheromones continues to be a difficult process, however to efficiently trap insects, the mass production of some specific pheromones on an industrial scale is now possible (Lo et al. 2015). Development of such an industrial pathway for crustacean pheromone production would benefit their control.

In most trials of novel control agents, the target is exposed directly to the biocide in a confined setting. Small-scale application methods such as these are not feasible at the invasion-site/farmland/fisheries/environmental scale. In aquatic environments the issue of solubility must also be addressed (Gill et al. 1992) and the quantity required must be considered to lower cost but maintain effectivity. Quantities can depend on the environment and application methods. Lakes can cause significant issues as large quantities of biocide may be required, however some application methods concentrate the biocide by using a medium that can contain the chemical such as providing food spiked with a biocide to attract the target (Stebbing et al. 2003).

Biocides could be packaged in degradable nanocarriers (small droplets of biodegradable materials) (Zheng et al. 2015); dsRNA can be altered to make it less degradable by nucleases through the use of an S-oligo backbone or addition of further chemical components (Gao et al. 1992); or the dsRNA could be produced by a prey item by being cloned into the prey as has been proven in genetically modified plants in agriculture (Huvenne and Smagghe, 2010). If the target is a parasite, the biocide could be introduced to the host through feed/injection instead of targeting the parasite directly; this has been adapted for the control of sheep intestinal parasites (Issa et al. 2005) and may have applications for fish lice (Katoch et al. 2013).

In agriculture, the use of nanocarriers has been used to deliver toxins to insect pests and could have applications for crustacean control (Zheng et al. 2015). The biobullet (a capsule containing a toxic substance), developed at Cambridge (Aldridge et al. 2006), holds a generalised toxic chemical (such as Chlorine) that concentrates in bivalves as it bio-accumulates, inducing mortality at high concentration. Other organisms tend not to be affected by the biobullet as they do not accumulate the substance as bivalves do (Aldridge et al. 2006). For Crustacea a similar method has not yet been developed.

1.4.6.4. Applications of genetic engineering to pest control

Genetic engineering has great potential to aid the control of harmful species but also introduces a certain degree of risk. Spread of genetically modified organisms (GMO) is

a constant worry for environmentalists and could pose a threat for biodiversity. In farmed settings the application of GMOs is in a controlled environment, but in the wild (an invasion site) there is less control over what happens to the GMO, such as where it can travel and if it can interbreed. This results in a low confidence in predicting how it will act. Despite the risks associated with this technology, it is important to state how it could be applied to help combat invasive and damaging Crustacea.

Documented examples of introducing GMOs into wild environments are few; however, success has been noted for some control attempts for insect pests (Benedict and Robinson, 2003). Mosquitos constitute a primary target for control and recent attempts have combined autocidal control efforts with genetic engineering to include both toxin genes (Thomas et al. 2000) and predispose infertility (Klein et al. 2012) to control populations. Genetically modified mosquitoes have also been (controversially) released into Malaysian territories, in an attempt to reduce the outbreak of vector borne disease (Lacroix et al. 2012).

Genetic engineering can benefit biocontrol (Leger and Wang, 2010). Applications have involved the inclusion of genes that allow genetically modified yeast to produce a lytic peptide, commonly found in bee venom, to control their invasive termite host (*Coptotermes formosanus*), first by killing symbiotic protozoa and bacteria in the gut of the termite and inducing mortality via inability to digest cellulose (Husseneder et al. 2016). Finally a more common use of the technology is to integrate biotoxin genes into plants to avoid consumption by herbivorous insect pests (Huvenne and Smagghe, 2010).

The application of gene-technologies to control crustacean pests has not been attempted, but a wide range of possibilities are available that could mimic the methods of the examples described above or create novel ways to control this group of pests. For example, crustaceans could be engineered to be infertile to apply autocidal control to a population. They could be provided with a ‘toxic’ gene as described above that is heritable, and would also reduce population size and fitness.

1.4.7. Concluding crustacean control

Pest crustaceans come in three forms: industrial crustacean pests; parasitic crustacean pests; and invasive crustacean pests. Each brings with them unique issues and impacts and provides a challenge for current control methods. A diversity of methods is available for the control of Crustacea; however few methods are specific enough to avoid harm to native and co-existing species. The control of these pests relies mainly on physical and

chemical control methods; however some areas have now begun to research a variety of methods, such as introducing RNAi as a potential tool for the field of crustacean control (Kato et al. 2011; Hirono et al. 2011; Nagaraju et al. 2011; Pamuru et al. 2012). Several new methods are now available based on novel discoveries and further understanding of crustacean biology; many pioneered by the field of insect control.

Areas that may one day provide a benefit to crustacean control are the application of RNAi, adaptation of the PirA/PirB complex, autocidal control and specific and regulated biological control. The specificity and effectivity of these forms of control show great promise for handling the threat posed by crustacean pests. Although some are very early in their discovery (RNAi, PirA/PirB), autocidal and biological control have present day applications. The development of species-specific control agents will allow for a targeted control mechanism for crustacean pests and prevent the further use of generalised chemicals, which themselves pose a threat to biodiversity. Control is only beneficial if it does not cause further damage to the environment and surrounding ecosystems; specificity is the key to preserving biodiversity from invaders, parasites and industrial pests.

Progression for crustacean biocontrol requires increased screening of high impact crustaceans to identify possible biocontrol agents. This constitutes the first step before progression onto lab-based assessment of agent host range.

1.5. Study systems

Within this thesis I use the globally invasive European shore crab, *Carcinus maenas* (Fig. 1.5) as an example study species, which has travelled from its native range to foreign environments, possibly carrying pathogens along with it. This system specifically looks at the invasion route between the UK, Faroe Islands and Atlantic Canada. This species has been the subject of several parasitological studies and is a good species to try and understand pathogen movement, pathogen acquisition and enemy release. In addition, a greater understanding of the symbionts carried by *C. maenas* may lead to better understanding of their risk to biodiversity and aquaculture.

Secondly, 11 amphipod species (Fig. 1.6) from the UK and Poland were selected as a second study group to better understand symbiont diversity and associated taxonomy, transmission and impact, which could travel along with their invasive host. These were selected because of their current or imminent threat to UK biodiversity. Poland sits along an invasion route for many invasive amphipods and better understanding of their symbionts may reveal possible invasion threats.

Figure 1.5: Dorsal and ventral images of *Carcinus maenas*, also known as the European shore crab or invasive green crab (https://commons.wikimedia.org/wiki/File:CSIRO_ScienceImage_864_Carcinus_maenas_European_Green_Crab.jpg and https://commons.wikimedia.org/wiki/File:Carcinus_maenas_(Portunidae_sp.),_Brouwersdam,_the_Netherlands_-_2.jpg). Scale = 1cm.

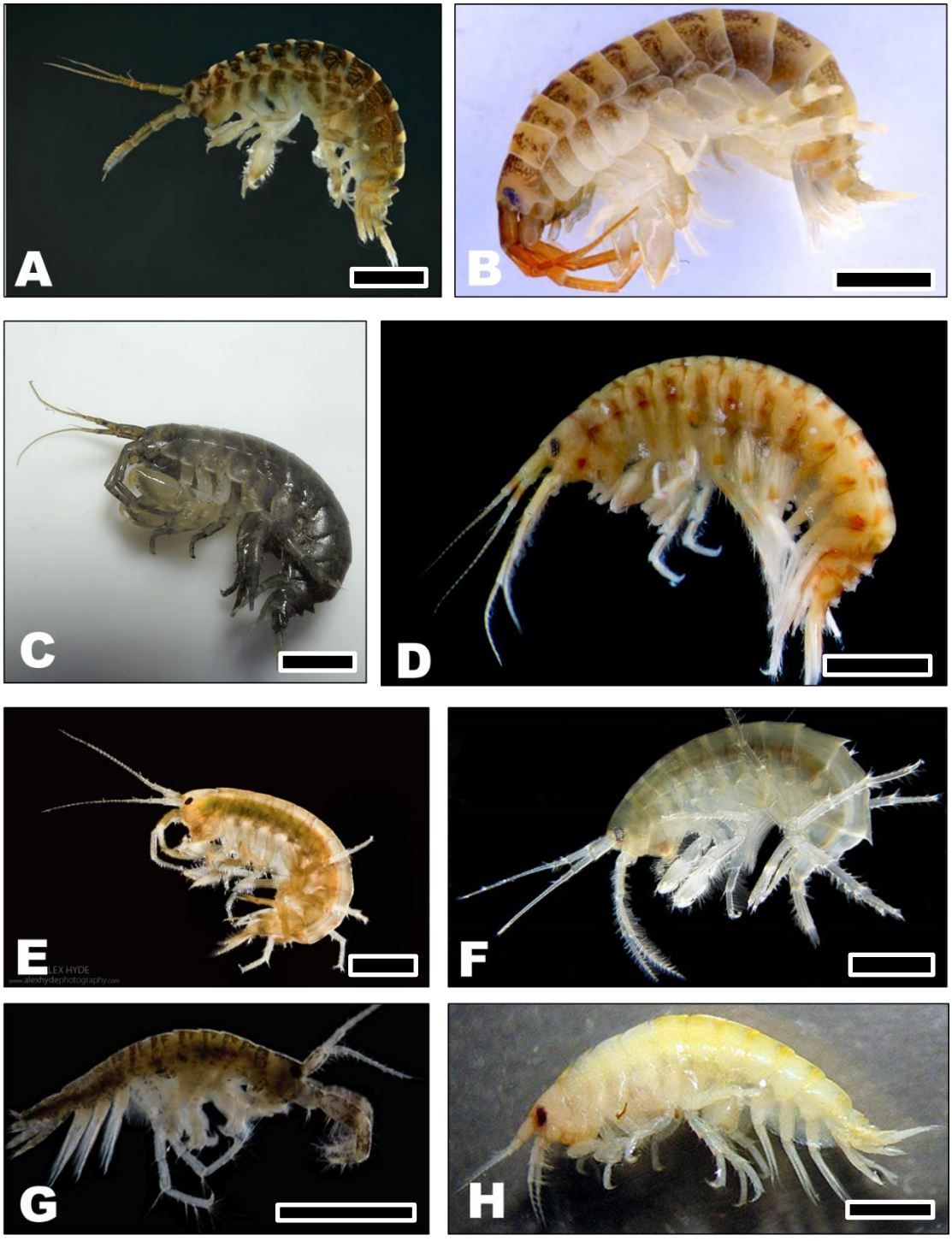


Figure 1.6: Amphipods used during the thesis, excluding *E. trichiatus* and *G. varsoviensis*. A) *D. villosus*. B) *D. haemobaphes*. C) *P. robustoides*. D) *G. tigrinus*. E) *G. pulex*. F) *G. roeselii*. G) *C. curvispinum*. H) *O. crassus*. I) *G. fossarum*. Picture credit to: www.vieraslajit.fi; alexhyde.photoshelter.com; www.hydra-institute.com; www.royalcanoeclub.com; zzb.umk.pl; www.flickr.com/photos/janhamrsky; and www.ias.by. Scales = 0.5cm.

1.6. Pathogen screening techniques

Surveying techniques exist that allow the specific detection of a given disease causing agent (e.g. specific PCR) and others that allow the generic discovery of disease agents, but give little detail to their taxonomy (e.g. histology). Using Figure 1.7 as a guideline to hunt for prospective invasive pathogens, it is important first to identify the invasive species you are working with. Many invaders have a cryptic life history and require both morphological and genetic identification to confirm their species, as has been seen in native and invasive *G. roeselii* populations across Europe (Grabowski et al. 2017).

Several technologies are available for screening invasive species for pathogens, from light microscopy through to next generation sequencing. Light microscopy (including: histology and wet-prepared material) can provide visual identification of several pathogen groups (Bojko et al. 2013) and can provide a strong basis for the application of other tools. Electron microscopy (scanning and transmission) is a technique that can provide high detail images of a given microbe and can aid in its taxonomic identification. However, to obtain good results and avoid wasting materials it is important to define the location of a heavy infection to better aim the electron microscopy process.

Molecular tools such as PCR, qPCR, RT-PCR, immunoassays and enzymatic digestions can all provide data on pathogen presence for both DNA and RNA based organisms, and sequencing of any DNA/RNA amplicons can better advance our understanding of pathogen taxonomy (Hsu et al. 1999; Cavender et al. 2004; Payungporn et al. 2006; Ovcharenko et al. 2010; Kulabhusan et al. 2017). Online databases, such as NCBI, can help in the identification of sequence data. Molecular techniques can also be used in tandem with histology in an immunohistochemistry effort to detect specific pathogens (Chaivisuthangkura et al. 2004).

The application of next generation sequencing can provide a ‘total screen’ whereby you can detect almost every organism present within a host by sequencing its genetic information and obtain a high quality understanding of the diversity present. Metagenomics and high throughput sequencing of PCR amplicons can give either a randomised dataset of available DNA (Pallen et al. 2014) or a dataset of PCR amplicons (e.g. 16S gene sequences) (Ranjan et al. 2016). These techniques can be applied through the use of eDNA to provide a better understanding of where invasive pathogens may be within the invasion site after their original introduction via an invasive host (Bass et al. 2015).

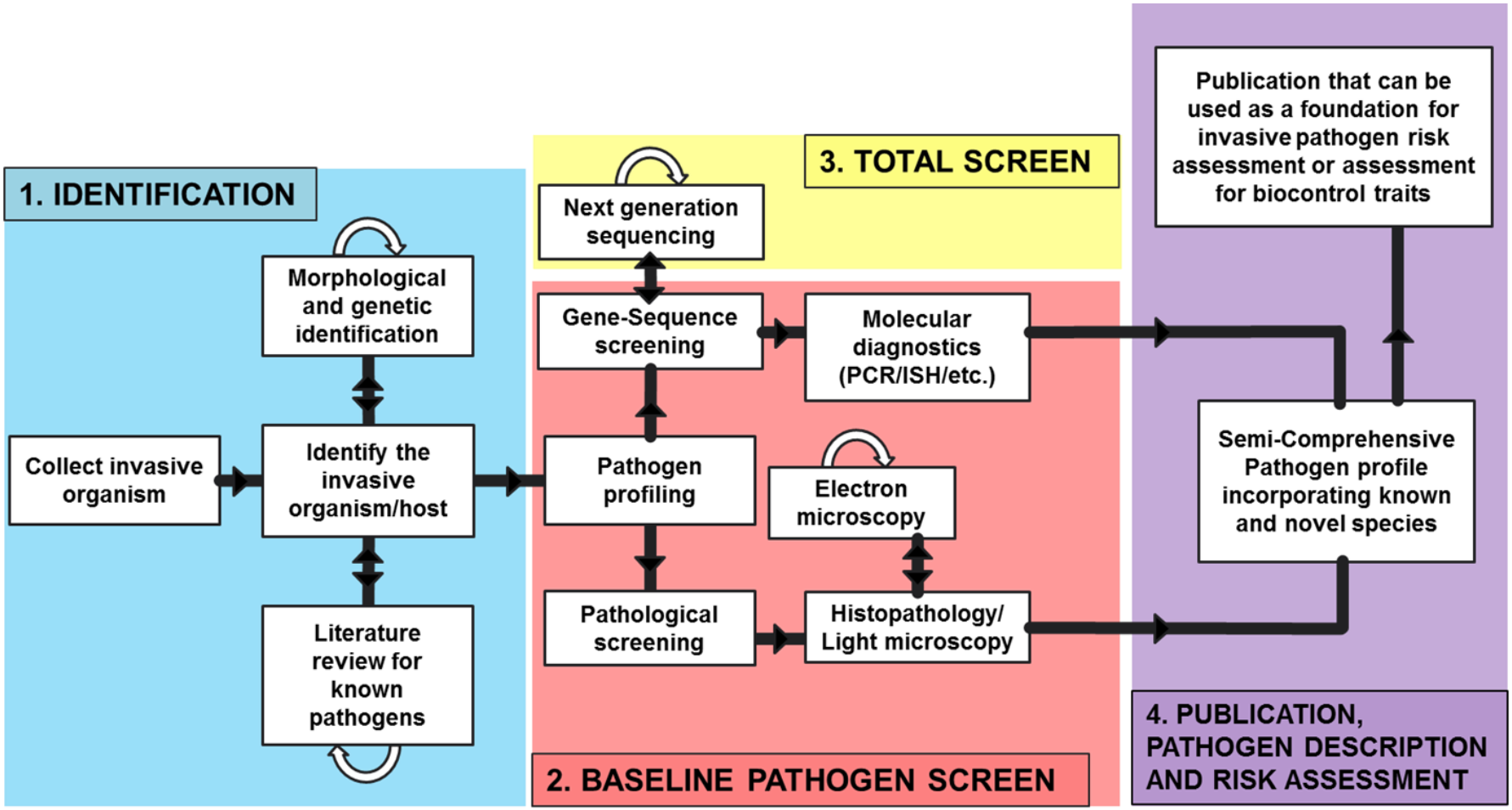


Figure 1.7: A process chart outlining the progression of identifying and screening an invader for novel pathogens, and the taxonomic identification of those pathogens. The process follows four main sections. The first is identification of the invasive host. The second involves several techniques to obtain detect pathogenic/symbiotic species. The third is an optional section, and involves the use of next generation sequencing to obtain a greater understanding of the hosts microbiome and pathobiome. Finally, publication and risk assessment needs to be considered to alert policy and wildlife consultants.

Once an invasive host has been screened for its microbial and organismal diversity, it is important to consider the risk that may be posed by these co-introduced species. Some species may share certain characteristics with closely related species, which may have a pre-existing risk assessment. In the majority of cases novel identification of an invasive pathogen requires an experimental assessment of its impact and risk (Roy et al. 2016). Some studies have experimented with infected hosts to better understand the impact of a pathogen upon its host's behaviour and survival (Bacela-Spychalska et al. 2014; Toscano et al. 2014). More studies exploring this aspect of invasive pathogen biology will help to define which species have the greatest potential to impact an invasion site and its inhabitants.

1.7. Thesis plan

In this thesis, I investigate the biocontrol potential and invasive potential of several pathogens to invasive amphipod and decapod crustaceans, firstly by screening large numbers from an invasive/native population, secondly identifying pathogens taxonomically, thirdly by testing the ability of the pathogens to manipulate their hosts' behaviour, lower or increase their hosts' survival rate, and finally by testing their host range. Figure 1.8 provides an overview of the thesis content by chapter, which is broadly categorised into three sub-sections: 'broad-scale screening'; 'invasive pathogen taxonomy'; and 'invasive pathogen impact and control potential'.

Chapter 2 explores the pathogen profile of the globally invasive *Carcinus maenas*, focussing on three populations from the UK (native range); Faroe Islands (native range) and Atlantic Canada (invasive range). Using histology, TEM and molecular diagnostics, the pathogens, parasites and commensals in each individual are identified morphologically in all cases, with further identification of some pathogens using TEM and molecular techniques. The presence or absence of pathogens along the invasion route is explored, directly linking the knowledge of pathogen transmission to vulnerable lobster fisheries and salmon aquaculture, and exploring the potential for biological control.

Chapter 3 involves the collection and screening of 11 separate amphipod species, which pose an invasion threat to the UK. Each species is screened for pathogens, parasites and commensals to identify species that may be useful as biological control agents or species that pose a threat as wildlife diseases. During the study, metazoans, protists, microsporidians, bacteria and viruses were all identified from native and invasive populations of amphipods in Poland.

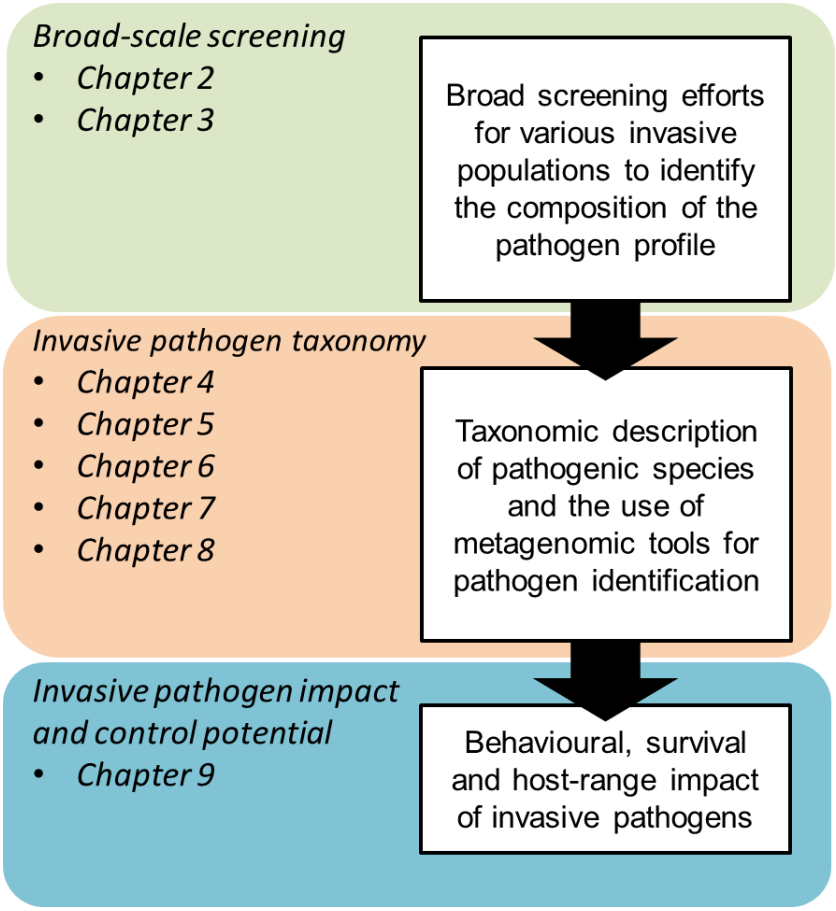


Figure 1.8: An outline of the thesis chapters within the three broad subsections: 'broad-scale screening'; 'invasive pathogen taxonomy'; and 'invasive pathogen impact and control potential'. A brief explanation is provided in the white boxes as to the work conducted in each section and how the various sections follow from each other to result in the taxonomic description of an invasive pathogen and the risks that pathogen may pose to native species, or the possibility for biological control.

Several of the pathogens observed in Chapters 2 and 3 were investigated in more detail. Chapter 4 identifies, taxonomically, a novel microsporidian species, *Parahepatospora carcini* n. gen. n. sp. observed during the collection and analysis of invasive *C. maenas* hepatopancreatic tissues.

Chapter 5 taxonomically characterises a novel member of the *Cucumispora*, *Cucumispora ornata* n. sp. from the tissues of the invasive demon shrimp, *Dikerogammarus haemobaphes*, sampled from UK freshwaters. The presence of this novel pathogen in UK freshwater ecosystems and its potential as either a control agent or wildlife disease are discussed.

Chapter 6 taxonomically characterises the third member of the *Cucumispora*, *Cucumispora roeselii* n. sp. from the musculature of *Gammarus roeselii*, along with several other pathogens present in this species. *Gammarus roeselii* is considered a low

impact non-native species across Europe, however this chapter identifies a wide range of pathogens, parasites and commensals to an invasive propagule (founding group of invasive individuals) from this species, identifying it as a high profile pathogen carrier with increased threat to invasion sites.

Chapter 7 uses next generation sequencing to provide a 51 scaffold, partial genome for the taxonomic erection of a novel bacterial genus and species, *Aquarickettsiella crustaci* n. gen. n. sp. isolated from the tissues of *Gammarus fossarum*, a native species in Poland but invasive in the UK. The detection of this novel pathogen is explored as a potential biocontrol agent for invasive propagules that have undergone enemy release.

Chapter 8 also uses next generation sequencing, but as a tool to identify hidden pathogens from two invaders in the UK, the demon shrimp (*D. haemobaphes*) and the killer shrimp (*D. villosus*).

Chapter 9 moves on to risk assess and explore the impacts of pathogens carried by *D. haemobaphes*, upon both itself and other potential hosts, using experimental survival challenges and behavioural assays.

In Chapter 10 I discuss the aforementioned chapters and studies in the context of invasive species control and the threats posed by newly discovered invasive pathogens.

CHAPTER 2

Symbiont profiling of the European shore crab, *Carcinus maenas*, along a North Atlantic invasion route

2.1. Abstract

The threats posed by invasive alien species (IAS) extend to those parasites and pathogens that the invader carries. The European shore crab, *Carcinus maenas*, is considered a high-impact invader on the Atlantic coast of Canada and the USA. In these locations, burgeoning populations have facilitated development of a legal industry in which *C. maenas* is used as a bait for capture of other economically important crustaceans, such as American lobster (*Homarus americanus*). The paucity of knowledge on pathogens and parasites of invasive *C. maenas*, and their potential transfer to lobsters via bait, poses a potential risk for unintended transmission via this practice. In this study I carried out a histological survey of pathogens, parasites and commensals of *C. maenas* populations sampled from their native range (UK and Faroe Islands) and from invasion sites on the shoreline of Atlantic Canada. The study design was based upon a proposed invasion route, previously defined by microsatellite analysis, from the UK, via the Faroe Islands, to Canada. In total, 19 separate symbiotic associations were identified in crab populations sampled from the three study areas, including numerous viral pathogens (putative parvovirus, putative herpes-like virus, putative iridovirus, *Carcinus maenas* Bacilliform Virus and a rod-shaped virus), bacteria (unidentified Rickettsia-like Organism, milky disease), microbial eukaryotes (ciliated epibionts, *Hematodinium* sp., *Haplosporidium littoralis*, *Nadelspora canceri*; *Parahepatospora carcini*, gregarines, amoebae) and metazoan parasites (nematodes, *Polymorphus botulus*, *Sacculina carcini, Microphallus similis*, isopods). The presence and prevalence of each differed markedly between populations with those from the Faroe Islands displaying greatest symbiont richness. Several pathogens, such as *Hematodinium* sp., were not observed in the Canadian population, suggesting enemy release. Several of those pathogens observed in populations of invasive European shore crab may pose a risk of transmission to other decapods via use of this host in the bait industry.

2.2. Introduction

Invasive alien species (IAS) have been identified as a pathway for the introduction of disease, and may carry their parasites to novel locations where they have the potential to infect native fauna, and lead to emerging wildlife diseases (Roy et al. 2016; Stebbing et al. 2012). Alternatively, maintaining or acquiring parasitic infections native to the introduced range may affect invasive population size, potentially lowering population size and limiting the impact of the invader (Colautti et al. 2004). Finally, invaders may leave their parasites behind as they progress along their invasion route, and become fitter in the process by escaping the need to immunologically defend against disease; a phenomenon broadly categorised as “enemy release” (Colautti et al. 2004).

The European shore crab, *Carcinus maenas*, is a crustacean species invasive across the globe (Darling et al. 2008). It has been found to decrease aquaculture productivity (Therriault et al. 2008) and decrease biodiversity (Therriault et al. 2008), at several invasion sites, including Canada and the United States of America (USA). The native range of *C. maenas* is large, spanning from the Atlantic and Mediterranean oceans around Northern Africa (Moroccan coast) and Central Europe up to the Baltic Sea around Northern Europe and the isolated islands of the Faroe Islands and Iceland (Darling et al. 2008). From here, populations have managed to colonise almost every coastline around the globe; excluding the Antarctic and New Zealand (Garside et al. 2014). One invasion route is defined by movement of *C. maenas* from the UK/mainland Europe, through the Faroe Islands into Atlantic Canada (the latter being considered the invasion range) (Darling et al. 2008). Accompanying this movement is the potential for symbiont transfer between populations, across a wide spatial and temporal dimension.

Carcinus maenas is associated with a wide range of parasitic and commensal fauna in both its native and invasive ranges, including: viruses (Vago, 1966; Bang, 1971; Bang, 1974; Bazin et al. 1974; Chassard-Bouchard et al. 1976; Bonami, 1976; Hoover and Bang, 1976; Hoover, 1977; Hoover and Bang, 1978; Johnson, 1983; Johnson, 1988; Sinderman, 1990); bacteria (Perkins, 1967; Spindler-Barth, 1976; Comely and Ansell, 1989; Eddy et al. 2007); protists (Chatton and Lwoff, 1935; Crothers, 1968; Sprague and Couch, 1971; Couch, 1983; Stentiford et al. 2004a; Stentiford and Feist, 2005; Hamilton et al. 2009; Stentiford et al. 2013a); fungi (Cuénot, 1895; Léger and Duboscq, 1905; Sprague and Couch, 1971; Azevedo, 1987; Stentiford et al. 2013b; Chapter 4); helminths (McIntosh, 1865; von Linstow, 1878; Monticelli, 1890; Vaullegeard, 1896; Hall, 1929; Rankin, 1940; Stunkard, 1957; Bourdon, 1965; Crothers, 1966; Deblock and Tran Van Ky, 1966; Crothers, 1968; James, 1969; Prévot and Deblock, 1970; Vivares, 1971; Liat

and Pike, 1980; Kuris et al. 2002; Pina et al. 2011); bryozoans (McIntosh, 1865; Duerden, 1893; Richard, 1899); crustaceans (Richard, 1899; Boschma, 1955; Bourdon, 1963; Crothers, 1966; Heath, 1976; Goudswaard, 1985; Choy, 1987); molluscs (Giard and Bonnier, 1887); and chordates (Crothers, 1966). Often, invasive organisms lack such well publicised parasite profiles (Roy et al. 2016) and as such, this data can be used to facilitate an understanding of enemy release (and potential acquisition) along invasion pathways. *Carcinus maenas* has successfully invaded a multitude of coastal habitats across the globe and genetic studies have defined the pathways via which this invader has spread (Darling et al. 2008). One such pathway involves movement between the United Kingdom, to the Faroe Islands and then to Atlantic Canada; as determined by host microsatellite analysis (Darling et al. 2008). Darling et al. (2008) identified several microsatellites from crab populations in the UK, a small number of which comprise the Faroese population. Several of those microsatellites present in the Faroese population are observed in invasive populations of European shore crab from Canada. Despite this low microsatellite diversity, the Faroe Islands are considered within the native range of this host. This invader significantly impacts native biodiversity, and aquaculture, across its invasive range (Therriault et al. 2008). In an attempt to reduce the population size of invasive *C. maenas*, the Canadian Government (Fisheries and Oceans Canada) issues 'green crab licences' that allows the harvesting of large numbers of crabs to use, and sell, as bait; particularly for use in the lobster (*Homarus americanus*) fishery industry (Fisheries and Oceans, Canada).

Given that no comprehensive surveys of symbionts have occurred in Canadian populations of *C. maenas* to date, it is pertinent to consider the potential risk of pathogen transfer (e.g. from crab to lobster) via the practice of bait use. Transmission of pathogens from an invasive to native host has been documented on several occasions, and includes the transmission of squirrel pox, gaffkaemia and crayfish plague (Stebbing et al. 2012; Chantrey et al. 2014; and Dunn and Hatcher, 2015); all of which have had a devastating impact on native populations. The lobster fishery industry in Atlantic Canada is of great economic importance and was worth $680.5 million in 2013 (Fisheries and Oceans Canada), providing an important incentive to assess the risk posed by invasive hosts and their parasites upon the native *H. americanus* population.

Although discrete pathogen surveys of *C. maenas* have occurred within the native range (Stentiford and Feist, 2005; Stentiford et al. 2013a; Stentiford et al. 2013b), to date, no comprehensive studies have been conducted across its invasive pathway. This study aimed to determine the symbiont (pathogen, parasite, commensal) profile of *C. maenas*

populations at three geographically distinct locations in the Northern Atlantic (UK, Faroe Islands and Atlantic Canada). By conducting a comprehensive screening programme based upon histology, transmission electron microscopy and molecular diagnostics, I demonstrate different presence and prevalence of symbionts across the invasive range and discuss their potential risk as invasive pathogens.

2.3. Materials and Methods

2.3.1. Sampling and dissection

Carcinus maenas were sampled from shoreline sites in the UK (n=15), Faroe Islands (n=5) and Atlantic Canada (n=7) (Table 2.1). In addition to samples collected during this study, I also utilised data relating to previous histopathology surveys of *C. maenas*, conducted in the UK by the Centre for Environment, Fisheries and Aquaculture Science (Cefas, UK), dating back to 2010 (Table 2.1). In all cases, crabs were either captured by baited traps set near to shore, or hand collected from the shoreline. After collection, animals were transported to one of three laboratories: Cefas (UK), Fiskaaling (Faroe Islands) or Dalhousie Agriculture Campus (Canada). Animals were euthanized on ice and dissected to provide gill, heart, muscle, hepatopancreas and gonad tissues for histology, electron microscopy and molecular diagnostics using procedures of the European Union Reference Laboratory (EURL) for Crustacean Diseases (www.crustaceancrl.eu). Animals collected post 2013 that were below 22mm carapace width were halved to provide histological and ethanol-fixed material. Animals below 15mm carapace width were fixed whole for histology.

Country	Sample site	Co-ordinates	Sample date	n=
UK 	Blakeney harbour, Norfolk	52.964, 0.964	07/2010 (Cefas historical data)	30
	Berwick upon Tweed	55.769, -2.009	08/2010 (Cefas historical data)	30
	North Shields	55.008, -1.433	08/2010 (Cefas historical data)	30
	Rye Harbour	50.930, 0.772	08/2010 (Cefas historical data)	30
	Poole Harbour	50.708, -2.000	08/2010 (Cefas historical data)	30
	Helford	50.096, -5.136	08/2010 (Cefas historical data)	30
	Newtons Cove, Weymouth	50.605, -2.449	08/2010 (Cefas historical data)	26
	Southend On Sea	51.533, 0.627	09/2010 (Cefas historical data)	30
	Menai Straights	53.246, -4.067	09/2010 (Cefas historical data)	30
	West Mersey	51.773, 0.900	10/2010 (Cefas historical data)	30
	Newtons Cove, Weymouth	50.605, -2.449	06/2012 (Cefas historical data)	188
	West Mersea Island	51.804, 1.000	10/2012 (Cefas historical data)	120
	Newtons Cove, Weymouth	50.605, -2.449	11/2012 (Cefas historical data)	8
	Newtons Cove, Weymouth	50.605, -2.449	02/2013 (Cefas historical data)	10
	Newtons Cove, Weymouth	50.605, -2.449	11/2013 – 03/2014 (This thesis)	146
Faroe Islands 	Kaldbaksfjørður	62.058, -6.875	07/2014 – 08/2014 (This thesis)	23
	Argir	61.997, -6.770	08/2014 (This thesis)	21
	Kirkjubøur	61.953, -6.798	08/2014 (This thesis)	25
	Nesvík	62.216, -7.016	08/2014 (This thesis)	181
	Tórshavn	62.018, -6.754	08/2014 (This thesis)	56
Canada (Nova Scotia) 	Port L'Hebert	43.801, -64.932	08/2014 (This thesis)	41
	Hubbards	44.642, -64.051	08/2014 (This thesis)	62
	Boutiliers Point	44.659, -63.952	08/2014 (This thesis)	20
	Fox Point	44.611, -64.058	08/2014 (This thesis)	22
	Pubnico	43.702, -65.783	08/2014 (This thesis)	111
	River Port	43.624, -65.484	08/2014 (This thesis)	42
	Malagash	45.813,-63.473	08/2014 (This thesis)	134

Table 2.1: Date, geographic location and sample size of *C. maenas* involved in the disease screening process. Each country is provided with a map, where the red spots identify the sampling locations listed in the table.

2.3.2. Histological processing and screening

All animals in this study underwent histological analysis. Post-dissection, organs and tissues were submerged in Davidson's seawater fixative (DSF) (Hopwood, 1996) for 48 h prior to their transfer to 70% ethanol or, industrial methylated spirit. Samples were wax infiltrated using an automated system (Peloris, Leica Microsystems, UK) prior to embedding in to wax blocks. Blocks were trimmed and then cut to provide a single section between 3-4µm thickness using a Finesse (E/NE) Rotary Microtome (Leica, UK). Sections were mounted on glass slides, stained with haematoxylin and alcoholic eosin (H&E) and cover-slipped with xylene. Stained slides were read and imaged via a Nikon-integrated Eclipse (E800) light microscope and digital imaging software at the Cefas Weymouth Laboratory.

2.3.3. Transmission electron microscopy (TEM)

Organ and tissue samples collected for TEM were fixed in 2.5% glutaraldehyde in 0.1% cacodylate buffer and stored until required. When a pathogen was identified via histology, the corresponding TEM sample for the same specimen was processed for TEM analysis. Briefly, samples were soaked in Sodium cacodylate buffer twice over a 10 min period and stained with 1% Osmium tetroxide (OsO_4) solution for 1 h prior to infiltration with acetone and infusion with Agar100 Resin. Individual samples were placed in to moulds (~1 cm^3) with fresh resin and polymerised at 60˚C for 16 h. The resulting blocks were trimmed with a razor blade to expose the surface of the sample and sectioned at 1µm thickness (stain: Toluidine Blue) with a glass knife. Ultra-thin sections were cut from the same block at ~80nm thickness using a diamond knife. Sections were stained with Uranyl acetate and Reynolds Lead citrate (Reynolds, 1963) prior to analysis on a Jeol JEM 1400 transmission electron microscope (Jeol, UK). In addition, one sample displaying a putative viral infection (for which a corresponding TEM sample was not available), was removed from the wax block using Histosolve and taken to water via an ethanol-water dilution series before being re-fixed in 2.5% glutaraldehyde in 0.1% cacodylate buffer. The process then continued as described above.

2.3.4. Molecular techniques

Where a pathogen of interest was identified via histology and TEM, a sample from the same specimen was processed for molecular diagnostics and systematics. DNA was extracted via a conventional Phenol-Chloroform method after initial digestion with Lifton's Buffer (0.1M Tris-HCl, 0.5% SDS, 0.1M EDTA), or via the EZ1 automated DNA extraction using manufacturer instructions (Qiagen, UK). The resulting DNA extract was tested with appropriate primer sets and reaction conditions for the pathogen type in question via a PCR diagnostic method detailed in Table 2.2. In all cases a single PCR reaction (50µl) included the following components: 1.25U of Taq Polymerase; 2.5mM $MgCl_2$; 0.25mM of each dNTP; 1µM of each primer; 1X flexi buffer; and 2.5µl of DNA template (30-100 ng/µl). Amplicons were visualised using a 2% agarose gel (120V, 45 min). Where appropriate, amplicons of correct size were extracted from the gel, purified for sequencing using spin columns and ethanol precipitation, and sequenced via the Eurofins sequencing barcode service (https://www.eurofinsgenomics.eu/).

Infection	Primers		Tc Settings (˚c)	Resulting amplicon	Reference
	Forward	Reverse			
Microsporidia	MF1: 5'-CCGGAGAGGGAGCCTGAGA-3'	MR1: 5'-GACGGGCGGTGTGTACAAA-3'	95-55-72	800-900bp	Tourtip et al. 2009
	V1F: 5'-CACCAGGTTGATTCTGCCTGAC-3'	1492r: 5'-CCATGTTACGACTTACATCC-3'	95-45-72	1400-1500bp	Vossbrinck et al. 1998
Amoebae 1st round	F1: 5'-TATGGTGAATCATGATAACTTWAC-3'	R1: 5'-TCTCCTTACTAGACTTTCAYK-3'	95-55-72	300-500bp	Kerr et al. Unpublished
Amoebae 2nd round	F2: 5'-AATCATGATAACTTWACGAATCG-3'	R1: 5'-TCTCCTTACTAGACTTTCAYK-3'	95-54-72	300-500bp	Kerr et al. Unpublished
Hematodinium 1st round	2009ITS1F: 5'-AACCTGCGGAAGGATCATTC-3'	2009its1&2R: 5'-TAGCCTTGCCTGACTCATG-3'	94-60-72	500bp	Small, Pers. Comm.
Hematodinium 2nd round	2009ITS1F: 5'-AACCTGCGGAAGGATCATTC-3'	2009ITS1R: 5'-CCGAGCCGAGGCATTCATCGCT-3'	94-60-72	350bp	Small, Pers. Comm.
RVCM polymerase	Pol3F: 5'-GTTACACACCCCTCCGATCA-3'	Pol3R: 5'-TCGCCGAACATTTTAGTGGG-3'	95-55-72	393bp	Unpublished

Table 2.2: The forward and reverse primer sequences used for the amplification of several parasite and pathogen groups using PCR from genomic template extracted from host and parasite/pathogen tissues.

2.3.5. Phylogenetic analysis of predicted protein sequence data

Materials collected from this study were used in a separate study to better understand the taxonomy of the rod-shaped virus from *C. maenas*. Here I include a phylogenetic tree based on the DNA polymerase amino acid sequence predicted from the genome of this virus. The evolutionary history was inferred by using the Maximum Likelihood method based on the Dayhoff matrix based model (Schwarz and Dayhoff, 1979) in MEGA 7 (Kumar et al. 2016). The tree represents 23 amino acid sequences from dsDNA viruses, all of varying length. There were a total of 2535 positions in the final dataset. Human alphaherpesvirus was used as an out group to root the tree.

2.3.6. Statistical analyses

Carcinus maenas symbiont data was obtained in a binomial manner, where the presence of a particular symbiont in an individual was allocated a score of '1' and a lack of that symbiont allocated a score of '0', irrelevant of the number of symbionts detected (symbiont profile). Data from each of the three field locations (UK, Faroe Islands, Canada) was analysed using R version 3.2.1 (R Core Team, 2014), via Rstudio interface, to apply the Marascuillo procedure to each population, which compares the prevalence of specific symbionts between sites and their respective sample sizes. The Marascuillo procedure highlights any significant differences ($P<0.05$) between specific populations,

and their population size, comparisons and their prevalence of a given symbiont via a rapid Chi squared assessment process. This system is comparable to the application of many Chi squared assessments but instead allows rapid assessment of the entire dataset without applying Chi squared individually to each population and each symbiont. Using the entire pooled dataset with known male or female sex, the crab population's sex ratios were compared with the presence of specific symbionts to identify any sex bias towards infection. This was conducted using a Pearson's Chi-squared test with Yates' continuity correction for each symbiont against the sex distribution of the host. Post analysis for normality, a Wilcoxon test was applied to count data to compare symbiont distribution amongst crab sexes.

Generalized linear models were used to assess whether the symbiont profiles of crab populations, on a country-wide basis, were significantly different to one another by comparing the prevalence/presence of symbionts across country-wide populations. The models utilised the Multcomp (Hothorn et al. 2009) and lme4 (Bates et al. 2007) packages and were adjusted using the Holm correction to counteract the problem of multiple comparisons. The GLM employed a Poisson error distribution model because the data was not over dispersed (residual deviance is less than the degrees of freedom).

2.4. Results

2.4.1. Symbiont profiles of C. maenas populations by Country

2.4.1.1. United Kingdom

Histological analyses revealed 14 symbionts in crabs collected from UK sites. Symbionts included metazoan parasites, single-celled eukaryotes, bacteria and viruses. The acanthocephalan parasite, *Polymorphus botulus*, was observed in one individual of the population sampled from Blakeney Harbour, Norfolk. Infection was noted prior to histological fixation. The mid-gut of infected specimens was filled with acanthocephala, presumably acquired from an avian host. Infection resulted in an enlarged gut, due to the presence of the parasite. *Sacculina carcini* was observed infecting crabs from 5 of the UK sites, at varying prevalence (Table 2.3). The trematode *Microphallus similis* was observed infecting crabs from all sites, often at high prevalence (Table 2.3). Unidentified nematode parasites were recorded at 8 of the UK sites (Table 2.3). Nematodes were encysted within a variety of tissues in their host [muscle (Fig. 2.1a), hepatopancreas, gonad, connective tissue] but no evidence of a host immune response was observed. The presence of ecto-parasitic isopods, of unknown identity but potentially *Priapion fraissei*, were noted in crabs collected from 2 UK sites (Table 2.3). Of particular note was the relatively high prevalence (20%) in crabs collected from the Menai Straights site.

Isopods (Fig. 2.1b) were also present at high burden, with 8-20 individuals between each gill filament, and were not associated with any observable host response.

Table 2.3a

	Collection site	Collection date	Sex distribution (M/F/U)	n=	Prevalence determined by histology (%)													
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Ciliated protists	*Nadelspora cancer*	Gregarines	CmBV	*Polymorphus botulus*	Nematode	*Mitrophaltus sinitis*	*Hematodinium sp.*	Gill-dwelling isopods	Milky disease	Parvovirus	HLV	*Haplosporidium littoralis*	*Saccultina carcini*
A	Blakeney Harbour, Norfolk	28/07/2010	13/17/0	30	83.3	3.3	0.0	16.7	3.3	0.0	96.7	6.7	0.0	0.0	0.0	0.0	6.7	0.0
B	Rye Harbour	06/08/2010	7/23/0	30	33.3	0.0	0.0	0.0	0.0	3.3	6.7	13.3	0.0	0.0	0.0	0.0	0.0	0.0
C	Helford	26/08/2010	12/18/0	30	83.3	6.7	0.0	0.0	0.0	0.0	30.0	3.3	0.0	0.0	0.0	0.0	0.0	16.7
D	Newtons cove, Weymouth	20/08/2010	8/18/0	26	73.1	3.8	0.0	0.0	0.0	0.0	65.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E	Berwick Upon Tweed	25/08/2010	10/20/0	30	43.3	0.0	0.0	0.0	0.0	3.3	23.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F	North Shields	26/08/2010	3/27/0	30	83.3	0.0	0.0	3.3	0.0	3.3	10.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0
G	Poole Harbour	31/08/2010	9/21/0	30	33.3	0.0	3.3	0.0	0.0	0.0	60.0	30.0	0.0	6.7	0.0	0.0	0.0	16.7
H	Southend on Sea	23/09/2010	30/0/0	30	100.0	0.0	0.0	0.0	0.0	3.3	63.3	3.3	0.0	3.3	0.0	0.0	0.0	0.0
I	Menai Straights	24/09/2010	16/14/0	30	60.0	0.0	0.0	0.0	0.0	10.0	40.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0
J	West Mersey	14/10/2010	21/9/0	30	63.3	3.3	0.0	0.0	0.0	3.3	50.0	50.0	3.3	0.0	0.0	0.0	3.3	0.0
K	Newtons cove, Weymouth	06/2012	80/108/0	188	0.0	1.6	0.0	2.1	0.0	0.5	46.3	1.6	0.0	3.2	0.0	3.7	1.1	5.9
L	West Mersea Island	10-11/2012	68/52/0	120	0.0	4.2	0.0	0.0	0.0	0.8	21.7	27.5	0.0	0.0	0.0	0.0	0.0	3.3
M	Newtons cove, Weymouth	14/11/2012	4/4/0	8	0.0	12.5	0.0	12.5	0.0	0.0	87.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N	Newtons cove, Weymouth	27/02/2013	5/5/0	10	10.0	0.0	0.0	0.0	0.0	0.0	90.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0
O	Newtons cove, Weymouth	11/2013 - 03/2014	70/76/0	146	0.0	1.4	1.4	2.7	0.0	0.0	76.7	0.7	0.0	0.0	1.4	0.0	0.0	4.1

Table 2.3b

Collection site	Collection site															
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
A															
B	7														
C	7														
D		7													
E	7														
F	7														
G		7													
H		1, 7				1, 7	1								
I	7														
J				8	8				8						
K	1, 7	7	1	1		1, 7		1	1	1, 8					
L	1, 7		1	1, 8	8	1		1	1, 8	1	8				
M	1	7	1	1		1, 7		1	1	1, 8		7, 8			
N	1	7	1	1	7	1, 7		1				7			
O	1	7	1, 7	1	7	1, 7		1	1	1, 8	7	7, 8			

Table 2.3: Prevalence percentages for each pathogen associated with *C. maenas* at each collection site in the United Kingdom. 2.3b) A display of the significant differences between populations holding different proportional prevalence's of commensals, parasites and pathogens. Significant associations are listed in the table and any non-significant associations are not listed in the table. Significance is calculated at a threshold of <0.05 using the Marascuillo procedure. The Yates correction was applied to negate any false positive results.

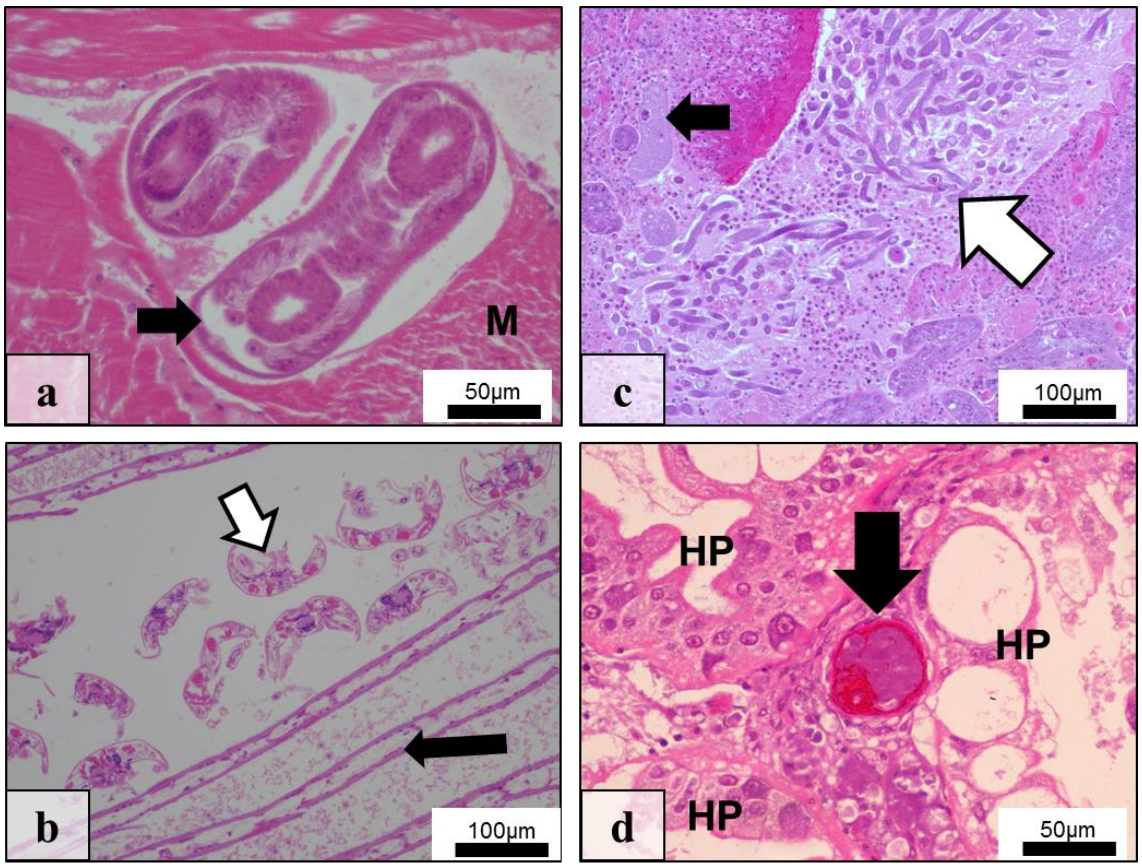


Figure 2.1: Parasites, pathogens and commensals inhabiting *C. maenas* from UK populations. a) A nematode (black arrow) encysted within the muscle tissues (M) of its host. b) Crustacean parasites (likely copepods or isopods) (white arrow) are present at high densities between many of the gill lamellae (black arrow) of the host. c) Gregarine parasites (white arrow) present at high densities in the gut lumen of the host. Most gregarines appear thin and elongate with some showing an enlarged physiology (black arrow). d) A bacterial plaque within the blood stream of the host (black arrow), between the tubules of the hepatopancreas (HP). The plaque featured in this image is undergoing melanisation (black arrow).

Several micro-eukaryote symbionts were observed. Gregarine parasites were recorded in crabs from 2 UK populations, at low prevalence (Table 2.3). Gregarines colonised the gut lumen, often at high burden (Fig. 2.1c). The presence of gregarines did not appear to illicit any observable immune response. A microsporidian resembling *Nadelspora canceri*, was observed infecting crabs from 7 sites, at varying prevalence (Table 2.3). This parasite infected its host in the same manner described by Stentiford et al (2013b); undergoing dimorphic development culminating in needle-like spores infecting mainly heart myofibres and oval *Ameson*-like spores in the skeletal musculature. Melanisation and phagocytic uptake of microsporidian spores was also observed. *Haplosporidium littoralis*, a haplosporidian parasite of *C. maenas*, was observed in crabs from 3 sites

(Table 2.3). The pathology caused by this parasite included infection of the musculature and blood stream and was identical to that described by Stentiford et al (2013a).

Hematodinium sp., a dinoflagellate parasite of *C. maenas*, was observed infecting crabs from 11 sites, at varying prevalence (Table 2.3). Ciliated protists, often alongside filamentous bacteria and detritus, were a common commensal observed colonising the space between gill lamellae and more generally on the carapace and appendages of crabs collected from 11 sites (Table 2.3). The presence of these commensals caused no discernible pathology.

Bacterial infections were characterised by a previously described condition termed 'Milky disease', a systemic bacterial infection of the haemolymph. It was detected in 3.2% of crabs collected from the Newtons Cove site in Weymouth. Large bacterial plaques occurred freely within the haemolymph and within fixed phagocytes of the hepatopancreas and gill (Fig. 2.1d). Infection was often accompanied by a pronounced host response, including melanisation (Fig. 2.1d).

Several viral pathogens were observed in crabs collected from UK sites. A Herpes-Like Virus (HLV) was recorded in 3.7% of animals sampled from the Newtons Cove site in Weymouth. Infection was apparently restricted to granulocytes and hematopoietic tissues and resulted in hypertrophy of the nucleus (Fig. 2.2a). In some cases, infected cells were binucleate. TEM revealed membrane-bound virions with a central genomic core (Fig. 2.2b, c). Virions measured 112.4nm ± 19.4nm (n=13) in diameter. The central genomic core measured 67.8nm ± 12.5nm (n=13) in length and 28.2nm ± 6.1nm (n=13) in width. This infection appeared not to elicit any visible host immune response. A putative Parvovirus infection was identified from 1.4% of specimens collected in the 2013/2014 sample from Newtons Cove, Weymouth. The virus caused nuclear hypertrophy in haemocytes and gill epithelial cells, often in the form of a Cowdry-like body (Fig. 2.2d). Under TEM, infected cells exhibited a viroplasm containing hexagonal virions that measured 89.6nm ± 18.9nm (n=15) in diameter (Fig. 2.2e, f). No immune response was observed toward infected host cells. Finally, *Carcinus maenas* Bacilliform Virus (CmBV) was located in the hepatopancreas of *C. maenas* sampled from 5 UK sites (Table 2.3). Infection was restricted to the nuclei of hepatopancreatic epithelial cells and although infected cells were observed sloughing from the basement membrane, no apparent immune response was observed.

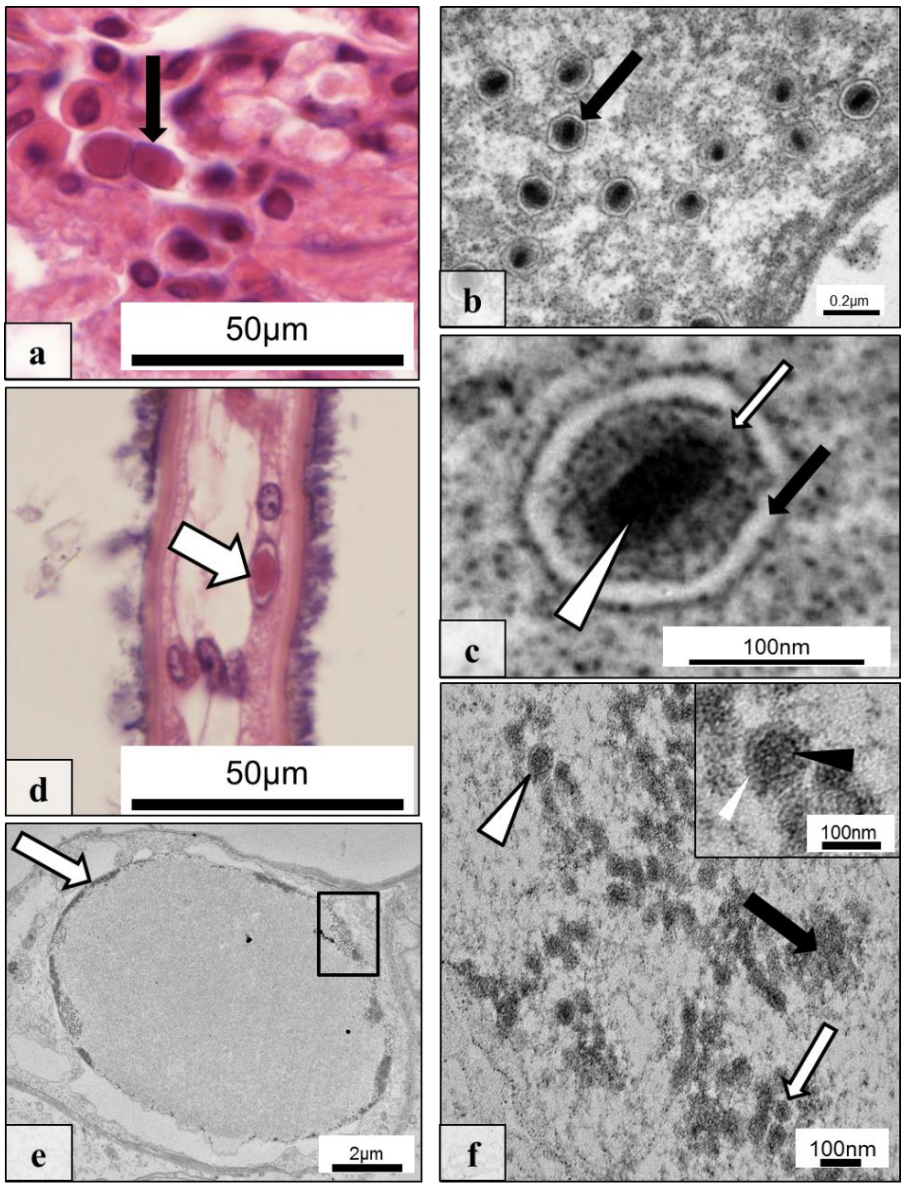


Figure 2.2: Viruses found in *C. maenas* collected from the UK. a) Histological section of infected (black arrow) and uninfected granulocytes in the haemolymph. b) Transmission micrograph of the nucleus of an infected granulocyte. Individual virions (black arrow) are present. c) High magnification image of a single virion, present with a genomic core (white triangle), capsid (white arrow), and lipid membrane (black arrow). d) Histological section of a gill lamella, where some epithelia are present with nuclei that possess cowdry bodies (white arrow). e) Transmission micrograph of an infected nucleus (white arrow), identifying the periphery of the cell where virions are developing (black square). f) A high magnification image of developing virions (white arrow) and viral proteins (black arrow); some which are developed (white triangle). The inset image identifies the core (black triangle) and extremity (white triangle) of the virus.

2.4.1.2. The Faroe Islands

Histological analyses revealed 13 symbionts in crabs collected from Faroe Island sites. Ten of these corresponded to those detected in crabs collected from sites in the UK. In

addition, I also identified two novel virus infections and colonisation by an amoeba, not detected in samples from the UK.

Table 2.4a

Collection site		Collection date	Sex distribution (M/F/U)	n=	Prevalence determined by histology (%)												
					1	2	3	4	5	6	7	8	9	10	11	12	13
					Ciliated protists	*Nadelspora cancer*	Gregarines	CmBV	*Polymorphus botulus*	Unidentified RLO	B-virus	*Microphallus similis*	*Hematodinium sp.*	Amoebae	Isopod	Parvovirus	Iridovirus
A	Kaldbaksfjørður	07-08/2014	6/11/6	23	69.6	0.0	0.0	0.0	13.0	0.0	0.0	0.0	26.1	8.7	0.0	0.0	0.0
B	Argir	08/2014	10/11/0	21	95.2	4.8	0.0	0.0	23.8	0.0	0.0	0.0	9.5	4.7	0.0	0.0	0.0
C	Kirkjubøur	08/2014	10/11/4	25	92.0	0.0	0.0	28.0	8.0	0.0	8.0	12.0	0.0	20.0	0.0	4.0	0.0
D	Nesvík	08/2014	53/79/49	181	81.8	1.7	10.5	13.3	6.1	3.9	7.2	61.3	22.7	9.9	1.1	1.1	1.1
E	Tórshavn	08/2014	29/15/12	56	83.9	1.8	0.0	16.1	1.8	16.1	3.6	16.1	0.0	19.6	3.6	0.0	0.0

Table 2.4b

Collection site	Collection site					
		A	B	C	D	E
A						
B						
C		4	4			
D		3, 4, 7, 8	3, 4, 7, 8	3, 8, 9		
E		4, 6, 8	4, 6, 8	6	3, 8, 9	

Table 2.4: 2.4a) Prevalence percentages for each pathogen associated with *C. maenas* at each collection site in the Faroe Islands. 2.4b) A display of the significant differences between populations holding different proportional prevalence's of commensals, parasites and pathogens. Significant associations are listed in the table and any non-significant associations are not listed in the table. Significance is calculated at a threshold of <0.05 using the Marascuillo procedure. The Yates correction was applied to negate any false positive results.

Metazoan parasites included an isopod infection (likely the same as that detected in UK samples) on the gills of crabs from the Nesvík and Tórshavn sites, at varying prevalence (Table 2.4) (Fig. 2.3a). The acanthocephalan *Polymorphus botulus* was detected in the gut of crabs collected at all sites, at varying prevalence (Table 2.4) (Fig. 2.3b). In histology, acanthocephala elicited a melanisation response in cases where infection breached the gut epithelium. The trematode *M. similis* was detected in crabs from 3 sites, at varying prevalence (Table 2.4).

Micro-eukaryote symbionts were frequently observed. Gut-dwelling gregarines were detected in 10.5% of animals from the Nesvík site (Fig. 2.3c). The taxonomic identity of the gregarines is currently unknown. Morphologically, gregarines were elongate with no clearly discernible epimerite, contained an eosinophilic nucleus and nucleolus and a granular, light blue-staining cytoplasm. Gregarines were often present at high density throughout the gut of infected hosts (Fig. 2.3c). No host immune response was noted to target these protists.

Ciliated protists were present at relatively high prevalence in crabs collected from all sites (Table 2.4) (Fig. 2.3d). Like those observed on the gills and appendages of specimens from the UK, ciliated protists from Faroese *C. maenas* were often present alongside filamentous bacteria and detritus and did not appear to elicit any pathology (or immune response) in their hosts.

Hematodinium sp. was detected in crabs from 3 sites (Table 2.4). Parasites colonised the haemolymph (Fig. 2.4a), a feature reflected in the opaque, white haemolymph of infected crabs upon dissection. Molecular diagnostics employing a nested PCR protocol provided a 345bp sequence including both the partial 18S gene and ITS region. BLASTn comparison of the sequence identified the 18S region to have 100% similarity to *Hematodinium* sp. isolated from *Chionoecetes opilio* (accession: FJ844422; e-value = $2e^{-92}$). The same analysis for the ITS region showed closest similarity (95%) to the same *Hematodinium* sp. isolated from *Chionoecetes opilio* (accession: FJ844422; e-value = $7e^{-22}$).

Amoebae were detected infecting crabs from all sites (Table 2.4). Amoebae were observed in open circulation, often at the end of the lacunae of individual gill lamellae (Fig. 2.4b). In one case, amoebae appeared to contain cytoplasmic inclusions of unknown identity (Fig. 2.4b). Amoebae elicited no observable immune response from the host despite their presence in the haemolymph. Analysis of the SSU rRNA gene, amplified from amoebae present within these infected crabs revealed two 100% similarity

(357bp/241bp) and a single 99% similarity (399bp) to *Neoparamoeba pemaquidensis* (EU884494), a parasite previously found infecting Atlantic salmon, sea urchins and lobsters. The heart and skeletal muscle-infecting microsporidian resembling *Nadelspora canceri* (=*Ameson pulvis*), detected in crabs from the UK, was also detected in crabs from 3 sites in the Faroe Islands, at varying prevalence (Table 2.4). Infection was confirmed by both histology and molecular phylogeny [amplification of the SSU rRNA gene providing a 901bp sequence with 99% similarity to *N. carcini* (accession: AF305708.1)].

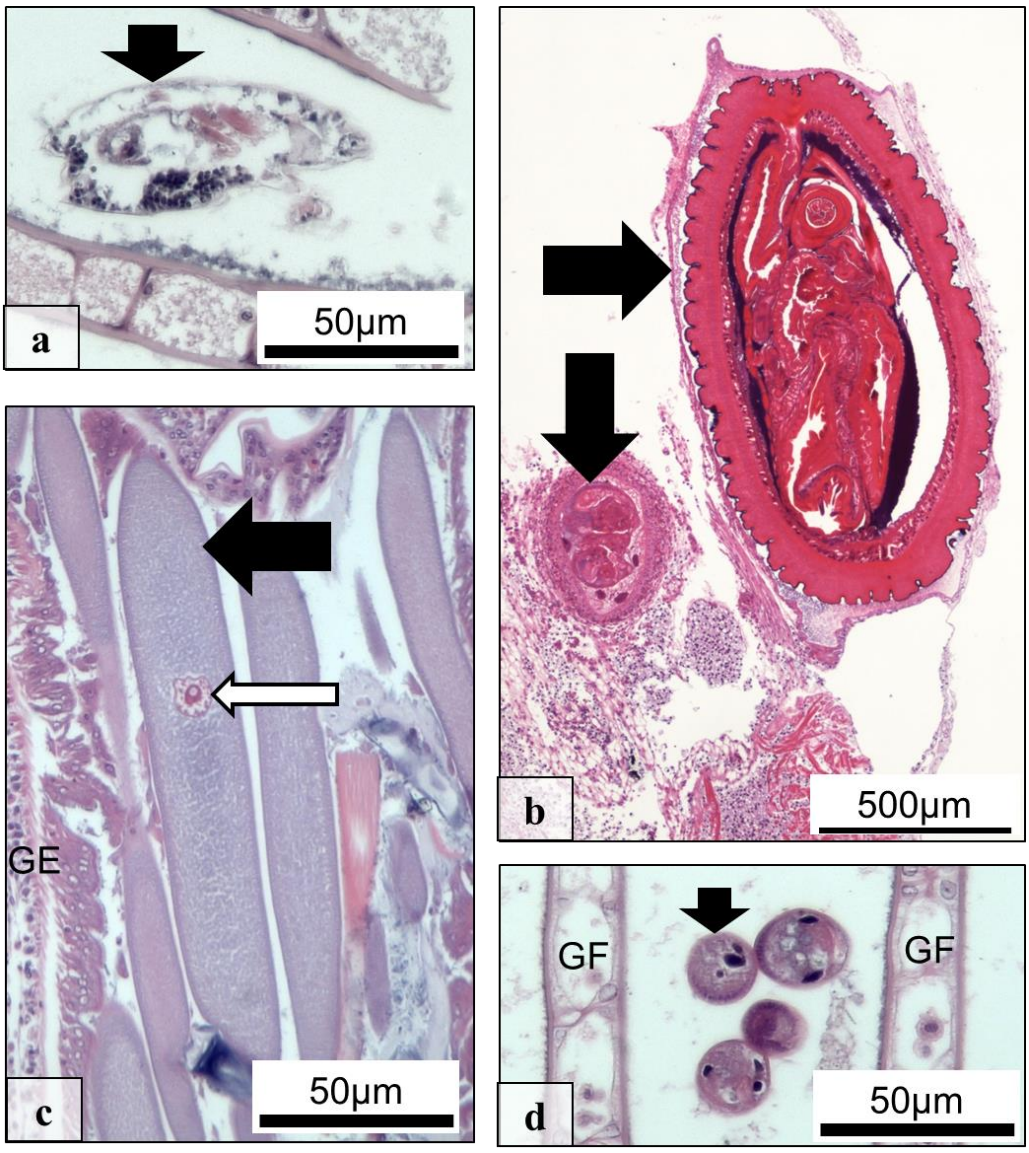


Figure 2.3: Parasites and commensals of *C. maenas* collected from the Faroe Islands. a) A crustacean (likely a copepod or isopod) (black arrow) between the gill lamellae of the host. b) *Polymorphus botulus* (black arrows) encysted into the gut wall of the host. c) Gregarine parasites (black arrow) with a distinguishable nucleus (white arrow) in the gut lumen of the host. d) Ciliated protists (black arrow) between the gill lamellae (GF) of the host.

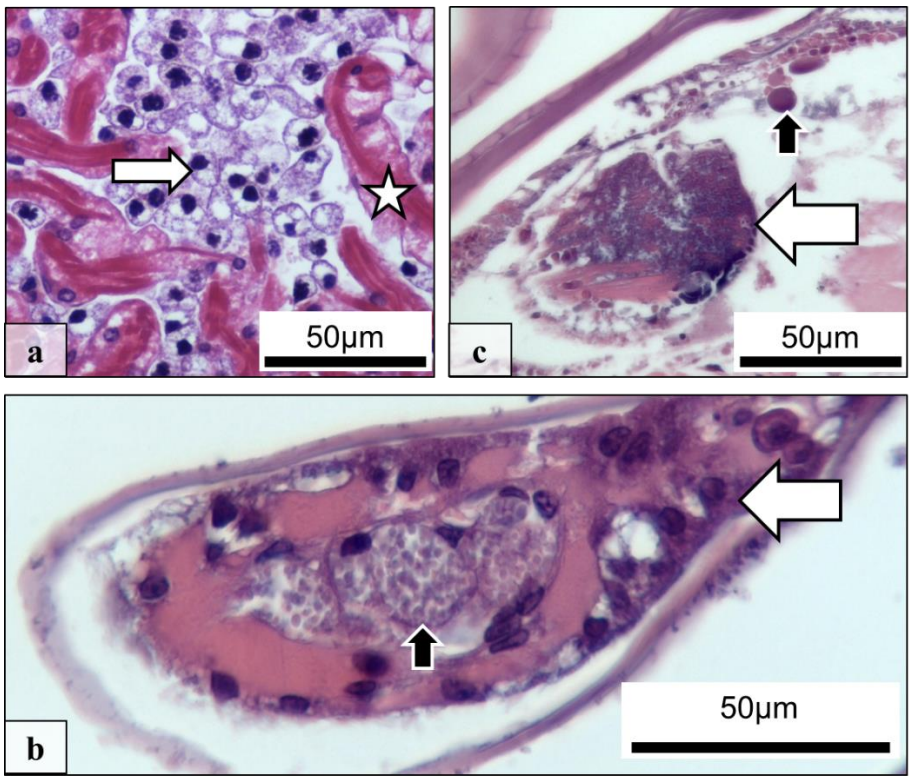


Figure 2.4: Parasites of *C. maenas* from the Faroe Islands. a) *Hematodinium* sp. (white arrow) in the haemolymph amongst the heart tissue (white star). b) Amoebae (black arrow), some with possible hyperparasites, present in the lumen of the gill filament (white arrow). c) An RLO developing within the musculature (white arrow) and haemolymph (black arrow) of the host.

The bacterial infection termed 'Milky Disease', observed in UK crab populations was not observed in animals collected from the Faroe Islands. I did however detect a putative Rickettsia-like organism (RLO) in crabs from 2 sites (Table 2.4). The putative RLO appeared to colonise the skeletal muscles of the host, forming plaques at the periphery of muscle fibres, in a region corresponding to the sarcolemmal space (Fig. 2.4c). Colonies of bacteria could also be identified in the histological section, present in the haemolymph (Fig. 2.4c). The presence of bacteria did not evoke an observable immune response from the host. Because the pathology extended to the muscle fibres I have identified this as a different pathology from that related to milky disease.

Several viral pathogens were observed in crabs collected from Faroe Island sites. CmBV was present in the hepatopancreas of individuals from 3 sites, at varying prevalence (Table 2.4). A putative parvovirus, with similarity to that observed infecting crabs in the UK was detected in specimens collected from 2 sites in the Faroe Islands (Table 2.4). Only the nuclei of haemocytes were infected, resulting in nuclear hypertrophy due to the presence of an amorphous "viroplasm" in the form of a Cowdry body (Fig. 2.5a). Under TEM, the viroplasm was packed with very small putative parvovirus particles, though

accurate measurement of individual “virions” was not possible (Fig. 2.5b). A novel Irido-like virus was observed to infect crabs (n=2, 1.1% site prevalence) from the Nesvík site. Infection appeared to be restricted to the connective tissues and tegmental glands of the primary gill lamellae (Fig. 2.6a). Infection elicited a distinctive eosinophilic staining characteristic of infected host cells (Fig. 2.6a). Under TEM, individual virions were shown to measure 96.6nm ± 12.2nm (n=50) in diameter, were arranged in a paracrystalline array (Fig. 2.6b, c) and occurred at high density in heavily infected cells. Individual virions were also observed transitioning through the membrane of infected cells (Fig. 2.6d). No immune response to infected host cells was observed. Finally, a rod-shaped virus was detected infecting crabs collected from 3 sites (Table 2.4). Histology revealed a deep-purple staining viroplasm in the infected nucleus of host haemocytes and haematopoietic organs (Fig. 2.7a). TEM revealed a rod-shaped virus, herein referred to as B-virus due to the similarity between this virus (Fig. 2.7b) and the pathogen previously noted by Bazin et al (1974) in *Carcinus* sp. from Europe. The TEM samples obtained in this study originated from wax-embedded materials originally fixed for histology. In this case, virions had the following dimensions: core width = 55.7nm ± 9.6nm, core length = 152.4nm ± 17.9nm, membrane width = 62.2nm ± 12.4nm and membrane length = 185.6nm ± 26.4nm (n=30). This viral infection elicited no observable immune response from the host.

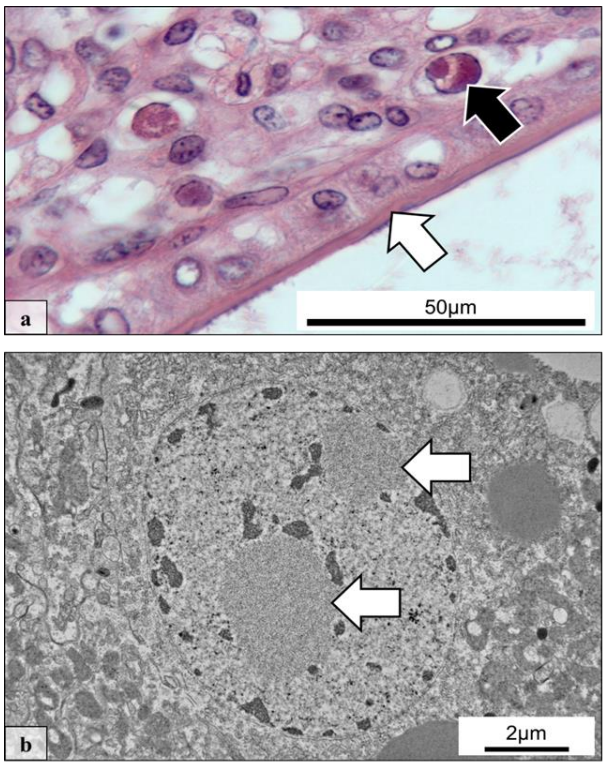


Figure 2.5: A putative parvoviral infection in the granulocytes of *C. maenas* from the Faroe Islands. a) Host granulocytes in the gill filament (white arrow) are present with a growing viroplasm, resulting in margination of host chromatin (black arrow). b) Transmission micrograph of an infected nucleus revealed a growing viroplasm (white arrow) without fully formed virions.

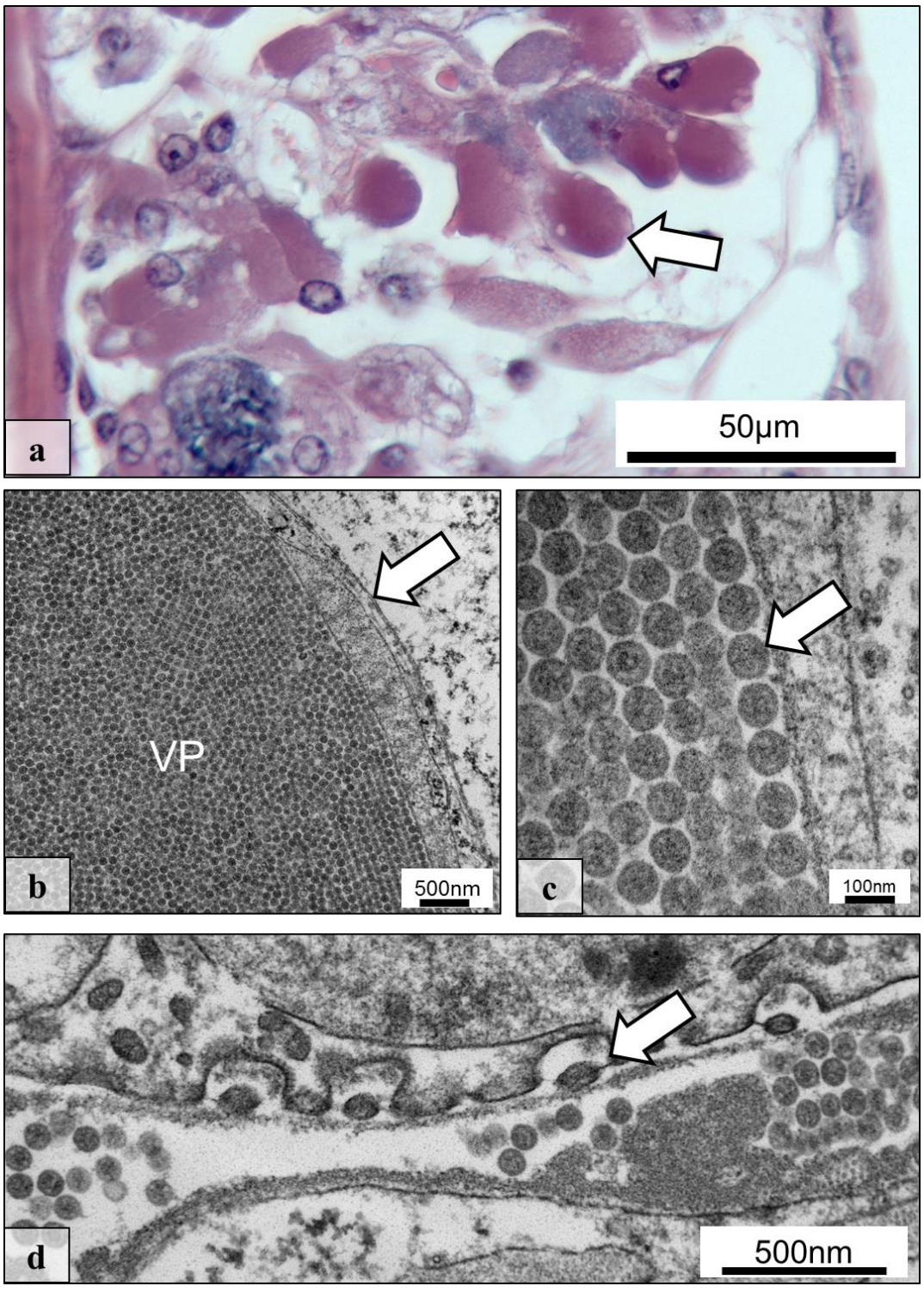


Figure 2.6: An iridovirus from the cytoplasm of gill epithelia in *C. maenas* collected from the Faroe Islands. a) Histologically, the virus produced a deep-pink staining viroplasm (white arrow) in the cells around the main gill stem. b) Transmission micrographs show virions in a para-crystalline arrangement (VP) in the cytoplasm of infected cells, reaching the cell membrane (white arrow). c) High magnification images revealed hexagonal virions (white arrow) arranged within the cytoplasm. d) In late infections the virions could be seen to move out of the host cell via exocytosis (white arrow) into the inter-cellular space.

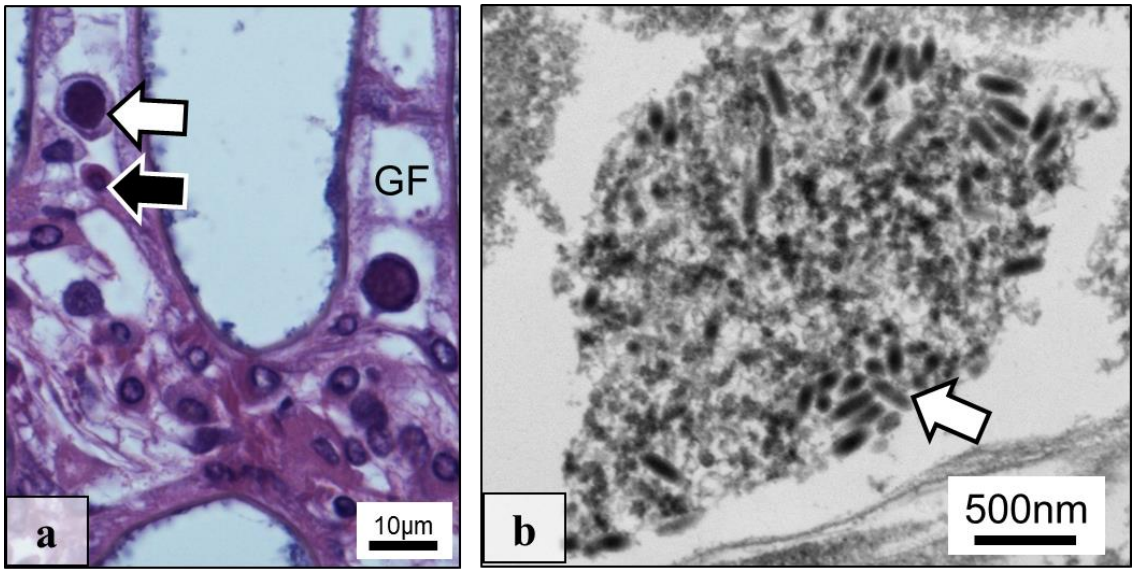


Figure 2.7: A rod-shaped virus in the granulocytes of the host with morphological similarity to B-virus. a) Uninfected (black arrow) and infected (white arrow) granulocytes are present in the gill filament (GF). b) A transmission micrograph from wax-embedded tissue revealed rod-shaped virions (white arrow) in the nucleus and cytoplasm of the host granulocytes.

2.4.1.3. Atlantic Canada

Histological analyses revealed 13 symbionts in crabs collected from the shoreline of Atlantic Canada. The survey revealed ten organisms also associated with crabs from the UK or Faroe Islands but also, a novel microsporidian parasite and potential re-discovery of a viral pathogen previously detected in invasive *C. maenas* from American waters.

Metazoan parasites included an isopod infection in crabs collected from 3 sites at varying prevalence (Table 2.5). Similar to that observed in infected crabs from the UK and Faroe Islands, isopods colonised the space between gill lamellae (Fig. 2.8a). *Polymorphus botulus* was detected in crabs from 2 sites, eliciting similar pathology to that observed at other geographic locations (Table 2.5). *Microphallus similis* was recorded in crabs from all Canadian sites, except for Fox Point, at varying prevalence (Table 2.5). A nematode infection was noted in a single specimen (0.9%) sampled from the Pubnaco site. Infection was localised to the connective tissues of the hepatopancreas (Fig. 2.8b). No immunological responses were observed to target this parasite.

Table 2.5a					Prevalence determined by histology (%)												
					1	2	3	4	5	6	7	8	9	10	11	12	13
Collection site		Collection date	Sex (M/F/U)	n=	Ciliated protists	*Nadelspora canceri*	Nematode	CmBV	*Polymorphus botulus*	Unidentified RLO	Milky disease	RV-CM	*Microphallus similis*	*Parahepatospora carcini*	Amoebae	Isopod	*Haplosporidium littoralis*
A	Port L'Hebert	08/2014	30/11/0	41	80.5	0.0	0.0	12.2	0.0	14.6	2.4	12.2	7.3	0.0	12.2	2.4	0.0
B	Hubbards	08/2014	20/17/25	62	48.4	0.0	0.0	29.0	0.0	0.0	0.0	0.0	11.3	0.0	38.7	0.0	0.0
C	Boutiliers Point	08/2014	2/7/11	20	20.0	0.0	0.0	25.0	0.0	0.0	0.0	15.0	20.0	0.0	0.0	0.0	0.0
D	Fox Point	08/2014	2/3/17	22	59.1	0.0	0.0	27.3	0.0	0.0	0.0	0.0	0.0	0.0	36.4	0.0	0.0
E	Pubnaco	08/2014	59/25/27	111	81.1	0.0	0.9	27.9	11.7	0.0	0.9	0.9	19.8	0.0	21.6	0.9	1.8
F	River Port	08/2014	34/8/0	42	81.0	0.0	0.0	23.8	2.4	1.8	0.0	0.0	21.4	0.0	0.0	9.5	0.0
G	Malagash	08/2014	55/77/2	134	70.1	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.7	0.7	0.7	0.0

Table 2.5b		Collection site						
		A	B	C	D	E	F	G
Collection site	A							
	B	1						
	C	1						
	D							
	E	5	1, 5	1, 5	5, 9			
	F		1, 11	1		11		
	G		4, 11	1		4, 5, 9, 11	4	

Table 2.5: 2.5a) Prevalence percentages for each pathogen type associated with *C. maenas* at each collection site in Canada. 2.5b) A display of the significant differences between populations holding different proportional prevalence's of commensals, parasites and pathogens. Significant associations are listed in the table and any non-significant associations are not listed in the table. Significance is calculated at a threshold of <0.05 using the Marascuillo procedure. The Yates correction was applied to negate the presence of false positive results.

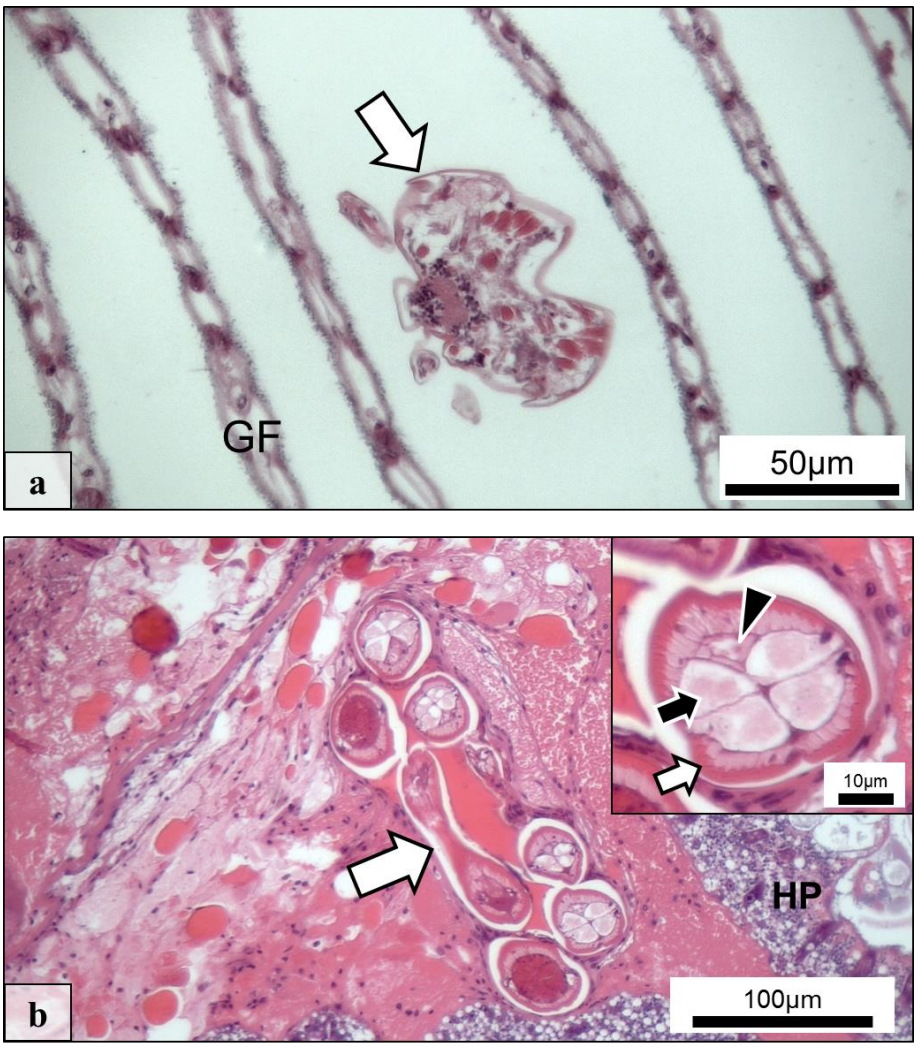


Figure 2.8: Commensals and parasites from *C. maenas* collected in Atlantic Canada. a) A crustacean (likely copepod or isopod) (white arrow) between the gill lamellae of the host (GF). b) A nematode (white arrow) encysted into the connective tissue of the host. The inset shows a section through the parasite in high detail, determining the five body cavities (black arrow/triangle) and surrounding smooth muscle (white arrow).

Micro-eukaryote symbionts were frequently observed. Ciliated protists (including stalked ciliated protists) were common in crabs collected from all Canadian sites (Table 2.5) (Fig. 2.9a). Amoebae, similar to those detected in crabs from the Faroe Islands, were observed infecting crabs from 5 sites, at varying prevalence (Table 2.5). The location and histological appearance of amoebae was as described above (Fig. 2.9b). Analysis of the SSU rRNA gene sequence from amoebae infecting crabs from Canada revealed potential for co-infection with two closely related parasites, *Neoparamoeba peraquidensis* (AY714363) (456bp - 99% identity) and *Neoparamoeba peruans* (EF216900) (356bp - 99% identity). These amoebae have previously been reported as

infections of *Homarus americanus* and *Salmo salar* (Mullen et al. 2004, 2005; Feehan et al. 2013). A haplosporidian resembling *Haplosporidium littoralis* was detected infecting crabs from the Pubnaco site, at low prevalence (n=2, 1.8%) (Fig. 2.10a). A microsporidian resembling *Nadelspora canceri* (=*Ameson pulvis*) was detected in 2.2% of crabs sampled from the Malagash site. A novel microsporidian parasite was detected infecting epithelial cells of the hepatopancreas of a single *C. maenas* (0.7%) from the Malagash site. Using histology, TEM and phylogenetics data, the parasite was named as *Parahepatospora carcini* n. gen. n. sp. in Chapter 4.

The putative RLO bacterial infection detected in crabs collected in the Faroe Islands was also observed infecting the musculature of *C. maenas* sampled from 2 Canadian sites (Table 2.5). Infection manifested as bacterial plaques formed in the sarcolemmal space of infected muscle fibres (Fig. 2.10b). Immune responses were noted to target plaques by an aggregation of granulocytes. Milky Disease, as recorded in crabs from the UK, was also observed in crabs collected from 2 sites in Canada (Table 2.5). High burdens of bacterial cells in the haemolymph resulted in a thick, opaque, white haemolymph, visible during dissection. Histologically, infection manifested as large, purple-pink staining bacterial plaques within the haemolymph and fixed phagocytes of the hepatopancreas (Fig. 2.10c), often associated with haemocyte aggregation and melanisation.

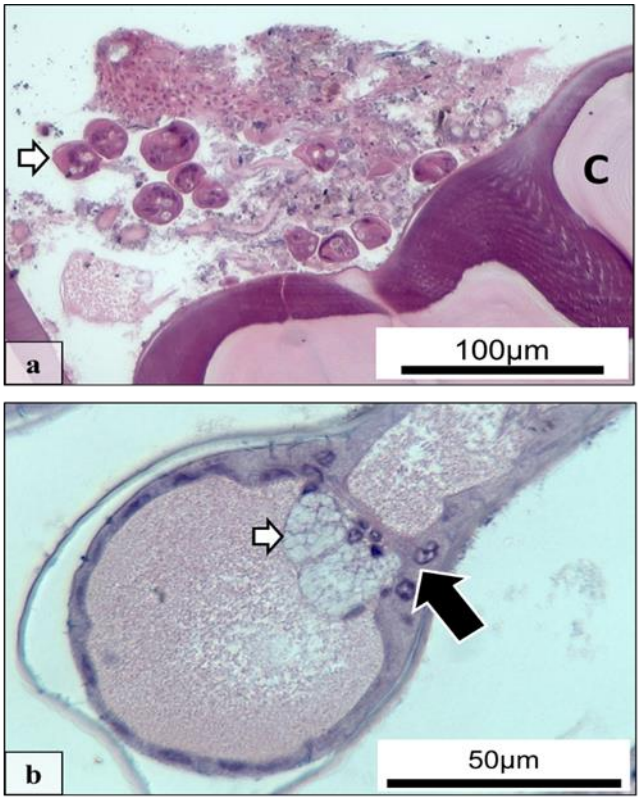


Figure 2.9: Ciliated protists and amoebae associated with *C. maenas* from Atlantic Canada. a) Stalked ciliated protists (white arrow) attached externally to the carapace (C) of the host. Amoebae (white arrow) staining light blue congregate at the ends of the host gill lamellae. Gill epithelia are defined by the black arrow.

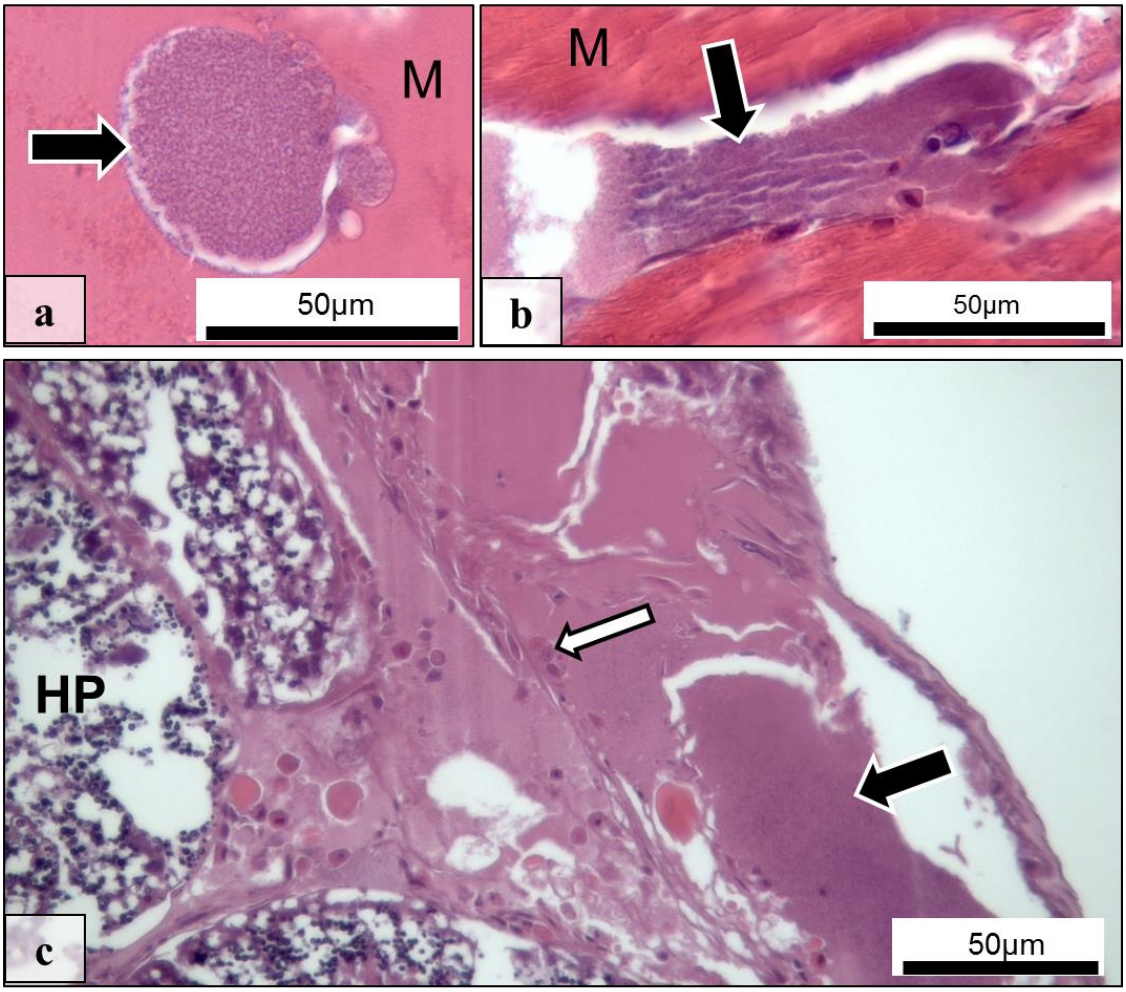


Figure 2.10: Haplosporidian and bacterial infections of *C. maenas* from Atlantic Canada. a) *Haplosporidium littoralis* (black arrow) in the musculature (M) of the host. b) A bacterial plaque (black arrow) forming on the musculature (M) of the host. c) Heavy bacterial colonisation of the blood stream (black arrow) surrounding the host haemocytes (white arrow) and hepatopancreas (HP).

Two viral pathogens were detected in crabs collected from Canadian sites. CmBV was observed infecting crabs collected from various sites (Table 2.5). Infection and pathology caused by infection with this virus mirrored that observed in crabs collected from other geographic locations within this study. A rod-shaped virus was detected in crabs collected from 3 sites in Canada, at varying prevalence (Table 2.5). Histological analysis revealed a deep-purple staining viroplasm within the nuclei of haemocytes and hematopoietic tissues (Fig. 2.11a). TEM revealed a rod-shaped virus, resembling both the B-virus reported in European crabs and, RV-CM, reported in invasive populations of *C. maenas* from the Atlantic coast of the USA (Johnson et al. 1988) (Fig. 2.11b, c). The rod-shaped virions contained condensed genomic material and a protein capsid along with a bi-laminar membrane (Fig. 2.11d). Dimensions of the virions were as follows: core

width = 100.3nm ± 13.3nm, core length = 245.6nm ± 42.1nm, membrane width = 219.8nm ± 36.3nm and membrane length = 306.2nm ± 34.7nm (n=30). This viral infection elicited no observable immune response from the host. Phylogenetic analysis of the DNA polymerase protein sequence suggests that this virus is part of the *Nimaviridae* (Fig. 2.12).

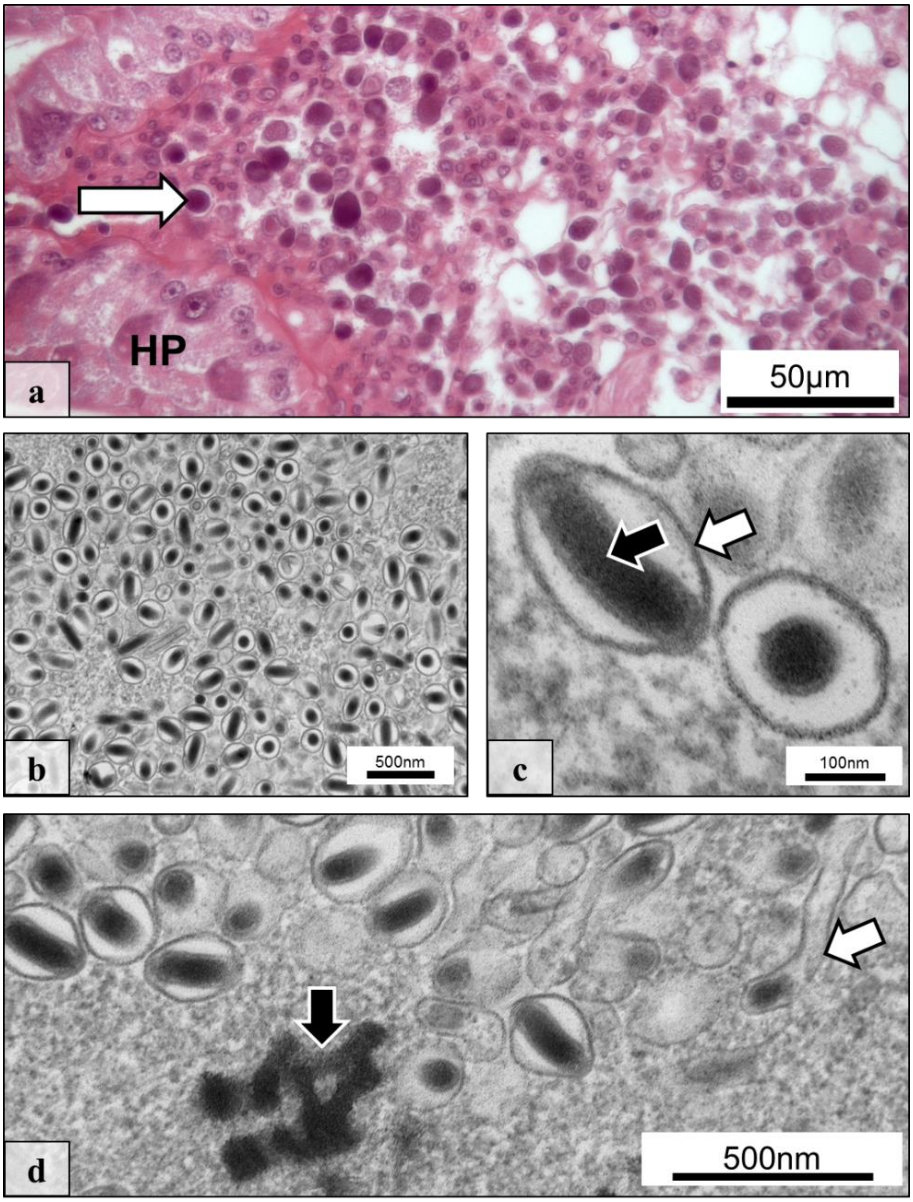


Figure 2.11: Re-discovery of RVCM, an intranuclear rod-shaped virus of *C. maenas* collected from Atlantic Canada. a) Histological sections identified haemocytes with hypertrophic, deep-purple-staining nuclei (white arrow) in the haemolymph around the hepatopancreas (HP). b) An electron micrograph of a portion of an infected nucleus displaying several developmental stages of RVCM. c) A high magnification image of a transverse and longitudinal section of two virions, identifying the genomic core (black arrow) and lipid membrane (white arrow). d) Developing genomic (black arrow) and lipid membrane (white arrow) material in the host nucleus.

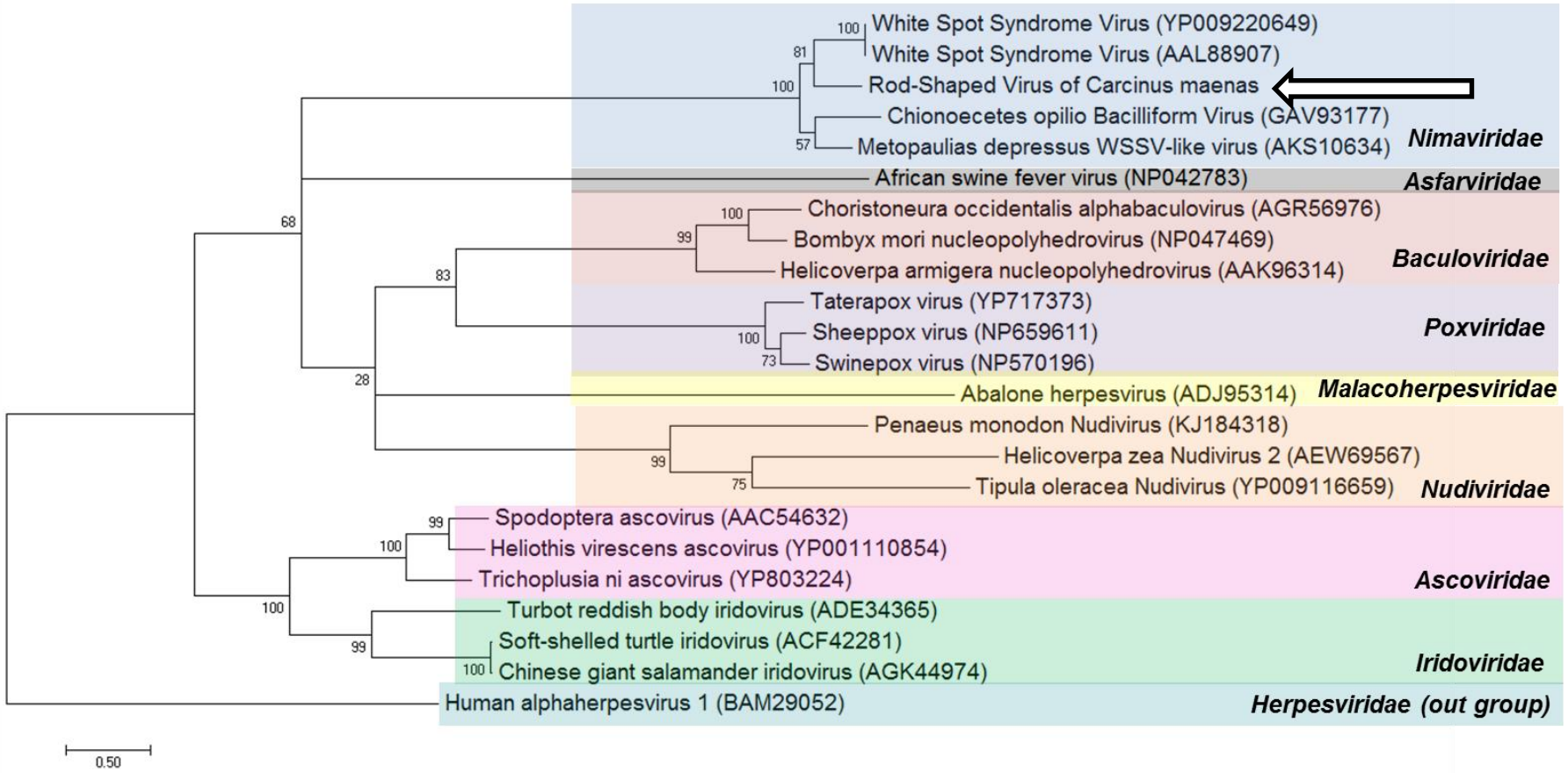


Figure 2.12: A phylogenetic tree including the DNA polymerase protein from several dsDNA viruses, including the rod-shaped virus identified from this study (white arrow). The evolutionary history was inferred by using the Maximum Likelihood method based on the Dayhoff matrix based model. The tree with the highest log likelihood (-64854.8617) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 amino acid sequences.

2.4.2. Statistical comparison of crab symbionts from the UK, Faroe Islands and Atlantic Canada

Data pertaining to 19 symbiont associations, from 1506 individual crabs collected from 23 sites (27 distinct sampling efforts: Table 2.1) in 3 distinctive geographical locations was utilised to compare combined symbiont profiles over the previously proposed invasion route of *C. maenas* from Europe/Faroe Islands to Atlantic Canada (Darling et al. 2008) (Table 2.6). Symbiont profiling revealed that discrete pathogens, parasites and commensals were shared between the three geographic locations, whereas others were more likely to have been acquired or lost in the invasive range (Table 2.6; Fig. 2.13; Fig. 2.14).

Using the Marascuillo procedure, an analysis was conducted to identify which symbionts were present at significantly different prevalence. This revealed a variety of significant associations detailed in Tables 2.3, 2.4, 2.5 and 2.6. Specifically, *Hematodinium* sp. was at a significantly higher prevalence in the Faroese population in comparison to the Canadian population ($P<0.05$), and the incidence of amoebae was significantly greater in the Canadian population relative to the other two countries ($P<0.05$). Ciliated protists were the most common symbiont in Canada and the Faroe Islands, however *M. similis* was most commonly observed in the UK (Fig. 2.13).

In addition to looking at the distribution and prevalence of the various symbionts across the sample populations, the factor of host sex was also assessed in comparison to symbiont presence. Analysis identified that Ciliates were more commonly associated with male *C. maenas* (Chi Squared test, $X^2_{df=1} = 15.341$, $P<0.001$); *P. botulus* were more commonly associated with male *C. maenas* (Chi Squared test, $X^2_{df=1} = 4.4475$, $P = 0.035$); and isopods were more commonly associated with male *C. maenas* in the UK (Chi Squared test, $X^2_{df=1} = 6.0116$, $P = 0.014$). All other symbionts revealed no preference for a particular sex of the host. Both sexes also show a similar co-infection rate, with males significantly holding a greater number of symbionts than females (Wilcoxon test, $W = 209470$, $P = 0.015$).

Table 2.6a		Prevalence determined by histology (%)																		
Country of collection	Collection date(s)	n=	1 Ciliated protists	2 *Hematodinium* sp.	3 *Haplosporidium littoralis*	4 *Nadelspora canceri*	5 Nematoda	6 CmBV	7 *Polymorphus botulus*	8 *Sacculina carcini*	9 Unidentified RLO	10 Gregarines	11 Parvovirus	12 Milky disease	13 HLV	14 *RV-CM / B-virus*	15 *Microphallus similis*	16 *Parahepatospora carcini*	17 Amoebae	18 Gill-dwelling isopods	19 Iridovirus
United Kingdom	2010-2014	768	25.4	10.4	0.7	1.9	1.3	2.0	0.1	4.0	0.0	0.4	0.3	1.7	0.9	0.0	48.4	0.0	0.0	0.9	0.0
Faroe Islands	07-08/2014	306	83.0	16.0	0.0	1.6	0.0	13.1	7.2	0.0	5.2	6.2	1.0	0.0	0.0	5.6	40.2	0.0	11.8	1.3	0.7
Atlantic Canada	08/2014	432	69.0	0.0	0.5	0.7	0.2	17.4	3.2	0.0	1.9	0.0	0.0	0.5	0.0	2.1	10.6	0.2	15.5	1.6	0.0

Table 2.6b		Country of collection		
		United Kingdom	Faroe Islands	Atlantic Canada
Country of collection	United Kingdom			
	Faroe Islands	1, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 17		
	Atlantic Canada	1, 2, 6, 7, 8, 13, 14, 15, 17, 18	1, 2, 10, 15	

*Table 2.6: 2.*6a) Prevalence percentages for each pathogen type observed in each country's population of *C. maenas*. 2.6b) The table highlights significant differences between collective populations in each country holding different proportional prevalence's of commensals, parasites and pathogens. Significant associations are listed in the table and any non-significant associations are not listed in the table. Significance is calculated at a threshold of <0.05 using the Marascuilo procedure. The Yates correction was applied to negate the presence of false positives.

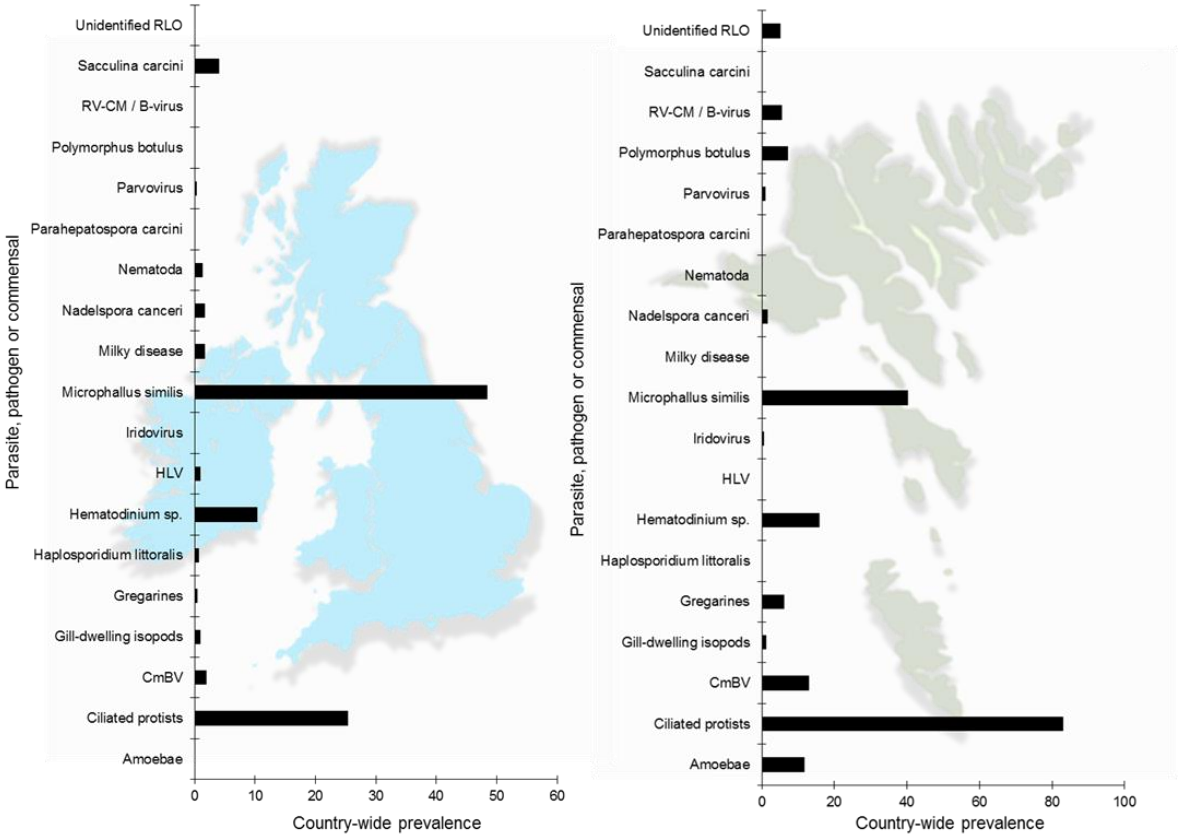


Figure 2.13: Bar graphs representing the UK, Faroe Islands and Nova Scotia populations of *C. maenas*, according to the prevalence of each commensal, parasitic or pathogenic association on a country-wide scale.

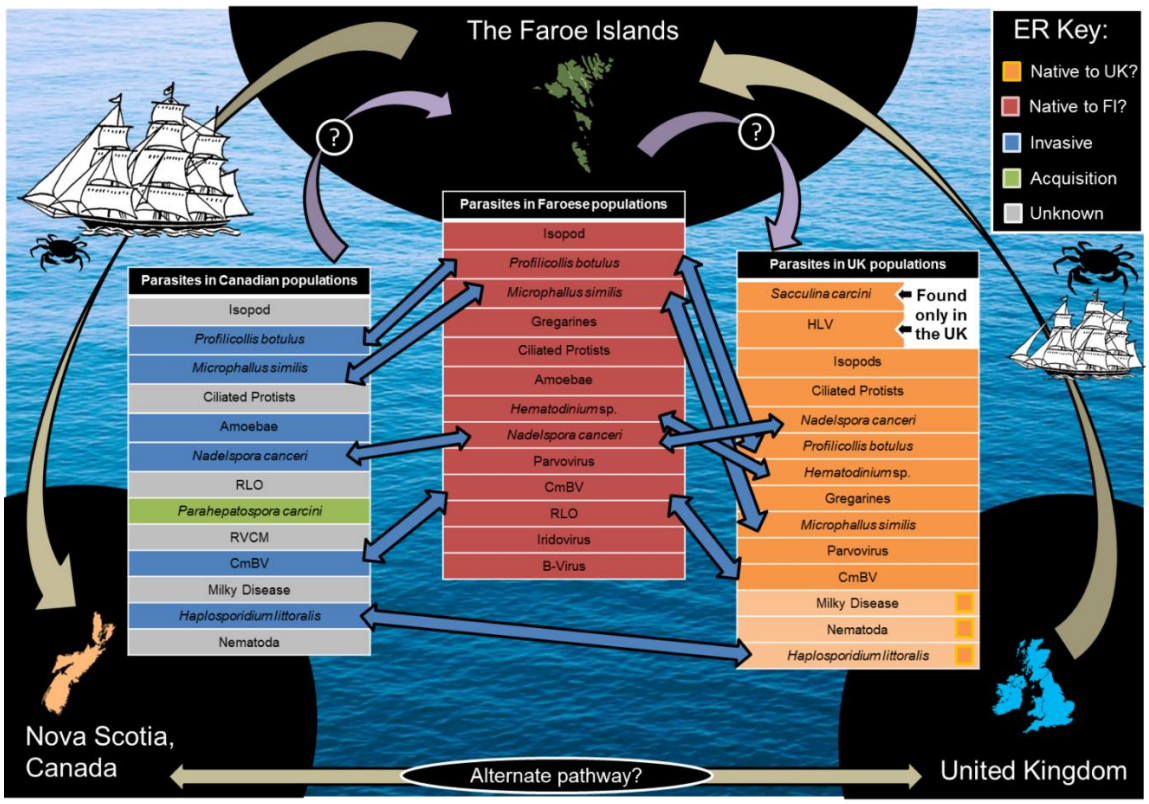


Figure 2.14: A figurative map of how *C. maenas* may have travelled between the UK, Faroe Islands and Atlantic Canada. Starting in the UK, *C. maenas* is considered native and therefore the pathogens it carries in this location are classed as native (orange). Those only found in UK populations are highlighted on the figure ("Found only in the UK"). An arrow with a ship and crab from the UK to the Faroe Islands signifies the first known movement of the invader. Here the pathogens are shown in red and considered native to the Faroe Islands, as the host is also considered native. A second arrow with a ship and crab represents the movement of *C. maenas* into its invasive territory in Nova Scotia, Canada. Here the pathogens the invader carries are either acquired (green), invasive along with the invader (blue) or have an unknown taxonomy and could be invasive or acquired (grey). The double ended blue arrows represent potential invasion. The purple, double ended, arrows with a "?" signify the possibility of crab movement in the reverse direction. Finally, some pathogens have been found in both the UK and Nova Scotia but not in the Faroe Islands, suggesting a possible movement from the UK to Nova Scotia irrelevant of the Faroe Islands (arrow: "Alternate pathway?").

Site	Sample size	Total pathogen richness	Average pathogen richness crab^{-1}
United Kingdom	768	754	0.98
Blakeney Harbour, Norfolk	30	65	2.17
Rye Harbour	30	17	0.57
Helford	30	42	1.40
Newtons cove, Weymouth, (2010)	30	37	1.23
Berwick Upon Tweed	30	21	0.70
North Shields	30	40	1.33
Poole Harbour	26	45	1.73
Southend on Sea	30	53	1.77
Menai Straights	30	39	1.30
West Mersey	30	53	1.77
Newtons cove, Weymouth (2012a)	188	124	0.66
West Mersea Island	120	69	0.58
Newtons cove, Weymouth (2012b)	8	9	1.13
Newtons cove, Weymouth (2013)	10	11	1.10
Newtons cove, Weymouth (2013-2014)	146	129	0.88
Faroe Islands	306	590	1.93
Kaldbaksfjørður	23	27	1.17
Argir	21	28	1.33
Kirkjubøur	25	43	1.72
Nesvík	181	401	2.22
Tórshavn	56	91	1.63
Atlantic Canada	432	533	1.23
Port L'Hebert	41	59	1.44
Hubbards	62	79	1.27
Boutiliers Point	20	21	1.05
Fox Point	22	27	1.23
Pubnaco	111	188	1.69
River Port	42	58	1.38
Malagash	134	101	0.75

Country-Comparison	Estimate	Std. Error	Z value	significance
FI-CA	**0.50705**	**0.06737**	**7.527**	**P<0.001**
UK-CA	**-0.18416**	**0.06098**	**-3.020**	**P = 0.003**
UK-FI	**-0.69121**	**0.05893**	**-11.730**	**P<0.001**

Table 2.7: The pathogen richness of each sample population, including the average richness crab^{-1} and the original population sample size are included in this table. Below are the results of a GLM (family = Poisson) (test adjusted = Holm), detailing how different each country-wide population is to one another from the perspective of pathogen richness.

Diseases that are considered as mortality-inducing were more common in the UK and Faroese populations (*Hematodinium* sp., Microsporidia, viruses) (Fig. 2.13). The Canadian populations showed a lower incidence of Microsporidia (0.7%) compared to

the UK and Faroe Islands (1.9%/1.6% respectively), along with a lower viral diversity. Amoebae in the Faroe Islands and Canada (fish and crustacean pathogens: *N. permaquidensis* and *N. peruans*) were at a significantly greater prevalence ($P<0.05$) than the UK, where no amoebal associations have yet been found.

The average pathogen richness calculated for each sample site, including a country-level analysis (Table 2.7), revealed that populations from the UK had an average pathogen richness of 0.98 crab^{-1}, compared to 1.93 crab^{-1} and 1.23 crab^{-1} in the Faroese and Canadian populations, respectively. Analysis, using generalised linear models, revealed that all the countries held a significantly different pathogen profile from each other, including the prevalence of each symbiont association (Table 2.7) and some associations that were specific to certain countries (Table 2.6; Fig. 2.13).

2.5. Discussion

Biological invasions are commonly associated with the introduction of parasites and pathogens (Dunn and Hatcher, 2015), however the success of those hitchhikers may be dependent on the invasive hosts' success; the environment they are transferred to; or the susceptibility (to infection and disease) of native species (Vilcinskas, 2015). Alternatively, invasive species can escape from their pathogens and benefit from increased fitness (Colautti et al. 2004). The invasive host may also become a sink for pathogens native in their new invasive range, leading to an increased threat of parasitism through 'spill-back' (Kelly et al. 2009).

In this study, I focused on a previously known northern Atlantic invasion pathway, determined by genomic microsatellite data (Darling et al. 2008) to investigate symbiont transfer, acquisition and loss in *C. maenas*. Utilising an existing comprehensive histopathology dataset relating to symbiont profiles of *C. maenas* in its native location (UK) coupled with additional surveys from UK, Faroese and Canadian populations of *C. maenas*, I compare symbiont profiles and reveal transferred, lost and potentially acquired symbionts in populations from the invasive range.

2.5.1. Potential symbiont transfer, loss and acquisition along the northern Atlantic invasion route

The UK dataset included animals sampled from 2010 through to 2014, collected over several seasons. It revealed 14 separate symbiont associations in the UK populations (Fig. 2.14), with 13 associations in populations from both the Faroe Islands and Atlantic

Canada (Fig. 2.14). Despite the lower number of pathogens identified, the Faroe Island populations (considered to reside within the native range for this host) were found to have the greatest average number of symbionts per crab (1.98 symbionts crab^{-1}), with Canadian populations displaying 1.23 symbionts crab^{-1}, and the UK having the lowest (0.98 symbionts crab^{-1}). Despite this information it is important to note that histology may be insensitive to an extent, and may not detect all the pathogens present – this is particularly important for latent pathogens, such as viruses or bacteria, which may be too small to see visibly, but would have been detectable through PCR or other molecular techniques. However, PCR techniques for many of the pathogens identified via histology are yet to be developed, and this study aimed to look at the diversity of symbionts present, not just specific groups. For this reason histology is highly useful as a general diagnostic.

As mentioned above, seasonality is also an important consideration and because the Faroe Islands and Canadian sampling efforts were restricted to the summer months (July, August, September), it could be that this survey has missed symbionts more prevalent in the winter. Increased screening during the winter months would benefit this dataset and allow for a detailed comparison of monthly symbiont prevalence between invasion sites. This increased screening may also identify whether certain pathogens are more likely to spread in warmer or colder months, and could advise biosecurity of areas during certain time periods.

The greater number of symbionts per crab in the Faroe Islands suggests that parasitism is more common here. When looking at the prevalence of specific symbionts in the Faroese populations, it is clear that some mortality driving pathogens, as well as other parasitic and commensal species (ciliated protists; *Hematodinium* sp.; gut gregarines; and *M. similis*), have been observed at greater relative prevalence to other countries (Table 2.6). Specifically, the species mentioned above were more common in the Faroese populations relative to the Atlantic Canadian populations. Similarly, some symbionts present in the UK were detected at significantly greater prevalence (*Hematodinium sp.*; *S. carcini*; isopods; HLV; and *M. similis*) than in Atlantic Canadian populations (Table 2.6). A higher prevalence of pathogens that lower host survival could be linked with the regulation of host population size (Patterson and Ruckstuhl, 2013). In combination with this possibility is the factor of symbiont 'preference' for host sex. I show here that males are significantly more likely to harbour more symbiont species than females, and this could identify them as a greater pathogen carrier risk. This specifically includes: *P. botulus*, ciliates protists, and isopods. If females are less likely to be invasive

due to behaviours such as brooding periods, when they are less active, this could hinder the movement symbionts to invasion sites. This theory would require studies on invasive capabilities of *C. maenas* males and females and would help to understand the patterns observed in this Chapter.

2.5.2. Viruses and bacteria

United Kingdom populations of *C. maenas* harboured three viruses (CmBV; parvovirus; HLV) and one bacterial disease (milky disease). Milky disease can be caused by a varied number of bacterial species and may be an opportunistic infection acquired through stress or co-infection (Eddy et al. 2007). This may mean that the aetiological agent of a clinical disease resembling 'milky disease' may differ between geographic locations. In contrast, the viral infections observed in this study are likely caused by specific agents; *Carcinus maenas* Bacilliform virus (CmBV) infecting the nuclei of the hepatopancreas (Stentiford and Feist, 2005), a putative parvovirus infecting the nuclei of gill epithelia and haemocytes (first reported here), and Herpes-like virus (HLV) infecting the nuclei of haemocytes (Bateman and Stentiford, 2017).

HLV was only detected in the UK at low prevalence (<1%), and specifically in the summer collection months from the Weymouth site – this pathogen is interesting from a seasonal perspective as discussed above. The apparent seasonal and site specificity of this infection may reduce its likelihood of spread to *C. maenas* invasion sites. Further, it may require suitable environmental and host-health conditions (temperature, stress) for infection, transmission and spread. Climate change and warming oceans may facilitate the spread of this virus amongst UK *C. maenas* populations, and potentially further (examples: Altizer et al. 2013). The Canadian populations were sampled in the summer and share similar sea temperatures with Weymouth, but no HLV infections were identified, suggesting it has not yet transferred to this location.

The putative parvovirus was detected at low prevalence (<1%) in crabs from both the UK and Faroese populations. Detection in the UK (Weymouth) occurred during winter, suggesting seasonality in susceptibility. Faroese populations, where the coast has a colder mean temperature than those in the south of England, presented a prevalence of 1%. This virus was not detected in the Canadian populations. Further assessment of the temperature effects on this virus are needed.

CmBV was detected in crabs sampled from all countries (UK: 2%; FI: 13%; CA: 17%) confirming its presence throughout this particular invasion pathway. The pathological

effects of this virus are well characterised, however its effects on the behaviour of the host are not (Stentiford and Feist, 2005). Recent studies have shown that the presence of similar viruses (*Nudiviridae*) in Crustacea may increase their host's activity (Bojko et al. Unpublished). Increased host activity has been related to the invasive potential of that host (Chapple et al. 2012).

In the Faroe Islands a putative iridovirus was detected at low prevalence (1%), however little is known about this virus other than the pathology and ultrastructure explored in this study. In both the Faroese and Canadian populations a rod-shaped virus was also detected. The virus resembles both B-virus, detected in crabs from the Faroes and previously, in crabs from mainland Europe Bazin et al (1974) and RVCM, a virus infecting invasive *C. maenas* on the Atlantic coast of the USA (Johnson, 1988). Morphologically, these viruses resemble white spot syndrome virus (WSSV) (*Nimaviridae*), an important pathogen of farmed penaeids (Stentiford et al. 2017), with a wide host range (Stentiford et al. 2009). Given that the rod-shaped virus detected here shares pathological characteristics with WSSV, further studies are required to investigate the susceptibility of native crustacean hosts in Canada (e.g. *Homarus americanus* is known to be susceptible to WSSV; Clark et al. 2013).

2.5.3. Microbial eukaryotes

Dinoflagellates, Haplosporidia, Microsporidia, ciliated protists and Apicomplexa have all previously been observed in the UK population of *C. maenas* (Stentiford and Feist, 2005; Stentiford et al. 2013a; Stentiford et al. 2013b). The current study has confirmed that ciliated protists, *Hematodinium* sp., *N. canceri (= A. pulvis)*, amoebae (*N. peruans* and *N. permaquidensis*) and gregarines in *C. maenas* from the Faroe Islands. The Canadian population is also colonised by ciliated protists, a haplosporidian resembling *H. littoralis* (<1%), a parasite resembling *N. canceri* (<1%), a *N. permaquidensis*-like parasite (15.5%), and a novel microsporidian parasite recently named as *Parahepatospora carcini* (<1%) (Chapter 4).

Ameson pulvis (=*Nadelspora canceri*) (Stentiford et al. 2013b) is now confirmed as an invasive species in *C. maenas* around Nova Scotia by both molecular and histological evidence and may threaten native populations of Crustacea. Molecular evidence is available to suggest that similar microsporidian species have been identified to infect rock crabs (*Cancer productus, Cancer magister*) (Amogan et al. Unpublished via NCBI). Rock crabs are common residents of Canadian and American coastlines and

susceptibility to transmission and infection may impact upon these species. It is possible that these initial identifications of *N. canceri* in *C. magister* and *C. productus* originated from the *C. maenas* invasion, and constitute an emerging wildlife disease. Detection of other microsporidia, such as *P. carcini*, that have not been detected in native locations could suggest an acquisition from the environment and lower the health and impact of the invasive populations (Chapter 4).

A parasitic dinoflagellate, *Hematodinium* sp. was detected in both the UK and Faroese populations at 10% and 16% prevalence respectively. In contrast, the parasite was not detected in the Canadian population, despite similar parasites known to infect native crustacean hosts from the Canadian marine environment (Shields et al. 2005). These dinoflagellate parasites are considered mortality drivers in crustacean populations, causing systemic infections that result in milky haemolymph, organ failure and eventually, host death (Shields and Squyars, 2000). The host range of *H. perezi* incorporates several crustacean hosts (MacLean and Ruddell, 1978; Small et al. 2012; Sullivan et al. 2016; O'Leary and Shields, 2017). The absence of *H. perezi* infection in those Canadian specimens in this study is intriguing and may reflect absence of this pathogen in its invasive range. However, given the pronounced seasonality of infection prevalence of *Hematodinium* dinoflagellates, repeat sampling in winter or spring would clarify the situation.

The amoebae (*Neoparamoeba* spp.) detected during this study may have originated from the environment, given that similar infections have not been detected to date in the UK population. Whether the infection is synonymous with the parasites known to infect salmon (where various *Neoparameoba* spp. have been implicated in amoebic gill disease (AGD) (Douglas-Helders et al. 2003; Feehan et al. 2013), remains to be shown.

The detection of *Neoparamoeba* spp. in the invasive *C. maenas* population in Canada (16% prevalence) could be the result of a 'spill-over' event, given that *N. permaquidensis* has been identified as the agent of a lethal disease of lobsters and sea urchins (Mullen et al. 2004; Mullen et al. 2005). The presence of this pathogen group in *C. maenas* populations without visible immunological response (as diagnosed via histology) or disease features suggests they may be a carrier of the disease. Work is now required to investigate synonymy between the pathogen detected in *C. maenas* and that known to infect *H. americanus* (Mullen et al. 2004; Mullen et al. 2005).

The prevalence of ciliated protists was observed to change between the cefas-acquired data and the data collected by myself in the UK. This could reflect a change in the

methods used upon historical Cefas samples; may reflect human error to not have noted this symbiont group; or could be a reflection of ciliate loss in the environment.

2.5.4. Metazoans

Several metazoan symbionts were identified in my study; including crustaceans, nematodes, Digenea and Acanthocephala. Populations from all countries and sites were infected with a digenean resembling *M. similis*, a trematode with a complex lifecycle involving snails, crabs and birds (Stunkard et al. 1957). Despite the complexity of this lifecycle, it appears adaptable to the specific conditions (hosts) encountered at these sites. The same phenomenon was observed in the case of *P. botulus*. No nematodes were detected in the Faroese populations, whilst infection in both the UK (1%) and Canada (<1%) was infrequent. It is likely these are opportunistic infections, however no molecular evidence is available to discern their taxonomy.

Isopods were detected on the gills of *C. maenas* from each country at low prevalence (1-2%). No genetic data is available to identify the isopods, however it is assumed they are commensal species likely native to the environment from which hosts were sampled. One has been identified in the past: *Priapion fraissei*. The absence of the parasitic barnacle *S. carcini* in Canadian populations is interesting given the relatively high prevalence observed in native populations by this survey. This reduced infection pressure may benefit *C. maenas* populations in Canada. *Sacculina carcini* has previously been reported as a potential biological control agent (Goddard et al. 2005). *Sacculina carcini* castrates and parasitizes its host, resulting in a combination of pathogen-based-biocontrol with the added benefits of autocidal control. A significant drawback includes the lack of host specificity: a common drawback of many biocontrol agents (Goddard et al. 2005).

2.5.5. Potential impact of C. maenas symbionts on native fauna in Canada

Atlantic Canada boasts a highly successful aquaculture trade, including a lobster fishery industry that is worth millions of dollars to their economy (Fisheries and Oceans Canada). The invasion of *C. maenas* and its pathogens pose significant risk to this economy (Chapter 4) and if transferable pathogens are introduced, a decline in the native populations could cause the country to lose a large amount of money to yield loss via emerging infectious disease.

Carcinus maenas have impacted aquaculture through competition and predation (Therriault et al. 2008) and our results identify that this invader also carries pathogens that could affect fisheries and the aquaculture industry. Some species could pose a significant pathological issue to native fauna, if *C. maenas* acts a reservoir; allowing the numbers of pathogens to build and spill back into the native populations. Such examples have been noted previously (Kelly et al. 2009) and the presence of *P. botulus* in *H. americanus*, an economically important fisheries asset, has already been identified with some parasite cross-over (Brattey and Campbell, 1986).

The use of *C. maenas* as a bait source for the capture of lobster could further facilitate pathogen and parasite transmission. Observation of particular taxa linked to disease in lobsters (*Neoparamoebae* sp.) (Mullen et al. 2004; Mullen et al. 2005), may be associated with the shore crab invasion. Other discoveries, such as the re-discovery of a haemocyte-infecting rod-shaped virus (Johnson, 1988), have been found in several farmed and fished Crustacea, and are strongly linked with mortality-causing disease (Bateman and Stentiford, 2017). One of the most economically devastating is white-spot syndrome virus (WSSV). The host range of WSSV is wide, encompassing some native Canadian species, such as *H. americanus* (Clark et al. 2013). The presence of RVCM, may prove to be a significant threat if transmissible to native, economically important Crustacea.

Carcinus maenas may obtain pathogens from native hosts. This survey identified *P. carcini*, a rare microsporidian pathogen that has likely been acquired due to a lack of detection in the native ranges of *C. maenas* (Chapter 4). Ciliated protists, gill-associated isopods, trematodes, acanthocephala, nematodes and bacterial diseases, are also likely acquisitions from natural Canadian fauna (birds, molluscs, crustaceans and other invertebrates) based on their commensal lifecycle, and opportunistic nature.

In total, the Atlantic Canadian populations of *C. maenas* include the following pathogens: ciliated protists; a haplosporidian; *N. canceri*; nematodes; *Cm*BV; *P. botulus*; an unidentified RLO; bacterial infections of the blood stream resulting in ‘milky disease’; RVCM; *M. similis*; *P. carcini*; amoebae; and commensal isopods (Table 2.5 and 2.6). Based on our survey, the invasive population is unlikely to harbour, or has an undetected low prevalence of, *Hematodinium*, *S. carcini*, gregarines, the putative parvovirus, HLV, or the iridovirus. It is yet to be determined whether the lack of these pathogens and parasites has an effect on the size and impact of the invasive population. The lack of these species could provide an opportunity for biocontrol, after host range, host survival and host behaviour analyses.

CHAPTER 3

Invasive pathogens on the horizon: screening Amphipoda to identify prospective wildlife pathogens and biological control agents

3.1. Abstract

Invasive non-native species (INNS) are one of the foremost drivers of biodiversity loss, and can result in the extinction of native species. A feature of invasion is disease introduction to new territories, which could infect native fauna. Alternatively, those diseases may help control the invasive host and limit its invasion impact. Horizon scanning for invasive pathogens provides an early warning system to better understand what may be carried by INNS.

Invasive and non-native freshwater amphipods threaten islands, such as the UK, and can colonise waterways at rapid rates. The Ponto-Caspian region is home to many species that now affect European environments and ecosystems. Amphipods from this region can pass through Poland via a "central invasion corridor" to reach Western Europe. In this chapter, I conduct a histological screen of amphipods from the Polish invasion corridor, with *ad hoc* application of molecular diagnostics and transmission electron microscopy (TEM) to identify parasitic, pathogenic, commensal or symbiotic organisms.

The screen revealed a range of associations, including: Metazoa (helminths and crustaceans); protists (ciliates, gregarines, *Haplosporidium*-like species); Microsporidia (*Cucumispora*; *Dictyocoela*); bacteria (bacilli; rickettsia-like organisms); and viruses (bacilliform viruses and viral-like pathologies). The taxonomy of some microsporidia, bacteria and viruses are explored further in Chapters 5 through 10. In chapters 5, 6 and 7 the figures relevant to that host or parasite species are included, but are mentioned in this chapter. *Dikerogammarus villosus* and *Pontogammarus robustoides* were collected from several sites in numbers large enough to apply statistical analyses for prevalence comparison.

The pathogen profile of each species, including the taxonomic composition of that profile, is discussed relative to possible biocontrol opportunities and wildlife pathogen introduction. I identify three species (taxonomically identified in Chapters 5, 6 and 7) that may be beneficial for control, including: microsporidians; rickettsiae; and viruses.

3.2. Introduction

Invasive species are capable of detrimentally affecting native habitats and their residents (Simberloff et al. 2005). Invasion sites often see a decrease in biodiversity as invaders replace vulnerable native species, which in turn can alter the services an ecosystem provides (Molnar et al. 2008). Invasive species can also alter the environmental stability and structure of the sites they invade (Pyšek and Richardson, 2010), and even impact upon human, livestock, and wildlife health via the introduction of pathogens and parasites (Roy et al. 2016).

The taxonomic order Amphipoda Latreille, 1816 is composed of >9,000 known species across terrestrial, freshwater and marine environments (Väinölä et al. 2008). Around 48 of these are listed to have become successful invaders (Rewicz et al. 2014; Chapter 1 – Appendix Table 3.3). The niche occupied by amphipods often involves nutrient recycling and an essential prey item at low trophic levels, meaning they are a keystone species for many ecological niches (Piscart et al. 2011; Boeker and Geist, 2015). Being present at a fundamental position in food-webs means that changes in amphipod population size and species structure can affect the environment and communities occupying all trophic levels and their function within the ecosystem (Boeker and Geist, 2015; Hellmann et al. 2017).

Amphipod population size and species diversity can be altered by an invasion (Hellmann et al. 2017). Localised extinction events (Mouritsen et al. 2005), competition (Pinkster et al. 1977), and increased predation (Strong, 1973) have all been reported to alter the survival rates and population sizes of native and invasive amphipods. Replacing a native amphipod with an invasive amphipod could have repercussions upon the environment due to relative change in predatory (Taylor and Dunn, 2017), competitive (MacNeil and Platvoet, 2005), and detritivorous behaviours (Piscart et al. 2011). Furthermore, the introduction of a pathogenic and parasitic cohort alongside an invasive host has the potential to change native amphipod populations by lowering the survival of their host (Duclos et al. 2006), changing their hosts behaviour (Arundell et al. 2014), or having further impacts upon an ecosystem. Invasive amphipods are known to carry viruses, bacteria, protists, microsporidians, helminths, and other crustaceans (Fig. 3.1), which all have the potential to invade alongside their host (Chapter 1 – Appendix Table 1.3).

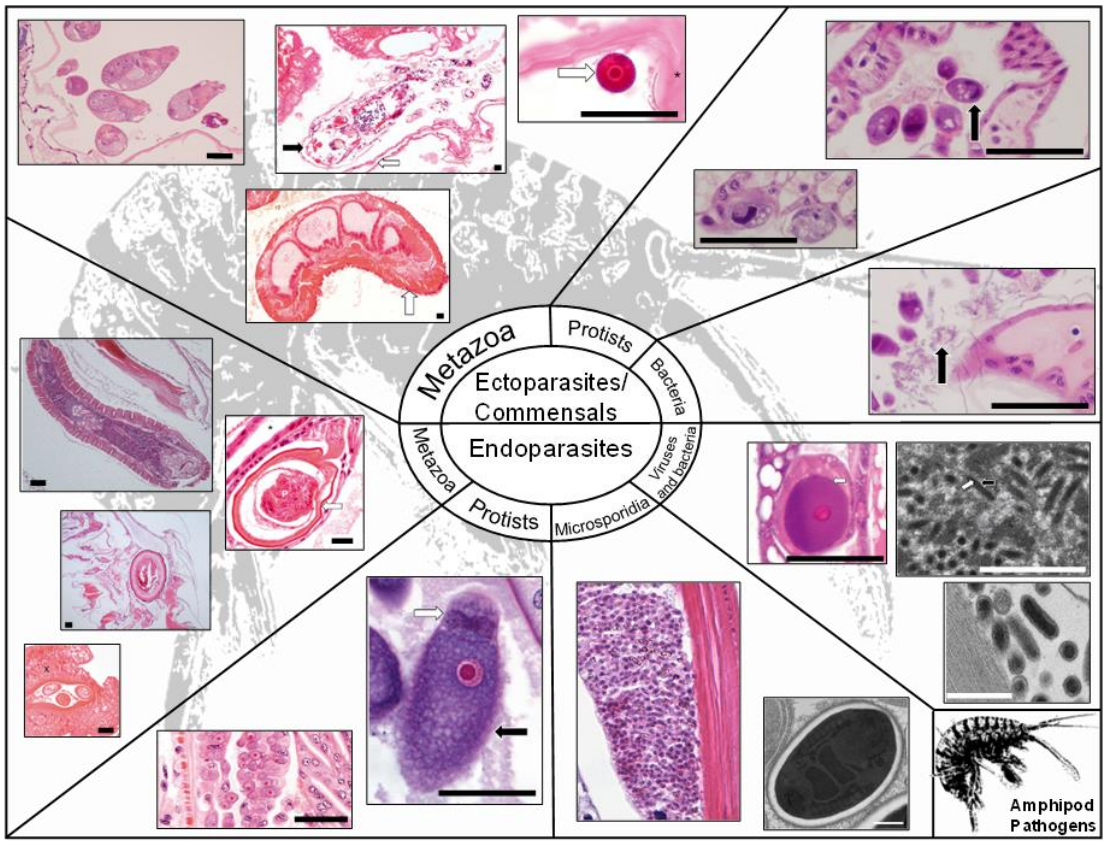


Figure 3.1: Parasites of invasive Amphipoda. From left to right: Ectoparasitic Metazoa: Oligochaete (from *Dikerogammarus villosus*); Rotifer (from *G. roeselii*)*;* Isopod (from *D. villosus*); Bryozoan (from *D. villosus*). Ectoparasitic Protists: Ciliated protist (from *G. roeselii*); stalked ciliated protist (from *G. roeselii*). Ectoparasitic Bacteria: Filamentous bacteria (from *G. roeselii*). Endoparasitic Viruses and Bacteria: *Dikerogammarus villosus* Bacilliform Virus pathology (from *D. villosus*); DvBV (from *D. villosus*); *Aquarickettsiella crustaci* (from *G. fossarum*). Endoparasitic Microsporidia: *C. ornata* (from *D. haemobaphes*); *C. ornata* (from *D. haemobaphes*). Endoparasitic Protists: gregarines (from *D. villosus*); gregarines (from *D. villosus*). Endoparasitic Metazoa: Acanthocephalan (from *D. villosus*); nematode (from *D. villosus*); *Polymorphus* sp. (from *G. pulex*); trematode (from *D. villosus*). Histology scale bars = 20µm. TEM scale bars = 500nm.

The UK has been invaded by several amphipod species over the past decade (Fig. 3.2). These include: *Dikerogammarus villosus*; *Dikerogammarus haemobaphes*; *Chelicorophium curvispinum*; *Gammarus fossarum*; *Crangonyx pseudogracillis*; *Echinogammarus ischnus*; and *Gammarus tigrinus*; with impending invasion from *Echinogammarus trichiatus*; *Pontogammarus robustoides*; *Gammarus roeselii* and several others (Roy et al. 2014a). The Ponto-Caspian region is the native range for many of the species listed above and constitutes a hot-spot of would-be invasive species and their pathogens (Gallardo and Aldridge, 2015) (Fig. 3.2). Poland constitutes part of the central invasion corridor, which many Ponto-Caspian invaders use to invade Western Europe, and particularly the UK (Bij de Vaate et al. 2002). This makes it an important place to screen invaders for their parasitic and pathogenic complement.

To gain a greater understanding of the pathogens, parasites and commensals carried by invasive amphipods destined for the UK, I carried out a histopathological screen augmented by targeted electron microscopy and molecular diagnostic analyses. Advancing our knowledge of invasive pathogens attributed to the Amphipoda provides a better standing for risk analysis without relying solely on the knowledge of the invasive host biology and behaviour. In addition, this information can provide a foundation for the development of biological control agents, and is a step forward in horizon scanning for the wildlife pathogens of the future.

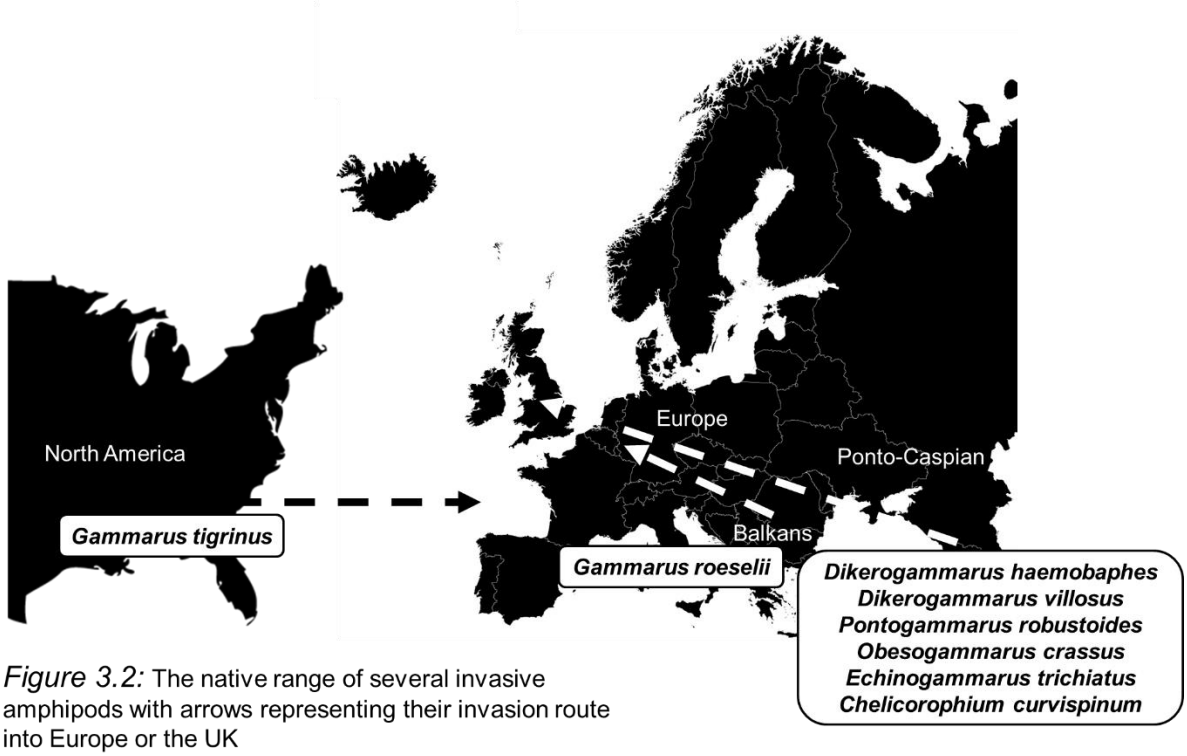


Figure 3.2: The native range of several invasive amphipods with arrows representing their invasion route into Europe or the UK

3.3. Materials and Methods

3.3.1. Sampling information

Amphipod specimens were collected using standard hydrobiological nets from the embankments of several rivers and lakes across Poland. To avoid bias the locations were each sampled in the same way, form the riverbank. In total, 15 sites were visited over an 8-day period between 16/06/2015 to 23/06/2015 and involved travelling over 2600km around Poland to reach the Vistula (9 sites), Bug (2 sites) and Oder River (4 sites) systems (Table 3.1). These sites showed a mixture of sites known only to harbour native species, whereas those sample sites from the Bug, Oder or Vistula Rivers are known to harbour invasive communities. This sampling regimen was chosen to attain a range of both native and invasive amphipods to look at any possible symbiont cross over.

Amphipods were identified based on a morphological key for genera and species of amphipods (Grabowski and Pöckl, 2010). Amphipods were either fixed on site for histology via injection of fixatives or were transported to a cold room, kept at 15˚C for up to three nights, before fixation or dissection. The specimens collected from this study cross over with the animals and symboints sampled for taxonomic descriptions in Chapters 6 and 7.

Sample site (Co-Ordinates) (Lat./Long.)	**Sample date**	**Sample site name**	**River system**	**Species sampled**	**n=**
52.49563, 19.44469	16/06/15	Lucień Lake in Lucień	Lake near Vistula	*D. haemobaphes*	123
				P. robustoides	211
52.584803, 19.479901	16/06/15	Włocławski Reservoir (Vistula River) in Nowy Duninów	Vistula River	*P. robustoides*	318
52.571839, 19.521571	16/06/15	Włocławski Reservoir (Vistula River) in Stary Duninów	Vistula River	*P. robustoides*	66
				D. villosus	27
52.611392, 19.561809	16/06/15	Skrwa Prawa River in Radotki	Vistula area	*None.*	-
52.653976, 19.541081	16/06/15	Skrwa Prawa River in Parzeń	Vistula area	*None.*	-
52.584056, 19.510798	16/06/15	stream in Murzynowo	Vistula area	*None.*	-
52.836048, 18.903723	16/06/15	Vistula River in Nieszawa	Vistula area	*P. robustoides*	8
				D. villosus	32
				C. curvispinum	37
51.31854, 21.914601	17/06/15	Vistula River in Janowiec	Vistula area	*D. haemobaphes*	1
51.824829, 19.459828	19/06/15	Bzura River in Łódź (Łagiewniki)	Vistula area	*G. fossarum*	140
52.460372, 21.01746	21/06/15	Zegrzynski Reservoir in Zegrze	Vistula area	*P. robustoides*	139
52.689838, 21.701035	21/06/15	Stream in Poręba-Koceby	Bug River area	*G. varsoviensis*	109
52.698281, 21.092706	21/06/15	Narew River in Pułtusk	Bug River area	*D. villosus*	68
52.66972, 14.46130	23/06/15	Oder in Porzecze	Oder River	*D. villosus*	13
52.966, 14.42906	23/06/15	stream in Chojna	Oder River area	*G. roeselii*	149
				G. pulex	49
53.25160, 14.47949	23/06/15	Oder in Gryfino	Oder River	*P. robustoides*	122
				O. crassus	4
				E. trichiatus	47
				G. tigrinus	15
53.69724, 14.54304	23/06/15	Szczecin Lagoon in Kopice	Oder River delta	*D. villosus*	1
				P. robustoides	287
				O. crassus	133
				E. trichiatus	6
				Total to screen:	**2105**

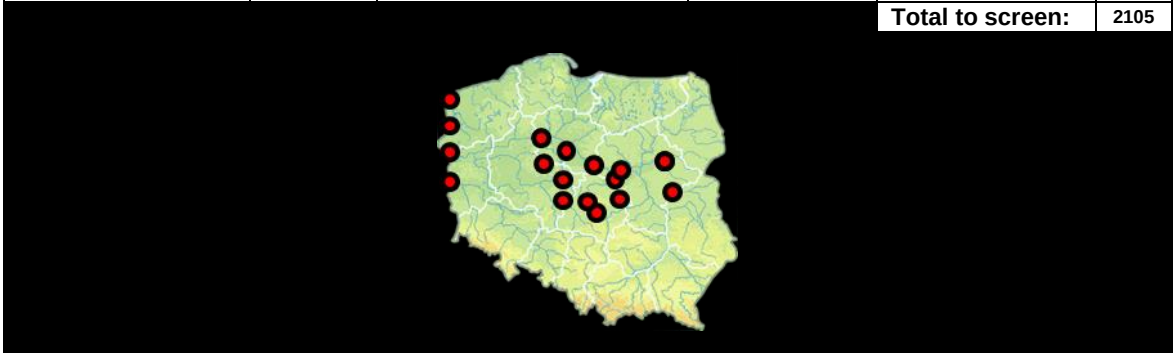


Table 3.1: The sites and river systems sampled from during the study with the number and diversity of each species collected for parasitological assessment for the presence of parasites, pathogens and commensals. The map included below the table outlines the sites visited across Poland.

3.3.2. Histopathology and electron microscopy

Amphipods (n=1978) were fixed on site in Davidson's freshwater fixative and were transferred to 70% industrial methylated spirit (IMS) after 48hr, and embedded into paraffin wax blocks using an automated tissue processor (Peloris, Leica Microsystems, UK). Material was sectioned on a Finesse E/NE rotary microtome (Thermofisher, UK) to produce 3µm thick sections of tissue. Specimen sections were stained using haematoxylin and alcoholic eosin (H&E) and slides examined using a Nikon Eclipse E800 light microscope. Images were captured using an integrated LEICA™ (Leica, UK) camera and edited/annotated using LuciaG software (Nikon, UK). This protocol is identical to that used in Chapter 5 with some small changes to account for different dissection and fixation techniques.

One hundred and twenty seven amphipods (*D. villosus = 104, G. fossarum = 13, G. roeselii = 9, G. pulex = 1*) were fully dissected to provide material for histology, TEM and DNA extraction, giving a total number of 2105 amphipods assessed during this study. Dissection involved removal of the gut and hepatopancreas, which was split for all three techniques with small muscle biopsies removed for fixation for TEM and DNA extraction. The main body of the animal and any remaining material was fixed for histology and transported to Cefas, Weymouth in ethanol.

Sample preparation for TEM followed that used in Chapter 5 starting with initial fixation in 2.5% glutaraldehyde before processing through two changes of 0.1M Sodium cacodylate buffer. Heavy metal staining was performed using Osmium tetroxide (OsO_4) followed by two 10 minute rinses in 0.1M Sodium cacodylate buffer. Samples were dehydrated through an ascending acetone dilution series (10%, 30%, 50%, 70%, 90%, 100%) before embedding in Agar100 resin using a resin:acetone dilution series (25%, 50%, 75%, 100%) (1 h per dilution). Tissues were placed into plastic moulds filled with resin and polymerised by heating to 60˚C for 16 h. Blocks were sectioned using a Reichart Ultracut Microtome equipped with glass blades (to cut sections at 1µm) or a diamond blade (to cut ultra-thin sections at around 80nm). Sections were stained using toluidine blue and checked using standard light microscopy and ultra-thin sections were stained using Uranyl acetate and Reynolds Lead citrate (Reynolds, 1963). Ultra-thin sections were observed using a Jeol JEM 1400 transmission electron microscope (Jeol, UK).

Scanning electron microscopy (SEM) was conducted on an individual *D. haemobaphes* collected from the Vistula River in Janoweic (17/06/2015) with visible features of advanced microsporidian infection. The process was conducted at the University of Łódź. To take individual spores from the animal, a small incision was made and gentle pressure

applied. Any liquid (liquefied muscle, particulate muscle, haemolymph) seeping from the incision was collected with a pipette. The drop of liquid (containing suspended spores) was placed onto an adhesive membrane and fixed in glutaraldehyde (2.5%) in cacodylate buffer (0.1 M). After 24 hours the spores were washed 4 times with distilled water (for 10 minutes each) then dehydrated by immersion for 15 min each in fresh solutions of ethanol 30%, 70%, 96%, and 3 x 100% and critical point dried. A muscle biopsy was also taken from the same individual and processed in the same way. Electron microscopy was conducted on a Phenom G2 pro (manufacturer: Phenom-World B.V.) scanning electron microscope.

3.3.3. Molecular diagnostics for microsporidian parasites

Molecular diagnostics were only conducted for microsporidian pathogens identified through histology. The anterior part of dissected amphipods were fixed in ethanol, and if histological analysis associated a microsporidian infection within the specimen it underwent DNA extraction using the EZ1 automated DNA tissue kit (Qiagen, UK). Amplification of the partial 18S gene of the microsporidian parasite was conducted using the MF1 (5'-CCGGAGAGGGAGCCTGAGA-3') and MR1 (5'-GACGGGCGGTGTGTACAAA-3') primers developed by Tourtip et al (2009). MF1/MR1 primers were used in a GoTaq flexi PCR reaction [1.25U/reaction of Taq polymerase, 1µM/reaction of each primer, 0.25mM/reaction of each dNTP, 2.5mM/reaction $MgCl_2$ and 2.5µl/reaction of DNA extract (10-30ng/µl)] in a 50µl volume. Thermocycler settings were: 94˚C (5 min); 94˚C-55˚C-72˚C (1 min per temperature) (40 cycles); 72˚C (10 min). Amplicons were visualised on a 2% agar gel using TAE buffer and 120V over 45 minutes. Any products were cut from the gel using a sterile scalpel. Those products were then frozen for a minimum of one hour, placed into a spin module and crushed against the side of the tube. The sample was spun at 13,000rpm and any liquid present after the centrifugation was made to 400µl using molecular grade water. This was placed into solution with Sodium acetate (5M) and 80% ethanol before being spun for a second time at full speed. Two further washes with 100% ethanol took place before pelleting the DNA and re-suspending in molecular grade water. The sample was diluted appropriately and sent for forward and reverse DNA sequencing using Eurofins (Eurofins Genomics, UK).

3.3.4. Statistical analyses

Amphipod symbiont data was recorded binomially, where the presence of a particular disease/commensal agent in an individual was allocated a score of '1' and a lack of the agent allocated a score of '0', irrelevant of the number of agents detected. Data from *D.*

villosus and *P. robustoides* collected throughout Poland was analysed using R version 3.2.1 (R Core Team, 2014), via Rstudio interface, to conduct the Marascuilo procedure to compare each population, which compares the prevalence of specific symbionts between sites and sample size. The Marascuilo procedure enables simultaneous testing of differences of all pairs of proportions when there are several populations under investigation. In this case, the Marascuilo procedure highlights significant differences ($P<0.05$) between populations, incorporating population size, and the prevalence of a given symbiont via a rapid Chi squared assessment process. This system is comparable to the application of many Chi squared assessments but instead allows rapid assessment of the entire dataset without applying Chi squared individually to each population and each symbiont. Statistical comparison of other amphipod populations was not feasible due to too few sample populations.

3.4. Results

The parasites, pathogens and commensals associated with the Polish Amphipoda cross a diverse array of taxonomic groups. Broadly, these break down into the Metazoa, Protista, Microsporidia, Prokaryota and viruses. Eleven host species were screened during this study (Table 3.1) and any organisms found to associate with each species are detailed in the relevant section below, according to their taxa (confirmed or predicted). The majority of sample sites harboured *P. robustoides* and *D. villosus* with high enough sample sizes to conduct a statistical comparison within each species, at each site, to compare pathogen prevalence.

3.4.1. Metazoan parasites of amphipod invaders

The amphipods carried metazoan parasites, identified through histological screening that were either acanthocephalans, trematodes, other helminths, rotifers, crustaceans, or of an undetermined taxonomy. Only *Gammarus tigrinus* was not identified with metazoan infections during the survey.

Acanthocephala were present in the following amphipod species and locations: *D. villosus* from the Bug River (1/18); *D. haemobaphes* from the Vistula River in Nieszawa (1/3); *Gammarus varsoviensis* from a stream in Poręba-Koceby (12/109); *G. roeselii* from Chonja (8/148); *G. fossarum* from Lagiewniki (3/140); and *G. pulex* from Chonja (1/48). In all cases the Acanthocephala held a *Polymorphus*-like anatomy (see Chapter 6: Fig. 3.1) and in rare cases were melanised by a host immune response.

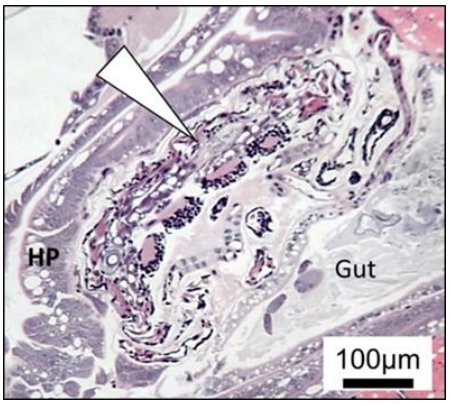
Trematodes were morphologically identified in *P. robustoides* from five of the sites (Table 3.2); *G. varsoviensis* from Poręba-Koceby (1/109); *O. crassus* from the Szczecin Lagoon in Kopice (5/133), and *G. roeselii* from Chonja (2/148). In all cases the trematodes encysted within the connective tissue of the body cavity and were surrounded by a proteinaceous, eosinophilic layer (Fig. 3.3).

Figure 3.3: Digenean trematodes from the connective tissues of *Pontogammarus robustoides* (white triangles). The centre of the cyst holds the parasite and the proteinaceous layer defends it from the host immune system. The specific species of these trematodes is unknown, and so is their lifecycle.

Helminth-like parasites were observed histologically in, or around, the body cavity of *D. villosus* from the Narew River in Pułtusk (1/50), *C. curvispinum* from the Vistula River at Nieszawa (1/33), and *G. pulex* from Chonja (4/48). In *D. villosus* and *G. pulex* the helminth was present in the body cavity, causing a displacement of the surrounding organs, however it did not elicit a histologically visible immune response. The helminth associated with *C. curvispinum* was present in the brood pouch of the host, around the eggs carried by a female of the species.

Rotifers were a common commensal association around the gills and appendages of *D. villosus* from several sites (Table 3.3), *D. haemobaphes* from Lucień Lake in Lucień (2/123), *P. robustoides* from several locations (Table 3.2), *G. varsoviensis* from Poręba-Koceby (62/109), *E. trichiatus* from the Szczecin Lagoon in Kopice (1/6), *G. fossarum* from the Bzura River in Łódź (Łagiewniki) (104/140), *G. pulex* from Chonja (10/48), and *G. roeselii* from Chonja (2/148).

Figure 3.4: An arthropod resembling an isopod (white triangle) was present in the body cavity of a *P. robustoides* with close association to the gut and hepatopancreas (HP).



						Pathogen prevalence determined by histology (%)										
						1	2	3	4	5	6	7	8	9	10	11
Collection site		Collection date	Species	Sex distribution (M/F/U)	n=	Fouling ciliates	Fouling rotifers	Gregarines	*P. robustoides* Bacilliform Virus	Putative HP cytoplasmic virus	Putative gut epithelia virus	Haemolymph protist	Microsporidia	Digenea	Bacterial infection	Isopod
A	Lucień Lake in Lucień	16/6/15	*P. robustoides*	65/117/29	211	58.3	6.2	40.8	38.4	0.0	0.0	0.0	8.0	0.5	0.0	0.0
B	Włocławski Reservoir (Vistula River) in Nowy Duninów	16/6/15	*P. robustoides*	106/159/52	318	81.4	3.5	35.5	11.0	0.0	0.0	0.0	7.5	0.9	0.0	0.0
C	Włocławski Reservoir (Vistula River) in Stary Duninów	16/6/15	*P. robustoides*	21/44/1	66	97.0	0.0	7.6	4.5	0.0	0.0	0.0	6.1	1.5	0.0	1.5
D	Vistula River in Nieszawa	16/6/15	*P. robustoides*	7/1/0	8	75.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E	Zegrznski Reservoir in Zegrze	21/6/15	*P. robustoides*	61/78/0	139	54.0	2.9	18.7	23.0	0.7	0.0	0.0	3.6	0.0	0.0	0.0
F	Oder in Gryfino	23/6/15	*P. robustoides*	45/59/18	122	51.6	1.6	56.6	9.0	4.9	0.0	3.3	4.1	6.6	0.0	0.0
G	Szczecin Lagoon in Kopice	23/6/15	*P. robustoides*	142/127/18	287	43.6	1.4	53.3	6.0	3.1	2.4	0.3	6.6	9.4	2.8	0.3

Table 3.2b		Collection site						
		A	B	C	D	E	F	G
Collection site	A							
	B	1, 4						
	C	1, 2, 3, 4	1, 3					
	D	3, 4, 8	3, 4, 8					
	E	3	1, 3	1, 4	3, 4			
	F	4	1, 3	1, 3,	3	3		
	G	4, 9	1, 3, 9	3	3, 4, 8, 9	3, 4, 9		

Table 3.2: *3.*2a) Prevalence percentages for each pathogen type associated with *P. robustoides* at each collection site. 3.2b) The significant differences between populations holding different proportional prevalence's of commensals, parasites and pathogens. Significant associations are listed in the table and any non-significant associations are not listed in the table. Significance is calculated at a threshold of <0.05 using the Marascuilo procedure. The Yates correction was applied to negate the presence of false positives.

An endoparasitic arthropod resembling a crustacean was present in *P. robustoides* from the Włocławski Reservoir (Vistula River) in Stary Duninów (1/66). The isopod was wrapped around the hepatopancreas of the host, present in the connective tissues (Fig. 3.4). Despite its large presence within the body cavity no observable immune responses were reacting to its presence. An isopod was also associated to *D. villosus* from Nieszawa, but on the outside of the animal (1/32).

The final metazoan association is of a currently undetermined ecto-parasite attached to the gills of *G. fossarum* from the Bzura River in Łódź (Łagiewniki), resembling a monogenean-like parasite. Several of the ecto-parasites were present on the gills of two infected individuals (2/140) (see Chapter 7: Fig. 3.3a).

3.4.2. Protist parasites of amphipod invaders

All amphipod species collected throughout Poland were associated with epibiotic ciliated protists and gut-dwelling gregarine parasites. Rare observations of an internal, haemolymph protist resembling a ciliated protist were observed in *G. roeselii*. Two amphipod species (*P. robustoides* and *G. varsoviensis*) were identified with a haemolymph infection displaying Haplosporidian-like parasites and pathological qualities.

Epibiotic ciliated protists appeared commensal to the host amphipods and were either attached to the gills or carapace (see Chapter 6: Fig. 6.1a, b; and Chapter 7: Fig. 7.2a, b) of their host without inciting any visible immune response. The diversity of species composing the ciliated protists upon each species is unknown, however some distinct morphotypes could be defined, including stalked and amorphous varieties. Their prevalence varied between different species: *D. villosus* (Table 3.3); *D. haemobaphes* from Lucień Lake and Vistula River (100/123 and 3/3 respectively); *P. robustoides* (Table 3.2); *C. curvispinum* (6/37); *G. varsoviensis* (68/109); *O. crassus* (39/133); *G. tigrinus* (14/15); *E. trichiatus* from the Oder and Szeczecin lagoon (45/47 and 5/6 respectively); *G. roeselii* (124/148); *G. fossarum* (115/140); and *G. pulex* (40/48). Their prevalence was seen to be significantly ($P<0.05$) different between some populations for *P. robustoides* and *D. villosus* (Table 3.2; Table 3.3). A ciliated protist circulating the haemolymph of a *G. roeselii* (1/148) is described in greater histological detail in Chapter 6.

Table 3.3a						Pathogen prevalence determined by histology (%)							
						1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Collection site		Collection date	Species	Sex distribution (M/F/U)	n=	Fouling ciliates	Fouling rotifers	Gregarines	*D. villosus* Bacilliform Virus	*Cucumispora dikerogammari*	Helminth	Acanthocephala	Isopod
A	Włocławski Reservoir (Vistula River) in Stary Duninów	16/06/2015	*D. villosus*	19/7/1	27	100.0	11.1	14.8	0.0	7.4	0.0	0.0	0.0
B	Vistula River in Nieszawa	16/06/2015	*D. villosus*	18/14/0	32	43.8	6.25	34.4	0.0	15.6	0.0	0.0	3.1
C	Narew River in Pułtusk	21/06/2015	*D. villosus*	41/19/8	68	89.9	2.9	30.9	1.5	42.6	1.5	1.5	0.0
D	Oder in Porzecze	23/06/2015	*D. villosus*	9/0/4	13	61.5	38.5	38.5	0.0	15.4	0.0	0.0	0.0

Table 3b		Collection site			
		A	B	C	D
Collection site	A				
	B	1			
	C	1	1		
	D				

Table 3.3: 3.3a) Prevalence percentages for each pathogen type associated with *D. villosus* at each collection site. 3.3b) The significant differences between populations holding different proportional prevalence's of commensals, parasites and pathogens. Significant associations are listed in the table and any non-significant associations are not listed in the table. Significance is calculated at a threshold of <0.05 using the Marascuilo procedure. The Yates correction was applied to negate the presence of false positives.

Gregarine parasitism (Apicomplexa) was also observed in all the host amphipod species, the parasites being present primarily in the gut lumen of the host (see Chapter 6: Fig. 6.1e, b; and Chapter 7: Fig. 7.2a, b) and occasionally in the hepatopancreas, without visible immune reactions. Several different morphologies of gregarine were observed but no specific characteristics could be used as taxonomic identifiers via histological screening, resulting in an overall prevalence for gregarine infection: *D. villosus* (Table 3.3); *D. haemobaphes* from Lucień Lake and Vistula River (20/123 and 2/3 respectively); *P. robustoides* (Table 3.2); *C. curvispinum* (9/37); *G. varsoviensis* (59/109); *O. crassus* (55/133); *G. tigrinus* (1/15); *E. trichiatus* from the Oder and Szczecin lagoon (15/47 and 3/6 respectively); *G. roeselii* (73/148); *G. fossarum* (23/140); and *G. pulex* (7/48). Their prevalence was significantly ($P<0.05$) different between some populations for *P. robustoides* and *D. villosus* (Table 3.2; Table 3.3), which could be assessed due to adequate sample size from several locations.

The protist parasites circulating the haemolymph of *P. robustoides* from the Oder River (4/122) and Szczecin Lagoon (1/287), and those from *G. varsoviensis* collected from Poręba-Koceby (1/109), had similar morphologies and pathologies (Fig. 3.5). The pathology was restricted to the hosts haemolymph, where multi-nucleated plasmodia could be seen circulating the blood stream. In the gill tissue of *P. robustoides*, fewer plasmodia were present and instead smaller micro-cells/spores could be identified circulating the blood stream. The protist lifecycle includes some life stages that show similarity to the Haplosporidia, such as the multi-nucleate life-stage, however a typical haplosporidian spore could not be determined from either host. The parasite has a multi-nucleate life stage as well as monokaryotic and diplokaryotic life stages, but further life stages could not be identified due to the limited quality of re-processed wax-embedded tissue for TEM. Some melanisation reactions could be seen to target the infection in *P. robustoides*, however no melanisation reactions or visible immune reactions were present in histological section for *G. varsoviensis*.

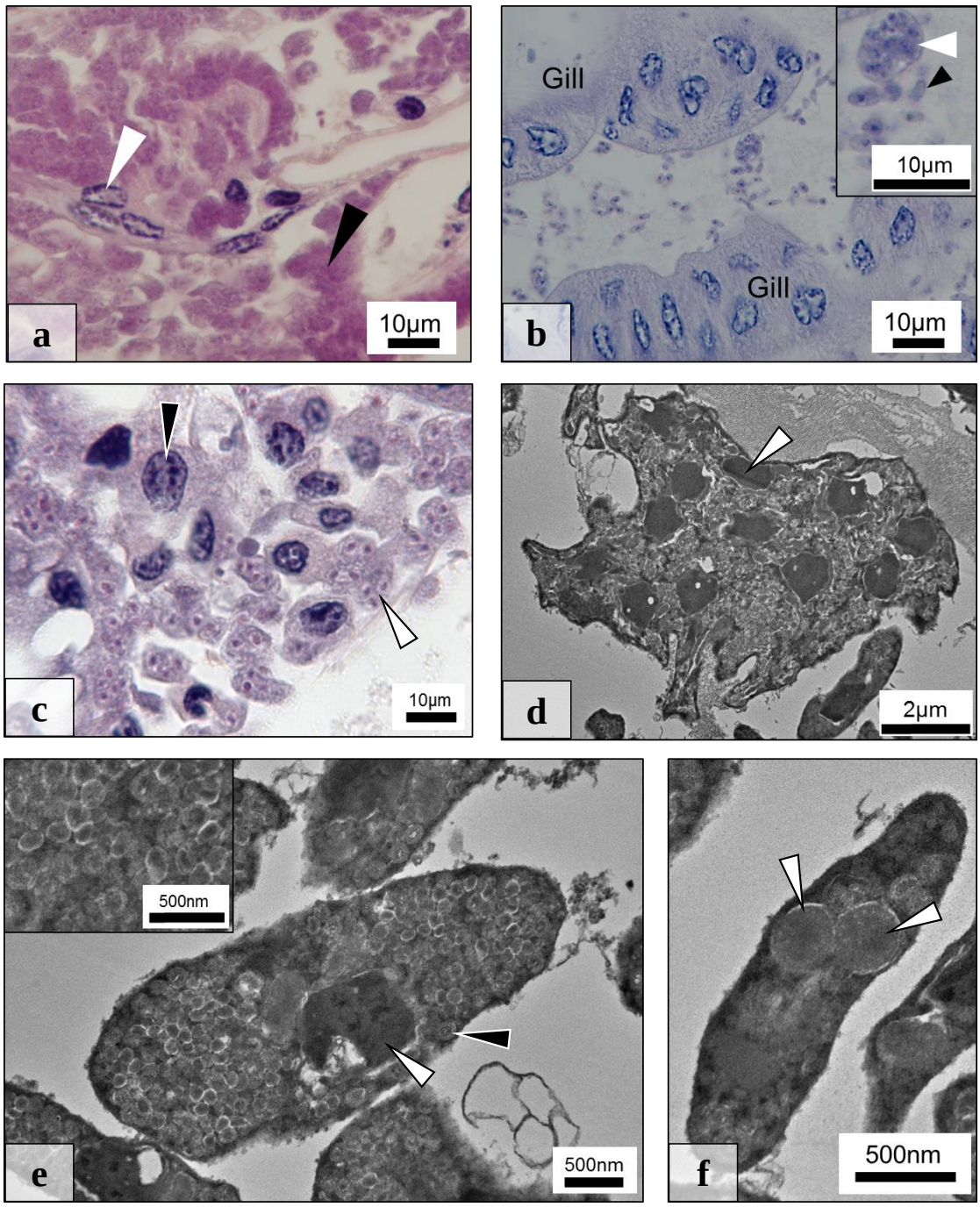


Figure 3.5: Haplosporidian-like parasites in the haemolymph of *P. robustoides*. a) Masses of eosinophilic plasmodia (black triangle) can be seen within the haemolymph of *P. robustoides* from the Oder River, and are closely connected to the host heart tissue (white triangle). b) In the gill lumen of the host the plasmodia appear to contain a multitude of spores (inset: white and black triangles), several of which are free in the gill haemolymph. c) A similar infection from the Szczecin Lagoon shows a marginally different infection with lower plasmodial (white triangle) density in the haemolymph, along with host haemocytes (black triangle). d) A TEM image from previously wax-embedded material identifies multi-nucleate (white triangle) plasmodia. e and f) Single protists contain 1-2 nuclei and a cytoplasm rich in a granular structure (black triangle) (e: inset).

3.4.3. Microsporidian parasites of amphipod invaders

Microsporidian pathogens infecting one or several of the host tissues (the musculature, gonad, connective tissues and hepatopancreas) were observed from several host species surveyed during the study. In addition, hyperparasitism of gregarines with microsporidian infections were identified from histological section for *P. robustoides* and *D. haemobaphes*.

Microsporidia infecting the musculature and connective tissues were observed in *Dikerogammarus villosus*, *D. haemobaphes*, *P. robustoides*, *G. varsoviensis*, *O. crassus*, *G. roeselii*, *G. fossarum* and *G. pulex.* The microsporidian infecting *D. villosus* at several of the invasion sites displayed similarity to *Cucumispora dikerogammari* (Table 3.3). The prevalence of *C. dikerogammari* at each of the collection sites did not differ significantly (Table 3.3). The microsporidian observed in *D. haemobaphes* is also present in the UK and is taxonomically described in Chapter 5 as a novel member of the *Cucumispora*. In Poland, this parasite was present in 32/123 individuals collected from Lucień Lake, but was not present in the Vistula River population sampled at Nieszawa. One individual collected from the Vistula River in Janowiec displayed a heavy infection and was taken for SEM analysis (Fig. 3.6).

Several microsporidian infections were detected via histology in the musculature of *P. robustoides*. One was observed to have an octosporous lifecycle via histology (Fig. 3.7), however greater detail is needed to identify this species. A second appeared to have a tetrasporous development stage. A third was ambiguous in histological section. In all cases a small number of melanisation reactions were visible for some infected hosts. The inability to confidently determine which microsporidian species is causing the infection via histology has resulted in a summed prevalence for each location (Table 3.2).

Microsporidia displaying octosporous development stages were found in 3/109 specimens and other microsporidia displaying an indeterminate pathway, via histology, were observed to infect the musculature of 7/109 *G. varsoviensis*. Microsporidian infections of the musculature were also observed from 6/133 *O. crassus*, 11/140 *G. fossarum* and 11/48 *G. pulex*. A single *G. pulex* had accompanying material fixed for molecular diagnostics, which provided a 414bp sequence and identified the microsporidian infection to be *Dictyocoela duebenum* (accession: KR871363; similarity: 99%; coverage: 100%; e-value = 0.0).

A microsporidian infection noted via histology from *G. roeselii* had accompanying tissues fixed for molecular and TEM analysis, and is taxonomically described in Chapter 6 as the third formal member of the *Cucumispora*.

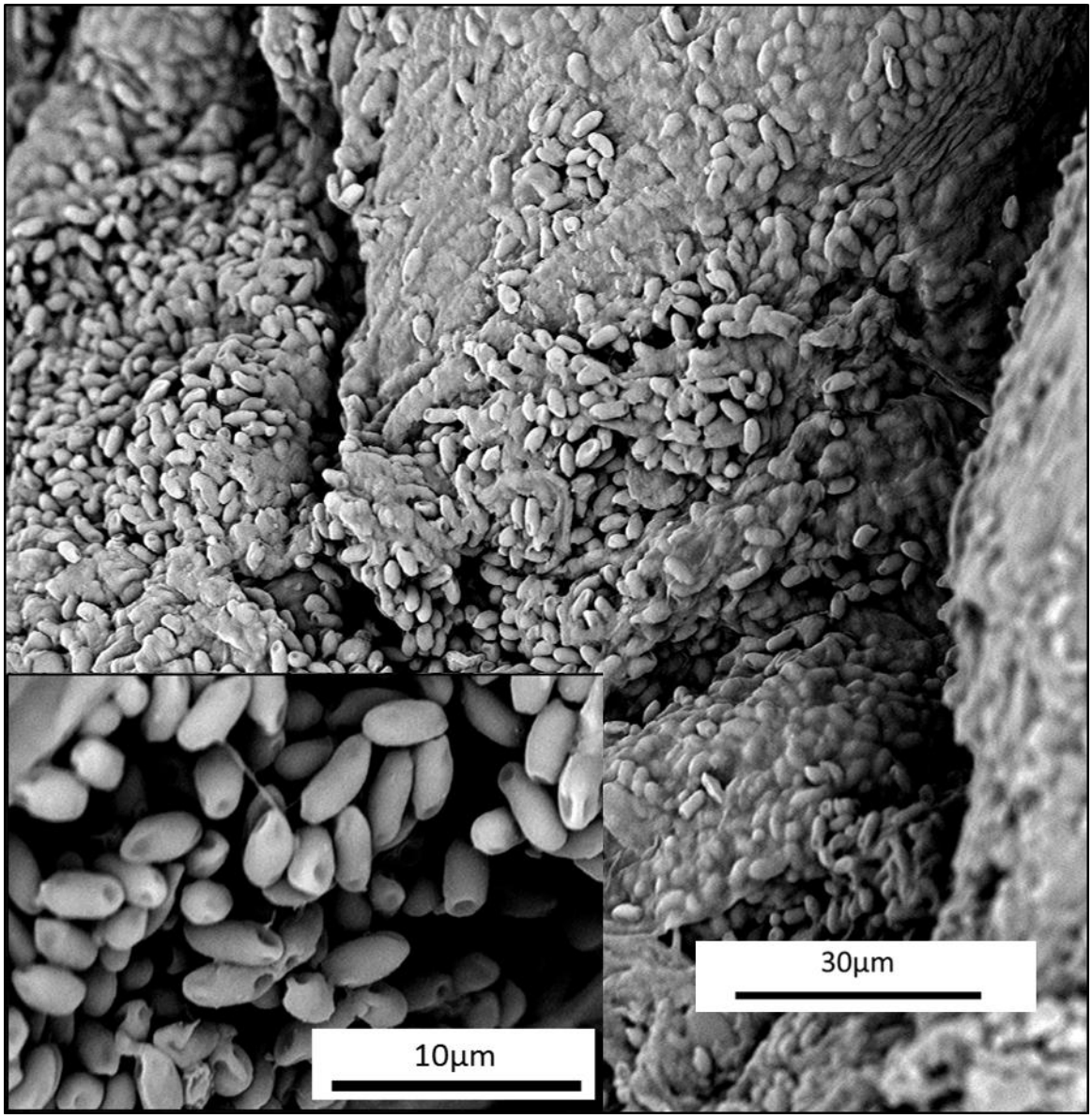


Figure 3.6: A scanning electron micrograph of a microsporidian infection (white arrow) of *D. haemobaphes*. The inset image is a 700X magnification of the microsporidian spores

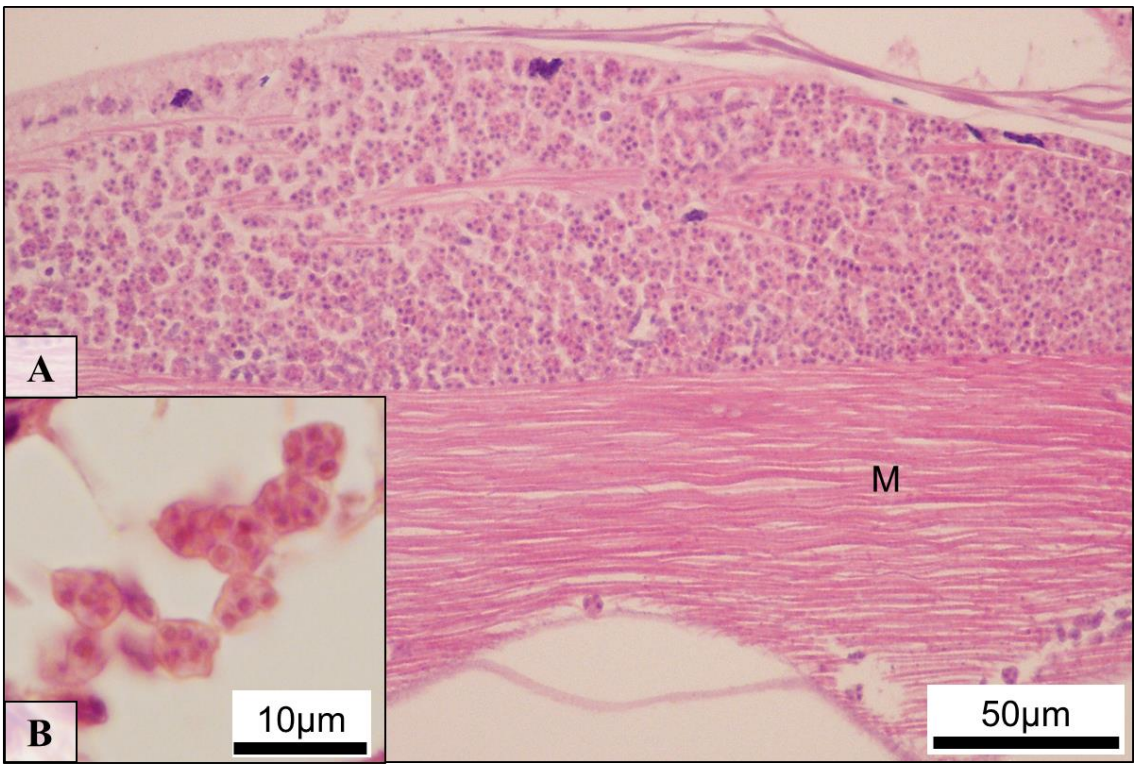


Figure 3.7: Histological observation of a microsporidian infection of *P. robustoides*. a) The infection is restricted to the musculature, specifically around the muscle (M) fibres and sarcolemma. b) High magnification reveals that a part of the development cycle for this parasite involves an octosporous life stage.

A microsporidian infection from *E. trichiatus* (4/47) was limited to colonisation of the connective tissues between the carapace and musculature of the host. The infection was observed in 4/47 specimens collected from the Oder River in Gryfino. This infection did not appear to elicit a visible immune response from the host. A second infection in this species was restricted to the cytoplasm within the oocytes of a single female (1/47) collected from the Oder River in Gryfino. No link can be made between these two microsporidian observations with current data. *Gammarus tigrinus* was also observed with a microsporidian infection restricted to the oocytes of the host (1/15) from the Oder in Gryfino. In each case the pathology was the same.

Microsporidia infecting the hepatopancreas of their host were identified from *G. varsoviensis* (1/109), *G. roeselii* (1/148), and *G. pulex* (4/48). In all cases the microsporidian life-stages were present in the cytoplasm of the hepatopancreatocyte (Chapter 6: Fig. 6.1j), and were not visibly targeted by any immune reaction.

The gregarine parasites of a single *D. haemobaphes* from Lucień Lake were infected with a putative microsporidian pathogen. Gregarines infecting *P. robustoides* from the Szczecin Lagoon in Kopice (6/287) and the Zegrznski Reservoir in Zegrze (5/139) also displayed microsporidian-like inclusions in their cytoplasm (Fig. 3.8).

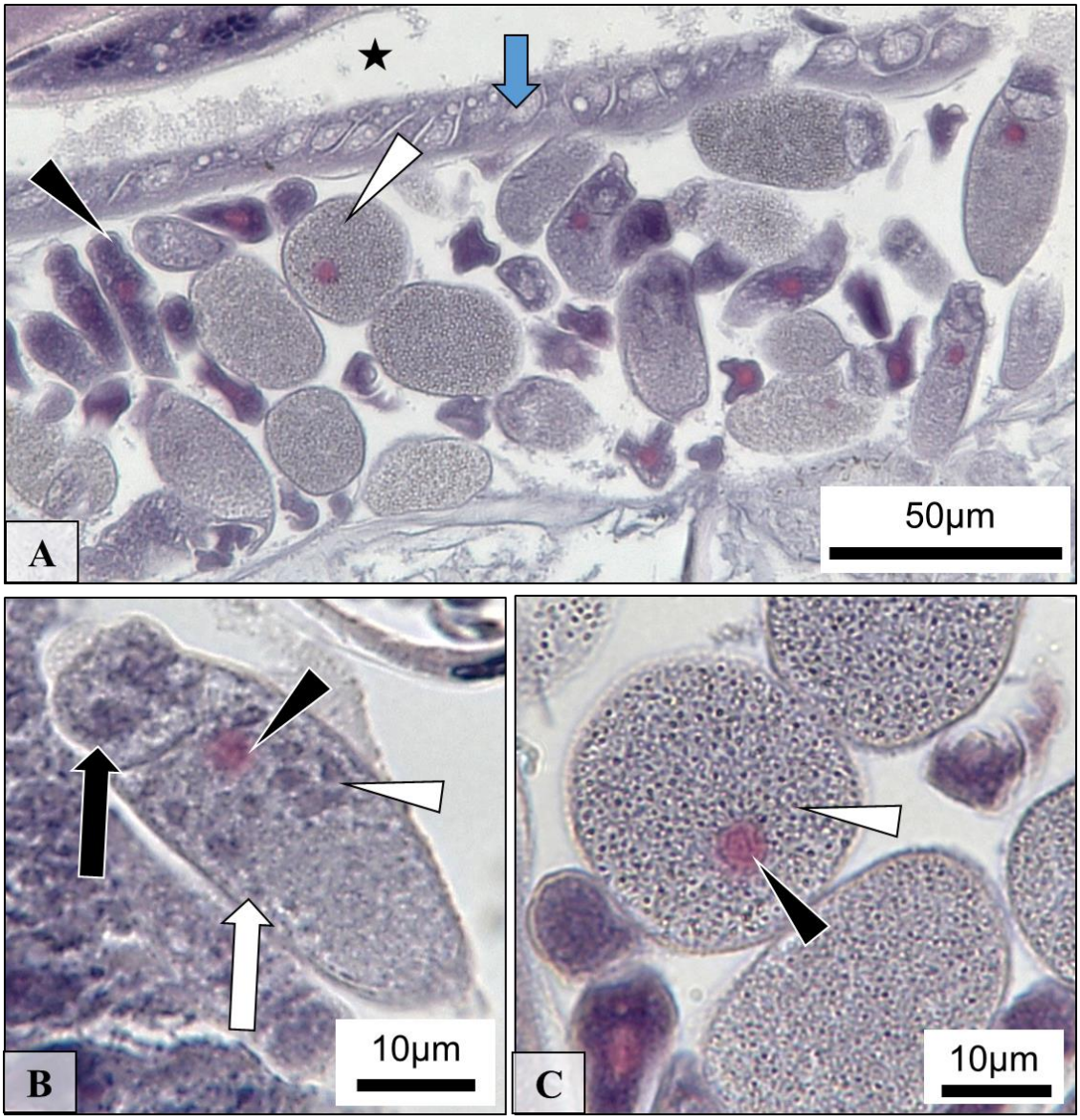


Figure 3.8: Microsporidian-like inclusions within the cytoplasm of gregarine parasites in the gut lumen of *P. robustoides*. a) Gregarine parasites (black triangle) lined up against the gut epithelia (blue arrow). The white triangle indicates one of the microsporidian-like infections in a gregarine. The black star indicates where the gut epithelia have moved away from the basal membrane. b) A gregarine displaying putative early development stages of infection (white triangle) in the epimerite (black arrow) and deuteromerite (white arrow). The black arrow indicates the host gregarines nucleus. c) Heavy putative infections result in the gregarine becoming enlarged and full of spores (white arrow).

3.4.4 Bacterial pathogens of amphipod invaders

Filamentous bacteria were common on the gills, carapace and appendages of all hosts, and were present upon all of the individuals screened. Bacterial infections of the haemolymph were observed from *P. robustoides* (Table 3.2), and *O. crassus* from the Szczecin Lagoon in Kopice (1/133). A rickettsia-like organism (RLO) targeting the haemocytes, musculature, gill and gonad was observed to infect *G. fossarum* (48/140)

and *G. varsoviensis* (17/109). RLO infections of the hepatopancreatic cell cytoplasm were observed from *D. haemobaphes* from Lucień Lake (21/123), *C. curvispinum* (4/33), *G. tigrinus* (3/15), *G. roeselii* (1/148), *G. fossarum* (22/140) and *G. pulex* (1/48).

Rod-shaped bacteria were free in the haemolymph of *P. robustoides* and *O. crassus*, often at high concentration in the heart (Fig. 3.9). The bacterial infection appeared to colonise the haemolymph and was targeted by haemocyte aggregations and melanisation reactions throughout the amphipods circulatory system (Fig. 3.9).

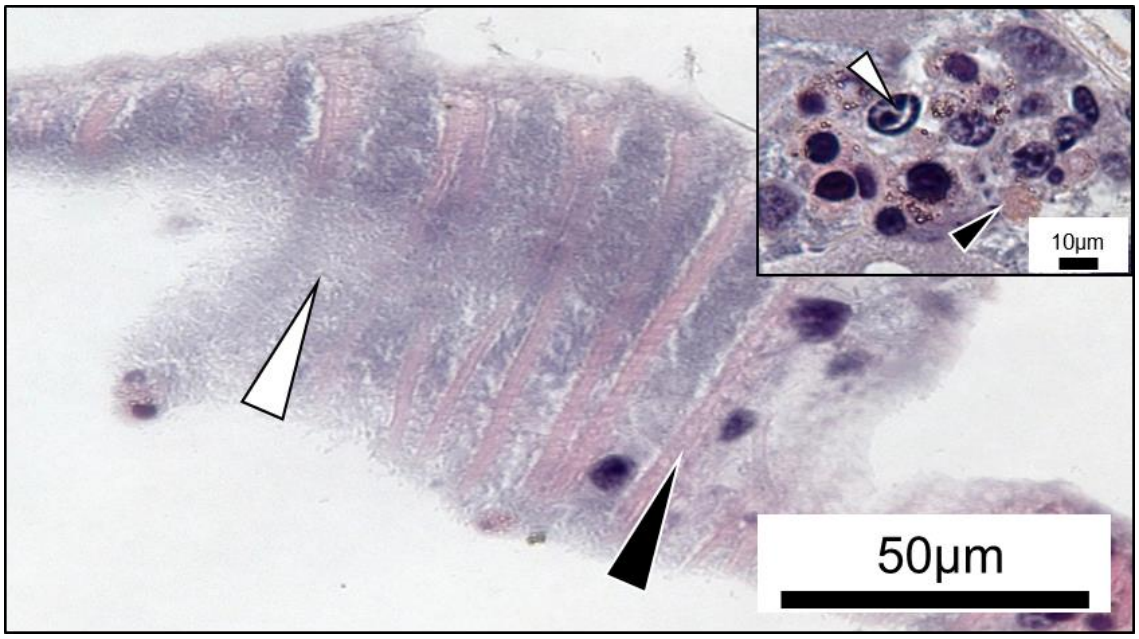


Figure 3.9: Bacilli in the blood stream of *P. robustoides*. The white arrow in the main image identifies the purple-staining bacterial infection. The black arrow in the main image indicates the myocardium of the host. The inset identifies a common melanisation reaction (black arrow) observed throughout the host, caused by the aggregation of haemocytes (white arrow).

An RLO infection within the cells of the haemolymph, musculature, gill and gonad was observed to infect *G. fossarum* (48/140) and *G. varsoviensis* (17/109). The pathogen infecting *G. fossarum* is taxonomically identified in Chapter 7 to belong to the novel genus, *Aquarickettsiella*. The infection within *G. varsoviensis* was pathologically similar to that observed in *G. fossarum*, however appropriately fixed materials were not available to identify the pathogen taxonomically. Wax embedded material was re-processed to produce TEM images of the infection, and identified it to be highly similar to that seen in *G. fossarum* (bacterial; *Aquarickettsiella*-like lifecycle; no proteinaceous fibres in the spherical body stage; highly condensed elementary bodies) (Fig. 3.10).

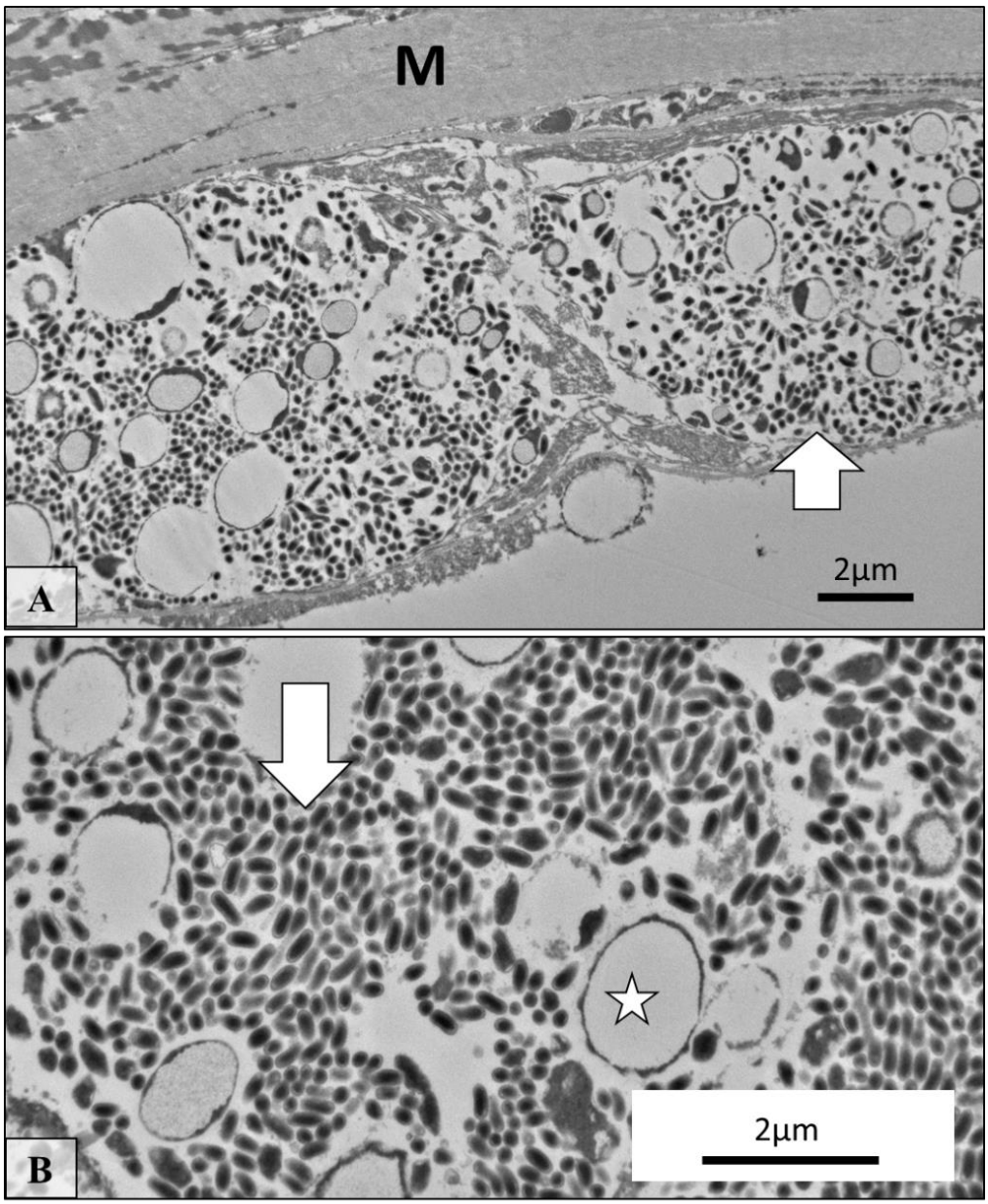


Figure 3.10: *Aquarickettsiella*-like bacterial infection from the muscle and haemocytes of *G. varsoviensis*. a) The muscle (M) sarcolemma is filled with developing bacteria (white arrow). b) The spherical bodies (white star) do not contain proteinaceous fibres. The white arrow indicates the condensed elementary bodies in the cytoplasm of an infected haemocyte.

RLOs from the cytoplasm of hepatopancreatocytes were histologically identified from six of the amphipod species and one was confirmed from *G. fossarum* using TEM (Chapter 7: Fig. 7.4). DNA sequence data could not be attained to taxonomically identify this hepatopancreatic RLO, however the TEM data revealed that the lifecycle and pathology of the bacterium was similar to the *Rhabdochlamydia* (Kostanjsek et al. 2004). Until greater detail is known about the other RLO infections of the hepatopancreas (e.g. TEM

and DNA sequence data) in the amphipod hosts, further taxonomic links cannot be made.

3.4.5. Viral pathogens of amphipod invaders

The amphipods sampled during the study were shown to be infected with a range of viral-like pathogens, termed herein as 'putative' unless TEM data is provided. The viruses identified cover bacilliform viruses confirmed from five different amphipod species and putative infections from the gut epithelia of five amphipods; from the cytoplasm of the hepatopancreatocytes of two amphipods; and a TEM image of a putative RNA virus in the hepatopancreas of *G. fossarum*.

Four bacilliform viruses were morphologically identified using histology and TEM from *D. haemobaphes* from Lucień Lake (18/123) (UK invasive virus presented in Chapters 8 and 10), *P. robustoides* (Table 3.2), *G. varsoviensis* from Poręba-Koceby (5/109); and *G. roeselii* (described in Chapter 6) (Fig. 3.11). A viral pathology was also observed from *G. pulex* but could not be followed up with TEM and remains putative for a bacilliform virus. DvBV was identified histologically from *D. villosus* (Table 3.3) in this study from comparisons with previously described histological data from Polish invasion sites (Bojko et al. 2013). The bacilliform virus from *P. robustoides,* termed *Pontogammarus robustoides* Bacilliform Virus (PrBV), is a novel discovery, measuring 37.5 ± 5.7nm core width and 166.4 ± 20.6nm core length, and 72.7 ± 8.0nm virion width and 217.8 ± 25.3nm virion length (Fig. 3.11). The viral pathology involves a growing pink staining viroplasm within the nuclei of hepatopancreatocytes, causing nuclear hypertrophy (Fig. 3.11). No immune responses were observed against the presence of the virus. The bacilliform virus from *G. varsoviensis* is termed *Gammarus varsoviensis* Bacilliform Virus (GvBV) and is also a novel discovery, measuring 35.6 ± 4.0nm core width and 161.5 ±14.0nm core length, and 60.6 ± 9.0nm virion width and 215.0 ± 12.0nm virion length (Fig. 3.11). The viral pathology involved a red-staining, growing viroplasm within the nuclei of hepatopancreatocytes, causing nuclear hypertrophy. No immune responses were observed against the presence of the virus.

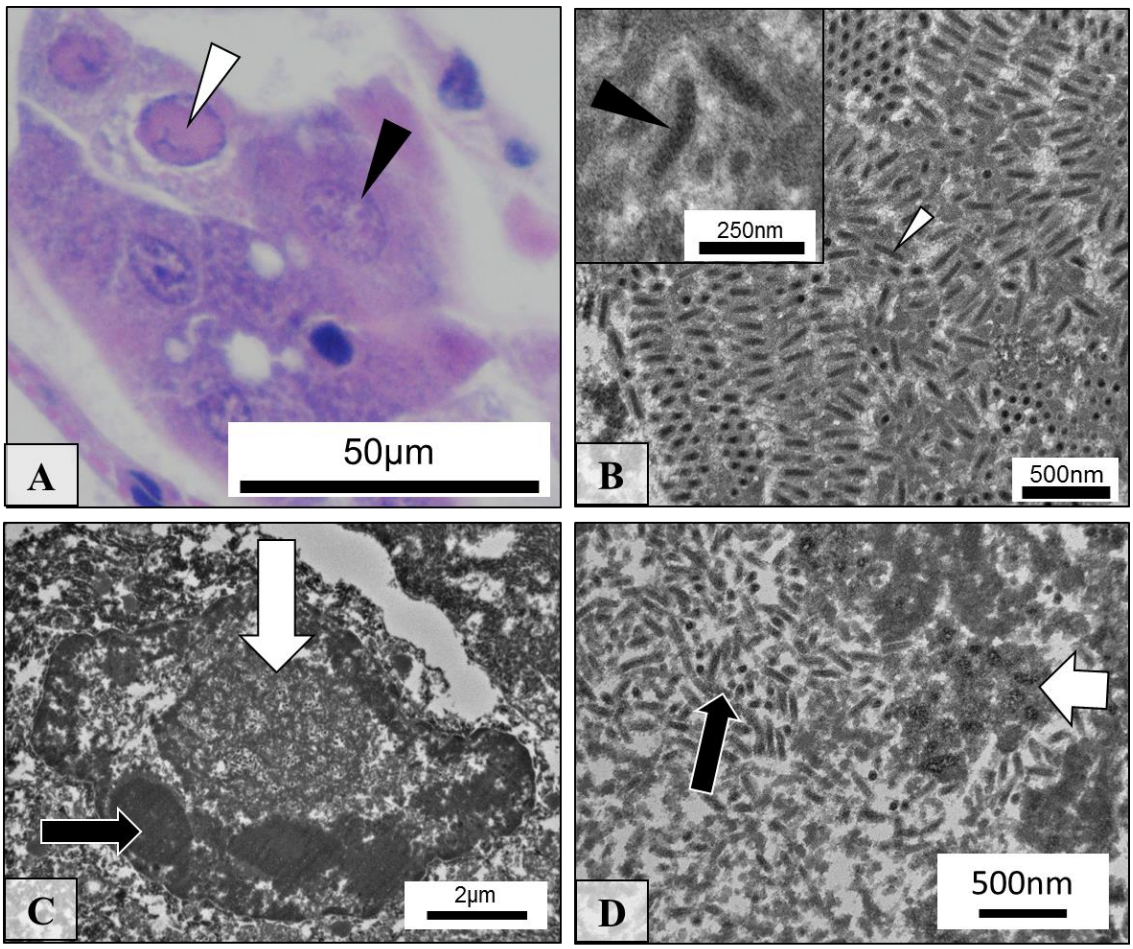


Figure 3.11: Bacilliform virus pathology and morphology in *P. robustoides* (PrBV) and *G. varsoviensis* (GvBV). a) A pink-staining viroplasm (white triangle) is growing within the nuclei of hepatopancreatocytes. An infected nucleus is shown (black triangle). b) TEM image of PrBV (white and black triangles). c) A TEM image from wax embedded material of an infected nucleus from *G. varsoviensis*, showing the growing central viroplasm (white arrow) and the condensed host chromatin (black arrow). d) A high magnification TEM image of the GvBV virions (black arrow) and free chromatin, likely the viral formation machinery (white arrow).

Four amphipods were identified with putative gut epithelial viruses, identified based on the presence of a growing viroplasm in the nuclei of gut epithelial cells in histological section. TEM images are yet to be obtained to confirm any of these viral pathologies morphologically. *Dikerogammarus haemobaphes* from Lucień Lake (14/123) contained hypertrophic nuclei in their gut epithelial cells, which did not appear to result in any host immune response. *Gammarus roeselii* (4/148) were identified with a similar pathology explored further in Chapter 6. *Gammarus fossarum* (3/140) were also identified with a putative gut epithelial virus, displaying the same pathological characteristics as stated above and described further in Chapter 7. *Pontogammarus robustoides* from the Szczecin Lagoon in Kopice (7/287) were identified with hypertrophic nuclei in their gut epithelial cells, which could be a growing viroplasm (Fig. 3.12).

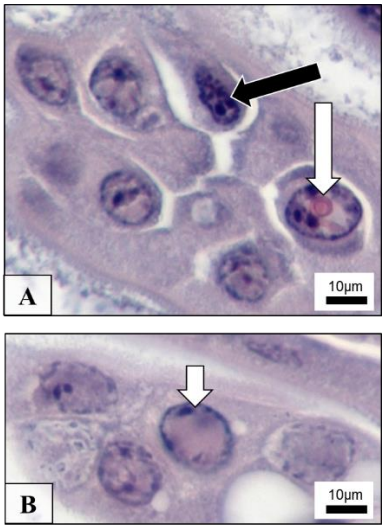


Figure 3.12: Gut epithelial cells of *P. robustoides* displaying hypertrophic nuclei with evidence of a viroplasm. a) The white arrow indicates a putative growing viroplasm within the nucleus of a gut epithelial cell from the mid-gut of *P. robustoides*. The black arrow indicates an uninfected nucleus. b) This image identifies a translucent/opaque inclusion which may also be linked to this infection.

Viral-like pathologies were also observed via histology in the hepatopancreas of *P. robustoides* (Table 3.2) and *G. varsoviensis* from Poręba-Koceby (4/109). A TEM image was obtained from *G. fossarum* which identifies a viral pathology from the cytoplasm of hepatopancreatocytes (Chapter 7: Fig. 7.5). However, the histology for the specimen did not display the same pathology noted for other putative hepatopancreas cytoplasm viruses (Chapter 7: Fig. 7.5a). Putative hepatopancreas cytoplasm viruses produced large pink/purple staining inclusions that could be both within the cytoplasm of the infected cell or span across several cells of the hepatopancreas (Fig. 3.13). In all cases the pathology did not seem to incite any detectable immune response from the host.

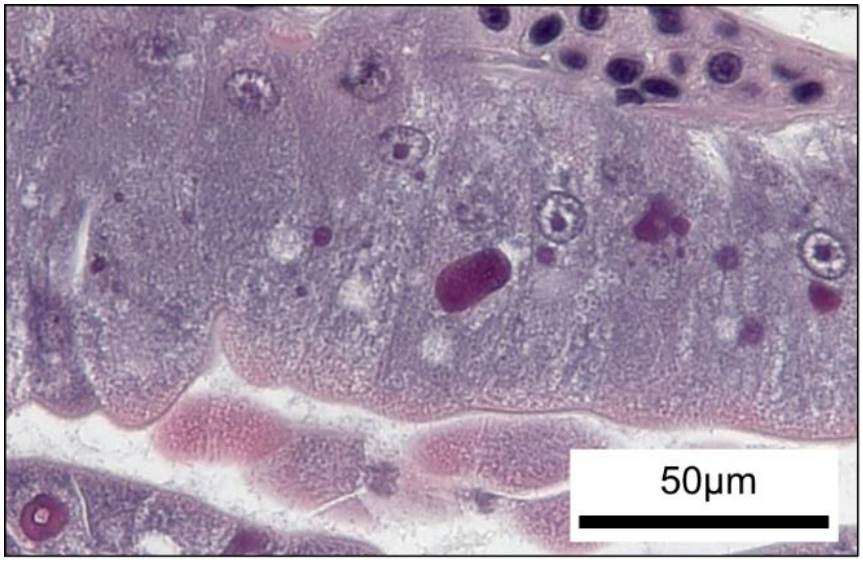


Figure 3.13: A putative pathology possibly relating to a viral pathology in the cytoplasm of the hepatopancreatocytes of *P. robustoides*. Deep purple staining inclusions (white arrow) can be seen across the cells with an unknown composition.

3.5. Discussion

INNS have complex relationships with their parasites and pathogens, which can be lost through enemy release (Colautti et al. 2004), be used as biological weapons to facilitate invasion and infect native species (Strauss et al. 2012), or could control the invaders impacts via biological control (Chapter 9). For amphipods, numerous pathogen groups have been associated to their invasion, including: viruses (Bojko et al. 2013); bacteria (Bojko et al. 2013); Protozoa (Ovcharenko et al. 2009); Microsporidia (Ovcharenko et al. 2009); Digenea (Bojko et al. 2013); and Acanthocephala (Bojko et al. 2013).

Here, I identify the pathogens and parasites in several species of Amphipoda. These newly identified associations belong to the Metazoa, Protozoa, Microsporidia, Prokaryota or viruses. Each group has members that could be used for biological control purposes, or include example species that have succeeded in infecting vulnerable native species.

3.5.1. Invasion routes for amphipods and their pathogens toward the UK

Dikerogammarus villosus, *D. haemobaphes* and *C. curvispinum* are all invaders present in the UK, each with a different invasion story. *Chelicorophium curvispinum* is thought to have invaded the UK in 1935 but has been linked with little ecological change and has been termed a low-impact non-native species in its UK range (Gallardo and Aldridge, 2015; EASIN). Knowledge of its pathogen complement during invasion, and within its native range, is little known (Chapter 1: Appendix Table 1.3). Other species, such as *D. villosus* and *D. haemobaphes* have had a great deal of parasitological study and are attributed to have undergone enemy release (Bojko et al. 2013; Fig. 3.14).

Dikerogammarus villosus was first reported in the UK in 2010 at Grafham Water, Cambridgeshire (MacNeil et al. 2010). Wattier et al (2007) found that *D. villosus* maintained their genetic diversity and parasitic diversity in their early invasion of Eastern Europe. This suggests a pattern of recurrent introductions, as opposed to single, infrequent invasive propagules. The alternative was detected in the UK by Bojko et al (2013) and Arundell et al (2015), who show a reduction in host genetic diversity in comparison to reference populations from the west coast of continental Europe, and that no co-evolved microsporidian parasites were detected through histological or molecular diagnostic methods, suggesting enemy release.

Populations of *D. villosus* in the UK were histologically screened and found to carry commensal microbes, such as: epibiotic ciliated protists; gregarines; bryozoans; helminths and isopods (Bojko et al. 2013). Histological screening of *D. villosus* from continental Europe detected the presence of viral, microsporidian and acanthocephalan

parasites that had not been carried into the UK (Bojko et al. 2013). This study adds fouling rotifers to this system. In one instance a microsporidian was histologically detected in the Grafham Water population (UK) (annual prevalence: 1/1937) but this observation included a morphology and lifecycle unlike any currently associated with this species, suggesting an acquisition from the invasion site. In conclusion, *D. villosus* is thought to have invaded the UK via small propagules and to have left many of its pathogens behind via enemy release (Fig. 3.14).

The Ponto-Caspian invader, *D. haemobaphes*, was identified in the UK in 2012 and has carried with it a microsporidian pathogen also observed during this study, and is taxonomically described in Chapter 5. Genetic isolates of this microsporidian have been identified from German and Polish populations of *D. haemobaphes* (Garbner et al. 2015; NCBI, BLAST), suggesting it is an invader in the UK along with its host. Further screening has identified gregarines, digeneans, microsporidia and viruses in UK *D. haemobaphes* populations (Chapter 9). In addition to these pathogens, this study has identified: epibiotic ciliated protists; rotifers; gregarines; bacteria and viruses, which could invade the UK alongside their host. In conclusion, *D. haemobaphes* also appears to have undergone enemy release when travelling into the UK, however it has lost fewer pathogen groups relative to *D. villosus*.

A diagrammatic breakdown of pathogens and parasites travelling with their hosts suggests enemy release has occurred to some extent in both amphipods; more significantly for *D. villosus* and less so for *D. haemobaphes* (Fig. 3.14).

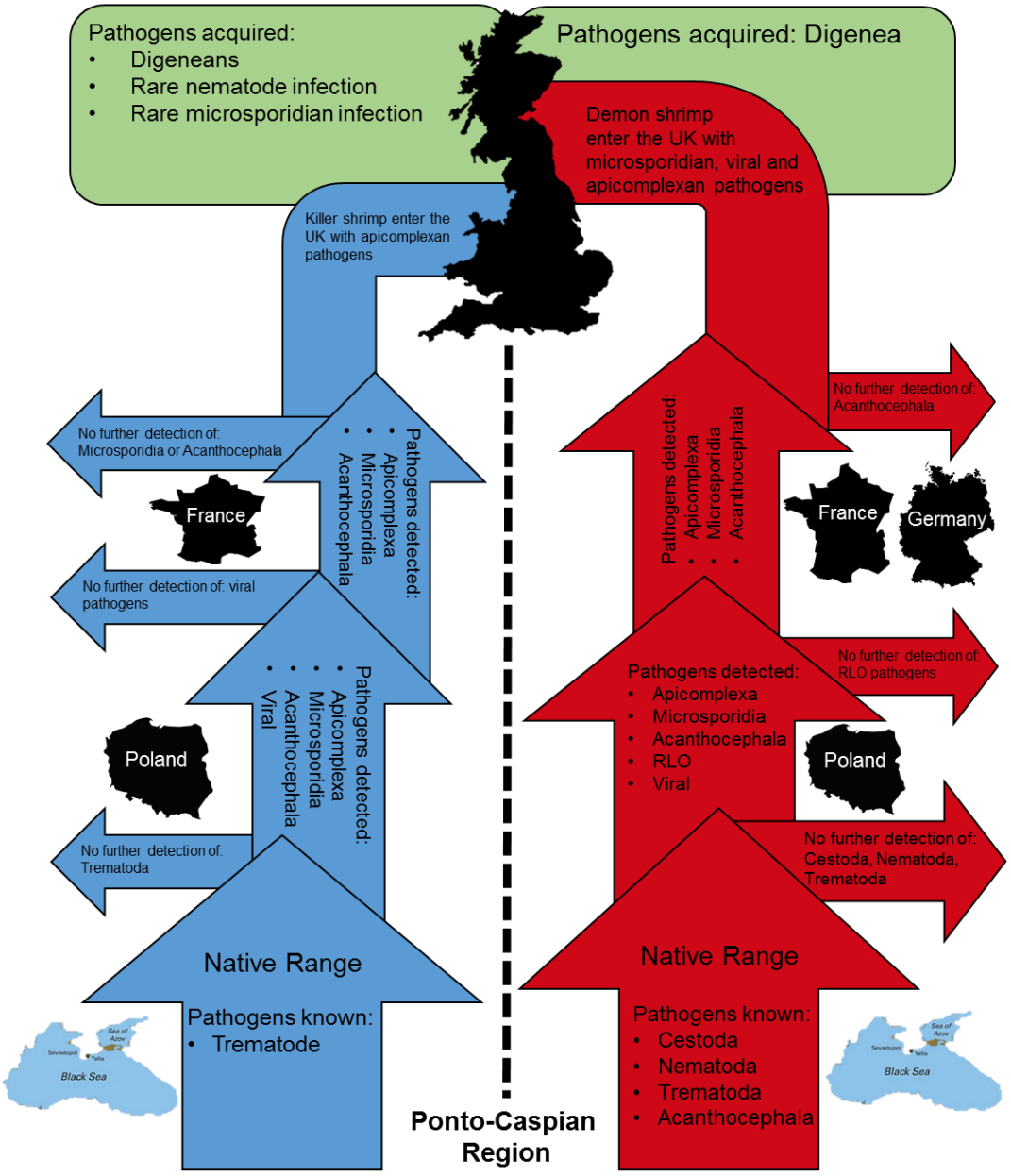


Figure 3.14: Invasion history of *D. villosus* and *D. haemobaphes* from the perspective of their pathogens and enemy release, as they move from the Black Sea (Rewicz et al. 2015), through Europe, via no specific route, to enter the UK. Only parasites and pathogens are accounted for in the diagram, not commensal or symbiotic species. The horizontal arrows indicate where pathogenic species have been lost and the vertical arrows indicate the movement of the invader. The history of each host and their parasitic profile along their invasion pathway is detailed on the left/blue for *D. villosus* and right/red for *D. haemobaphes*. Pathogens that appear to be acquired from the UK are detailed in the green boxes. Based on current pathogen profiling efforts it appears that *D. villosus* has undergone enemy release, leaving behind almost all known pathogens during its invasion of the UK (Wattier et al. 2007; Ovcharenko et al. 2009; Ovcharenko et al. 2010; Wilkinson et al. 2011; Bojko et al. 2013; Arundell et al. 2015). Non-native *D. haemobaphes* have carried its viral and microsporidian pathogens to the UK (Komarova et al. 1969; Bauer et al. 2002; Ovcharenko et al. 2009; Đikanovic et al. 2010; Kirin et al. 2013; Green-Extabe et al. 2015). Absence of evidence is not evidence of absence, however, even if parasites are present at low levels the effects may be relatively minimal.

3.5.2. Other invasive amphipods and their invasive pathogens

During the survey I also screened *E. trichiatus*, *O. crassus* and *P. robustoides*; all of which are from the Ponto-Caspian region and possible future invaders of the UK (Roy et al. 2014a) and have now been identified with several pathogen groups that may co-invade to reach UK freshwaters. *Echinogammarus trichiatus* were identified with epibiotic ciliated protists, rotifers, gregarines, and microsporidia infecting the oocytes and connective tissues. These groups may pose little threat to native fauna because they have not been associated with mortality in amphipods, and have a more commensal lifestyle (Bojko et al. 2013). Microsporidia that infect the oocytes of their host have been linked with vertical transmission, and may belong to the *Dictyocoela* (Terry et al. 2004). Alternatively, microsporidia have been identified to infect both the gonad and connective tissues of their host, such as *Areospora rohanae*; a pathogen of the king crab, *Lithodes santolla* (Stentiford et al. 2014) and *Agmasoma penaeii* a pathogen of the pacific white shrimp, *Litopenaeus setiferus* (Sokolova et al. 2015); such pathogens may pose a greater threat.

The pathogens associated with *O. crassus* that pose the greatest threat to native wildlife include the microsporidia and digenean trematodes. Digenea have a complex lifecycle, which may hinder their ability to invade novel areas, however if alternative host species are present in the new environment the native fauna could face infection and behavioural alteration (Poulin, 2000). Microsporidia associated with Ponto-Caspian invaders have been shown to have a varied host range, behavioural impact and lower host survival rates (Bacela-Spychalska et al. 2014; Chapter 9). If the microsporidia carried by *O. crassus* share these characteristics they may also pose a threat to native fauna.

Invasive populations of *P. robustoides* have been previously found to carry gregarines (*Uradiophora* sp. and *Cephaloidophora* sp.) and microsporidia (*Nosema pontogammari* and *Thelohania* sp.) (Ovcharenko et al. 2009). The profile of this species now includes: ciliated protists; rotifers; digeneans; uncharacterised bacterial infections; isopods; viruses; and a *Haplosporidium*-like protist from the haemolymph. The microsporidia I have detected using histopathology likely link with *N. pontogammari* and *Thelohania* sp., but without appropriate material to acquire the SSU DNA sequence or ultrastructure and lifecycle of the parasite it is impossible to be sure. *Cucumispora dikerogammari* (=*Nosema dikerogammari*) has been taxonomically re-identified to fit into the *Cucumispora*, and if a similar taxonomic alteration is needed for *N. pontogammari*, which shares a similar pathology (Ovcharenko et al. 2009), it could link with a higher risk of wildlife disease introduction due to knowledge of host behaviour alteration and survival in infected amphipods (Bacela-Spychalska et al. 2012; Chapter 9).

The invasive *G. roeselii*, originally from the Balkans, was associated with ~12 symbionts and is discussed in greater detail in Chapter 6. The recently detected UK invader *G. fossarum* is also described in a separate chapter in greater detail (Chapter 7). These species are low-impact non-native species and do not appear to have a high impact upon their invasion sites. Each provides an example of how low impact non-natives can carry a high number of pathogenic agents that could threaten wildlife in novel locations (Roy et al. 2016; Chapter 6).

Another invader, *G. tigrinus* from North America, was little represented in the survey (n=15), however those few specimens were found to associate with ciliated protists, gregarines, an RLO and a microsporidian within the oocytes of the host. Feminising microsporidia have been identified as a benefit for invaders by skewing host-sex ratios, and could aid the growth of invasive propagules; this mechanism of causing an increased female to male ratio is thought to provide a greater population fecundity because females are considered a limiting factor when reproducing (Slothouber-Galbreath et al. 2004). Little is known about the hepatopancreatic RLOs of amphipods and they require greater research and understanding before determining them as harmful co-invasives (Chapter 6).

3.5.3. Potential for biological control of invasive amphipods

This study identified a range of pathogenic, parasitic and commensal species carried by several invasive and native amphipods, which may pose a threat to native fauna, but could have the potential to be utilised as biological control agents of high impact invaders. Populations of agricultural/aquaculture pests have been controlled using their parasites and pathogens in the past, to decrease their effects on crops and livestock (Hajek and Delalibera, 2010). It has been suggested that invasive amphipods could be a target for biological control to lessen their impact (Bojko et al. 2013). Fungi, nematodes, microsporidia, rickettsiae and viruses have all been suggested, and/or applied, as control agents in agriculture (Hajek and Delalibera, 2010) and parallel procedures applying amphipod pathogens could help to control invasive population size and environmental affect. Using viral pathogens as an example group, and one that is commonly applied in agriculture (Hajek and Delalibera, 2010), pests are often inundated with the pathogen to cause a rapid epizootic (high increase in viral prevalence) to induce mortality in a large proportion of the pest population. Similar mechanisms, if applied to aquatic habitats with invasive amphipods, could result in the same outcome.

The primary discoveries from this study include the microsporidian, rickettsia and viral pathogens from Ponto-Caspian and native hosts. Ponto-Caspian invaders have been

noted to have a high impact on the environments they encounter, and forecasting has predicted their capability to spread throughout the UK (Gallardo and Aldridge, 2015). Species such as *D. villosus*, which has impacted upon UK ecosystems (MacNeil et al. 2013), and has escaped many of its native pathogens (Bojko et al. 2013).

The microsporidian parasite, *C. dikerogammari*, is a species described from *D. villosus* and is not currently present in the UK (Bojko et al. 2013; Arundell et al. 2015), but has been noted as a potential control agent for this species (Bacela-Spychalska et al. 2014). This microsporidian has been noted to have a varied host range, and has been detected in the wild to infect native Polish amphipods at low prevalence, possibly through intraguild predation (Bacela-Spychalska et al. 2014). No other pathogens have been identified that are associated with decreased mortality in this species (Bacela-Spychalska et al. 2014), and without this parasite in UK waterways *D. villosus* may experience increased fitness. Lack of *C. dikerogammari* in the UK may be beneficial if vulnerable native species can avoid infection. Continued screening is needed to identify rare, mortality causing pathogens with specific host ranges to help control this species.

It may be possible to control a target species with the pathogens of another, closely related species. Close relatives to *D. villosus*, such as *D. haemobaphes*, may have parasites that can transmit to *D. villosus* but not infect native species. One such parasite is the novel microsporidian identified in this study and taxonomically described in Chapter 5. Whether this pathogen can infect *D. villosus* and incur biological control over the population is tested in Chapter 9.

Rickettsiae (RLOs) are another group of pathogens that could be useful as control agents. This study has identified a novel bacterial pathogen from *G. fossarum*, which is taxonomically identified in Chapter 7. A similar bacterial pathogen has also been detected in *G. varsoviensis*, which may have a similar taxonomic lineage. The pathology caused by these bacterial pathogens is systemic, resulting in the infection of haemocytes, muscle tissue and nerve tissue, suggesting that it may cause mortality in the host and a decrease in activity. These traits require experimental understanding, but if confirmed such a pathogen could benefit biological control. *Gammarus fossarum* has now been identified as an invasive non-native in the UK and this pathogen could be utilised as a control agent. The detection of such pathogens in amphipods assumes that other species may also hold RLOs that could benefit the control of their host. Increased screening of high-impact invaders, such as *D. villosus*, for RLOs could benefit the discovery of a viable control agent.

Finally, viruses of amphipods may be suitable as control agents (Hajek and Delalibera, 2007). Bacilliform viruses have now been confirmed from five of the hosts, including *D.*

villosus, *P. robustoides*, and *D. haemobaphes*. Recent data has identified these viruses from the hepatopancreas to be likely members of the *Nudiviridae* (Yang et al. 2014; Chapter 6), and related to the baculoviruses, which have been used in biological control efforts in the past (Hajek and Delalibera, 2007). Whether these viruses also impact the behaviour and survival of these amphipod hosts is required, and explored from a behavioural aspect in Chapter 9.

CHAPTER 4

Parahepatospora carcini n. gen., n. sp., a parasite of invasive *Carcinus maenas* with intermediate features of sporogony between the Enterocytozoon clade and other Microsporidia

4.1. Abstract

Parahepatospora carcini n. gen. n. sp., is a novel microsporidian parasite from the cytoplasm of the epithelial cells of the hepatopancreas of a single *Carcinus maenas* specimen. The crab was sampled from within its invasive range in Atlantic Canada (Nova Scotia). Histopathology and transmission electron microscopy were used to show the development of the parasite within a simple interfacial membrane, culminating in the formation of unikaryotic spores with 5-6 turns of an isofilar polar filament. Formation of a multinucleate meront (>12 nuclei observed) preceded thickening and invagination of the plasmodial membrane, and in many cases, formation of spore extrusion precursors (polar filaments, anchoring disk) prior to complete separation of pre-sporoblasts from the sporogonial plasmodium. This developmental feature is intermediate between the Enterocytozoonidae (formation of spore extrusion precursors within the sporont plasmodium) and all other Microsporidia (formation of spore extrusion precursors after separation of sporont from the sporont plasmodium). SSU rDNA-based gene phylogenies place *P. carcini* within microsporidian Clade IV, between the Enterocytozoonidae and the so-called *Enterocytospora*-clade, which includes *Enterocytospora artemiae* and *Globulispora mitoportans*. Both of these groups contain gut-infecting microsporidians of aquatic invertebrates, fish and humans. According to morphological and phylogenetic characters, I propose that *P. carcini* occupies a basal position to the Enterocytozoonidae. I discuss the discovery of this parasite from a taxonomic perspective and consider its origins and presence within a high profile invasive host on the Atlantic Canadian coastline.

4.2. Introduction

Microsporidia are a highly diverse group of obligate intracellular parasites, belonging to a sister clade to the Fungi Kingdom, which also includes the Aphelids and Cryptomycota (Haag et al. 2014; Corsaro et al. 2014; Karpov et al. 2015). Their diversity remains highly under-sampled, but known microsporidia infect a wide array of host taxa, many of which occur in aquatic habitats (Stentiford et al. 2013c). Molecular-phylogenetic approaches

are not only clarifying the position of the Microsporidia amongst the eukaryotes, but are also increasingly defining within-phylum taxonomy (Stentiford et al. 2016).

Microsporidian phylogenies built upon ribosomal gene sequence data have led to proposals for five taxonomically distinctive microsporidian clades (I, II, III, IV, V), each of which can be further aligned to three broad ecological groupings; the Marinosporidia (V); Terresporidia (II, IV); and Aquasporidia (I, III) (Vossbrinck and Debrunner-Vossbrinck, 2005). Clade IV forms a particularly interesting group due to the fact that it contains the family Enterocytozoonidae, where all known taxa infect aquatic invertebrates or fish hosts; with the exception of a single species complex (*Enterocytozoon bieneusi*). *Enterocytozoon bieneusi* is the most common microsporidian pathogen infecting immune-suppressed humans (Stentiford et al. 2013c; Stentiford et al. 2016). Other genera within the Enterocytozoonidae include: *Desmozoon* (=*Paranucleospora*), *Obruspora, Nucleospora, and Enterospora*. Other species, such as *Enterocytozoon hepatopenaei*, which infect fish and shrimp, appear to have been assigned to the genus *Enterocytozoon* erroneously, using relatively low SSU sequence similarity (~88%) and similar development pattern contrary to a closer SSU sequence similarity to the Enterospora genus (~93%) (Tourtip et al. 2009). Based upon its phylogenetic position, *E. bieneusi* is almost certainly a zoonotic pathogen of humans, likely with origins in aquatic habitats (Stentiford et al. 2016). This makes the phylogeny of existing and novel microsporidians within, and related to, the family Enterocytozoonidae an intriguing research topic. Aquatic crustaceans may offer a likely evolutionary origin to current day human infections by *E. bieneusi* (Stentiford et al. 2016).

The microsporidium *Hepatospora eriocheir* was recently discovered infecting the hepatopancreas of aquatic crustaceans (Stentiford et al. 2011; Bateman et al. 2016). Morphological characters and phylogenetic analysis found that *H. eriocheir* was related to the Enterocytozoonidae; grouping as a sister group to this family on SSU rRNA gene trees (Stentiford et al. 2011). *Hepatospora eriocheir* displayed somewhat intermediate characters between the Enterocytozoonidae and all other known taxa (e.g. potential to form spore extrusion precursors in bi-nucleate sporonts prior to their separation and, to uninucleate sporoblast and spore formation) even though the distinctive morphological characters of the Enterocytozoonidae were not observed (e.g. presence of spore extrusion precursors in multi-nucleate sporonts). Spore extrusion precursors develop after final separation of pre-sporoblasts from sporont plasmodia in all other microsporidians. The discovery of the genus *Hepatospora* led to the proposal of a sister family to the Enterocytozoonidae with intermediate traits between this family and other existing taxa. The family was tentatively assigned as the Hepatosporidae with *H.*

eriocheir (and the newly erected genus *Hepatospora*), as its type member, pending discovery of further members (Stentiford et al. 2011).

In this study I describe a novel microsporidian infecting the hepatopancreas of *Carcinus maenas* (European shore crab, or invasive green crab), commonly referred to as the green crab in North America, collected from within its invasive range in Nova Scotia, Canada. I determined that this parasite falls at the base of the Enterocytozoonidae, *Enterocytospora-like* clade and the tentatively proposed Hepatosporidae, based upon morphological, ultrastructural and phylogenetic evidence. The new parasite is distinct from *Abelspora portucalensis* (a previously described microsporidian infecting the hepatopancreas of *C. maenas,* but without available genetic data), and three other microsporidians, known to infect *C. maenas* from its native range in Europe (Sprague and Couch, 1971; Azevedo, 1987; Stentiford et al. 2013b). Given that the new parasite was not discovered within its host's native range, it is possible that it represents a case of parasite acquisition from the host community in which this non-native crab now resides. I erect the genus *Parahepatospora* n. gen. and species *Parahepatospora carcini* n. sp. to contain this novel parasite.

4.3. Materials and Methods

4.3.1. Sample collection

Carcinus maenas were sampled from Malagash Harbour on the north shore of Nova Scotia, Canada (45.815154, -63.473768) on 26/08/2014 using a mackerel-baited Nickerson green crab trap. In total, 134 *C. maenas* were collected from this site and transported to the Dalhousie University Agricultural Campus where they were kept overnight in damp conditions. Animals were euthanized, then necropsied with muscle, hepatopancreas, heart, gonad and gill tissue, preserved for DNA extraction (100% ethanol), transmission electron microscopy (2.5% glutaraldehyde) and histopathology (Davidson's saltwater fixative) using protocols defined by the European Union Reference Laboratory for Crustacean Diseases (www.crustaceancrl.eu).

4.3.2. Histology

Tissues were submerged in Davidson's saltwater fixative (Hopwood, 1996) for 24-48 hours then immersed in 70% ethanol prior to transportation to the Cefas Weymouth Laboratory, UK. Samples were prepared for histological analysis by wax infiltration using a robotic tissue processor (Peloris, Leica Microsystems, United Kingdom) before being embedded into wax blocks. Specimens were sectioned a single time at 3-4µm (Finesse

E/NE rotary microtome) and placed onto glass slides, prior to staining with haematoxylin and alcoholic eosin (H&E). Data collection and imaging took place on a Nikon-integrated Eclipse (E800) light microscope and digital imaging software at the Cefas laboratory (Weymouth).

4.3.3. Transmission electron microscopy (TEM)

Glutaraldehyde-fixed tissue biopsies were soaked in Sodium cacodylate buffer twice (10 min) and placed into 1% Osmium tetroxide (OsO_4) solution for 1 hour. Osmium stained material underwent an acetone dilution series as follows: 10% (10 min); 30% (10 min); 50% (10 min); 70% (10 min); 90% (10 min); 100% (x3) (10 min). Samples were then permeated with Agar100 Resin using a resin:acetone dilution series: 1:4; 1:1; 4:1; 100% resin (x2). Each sample was placed into a cylindrical mould (1 cm^3) along with fresh resin and polymerised in an oven (60˚C) for 16 hours. The resulting blocks were cropped to expose the tissue using a razor blade and sectioned at 1µm thickness (stain: Toluidine Blue) using a glass knife before being read on an Eclipse E800 light microscope to confirm infection. Ultra-thin sections were taken at ~80nm thickness using a diamond knife, stained with Uranyl acetate and Reynolds Lead citrate (Reynolds, 1963), and read/annotated on a Jeol JEM 1400 transmission electron microscope (Jeol, UK).

4.3.4. PCR and sequencing

DNA was extracted from ethanol-fixed samples of hepatopancreas using an automatic EZ1 DNA extraction kit (Qiagen). Primers: MF1 (5'-CCGGAGAGGGAGCCTGAGA-3') and MR1 (5'-GACGGGCGGTGTGTACAAA-3') (Tourtip et al. 2009), were used to amplify a fragment of the microsporidian SSU rRNA gene using a GoTaq flexi PCR reaction [1.25U of Taq polymerase, 2.5mM $MgCl_2$, 0.25mM of each dNTP, 100pMol of each primer and 2.5µl of DNA template (10-30ng/µl) in a 50µl reaction volume]. Thermocycler settings were as follows: 94˚C (1 min) followed by 30 cycles of 94˚C (1 min), 55˚C (1 min), 72˚C (1 min) and then a final 72˚C (10 min) step. Electrophoresis through a 2% Agarose gel (120V, 45min) was used to separate and visualise a resulting 939bp amplicon. Amplicons were purified from the gel and sent for forward and reverse DNA sequencing (Eurofins genomics sequencing services: https://www.eurofinsgenomics.eu/).

4.3.5. Phylogenetic tree construction

Several microsporidian sequences were downloaded from NCBI (GenBank), biased towards clade IV (Vossbrinck and Debrunner-Vossbrinck, 2005), but also including

members of clade III, and the genus *Glugea* (clade V) as an out-group. BLASTn searches were used to retrieve the closest related sequences to the *C. maenas* parasite. The consensus sequence of the SSU rRNA gene of the new parasite (939 bp) was added and aligned with the aforementioned dataset using the E-ins-I algorithm within mafft version 7 (Katoh and Standley, 2013). The resulting alignment, (65 sequences, 1812 positions analysed) was refined manually and analysed firstly using Maximum Likelihood (ML) in RAxML BlackBox version 8 (Stamatakis, 2014) [Generalized time-reversible (GTR) model with CAT approximation (all parameters estimated from the data)]; an average of 10,000 bootstrap values was mapped onto the tree with the highest likelihood value. A Bayesian consensus tree was then constructed using MrBayes v3.2.5 for a secondary comparative tree (Ronquist et al. 2012). Two separate MC^3 runs with randomly generated starting trees were carried out for 5 million generations, each with one cold and three heated chains. The evolutionary model used by this study included a GTR substitution matrix, a four-category auto-correlated gamma correction, and the covarion model. All parameters were estimated from the data. Trees were sampled every 1,000 generations. The first 1.25 M generations were discarded as burn-in (trees sampled before the likelihood plots reached stationarity) and a consensus tree was constructed from the remaining sample. The 18S rDNA sequence generated by this study is available from NCBI (accession number: KX757849).

4.4. Results

4.4.1. Histopathology

Of the 134 individuals sampled from the shoreline at Malagash, a single individual (trap-caught male) was found to be parasitized by a microsporidian parasite targeting the epithelial cells of the hepatopancreatic tubules (1/134; 0.75%). The hepatopancreas of the infected individual appeared to be healthy without clearly visible clinical signs of infection at the time of necropsy. Histopathological analysis revealed the microsporidian infection to be contained within the cytoplasm of infected hepatopancreatocytes (Fig. 4.1a-c). Presumed early life stages of the parasites (meronts and sporont plasmodia) stained dark blue/purple under H&E whilst apparent later life stages (sporoblasts, spores) became eosinophilic and refractile (Fig. 4.1b). In general, early life-stages of the parasite were observed to develop at the periphery of the infected cell, while spores generally occupied more central positions (Fig. 4.1b). In late stages of cellular colonisation, infected host cells appeared to lose contact with neighbour cells and the basement membrane for presumed expulsion to the tubule lumen (hepatopancreatic tubules empty to the intestine) (Fig. 4.1c). Infected hepatopancreatic tubules appeared

heavily degraded during late stage infection due to the sloughing of infected cells from the basal membrane (Fig. 4.1a-c).

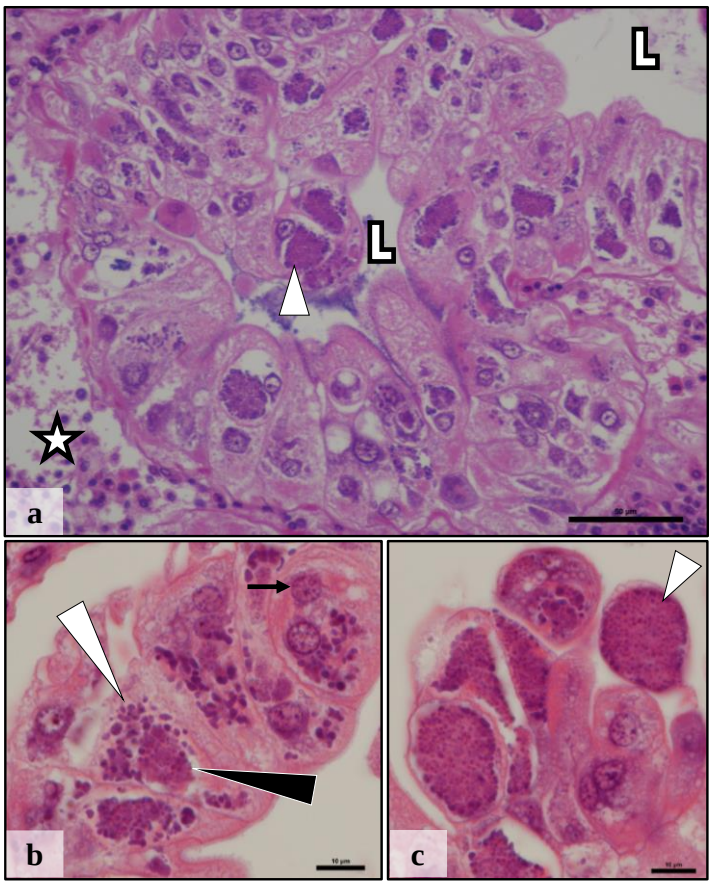


Figure 4.1: Histology of a *Parahepatospora carcini* n. gen n. sp. infection in the hepatopancreas of *Carcinus maenas*. a) A cross-section of a hepatopancreatic tubule infected with *P. carcini* (white arrow). The star indicates a blood vessel and 'L' represent the lumen of two tubules. b) A high magnification image of early infected cells. Development of early sporonts occurs as the periphery of the cell cytoplasm (white arrow) and spores appear to aggregate in the centre (black arrow). c) Cells can be seen sloughing from the basal membrane (white arrow) into the lumen, filled with microsporidian spores.

4.4.2. Microsporidian ultrastructure and lifecycle

All stages of the microsporidian parasite occurred within a simple interfacial membrane, which separated parasite development stages from the host cell cytoplasm. Earliest observed life stages, apparent uninucleate meronts, contained a thin cell membrane and were present at the periphery of the interfacial membrane (Fig. 4.2a). Unikaryotic meronts appeared to undergo nuclear division without cytokinesis, leading to a diplokaryotic meront, again occurring predominantly at the periphery of the interfacial membrane (Fig. 4.2b). Darkening of the diplokaryotic cell cytoplasm and separation of the adjoined nuclei, possibly via nuclear dissociation, preceded further nuclear divisions to form multinucleate meronts, with the greatest number of (visible) nuclei observed being 12 (Fig. 4.2c-d). The multinucleate plasmodia appear to invaginate and elongate (Fig. 4.2d). Following thickening of the multinucleate plasmodial wall, primary spore organelle formation (polar filament and anchoring disk precursors) occurred prior to the

separation of pre-sporoblasts from the sporont plasmodium in most cases (primary pathway); only in a few cases were spore pre-curser organelles not present (Fig. 4.2e-f). Other sporonts appeared to progress to sporoblasts by forming precursor spore organelles after separation from the multinucleate sporont plasmodium. Each sporoblast contained a single nucleus (Fig. 4.2f). Sporoblasts displayed noticeable thickening of the endospore and electron lucent zones of their walls (Fig. 4.3a). Mature spores contained an electron dense cytoplasm and were oval shaped with a length of 1.50µm ± 0.107µm (n=10) and a width of 1.12µm ± 0.028µm (n=16). Spores were unikaryotic, and possessed a relatively thin spore wall, consisting of a thin endospore [39.21nm ± 8.674 (n=30)], exospore [26.47nm ± 2.301nm (n=30)] and internal cell membrane. The polar filament was layered with electron lucent and electron dense rings resulting in an overall diameter of 64.18nm ± 5.495nm (n=22). The polar filament underwent 5 to 6 turns (Fig. 4.3b-d) and was terminated with an anchoring disk [width: 292.20nm ± 19.169nm (n=5)]. The endospore appeared slightly thinner in the vicinity of the anchoring disk. A highly membranous polaroplast and electron lucent polar vacuole were observed at the anterior and posterior of the spore, respectively (Fig. 4.3b-d). A depiction of the full lifecycle is presented in Fig. 4.4.

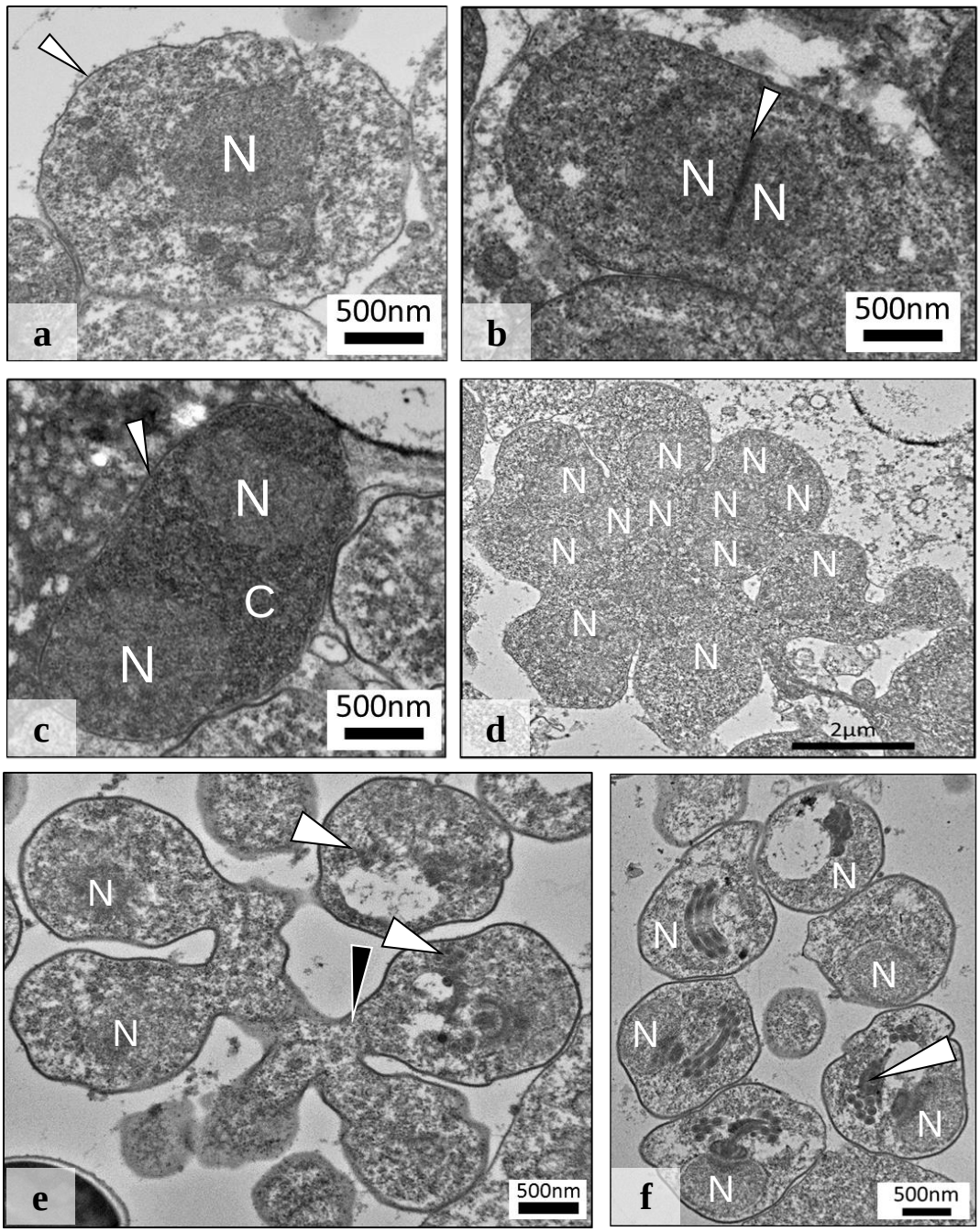


Figure 4.2: Transmission electron micrograph of the early developmental stages of *Parahepatospora carcini* n. gen. n. sp. a) Unikaryotic meront with thin cell membrane (white arrow) and single nucleus (N). b) Diplokaryotic meront with connected nuclei (N/N). c) Separation of the nuclei (N) within the diplokaryotic cell in preparation for multinucleate cell formation. Note the darkening of cytoplasm (C) and thickening cell membrane (white arrow). d) Multinucleate plasmodium containing 12 nuclei (N). e) Plasmodium cell division. Individual pre-sporoblasts bud from the main plasmodium (black arrow). Early polar filament and anchoring disks can be seen (white arrow) alongside further cell membrane thickening. f) Sporoblast formation after multinucleate cell division. Each sporoblast contains a single nucleus (N) and polar filament with an anchoring disk (white arrows).

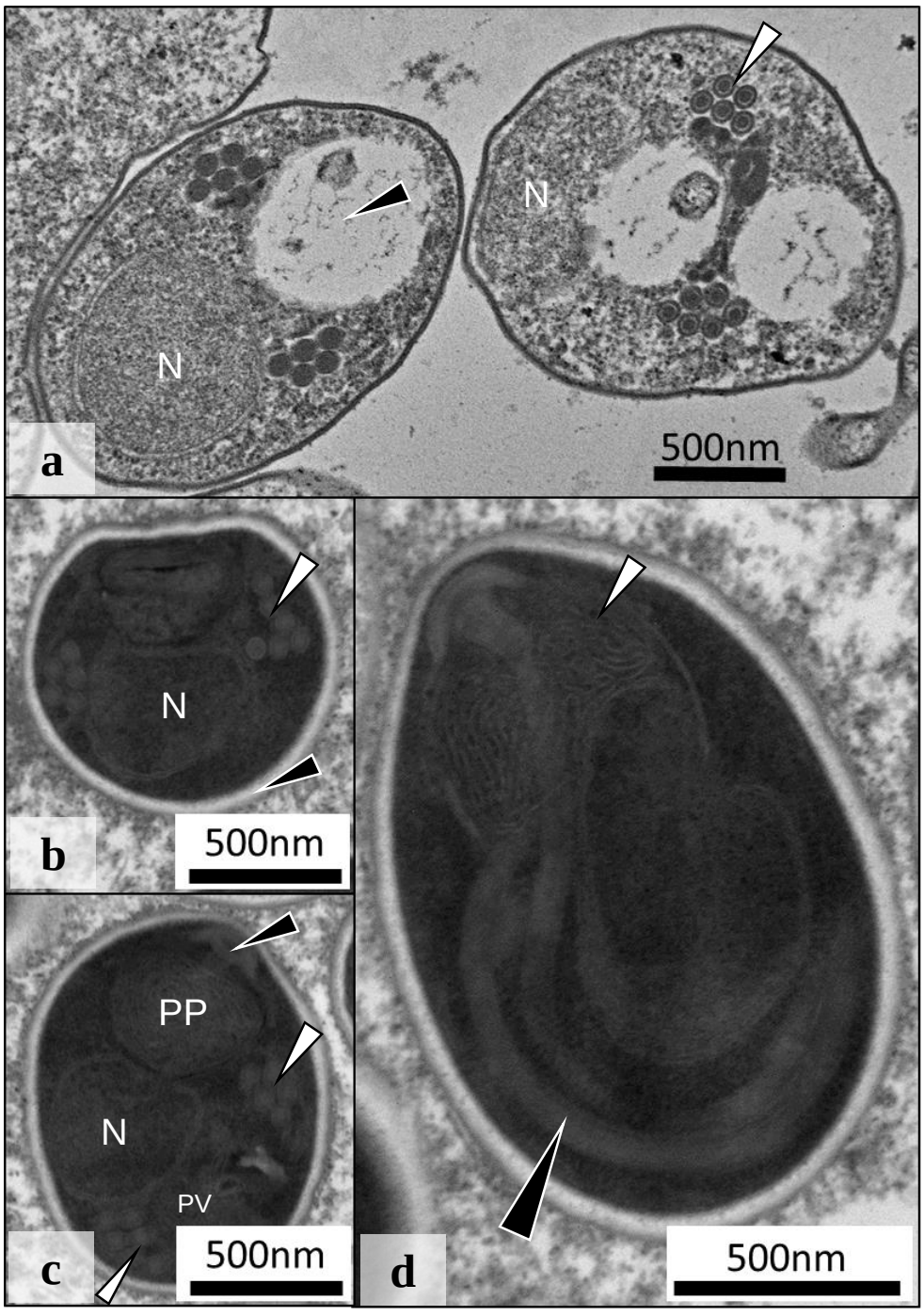


Figure 4.3: Final spore development of *Parahepatospora carcini* n. gen. n. sp. a) Sporoblasts of *P. carcini* hold 5-6 turns of the polar filament, a single nucleus and an electron lucent organelle, suspected to develop into the polaroplast (black arrow). b) Cross section of a fully developed spore displaying a single nucleus (N) and 5-6 turns of the polar filament (white arrow). Note the fully thickened, electron lucent endospore (black arrow). c) Cross section of a fully formed spore depicting a single nucleus (N), polaroplast (PP), polar vacuole (PV), cross sections of the polar filament (white arrow) and anchoring disk (black arrow). d) The final spore of *P. carcini* with a membranous polaroplast (white arrow) and curving, right-leaning, polar filament with anchoring disk (black arrows). Note the thinner endospore at the point closest to the anchoring disk.

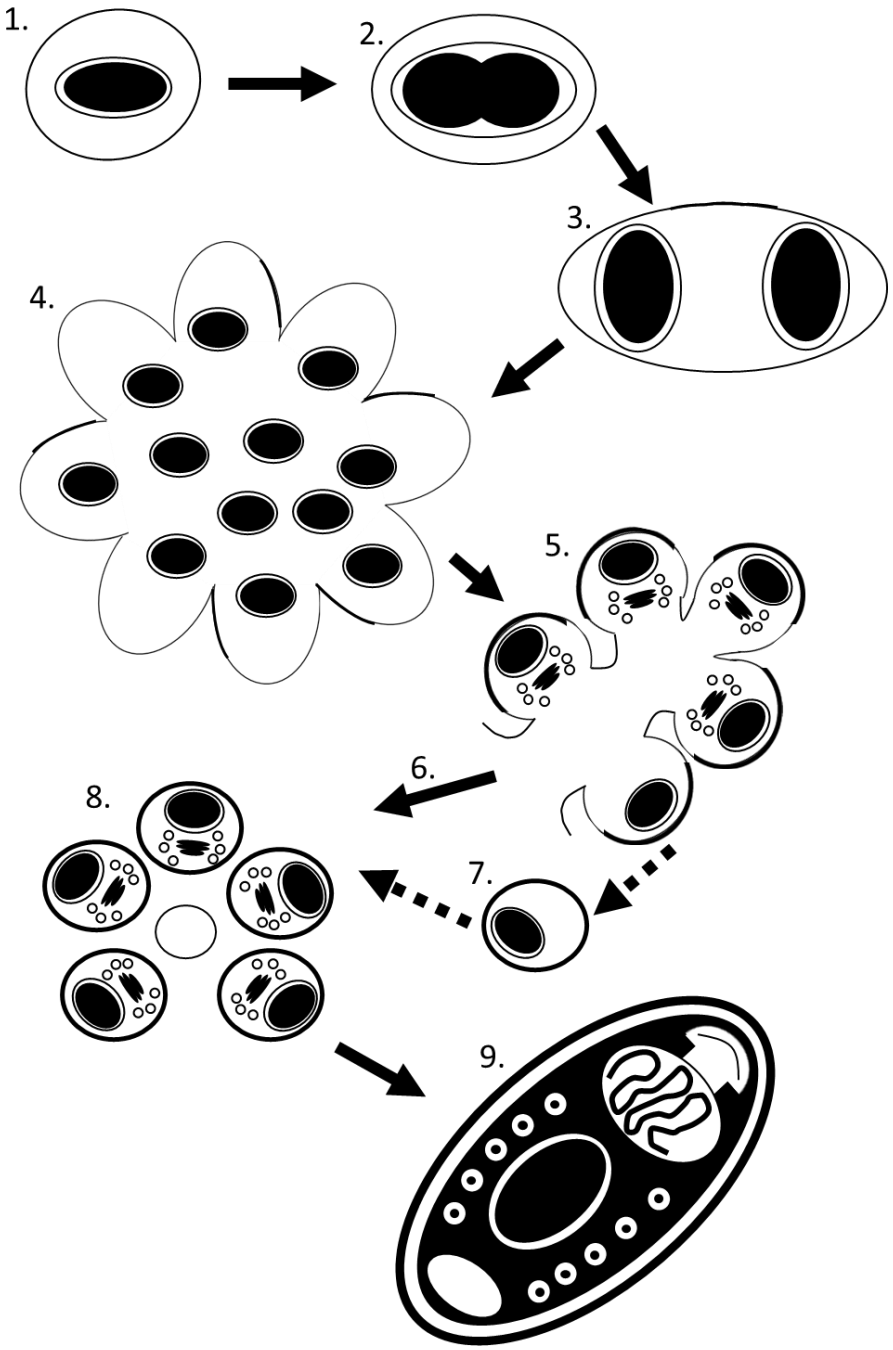


Figure 4.4: Predicted lifecycle of *Parahepatospora carcini* n. gen. n. sp. 1) The lifecycle begins with a uninucleate meront. 2) The nucleus of the meront divides to form a diplokaryotic meront. 3) The diplokaryotic nucleus divides, eventually forming a large meront plasmodium. 4) The meront plasmodium shows cytoplasmic invagination before early sporont formation. 5) A cytoplasmic elongation from a sporogonial plasmodium coupled with budding sporonts; most with early spore-organelle formation following the primary development pathway. 6) Sporonts equipped with early spore-organelles mature to sporoblasts. 7) Sporonts without early spore-organelles now develop these organelles to become sporoblasts; a secondary, uncommon pathway of development. 8) Sporoblasts mature with further thickening of the cell wall and completely separate from the sporogonial plasmodium. 9) The final, infective, uninucleate spore is formed, completing the lifecycle.

4.4.3. Phylogeny of the novel microsporidian infecting C. maenas

A single consensus DNA sequence (939bp) from the microsporidian parasite was obtained and utilised to assess the phylogeny of the novel taxon. BLASTn results revealed the highest scored hit belonged to *Globulispora mitoportans* (KT762153.1; 83% identity; 99% coverage; total score = 815; e-value = 0.0). The closest overall identity match belonged to '*Microsporidium sp. BPAR2 TUB1*' (FJ756098.1; 85% identity; 57% coverage; total score = 527; e-value = 2e-145). This suggested that the new parasite belonged in Clade IV of the Microsporidia (Vossbrinck and Debrunner-Vossbrinck, 2005) but, with distinction from all described taxa to date.

Maximum Likelihood (ML) and Bayesian (PP) analyses grouped the new parasite within the Clade IV of the microsporidia and was positioned basally to the Enterocytozoonidae, *Enterocytospora*-like clade, putative Hepatosporidae and other taxonomic families (indicated on Fig. 4.5), at weak confidence: 0.30 (ML) and 0.53 (Pp) (Fig. 5). This provides a rough estimate of its phylogeny but with little confidence as to its true position and association to the families represented in the tree.

A second tree representing microsporidian taxa that have been taxonomically described (including developmental, morphological and SSU rDNA sequence data) is presented in Fig. 4.6. This tree is annotated with developmental traits at the pre-sporoblastic (sporont) divisional level and identifies that *H. eriocheir* and *P. carcini* show intermediate development pathways between the Enterocytozoonidae and the *Enterocytospora*-like clade, supported weakly [0.38 (ML), 0.42 (Pp)] by the 18S phylogenetics. *Parahepatospora carcini* branched between the formally described *Agmasoma penaei* and *H. eriocheir*: both parasites of Crustacea but each with different developmental strategies at the pre-sporoblastic level (Fig. 4.6).

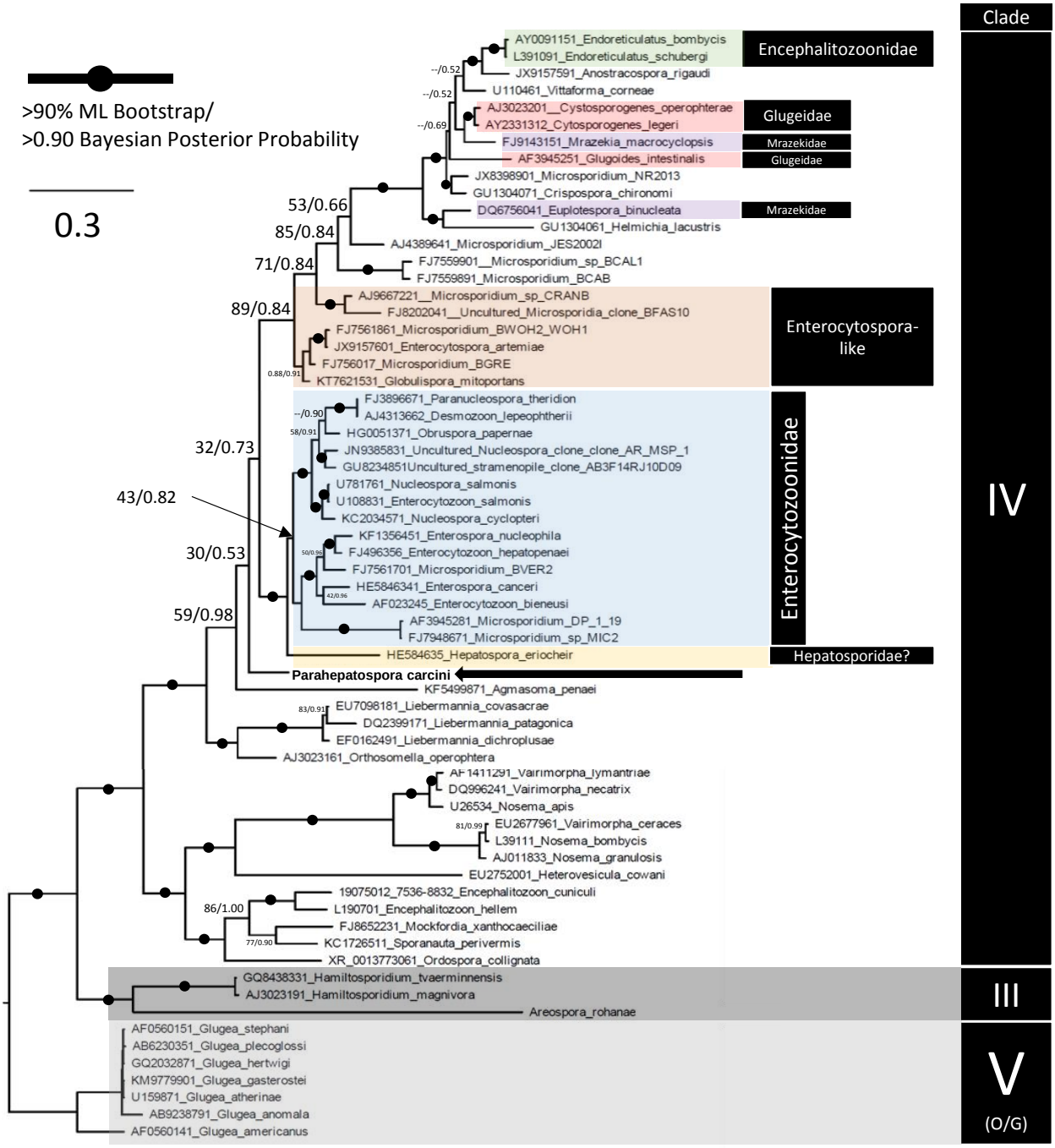


Figure 4.5: Bayesian SSU rDNA phylogeny showing the branching position of *Parahepatospora carcini* n. gen. n. sp. in microsporidian clade IV. Both Maximum Likelihood bootstrap values and Bayesian Posterior Probabilities are indicated at the nodes (ML/PP). Nodes supported by >90% bootstrap/0.90 PP are represented by a black circle on the branch leading to the node. The numbered microsporidian clades are indicated to the right of the tree. Important microsporidian families and groups are also highlighted with accompanying colours (Enterocytozoonidae, *Enterocytospora*-like, Hepatosporidae, etc.). Members of the genus *Glugea* (Clade V) are utilised as an out-group (O/G). Scale = 0.3 Units.

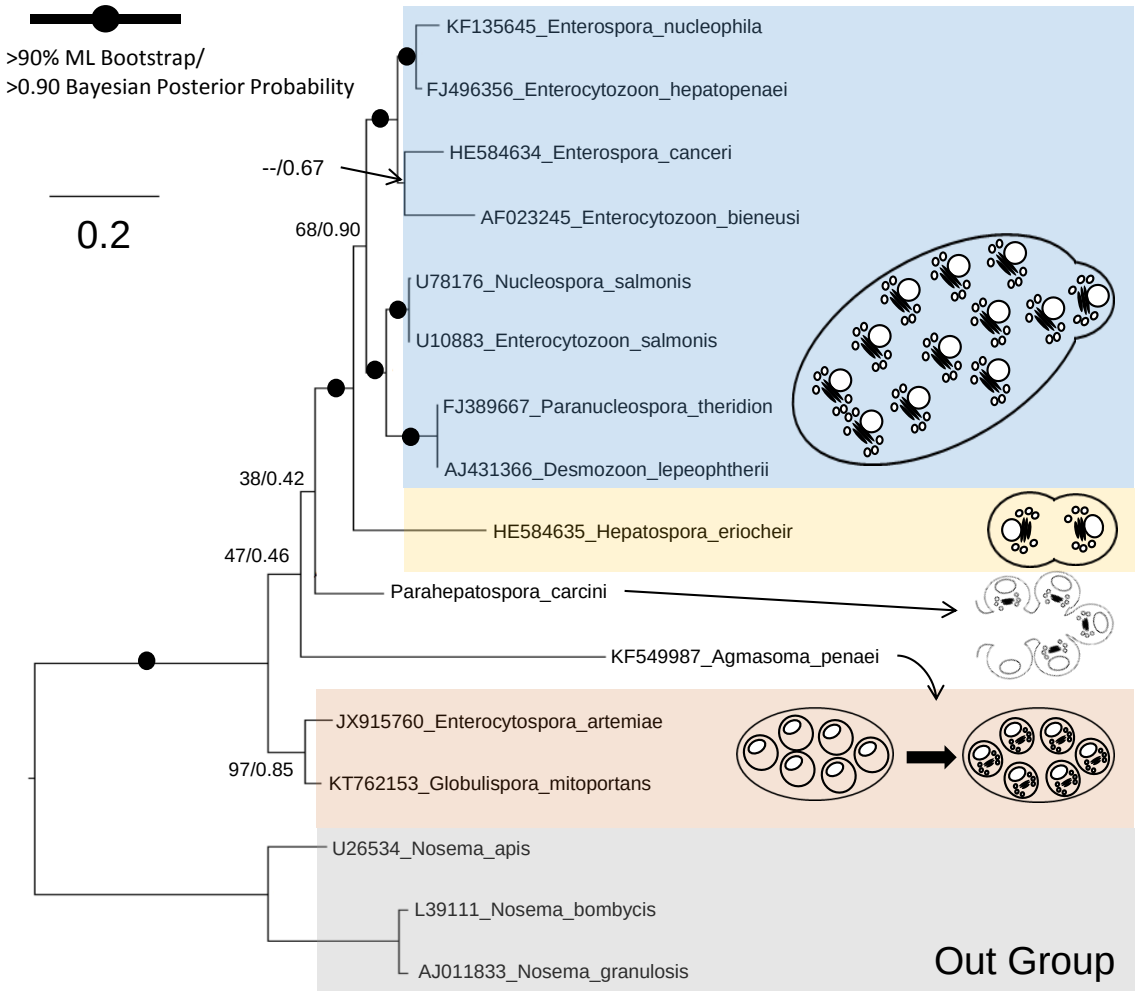


Figure 4.6: Bayesian SSU rDNA phylogeny showing the branching position of *Parahepatospora carcini* n. gen. n. sp. in microsporidian clade IV alongside microsporidia with available development pathways. Both Maximum Likelihood bootstrap values and Bayesian Posterior Probabilities are indicated at the nodes (ML/PP). Nodes supported by >90% bootstrap/0.90 PP are represented by a black circle on the branch leading to the node. The blue group (Enterocytozoonidae) all utilise large plasmodia with polar-filament development at the pre-sporoblastic divisional level. The yellow group (Hepatosporidae) show precursor development to the aforementioned trait. The orange group (*Enterocytospora*-like clade) develop the polar filament post-sporoblastic division; considered a conventional microsporidian development method. *Parahepatospora carcini* development is included alongside as an intermediate feature. *Nosema* spp. act as an out-group. Scale = 0.2 Units.

4.5. Taxonomic Description

4.5.1. Higher taxonomic rankings

Super-group: Opisthokonta

Super-Phylum: Opisthosporidia (Karpov et al. 2015)

Phylum: Microsporidia (Balbiani, 1882)

Class: Terresporidia (Clade IV) (nomina nuda) (Vossbrinck and Debrunner-Vossbrinck, 2005)

4.5.2. Novel taxonomic rankings

***Genus*:** *Parahepatospora* gen. nov.

Genus description: Morphological features are yet to be truly defined as this is currently a monotypic genus. Developmental characteristics may include: polar-filament development prior to budding from the multinucleate plasmodium; multinucleate cell formation; nuclear division without cytokinesis at the meront stage; and budding from a plasmodial filament, would increase the confidence of correct taxonomic placement. Importantly, sporonts (pre-sporoblasts) have the capacity to develop precursors of the spore extrusion apparatus prior to their separation from the sporont plasmodium. Novel taxa placed within this genus will likely have affinity to infect the hepatopancreas (gut) of their host and clade closely to the type species *P. carcini* (accession number: KX757849 serves as a reference sequence for this genus).

Type species: *Parahepatospora carcini* n. gen. n. sp.

Description: All life stages develop within a simple interfacial membrane in the cytoplasm of host cells. Spores appear oval shaped (L: 1.5µm ± 0.107µm, W: 1.1µm ± 0.028µm), and have an electron lucent endospore (thickness: 39.21nm ± 8.674nm) coupled with an electron dense exospore (thickness: 26.47nm ± 2.3nm) by TEM. The polar filament turns 5-6 times and the polaroplast of the spore is highly membranous. The spores are unikaryotic with unikaryotic merogonic stages during early development, which progress through a diplokaryotic meront stage to a multinucleate plasmodium stage in which spore extrusion precursors primarily form prior to the separation of sporonts (pre-sporoblasts). Sporonts bud from the plasmodium via an elongation of the cytoplasm. *Parahepatospora carcini* SSU rDNA sequence data is represented by accession number: KX757849.

Type host: *Carcinus maenas*, Family: *Portunidae*. Common names include: European shore crab and invasive green crab.

Type locality: Malagash (invasive range) (Canada, Nova Scotia) (45.815154, -63.473768).

Site of infection: Cytoplasm of hepatopancreatocytes.

Etymology: "*Parahepatospora*" is named in accordance to the genus "*Hepatospora*" based upon a similar tissue tropism (hepatopancreas) and certain shared morphological characters. The specific epithet "*carcini*" refers to the type host (*Carcinus maenas*) in which the parasite was detected.

Type material: Histological sections and TEM resin blocks from the infected Canadian specimen is deposited in the Registry of Aquatic Pathology (RAP) at the Cefas Weymouth Laboratory, UK. The SSU rRNA gene sequence belonging to *P. carcini* has been deposited in Gen-Bank (NCBI) (accession number: KX757849).

4.6. Discussion

In this study I describe a novel microsporidian parasite infecting the hepatopancreas of a European shore crab (*Carcinus maenas*), from an invasive population in Atlantic Canada (Malagash, Nova Scotia). The SSU rRNA phylogenies place *Parahepatospora carcini* within Clade IV of the Microsporidia, and specifically at the base of the Enterocytozoonidae (containing *Enterocytozoon bieneusi*) and recently-described *Enterocytospora*-like clade (infecting aquatic invertebrates) (Vavra et al. 2016). Its appearance at the base of these clades coupled with its host pathology and development, suggest that this species falls within the Hepatosporidae. However, this cannot be confirmed with current genetic and morphological data. Collection of further genetic data in the form of more genes from both this novel species and other closely related species, will help to infer a more confident placement in future. *Parahepatospora carcini* n. gen. n. sp. is morphologically distinct from the microsporidian *Abelspora portucalensis*, which parasitizes the hepatopancreas of *C. maenas* from its native range in Europe (Azevedo, 1987). It is important here to consider whether *P. carcini* n. gen. n.

sp. has been acquired in the invasive range of the host, or whether this novel microsporidian is an invasive pathogen carried by its host from its native range.

4.6.1. Could Parahepatospora carcini *n. gen. n. sp. be* Abelspora portucalensis *Azevedo, 1987?*

Abelspora portucalensis was initially described as a common microsporidian parasite of *C. maenas* native to the Portuguese coast (Azevedo, 1987). While *A. portucalensis* and *P. carcini* infect the same organ (hepatopancreas), and both develop within interfacial membranes separating them from the cytoplasm of infected cells, the two parasites do not resemble one another morphologically. No visible pathology was noted for *P. carcini* whereas *A. portucalensis* leads to the development of 'white cysts' on the surface of the hepatopancreas, visible upon dissection. In contrast to the high prevalence of *A. portucalensis* in crabs collected from the Portuguese coast, *P. carcini* infection was rare (<1%) in crabs collected from the Malagash site.

The parasites share some ultrastructural characteristics, such as: a uninucleate spore with 5-6 turns of a polar filament and a thin endospore. However, the ellipsoid spore of each species shows dissimilar dimensions [*A. portucalensis* (L: "3.1 - 3.2µm", W: "1.2 – 1.4µm") Azevedo, 1987] [*P. carcini* (L: 1.5µm ± 0.107µm, W: 1.1µm ± 0.028µm)]. In addition, *A. portucalensis* spores were observed to develop in pairs, within a sporophorous vesicle whilst life stages of *P. carcini* develop asynchronously within an interfacial membrane (Fig. 4.2 and4.3). *Parahepatospora carcini* undergoes nuclear division to form a diplokaryotic meront without cytokinesis (Fig. 4.2b) where both *A. portucalensis* and *H. eriocheir* undergo nuclear division with cytokinesis at this developmental step; further distinguishing these two species from *P. carcini*. *Parahepatospora carcini* also possesses a characteristically distinctive development stage in which multinucleate plasmodia lead to the production of early sporoblasts. These sporoblasts develop spore extrusion organelles prior to their separation from the plasmodium (Fig. 4.2e-f). This critical developmental step, characteristic of all known members of the Enterocytozoonidae (Stentiford et al. 2007) has also been observed (albeit in reduced form) in *H. eriocheir*, the type species of the Hepatosporidae (Stentiford et al. 2011). This feature was not reported by Azevedo (1987) for *A. portucalensis*, providing further support that *P. carcini* and *A. portucalensis* are separate.

Because of these differences, and in the absence of DNA sequence data for *A. portucalensis*, I propose that *P. carcini* n. gen. n. sp. is the type species of a novel genus (*Parahepatospora*) with affinities to both *Hepatospora* (Hepatosporidae) and members of the Enterocytozoonidae. However, given the propensity for significant morphological

plasticity in some microsporidian taxa (Stentiford et al. 2013b), I note that this interpretation may change in light of comparative DNA sequence data becoming available for *A. portucalensis*.

4.6.2. Could Parahepatospora carcini *n. gen n. sp. belong within the Hepatosporidae?*

The Hepatosporidae was tentatively proposed to contain parasites infecting the hepatopancreas of crustacean hosts (Stentiford et al. 2011). To date, it contains a single taxon, *H. eriocheir*, infecting Chinese mitten crabs (*Eriocheir sinensis*) from the UK (Stentiford et al. 2011), and from China (Wang et al. 2007). The Hepatosporidae (labelled within Fig. 4.5) is apparently a close sister to the Enterocytozoonidae. As outlined above, *P. carcini, H. eriocheir* and all members of the Enterocytozoonidae share the developmental characteristic of early spore organelle formation (such as the polar filament and anchoring disk) within the pre-divisional sporont plasmodium. In contrast, members of the *Enterocytospora*-like clade display developmental features consistent with all other known microsporidian taxa (i.e. spore precursor organelles form after the separation of the sporont from the plasmodium, Rode et al. 2013a). Like *H. eriocheir*, *P. carcini* displays early spore-organelle formation both pre- and post- sporont separation from the sporont plasmodium. It is tempting to propose that this characteristic is an intermediate trait between the Enterocytozoonidae and all other Microsporidia and, that this trait is possibly definitive for members of the Hepatosporidae; but further SSU rRNA gene phylogeny data is required to further confirm this, and to link these observations. Intriguingly, *Agmasoma penaei* (branching below *P. carcini*), a pathogen of the muscle and gonad (only gonad in type host), which is closely associated to *P. carcini* phylogenetically (Fig. 4.5 and 4.6), shows tubular inclusions at the plasmodium developmental stage; however polar filament precursors do not fully develop until after sporont division (Sokolova et al. 2015); this could indicate a further remnant of the developmental pathways seen in *P. carcini*, *H. eriocheir* and members of the Enterocytozoonidae.

The shared developmental and pathological characteristics of *P. carcini* and *H. eriocheir* suggest a taxonomic link; however this is not clearly supported by the SSU rRNA gene phylogenies (Fig. 4.5 and 4.6). Confidence intervals supporting the placement of *P. carcini* outside of both the Enterocytozoonidae, the *Enterocytospora*-like clade and the Hepatosporidae are low (Fig. 4.5 and 4.6) forcing me to suggest that additional data in the form of further gene sequencing of this novel parasite, or possibly from others more

closely related through diversity studies, is required before confirming a familial taxonomic rank for this new taxon.

4.6.3. Is Parahepatospora carcini *n. gen. n. sp. an invasive pathogen or novel acquisition?*

The 'enemy release' concept proposes that invasive hosts may benefit from escaping their natural enemies (including parasites) (Colautti et al. 2004). Invasive species may also introduce pathogens to the newly invaded range, as illustrated by spill-over of crayfish plague (Jussila et al. 2015) to endangered native crayfish in Europe. Invaders can also provide new hosts for endemic parasites through parasite acquisition (e.g. Dunn and Hatcher, 2015).

Invasive populations of *C. maenas* in Canada are thought to have originated from donor populations in Northern Europe, specifically: Scandinavia, the Faroe Islands and Iceland, based on microsatellite analysis (Darling et al. 2008). *Carcinus maenas* are yet to be screened for microsporidian parasites within some of these ancestor populations and they may prove to be a good geographic starting point for studies to screen for *P. carcini*. The Faroe Islands have had some screening and *P. carcini* was not detected (Chapter 2). Alternatively, the recent discovery of *P. carcini* at low prevalence in *C. maenas* from the invasive range in Canada could indicate that the parasite has been acquired from the Canadian environment via transfer from an unknown sympatric host. The low prevalence (a single infected specimen) of infection could suggest the single *C. maenas* in this study was infected opportunistically, however the potential remains for *P. carcini* to be present at low prevalence, with gross pathology, as a mortality driver and emerging disease in *C. maenas* on the Canadian coastline. Currently, no evidence is available to confirm whether *P. carcini* is non-native or endemic.

For future studies it is important to consider whether *P. carcini* may be a risk to native wildlife (Roy et al. 2016), or, if the parasite has been acquired from the invasive range (pathogen acquisition), how it was acquired. If invasive, important questions about the invasion pathway of *P. carcini* would help to indicate its risk and invasive pathogen status (Roy et al. 2016). Finally, assessing the behavioural and life-span implications of infection could address whether *P. carcini* has the potential to be used to control invasive *C. maenas* on the Canadian coastline (potential biological control agent).

CHAPTER 5

Cucumispora ornata n. sp. (Fungi: Microsporidia) infecting invasive 'demon shrimp' (*Dikerogammarus haemobaphes*) in the United Kingdom

5.1. Abstract

Dikerogammarus haemobaphes, the 'demon shrimp', is an amphipod native to the Ponto-Caspian region. This species invaded the UK in 2012 and has become widely established. *Dikerogammarus haemobaphes* has the potential to introduce non-native pathogens into the UK, creating a potential threat to native fauna. In this study I describe a novel species of microsporidian parasite infecting 72.8% of invasive *D. haemobaphes* located in the River Trent, UK. The microsporidium infection was systemic throughout the host; mainly targeting the sarcolemma of muscle tissues. Electron microscopy revealed these parasite to be diplokaryotic and have 7-9 turns of the polar filament. The microsporidium is placed into the *Cucumispora* based on host histopathology, fine detail parasite ultrastructure, a highly similar life cycle and SSU rDNA sequence phylogeny. Using this data this novel microsporidian species is named *Cucumispora ornata*, where 'ornata' refers to the external beading present on the mature spore stage of this organism. Alongside a taxonomic discussion, the presence of a novel *Cucumispora* sp. in the United Kingdom is discussed and related to the potential control of invasive *Dikerogammarus* spp. in the UK and the health of native species which may come into contact with this parasite.

5.2. Introduction

The Microsporidia are a diverse group of obligate parasites within the Kingdom Fungi (Capella-Guitiérrez et al. 2012; Haag et al. 2014). They infect hosts from all animal phyla and from all habitats; are genetically diverse; use a variety of transmission methods; can infect a range of different tissue and organ types; and exhibit high developmental and morphological plasticity (Dunn et al. 2001; Stentiford et al. 2013a; Stentiford et al. 2013c). Plasticity in parasite morphology has led to the formation of polyphyletic taxa whose inter-relationships are now being clarified by application of molecular phylogenetic approaches (e.g. Vossbrinck and Debrunner-Vossbrinck, 2005; Stentiford et al. 2013c).

Furthermore, similar approaches are being applied to increase the confidence in placement of the Microsporidia at the base of the Fungi (Capella-Guitiérrez et al. 2012). The discovery and description of novel taxa, such as *Mitosporidium daphniae*, emphasise this positioning by essentially bridging the gap between true Fungi, the Cryptomycota (e.g. *Rozella* spp.) and the Microsporidia (Haag et al. 2014). Novel taxonomic descriptions now combine data pertaining to ultrastructural features, lifecycle characteristics, host type and habitat type, and conclusively, phylogenetics (Stentiford et al. 2013c).

Microsporidia were first identified infecting members of the Gammaridae (a family of omnivorous amphipods found across the world in freshwater and marine habitats), specifically *Gammarus pulex*, by Pfeiffer (1895). Since this initial discovery, gammarids have been shown to play host to a wide diversity of Microsporidia (Bulnheim, 1975; Terry et al. 2003). Ten microsporidium genera are currently known to infect gammarid hosts including: *Dictyocoela* (unofficially presented by Terry et al. 2004); *Nosema* (Nägeli, 1857); *Fibrillanosema* (Slothouber-Galbreath et al. 2004); *Thelohania* (Henneguy and Thélohan, 1892); *Stempillia* (Pfeiffer, 1895); *Pleistophora* (Canning and Hazard, 1893); *Octosporea* (Chatton and Krempf, 1911); *Bacillidium* (Janda, 1928); *Gurleya* (Hesse, 1903); *Glugea* (Thélohan, 1891); *Amblyospora* (Hazard and Oldacre, 1975) and *Cucumispora* (Ovcharenko and Kurandina, 1987). Based on phylogenetic analysis and tree construction, these gammarid-infecting microsporidia appear alongside those infecting fish, insects and other crustacean hosts from marine and freshwater environments (Stentiford et al. 2013c). Members of these genera utilise either horizontal or vertical transmission pathways, or a combination of the two, to maintain infections within populations of target hosts (Smith, 2009). *Dictyocoela berillonum* (vertical transmission), *Pleistophora mulleri* (vertical and horizontal transmission) and *Gurleya polonica* (horizontal transmission solely) provide examples of these transmission methods (Czaplinska et al. 1999; Terry et al. 2003; Terry et al. 2004; Wattier et al. 2007).

Most organs and tissues of gammarids can become infected by microsporidia. Whilst some taxa cause systemic infections (e.g. *Cucumispora dikerogammari*), others target specific tissue types such as muscle fibres (e.g. *G. polonica* in *Orchestia* sp.). In general, vertically transmitted microsporidia infect gonadal tissues and often elicit only minor pathologies unless they are also capable of horizontal transmission (Terry et al. 2003). Horizontally transmitted microsporidia on the other hand can elicit negative effects on feeding and locomotion and often result in host mortality (Bacela-Spychalska et al. 2014). For these reasons, horizontally transmitted microsporidia are considered a useful target

for biological control strategies against agriculturally-important insect pests (Hajek and Delalibera Jr, 2010).

Members of the genus *Dikerogammarus* are a group of freshwater amphipods, native to the Ponto-Caspian region. Within the genus, two taxa have received considerable attention as invasive non-native species (INNS) within Europe: the 'killer shrimp' *Dikerogammarus villosus* (Rewicz et al. 2014) and the 'demon shrimp' *Dikerogammarus haemobaphes* (Bovy et al. 2014). *Dikerogammarus villosus* is listed in the 'top 100 worst invasive species in Europe' (DAISIE, 2014) due to its widely documented detrimental impact on native invertebrate fauna and its ability to spread parasites to novel locations (Wattier et al. 2007). In 2010, populations of *D. villosus* were discovered in several locations within the UK where they have subsequently caused significant issues to both native fauna and the environment (MacNeil et al. 2013). Subsequent to the invasion by *D. villosus*, in 2012, a second invader, *D. haemobaphes*, was also detected in UK freshwater habitats and has since been detected at numerous sites across a wide geographic space (Bovy et al. 2014; Green-Etxabe et al. 2015).

An extensive survey of *D. villosus* using histopathology revealed a distinct lack of pathogens and parasites in populations of *D. villosus* in UK sites (Bojko et al. 2013). These data were reinforced in a subsequent study by Arundell et al (2015), which demonstrated an absence of microsporidium pathogens in invasive *D. villosus* using a PCR-based surveillance approach. Parasites may alter the outcome or impact of invasions as they are either introduced into new communities along with invading species, or left behind in the host's ancestral range, affording the host "enemy release" (Dunn, 2009). In the case of *D. villosus*, its native microsporidium parasite, *C. dikerogammari*, was found to have hitchhiked along an invasion pathway in continental Europe, entering Poland (via the River Vistula), France and Germany (via the River Rhine) (Wattier et al. 2007; Ovcharenko et al. 2009; Ovcharenko et al. 2010). In these countries, *C. dikerogammari* has also been detected infecting native gammarids (Bacela-Spychalska et al. 2012), presumably via transmission from proximity to infected *D. villosus*. Conversely, studies of UK populations of *D. villosus* have found little evidence for the presence of this microsporidium, or indeed other pathogens; suggesting that at least in this location, *D. villosus* may be benefiting from enemy release (Bojko et al. 2013; MacNeil et al. 2013; Arundell et al. 2014).

In addition to *C. dikerogammari*, several microsporidia are known to infect *D. villosus* and *D. haemobaphes* across their invasive and native ranges (Table 5.1) (Bojko et al. 2013). It has been suggested that *C. dikerogammari*, may pose a significant risk to native range amphipods due to its potential for cross-taxa transmission (Bacela-Spychalska et

al. 2012). In the current study I describe a novel microsporidium pathogen infecting *D. haemobaphes* collected from the River Trent, UK. Histological, ultrastructural and phylogenetic evidence is used to propose a novel species within the genus *Cucumispora*. My findings are discussed in relation to the invasion pathway for this pathogen to the UK, the relationship to sister taxa within the genus and the potential for the novel pathogen to spread to both native hosts, and to the invasive sister species *D. villosus*.

Microsporidia infecting *Dikerogammarus haemobaphes*	**Species:**	**Location**	**Reference**
	Cucumispora (=*Nosema*) *dikerogammari*	Goslawski Lake and Bug in Wyszków	Ovcharenko et al. 2010
	Thelohania brevilovum	Goslawski Lake, Poland	Ovcharenko et al. 2009
	Dictyocoela mulleri	Goslawski Lake, Poland	Ovcharenko et al. 2009
	Dictyocoela spp. ('Haplotype: 30-33')	Goslawski Lake, Poland	Wilkinson et al. 2011
	Dictyocoela berillonum	Unknown	Wroblewski and Ovcharenko (BLAST)
		Wallingford Bridge and Bell Weir, UK	Green-Etxabe et al. 2015

Table 5.1: Microsporidian parasites known to infect *Dikerogammarus haemobaphes*.

5.3. Materials and Methods

5.3.1. Sample collection

Dikerogammarus haemobaphes (n=81) were sampled using nets from two sites on the River Trent, United Kingdom (grid ref.: SK3870004400 and SK1370013700) in March 2014. Animals were identified based on their morphology and placed on ice before dividing into three parts using a sterile razor blade. The 'head' and urosome were removed and placed into 100% ethanol for later DNA extraction. Sections 2 and 3 of the pereon, including the gnathopods, were dissected along with internal organs and placed into 2.5% glutaraldehyde for transmission electron microscopy (TEM). The remainder of the animal (pereon 4 to the pleosome) was fixed for histology in Davidson's freshwater fixative (Hopwood, 1996).

5.3.2. Histology

After 24 h, samples in Davidson's freshwater fixative were transferred to 70% industrial methylated spirit (IMS) before processing to paraffin wax blocks using an automated tissue processor (Peloris, Leica Microsystems, UK) and sectioned on a Finesse E/NE

rotary microtome (Thermofisher, UK). Specimens were stained using haematoxylin and alcoholic eosin (H&E) and slides examined using a Nikon Eclipse E800 light microscope at a range of magnifications. Images were obtained using an integrated LEICA™ (Leica, UK) camera and edited/annotated using LuciaG software (Nikon, UK). Animal processing protocol here is identical to that described in Bojko et al. (2013).

5.3.3. Transmission electron microscopy (TEM)

Samples fixed for TEM (present in 2.5% Glutaraldehyde) were processed through 2 changes of 0.1M Sodium cacodylate buffer over 15 min periods. Secondary fixation was performed using Osmium tetroxide (OsO_4) (1 hour) followed by two 10 minute rinses in 0.1M Sodium cacodylate buffer. Samples were dehydrated through an ascending acetone dilution series (10%, 30%, 50%, 70%, 90%, 100%) before embedding in Agar100 resin using a resin:acetone dilution series (25%, 50%, 75%, 100%) (1 h per dilution). The tissues were placed into plastic moulds filled with resin and polymerised by heating to 60˚C for 16 h. Blocks were sectioned using a Reichart Ultracut Microtome equipped with glass blades [semi-thin sections (1µm)] or a diamond blade [ultra-thin sections (around 80nm)]. Semi-thin sections were stained using toluidine blue and checked using standard light microscopy. Ultra-thin sections were stained using Uranyl acetate and Reynolds Lead citrate (Reynolds, 1963). Ultra-thin sections were observed using a Jeol JEM 1400 transmission electron microscope (Jeol, UK).

5.3.4. DNA extraction, PCR and sequencing

The head and urosome of each amphipod, fixed in ethanol, underwent DNA extraction using the EZ1 DNA tissue kit (Qiagen, UK). Amplification of the partial SSU rRNA gene was accomplished using two previously identified PCR primer sets (Vossbrinck et al., 1987; Baker et al. 1995; Tourtip et al. 2009) (Table 5.2). V1F/530r and MF1/MR1 primer protocols were used in a GoTaq flexi PCR reaction including 1.25U/reaction of Taq polymerase, 1µM/reaction of each primer, 0.25mM/reaction of each dNTP, 2.5mM/reaction $MgCl_2$ and 2.5µl/reaction of DNA extract (10-30ng/µl) in a 50µl reaction volume. Thermocycler settings for V1F/530r were; 95˚C (5 min), 95˚C (50 sec)-60˚C (70 sec)-72˚C (90 sec) (40 cycles), 72˚C (10 min). Thermocycler settings for MF1/MR1 were; 94˚C (5 min), 94˚C-55˚C-72˚C (1 min per temperature) (40 cycles), 72˚C (10 min). Amplifications were run on a 1.5% agar gel (120V / 45 minutes) and products were excised from the gel and purified using freeze-and-squeeze purification before sequencing on an ABI PRISM 3130xl Genetic Analyser (Applied Biosystems, UK) or sequencing via Eurofins (Eurofins Genomics, UK).

Forward Primer		Reverse Primer		Fragment size	Reference
V1F	5'-CACCAGGTTGATTCTGCCTGAC-3'	530r	5'-CCGCGGCTGCTGGCAC-3'	530bp	Vossbrinck et al. 1987; Baker et al. 1995
MF1	5'-CCGGAGAGGGAGCCTGAGA-3'	MR1	5'-GACGGGCGGTGTGTACAAA-3'	900bp	Tourtip et al. 2009

Table 5.2: Primer sets used to partially amplify the microsporidian SSU rRNA gene.

5.3.5. Phylogenetic analysis

Gene sequences retrieved from microsporidium-infected demon shrimp were analysed using CLC Main Workbench (7.0.3) where a neighbour joining tree was produced, incorporating my own acquired sequences with other closely related microsporidium sequences, and in particular, those used in the analysis by Ovcharenko et al. (2010). The analysis included 1000 bootstrap replicates and utilised the Jukes-Cantor evolution model (Jukes and Cantor, 1969). Similar BLAST hit sequences from several undetermined "*Microsporidium* sp." were also incorporated in to the phylogenetic analysis. The tree underwent 100 bootstrap replicates to test robustness. *Basidiobolus ranarum* (AY635841), *Heterococcus pleurococcoides* (AJ579335.1) and *Conidiobolus coronatus* (AF296753) were used as a fungal out-group.

5.4. Results

5.4.1. Pathology and ultrastructure

Prior to fixation, live animals did not display obvious clinical signs of infection. Despite this, histology revealed a microsporidium infection in 72.8% of animals obtained from the River Trent population. Infection was observed in the skeletal musculature (located mainly within the space immediately beneath the sarcolemma), nervous tissues, oocytes and connective tissues. Infections by spore life-stages of the microsporidia were clearly visible via light microscopy, and often seen to begin infection in the sarcolemma of muscle blocks (Fig. 5.1a). In advanced infections, the majority of the skeletal musculature was replaced with microsporidian life stages, moving from the sarcolemma to infect the rest of the muscle block (Fig. 5.1b). Under high magnification, spores appeared somewhat elongate and were apparently in direct contact with the host cell cytoplasm (Fig. 5.1c). Infections in connective tissue cells appeared to lead to formation of cysts (multi-nucleated syncitia), potentially due to fusion of adjacent infected host cells

(Fig. 5.1d). In female hosts, the gonad was sometimes targeted by the parasite, with microsporidian spores occasionally visible within oocytes. Limited host encapsulation of parasite life stages was observed, although in advanced infections, presumably related to host cell rupture, small melanised haemocyte aggregates were seen. In other cases, liberated spores were seen to be phagocytised by host haemocytes (Fig. 5.1e).

TEM of infected muscle tissues revealed merogonial and sporogonial life stages of a microsporidium pathogen developing in direct contact with the host cell cytoplasm. In early stages, the pathogen occupied the sub-sarcolemmal region at the periphery of infected muscle fibres with progression to the main muscle fibre in later stages of infection. The lifecycle began with a diplokaryotic meront (Fig. 5.2a), which followed one of two possible pathways; the first involving direct development to the diplokaryotic sporont, depicted by regional, and eventually complete, thickening of the cell membrane and darkening of the cell cytoplasm (Fig. 5.2b, c). The second pathway involved nuclear division to form a tetranucleate (2 x 2n) meront plasmodium which then divided through binary fission to form two diplokaryotic sporoblasts (Fig. 5.2d, e, f) (as seen by *C. dikerogammari* in Ovcharenko et al. 2010). In rare cases, unikaryotic meronts were observed, however they were assumed to be non-representative cross-sections of diplokaryotic cells (cross-sections through a diplokaryotic meront due to the use of TEM gives the appearance of a unikaryotic cell). No sporophores vesicles were observed throughout this study.

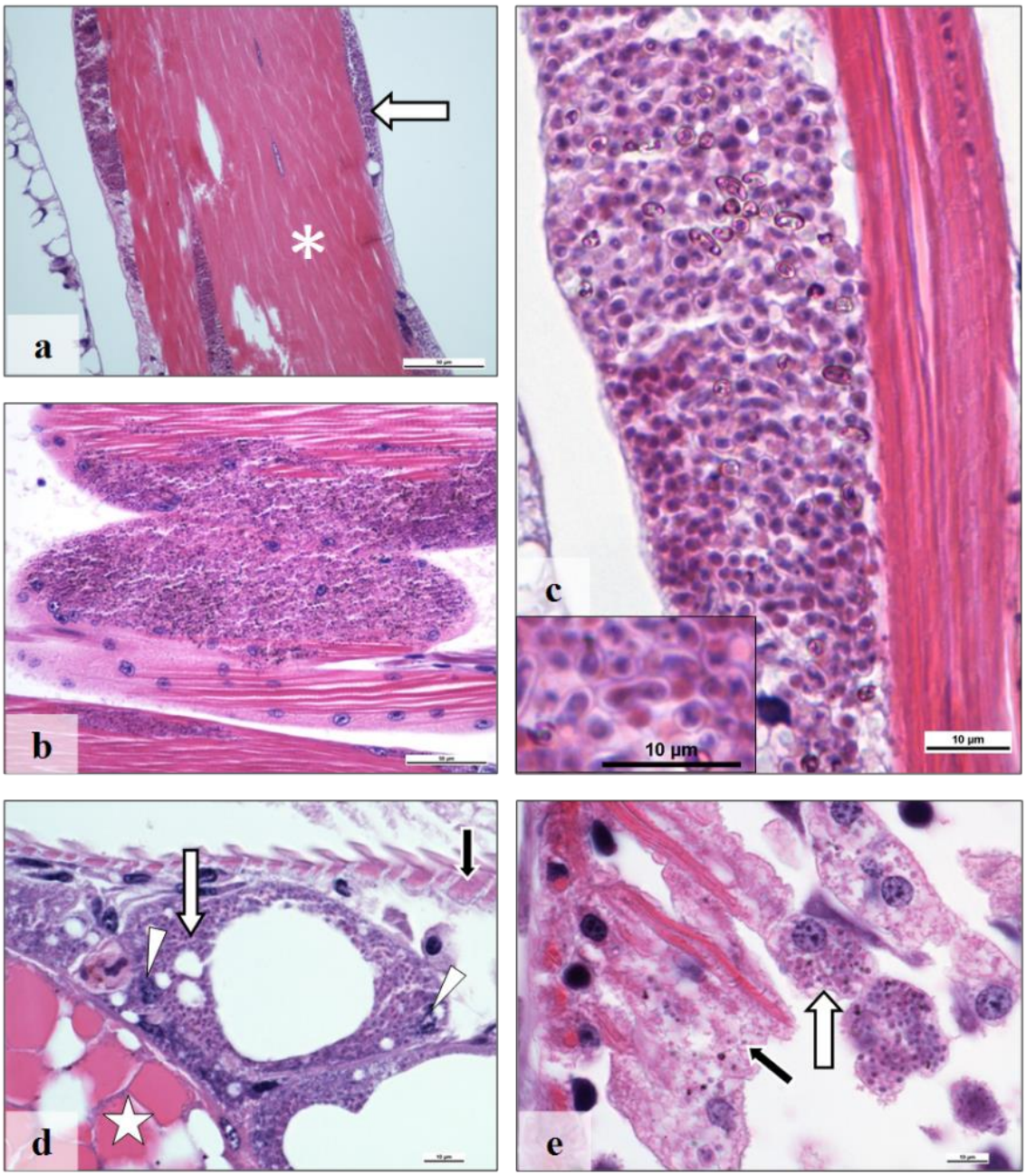


Figure 5.1: *Cucumispora ornata* n. sp. associated histopathology in *D. haemobaphes*. a) Microsporidian infection colonising the sarcolemma and muscle cells of available muscle blocks (white arrow). Some muscle remains uninfected (*). Scale = 100µm. b) Large infection replacing areas of the muscle block within the leg of *D. haemobaphes*. Scale = 10µm. c) A high magnification image of microsporidian spores under histology. The inset sows both laterally and longitudinally sectioned spores. Scale = 10µm. d) Microsporidian filled cells (white arrow) in the connective tissue between the gut smooth muscle (black arrow) and gonad (white star) of *D. haemobaphes*. Individual nuclei are depicted with a white triangle. Scale = 10µm. e) Granulocytes in the heart are present with phagocytised microsporidian spores (white arrow). The sarcolemma of the heart muscle also appears infected (black arrow). Scale = 10µm.

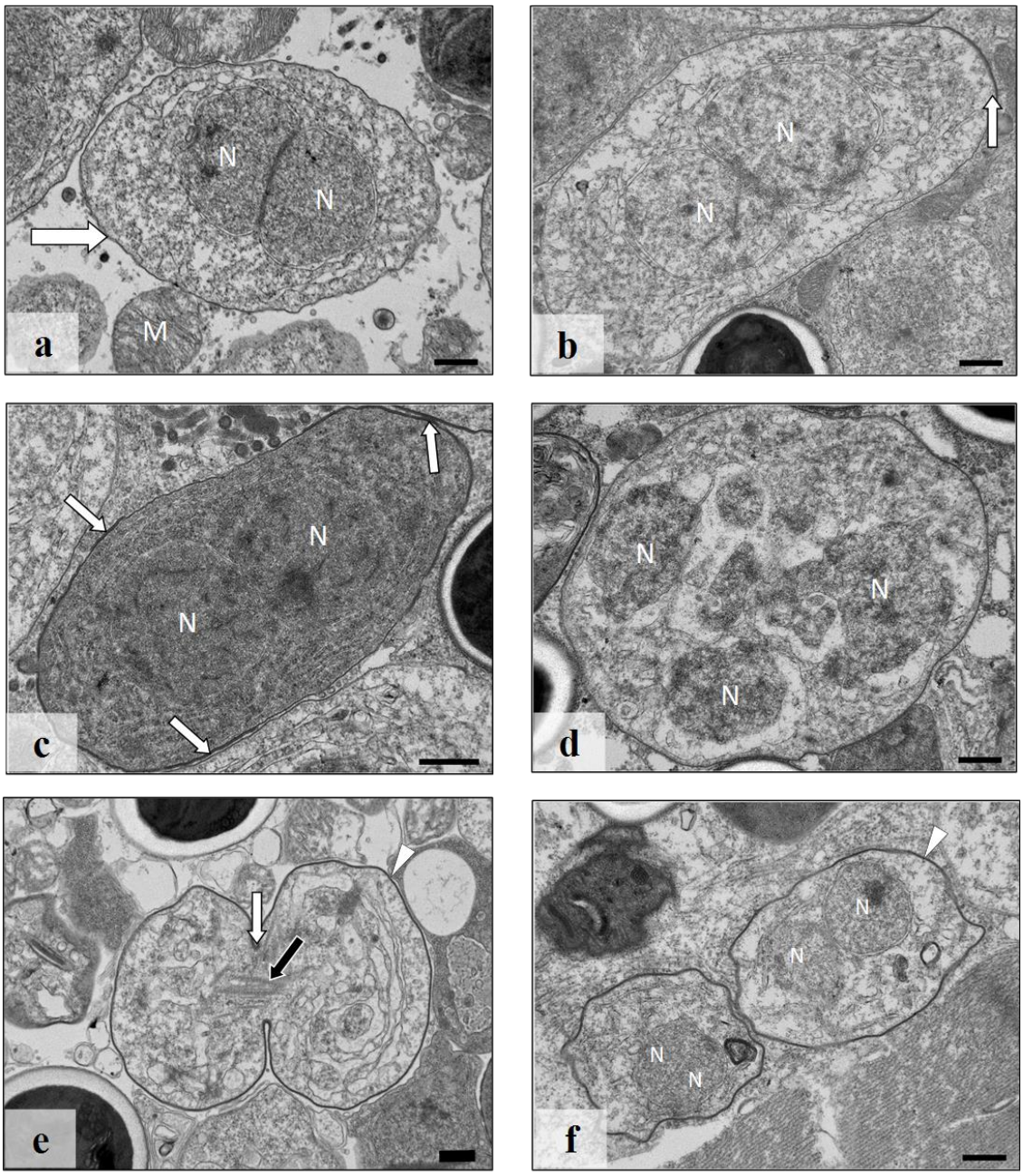


Figure 5.2: Merogony of *Cucumispora ornata* n. sp. in the musculature of *Dikerogammarus haemobaphes*. a) Diplokaryotic meront. Host mitochondria (M) appear in close association. Scale = 500nm. b) Diplokaryotic meront with initial wall thickening (white arrow). Scale = 500nm. c) Diplokaryotic meront to diplokaryotic sporont transition. White arrows indicate thickening cell membranes. Scale = 500nm. d) A tetranucleate cell. Scale = 500nm. e) Binary fission of a tetranucleate cell. The white arrow indicates where the division is occurring and the black arrow indicates the microtubules present. The white triangle highlights the ever thickening cell wall. Scale = 500nm. f) Post-separation of the tetranucleate sporont to two diplokaryotic sporonts. The white triangle highlights the thickness of the cell wall at this developmental stage. Scale = 500nm.

The second pathway, which involves a tetranucleate meront plasmodium stage, served as a multiplication step for the parasite (Fig. 5.2d, e, f) which is skipped during direct formation of the 2n meront to the 2n sporont, seen in pathway one (Fig. 5.2c, d). Both of these pathways appear to lead to the same eventual spore type. In both cases, diplokaryotic sporonts, with thickened cell wall and increasingly electron dense cytoplasm initiate development of spore extrusion precursors, which mark the transition to the diplokaryotic sporoblast (Fig. 5.3a).

Organelles including the anchoring disk, polar filament and condensed polaroplast began to form during development of the sporoblast (Fig. 5.3a). This was followed by thickening of the endospore (Fig. 5.3b) and eventual development of the mature spore (Fig. 5.3c). The mature spore was diplokaryotic, contained an electron dense cytoplasm and 7-9 turns of an isofilar polar filament, arranged in a linear rank at the periphery of the spore (Fig. 5.3c). The polar filament was 115.03nm +/- 3.4nm (n=4) in diameter and comprised of concentric rings of varying electron density (Fig. 5.3d). The manubrial region of the polar filament passed through a bilaminar polaroplast and terminated at an anchoring disk (Fig. 5.3e). The bilaminar polaroplast at the anterior of the spore contained an electron dense outer layer in contact with the plasmalemma, and an electron lucent, folded layer surrounding the polar filament. The polar vacuole occupied approximately 20% of the spore volume at the posterior end and was contained within an electron lucent membrane. Mature spores measured approximately 4.24µm +/- 0.43µm (n=19) in length and 2.03µm +/- 0.19µm (n=23) in width using histologically fixed material and TEM. The spore wall was comprised of a plasmalemma, endospore, exospore and external protein beading (Fig. 5.3f). The endospore was electron lucent, measuring 186.33nm +/- 33.5nm [n=115 (23 spores measured 5 times)] around the majority of the spore, however at the anchoring disk the endospore thinned to a third of its normal thickness (Fig. 5.3e). The exospore measured 39.9nm +/- 11.2nm [n=115 (23 spores)] and the external beads extended approximately 29.05nm +/- 4.5nm (n=15) from the exospore into the host cell cytoplasm (Fig. 5.3f).

On occasion small, electron dense, diplokaryotic cells, often attached to an undefined remnant were observed (Fig. 5.4a, b). Remnants seen in figures 5.4a and 5.4b are only ever present once on these unknown cells and have the appearance of type 1 tubular secretions (as seen in Takvorian and Cali, 1983). Takvorian and Cali (1983), state these secretions are associated with the sporoblast life stage; however these unknown cells in figure 5.4a and 5.4b lack the relevant organelles to be sporoblasts. The cells depicted here (Fig. 5.4a, b) and their accompanying remnants could be an early sporoplasm with

a remnant of the polar filament, aberrant stages of development, or possibly degraded life stages. A diagrammatic representation of the lifecycle is presented in Figure 5.5.

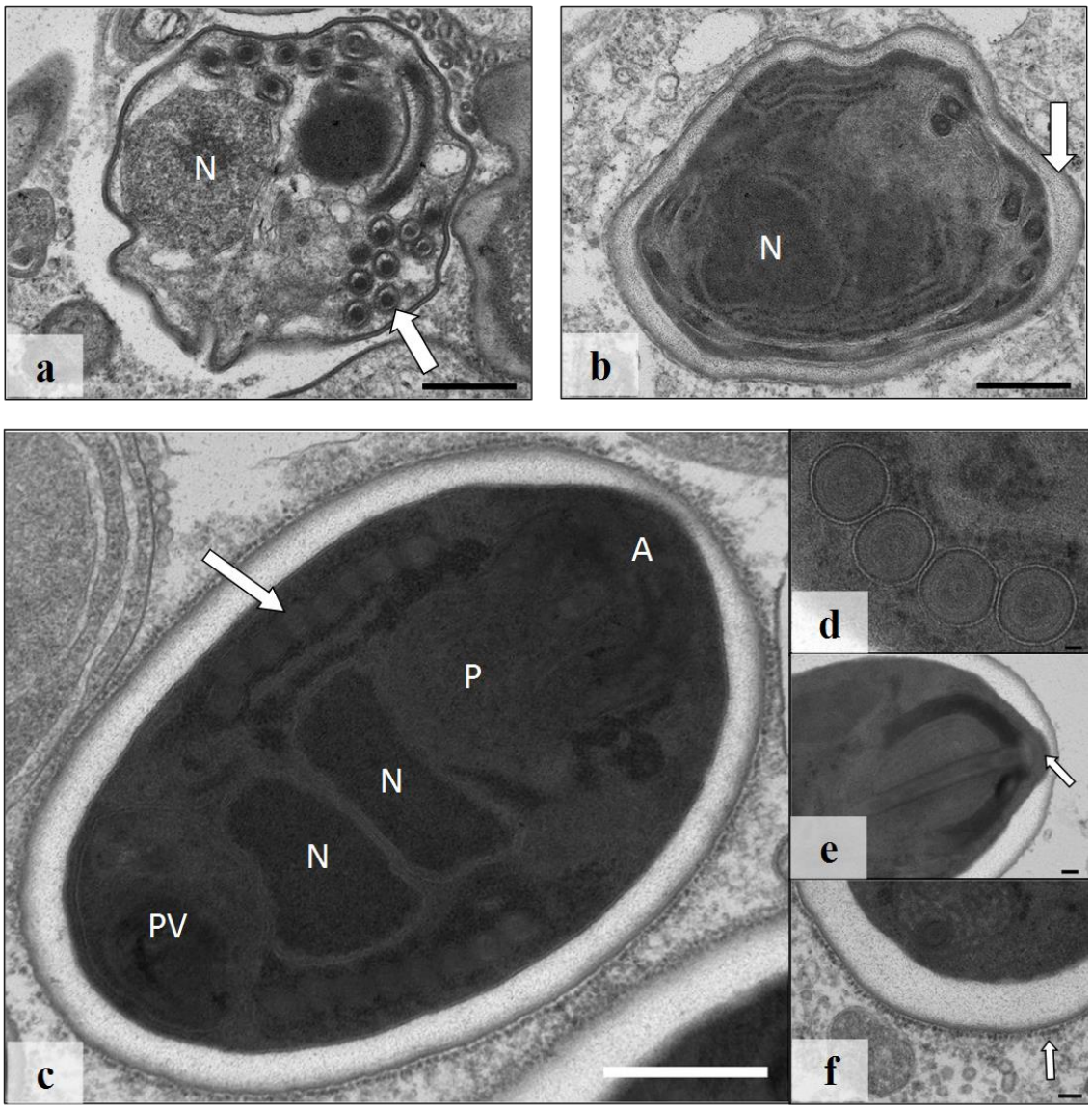


Figure 5.3: *Cucumispora ornata* n. sp. lifecycle progression from the sporoblast to final mature spore. a) The sporoblast, present with nuclei (N) and developing polar filament (white arrow). Scale = 500nm. b) Thickening of the sporoblast endospore (white arrow). Scale = 500nm. c) The final diplokaryotic spore life stage with darkened cytoplasm, polar vacuole (PV), nuclei (N), polar filaments (white arrow), polaroplast (P) and anchoring disk (A). Scale = 500nm. d) High magnification of individual turns of the polar filament. Scale = 20nm. e) High magnification image of the anchoring disk and associated thinning of the endospore (white arrow). Scale = 100nm. f) External beading on the exospore. Scale = 100nm.

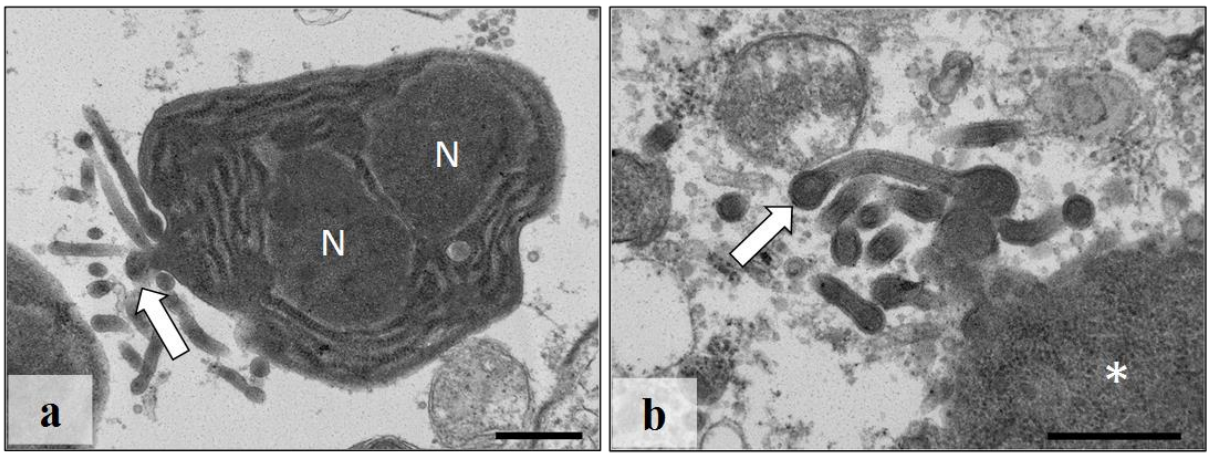


Figure 5.4: Images of the commonly seen, unidentified cells. a) An example cell, present with nuclei (N) and electron dense cytoplasm, was commonly seen during the study. A currently undefined cytoplasmic extrusion is highlighted by a white arrow. Scale = 500nm. b) High magnification image of the cytoplasmic remnant (white arrow) attached to the cytoplasm (*) of the undefined cell. Scale = 500nm.

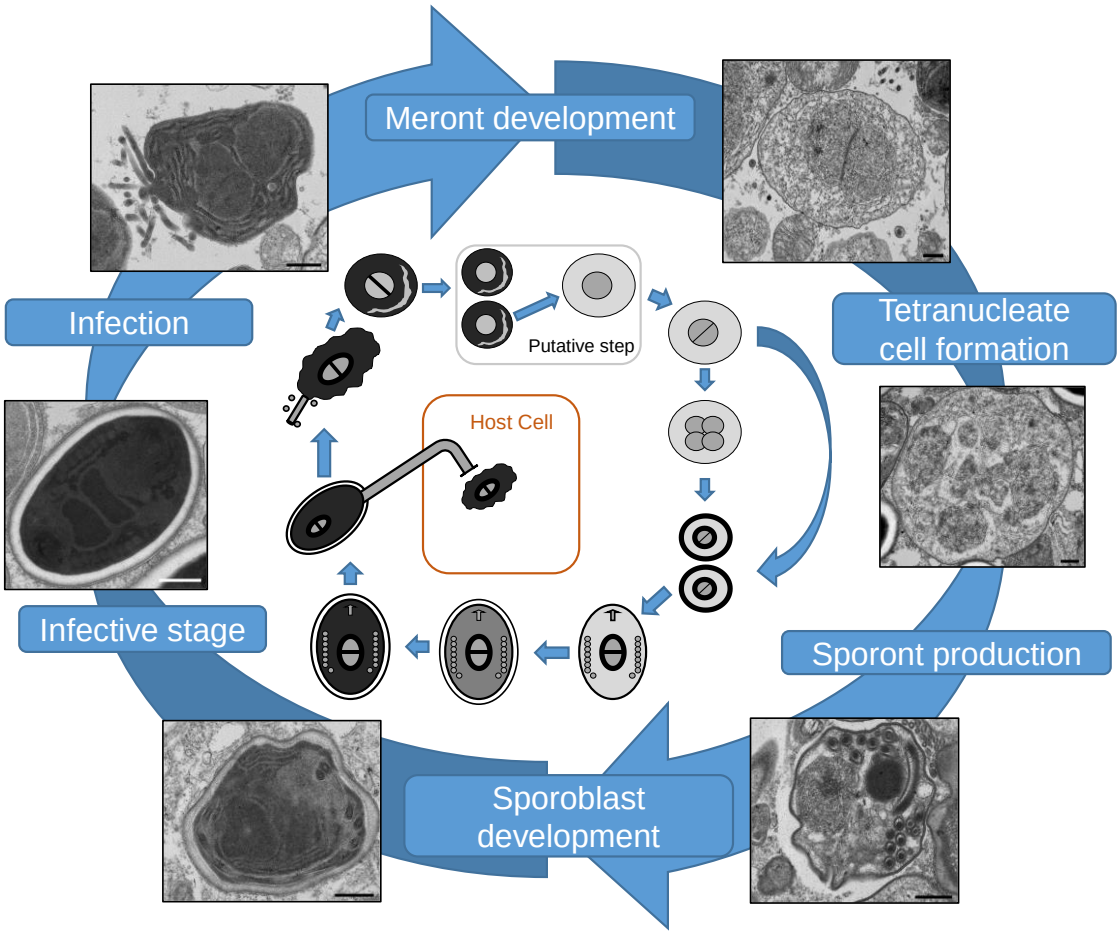


Figure 5.5: A depiction of the lifecycle of *C. ornata* within the host cell.

5.4.2. Molecular phylogeny

Molecular phylogeny of the microsporidium parasite infecting *D. haemobaphes* was based upon a partial sequence of the SSU rRNA gene retrieved from histopathologically confirmed infected host material. A 1186bp sequence of the SSU rRNA gene retrieved BLAST (NCBI) comparisons with 98% similarity to "*Microsporidium sp.* JES2002G" (AJ438962.1) (query cover = 99%, ident.= 98%), a parasite infecting *Gammarus chevreuxi* from the UK, and to *Cucumispora dikerogammari* (91% sequence identity), a microsporidium parasite infecting *D. villosus* from continental Europe (Ovcharenko et al. 2010) - a close taxonomic relation to *D. haemobaphes*. Phylogenetic assessment using a neighbour joining analysis grouped this parasite (to be named *Cucumispora ornata*) with closely related BLAST hits (*Microsporidium* sp.) and *C. dikerogammari* (Fig. 5.6) (bootstrap value of 100). The phylogenetic analysis presented here utilised the majority of the microsporidium sequences presented by Ovcharenko et al (2010) in their description of *C. dikerogammari*. The closely related *Microsporidium sp.* JES2002G (98% sequence identity) is distanced from *C. ornata* by a short branch length of 0.009 (relative genetic change), highlighting their similar sequence identity. *Cucumispora dikerogammari* and the parasite observed here are parted by a distance of 0.086 on the phylogenetic tree, with the closest member outside this group being *Spraguea lophii* (AF056013) with a branch distance, from the parasite, of 0.222.

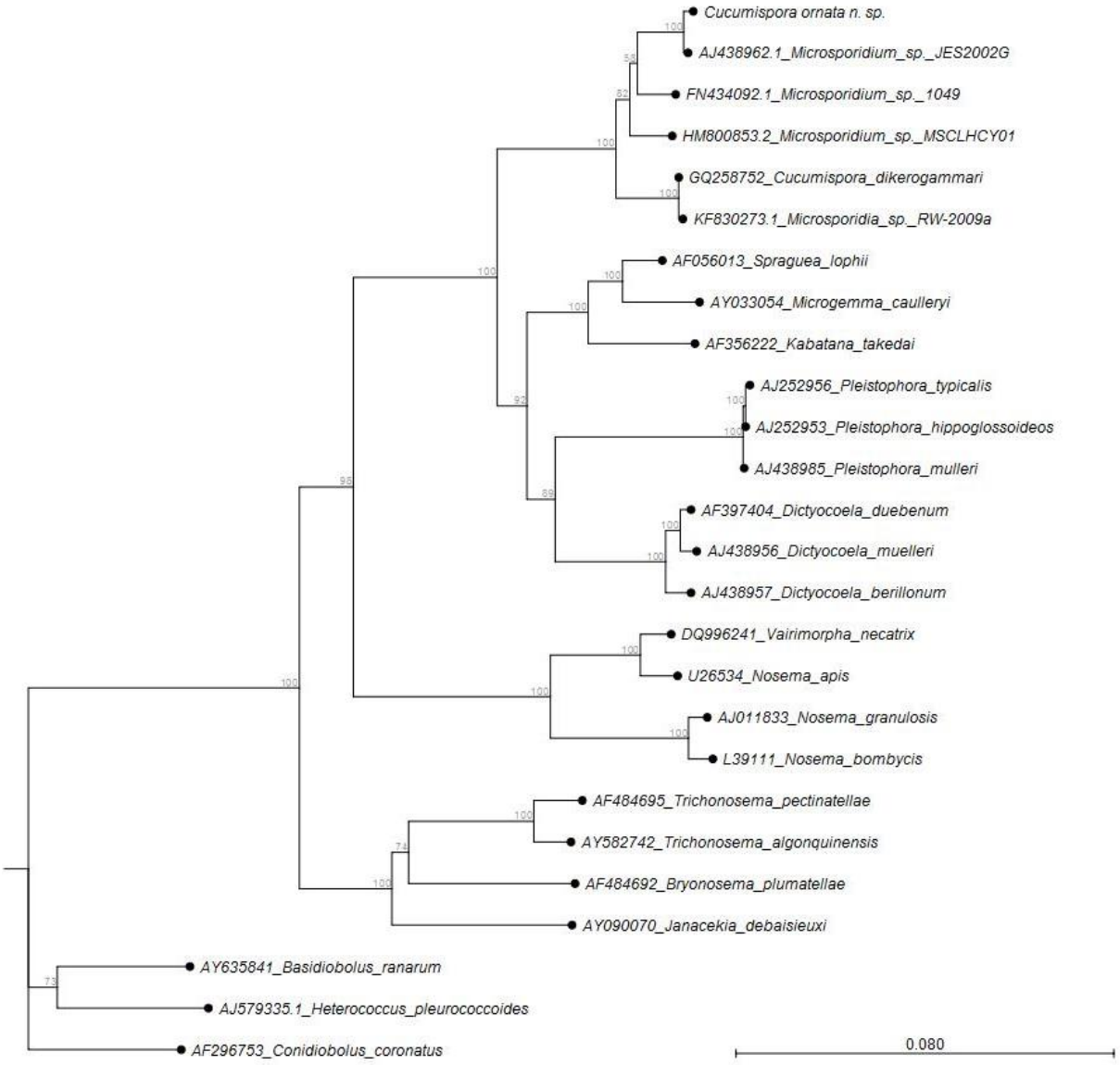


Figure 5.6: Neighbour joining phylogenetic tree using partial SSU rRNA gene sequences from microsporidia in CLC workbench. *Basidiobolus ranarum* (AY635841), *Heterococcus pleurococcoides* (AJ579335.1) and *Conidiobolus coronatus* (AF296753) are used as out-group species.

5.5. Taxonomic Summary

***Genus*:** *Cucumispora* (Ovcharenko et al. 2010)

In all developmental stages the nuclei are diplokaryotic and develop in direct contact with the host cell cytoplasm. Merogonic and sporogonic stages divide by binary fission. Each sporont produces 2 elongate sporoblasts which develop into 2 elongate spores with thin spore walls, uniform exospores and isofilar polar filaments arranged in 6–8 coils. The angle of the anterior 3 coils differs from that of subsequent coils. A thin, umbrella-shaped, anchoring disc covers the anterior region of the polaroplast, which has 2 distinct lamellar

regions, occupying approximately one fourth of the spore volume. The parasite infects gammaridean hosts and infects primarily muscle tissue but can also occur in other tissues (adapted from Ovcharenko et al. 2010).

Type species: *Cucumispora ornata* n. sp.

Species description: Using histology and TEM, spores appear ellipsoid (4.24µm +/- 0.43µm in length and 2.025µm +/- 0.19µm in width), with an endospore (186.33 nm +/- 33.5nm) and externally beaded (decorated) exospore (40nm +/- 11.2nm). The polar filament turns between 7-9 times. The spores are diplokaryotic with a diplokaryotic lifecycle except for the putative presence of a unikaryotic meront. The lifecycle follows closely that of the initially described species *C. dikerogammari* but is morphologically dissimilar in some aspects, including a shorter spore length, coil turns and external beading. Relation by SSU rDNA phylogeny to *C. dikerogammari* is 91%. No transmission information is currently available. *Dikerogammarus haemobaphes* is currently the only known host but falls within the Gammaridae.

5.5.1. Cucumispora ornata *n. sp. taxonomy*

Type host: *Dikerogammarus haemobaphes* Eichwald, 1841 (common name: demon shrimp)

Type locality: The River Trent (United Kingdom) and adjacent, connected waterways (SK3870004400 and SK1370013700). A confirmed site of an invasive population of *Dikerogammarus haemobaphes*. It is unknown whether this parasite exists in populations of *D. haemobaphes* in their native range.

Site of infection: Infections appear systemic, but infecting the musculature primarily. Connective tissues between the gut and gonad, musculature, nervous system and carapace are often infected in advanced cases.

Etymology: "*Cucumispora*" (Ovcharenko et al. 2010) is so named due to the elongated, "cucumiform" spore morphology of initially described species *Cucumispora dikerogammari* (Ovcharenko and Kurandina, 1987; Ovcharenko et al. 2010). The specific epithet "*ornata*" is derived from the Latin word "ornatum" which means "adorned" in English. This refers to the external beading covering the exterior of the spore life stages of this organism.

Type material: Histological sections and TEM resin blocks from the UK specimens are deposited in the Registry of Aquatic Pathology at the Cefas Weymouth Laboratory, UK.

Cucumispora ornata SSU rRNA gene sequences from samples collected in the United Kingdom have been deposited in Gen-Bank (accession number: KR190602).

5.6. Discussion

In this study I describe a novel microsporidium parasite infecting an invasive gammarid, *D. haemobaphes*, from UK fresh waters. The parasite is herein named as *Cucumispora ornata* n. sp. based upon host ecology, histological and ultrastructural pathology, and partial sequencing of the SSU rRNA gene of the parasite. Given that *C. ornata* has not previously been described infecting gammarids (or other hosts) from UK waters it is presumed that it was similarly introduced during the invasion of its host after 2012. Since initial description of this microsporidian, Grabner et al (2015) have identified the species from German territories, and Polish researchers have placed identical SSu sequence data onto BLAST from Polish sources. In addition this microsporidian was also detected via histology in Chapter 3. Whether *C. ornata* n. sp. is present within the hosts native range (Ponto-Caspian Region) has yet to be determined.

5.6.1. Taxonomy of Cucumispora ornata *n. sp.*

Sequencing of the partial SSU rRNA gene of *C. ornata* revealed a closely related branch containing this parasite, three unassigned '*Microsporidium*' species infecting other Crustacea ('*Microsporidium'* is a holding genus according to Becnel et al. 2014 until further information is acquired) and *C. dikerogammari* infecting the sister gammarid *D. villosus* (Fig. 5.6). The close similarity and cladding of the 98% similar "*Microsporidium* sp*.* JES2002G" does suggest that these species could be the same microsporidian. However, without histological and morphological identity it is impossible to be sure at this time. *Cucumispora ornata* n. sp. is now known to infect *Gammarus* sp. (from which *Microsporidium* sp*.* JES2002G SSU was originally identified) (Chapter 8), meaning this could likely harbour infection. Detailed studies of the species *Microsporidium* sp*.* JES2002G was identified from could help to identify if this is *C. ornata* n. sp.

Within the phylogenetic tree, *C. dikerogammari* and *C. ornata* shared 91% sequence identity, with higher similarity between *C. ornata* and the unassigned *Microsporidium* taxa available in BLAST. Although I acknowledge the relatively low similarity between the partial SSU rRNA gene sequence between *C. ornata* and *C. dikerogammari*, since both have a similar lifecycle, are muscle-infecting parasites of congeneric hosts, with an additional three unassigned parasites (also in gammarids and copepods) as branch relatives, I have elected to assign the parasite described herein to the genus

Cucumispora. A quickly evolving SSU rRNA gene may account for the relatively low genetic similarity between *C. ornata* and *C. dikerogammari*. Relative gene sequence evolution, primarily in the SSU genes, is known to vary between microsporidia (Philippe, 2000; Embley and Martin, 2006). Considering this, I propose that the remaining three *Microsporidium* taxa described in studies by Terry et al. (2004), Jones et al. (2010) and Krebes et al. (2010) are also likely to be members of this genus given their (relatively) close SSU sequence identity and shared choice of crustacean hosts.

The placement of this novel parasite in to the genus *Cucumispora* is largely supported by ultrastructural and lifecycle characteristics such as a diplokaryotic spore, development in direct contact with the host cell cytoplasm, some similar spore features (bilaminar polaroplast and thin anchoring disk) and predilection for similar host tissues and organs are shared between *C. dikerogammari* (Ovcharenko et al. 2010) and the parasite described herein. Although I report putative uninucleate (1n) meronts in *C. ornata* (a feature not observed in *C. dikerogammari*), my confidence in reporting this trait is low given the limitations of TEM for detection of uninucleate life stages. However, diplokaryotic stages predominate the lifecycle and follow the development process observed for *C. dikerogammari*. The morphology of *C. ornata* does differ from *C. dikerogammari* in respect to spore length, the presence of a beaded exospore and a thicker endospore, however morphology is often not a reliable tool for microsporidian taxonomy (Stentiford et al. 2013b). Differing features, such as the beaded exospore, when taken together with reasonable genetic variation in the SSU rRNA gene (9% difference between *C. ornata* and *C. dikerogammari*) may eventually be revealed to be sufficient for the erection of a novel genus to contain this parasite, but further information may be needed from other members of the *Cucumispora* before this can be reassessed. Concatenated phylogenies, based upon non-ribosomal protein coding genes and studies on fresh (live) material (not histologically processed) have the potential to assist definition and answer developmental queries of novel taxa in such instances and may prove fruitful for further study of this parasite (Stentiford et al. 2013b).

5.6.2. Cucumispora ornata *n. sp. as an invasive species*

Parasites that are transferred from ‘exotic’ locations can also be deemed as invasive (Dunn, 2009). Just like their hosts, invasive parasites have been shown in the past to cause negative effects on native fauna and ecosystems by either infecting native species or facilitating their hosts’ invasive capabilities (Prenter et al. 2004; Dunn et al. 2009). The ecological impact of *C. ornata* n. sp. is likely to be of considerable interest for the invasion of the host, and for the invaded freshwater community. The parasite reaches high burden

in the host and causes a systemic pathology, primarily targeting the muscle tissues. Prevalence was also relatively high (72.8%). It is probable therefore that this parasite has a regulatory effect on the *D. haemobaphes* host population which may, in turn, moderate the potential impact of the invader (explored further in Chapter 9). Alternatively, *C. ornata* could have a detrimental impact on native species should transmission to new species occur, and in Chapter 9 it is identified as a pathogen of native *Gammarus pulex*. High spore densities were observed in the muscle of infected individuals suggesting that intraguild predation may provide opportunities for transmission. The related microsporidium species, *C. dikerogammari* preferentially infects Ponto-Caspian amphipods but has been found to infect a variety of other amphipod species at low prevalence (Ovcharenko et al. 2010; Bacela-Spychalska et al. 2012; Bacela-Spychalska et al. 2014), and it is possible that *C. ornata* may be similarly generalist. It is important therefore that future work investigates the specificity of *C. ornata* and its virulence should it infect native hosts.

5.6.3. The future of Cucumispora ornata *n. sp. in the UK*

Future assessment of *C. ornata* should include host range and capability for invasive species control (followed up in Chapter 9). Movement of these invaders facilitates the movement of their pathogens so tracking the spread of this invasion is an important endeavour (Anderson et al. 2014). It may be interesting to consider that demon shrimp and killer shrimp do not currently co-exist in the UK. Were they to co-habit a location, it would provide the opportunity to transfer parasites. The introduction of microsporidia to killer shrimp populations in the UK has been suggested as a future possibility for controlling, otherwise unmanageable, populations that lack these parasites (Bojko et al. 2013). The presence of *C. ornata* in UK waterways may provide such an opportunity. Microsporidia have been adapted as biocontrol agents in the past and have shown to be effective in this role (Hajek and Delalibera Jr, 2010) however the application of microsporidian biological control agents to control an invasive species in an ecosystem setting has not been previously attempted.

CHAPTER 6

Parasites, pathogens and commensals in the "low-impact" non-native amphipod host *Gammarus roeselii*

6.1. Abstract

Whilst vastly understudied, pathogens of non-native species (NNS) are increasingly recognised as important threats to native wildlife. This study builds upon recent recommendations for improved screening for pathogens in NNS by focusing on populations of *Gammarus roeselii* in Chojna, north-western Poland. At this location, and in other parts of Continental Europe, *G. roeselii* is considered a well-established and relatively 'low-impact' invader, with little known about its underlying pathogen profile and even less on potential spill-over of these pathogens to native species.

Using a combination of histological, ultrastructural and phylogenetic approaches, I define a pathogen profile for non-native populations of *G. roeselii* in Poland. This profile comprised Acanthocephala (*Polymorphus minutus*, *Pomphorhynchus* sp.), digenean trematodes, commensal rotifers, commensal and parasitic ciliated protists, gregarines, microsporidia, a putative rickettsia-like organism, filamentous bacteria and two viral pathogens, the majority of which are previously unknown to science. To demonstrate potential for such pathogenic risks to be characterised from a taxonomic perspective, one of the pathogens, a novel microsporidian, is described based upon its pathology, developmental cycle and SSU rRNA gene phylogeny. The novel microsporidian is named *Cucumispora roeselii* n. sp. and displayed morphological and phylogenetic similarity to two previously described taxa, *Cucumispora dikerogammari* and *Cucumispora ornata*.

In addition to this discovery extending the host range for the genus *Cucumispora* outside of the amphipod host genus *Dikerogammarus*, I reveal significant potential for the co-transfer of (previously unknown) pathogens alongside this host when invading novel locations. This study highlights the importance of pre-invasion screening of low-impact NNS and, provides a means to document and potentially mitigate the additional risks posed by previously unknown pathogens.

6.2. Introduction

Understanding and interpreting the role played by pathogens in the invasion mechanisms of their hosts is becoming increasingly important as legislative pressure is placed upon managers to prevent and control wildlife disease (Dunn and Hatcher, 2015; Roy et al. 2016). Often, the pathogens of invasive hosts are little known or cryptic, requiring dedicated screening efforts to elucidate underlying parasites and pathogens that may be vectored to new habitats by non-native species (NNS) (Bojko et al. 2013; Roy et al. 2016).

The Amphipoda constitute a diverse crustacean group with many species displaying invasive characteristics that have spread throughout Europe via invasion corridors (Bij de Vaate et al. 2002). Poland is considered part of one such invasion corridor connecting the Ponto-Caspian region to Western Europe (Bij de Vaate et al. 2002; Grabowski et al. 2007), making it an important study site for both recipient and donor populations of amphipods destined to reach other parts of Europe. Most non-native amphipod taxa found in Poland originate from the Ponto-Caspian region, however some exceptions exist. One example is *Gammarus roeselii* Gervais, 1835, of Balkan origin and documented to have invaded Western Europe (including Poland, Italy, France and Germany over a century ago), with relatively low impact (Karaman and Pinkster, 1977; Jażdżewski, 1980; Barnard and Barnard, 1983; Médoc et al. 2011; Lagrue et al. 2011). This species continues to extend its non-native range, now encompassing the Apennine Peninsula (Paganelli et al. 2015). Although the host *per se* is considered a low impact NNS (Trombetti et al. 2013), current risk assessments associated with its spread do not take account of its underlying pathogen profile, nor the effect of these pathogens on receiving hosts and habitats.

Several pathogens of *Gammarus roeselii* are known, including the acanthocephalans *Polymorphus minutus* (Médoc et al. 2006); *Pomphorhynchus laevis* (Bauer et al. 2000) and *Pomphorhynchus tereticollis* (Špakulová, et al. 2011); and the microsporidians *Dictyocoela muelleri* (Haine et al. 2004); *Dictyocoela roeselii* (Haine et al. 2004); *Nosema granulosis* (Haine et al. 2004); and several *Microsporidium* spp. (Grabner et al. 2015; Grabner et al. 2016) (Table 6.1).

Parasite Taxa:	Species:	Location:	Available Data:	Reference:
Acanthocephala	*Polymorphus minutus*	France	Visual	Médoc et al. 2006
	Pomphorhynchus tereticollis	Denmark	DNA seq. and visual	Špakulová et al. 2011
	Pomphorhynchus laevis	France	Visual	Bauer et al. 2000
Microsporidia	*Dictyocoela muelleri*	France	DNA seq.	Haine et al. 2004
	Dictyocoela roeselii	France	DNA seq.	Haine et al. 2004
	Nosema granulosis	France	DNA seq.	Haine et al. 2004
	Microsporidium sp. G	Germany	DNA seq.	Grabner et al. 2015
	Microsporidium sp. 505	Germany	DNA seq.	Grabner et al. 2015
	Microsporidium sp. nov. RR2	Germany	DNA seq.	Grabner et al. 2015
	Microsporidium sp. nov. RR1	Germany	DNA seq.	Grabner et al. 2015
	Microsporidium sp. group F	Germany	DNA seq.	Grabner, 2016
	Microsporidium sp. group E	Germany	DNA seq.	Grabner, 2016
	Microsporidium sp. 2	Germany	DNA seq.	Grabner, 2016

Table 6.1: Species associated with *Gammarus roeselii* and available reference for each association.

Acanthocephala infecting *G. roeselii* cause various behavioural (Bauer et al. 2000), physiological (Rampus and Kennedy, 1974) and transcriptomic changes (Sures and Radszuweit, 2007), which may alter their host's invasive capability. Some of the microsporidia infecting *G. roeselii* (Table 6.1) are associated with other invasive amphipod hosts (Terry et al. 2004; Bojko et al. 2015; Grabner et al. 2015). '*Microsporidium* spp.*'* infecting *G. roeselii* may reside within the genus *Cucumispora*. This genus contains two species isolated from amphipods: *Cucumispora dikerogammari* (Ovcharenko et al. 2010) and *Cucumispora ornata* (Bojko et al. 2015). Like their hosts, members of the genus *Cucumispora* may be of Ponto-Caspian origin due to their identification within tissues of *Dikerogammarus* spp*.* native to that region (Ovcharenko et al. 2010). The detection of *Cucumispora*-like sequences (based upon PCR diagnostics and sequencing) in non-native *G. roeselii* originating from the Balkans, suggests that microsporidia belonging to the *Cucumispora* have a range extending further than the Ponto-Caspian region depending on whether *G. roeselii* is a co-evolved host (Grabner et al. 2015). *Cucumispora* spp*.* are associated with a variable host range, inferring there is a possibility for transmission from Ponto-Caspian invaders meaning *Cucumispora* spp. are likely emerging diseases among amphipods (Bacela-Spychalska et al. 2012).

In order to understand the pathogen profile of a low-impact non-native species and assess the risk of pathogen introduction from such an invader, I surveyed a population of *G. roeselii* in north-western Poland with an aim to understand which pathogen groups were present, whether the pathogen profile of a low-impact invader was different from high-impact invaders and, whether these pathogens pose a significant threat to native wildlife. I present the outcome of that survey here as the first comprehensive pathogen survey of *G. roeselii*. I define an array of novel pathogens associated with this host and

taxonomically define a new member of the microsporidian genus *Cucumispora* (hereby, *Cucumispora roeselii* n. sp.) infecting *G. roeselii*. I discuss these results relative to the impact of these pathogens on population success and impact in Poland, their potential risk of transfer with further spread of this host across Europe and the importance of screening low-impact, non-native species for pathogens without simply focussing on screening high-impact invasive hosts.

6.3. Materials and Methods

6.3.1. Collection, dissection and fixation of Gammarus roeselii

Gammarus roeselii were sampled using standard hydrobiological nets and kick-sampling from the banks of a stream in Chojna, north-western Poland (Oder river catchment) (52.966, 14.42906) on 23/06/2015, as described in Chapter 3. A total of 156 specimens were collected: 8 were fully dissected to remove muscle and hepatopancreas to fix for histology (Davidson's freshwater fixative), transmission electron microscopy (TEM) (2.5% Glutaraldehyde) and molecular diagnostics (96% Ethanol), and 148 were injected on site with fixative for histological screening. Carcasses in fixative, or live animals, were transported to Łódź University, Poland for storage and/or dissection. The samples used in this chapter also cross over with the *G. fossarum* collected in Chapter 3.

6.3.2. Histopathology and transmission electron microscopy

Specimens preserved in Davidson's freshwater fixative were transferred to 70% methylated spirit after 24 - 48 hr and infiltrated with paraffin wax using an automated tissue processor (Peloris, Leica Microsystems, UK). Wax embedded tissues were then sectioned a single time through the centre of the specimen on a Finesse E/NE rotary microtome (Thermofisher, UK) (3-4µm thickness). Sections were glass mounted and stained using haematoxylin and alcoholic eosin (H&E) and examined using a Nikon Eclipse E800 light microscope. Images were captured using an integrated LEICA™ (Leica, UK) camera.

Sample preparation and observation for transmission electron microscopy (TEM) followed that used in Chapter 5 for muscle and hepatopancreas tissues dissected from *G. roeselii* and should be referred to for the full-detail TEM process.

6.3.3. Molecular diagnostics

Muscle tissue dissected from a single infected *G. roeselii* was confirmed positive, via visual, histology and TEM diagnostics, for microsporidiosis. Sympatric tissues from the same individual were fixed in ethanol upon dissection, and used for DNA extraction. DNA extraction was performed using a standard phenol-chloroform method. SSU rRNA gene amplification was performed using the MF1 (5'- CCGGAGAGGGAGCCTGAGA -3') and MR1 (5'- GACGGGCGGTGTGTACAAA -3') primers developed by Tourtip et al. (2009) and 2.5µl of DNA template (~30ng/µl) in a GoTaq flexi PCR reaction (reaction^{-1}: 1µM of each primer; 0.25M of each dNTP; 1.25U of Taq Polymerase; 2.5mM $MgCl_2$) at 50µl total volume. T_c settings were: 94˚C (5 min), 94˚C-60˚C-72˚C (each 1 min; 35 cycles), 72˚C (10 min). Amplicons were observed using gel electrophoresis on a 2% agarose gel (30min/120V) producing a microsporidian band at ~800bp. This band was excised and purified for forward and reverse sequencing via Eurofins genomics barcode-based sequencing service (Eurofinsgenomics, UK).

6.3.4. Phylogenetics and sequence analysis

The final SSU rRNA gene sequence for this microsporidian consisted of an 825bp sequence, which was placed into BLASTn (NCBI) to retrieve identical or close hits. The sequence was placed alongside several SSU rRNA gene sequences used by Ovcharenko et al. (2010) to form the initial description of *C. dikerogammari* (GQ246188.1), as well as some closely linked, recently described microsporidian sequences [*C. ornata* (KR190602.1); *Paradoxium irvingi* (KU163282.1); *Hyperspora aquatica* (KX364284.1), *Unikaryon legeri* (KX364285.1)], and all available partial or complete sequences from BLAST that link with close similarity to *C. dikerogammari* (GQ246188.1) and could potentially be candidates for the genus *Cucumispora*.

The sequences were aligned with MAFFT 7.017 (Katoh et al. 2002) using default values, in Geneious 6.1.8 (Biomatters Inc., 2013). The phylogeny reconstruction was performed in MEGA 7 (Kumar et al. 2016) using the Maximum-Likelihood (Saitou and Nei, 1987a) and Neighbour-Joining (Saitou and Nei, 1987b) methods. Clade credibility was assessed using bootstrap tests with 1000 replicates (Felsenstein, 1985). The T92 model of evolution with gamma-distributed rate heterogeneity (G) was selected for the data set using the complete deletion model selection algorithm implemented in MEGA 7. Clade IV microsporidian species were used as an out-group to root the tree.

6.4. Results

6.4.1. Histological observations

Overall, 156 *G. roeselii* specimens were histologically screened from Chojna, revealing several parasite and pathogen associations. Altogether, 14 associations were catalogued. These included: epibiotic stalked ciliated protists (Fig. 6.1a-b); epibiotic, gill-embedded ciliated protists (Fig. 6.1c); epibiotic filamentous bacteria (Fig. 6.1b); epibiotic rotifers (Fig. 6.1a); a parasitic peritrichioius protist (Fig. 6.1d); gut-dwelling gregarines (Fig. 6.1e); a putative gut virus (Fig. 6.1f); a putative rickettsia-like organism (RLO) in the hepatopancreas (Fig. 6.1g); digenean trematodes (Fig. 6.1h); acanthocephala [including: *Polymorphus minutus* (Fig. 1i) and *Pomphorhynchus* sp. (no image)]; a microsporidian restricted to the hepatopancreas (Fig. 6.1j); a bacilliform virus from the nuclei of the hepatopancreas with confirmed morphological information (Fig. 6.2); and a muscle-targeting microsporidian, which is also taxonomically identified herein using histology (Fig. 6.3), TEM (Fig. 6.4 and 6.5) and phylogenetic analysis (Fig. 6.6). Prevalence information for all parasites and pathogens is contained in Table 6.2.

Parasite group:	Species/Disease	Prevalence	Image Ref.
Viruses	*Gammarus roeselii* Bacilliform Virus	12.2%	Fig. 6.2
	Putative gut virus	2.7%	Fig. 6.1f
Bacteria	Epibiotic filamentous bacteria	100%	Fig. 6.1b
	Putative rickettsia-like organism	<1%	Fig. 6.1g
Microsporidia	*Cucumispora roeselii* n. sp.	12.2%	Fig. 6.3, 6.4, 6.5
	Microsporidium sp. from the hepatopancreas	<1%	Fig. 6.1j
Protists	Epibiotic, stalked, ciliated protists	83.9%	Fig. 6.1a-b
	Epibiotic embedded ciliated protists	83.9%	Fig. 6.1c
	Parasitic ciliated protists	<1%	Fig. 6.1d
	Gut-dwelling gregarines	50.0%	Fig. 6.1e
Metazoa	Epibiotic rotifer	48.6%	Fig. 6.1a
	Digenean trematodes	1.4%	Fig. 6.1h
	Polymorphus minutus	1.4%	Fig. 6.1i
	Pomphorhynchus sp.	4.1%	No image

Table 6.2. Parasites and pathogens associated with *Gammarus roeselii* during this study. The prevalence of each pathogen and parasite in the population sampled from Chojna, Poland, is stated alongside the reference image, if available.

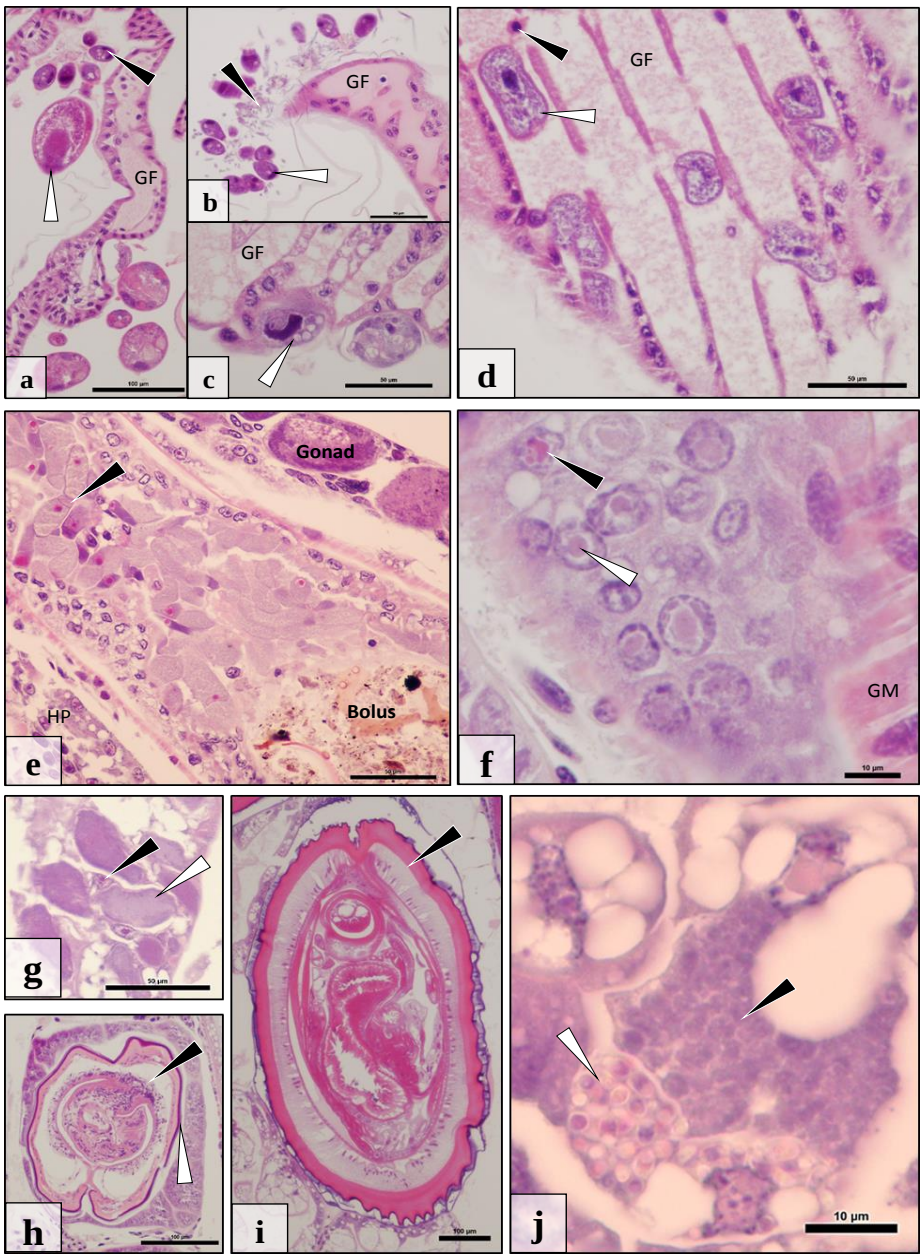


Figure 6.1: Parasites of *Gammarus roeselii*. a) External rotifers (white arrow) and ciliated protists (black arrow) clustered around a gill filament (GF). Scale = 100µm. b) Ciliated protists (white arrow) and filamentous bacteria (black arrow) clustered around a gill filament (GF). Scale = 50µm. c) Ciliated protists (white arrow) embedded into the gill filament (GF). Scale = 50µm. d) Ciliated protists (white arrow) present in the blood stream (blood cell = black arrow) of the gill filament (GF). Scale = 50µm. e) Dense cluster of gregarines (black arrow) in the gut alongside bolus, gonad and hepatopancreas (HP). Scale = 50µm. f) Putative nuclei-targeting gut epithelia virus displaying nuclear hypertrophy due to expanding viroplasm (black and white arrows) (GM = gut muscle). Scale = 10µm. g) Putative rickettsia-like organism in the cytoplasm of hepatopancreatocytes (white arrow). Nucleus (black arrow). Scale = 50µm. h) Digenean (black arrow), present with external pearling (white arrow), encysted internally within *G. roeselii*. Scale = 100µm. i) *Polymorphus* sp. encysted internally within *G. roeselii*. Scale = 100µm. j) Microsporidian pathogen in the cytoplasm of infected hepatopancreatocytes. Developing (black arrow) and spore stages (white arrow) of the pathogen can be clearly identified in separate cells. Scale = 10µm.

The carapace and appendages of *G. roeselii* were often coated with stalked ciliates and epibiotic rotifers (Fig. 6.1a), however the gills and brood pouch were commonly associated will all epibiotic commensals. All epibiotic commensals induced no immune response from the host and were common throughout the *G. roeselii* population (Table 6.2).

A single animal was observed with a ciliated protist infection in the haemolymph, with accumulations of the parasite in the antennal gland, gills (Fig. 6.1d), heart and appendages. No immune response toward the parasitic protist was noted throughout the histological screen.

Gregarines (Apicomplexa) were commonly associated with the gut (50% prevalence) (Fig. 6.1e) and less frequently, the hepatopancreatic tubules (<1%). Gregarines were often seen in large numbers in the gut with both extracellular and intracellular developmental stages with occasional observation of syzygy. Gregarines elicited no apparent immune response from the host but were detected in significant numbers in the gut lumen.

A putative gut-epithelial virus was observed in four individuals where gut nuclei were present with an expanded, eosinophilic viroplasm, resulting in nuclear hypertrophy and marginated host chromatin (Fig. 6.1f). No immune response was observed against this virus in the histology.

A putative RLO in the cytoplasm of hepatopancreatocytes was observed in a single individual (Fig. 6.1g). The cytoplasm of infected cells appeared dense, granular and purple in colour (H&E stain), a common feature of RLO infections in other hosts. Host nuclei were unaffected and no immune responses were observed in affected tissues.

Three metazoa were observed to infect *G. roeselii* (see Table 6.2 for prevalence details). Digenea were encysted in the gut, gonad and hepatopancreas (Fig. 6.1h). Large acanthocephala such as *Polymorphus minutus* (Fig. 6.1i) and *Pomphorhynchus* sp. were present in the same tissue types but not together in the same host. No helminths elicited an immune response from the host.

Two microsporidian infections were observed during screening; the first from the hepatopancreas and the second from the muscle. The microsporidian from the hepatopancreas was observed in a single specimen fixed for histology, meaning that no ethanol or glutaraldehyde fixed materials were taken, resulting in a lack of information for full taxonomic analysis for this species. This microsporidian was present only in the hepatopancreas; specifically, in the cytoplasm of infected cells where several

development stages could be seen in low-detail (Fig. 6.1j) and disintegration of infected tubules was observed. No immune response was observed against this microsporidian.

6.4.2. Gammarus roeselii *Bacilliform Virus: histopathology and TEM*

A novel virus infecting the nuclei of hepatopancreatocytes was observed using histology and TEM. Histologically, the virus was present only in the nuclei of infected hepatopancreatocytes (Fig. 6.2a) and caused host chromatin margination and nuclear hypertrophy due to an expanded viroplasm. Uninfected cell nuclei showed normal chromatin configuration without expanded viroplasm (Fig. 6.2a inset). This viral pathology was present in 12.2% of specimens.

TEM of an infected hepatopancreas tubule and associated cells revealed a viroplasm consisting of large bacilliform virus particles in the host cell nucleus (Fig. 6.2b). Virions were rod-shaped and consisted of an electron dense, cylindrical core (L: 177.4nm ± 18nm, W: 35.9nm ± 6nm) and, were surrounded by a single membrane (L: 224.0nm ± 17nm, W: 70.0nm ± 13nm) (Fig. 6.2c). Currently no genetic data is available for this virus. This novel virus is termed *Gammarus roeselii Bacilliform Virus* (GrBV) until further data can be acquired, to allow for taxonomic identification.

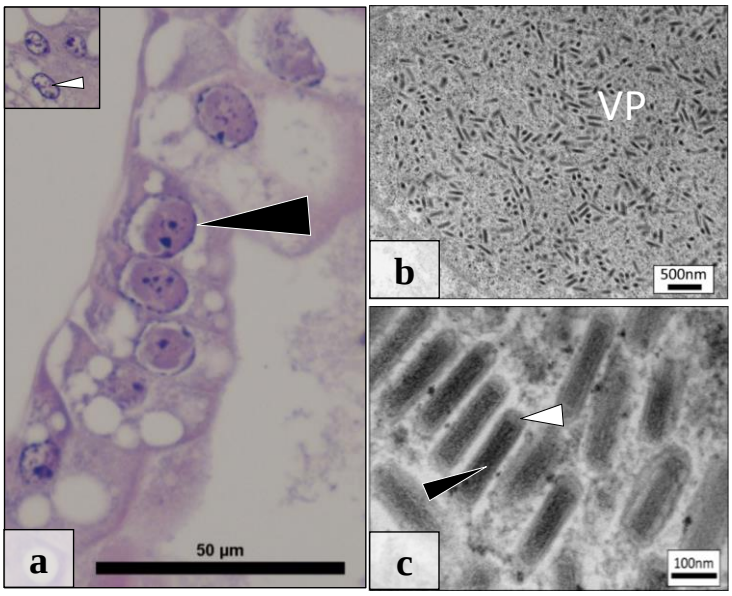


Figure 6.2: *Gammarus roeselii Bacilliform Virus* histopathology and ultrastructure. a) Several virally infected, hypertrophic, nuclei (black arrow) in the hepatopancreas. The inset shares the same magnification and details a cluster of uninfected nuclei (white arrow). Scale = 50µm. b) An electron micrograph detailing a growing viroplasm (VP) in a nucleus of the hepatopancreas. Scale = 500nm. c) High magnification image of the bacilliform virus present with electron dense core (black arrow) and membrane (white arrow) in a paracrystalline array within a heavily infected cell nucleus. Scale = 100nm.

6.4.3. Microsporidian histopathology, TEM and molecular phylogeny

6.4.3.1. Microsporidian histopathology

The microsporidian present in the musculature of *G. roeselii* causes an externally visible opacity in infected amphipods due replacement of muscle fibres with masses of parasites. Histologically, microsporidian spores were seen throughout the musculature of 12.2% of individuals (Fig. 6.3a), with early-stage infections apparently limited to the muscle fibre periphery (Fig. 6.3b). No microsporidian spores were observed in other host organs or tissues. Often, melanisation reactions and, haemocyte aggregation were associated with clusters of spores (Fig. 6.3c) with some evidence of spore phagocytosis by haemocytes. Via histology, mature spores appeared eosinophilic (pink) (Fig. 6.3a) with earlier developmental stages (e.g. meronts) appearing blue-purple in section (Fig. 6.3b).

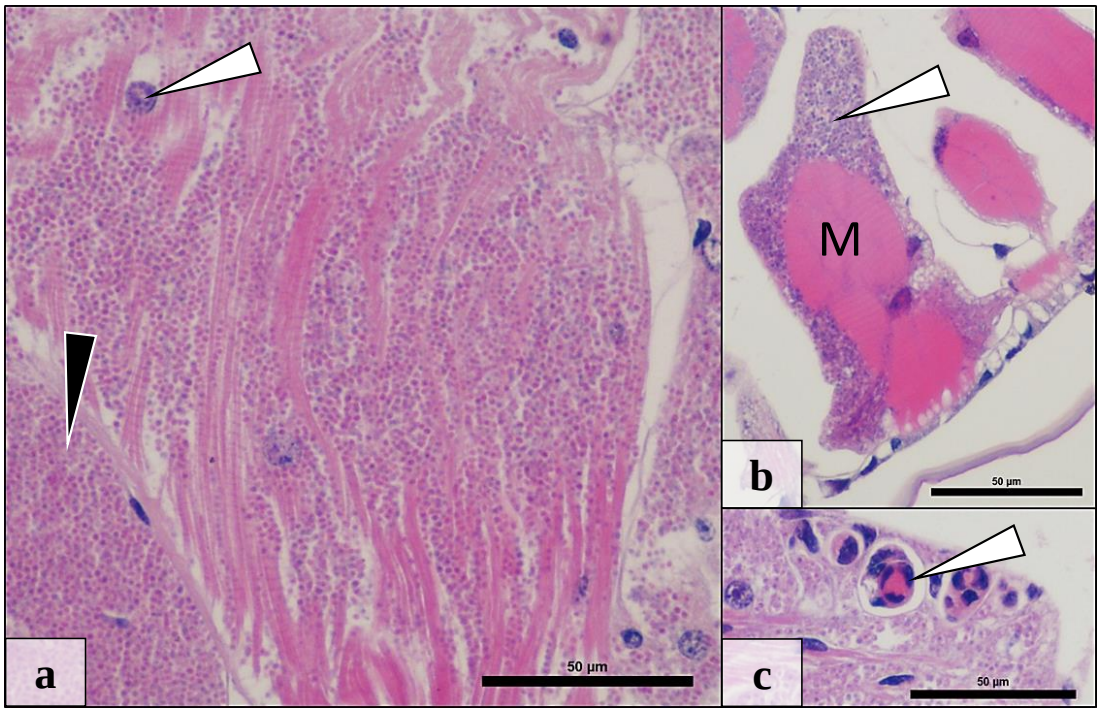


Figure 6.3: *Cucumispora roeselii* n. sp. histopathology. a) Microsporidian spores (black arrow) can be seen throughout the musculature in heavy infections. Muscle nuclei (white arrow) can be seen amongst parasite spores. Scale = 50µm. b) Early stage microsporidian infected muscle blocks (M) demonstrate initial sarcolemma infection (white arrow). Scale = 50µm. c) Immune reactions (white arrow) towards microsporidian infection. Scale = 50µm.

6.4.3.2. Microsporidian life cycle and ultrastructure

Ultrastructurally, the developmental cycle of the microsporidian in *G. roeselii* resembled that observed by Ovcharenko et al. (2010) and, Bojko et al. (2015) for *C. dikerogammari* and *C. ornata*. Infected muscle fibres contained tightly packed merogonial and sporogonial life stages, which developed in direct contact with the host muscle cytoplasm, often in the sarcolemmal space. The microsporidian development began with a diplokaryotic meront (2n) bound by a thin cell membrane (Fig. 6.4a). Nuclear division of the diplokaryotic meront formed a tetranucleate meront plasmodium (2 x 2n) present with a string of four nuclei separated by a thin membrane (Fig. 6.4b). The tetranucleate meront plasmodium can show early thickening of the cell membrane (Fig. 6.4b) prior to its division to form two diplokaryotic sporonts (2n), which show further thickening of the cell membrane prior to any formation of spore extrusion apparatus (Fig. 6.4c-d). Later stage sporonts developed an electron dense cytoplasm prior to formation of early spore extrusion apparatus (Fig. 6.4e). The maturing sporoblast became electron dense and cucumiform in shape, with an early anchoring disk and coiled, irregular-shaped, polar filament in cross-section (Fig. 6.4f). The condensed sporoblast displayed the earliest development of an electron lucent endospore (Fig. 6.4f) and became increasingly turgid during spore maturation (to presume an oval shape) (Fig. 6.5a-b). Further thickening of the electron-lucent endospore, circularisation of the polar filament cross-sections and, development of spore organelles such as the polaroplast and polar vacuole occurred in the late sporoblast (Fig. 6.5a-b). At this stage, the exospores resumed an irregular surface (most clearly seen in the image of the final spore, Fig. 6.5c).

The final diplokaryotic spore was 2.2 µm ± 0.1 µm in length (n=30) and 1.5 µm ± 0.1 µm in width (n=30), contained an anchoring disk, bi-laminar polaroplast, 9-10 turns of the polar filament [cross-sectional diameter: 92nm ± 13nm (n=30)] with rings of proteins at varying electron density, thickened spore wall (plasmalemma, endospore, exospore) and, a ribosome-rich electron dense cytoplasm (Fig. 6.5c). The spore wall was of variable thickness according to location; thinnest at the terminal point of the anchoring disk (40 nm ± 6 nm) and thicker elsewhere (up to 185 nm ± 50 nm).

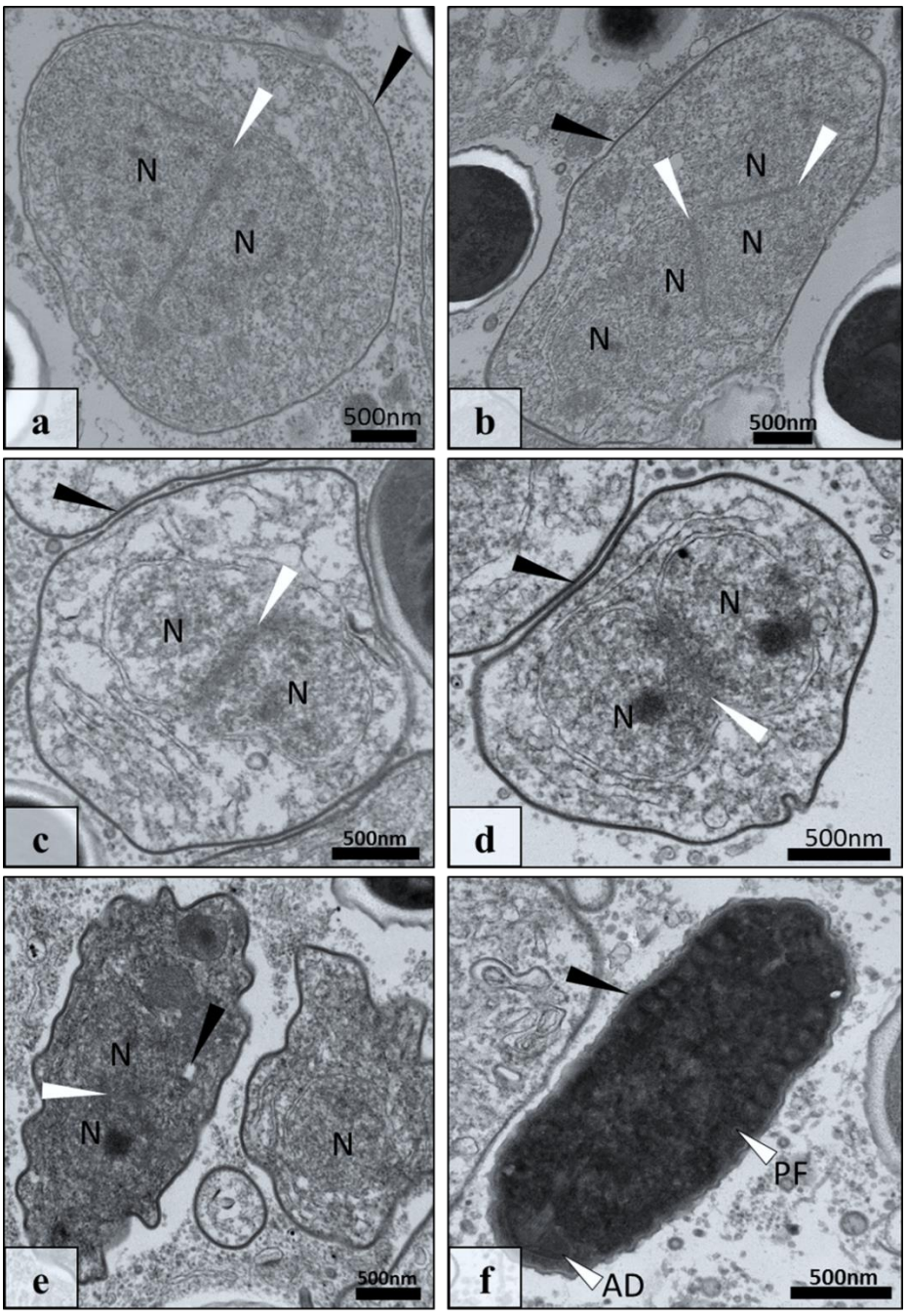


Figure 6.4: Transmission electron micrograph of early spore development for *Cucumispora roeselii* n. sp. a) Diplokaryotic meront displaying attached nuclei (N; white arrow). Note the thin cell membrane (black arrow). Scale = 500nm. b) Tetranucleate cell displaying four attached nuclei (N; white arrows) with a thickening cell wall (black arrow). Scale = 500nm. c) After division, two early diplokaryotic (N; white arrow) sporoblasts are produced with further cell membrane thickening (black arrow). Scale = 500nm. d) Early diplokaryotic (N; white arrow) sporoblast displaying further thickening of the cell membrane (black arrow). Scale = 500nm. e) The early sporoblast begins to become electron dense and condense with some early development of spore organelles such as the polar filament (black arrow). Scale = 500nm. f) Fully condensed sporoblast development stage present with electron dense cytoplasm and coiled polar filament (PF) and anchoring disk (AD). At this stage the formation of the early endospore is visible (white arrow). Scale = 500nm.

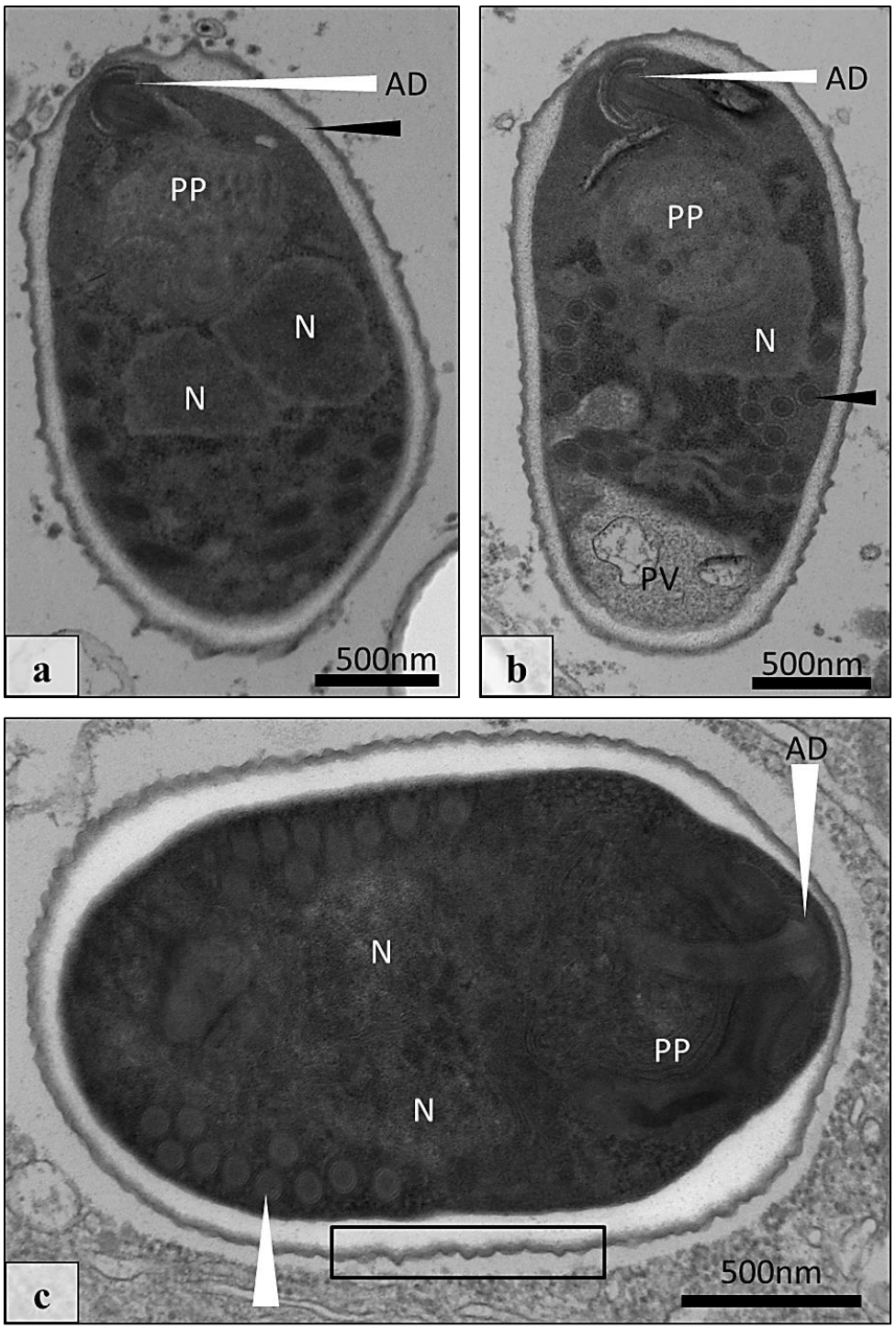


Figure 6.5: Final development stages of *Cucumispora roeselii* n. sp. a) Diplokaryotic sporoblast (N) with anchoring disk (AD), polaroplast (PP) and thickened endospore (black arrow). Scale = 500nm. b) A second sporoblast displaying a clear polar vacuole (PV) and polar filament with rings of varying electron density (black arrow). Scale = 500nm. c) The final diplokaryotic (N) spore with bilaminar polaroplast (PP), anchoring disk (AD) and polar filament (9-10 turns; white arrow). The spore wall thins at the anchoring disk (AD) whilst being thickest at the periphery of the anchoring disk. Note the 'thorned' spore exterior (black rectangle). Scale = 500nm.

6.4.3.3. Microsporidian phylogeny

The amplicon derived from the microsporidian infecting the musculature of *G. roeselii* provided an 825bp sequence of the SSU rRNA gene. This sequence showed closest similarity to *Microsporidium* sp. 1049 (FN434092.1: 98% similarity; query cover: 99%; e-value = 0.0) a microsporidian isolated from *Gammarus duebeni duebeni* from Dunstaffnage Castle (Scotland, UK), and *Microsporidium* sp. MSCLHCY01 (HM800853.2: 96% similarity; query cover: 96%; e-value = 0.0) a microsporidian isolated from the copepod (*Lepeophtheirus hospitalis*) parasitizing the starry flounder (*Platichthys stellatus*) from British Columbia, Canada. The closest fully described species were *C. ornata* (KR190602.1: 95% similarity; query cover: 99%; e-value = 0.0) a microsporidian pathogen isolated from the invasive demon shrimp, *Dikerogammarus haemobaphes*, from the Carlton Brook invasion site, UK, and *C. dikerogammari* (GQ246188.1: 93% similarity; query cover: 96%; e-value = 0.0) a microsporidian isolated from the killer shrimp, *Dikerogammarus villosus*, from an invasion site in France. Several microsporidian SSU sequences show high similarity (~90-100%) to those corresponding to the *Cucumispora* genus and are included in Table 6.3, depicting their host and geographic origin.

This novel microsporidian sequence branches at the base of the *Cucumispora* with mid to low bootstrap confidence (Fig. 6.6). The closest phylogenetic associations are with *Microsporidium* sp. 1049, *Microsporidium* sp. BCYA2 CYA1 (FJ756003.1: 98% similarity; query cover: 63%; e-value = 0.0) and *Microsporidium* sp. BCYA2 CYA2 (FJ756004.1: 98% similarity; query cover: 63%; e-value = 0.0). Each "*Microsporidium* sp." has no supporting developmental or morphological data. The clade identified as "*Cucumispora* candidates" (highlighted in Fig. 6.6) is differentiated (bootstrap support = 90-37%) from the closest taxonomically identified genus: *Hyperspora* (which includes a hyperparasitic microsporidian). Some of the SSU sequences present in the "*Cucumispora* candidates" may be associated with this genus but without developmental or ultrastructural information it is difficult to be sure. The microsporidian sequence isolated by this study is separate from *Microsporidium* sp. MSCLHCY01 (an isolate closely associated with *H. aquatica* at 95-99%) on the tree, despite the overall sequence similarity (96%) (Fig. 6.6).

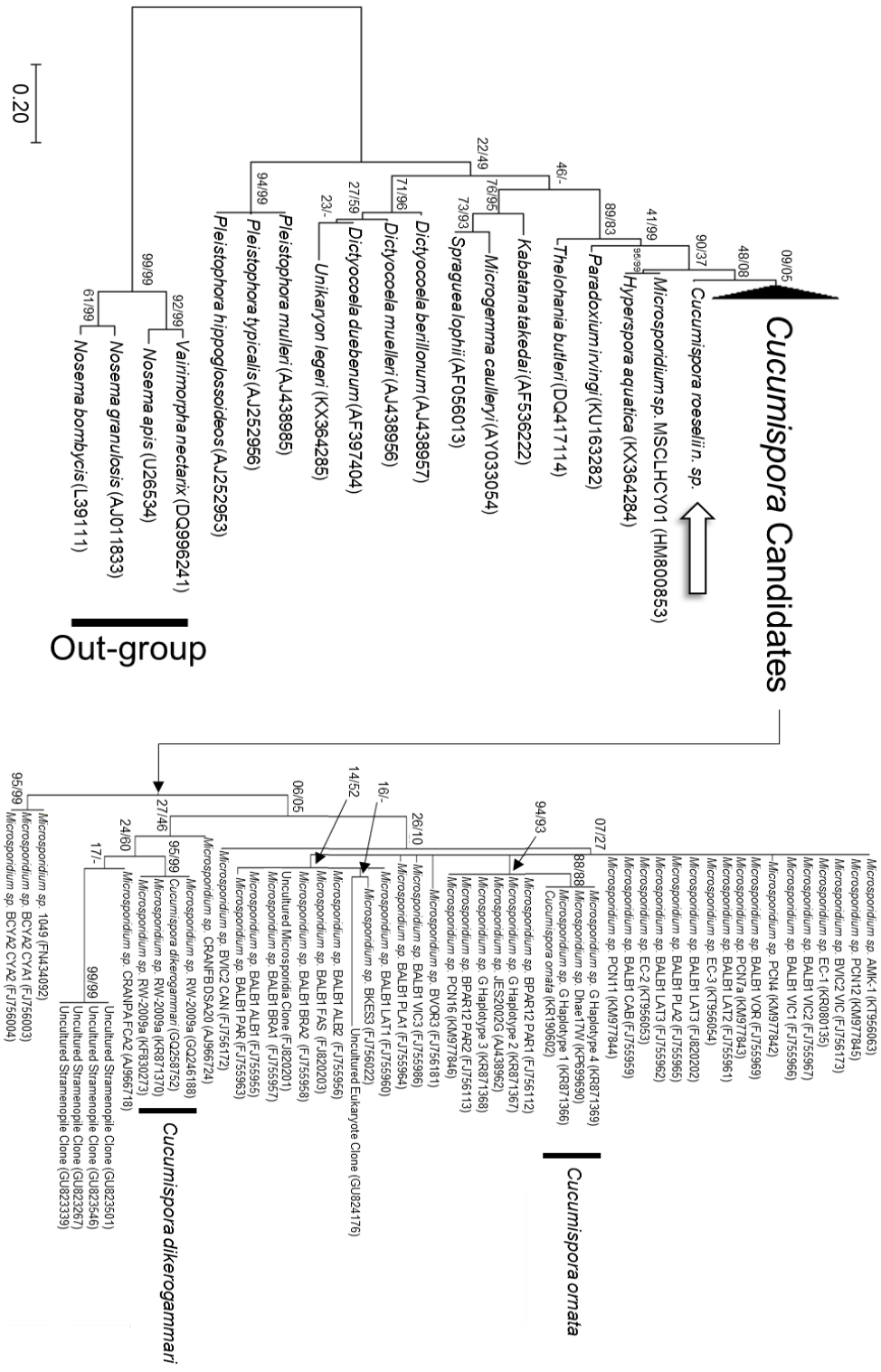


Figure 6.6: A Maximum-Likelihood tree including the bootstrap confidence for ML/NJ phylogenies. If the Neighbour Joining phylogeny did not produce a branch observed on the Maximum-Likelihood tree, a '-' is noted. The tree is displaying the position of *Cucumispora roeselii* n. sp. (white arrow), *Cucumispora*-related SSU isolates ("*Cucumispora* Candidates"), various 'Clade V' representatives, and various 'Clade IV' representatives (Vossbrinck and Debrunner-Vossbrinck, 2005) as an out-group. Sequences belonging to existing members of the *Cucumispora* are labelled with the scientific name after a black line.

Microsporidian SSU isolate	Host	Geographic location	Hosts range	Reference
Microsporidium sp. BALB1 PLA1	*Micruropus platycercus*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 VIC2	*Acanthogammarus victorii*	Russia: Lake Baikal	Native range	Unpublished
Microsporidia clone BALB1 LAT3	*Gmelinoides fasciata*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 PLA2	*Micruropus platycercus*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 LAT3	*Brandtia latissima latior*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 CAB	*Garjajewia cabanisii*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. PCN11	*Pallasea cancellus*	Russia: Lake Baikal	Native range	Adelshin et al. 2015
Microsporidia sp. EC-1	*Eulimnogammarus cyaneus*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. PCN4	*Pallasea cancellus*	Russia: Lake Baikal	Native range	Adelshin et al. 2015
Microsporidium sp. PCN7a	*Pallasea cancellus*	Russia: Lake Baikal	Native range	Adelshin et al. 2015
Microsporidium sp. PCN12	*Pallasea cancellus*	Russia: Lake Baikal	Native range	Adelshin et al. 2015
Microsporidium sp. BALB1 VOR	*Linevichella vortex*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 LAT2	*Brachyuropus grewingkii*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BVOR3	*Linevichella vortex*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 VIC1	*Acanthogammarus victorii*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 BRA1	*Macrohectopus branickii*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 BRA2	*Macrohectopus branickii*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BKES3	*Pallaseopsis kessleri*	Russia: Lake Baikal	Native range	Unpublished
Microsporidia clone BALB1 FAS	*Gmelinoides fasciata*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 PAR	*Dorogostaiskia parasitica*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 ALB2	*Ommatogammarus albinus*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 ALB1	*Ommatogammarus albinus*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BALB1 LAT1	*Brandtia latissima latior*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BVIC2 CAN	*Pallasea cancellus*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BVIC2 VIC	*Acanthogammarus victorii*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. G (Dh4-6)	*D. haemobaphes*	Germany	Invasive range	Grabner et al. 2015
Microsporidium sp. G (Dh2-10)	*D. haemobaphes*	Germany	Invasive range	Grabner et al. 2015
Microsporidium sp. G (Dh2-3)	*D. haemobaphes*	Germany	Invasive range	Grabner et al. 2015
Cucumispora ornata	*D. haemobaphes*	UK: River Trent	Invasive range	Bojko et al. 2015
Microsporidium sp. PCN16	*Pallasea cancellus*	Russia: Lake Baikal	Native range	Adelshin et al. 2015
Microsporidium sp. BPAR12 PAR1	*Dorogostaiskia parasitica*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BPAR12 PAR2	*Dorogostaiskia parasitica*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. G (Gr2-10)	*G. roeselii*	Germany	Invasive range	Grabner et al. 2015
Microsporidium sp. G (Gr2-12)	*G. roeselii*	Germany	Invasive range	Grabner et al. 2015
Microsporidium sp. JES2002G	*Gammarus chevreuxi*	UK: River Avon	Native range	Terry et al. 2004
Microsporidia clone BFAS11	*Gmelinoides fasciata*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. BCYA2 CYA1	*Eulimnogammarus cyaneus*	Russia: Lake Baikal	Native range	Unpublished
Microsporidium sp. 1049	*Gammarus duebeni duebeni*	UK: Scotland	Native range	Krebes et al. 2010
Microsporidium sp. BCYA2 CYA2	*Eulimnogammarus cyaneus*	Russia: Lake Baikal	Native range	Unpublished
Cucumispora roeselii n. sp.	*G. roeselii*	Poland: Chonja	Invasive range	This Study
Microsporidium sp. CRANFB	*Crangonyx floridanus*	USA: River Styx	Native range	Galbreath et al. 2010
Microsporidium sp. CRANPA	*Crangonyx pseudogracilis*	France: Beuvron	Invasive range	Galbreath et al. 2010
Microsporidia sp. RW-2009a	*Dikerogammarus villosus*	France	Invasive range	Ovcharenko, 2010
Microsporidia sp. RW-2009a	*Dikerogammarus villosus*	Poland	Invasive range	Ovcharenko, 2010
Microsporidium sp. RW-2009a	*Dikerogammarus villosus*	Germany	Invasive range	Grabner et al. 2015
Uncultured Stramenopile clone	Water sample	Caribbean Sea	N/A	Edgcomb et al. 2011
Uncultured Stramenopile clone	Water sample	Caribbean Sea	N/A	Edgcomb et al. 2011
Uncultured Stramenopile clone	Water sample	Caribbean Sea	N/A	Edgcomb et al. 2011
Uncultured Stramenopile clone	Water sample	Caribbean Sea	N/A	Edgcomb et al. 2011
Uncultured Stramenopile clone	Water sample	Caribbean Sea	N/A	Edgcomb et al. 2011

Table 6.3: Geographic and host data for those microsporidian gene isolates that clade within the "*Cucumispora* candidates" group in Figure 6.6.

6.5. Taxonomic description for *Cucumispora roeselii* n. sp.

6.5.1. Higher taxonomic rankings

Super-Phylum: Opisthosporidia (Karpov et al. 2014)

Phylum: Microsporidia (Balbiani, 1882)

Class: Marinosporidia (Clade V) (nomina nuda) (Vossbrinck and Debrunner-Vossbrinck, 2005)

Order: Crustaceacida (Stentiford et al. 2010)

Family: Myosporidae (Stentiford et al. 2010)

Genus: *Cucumispora* (Ovcharenko et al. 2010)

6.5.2. Type species: Cucumispora roeselii n. sp.

Species description: Ultrastructurally, spores appear oval (L: 2.2 µm ± 0.1 µm; W: 1.5 µm ± 0.1 µm), with a "thorned" spore wall consisting of an electron lucent endospore and electron dense exospore at varying thicknesses either around the spore (138 nm ± 27 nm), at the point of the anchoring disk (40 nm ± 6 nm), or at the periphery of the anchoring disk (185 nm ± 50 nm). The polar filament turns between 9–10 times around the centre and posterior of the spore. This parasite is diplokaryotic throughout its lifecycle. Similarity of the SSU rDNA sequence to the type species: *C. dikerogammari*, is 93%. Transmission information is currently unavailable but predicted to be horizontal as derived from the pathology – no infection of the gonad was observed.

Type host: *Gammarus roeselii* (Gammaridae) collected from outside its native range.

Type locality: Chojna, Poland (52.966, 14.42906), Oder River Basin.

Site of infection: Infections are restricted to the musculature of *G. roeselii*. Microsporidian spores can be seen in haemocytes likely due to phagocytosis.

Etymology: The *Cucumispora* genus (Ovcharenko et al. 2010) is named due to the elongate, "cucumiform" spore shape in the type species: *Cucumispora dikerogammari*. The specific epithet "*roeselii*" is derived from the host species, which is named for the German taxonomist, Roesel.

Type material: Histological sections and TEM resin blocks of the *C. roeselii* n. sp. infected *G. roeselii* tissues are deposited in the Registry of Aquatic Pathology (RAP) at

the Cefas Laboratory, Weymouth, UK. *Cucumispora roeselii* n. sp. SSU rRNA sequence data are deposited in NCBI (KY200851).

6.6. Discussion

This study presents the first comprehensive pathogen screen of the non-native gammarid, *G. roeselii*, outside of its native range and includes a taxonomic description of a novel species of microsporidian belonging to the *Cucumispora* genus. The novel microsporidian is named herein as *Cucumispora roeselii* n. sp. Studies such as this one are important to advise risk assessment criteria for invasive and non-native species, specifically in the light of little information on the pathogens and parasites of invasive and non-native species (Roy et al. 2016). While *G. roeselii* has previously been considered as a low-impact invader, in this case I identify *G. roeselii* as a potentially high-profile invader because of its status as a pathogen carrier, transferring pathogens along its route of introduction and spread. It is important to consider if these pathogens could transmit to native wildlife, if they act as a regulator for the host species; limiting its potential impact when present, or if they could be used against the invader in a targeted biological control approach.

6.6.1. Cucumispora roeselii *n. sp. and the genus:* Cucumispora

The evidence provided by this study recognises a novel aquatic microsporidian parasite that shows ultrastructural (9-10 turns of polar filament; bi-laminar polaroplast), developmental (diplokaryotic life cycle), histopathological (muscle infecting) and genetic (SSU similarity of 93%) similarities to the type species of the *Cucumispora* genus: *C. dikerogammari* (Ovcharenko et al. 2010).

Interestingly, the amphipod host of *C. roeselii* n. sp. is not of Ponto-Caspian origin or part of the genus *Dikerogammarus*, as both previously described host species are (Ovcharenko et al. 2010; Bojko et al. 2015). *Cucumispora dikerogammari* and *C. ornata* are both thought to originate in the same native range as their hosts however the inclusion of *C. roeselii* n. sp. in this genus requires reconsideration of the origins and range of *Cucumispora* species. Were this parasite to have originated from the hosts native range (The Balkans) it could indicate an interesting phylogeographic spread of microsporidia from this genus. There is a possibility that this parasite has been acquired from the Polish environment from other invaders, but without previous documentation it is impossible to be certain.

Several genetic isolates have been studied in the past that provide strong sequence similarity to members of the *Cucumispora* (Terry et al. 2004; Wattier et al. 2007; Krebes et al. 2010; Ovcharenko et al. 2010; Orsi et al. 2011; Jones et al. 2012; Bojko et al. 2015; Grabner et al. 2015; Unpublished works through BLASTn) (Table 6.3, Fig. 6.6). The ranges of these parasite sequences belong mainly to European territories, but some studies demonstrate isolates from Caribbean and Canadian waters (Orsi et al. 2011; Jones et al. 2012). This information suggests that the *Cucumispora* genus may be present around the globe, and their recent identification further suggests their role as emergent pathogens, not only in gammarids but in copepods as well (Jones et al. 2012). However, recently published information suggests that hyperparasitic microsporidia with the capability to infect protists appear to have similar SSU sequences to the *Cucumispora* and have been placed into the newly erected genus: *Hyperspora* (Stentiford et al. 2016b). Until further information is provided in the form of legitimate taxonomic descriptions from more of the SSU isolates in Figure 6.6, the native/invasive range and host range of many potential *Cucumispora* spp*.* remains an interesting phenomenon.

Some isolates show close relatedness to taxonomically described *Cucumispora* spp*.* (Fig. 6.6). *Microsporidium* sp*. G* (haplotypes 1, 2, 3 and 4) isolated from *D. haemobaphes* (Germany) is 99% similar to *Cucumispora ornata* and clades closely in the tree presented in Figure 6.6. It is likely these are the same parasite and should be synonymised (Grabner et al. 2015). However, determining a taxonomic basis on a single gene does not propagate a strong scientific standing and histological and TEM evidence for *Microsporidium* sp*. G* from both *D. haemobaphes* and *G. roeselii* should be confirmed in each host before amalgamating.

6.6.2. Parasites, pathogens and invasion biology of Gammarus roeselii

Several pathogens were identified histologically in this study. *Polymorphus minutus* and *Pomphorhynchus* sp*.* represent two known acanthocephalan parasites of *G. roeselii* (Table 6.1) also observed in this sample from Chojna. Epibiotic rotifers, ciliated protists and filamentous bacteria are commonly associated with aquatic species (Stentiford and Feist, 2005; Bojko et al. 2013) as are gut dwelling gregarines in amphipod hosts (Ovcharenko et al. 2009; Bojko et al. 2013).

Digenean associations with amphipods are also common and several are known to utilise amphipods as intermediate hosts before entering further hosts where they can reach sexual maturity (Mouritsen et al. 1997). Digenea detected in this study were of an

undetermined species and their lifecycle and reason for parasitizing *G. roeselii* is currently unknown.

The parasitic ciliated protist (Fig. 6.1d) has not been noted from *G. roeselii* in the past and is likely a novel association for this species. Without DNA sequence data it is uncertain whether this parasite is taxonomically novel or not. Parasitic ciliates have been noted in amphipods in the past, such as *Fusiforma themisticola*, which parasitizes *Themisto libellula* (Chantangsi et al. 2013).

A second microsporidian association in this study was of a rare parasite (<1% prevalence) targeting the hepatopancreas of *G. roeselii*. Most microsporidia that target the hepatopancreas of Crustacea fall into the clade IV of microsporidian taxonomy (Terresporidia: Vossbrinck and Debrunner-Vossbrinck, 2005) and further into the *Hepatosporidae* (Stentiford et al. 2011; Bojko et al. 2016). Obtaining TEM and SSU sequence data would help to taxonomically identify this species. A recent study by Grabner et al (2015) revealed two microsporidian SSU sequences, isolated from *G. roeselii*, that correspond to microsporidia from Group IV (Terresporidia); the histopathology presented by this study may link to one of these isolates and further tests should be carried out to confirm this.

A single observation of a putative RLO in the cytoplasm of infected hepatopancreatocytes is an interesting association, as few RLOs have been noted from amphipods in the past. To date, the only examples include putative Rickettsiella-like SSU rDNA sequences available from BLASTn (NCBI) and systemic haemolymph infections caused by RLOs in *Gammarus pulex* (Larsson, 1982) and *Crangonyx floridanus* (Federici, 1974).

6.6.3. Viruses in the Amphipoda

A variety of viruses have been identified from Crustacea either morphologically, via DNA sequence data, or through searching for endogenous viral elements in the genome of crustacean hosts (Johnson, 1983; Bonami and Lightner, 1991; Thézé et al. 2014). Despite this diversity, few have ever been identified from hosts belonging to the Order: Amphipoda. To date only three published viral associations have been made from amphipods: the first is in the form of histology and TEM images of a bacilliform virus from the hepatopancreas of *Dikerogammarus villosus* and referred to as *Dikerogammarus villosus Bacilliform Virus* (DvBV) (Bojko et al. 2013); the second, an unassigned circovirus from a *Gammarus* sp. (Rosario et al. 2015); and the third includes various circular-virus associations to *Diporeia* spp. (Hewson et al. 2013).

Although DvBV was, previous to this study, the only visually confirmed virus from an amphipod, bacilliform viruses from the hepatopancreas of crustaceans are common and several have been identified morphologically (Table 6.4). One of these viruses has been the focus of genome sequencing efforts, revealing that this group of morphologically-similar viruses are likely nudiviruses (*Nudiviridae*) (Yang et al. 2014). Further genome sequencing and generalised primer-designs for nudivirus genes would benefit this area greatly and allow further taxonomic insight into these virus's life history.

Organism	Host species	Bacilliform Virus from the HP	Reference
Crayfish	*Astacus astacus*	AaBV	Edgerton et al. 1996a
	Cherax quadricarinatus	CqBV	Anderson et al. 1992
	Pacifasticus leniusculus	PlBV	Hedrick et al. 1995
	Cherax destructor	CdBV	Edgerton, 1996b
	Austropotamobius pallipes	ApBV	Edgerton et al. 2002
Crab	*Cancer pagurus*	CpBV	Bateman and Stentiford, 2008
	Carcinus maenas	CmBV	Stentiford and Feist, 2005
	Pinnotheres pisum	PpBV	Longshaw et al. 2012
Shrimp	*Crangon crangon*	CcBV	Stentiford et al. 2004b
	Penaeus monodon	PmNV	Yang et al. 2014
Amphipod	*Dikerogammarus villosus*	DvBV	Bojko et al. 2013
	Gammarus roeselii	GrBV	This Study

Table 6.4: Bacilliform viruses from the hepatopancreas of several Crustacea.

GrBV, isolated from the hepatopancreas of *G. roeselii* in this study fits morphologically and pathologically alongside the viruses in Table 6.4. Discovery of this virus classes it as the second bacilliform virus to be discovered from an amphipod.

The viral pathology in the gut of *G. roeselii* remains putative due to a lack of appropriately fixed material to observe virions via TEM. Pathologically however the presence of the infection (nuclei of gut epithelia) suggests a DNA virus. It is uncertain at this point whether this infection is caused by GrBV simply infecting a separate tissue type; this cannot be tested for using my current data and materials. Re-sampling and TEM processing should provide important data, however genetic data would be most beneficial; a valid point for many of the viruses in Table 6.4.

6.6.4. Cucumispora roeselii n. sp. invasion threat or beneficial for control?

Although the prospect of invaders carrying pathogens poses a potential problem (Strauss et al. 2012; Dunn and Hatcher, 2015), in some instances parasites can act as controlling agents (Hajek and Delalibera, 2010). This phenomenon may be taking place with the *D. haemobaphes* invasion of the UK, where the microsporidian pathogen, *C. ornata,* may

limit the health of the invasive population (Chapter 9). Amphipod populations without microsporidian pathogens are not regulated as they would be in their native range, and loss of their “enemies” may result in greater fitness and impact on the environment; as with the killer shrimp in the UK (MacNeil et al. 2013; Bojko et al. 2013).

Gammarus roeselii is considered to be a low impact non-native species (European Alien Species Information Network) in freshwater systems across Europe (Karaman and Pinkster, 1977; Barnard and Barnard, 1983; Médoc et al. 2011; Lagrue et al. 2011; EASIN Database). It is important however to understand that in some cases, the non-native host may not be the main issue but instead its pathogens can act as “biological weapons” to facilitate invasion and harm wildlife (Strauss et al. 2012; Dunn and Hatcher, 2015; Roy et al. 2016). The concept of being a pathogen carrier is often ignored in risk assessment, often due to a lack of information around the capability to accurately assess the risk invasive pathogens pose (Roy et al. 2016). Possible parasite transmission from *G. roeselii* to native fauna is high, based on the large diversity of parasites and pathogens observed by this study. Due to limited records, it is difficult to be certain which pathogens and parasites are from the native range of *G. roeselii* and which have been acquired during its introduction and spread. Further assessment of co-evolved pathogens in the native range of *G. roeselii* could increase our understanding of the origins of *C. roeselii* n. sp. and other pathogens observed during this study. Examples of enemy release in gammarids are available, including: the loss of pathogens during the introduction process (Bojko et al. 2013) and of gammarids carrying pathogens into novel invasion sites (Wattier et al. 2007; Chapter 5).

It may be possible that the pathogens regulate the host species, and escape from these regulators could increase the impact and risk of *G. roeselii*. Understanding the associated mortality rate, host range, behavioural alterations and physiological changes these pathogens impose upon their host would allow further assessment of whether these pathogens are regulating non-native *G. roeselii* populations in Chojna and elsewhere within Europe. Information gleaned from such studies could define whether *C. roeselii*, and other pathogens associated with *G. roeselii*, could be useful as biocontrol agents, or if they are emerging diseases and detrimental for vulnerable wildlife.

CHAPTER 7

Aquarickettsiella crustaci n. gen. n. sp. (Gammaproteobacteria: Legionellales: Coxiellaceae); a bacterial pathogen of the freshwater crustacean: *Gammarus fossarum* (Malacostraca: Amphipoda)

7.1. Abstract

The pathogens and parasites of crustaceans are of particular interest for their prospective adaptation into biological control agents to regulate invasive populations. Viruses, bacterial species and microsporidia constitute some of the most viable options as control agents, however few have been identified from invasive or native populations of amphipods; particularly the bacterial pathogens. The native range of invasive species is predicted to have the greatest diversity of co-evolved parasite and pathogen species.

In this study a novel bacterial species and genus (*Aquarickettsiella crustaci* n. gen. n. sp.) is erected through the use of metagenomics to assemble 51 contiguous sequences associating to the novel species; phylogenetics to compare the relative sequence data to other known species and isolates; histopathology and transmission electron microscopy tools to identify the species pathology, ultrastructure and development. This novel rickettsia-like organism is an intracellular pathogen. The developmental cycle includes an elementary body (496.73nm ± 37.56nm in length, and 176.89nm ± 36.29nm in width), an elliptical, condensed sphere stage (737.61nm ± 44.51nm in length and 300.07nm ± 44.02nm in width), a divisional stage, and a spherical initial body stage (1397.59nm ± 21.26nm in diameter). The pathogen was found to infect the haemal, muscle, nerve, gill and gonad tissues of the host, *Gammarus fossarum*, from its native range in Poland. This host has recently been detected in the UK and little is known about its pathogens and parasites.

Phylogenetic information for the 16S gene phylogeny and multi-gene phylogeny of the bacterial pathogen suggest that it is related closest to the *Rickettsiella*, a genus including bacterial species that infect terrestrial insects and isopods. A clear split can be seen between the aquatic, crustacean-infecting RLO's and the *Rickettsiella* alongside ultrastructural and morphological differences and the choice of host, providing the incentive to develop a new genus and species.

Metagenomic and histological analysis of *G. fossarum* tissues also identified other species that use *G. fossarum* as a host. The importance of understanding the pathogens and parasites of native and invasive amphipods is explored as is the taxonomic identification of *A. crustaci* n. gen. n. sp. and its potential use as a biological control agent.

7.2. Introduction

The Prokaryotes comprise one of the simplest, but most diverse, groups of organisms on the planet (Hugenholtz, 2002; Logares et al. 2014). They are found in a wide range of environments, from ice-sheets to volcanoes, and within diverse hosts, from humans to protists, and are considered one of the most ancient lineages of life (3-4 Gya) (Poole et al. 1999; DeLong and Pace, 2001). Many bacterial taxa have adapted to survive through colonisation of a host; acting either as parasite or symbiont to survive (Bhavsar et al. 2007; Chow et al. 2010). The taxonomy of bacteria is being revolutionised through wider application of DNA sequencing techniques and development of improved phylogenetic tools to resolve their taxonomic position (Konstantinidis and Tiedje, 2007).

Some bacterial taxa reside within the cells of their host, utilising resources within the cell for their own division and development. One such group are the Rickettsia-Like Organisms (RLO); including well-known examples such as *Chlamydia trachomatis*, a common sexually transmitted disease in humans (Campbell et al. 1987; Stephens et al. 1998). Several others are either medically or economically important; resulting in diseases that cause significant healthcare costs, or crop yield losses, respectively (Pospischil et al. 2002). Others are interesting from a biodiversity and wildlife pathogen perspective (Duron et al. 2015).

The genus *Rickettsiella* (Philip, 1956) comprises an important group of arthropod-infecting RLOs. *Rickettsiella* resides within the family Coxiellaceae (Garrity et al. 2007) with the genera *Aquicella* (Santos et al. 2003); candidatus *Berkiella* (Mehari et al. 2015); *Coxiella* (Philip, 1948); and *Diplorickettsia* (Mediannikov et al. 2010). Many of these genera include pathogens of invertebrates. The type description of *Rickettsiella* came from *Rickettsiella popilliae* infection of the fat body of *Popillia japonica* (Japanese beetle) and two species of June beetle (Phyllophaga) (Dutky and Gooden, 1952; Philip, 1956). However, despite subsequent co-generic placements, this type species still requires DNA sequence phylogeny along with many others that are currently assigned to the genus (*Rickettsiella chironomi*) (Philip, 1956).

The *Rickettsiella* are thought to have diverged from *Coxiella* ~350 million years ago (Cordaux et al. 2007) and currently nine *Rickettsiella* species are considered adequately described using genetic, morphological and pathological information. All are obligate intracellular bacterial pathogens of arthropods. *Rickettsiella agriotidis* (Leclerque et al. 2011) (host: *Agriotes* sp.), *Rickettsiella pyronotae* (Kleespies et al. 2011) (host: *Pyronota* spp.), *Rickettsiella costelytrae* (Leclerque et al. 2012) (host: *Costelytrae zealandica*) and *Rickettsiella melolonthae* (Kreig, 1955) (host: *Melolontha melolontha*) all infect the cells of beetles (Insecta: Coleoptera). *Rickettsiella grylli* (Roux et al. 1997) (host: *Gryllus bimaculatus*) infects cells of crickets (Insecta: Orthoptera). *Rickettsiella viridis* (Tsuchida et al. 2014) (host: *Acyrthosiphon pisum*) infects cells of aphids (Insecta: Hemiptera). *Rickettsiella isopodorum* (Kleespies et al. 2014) (host: *Porcellio scaber*) and *Rickettsiella armadillidii* (Cordaux et al. 2007) (host: *Armadillidium vulgare*) infect cells of isopods (Crustacea: Isopoda). To date, all described taxa within the genus are from terrestrial hosts although *Rickettsiella tipulae* (Leclerque and Kleespies, 2008) infects the crane fly, *Tipula paludosa*, an insect with a semi-aquatic life history.

Several other *Rickettsiella*/RLO-like taxa have been described infecting the cells of aquatic hosts but description is only based on morphological information. These include those infecting the aquatic crustaceans: *Carcinus mediterraneus* (Bonami and Pappalardo, 1980); *Paralithoides platypus* (Johnson, 1984); *Cherax quadricarinatus* (Romero et al. 2000); *Eriocheir sinensis* (Wang and Gu, 2002); three species of penaeid shrimp (Anderson et al. 1987; Brock, 1988; Krol et al. 1991); and the two amphipods, *Gammarus pulex* (Larsson, 1982) and *Crangonyx floridanus* (Federici, 1974). Over 100 rDNA gene sequence accessions exist within online databases for bacterial isolates linked to the *Rickettsiella* and these include taxa infecting a wide diversity of arthropod hosts, including isolates from aquatic species (NCBI). An example from an aquatic host includes an isolate from *Asellus aquaticus*, an aquatic isopod (NCBI: AY447041), that lacks morphological and ultrastructural information.

Rickettsiella spp. are considered to have a slow developmental cycle, which involves initially entering a host cell through phagocytosis, dividing within a vesicle, and eventually lysing the cell before completing its life cycle (Cordaux et al. 2007). Small, dense elementary bodies are first phagocytosed by the host cell, prior to their enlargement (Kleespies et al. 2014). In insects at least, these enlarged cells often contain a crystalline substance that has not yet been observed in those *Rickettsiella* infecting crustaceans (Kleespies et al. 2014). Finally, these enlarged cells condense and divide (Kleespies et al. 2014).

Rickettsiella spp. often cause disease in their host. Some have been associated with clinical signs, leading to descriptions such as “Blue Disease” or “Milky Disease” (Dutky and Gooden, 1952; Kleespies et al. 2011). In insects, disease often results in an iridescent appearance to the infected tissues (Dutky and Gooden, 1952; Kleespies et al. 2011). In crustaceans, clinical signs include an opaque white appearance of fluids and intersegmental membranes (Vago et al. 1970; Federici, 1974). In all cases, bacterial colonies are observed in the cytoplasm causing displacement of organelles and cellular hypertrophy (Federici, 1974; Kleespies et al. 2014). Although genomic information is not available for many taxa, a full genome sequence is available for *R. grylli* (Leclerque, 2008) along with several others from closely related genera (Seshadri et al. 2003; Mehari et al. 2015).

As part of a survey of natural populations of the amphipod *Gammarus fossarum* for pathogens and symbionts, I discovered infection and disease associated with a novel RLO. I utilise high throughput sequencing data to construct a partial genome of the pathogen and further information obtained from transmission electron microscopy and histopathology to describe a novel genus and species, *Aquarickettsiella crustaci* n. gen. n. sp., as a sister taxon to *Rickettsiella*. The pathogen infects the cytoplasm of circulating haemocytes and cells of the gill, gonad, nerve and musculature of the amphipod. Genomic information derived from *A. crustaci* n. gen. n. sp. is presented and annotated alongside genetic information attained from its amphipod host.

7.3. Materials and Methods

7.3.1. Animal Collection

Gammarus fossarum (n=140) were collected from the Bzura River in Łódź (Łagiewniki), Poland (N51.824829, E19.459828) in June 2015. One hundred and twenty seven individuals were fixed for histology on site while 13 were transported live to the University of Łódź for dissection. Dissection involved initial cooling to anaesthetise the individual before removing and dividing the hepatopancreas, gut and muscle tissue for fixing for molecular diagnostics (96% Ethanol), histology [Davidson’s freshwater fixative (Hopwood, 1996)] and, transmission electron microscopy (2.5% glutaraldehyde in Sodium cacodylate buffer) according to Chapter 5. The collection of *G. fossarum* specimens in this case is the same as that described for Chapter 3, where this chapter goes into greater detail about this species (*G. fossarum*) and its symbionts, focussing on the presence of a novel bacterial species.

7.3.2. Histopathology and transmission electron microscopy (TEM)

For histology, whole animals or dissected organs and tissues were initially fixed in Davidson's freshwater fixative for 48 hr. After fixation, the tissues were submerged in 70% ethanol and transported to the Cefas Weymouth Laboratory, UK for histological processing. Specimens were decalcified for 30 min before placement in 70% industrial methylated spirit and transfer to an automated tissue processor (Leica, UK) for wax infiltration. Whole animals, or dissected organs and tissues were embedded in wax blocks and sectioned at 3µm before transfer to glass slides. Sections were stained using haematoxylin and alcoholic eosin (H&E) and mounted with a glass coverslip using DPX. All slides were read using standard light microscopy (Nikon E800, Nikon, UK). Digital images were captured using an integrated camera (Leica, UK) and Lucia Image Capture software. For TEM, dissected tissues were processed and analysed according to Bojko et al. (2015). Digital images were obtained on a Jeol JEM 1400 transmission electron microscope using on-board camera and software (Jeol, UK). These two techniques identified the RLO in section, providing the incentive to apply molecular tools for bacterial diagnostics.

7.3.3. DNA extraction, PCR and sequencing of 16S rDNA

Ethanol-fixed tissues from infected amphipods were initially digested using proteinase K (10mg/ml) in solution with Lifton's Buffer (0.1M Tris-HCl, 0.5% SDS, 0.1M EDTA). The solution underwent a phenol cleaning step followed by a chloroform cleaning step before adding the same volume of 100% ethanol. After an hour cooling to -20˚C, all the liquid was removed to leave a DNA pellet. The DNA pellet was re-suspended in ethanol, TE buffer and 5.0M Ammonium Acetate and underwent a second cooling step at -20˚C. The resulting DNA pellet was suspended in molecular grade water. Extracts were analysed for 16S rDNA in a single round Taq polymerase PCR protocol using the general bacterial 16S primers DD1 and FD2 according to Weisburg et al. (1991). Amplicons (~900bp) were excised from the gel and forward and reverse sequenced using 'eurofinsgenomics' services (www.eurofinsgenomics.eu).

7.3.4. Genome sequencing, assembly and annotation

A single infected *G. fossarum* carcass, initially fixed in 96% ethanol, was prepared for metagenomic analysis using the Illumina MiSeq platform (Illumina, UK). The specimen was split into 3 sub-samples with 1 ng of DNA from each sub-sample prepared for sequencing by Nextera XT library preparation per manufacturer's protocol (Illumina;

www.illumina.com). Libraries were quality and size checked by bioanalyzer (Agilent; www.agilent.com/) and quantified by QuantiFluor fluorimeter (Promega, www.promega.com) before being pooled in equimolar concentrations, denatured by Sodium Hydroxide, and diluted to 10 pM in Illumina HT1 hybridisation buffer for sequencing. Sequencing was done on an Illumina MiSeq system with a V2-500 cartridge. All bioinformatics analyses were conducted through BioLinux (Field et al. 2006). Cumulatively this provided 9.9Gbp of pooled data, which was trimmed using Illuminaclip (Trimmomatic- Illumina) (Bolger et al. 2014), pre-assigned to associate forward and reverse reads using PEAR (Zhang et al. 2014) (99.7% sequence-pairs) and assembled using MetaSpades (Nurk et al. 2016) to provide 69212 scaffolds. Scaffolds were annotated using PROKKA (Seemann et al. 2014) and DIAMOND (Buchfink et al. 2015), and were compared for sequence similarity in BLAST (NCBI) to available members of the Coxiellaceae. The annotated genome of *R. grylli* (NZ_MCRF00000000) was used in combination with MAUVE (Darling et al. 2004) to associate non-coding sequence data. Post-analysis, a list of 51 scaffolds were identified for *A. crustaci* n. gen. n. sp.

In addition to the annotation of the *A. crustaci* n. gen. n. sp. genome, the mitochondrial genome of the host was also sequenced and annotated. Some host nuclear genes were also identified using GlimmerHMM (Majoros et al. 2004) to identify available scaffolds with intron-including genetic information.

The program Metaxa2 (Bengtsson-Palme et al. 2015) was applied to raw read data as well as assembled data to detect further pathogen diversity alongside genome assembly of the target RLO.

7.3.5. Phylogenetics

Gene sequence data acquired from targeted PCR and generalized metagenomics analyses were utilised in combination with available sequence data from NCBI to provide two Maximum-Likelihood phylogenetic trees. The first utilised the 16S gene (~900bp) of various RLOs/bacteria, including two *Chlamydophila* sp. that act as an out-group to root the tree. The sequences were aligned and trimmed in MEGA 7.0.21 (Kumar et al. 2016) using ClustalW, and phylogenetically compared using the Tamura-3 parameter model (Tamura, 1992) (100 bootstraps) to form a final tree. A concatenated phylogeny was also conducted using 19 end-to-end gene sequences [16S, 50S L1-5, 30S S1-5, DNA Pol III alpha/beta/tau/delta/epsilon subunit, DNA primase, Replicative DNA Helicase (DnaB), DNA Pol I] for 7 individual bacterial taxa for which data was available, including

Chlamydophila pneumoniae to root the tree. Development of the concatenated tree used the same parameters as specified above.

7.4. Results

7.4.1. Histopathology and ultrastructure of a novel RLO and other microbial associates of G. fossarum

Gammarus fossarum were found to harbour at least 10 different microbial associations, including: Acanthocephala in 2.4% of the population (Fig. 7.1); stalked ciliated protist upon 90.6% of the host population (Fig. 7.2A); gill-embedded ciliated protists upon 47.2% of the host population (Fig. 7.2B); rotifers upon 81.9% of hosts (Fig. 7.2C); undetermined gill ectoparasites upon 4.7% of hosts (Fig. 7.3A); gut-dwelling gregarines in 18.1% of hosts (Fig. 7.3B); a muscle-infecting microsporidian in 8.7% of hosts (Fig. 7.3C); An RLO in the hepatopancreas of 14.2% of hosts, morphologically discernible from the RLO focused upon in this study (Fig. 7.4); a putative RNA virus observed in the hepatopancreas of <1% of hosts during TEM analysis (Fig. 7.5A); a putative DNA virus in the nuclei of gut epithelial cells in 2.4% of hosts (Fig. 7.5B); and a second RLO infecting the muscle, haemocytes, gonad and nerve tissue, present in 37.8% of hosts and taxonomically identified herein as *Aquarickettsiella crustaci* n. gen. n. sp.

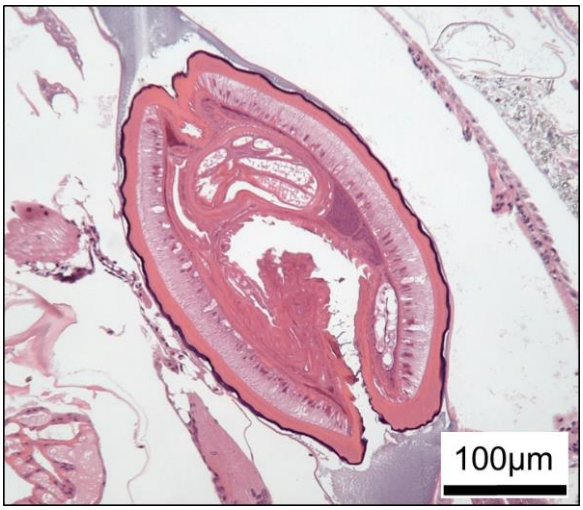


Figure 7.1: An acanthocephalan cyst in the body cavity of *G. fossarum*.

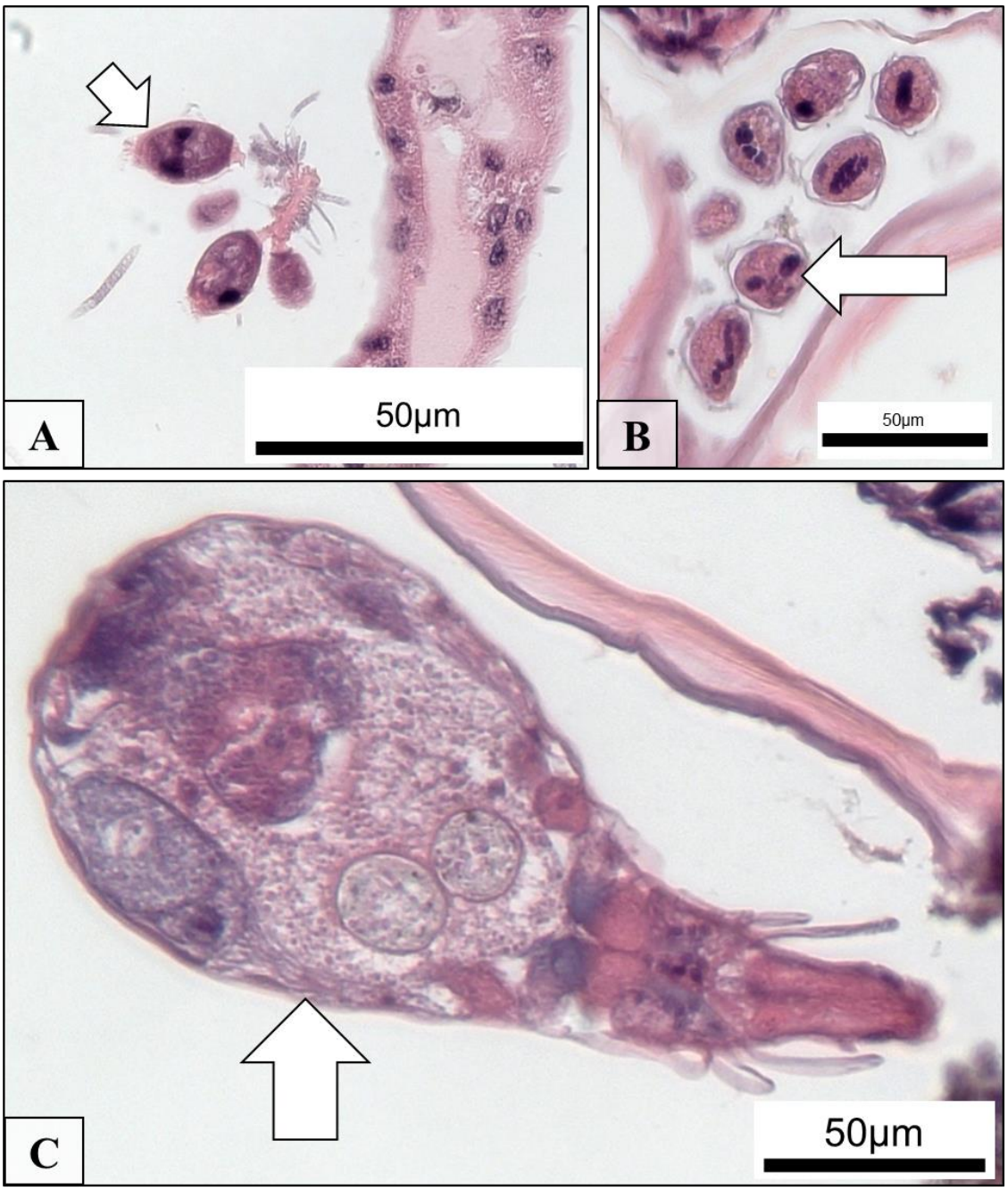


Figure 7.2: The commensal ectofauna of *G. fossarum*. A) Stalked ciliated protists (white arrow) attached to a gill filament. B) Ciliated protists that secrete an external layer (white arrow), here attached to the carapace of the host. C) A rotifer (white arrow) closely associated with the carapace of the host.

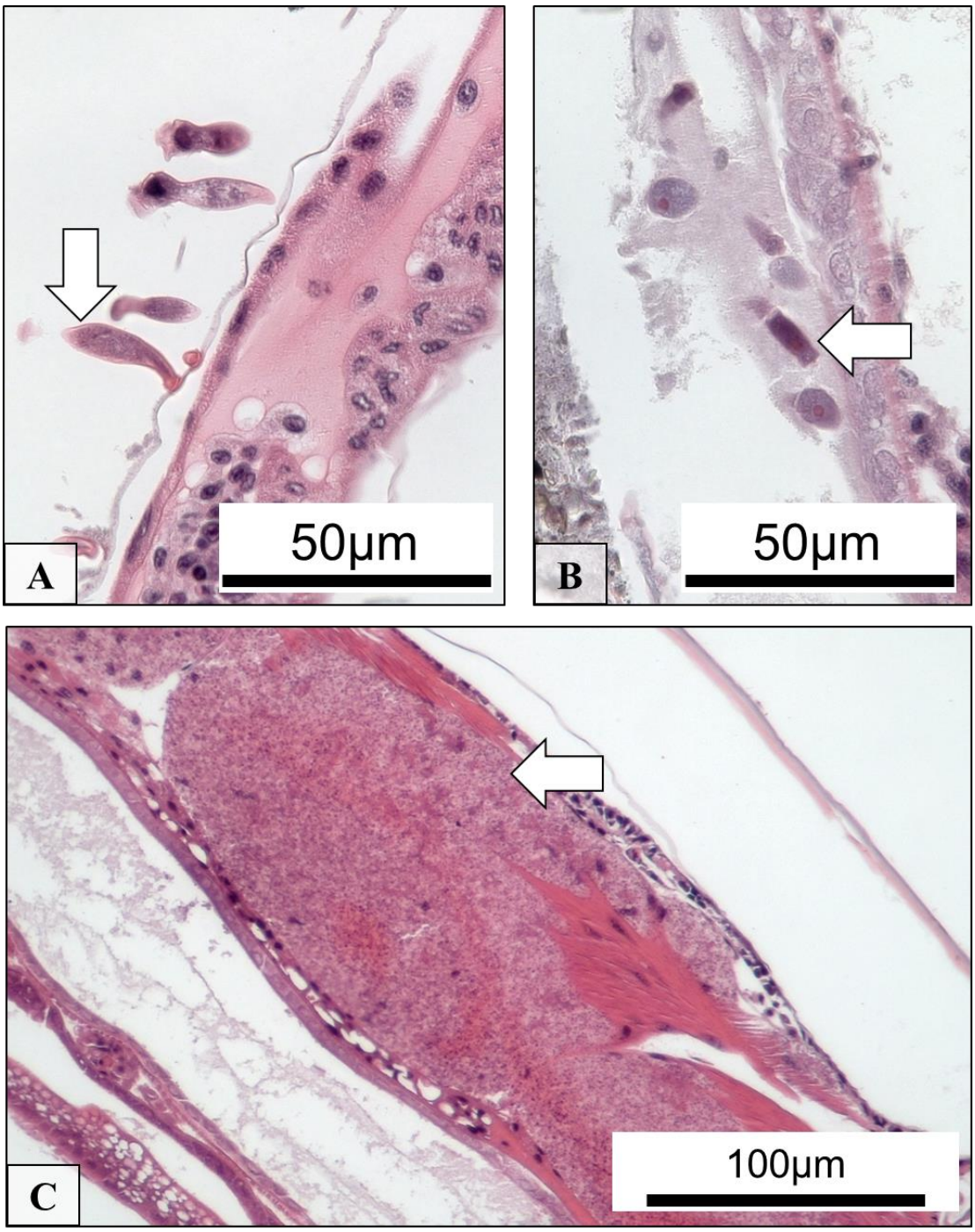


Figure 7.3: Parasites and commensals of *G. fossarum*. A) Undetermined ectoparasites (white arrow) attached to the gill filament of the host. B) Gregarine parasites (Apicomplexa) (white arrow) in the gut lumen of the host. C) Microsporidian colonisation of the host musculature (white arrow).

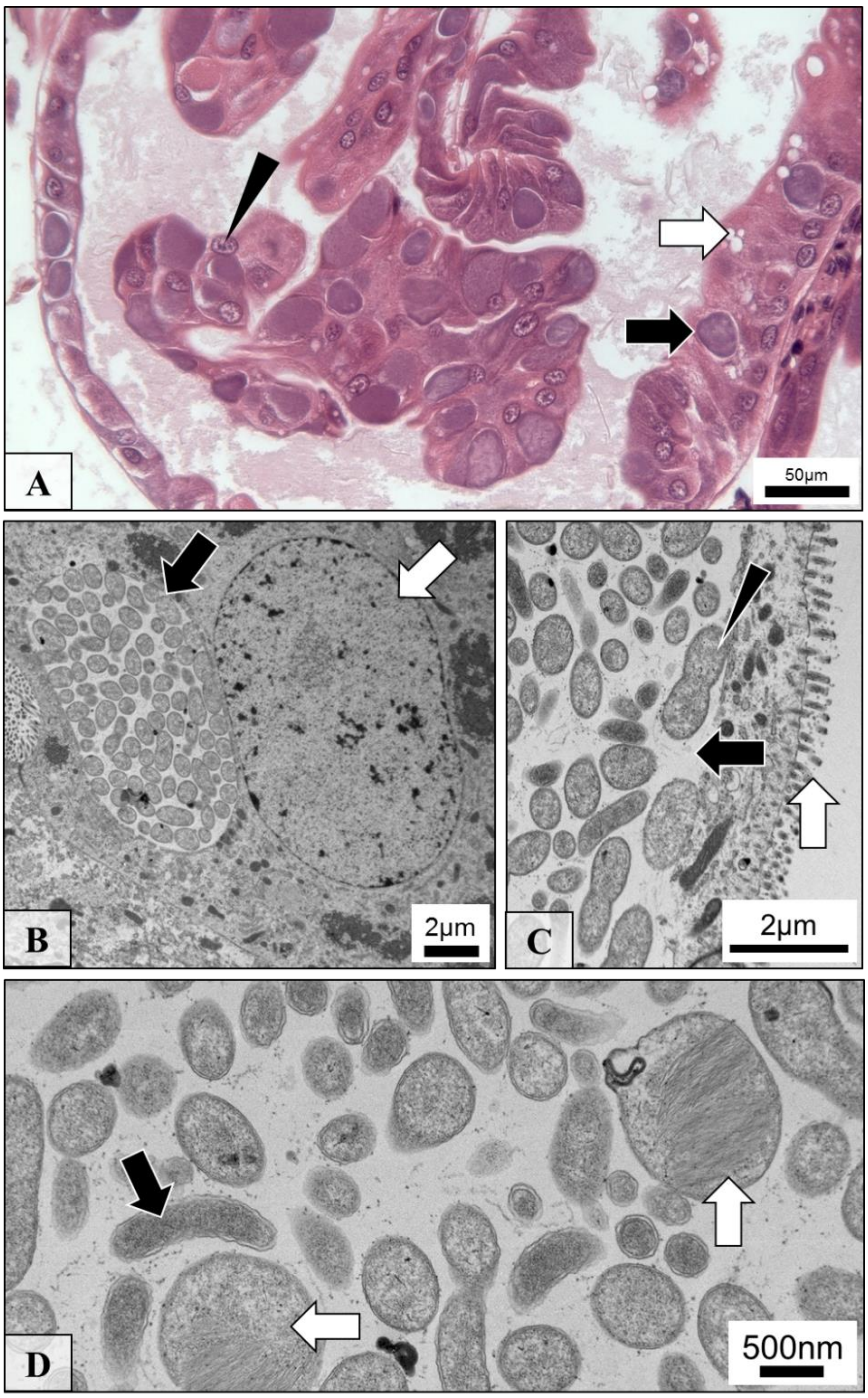


Figure 7.4: A bacterial pathogen infecting the hepatopancreas of the host, *G. fossarum*. This bacterial pathogen is present in a different site of infection and displays morphological dissimilarity from the RLO taxonomically described herein. A) Histologically derived image of the pathology, where the cytoplasm of alpha and beta cells in the hepatopancreas display intracytoplasmic bacterial plaques (black arrow) which does not physically interact with the nucleus (black triangle). An uninfected cell is indicated with a white arrow. B) Transmission electron micrograph of a vesicle containing the unidentified bacteria (black arrow) next to the nucleus (white arrow). C) Various bacterial developmental stages, including bacterial division (black triangle). The vesicle is electron lucent (black arrow) and pressing up against the hepatopancreatic villi (white arrow). D) Elementary body (black arrow) and spherical bodies, containing fibrous inclusions, (white arrow) development stages of bacteria within the hepatopancreas.

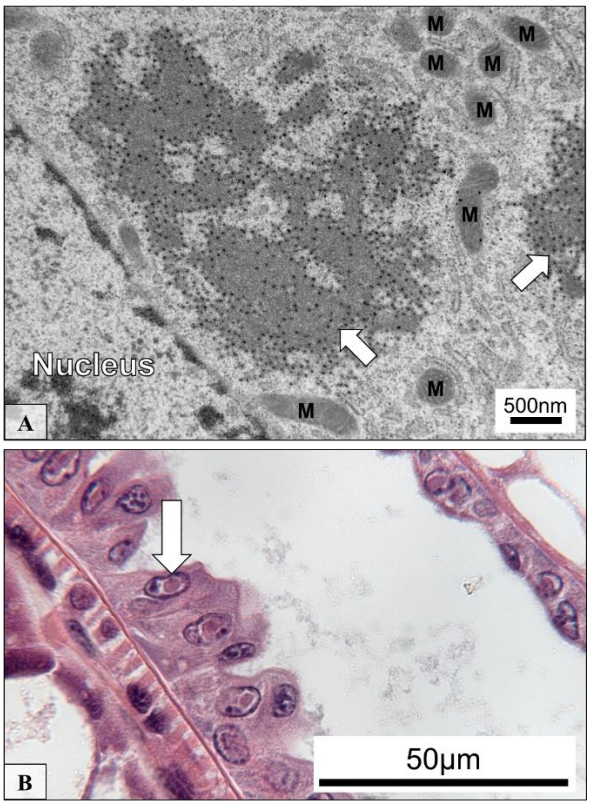


Figure 7.5: Putative viral pathogens detected in the tissues of *G. fossarum.* A) A putative RNA virus observed via TEM, in the cytoplasm of an hepatopancreatocyte. The viroplasm (white arrow) is surrounded by mitochondria ('M') and is located near the nucleus ('Nucleus'). B) Gut epithelial cells with hypertrophic nuclei, which display a putative, eosinophilic, viroplasm.

Histopathology and TEM revealed systemic infection with *A. crustaci* n. gen. n. sp., which colonised cells within the haemolymph, (Fig. 7.6A), nervous system (Fig. 7.6B-C), gill, gonad, and musculature (Fig. 7.6D). This bacterial infection was detected in 37.8% of the animals processed for histology. TEM revealed an intracellular RLO in both the sarcolemma of muscle cells (Fig. 7.7A) and in the cytoplasm of haemocytes (Fig. 7.7B). Bacteria with a highly condensed cytoplasm measured 496.73nm ± 37.56nm (n=20) in length, and 176.89nm ± 36.29nm in width, contained an electron dense core (Fig. 7.6C-D) and electron lucent lamella (D). The bacteria apparently develop through four main stages (Fig. 7.6E-H). The first stage being the electron dense elementary body (Fig. 7.6E), followed by an elliptical, condensed sphere stage [737.61nm ± 44.51nm (n=10) in length and 300.07nm ± 44.02nm in width (n=17)], with and electron lucent cytoplasm (Fig. 7.6F), which then underwent division (Fig. 7.6G). Spherical initial bodies were the largest stages observed, measuring 1397.59nm ± 21.26nm (n=10) in diameter (Fig. 7.6H), though their position in the developmental cycle is uncertain. It is likely they sit between the elementary body and elliptical condensed sphere stage. In 12.5% of infections with *A. crustaci* n. gen. n. sp. infection of the hepatopancreas was also observed, however there is uncertainty due to pathological and morphological difference (Fig. 7.4) that cannot be determined with current data and materials.

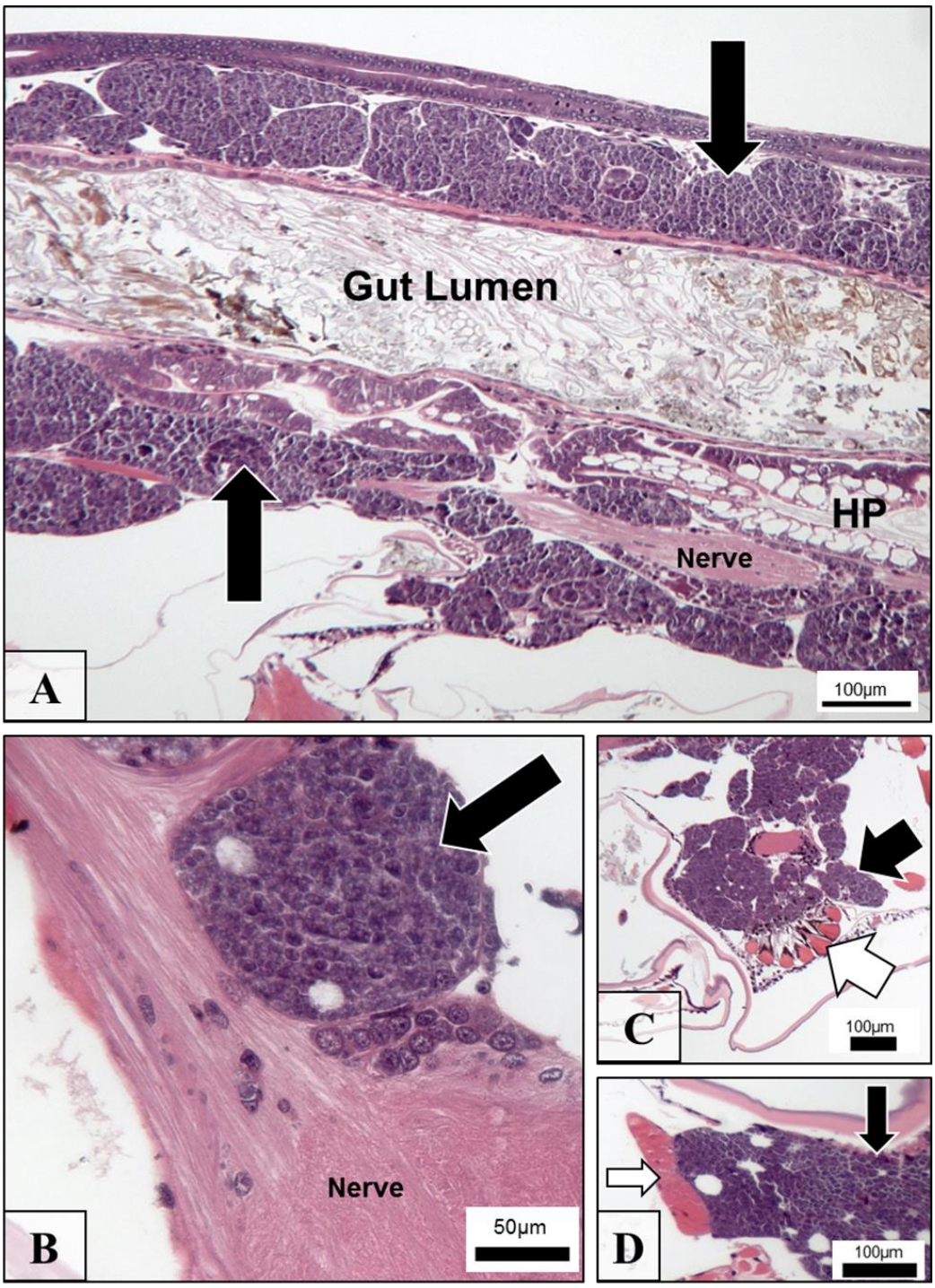


Figure 7.6: *Aquarickettsiella crustaci* n. gen. n. sp. histopathology in its host, *G. fossarum*. A) A low magnification histology image of the pereon of an infected *G. fossarum.* The gut lumen and hepatopancreas ('HP') are uninfected with bacteria (black arrow). The blood stream, nerve tissue ('Nerve') and muscle are all heavily burdened by growing intracellular bacterial plaques (black arrow). B) A detailed histological image of the bacterial pathology (black arrow) upon nerve tissue. The infection forms plaques within the nerve fibres and neurosecretory cells. C) The eye (white arrow) and surrounding nerve tissue (black arrow) is infected, possibly resulting in decreased vision. Scale = 100µm. D) The muscle (white arrow) sarcolemma is colonised by the bacterial infection and over proliferated (black arrow).

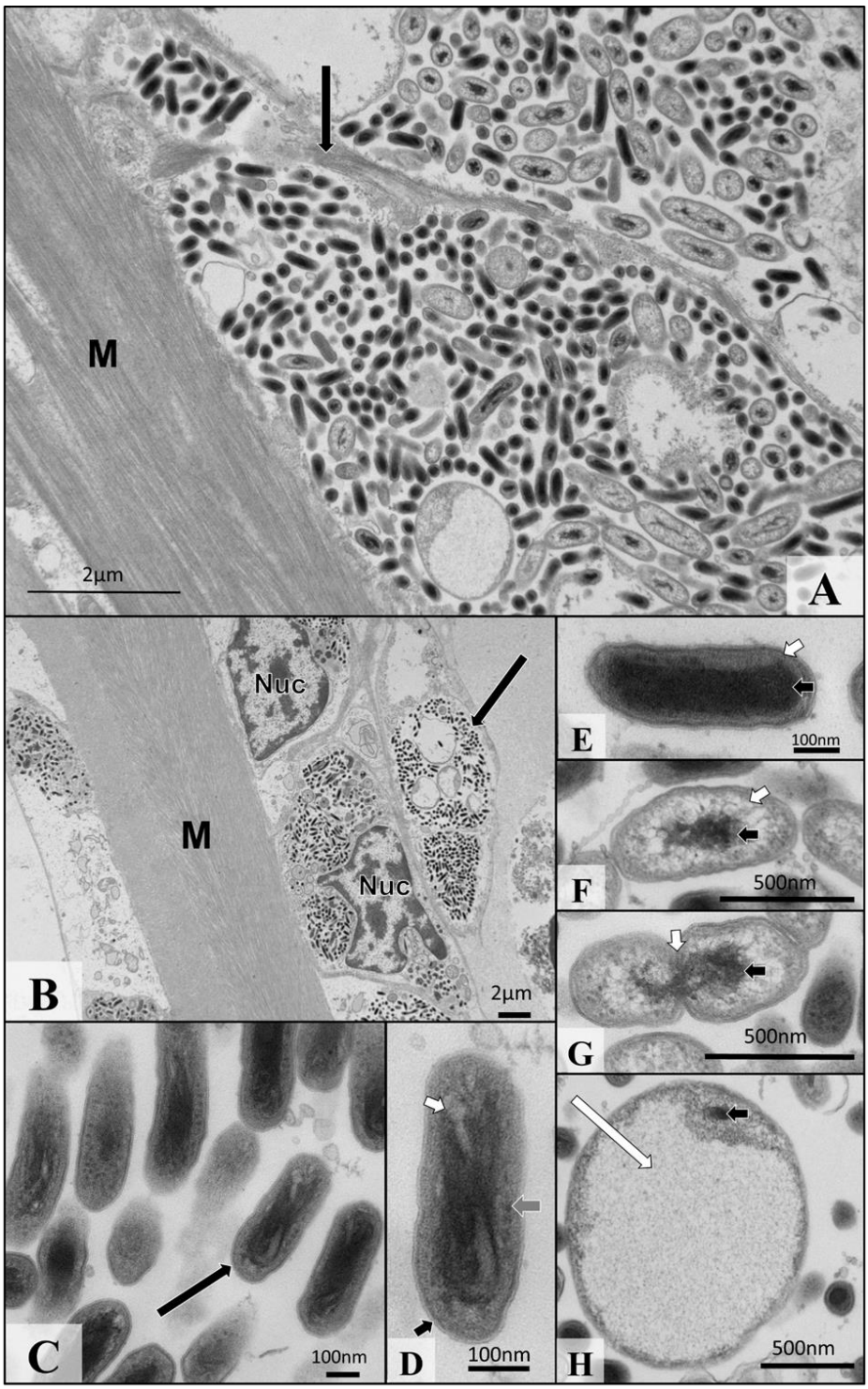


Figure 7.7: *Aquarickettsiella crustaci* n. gen. n. sp. ultrastructure and development cycle. A/B) TEM images of the pathology reveal that the sarcolemma of the muscle ('M') and the haemocytes (nuclei = 'Nuc') are infected with a rickettsia-like organism displaying four developmental stages. C) High magnification TEM images of the arranged elementary bodies (black arrow) detail the bacterial ultrastructure. D) The elementary bodies are present with an electron lucent lamellae (white arrow), condensed, electron dense bodies in the bacterial cytoplasm (grey arrow), a bi-laminar outer membrane (black arrow) and an electron dense core. The lifecycle of *A. crustaci* n. gen. n. sp. includes images E (condensed elementary body), F (elliptical condensed sphere stage), G (division), and H (spherical body).

7.4.2. Aquarickettsiella crustaci n. gen. n. sp. genome sequence and annotation

A total of 51 contiguous scaffolds, totalling 1,489,566bp were attributed to *A. crustaci* n. gen. n. sp. based on the presence of similar gene sequence data to existing Coxiellaceae, or through genomic mapping to the *Rickettsiella grylli* genome (NZAAQJ02000001) (Fig. 7.8). In total, PROKKA analysis across the 51 combined contigs revealed 1396 predicted genes belonging to *A. crustaci* n. gen. n. sp. (Appendix Table 1). One thousand and sixty of these genes have homologues that most closely associate with those present in *R. grylli* (Appendix Table 1). Thirteen genes share 98.5-100% similarity with their *R. grylli* homologue (Appendix Table 1). Three hundred and fifty of the genes identified by PROKKA are hypothetical genes and have not yet been fully characterised in this and other organisms. The 16S, 23S and 5S rDNAs are also featured within the 51 contigs, including 16 tRNAs except for Asparagine, Cytesine, Isoleucine and Phenylalanine (see NCBI submission: accession to be assigned). The genes included on the 51 contigs suggest a wide range of metabolic and physiological capabilities; of interest, are those that may be involved in virulence. These include secretion systems (Vir, Dot, Icm) and conjugal transfer proteins (Tra), which may aid horizontal gene transfer to conspecifics and host cells.

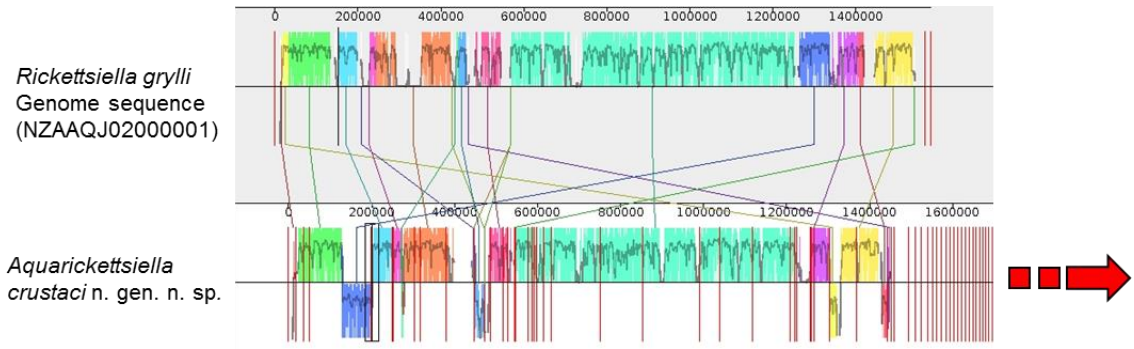


Figure 7.8: *Aquarickettsiella crustaci* n. gen. n. sp. scaffold comparison to the closest available genome, *Rickettsiella grylli* (NZAAQJ02000001). Overall the two species share 12 broad sections of spatial genomic sequence conservation that have shuffled around within the genome to occupy a different genomic order over evolutionary time. The red arrow indicates the other contiguous scaffolds produced from the sequence data that did not associate with the *R. grylli* genome.

7.4.3. Phylogeny of Aquarickettsiella crustaci n. gen. n. sp.

The 16S gene of *A. crustaci* n. gen. n. sp. was used to screen the NCBI database for similar species, determining that the closest known relative belonged to a *Rickettsiella* symbiont of *Asellus aquaticus* (similarity = 99%; e-value = 0.0) (AY447040) and that the

most closely related species with full taxonomic description was *R. isopodorum* (similarity = 97%; e-value = 0.0) (JX406180).

The 19-gene concatenated phylogeny determined that *R. grylli* is the most similar known taxon with complete genome sequence data, to *A. crustaci* n. gen. n. sp. (Fig. 7.9). The two isolates group together with 100% bootstrap confidence, but are separated by a branch distance of 0.298 substitutions per site. The phylogenetic tree representing the 16S genes of many available uncategorised isolates, *Rickettsiella* sp., or other Coxiellaceae, outlines a similar result whereby *A. crustaci* n. gen. n. sp. sits outside of the terrestrial *Rickettsiella*, grouping with aquatic examples of RLO isolates (Fig. 7.10). The single gene phylogeny showed strong support for the separation (77% bootstrap confidence) between the *Rickettsiella* spp. isolated from terrestrial environments/hosts and those isolated from aquatic environments/hosts (Fig. 7.10). The 16S phylogeny also determined that *R. isopodorum* and *R. armidillidii* branch separately to those *Rickettsiella* sp. that infect insect hosts (63% bootstrap confidence).

One species, *R. viridis*, branches early within the tree, and outside of the *Rickettsiella*, with 100% bootstrap confidence. The closest branching species on the tree to *R. viridis* is *Diplorickettsia massiliensis* (0.126 substitutions per site), which sits between *R. viridis* and the *Rickettsiella* and *Aquarickettsiella* n. gen.

Based upon the rDNA gene sequence of this novel RLO and closely related rDNA sequences from NCBI, along with ultrastructural differences (such as the lack of crystalline protein formation at the spherical initial body stage) between the terrestrial insect-infecting *Rickettsiella* and the aquatic crustacean-infecting RLO described here, it seems prudent to erect the novel genus, *Aquarickettsiella*, to hold this group of aquatic, crustacean-infecting RLOs.

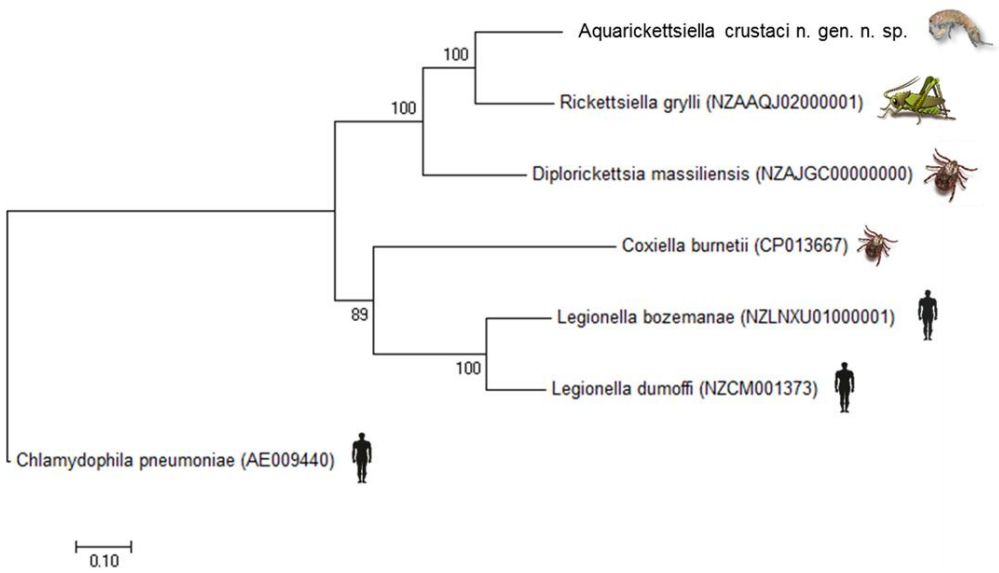


Figure 7.9: Phylogenetic placement of *Aquarickettsiella crustaci* n. gen. n. sp. using a 19 gene concatenated phylogeny, relative to other related bacterial species with the available gene complement for sequence analysis. The evolutionary history was inferred by Maximum Likelihood based on the Tamura 3-parameter model. The tree with the highest log likelihood (-160585.0007) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is to scale, with branch lengths measured in the number of substitutions per site. There were a total of 24736 positions in the final dataset.

7.4.4 Metagenomic identification of other species and host genetic data

Using the metagenomics data from the MiSeq analysis and genome assembly of *A. crustaci* n. gen. n. sp., several rDNA sequences were identified via the Metaxa2 software. Analysis of the assembled data revealed only three different sequences; a bacterial rRNA associating to *A. crustaci* n. gen. n. sp.; a mitochondrial 16S associating to the host, *G. fossarum*; and an 18S sequence also associating to the host, *G. fossarum*. Individual forward and reverse reads (23090904 individual reads) revealed 24 Archaea, 6828 Bacteria, 1962 Eukaryote, 2320 chloroplast and 5145 mitochondrial rDNA sequences in total. A BLASTn summary of the sequences is presented in additional Appendix files 1 and 2, and revealed that all Archaea and chloroplast sequences were bacterial. The bacterial sequences, aside from the Coxiellaceae, were composed of sequences relating to: *Methylomicrobium* sp.; *Oceanisphaera* sp.; *Cyclolasticus* sp.; *Bathymodiolus* sp.; *Xanthomonas* sp.; *Brugia* sp.; *Rhodanobacter* sp.; *Dyella* sp.; *Erwinia* sp.; or belonging to a taxonomically unassigned bacterial isolate or clone. The eukaryotic rDNA associations were only to the host (Amphipoda).

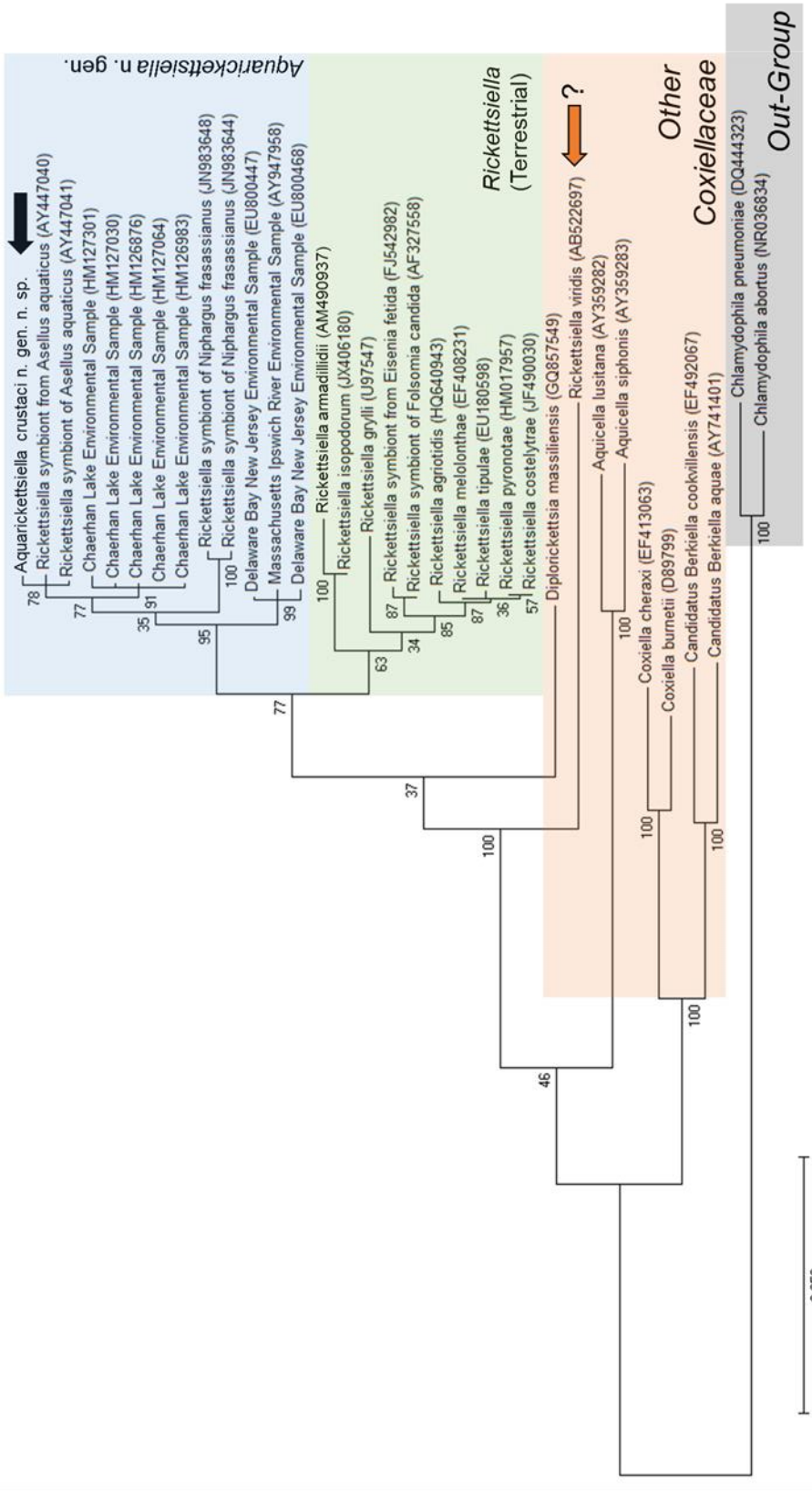


Figure 7.10: A phylogenetic tree of the available 16S gene sequences for several bacterial species, closely associated to *A. crustaci* n. gen. n. sp. (black arrow). The evolutionary history was inferred using Maximum Likelihood based on the Tamura 3-parameter model. The tree with the highest log likelihood (-8909.0296) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 33 nucleotide sequences. There were a total of 1643 positions in the final dataset. The orange arrow indicates *Rickettsiella viridis*, which sits outside the *Rickettsiella*.

The predicted mitochondrial genome of the host and several nuclear genes were also isolated from the metagenomics analysis. The mitochondrial and nuclear genes isolated

from the analysis are displayed in Appendix Table 2, and include the host 18S rDNA and 28S rDNA sequences along with any identifiable mitochondrial genes.

7.5. Taxonomic description

Domain: Prokaryota

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Legionellales

Family: Coxiellaceae

Genus: *Aquarickettsiella* n. gen.

Intracellular, rickettsia-like organisms, which are pathogenic for crustaceans in aquatic environments. Crystalline inclusions, present in insect-infecting *Rickettsiella*, are not present in crustacean-infecting *Aquarickettsiella*. The RLO infects the cell cytoplasm of host muscle, gill, gonad, nerve and haemal cells, resulting in a systemic infection. Externally visible pathologies include a white iridescent appearance to infected Crustacea, particularly their muscle tissues. The RLO will pass through a four-step development cycle including: the elementary body (smallest development stage); an elliptical, condensed sphere stage; division; and a spherical initial body. All developmental stages take place in the host cytoplasm, however the elementary body (infective stage) is predicted to be able to survive outside the host cell. Genome sequence data of novel species must show close relatedness through the phylogenetic methods used by this study, and gene conservation relative to the type species.

Type species: *Aquarickettsiella crustaci* n. gen. n. sp.

This species is intracellular in the tissues of the host, *Gammarus fossarum*, including the musculature, nervous system, gonad, gill and haemolymph. Heavy infection burden causes the animal to become white in colour, often iridescent with orange beads running along either side of its pereon. The ultrastructure of the elementary body is composed of an outer membrane measuring 496.73nm ± 37.56nm (n=20) in length, and 176.89nm ± 36.29nm in width, and is present with an electron dense core and electron lucent lamella. Development progresses from the elementary body, to an elliptical condensed sphere stage which undergoes division and includes an initial spherical body stage. Initial spherical body stages do not appear to contain crystalline substances observed in other

members of the family. *Aquarickettsiella crustaci* can be discriminated from others members of the family and presumably newly discovered members of the genus by 16S rDNA phylogenies, or construction of concatenated phylogenies based upon the multi-gene sequences as described herein.

Type host: *Gammarus fossarum* (Gammaridae).

Type locality: Bzura River in Łódź (Łagiewniki) (N51.824829, E19.459828).

Site of infection: Commonly intracellular within haemocytes, nerve cells, and muscle sarcolemma but can be identified within/around the gill and gonad.

Etymology: The genus name "*Aquarickettsiella*" is based upon the similarity between this genus and the sister genus *Rickettsiella*, whilst referring to the aquatic habitat and host in which the type species was detected. The specific epithet "*crustaci*" refers to the aquatic crustacean host of *Aquarickettsiella crustaci* n. gen. n. sp.

Type material: Histological, TEM and ethanol-fixed material is deposited within the Registry of Aquatic Pathology, Cefas, UK. Data pertaining to the 16S rDNA gene, MiSeq data for pathogen, host, etc., is deposited at the NCBI database (accession numbers to be assigned).

7.6. Discussion

This study explores the parasites, pathogens and commensals present in an amphipod species native to continental Europe (Poland), focussing specifically on a novel intracellular bacterial species named herein as *Aquarickettsiella crustaci* n. gen. n. sp. using histology, TEM, next generation sequencing and phylogenetics. *Aquarickettsiella crustaci* n. gen. n. sp. forms an interesting novel association between the pathogens of insects and crustaceans. It is important to consider the presence of *Aquarickettsiella* sp. in the native ecology and how this study may pave the way for further discoveries of similar species that may be applied as biocontrol agents to regulate the populations of high-profile invasive species, such as the killer shrimp, *Dikerogammarus villosus*. A greater understanding of the pathogens known to infect amphipods can advise control and biosecurity processes for invasive amphipods and their prospective diversity of hitchhikers (pathogens, parasites, commensals).

7.6.1. Taxonomic ranking of Aquarickettsiella crustaci n. gen. n. sp.

Considering the data provided by this study, the aquatic relations of the *Rickettsiella* display some significant differences to terrestrial species. Several insects have been found to include *Rickettsiella* spp. within their pathogen profile (Kreig, 1955; Roux et al. 1997; Leclerque and Kleespies, 2008; Leclerque et al. 2011; Kleespies et al. 2011; Leclerque et al. 2012; Tsuchida et al. 2014) as well as some terrestrial isopods (Cordaux et al. 2007; Kleespies et al. 2014). The phylogenetics conducted by this study suggests that, within the *Rickettsiella*, a divergence (63% bootstrap support) is seen between those species infecting crustaceans and those infecting insects (Fig. 7.10). Expanding upon this, a divergence (77% bootstrap support) is seen between RLOs isolated from aquatic hosts/environments relative to those from terrestrial hosts/environments (Fig. 7.9).

When bacterial physiology is considered, one primary feature mentioned in the initial genus description (Philip, 1956) is the crystalline protein production of the 'initial body' development stage of the *Rickettsiella*. This is missing from those relations that infect aquatic Crustacea (Federici, 1974; Larsson, 1982; This Study), but is observable for all the currently described terrestrial species, including the two terrestrial isopods (Vago et al. 1970; Kleespies et al. 2014).

Therefore, it seems prudent to erect a novel genus to include the aquatic crustacean-infecting species described herein. The primary reasons for this being phylogenetic and physiological reasoning, such as: the lack of crystalline protein formation in the initial body development, which is seen in the *Rickettsiella*; the divergence noted in the 16S phylogeny of aquatic and terrestrial isolates (Fig. 7.10); and the branching distance between *A. crusaci* n. gen. n. sp. and *R. grylli* (Fig. 7.9). As more *Aquarickettsiella* spp. are characterised, such as the two *Rickettsiella* symbionts isolated from *Asellus aquaticus* (AY447040 and AY447041) (Fig. 7.10), or those from *G. pulex* and *C. floridanus*, the solidarity of this genus should be reassessed.

7.6.2. Genome composition and annotation

This study identified 51 contigs associated with *A. crustaci* n. gen. n. sp. from the tissues of *G. fossarum*. Several of the genes isolated from the genomic fragments have homologues that associate to well-characterised pathogens, such as *Legionella* sp. (Edelstein et al. 1999). *Legionella* sp. have been used in model systems to identify which genes are involved in the infection process and several studies like the one by Edelstein et al (1999) have identified that Type IV secretion systems and conjugal transfer proteins are important for the virulence of *Legionella*. Such studies are yet to be conducted in

bacterial species that are more closely related to the *Aquarickettsiella*, however parallels can be drawn for certain homologues in both *A. crustaci* n. gen. n. sp. and *R. grylli*. Both species include Dot-like genes, Icm-like genes and conjugal transfer proteins (Tra) that are homologous to those found in *Legionella*. Only *A. crustaci* n. gen. n. sp. encodes Vir-like proteins homologous to those found in *Legionella*, *Tatlockia* and *Diplorickettsia*.

The presence of several genes associating to the Type IV secretion system in the genome of *A. crustaci* n. gen. n. sp. suggests it has the capability to introduce genetic material to its hosts cells, a process which may be similar to the well-characterised pathway used by *Agrobacterium tumefaciens* to engineer its hosts cell cycle to suit the needs of the bacteria (Wood et al. 2001; Tzfira and Citovsky, 2006). Pathologically, plants infected with the wild-type, pathogenic, *A. tumefaciens* result in localised cellular growth to form a "gall" (Wood et al. 2001; Tzfira and Citovsky, 2006). For *A. crustaci* n. gen. n. sp., the histopathology data revealed several infected tissue types, all of which were undergoing hypertrophy; in particular, the infected haemocytes had adhered to one another forming a large mass in the circulatory system of the host (Fig. 7.6a). High detail TEM images show a large number of bacteria in the haemocytes but not in any paracrystalline fashion (Fig. 7.7), suggesting that cellular hypertrophy may not be solely due to the overwhelming presence of bacteria. Although speculation at this point, this species and the systems encoded by its genome may provide a useful insight for future studies exploring the introduction of genetic material to crustacean tissues.

7.6.3. Why characterise the pathogens of native amphipod hosts?

Most species on the planet are evolutionarily adapted to survive in particular settings, but when transferred to new surroundings those species may either thrive and become invasive, or perish and are removed from the ecology. Amphipods are renowned for their capability to spread and colonise water systems, and several studies have assessed their hardiness (Bruijs et al. 2001), behaviour (Dick et al. 2002) and ability to spread (Bacela-Spychalska, 2016); even suggesting some are "perfect invaders" (Rewicz et al. 2014). With impending invasion comes the possibility to co-introduce disease (Dunn and Hatcher, 2015), or escape from disease, allowing the host to become fitter and more competitive in its new territory (Colautti et al. 2004). As these biological invasions are one of the major threats to biological diversity, finding natural enemies that may control the invasive species is an important task to achieve.

When a species escapes its native parasites and pathogens it is suspected that those disease-causing agents that are present at the lowest prevalence in the native range are

the most likely to be left behind. This means that when an invasive species moves to a new area it has likely lost a lot of its pathogen diversity (according to Enemy Release Hypothesis, e.g. Torchin et al. 2004), and with this a range of microbial agents that could be beneficial to biologically control the invasive species. *Gammarus fossarum* has now been detected in the UK and could be an invasive species that requires control (Blackman et al. 2017). This novel pathogen has the potential to be adapted into a control agent for this species.

By looking at a native amphipod in its co-evolved environment, it is more feasible to consider that the pathogens found are those that have co-evolved with the host. In this study, the identification of *A. crustaci* n. gen. n. sp. provides an example of a novel organism similar to agents that have been suggested as useful for biological control in the past (McNeill et al. 2014). *Aquarickettsiella crustaci* n. gen. n. sp. is the first fully characterised RLO from amphipods and this novel genus likely includes the RLOs identified from *C. floridanus* (Federici, 1974) and *G. pulex* (Larsson, 1982). This new discovery suggests that the native environments of high profile invasive amphipods, such as *D. villosus* and *Pontogammarus robustoides*, may hold a high diversity of microbial agents, perhaps even *Aquarickettsiella* spp., that are yet to be discovered from these amphipods and could benefit the biological control of these invaders. In addition, when invaders co-occur with native fauna, including *G. fossarum* inhabiting the lowland rivers of Central Europe, these invaders may face new pathogens, such as the one descried in this study, which could be contracted and may also play a role as a control agent.

CHAPTER 8

Metagenomics helps to expose the invasive pathogens associated with the demon shrimp (*Dikerogammarus haemobaphes*) and killer shrimp (*Dikerogammarus villosus*)

8.1. Abstract

Invasive species constitute a high risk for biodiversity conservation and have been recognised as a pathway for the introduction of pathogens and parasites. Understanding the parasitic complement of an invader benefits the risk assessment of the species and may inform policy makers to take the appropriate action to control invaders and their pathogens. Metagenomics is a highly adaptable tool to research the organisms living within hosts, including those carried by invasive and non-native species.

Invasive amphipods in the UK are carriers for several pathogen groups, including: Metazoa; Protozoa; Microsporidia; bacteria; and viruses. Our current knowledge of these pathogens has been derived from microscopy and PCR based studies. Herein I apply metagenomics to screen the demon shrimp, *Dikerogammarus haemobaphes*, and killer shrimp, *Dikerogammarus villosus*, for the presence of other organisms.

The application of metagenomic tools has further increased our knowledge of the species residing within these invasive amphipods. The demon shrimp was found to contain SSU rDNA sequence data with similarity to a range of species, including: bacteria (*Krokinobacter*; *Thiothrix*; *Deefgea rivuli*); Euglenoids (*Trachelomonas*); Oomycetes (*Saprolegnia parasitica*); and Microsporidia (*Cucumispora ornata*; *Dictyocoela berillonum*). Annotated protein and DNA sequence data identified three viral families present in the dataset: *Nudiviridae*; *Circoviridae*; *Ascoviridae/Iridoviridae*. *Paenibacillius*, putative symbiotic bacteria, various protists, fungal, microsporidian and nematode signals were also identified via protein similarity.

The killer shrimp samples contained SSU sequence data relating to 34 bacterial species. Protein annotation and similarity identified the presence of three viral families: *Nudiviridae*; *Circoviridae*; and *Nimaviridae*; one with protein similarity to white spot syndrome virus. Bacteria (*Burkholderia*; *Rickettsiales*) amoebae; and fungi were also detected through protein similarity searches.

Identification of these species increases the arsenal of potential biocontrol agents for these amphipods whilst providing an assessment for novel emerging disease. The increased knowledge gained through metagenomics can also provide an increased taxonomic understanding of invasive pathogen groups, can identify species that have been undetectable to conventional microscopy and PCR based studies, and can better advise policy on emerging wildlife diseases.

8.2. Introduction

Metagenomics, the *ad hoc* high-throughput sequencing of DNA, has revolutionised how researchers can assess, understand and characterise biodiversity (Tringe and Rubin, 2005). Its application has recently seen the discovery of novel taxonomic groups (Men et al. 2011), it has been involved in the diagnosis of human diseases and in the characterisation of the human gut microbiome (Turnbaugh et al. 2007), and has been applied as an environmental DNA (eDNA) diagnostic method to detect whether an environment is concealing invasive alien species (IAS) (Nathan et al. 2014; Rees et al. 2014). Metagenomics has wide applications in invasion biology and can help to provide a greater understanding of which IAS are present in an environment and what microbial complement they may be carrying. This tool can be adapted to identify the symbionts carried by IAS, and could provide a rapid screening tool for incoming invaders and their invasive pathogens (Roy et al. 2016; Chapter 1). Many IAS lack pathogen profiles and the use of metagenomics could rapidly build data upon this lack of knowledge. Despite this, understanding the level of diversity present does not reflect risk. Further characterisation of those symbionts is required to understand their pathological impact upon their host and their host range (Chapter 9).

IAS are one of the major causes of biodiversity loss and are a hindrance for conservation efforts (Russell and Blackburn, 2017). Anthropogenic activities transport IAS across the world and it is now a global priority to prevent their spread and impact (Singh et al. 2015). A major threat from invasion, observed in over 25% of cases, is the co-introduction of invasive pathogens, which result in wildlife health issues (Roy et al. 2016).

Squirrel pox (*Squirrelpox virus*) (Chantrey et al. 2014), Crayfish Plague (*Aphanomyces astaci*) (Jussila et al. 2015) and Chitrid Fungus (*Batrachochytrium dendrobatidis*) (McMahon et al. 2013) are all examples of high-impact invasive pathogens (Roy et al. 2016). The detection of each of these pathogens was only after their effects had been observed due to spill-over and the decline of native/vulnerable species. To identify and potentially prevent invasive pathogens from reaching native hosts in future invasions it

is important to screen invasive populations (low impact or high impact IAS) for pathogens (Chapter 6). In the past, invaders have been screened for pathogens using a wide suite of techniques. These primarily include histological analysis (Bojko et al. 2013) and the application of specific/degenerate molecular diagnostics (Arundell et al. 2015).

The UK suffers from a diversity of IAS, however a recent "high-impact" amphipod invader known as the killer shrimp, *Dikerogammarus villosus*, is a priority species and is considered to be a "perfect invader" (Rewicz et al. 2015). This species is co-invasive along with its pathogens in continental Europe (Wattier et al. 2007) but has escaped several of its native parasites (including acanthocephalan, microsporidian and viral agents) during its invasion of the UK but still harbours some of its more commensal associations (Wattier et al. 2007; Bojko et al. 2013; Arundell et al. 2015).

A congeneric of *D. villosus*, the demon shrimp (*Dikerogammarus haemobaphes*) tells a different parasitological story in its invasion of the UK. This invader has carried with it a suite of parasites and pathogens, including: viruses; microsporidia; gregarines; nematodes; and trematodes, all detected through the application of histology, electron microscopy and molecular diagnostics (Green-Extabe et al. 2015; Chapter 5; Chapter 7). *Dikerogammarus haemobaphes* has a lower predatory impact than *D. villosus* (Bovy et al. 2014), however *D. haemobaphes* harbours a higher diversity of parasites and pathogens, which may pose a risk to native species (Chapter 5).

This study utilises metagenomics to detect the hidden microbial diversity in two invasive species: *D. villosus* and *D. haemobaphes*, which continue to spread throughout the UK. Although this study involves a specific case study using these two amphipods it has wider applications to how invasive species should be screened for pathogens in the future to avoid/detect the introduction of invasive pathogens and identify which species show the greatest risk as pathogen carriers.

8.3. Materials and Methods

8.3.1. Sample collection

In total, six whole animals were analysed using metagenomics; three *D. villosus* and three *D. haemobaphes*. Two *D. villosus* were taken from archived ethanol-fixed material collected from Grafham Water (September 2011 and August 2012). The final *D. villosus* was collected from Grafham Water in June 2014 and snap-frozen in liquid nitrogen. Two *D. haemobaphes* were collected form Carlton Brook (Leicestershire) in June 2015, and fixed onsite in 99% ethanol. The urosome of a third specimen, observed to harbour two

viruses via histology from separate studies (Chapters 3 and 10), was collected in May 2015 and was maintained in the laboratory for two days before dissection and fixation in 99% ethanol.

8.3.2. Sample preparation, sequence assembly and analysis

Each separate animal underwent DNA extraction via a Phenol-Chloroform method resulting in six high-quality DNA extracts. Preparation followed that specified by the Illumina protocol for indexing via a NEXTERA XT DNA library preparation kit (Illumina) for use with a 'V3 600' Illumina MiSeq cartridge (Illumina). The specimens were run in tandem on a single Illumina MiSeq run and were attributed to their specific barcode after the process. Cumulatively this provided 4.5Gbp of sequence data; 1.9Gbp belonging to *D. villosus* specimens and 2.6Gbp belonging to *D. haemobaphes* specimens.

All bioinformatics analyses were conducted through BioLinux (Field et al. 2006). The sequence data was initially trimmed using Illuminaclip (Trimmomatic-Illumina) (Bolger et al. 2014) and assembled using the a5 pipeline (Coil et al. 2014) to provide 35574 individual scaffolds attributed to the *D. villosus* specimens, and 64782 individual scaffolds for the *D. haemobaphes* specimens. Scaffolds were annotated using PROKKA (Seemann et al. 2014) and GlimmerHMM (Majoros et al. 2004) to distinguish between protein-coding genes that may include introns, and analysed using DIAMOND (Buchfink et al. 2015) in combination with MEGAN6 (Huson et al. 2007) to visualise the taxonomic distribution of predicted-protein sequence data. MEGAN6 inference of taxonomy is limited and often incorrect so confirmation of sequence similarity using BLASTp was conducted and the results are available in the Appendix files. Predicted protein sequences for the viral taxa were analysed for function and domain presence/structure using UniProt (UniProt consortium, 2017), InterPro (Quevillon et al. 2005) and BLASTp.

The program Metaxa2 (Bengtsson-Palme et al. 2015) was applied to raw read data as well as assembled data to detect pathogen diversity based on the presence of rDNA sequences. In addition to the collection of microbial diversity data, any nuclear or mitochondrial host genes that could be distinguished from the assembly were also characterised. Raw read data is used to detect any SSU information lost during assembly cut-off at 300bp.

8.3.3. Phylogenetics

All phylogenetic analyses were conducted in MEGA version 7.0 (Kumar et al. 2016). Phylogenetic analysis of *Dh*BV (PIF-1: 500aa), DvBV (PIF-2: 406aa), *Dikerogammarus*

haemobaphes bi-facies-like virus (DhbflV) (Helicase: ~150aa) and the *Dikerogammarus villosus* WSSV-like virus (DNA polymerase: 2495aa) involved Clustal W alignment with the Gonnet weight matrix and a delay divergent cut off of 30%. The maximum likelihood tree topography was based on 100 bootstraps using the Dayhoff model (Schwarz and Dayhoff, 1979). The REP proteins of *Dikerogammarus haemobaphes* circovirus (~320aa) and *Dikerogammarus villosus* Circovirus (~430aa), along with the REP proteins of other *Circoviridae*, were aligned using Clustal W, as described above. The maximum likelihood tree was developed using 100 bootstraps and based on the Poisson correction model (Zuckerkandl and Pauling, 1965).

8.4. Results

8.4.1. Taxonomic output from Metaxa2 (SSU rDNA sequence diversity)

The forward, reverse and assembled reads for each species were used to search for rDNA sequences that would conform to the host or any other organisms that also encoded an rDNA gene. The number of sequences with similarity to other species were used to determine the diversity of the microbial presence within the demon and killer shrimp.

8.4.1.1. SSU rDNA diversity in the D. haemobaphes *microbiome*

94,392 DNA scaffolds (minimum length of 300bp) consisting of 59,256kbp were assembled for the cumulative demon shrimp samples, from an original 1,142,175kbp of forward raw reads and 1,489,302kbp of reverse raw reads. Metaxa2 analysis of the assembled reads revealed 11 bacterial, 10 eukaryotic and 1 mitochondrial SSU sequence(s). The bacterial sequences showed closest similarity to *Krokinobacter* sp., *Thiothrix* sp., *Deefgea rivuli*, and two uncultured bacterial clones (Appendix Table 8.1). The eukaryotic sequences showed the closest similarity to the host (*Dikerogammarus* sp.), *Trachelomonas* sp., *Saprolegnia parasitica*, *Saprolegnia* sp., *Cucumispora ornata* (*Microsporidium* sp. Dhae17W) and *Dictyocoela berillonum* (Appendix Table 8.2). Finally, the single mitochondrial sequence showed closest similarity to *Dikerogammarus haemobaphes* (AJ440890; 98.5% similarity; e-value: $2e^{-158}$). The combined raw reads identified 503 predicted bacterial sequences (Appendix Table 8.3), 1524 predicted eukaryotic sequences (Appendix Table 8.4) and 6 predicted mitochondrial sequences (Appendix Table 8.5).

8.4.1.2. SSU rDNA diversity in the D. villosus *microbiome*

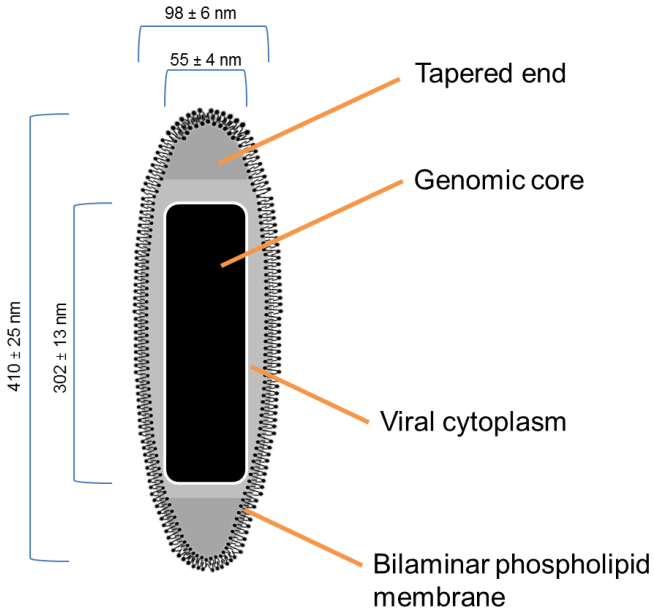
22,141 DNA scaffolds (minimum length of 300bp) consisting of 32,984kbp were assembled for the cumulative killer shrimp samples, from an original 2,216,565kbp of forward raw reads and 1,992,039kbp of reverse raw reads. The assembled reads gave only host-specific sequences for both the 18S and mitochondrial 16S genes. The raw forward and reverse reads identified a total 34 bacterial, 2131 eukaryotic and 54 mitochondrial SSU sequences. The 34 bacterial sequences link specifically to the *Flavobacterium* sp*., Sporichthya* sp*., Piscinibacter* sp*., Pseudomonas baetica, Parasegetibacter* sp., *Bacteroidetes* sp., *Delftia tsuruhatensis,* several uncultured proteobacteria, and several uncultured bacterial clones (Appendix Table 8.6). All of the eukaryotic SSU sequences link closest to host sequences as did all of the mitochondrial sequences (Appendix Table 8.7).

8.4.2. Taxonomic output from MEGAN6 (protein-coding gene sequence diversity)

The DNA scaffolds were each annotated to search for viral, bacterial and eukaryotic gene sequences using a combination of different protein-coding gene annotators. Each batch of predicted genes were visualised in MEGAN6, which attributes them to a particular species. MEGAN6 inference of taxonomy is limited and often incorrect so confirmation of sequence similarity using BLASTp was conducted and the results are available in the Appendix files.

8.4.2.1. Dikerogammarus haemobaphes *viral diversity*

Sequence data belonging to three viral families were detected through protein sequence similarity: *Nudiviridae*; *Circoviridae* and *Iridoviridae*/*Ascoviridae*. The first included 16 different genes across 10 scaffolds that associate to the *Nudiviridae* and belong to *Dikerogammarus haemobaphes* Bacilliform Virus (*Dh*BV) (Appendix Table 8.8; Fig. 8.1). The 16 genes encode proteins for replication, lifecycle, viral structure, infectivity and carbohydrate metabolism (Appendix Table 8; Fig. 8.1). Phylogenetic analysis identified that *Dh*BV is most closely related to *Penaeus monodon* Nudivirus (*Pm*NV) a virus of the decapod *P. monodon*, using the PIF-1 gene (per os infectivity factor) (Fig. 8.2).



PROKKA-predicted ORF's and annotation:

Protein label	Length (bp)	Length (aa)	Predicted protein	Predicted function	Predicted location
PROKKA_02100	2364	787	Unknown	Unknown	Unknown
PROKKA_02101	1653	550	Polysaccharide lyase	Carbohydrate metabolism	Unknown
PROKKA_02847	1362	435	Baculovirus envelope (E56)	Unknown	Viral envelope
PROKKA_03129	1401	466	Unknown	Unknown	Unknown
PROKKA_03548	279	92	Unknown	Unknown	Unknown
PROKKA_03549	1959	652	LEF-8	DNA-templated transcription	Unknown
PROKKA_05984	591	196	Baculoviridae P74	Viral life-cycle	Unknown
PROKKA_05985	1371	456	Unknown	Unknown	Unknown
PROKKA_07216	1716	571	Polysaccharide lyase	Carbohydrate metabolism	Unknown
PROKKA_09164	1503	500	PIF-1	Infectivity factor	Unknown
PROKKA_12086	459	152	VLF-1	DNA recombination and integration	Unknown
PROKKA_12087	711	236	LEF-9	RNA polymerase	Unknown
PROKKA_14566	1071	356	Helicase	DNA unwinding	Unknown
PROKKA_15365	990	329	p-loop NTPase	Molecule conformation alteration	Unknown

Figure 8.1: A morphological representation of *Dikerogammarus haemobaphes* Bacilliform virus along with the predicted gene and protein annotations, and their various sizes and functions, which associate to this virus.

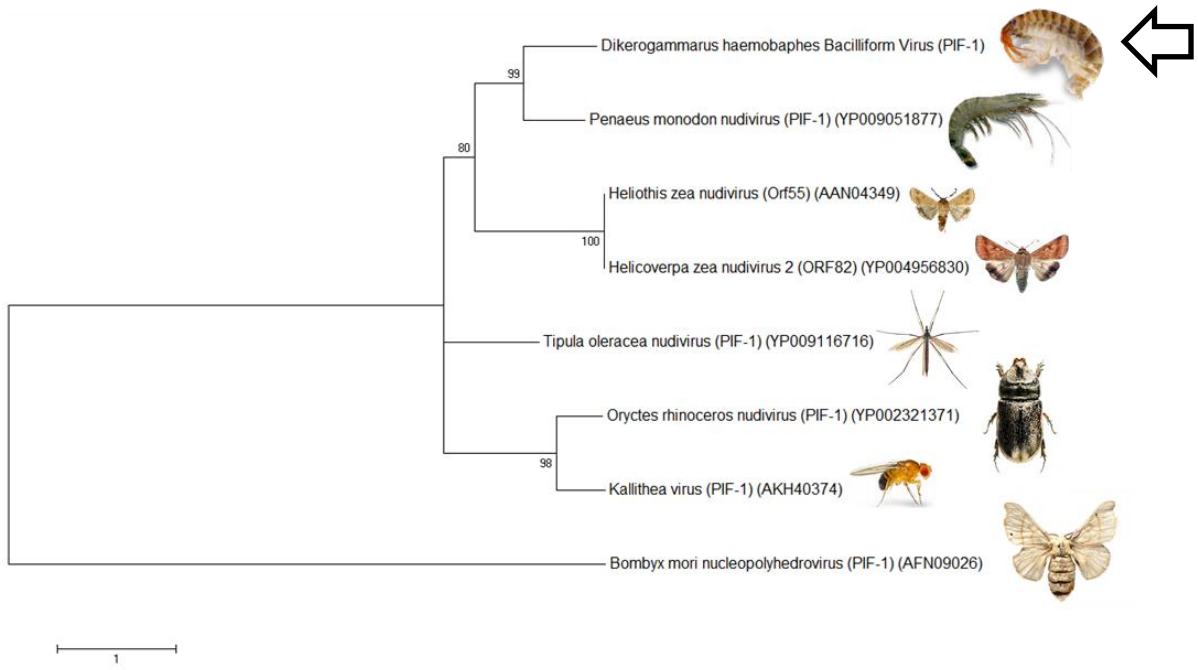


Figure 8.2: A phylogenetic tree representing DhBV (white arrow) relative to other nudiviruses, based on the PIF-1 protein. The evolutionary history of this tree was inferred by using the Maximum Likelihood method based on the Dayhoff matrix based model. The tree with the highest log likelihood (-9219.6279) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 amino acid sequences. There were a total of 611 positions in the final dataset.

Three scaffolds were annotated with genes that relate to the *Circoviridae*, specifically the Rep gene (replication-associated) and resultant protein. One scaffold encoded the conserved nonanucleotide sequence (AGTATTAC), where ssDNA synthesis is initiated, however the capsid protein could not be identified through annotation or otherwise. Phylogenetic analysis of the amino acid sequence for the REP protein revealed that the closest identified branching relative to the three sequences was from a circular virus infecting the hermit crab, *Petrochinus diogenes* (accession: YP 009163897; sequence similarity: 33%; sequence coverage: 78%; e-value: $2e^{-42}$) (Fig. 8.3). However, overall the sequence identified closest with an uncharacterised protein from *Hyalella azteca* (accession: XP 018015067; sequence similarity: 45%; sequence coverage: 91%; e-value: $7e^{-74}$) and the REP protein of a 'Dragonfly orbiculatusvirus' (accession: YP 009021243; sequence similarity: 39%; sequence coverage: 78%; e-value: $2e^{-50}$).

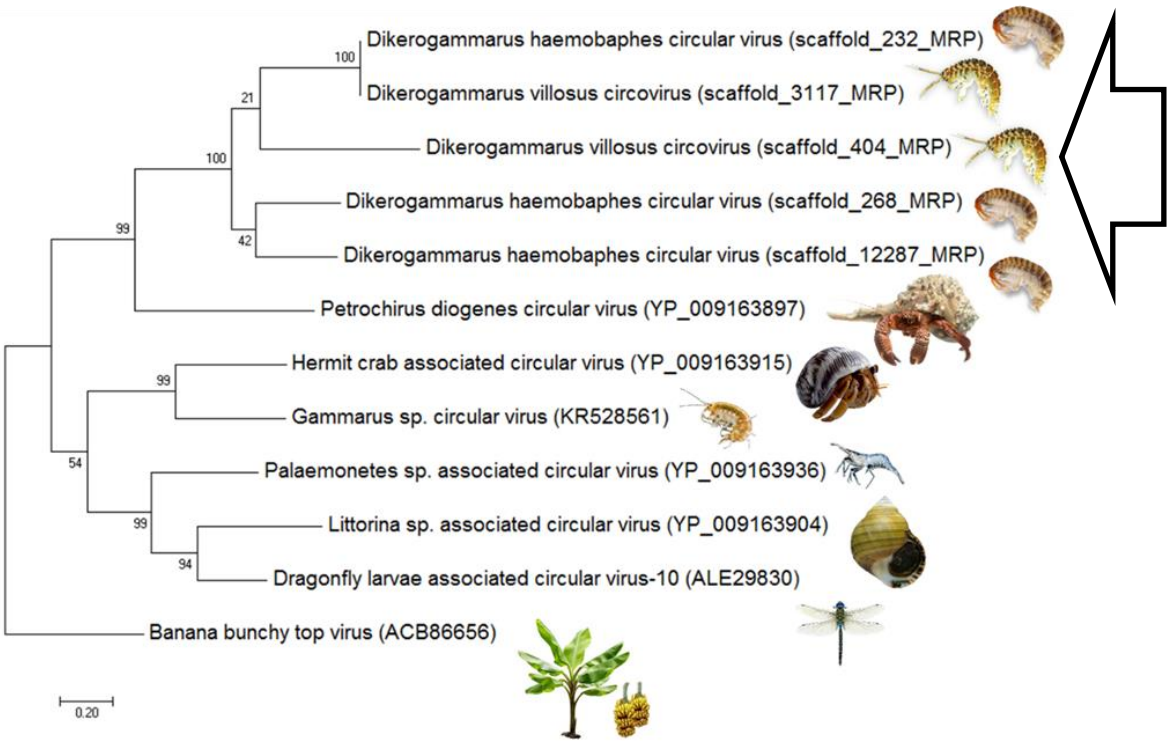
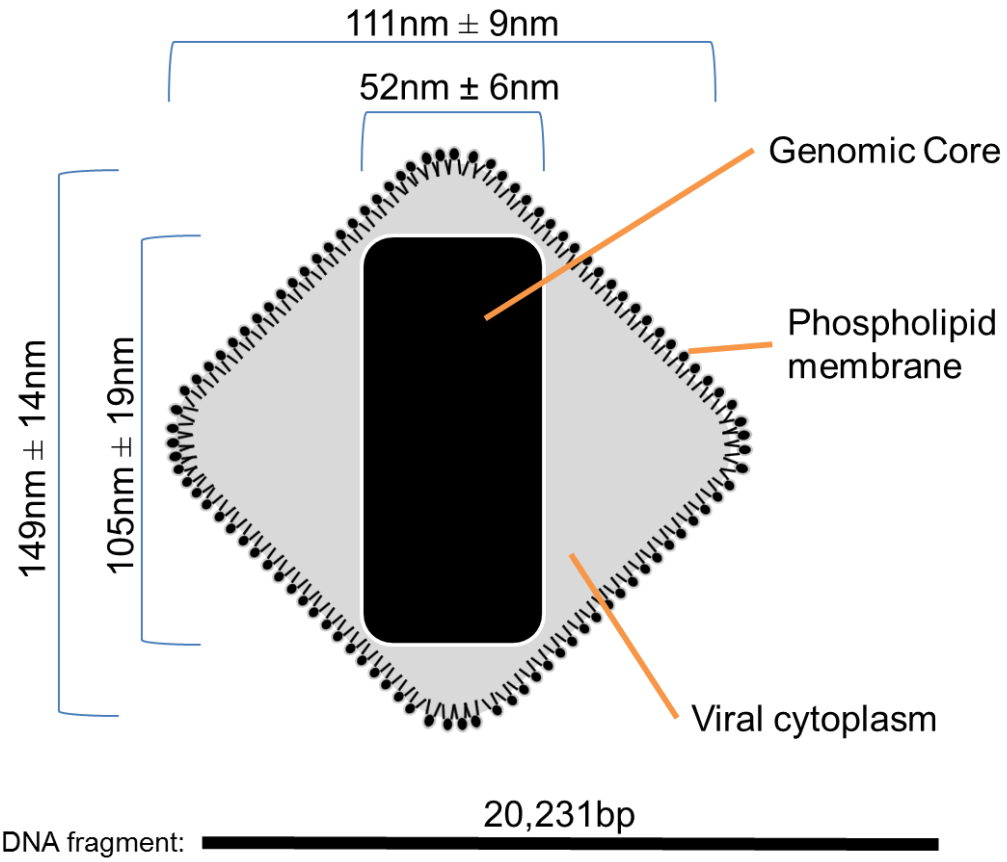


Figure 8.3: A phylogenetic tree comparing the circovirus replication proteins from *Dikerogammarus* spp. (white arrow) metagenomics analyses. The evolutionary history was inferred by using the Maximum Likelihood method based on the Poisson correction model. The tree with the highest log likelihood (-8955.9982) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 12 amino acid sequences. There were a total of 456 positions in the final dataset.

A single scaffold of 20,231bp included a protein coding gene that associated closest to *Panulirus argus* Virus 1 (PAV-1), a virus distantly related to the *Iridoviridae*/*Ascoviridae* and known to infect the Caribbean spiny lobster, *Panulirus argus*. This scaffold was annotated with 18 putative protein coding genes with predicted functions to include: short RNA synthesis; DNA unwinding; host cell apoptosis; transcription; viral capsid structure; and DNA replication (Appendix Table 8.9; Fig. 8.4). Phylogenetic comparison, using the helicase gene of DhbfIV, grouped this virus with PAV-1 at 96% confidence (Fig. 8.5).



PROKKA ORF detection and annotation:

Protein label	Length (bp)	Length (aa)	Predicted protein	Predicted function	Predicted location
PROKKA_00064	219	72	Unknown	Unknown	Unknown
PROKKA_00065	243	80	Unknown	Unknown	Transmembrane
PROKKA_00066	2937	978	Primase/Helicase	Short RNA synthesis	Unknown
PROKKA_00067	405	134	Unknown	Unknown	Unknown
PROKKA_00068	465	154	Helicase	DNA Unwinding	Unknown
PROKKA_00069	306	101	Unknown	Unknown	Unknown
PROKKA_00070	864	287	Unknown	Unknown	Unknown
PROKKA_00071	762	253	Unknown	Unknown	Unknown
PROKKA_00072	618	205	Unknown	Unknown	Unknown
PROKKA_00073	192	63	Unknown	Unknown	Unknown
PROKKA_00074	1068	355	ADP-Ribosylation	Host Cell Apoptosis	Unknown
PROKKA_00075	249	82	Unknown	Unknown	Unknown
PROKKA_00076	1011	336	Unknown	Unknown	Unknown
PROKKA_00077	267	88	Unknown	Unknown	Unknown
PROKKA_00078	3084	1027	DNA-directed RNA polymerase	Transcription	Unknown
PROKKA_00079	645	214	Unknown	Unknown	Unknown
PROKKA_00080	1533	510	Hexon coat protein	Structural protein	Viral capsid
PROKKA_00081	2538	845	DNA-Directed DNA Polymerase	DNA Replication	Unknown

Figure 8.4: A morphological representation of *Dikerogammarus haemobaphes* bi-facies-like virus along with the predicted gene and protein annotations, and their various sizes and functions, which associate to this virus.

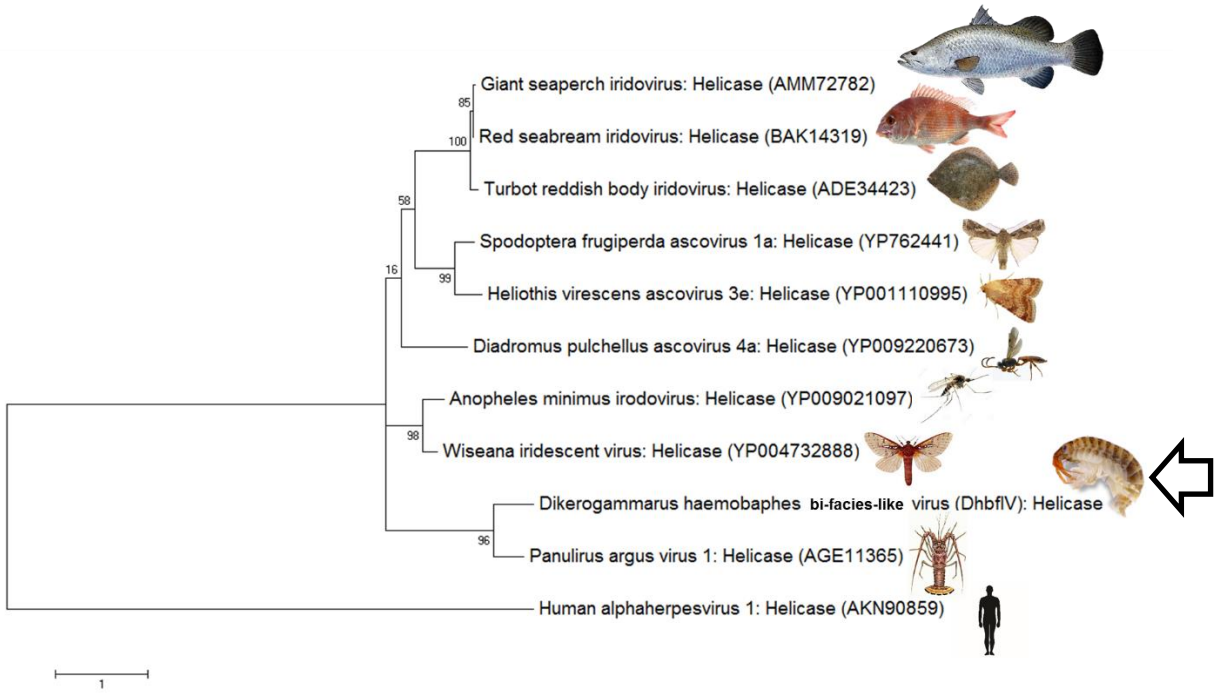


Figure 8.5: A phylogenetic comparison between DhbfIV and related viruses from the *Ascoviridae* and *Iridoviridae* using the helicase protein. The evolutionary history was inferred by using the Maximum Likelihood method based on the Dayhoff matrix based model. The tree with the highest log likelihood (-5754.9049) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 11 amino acid sequences. There were a total of 886 positions in the final dataset.

8.4.2.2. Dikerogammarus haemobaphes *bacterial diversity*

Those bacterial groups best represented through the protein analysis referred to the *Paenibacillus* (11 proteins over 7 scaffolds), a 'gill symbiontic bacteria' from a mollusc (8 proteins over 8 scaffolds), *Thiothrix* (27 proteins over 27 scaffolds), *Burkholderia* (9 proteins over 9 scaffolds) and *Flavobacterium* (9 proteins over 9 scaffolds). *Thiothix* sp., *Burkholderia* sp. and *Flavobacterium* sp. are commonly found in water systems however the other two bacteria detected through protein annotation are of particular interest.

The predicted proteins associating to *Paenibacillus* sp. all annotate as hypothetical except for one which identifies as a LexA DNA binding protein (280aa). After BLASTp analysis a single hypothetical protein was found to relate closest to a hypothetical protein of *Paenibacillus pini* (accession: WP036653661; similarity: 39%; coverage: 79%; e-value: 4e-13). The other proteins were found to be linked to other organisms (Appendix File 8.1).

The 8 predicted proteins associating to the ‘gill symbiotic bacteria’ show a predicted functionality as reverse transcriptases (3), pol-like proteins (2), ribonucleases (2), and a hypothetical protein (Appendix File 8.2).

8.4.2.3. Dikerogammarus haemobaphes *protist, microsporidian, fungal and metazoan diversity*

MEGAN6 scaffold annotation and representation revealed a variety of predicted proteins associated with the Viridiplantae (120), Stramenopiles (39), Opisthokonta (42), Acrasiomycetes (994), Rhabditida (59), Deuterostomia (3166), Fungi (389), Amoebozoa (128), and Microsporidia (95). It was assumed that the Viridiplantae and Stramenopiles were likely environmental contamination from gut material or attached to the carapace.

The protistan groups include the Opisthokonta, Acrasiomycetes, and Amoebozoa. The 42 proteins associating with the Opisthokonta are detailed in Appendix files (Appendix File 8.3). Some sequences show similarity to *Capsaspora owczarzaki*, the closest known unicellular organism to the metazoa. The Acrasiomycetes are represented by 994 predicted proteins (Appendix File 8.4), some associating to *Fonticula alba*, a slime mould. Those proteins grouping within the Amoebozoa (Appendix File 8.5) include reference to *Dictyostelium fasciculatum*.

The microsporidian proteins were identified by bacterial protein annotation due to their prokaryotic-like splicing patterns, providing 95 representative protein sequences (Appendix File 8.6). These sequences related closest to a range of different microsporidian species, including: *Anncaliia algerae*; *Encephalitozoon* sp.; *Edhazardia aedis*; *Pseudoloma neurophilia*; *Trachipleistophora hominis*; *Vavraia culicis*; *Nosema* sp.; *Spraguea lophii*; and *Ordospora colligata*.

The fungi were represented in the annotated dataset by 389 predicted proteins (Appendix File 8.7) crossing a wide range of fungal groups (Dikarya; Saccharomycetales; Sordariomyceta; Eurotiomycetidae; and Dothideomycetes), but were primarily associated with four species: *Trichophyton tonsurans* (172 associated proteins); *Trichophyton equinum* (41 associated proteins); *Podospora anserine* (26 associated proteins); and *Ophiocordyceps sinensis* (17 associated proteins), according to MEGAN6. BLASTp analysis suggested that many of the sequences relating to the fungi through MEGAN6 were in fact more closely related to other organisms (Appendix File 8.7) with one showing similarity to *Trichophyton*.

The metazoan parasites were represented by proteins associating to the Rhabditida (Appendix File 8.8) in MEGAN6. BLASTp analysis confirmed sequence similarity to *Caenorhabditis elegans* for some of the proteins.

8.4.2.4 Dikerogammarus villosus *viral diversity*

Sequence data associating to viruses from the killer shrimp material showed closest identity to three viral families: *Nimaviridae* (*Whispovirus*); *Nudiviridae*; and *Circoviridae*. A single scaffold of 56,544bp was annotated with 36 predicted protein coding genes (Appendix Table 8.10). The predicted function of each gene is presented in Appendix Table 8.11. Broadly, the genes annotated on this scaffold correlate with protein domains involved in nucleotide binding, viral lifecycle, DNA repair, inhibition of apoptosis, viral DNA replication, phosphorylation, transmembrane proteins, and others of unknown function. Phylogenetic comparison of the DNA-directed DNA polymerase protein sequence on this scaffold relative to other dsDNA viral species is presented in Figure 8.6. The dsDNA virus families represented on the tree show clear grouping using the DNA polymerase amino acid sequence for the representatives of each family. *Dikerogammarus villosus* WSSV-like virus DNA polymerase branches before the primary members of the *Nimaviridae* [WSSV, RVCM and *Metopaulias depressus* WSSV-like virus, *Chionoecetes opilio* Bacilliform Virus (*Co*BV) (100% bootstrap confidence)] with a bootstrap confidence of 92%. *Dikerogammarus villosus* WSSV-like virus DNA polymerase is 5.217 substitutions per site away from WSSV, where the most distant member of this family (*Co*BV) is 0.869 substitutions per site away from WSSV.

Six predicted protein coding genes were annotated on the dataset that correspond to the *Nudiviridae*, and belong to *Dikerogammarus villosus* Bacilliform Virus (DvBV). These genes relate closest to PmNV (Appendix Table 8.12) and their function corresponds to p-loop NTPase activity (nucleotide binding), per os infectivity and several of undefined function (Appendix Table 8.13). Using the PIF-2 gene, a phylogenetic analysis of the relative taxonomic position of this virus was tested, revealing that this virus groups with PmNV at 100% bootstrap confidence (Fig. 8.7).

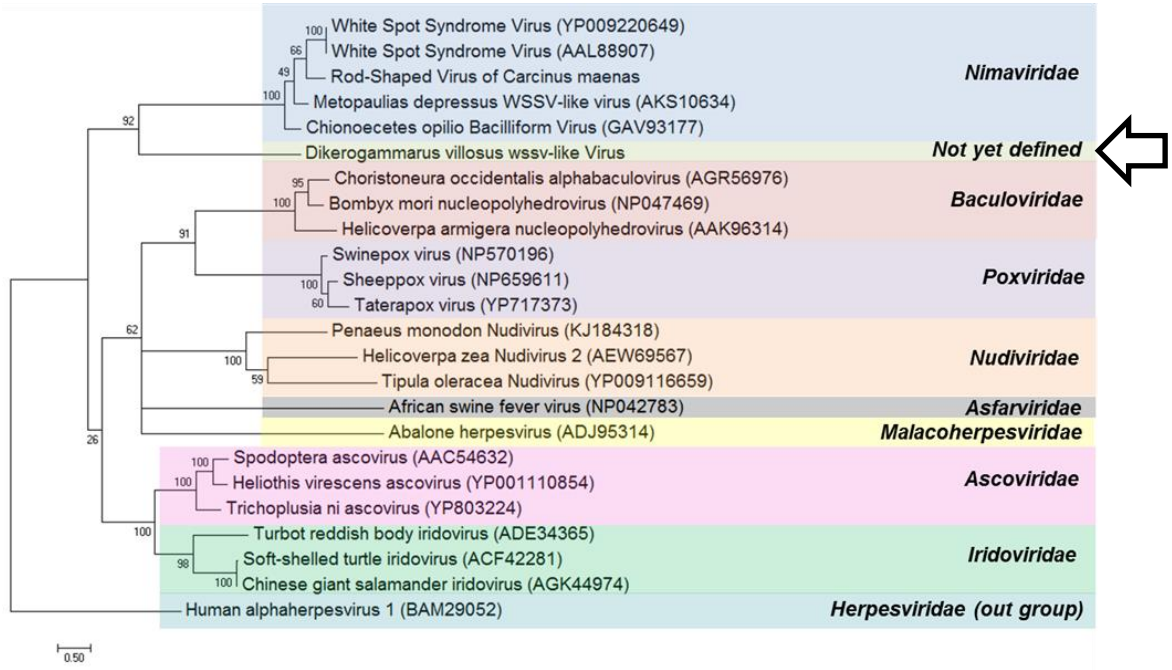


Figure 8.6: A phylogenetic tree representing the dsDNA viruses, including the novel WSSV-like virus DNA polymerase protein sequence from *D. villosus* (white arrow). Each group is defined by a separate colour and the viral family, if available, is named. The evolutionary history was inferred by using the Maximum Likelihood method based on the Dayhoff matrix based model. The tree with the highest log likelihood (-72173.2962) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The analysis involved 24 amino acid sequences. There were a total of 2761 positions in the final dataset.

Two scaffolds (3322bp, 1462bp) were found to contain Rep genes associating with the *Circoviridae*. One scaffold was also annotated with a second hypothetical protein. BLASTp analysis revealed that scaffold 1 (3322bp) REP protein was most similar to an uncharacterised protein from *H. azteca* (XP018015067; similarity: 41%; coverage: 87%; e-value: 2e-80). Scaffold 2 (1462bp) REP protein was also most similar to an uncharacterised protein from *H. azteca* (XP018015067; similarity: 40%; coverage: 80%; e-value: 4e-77). The hypothetical protein on Scaffold 1 did not show close affinity to any other known protein on NCBI. Incorporation of the two REP proteins into the Circovirus phylogenetic tree including *Dikerogammarus haemobaphes* circovirus revealed that these two proteins grouped together with those from *D. haemobaphes* (Fig. 8.3).

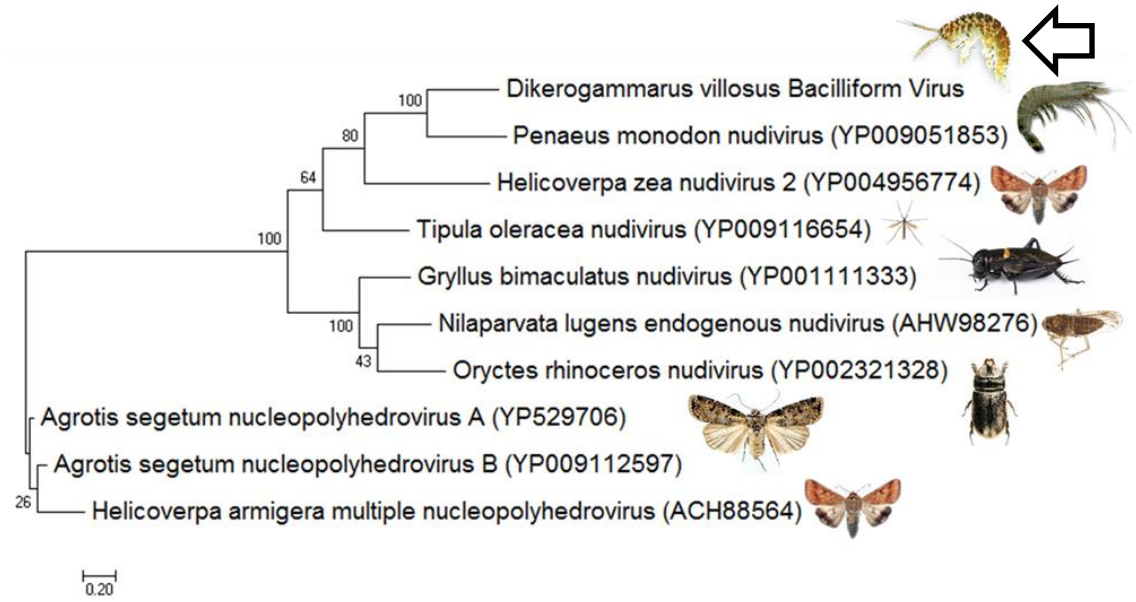


Figure 8.7: A phylogenetic tree representing DvBV (white arrow) relative to other nudiviruses, based on the PIF-2 protein. The evolutionary history was inferred by using the Maximum Likelihood method based on the Dayhoff matrix based model. The tree with the highest log likelihood (-8082.3528) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 10 amino acid sequences. There were a total of 486 positions in the final dataset.

8.4.2.5. Dikerogammarus villosus *bacterial diversity*

Proteins with similarity to *Burkholderia* spp., and a group of proteins referring to the *Rickettsiales* were identified as the most prominent bacterial organisms among the protein similarity analysis in MEGAN6.

Burkholderia spp. were identified from 11 different scaffolds to hold 32 predicted protein sequences in MEGAN6, however only one protein was found to have significant similarity with *Burkholderia multivorans* (Appendix File 8.9).

Those annotations referring to the *Rickettsiales* covered 6 scaffolds and included 11 predicted proteins (Appendix File 8.10), some showing similarity to the hypothetical proteins of *Anaplasma phagocytophilum* and *Rickettsia amblyommii*.

8.4.2.6. Dikerogammarus villosus *protist, microsporidian, fungal and metazoan diversity*

MEGAN6 associated a variety of predicted proteins with the Viridiplantae (105), Stramenopiles (31), Acrasiomycetes (775), Rhabditida (62), Fungi (250), and Amoebozoa (82). It was assumed that the Viridiplantae and Stramenopiles were likely environmental contamination from gut material or attached to the carapace.

After BLASTp confirmation, the protistan groups associated with the killer shrimp included only the Amoebozoa. Some proteins grouping within the Amoebozoa (Appendix File 8.11) show similarity to hypothetical proteins of *Dictyostelium* sp.

The fungi were represented by MEGAN6 to include 250 predicted proteins (Appendix File 8.12), which after BLASTp analysis were primarily associated with other organisms, except for one protein showing similarity to link to *Aspergillus flavus*.

No metazoan parasites could be determined from the dataset.

8.4.3 Host sequence data

The DNA scaffolds containing nuclear genes for each host species were detected using BLASTp on post-assembled scaffolds annotated using GlimmerHM, to assess for their closest eukaryotic taxa and predicted function of any proteins or RNA produced. The partial mitochondrial genomes of *D. haemobaphes* and *D. villosus* were also assembled (accession numbers to be assigned).

8.4.3.1. Dikerogammarus haemobaphes *nuclear and mitochondrial genes*

The assembly data primarily consisted of host sequences that were annotated to contain over 100 genes showing similarity to homologues in other species (Appendix Table 8.14). The 28S, 18S and 5.8S genes of the host were all identified along with several genes that show similarity to snRNAs of *Parhyale hawaiensis*. The genes detected encoded proteins with various function, such as: histone proteins; DNA-repair/replication proteins; oxygen-carriers; phosphorylation enzymes; hormones; metabolic enzymes/proteins; or proteins with other predicted functions (Appendix Table 8.14). Various heat shock proteins, a cadherin-related protein, and a double-stranded RNA-binding protein were also identified. Observation of such proteins provides detail to possible stress responses, susceptibility to delta-endotoxins and the presence of an RNAi pathway in this host.

8.4.3.2. Dikerogammarus villosus *nuclear and mitochondrial genes*

Genes predicted to belong to the host included functions as: energy production (mitochondrial genes); histone proteins; developmental proteins; DNA-repair/replication proteins; oxygen-carriers; phosphorylation enzymes; hormones; muscle structural proteins; nerve system and sight related proteins; RNAi pathway-related proteins; transcription factors; heat-shock response proteins; metabolic enzymes/proteins; or proteins with other predicted functions (Appendix Table 8.15). Among the scaffolds, the 5.8S, 18S, 28S and various snRNAs were also identified, including a specific link to *D. villosus* via 100% similarity in the 18S gene.

8.5. Discussion

Understanding the multitude of hitchhiking species travelling along with an invasive host is paramount to best understand the extended impact of an invasion and predict the impacts novel invasive diseases may cause to a naïve ecosystem (Roy et al. 2016). *Dikerogammarus* spp. in the UK have been found to harbour a range of pathogens through histological and molecular identification (Bojko et al. 2013; Green-Etxabe et al. 2015; Chapter 5), however detailed screening techniques, such as the application of next generation sequencing, have the potential to unveil a greater diversity of associated pathogens; primarily those that are asymptomatic or latent with the genome of an invasive host. Prior to this study, the killer shrimp was thought to have the greatest impact as an invasive predator (Dick et al. 2002), however the detection of a novel virus linked to the *Nimaviridae* may mean this amphipod holds a greater risk as a disease carrier. Dedicated parasitological screening efforts comprise a worthwhile addition to the risk assessment regimen of invasive species, irrelevant of their low or high impact status (Chapter 6).

8.5.1. The microbiome of the demon shrimp

Dikerogammarus haemobaphes has been categorised as a low-impact non-native species relative to other invasive amphipods in the UK (Bovy et al. 2014). Despite this, the species appears to be an invasive pathogen carrier, and the invasive hosts low impact is likely due to the presence of mortality inducing pathogens (Chapters 5 and 9). Metagenomic analysis of the species has identified a range of known and novel parasites and pathogens, including DNA sequence identification of: bacteria; *Saprolegnia* sp.; and microsporidians. Protein sequence similarity comparison identified three viral groups (*Nudiviridae*, *Iridoviridae*/*Ascoviridae*, and *Circoviridae*), bacteria (*Paenibacillus*,

symbiotic bacteria, etc.); increased confidence in microsporidian detection, fungi (primary similarity to *Trichophyton*), protistan-like protein signals (amoebae, slime moulds and *Capsaspora-like* proteins), and finally some protein similarity to the Rhabditida.

A single protein sequence showed closest similarity with *C. elegans*, a nematode, indicating that a nematode species may have been present in the study specimens. Nematodes have been detected from *D. haemobaphes* (*Hysterothylacium deardorffoverstreetorum* and *Cystoopsis acipenseris*) (Bauer et al. 2002; Green-Extabe et al. 2015), and this sequence could identify with the presence of these species.

Genetic and protein similarity data to *Saprolegnia* spp., with specific 99% similarity to *S. parasitica*, indicates that *D. haemobaphes* may be a carrier, or host, of this pathogen group. *Saprolegnia parasitica* is an oomycete pathogen of freshwater fish species (van West, 2006) and related oomycete parasites, such as *Aphanomyces astaci* (crayfish plague), are lethal pathogens of endangered crayfish species (Svoboda et al. 2014). Further work is needed to identify the oomycete entourage of *D. haemobaphes* taxonomically and determine if this pathogen is a risk to native species, or if it has the potential to control this invader.

The high number of genes associating to the *Trichophyton* indicates the presence of a fungal species. The *Trichophyton* genus includes both soil dwelling and parasitic species, meaning that taxonomic identification of fungi from *D. haemobaphes* could be a worthwhile endeavour in the search for biocontrol agents (Hajek and Delalibera, 2010). *Dictyocoela berillonum* and *C. ornata* are known to be present in this invasive population and the microsporidian protein signals detected during this study likely attribute to either parasite. SSU identification of euglean, *Trachelomonas*, is likely an environmental observation from the host gut.

The SSU sequences of *Krokinobacter, Thiothrix,* and *Deefgea* were all acquired from Metaxa2 analysis, and further detection of bacteria through protein sequence similarity (*Paenibacillus*, *Burkholderia* and *Flavobacterium*) provide an insight into the microbiome of this host. *Krokinobacter* and *Flavobacterium* are similar taxa and commonly isolated from environmental samples and associated with biogeochemical processes (Khan et al. 2006). *Thiothrix* sp. are thought to have a similar role, but as Sulphur-oxidising organisms (Rubio-Rincon et al. 2017). *Deefgea* sp. are common aquatic anaerobes, however they have been commonly associated with disease in fish (Jung and Jung-Schroers, 2011). Bacteria belonging to the *Burkholderia* have been isolated from humans, animals and plants, as pathogenic and symbiotic species (Eberl and Vandamme, 2016; Limmathurotsakul et al. 2016). Finally, *Paenibacillus larvae* is associated with ‘foulbrood

disease' in honey bees (*Apis* sp.), resulting in a limited capability to reproduce (Descamps et al. 2016). Identification of similar bacteria that could reduce the reproductive capability of invasive *D. haemobaphes* would provide insight into new biocontrol potential.

Dikerogammarus haemobaphes Bacilliform Virus has morphological (bacilliform shape; membrane-bound; size; genome composition) and pathological features (hepatopancreatits-inducing; nucleus-bound) putatively attributing this virus to the *Nudiviridae* (Yang et al. 2014; Chapter 9). This study has now associated 16 novel gene sequences to the *Nudiviridae*, which likely associate with *Dh*BV, and phylogenetic assessment using the PIF-1 gene has confirmed this virus sits closest to a second crustacean nudivirus, PmNV (Yang et al. 2014). This virus is known to infect *D. haemobaphes* in its invasive ranges, including the UK and Poland (Chapters 3 and 10).

Three protein sequences with similarity to circoviral replication genes may indicate another viral association with this species. Phylogenetic analyses show that this virus, along with a similar virus identified from *D. villosus*, groups with other *Circoviridae* from marine crustaceans. Protein sequence similarity assessment using BLASTp identified that a gene from the amphipod, *H. azteca* (XP 018015067) did show relatively close association to the proteins identified from *Dikerogammarus* spp. This could indicate that these proteins may be present in the genome of these hosts, however no other host genes were present on the contiguous sequences upon which the annotation took place. Alternatively, this could indicate that the *H. azeta* specimen that underwent genome sequencing may have been infected with a circovirus, which was either endogenous or may have been incorrectly incorporated into the genome of the host during *in silico* assembly (Murali et al. Unpublished; NCBI – direct submission).

Viruses relating to the *Ascoviridae* and *Iridoviridae* have been isolated from several crustacean hosts, including *Panulirus argus* virus 1 (PAV-1), various herpes-like viruses, and 'bi-facies virus' from *Callinectes sapidus* (Bateman and Stentiford, 2017). Only PAV-1 has any related genetic information. The partial genome for *DhbflV* presented in this study has one gene that shows high similarity and phylogenetic association to PAV-1, as well as morphological and pathological similarity, indicating they are likely related viral species. The PAV-1 virus has been associated with high mortality rates in Caribbean *P. argus* populations (Butler et al. 2008) and if *DhbflV* shares a similar mortality-inducing trait, this virus could be an important control agent of *D. haemobaphes* and may provide further reasoning as to why this species has a lower environmental impact in the UK.

8.5.2. The microbiome of the killer shrimp

Invasive and native *D. villosus* populations are associated with specific groups of pathogens, including: helminths (acanthocephala, trematodes); protists (apicomplexans); microsporidia (opisthosporidians); and viruses (dsDNA) (Bojko et al. 2013; Rewicz et al. 2014). Through next generation sequencing, several novel groups, such as a range of novel viral, bacterial, amoebal, and nematode associations have also been made. Retrospectively, this technique did not detect several of the parasites previously identified from this species, such as the gregarines (common in UK specimens) or microsporidian pathogens (thought to have been lost through enemy release) and use of this technique in tandem with histological and TEM evidence is paramount for future studies involving the pathological screening of invaders. Increased sample size of animals screened via metagenomic analysis may increase the detectable diversity, where this study was limited through the use of six individuals.

The detection of amoebae through protein sequence similarity requires a follow-up study to identify and confirm the presence of these pathogen groups. Amoebae have been associated with mortality in crustacean species in the past (Mullen et al. 2004; Mullen et al. 2005) and this amoebae could be a risk to native wildlife, or a potential control agent for *D. villosus*.

The bacterial diversity identified from the metagenomics dataset seems limited to commensal species, without any of the 16S sequences detected through the Metaxa2 analysis linking to any known pathogenic bacterial groups. The identification of bacterial species through protein sequence data detected some bacteria that correspond to rickettsia-like organisms (RLO). RLOs have been identified from crustacea in the past and may be suitable as biocontrol agents (Chapters 3, 6 and 7). Taxonomic identification and pathological description of RLOs from *D. villosus* would increase the repertoire of available control agents for this species.

This study has shed greater taxonomic detail on the viral entourage carried by this species, identifying that viruses with similarity to the *Nimaviridae*, *Nudiviridae*, and *Circoviridae* can be identified from invasive populations.

Detection of six nudiviral genes likely associate with the morphologically described *Dv*BV, which holds morphological and pathological similarity to PmNV, a nudivirus from *Penaeus monodon* (Bojko et al. 2013; Yang et al. 2014). This virus has been detected from the Polish invasive range and was not detected in the UK via histology (Bojko et al. 2013). Metagenomic analysis has now detected this virus in the UK meaning that it has avoided detection through histological screening (Bojko et al. 2013). The presence of a

virus linking to the *Nimaviridae* is discussed below. The circovirus identifies closest with other crustacean-infecting ssDNA viruses, however little is known about the morphology and pathology of this virus. Now that gene sequence data is available for these viruses it provides the incentive to develop diagnostic tools to assess both invasive populations and vulnerable native species for positive infection status. Development of a detection method also provides a basis to taxonomically identify these viruses in future studies.

8.5.3. Metagenomic discovery of a related member of the Nimaviridae in the Killer Shrimp

A 56,544bp DNA scaffold was assembled with genes that have similarity to WSSV, a high impact aquaculture disease, and related viruses. White spot syndrome virus has the greatest impact of any disease upon penaeid aquaculture, contributing to gross economic losses of over $3bn (Stentiford et al. 2012). This virus is known to have a wide host range (Rajendran et al. 1999), and can induce mortality in aquaculture species in less than a day (Kim et al. 2007). Viruses related to WSSV and unofficial members of the *Nimaviridae* have been morphologically described in the past, including: B-virus (Bazin et al. 1974); RVCM (Johnson, 1988); B2-Virus (Mari and Bomani, 1986); Baculo-B virus (Johnson, 1988); Baculo-A virus (Johnson, 1976); Tau virus (Pappalardo et al. 1986); and *Chionoecetes opilio* Bacilliform Virus (Kon et al. 2011). Each of these is associated with haemolymph infection in the host, however the host range of these unofficial *Nimaviridae* is not reported.

The presence of a WSSV-like virus travelling alongside the killer shrimp throughout Europe could constitute a major threat to susceptible wildlife and aquaculture. Without pathological information to corroborate with the metagenomics detection of this virus it is difficult to be sure of the pathology associated, and whether it shares a pathological impact similar to its relatives listed above. The development of a diagnostic tool, like a sensitive PCR or biosensor, would provide the necessary equipment to rapidly detect this virus in *D. villosus* and any other hosts. This information would also contribute to the taxonomic description of this virus.

8.5.4. The potential for pest control

Dikerogammarus villosus has had a large impact on native ecology in the UK (MacNeil et al. 2013) and requires control and/or eradication to preserve the environment and native ecosystem. Avenues for the control of this species span physical, chemical and biological possibilities. Chemical control methods have had laboratory trialling (Stebbing

et al. Unpublished) and include the use of a hot-water treatment system to aid biosecurity (Anderson et al. 2015). The potential for biological control for this species is an advancing field, with the continued detection of novel pathogenic species (Ovcharenko et al. 2010; Bojko et al. 2013) and experimentation with those species to better understand their impact upon the hosts' behaviour and survival (Bacela-Spychalska et al. 2014). This study has now increased the range of possible biocontrol agents for the demon and killer shrimp, which require host range and survival testing. In particular, the detection of oomycetes, microsporidia and viruses may hold the greatest potential as control agents due to the impacts of related species upon their hosts life-span (crayfish plague; *Cucumispora dikerogammari*; WSSV) (Ovcharenko et al. 2010; Svoboda et al. 2014; Kim et al. 2007). However, caution must be taken because of the possibility that these novel pathogens may affect non-target hosts.

Alternate possibilities include the development of endotoxins, like Bt toxin (*Bacillus thuringiensis*), that can reduce the survival of some Crustacea. These have recently been identified from emerging aquaculture diseases (Han et al. 2015). Re-adaptation of such toxins to combat invasive species is a possible avenue for control, but also one that requires much research: firstly to understand the Pir-toxin mechanism; and secondly the susceptibility of target and non-target species. The host genetic data provided here could help to advance control options by providing genetic and protein sequence data that could link to the Pir-toxin mechanism. For example, a cadherin-like gene was found on scaffolds associating to *D. haemobaphes*; cadherin is involved in the Bt toxin mechanism.

A second method that benefits from the presence of host gene data is RNA interference as a control tool (Katoch et al. 2013). Genetic data from both *Dikerogammarus* spp. has identified dsRNA-interacting proteins that may be involved in the host's natural RNAi pathway to protect it from viral infection. This method has been adapted to control insects and can also control other pests (Katoch et al. 2013). RNAi is a specific method and works by providing dsRNA complementary to mRNA produced by the host to result in excision and breakdown of the translation pathway for a crucial host gene. Without expression of a crucial gene, a cell will undergo apoptosis. On a large scale, this can result in the death of an organism (Katoch et al. 2013). Developing RNAi targets for *D. villosus* and *D. haemobaphes* genes is a viable possibility to control these invasive species.

8.5.5. Concluding remarks and the use of metagenomics to understand the co-invasive microbiome of IAS

Metagenomics has proven to be a useful tool for characterising biodiversity (Tringe and Rubin, 2005) and detecting novel taxonomic groups (Men et al. 2011). It has been involved in disease diagnosis (Turnbaugh et al. 2007), and applied as an eDNA tool (Bass et al. 2015), and here I have shown metagenomics to be a highly informative tool for the parasitological screening of invasive species. Despite this it is important to address some limitations to the use of this technique. Firstly is sample size, which if increased would provide a greater understanding of the diversity of symbionts but which is limited by the costs of the technique. The use of power analyses could identify how many animals require screening to be certain of the presence/loss of a symbiont. In this study I utilised whole animals because of interests of symbionts present throughout the individuals, not just specific tissues; however this predisposes to environmental contamination that could result in the identification of fouling organisms and not true symbionts. I also employ the use of genetic and protein data to screen the dataset. This is highly informative for genetic data but less so for protein sequence data, because proteins can be similarly produced from different gene sequences. Despite this, the viruses identified from this study are so diverse that without protein comparison it would have been impossible to identify them from the data via similarity comparison. Error rate within sequencing is relatively low for Illumina technologies (76% correct base calls) (Quail et al. 2012) but is a limitation to the use of the technique – due to this it is important to rely primarily on assembled data and to quality check as has been conducted herein.

Despite these limitations this tool has identified a wide range of symbionts present upon the IAS from a wide range of taxonomic groups and allows their characterisation to species level on a genetic level. This technique is more general than PCR and is capable of sequencing all the genetic material available, not just specified primer-flanked regions. It also provides a greater screening method than histological assessment, despite lacking the ability to provide pathological information.

Its common application is much needed to advance our understanding of the pathogens, parasites and commensals carried by invasive species. In addition, the application of this tool can further increase our knowledge about the invasive hosts' genome composition and identify possible targets for control.

CHAPTER 9

Pathogens carried to Great Britain by invasive *Dikerogammarus haemobaphes* alter their hosts' activity and survival, but may also pose a threat to native amphipod populations

9.1. Abstract

Non-native species that are introduced without their natural enemies can become invasive due to the absence of population regulation, benefiting spread and population growth. When non-native species are introduced with their natural enemies, these enemies may limit the impact of the invader, but may also pose a risk to native taxa. *Dikerogammarus haemobaphes* is a low-impact non-native species, widespread in the UK, and was introduced with a microsporidian pathogen (*Cucumispora ornata*). Here, I describe three complementary studies that explore the impacts of *D. haemobaphes* pathogen communities on native and invasive species.

The first study is a broad screen for pathogens carried by *D. haemobaphes* using histology, electron microscopy and molecular diagnostics. The results show two novel viruses [*Dikerogammarus haemobaphes* bi-facies-like virus (DhbflV), *Dikerogammarus haemobaphes* Bacilliform Virus (DhBV)], along with microsporidians, apicomplexans, and digeneans.

In the second study the effect of parasitism on the host was explored. *Dikerogammarus haemobaphes* were tested using two behavioural assays that measured (i) relative activity and (ii) aggregation behaviour. Hosts were then screened using histology to identify their individual pathogen profile and compare it to the activity and social aggregation behaviour of their host. The results show that infection with DhBV was correlated with increased host activity, and that high burden infections of *C. ornata* reduced host activity.

In the third study, feed containing the microsporidian *C. ornata* was provided to *D. haemobaphes*, a second invader *Dikerogammarus villosus*, and the native amphipod *Gammarus pulex*, in a laboratory trial. Additionally, *ad hoc* samples of macroinvertebrates were collected to screen for *C. ornata* in wild populations. *Dikerogammarus haemobaphes* and *G. pulex* were both PCR positive for *C. ornata*

infection after the laboratory trial, and *D. villosus* was not. Survival analysis revealed that *C. ornata* significantly decreased survival in *D. haemobaphes* and *G. pulex*. Further screening for DhbflV infection in *D. haemobaphes* revealed that this virus also reduced survival.

In conclusion, *C. ornata* was detected in native and invasive fauna and was observed to transmit to *G. pulex* experimentally, with evidence of spores in the musculature via histological analysis. This suggests *C. ornata* is not a suitable biocontrol agent and may constitute a threat to native wildlife, including to a keystone shredder in aquatic ecosystems.

9.2. Introduction

Invasive alien species (IAS) can impact negatively on the environments they encounter, causing damage to biodiversity (Molnar et al. 2008), ecosystem services (Dukes and Mooney, 2004) and environmental and man-made structures (Dutton and Conroy, 1998). An often-overlooked concept in invasion biology, particularly in behavioural assessment, is the complex relationships that IAS share with their parasites and pathogens (Vilcinskas, 2015). Parasites and pathogens can accompany their host along its invasion route (Dunn, 2009) or can be left behind (enemy release) increasing the fitness of the invasive propagules (Lee and Klasing, 2004; Heger and Jeschke, 2014; Prior and Hellmann, 2014). If pathogens persist along invasion pathways and in introduced populations, the possibility of disease introduction becomes feasible, resulting in the potential for host switching events (Roy et al. 2016). Alternatively, the pathogens introduced by an invader can control its population size and impact through infection (Dunn and Hatcher, 2015); the mechanisms involved in this process are similar to those involved with biological control.

Biological control is a process which utilises ‘enemies’ of a target organism (such as a parasite or pathogen) to regulate that organism’s behaviour and/or population size through introduction, augmentation or conservation of a biological agent (Hajek et al. 2007; Lacey et al. 2015). The use of pathogens as biocontrol agents is a well-studied subject area common within the agricultural industry (McFadyen, 1998; Lacey et al. 2001; De Faria and Wraight, 2007). Managed environments, such as farmland, are often protected from pests through application of pathogenic agents, such as microsporidians and baculoviruses (Lacey et al. 2001; De Faria and Wraight, 2007). If appropriate control agents can be found or developed, it is reasonable to consider that such mechanisms could be applied to control invasive crustacean species.

The invasive ‘demon shrimp’, *Dikerogammarus haemobaphes*, carried a microsporidian parasite (*Cucumispora ornata*) into the UK in 2012 (Chapter 5). Whether this parasite regulates the populations of *D. haemobaphes* is unclear. *Dikerogammarus haemobaphes* is thought to pose a lesser impact on invaded communities than its congener, *Dikerogammarus villosus* (the ‘killer shrimp’), which invaded the UK in 2010 without its microsporidian parasites (MacNeil et al. 2010; Bojko et al. 2013; Bovy et al. 2014; Dodd et al. 2014). However, by carrying pathogens to new habitats, the demon shrimp could act as a high-profile invader due to its status as a pathogen carrier (Chapter 6).

Identifying the pathogens present in *D. haemobaphes*, and their affects upon their host, as well as alternative native and invasive species, will help to better understand their role as either a control agent or wildlife threat. If the diseases carried by *D. haemobaphes* limit its behaviour and survival rate they may make good biocontrol agents. Alternatively, if their host range includes non-target species, and infection results in mortality, they may be more of a threat to native species than a prospective control agent for IAS.

In this study I compare the activity, aggregation, and rate of survival for healthy and infected *D. haemobaphes*, taken directly from their invasive habitat. *Cucumispora ornata*, two novel viruses [*Dikerogammarus haemobaphes* bi-facies-like virus (DhbflV)] [*Dikerogammarus haemobaphes* Bacilliform Virus (DhBV)], Digenea, and gut gregarines were all shown to infect *D. haemobaphes* using histology, transmission electron microscopy (TEM) and molecular diagnostics, or a combination of those tools. DhBV and DhbflV are described morphologically using histopathology and TEM. The host range of *C. ornata* within UK freshwater taxa is tested using a nested PCR procedure, and the impact of this parasite on type (*D. haemobaphes*) and alternative (*Gammarus pulex*; *D. villosus*) host survival, is assessed using an experimental transmission trial.

9.3. Materials and Methods

9.3.1. Sampling and acclimatisation of test subjects

Dikerogammarus haemobaphes were collected via kick sampling (18/05/2015, 19/07/2015, 27/07/2015, 03/08/2015) from Carlton Brook (Leicestershire, UK) (grid ref: SK3870004400) for behavioural assessment, physiological analysis and pathogen screening. A second collection was conducted from the same area on 14/08/2016 for individuals for use in pathogen transmission trials. *Dikerogammarus villosus* were collected from Grafham Water (TL1442767283) for use in the transmission trials (20/09/2016). Two collections of *Gammarus pulex* were conducted, one group found co-

occurring in Carlton Brook alongside *D. haemobaphes* were sampled (14/08/2016) and a second naïve population of *G. pulex* from Meanwood park, Leeds (SE2803737255) (01/11/2016), which have not encountered the invader before.

9.3.2. Experimental transmission trial and survival data collection

An inoculum was produced by homogenising the carcasses of *D. haemobaphes*, visibly infected with *C. ornata*, which was fed to the animals included in the exposure trial. The inoculum was not quantified in terms of the number of spores, meaning that individuals may have received different concentrations of pathogen. The composition of animals in each trial is outlined in Table 9.1, where animals collected on site were immediately fixed in ethanol to identify the background prevalence of *C. ornata* in the wild population. In addition to these amphipod specimens, bivalves, beetle larvae, fly larvae, isopods, leeches and snails were also obtained during the visit and were tested with both general and specific microsporidian primers.

Species/Population	Sample site	Collected on site	Control trial	Exposure trial
D. haemobaphes	Carlton Brook	30	29	27
D. villosus	Grafham Water	30	29	28
G. pulex	Carlton Brook	17	9	10
G. pulex	Meanwood Park	30	13	14

Table 9.1: A breakdown of the animals used in each transmission trial to allow exposure to *C. ornata* spores. The "collected on site" column outlines the number of animals collected for microsporidian screening prior to conducting the survival challenge, to obtain an understanding of background prevalence on site at the time of collection. The control trial were fed uninfected material. The exposure trial were fed the same amount of food which was composed of homogenate infected tissue (confirmed by PCR to contain *C. ornata*).

Each animal used in the transmission trial was separated into individual petri-dishes which were split into oxygenated tanks. The trials consisted of a 48hr starvation period before providing 15mg of food pellets (uninfected material) to each petri-dish in the control group and 15mg of demon shrimp homogenate (infected tissue positive for *C. ornata* via nested PCR, but not for virus via PCR) to the exposure group. Each group was cultured for 30 days after initial starvation and survival rate was measured at 12:00pm on a daily basis. During (if mortality occurred) or after the trial, *D. haemobaphes* were cut in two, one half fixed in 100% ethanol for molecular diagnostics to assess for pathogen presence and the second used to produce more homogenate to feed alternative species. *Dikerogammarus villosus* and *G. pulex* were cut in half for dissection to allow pathogenic assessment using both molecular diagnostics (head and I-III pereon segment) and histology (IV pereon segment to telson) to detect infection.

9.3.3. Impact of natural infection on the behaviour and fitness of field collected *D. haemobaphes*

Dikerogammarus haemobaphes (n=282) underwent measurement of various morphological characteristics, including: sex; presence and number of offspring; length; weight; and pair status. After collection, animals were transported to the University of Leeds and acclimatised in canal water with vegetation at 14˚C for a minimum of 24 hours before use in behaviour trials. Each animal was only used once, and upon completion of the behavioural trial were fixed for histology.

9.3.3.1. Activity assessment

Dikerogammarus haemobaphes (n=120) were placed into uniform transparent pots bisected equally with a black line. Animals were placed on this line at 00:00min and provided with 02:00min to acclimatize to the new surroundings. After 02:00min, activity (crosses of the black line) was recorded between 02:00-04:00min, 06:00-08:00min and 10:00-12:00min providing a total 6 minutes of activity data collection per individual. Animal activity was not recorded between 00:00-02:00min (acclimatisation period), 04:00-06:00min and 08:00-10:00min. After each experiment the test subject was measured for size, weight, gravidity, egg clutch size, mating pair status, and if visibly infected with microsporidia. Similar methods were applied by Bacela-Spychalska et al. (2014).

9.3.3.2. Aggregation assessment

Dikerogammarus haemobaphes (n=63) were assessed for their aggregative behaviour (amount of time aggregating in either a social or null zone) using an experimental set-up that consisted of a white tray which was bisected by a black line complete with buffer zone (2cm locus). This white tray contained two gauze cages of 8cm^3 volume with 0.5mm mesh size, one containing with four male *D. haemobaphes* and the second empty at either end of the tray. Gauze cages were placed equidistant to the black line. The side of the tray containing the gauze cages present with animals was designated the ‘social zone’ and the side without animals the ‘null zone’. De-chlorinated water was changed before each experiment which included 03:00min with gauze cages in the water to allow the scent of the males to spread equally before each experiment. The test subject was placed into a black tube on the buffer zone to acclimatize for a further 02:00min. Once acclimatised, the test subject was released from the black tube and its time spent in either zone was measured over a 10:00min period. Time data collected from this

experiment was used to create a percentage of time spent in each area. Time spent in the buffer zone was excluded to ensure that the preferences corresponded to a strong choice between the social and null zones.

9.3.4. Histology and transmission electron microscopy

Specimens were anaesthetised using carbonated water and dissected; removing the urosome for DNA extraction and molecular diagnostics with the rest of the animal being fixed for histological analysis. This same procedure took place after each behavioural experiment for each test subject. A single specimen displaying a rare viral infection was cut from wax block it was initially preserved in for histology, to be re-processed for TEM analysis. A stock specimen collected from Chapter 5 was used to gather TEM evidence for the Bacilliform Virus infection of the hepatopancreas.

Dikerogammarus haemobaphes displaying *C. ornata* infection in the histology were assigned a burden intensity ranging from uninfected (score = 0) through to heavy infection (score = 3) (see: Fig. 9.1). Animals displaying Bacilliform Virus infection were assigned a percentage burden estimation using the number of infected nuclei of the hepatopancreas divided by the total number of nuclei in the hepatopancreas. Other infections were not assessed for burden but recorded in binary as infected or uninfected (0-1).

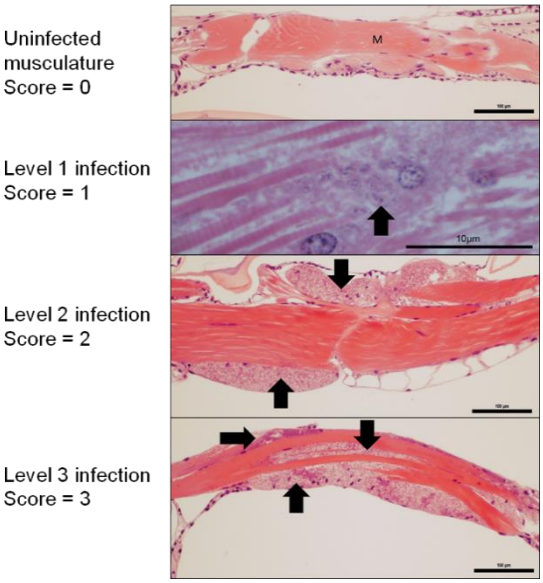


Figure 9.1: The microsporidian intensity scale used to histologically quantify the burden of a microsporidian infection. The scale starts at 0 (uninfected) and moves through to level 3 (heavy burden infection) as shown to the left of the diagram. The black arrows indicate the infected areas in all images. Scale 1 identifies the presence of microsporidian development stages at the lowest burden, perhaps even without spore formation as shown. Scale 2 shows sarcolemma infection (can include connective tissue infection). Scale 3 shows the highest burden where myofibrils and sarcolemma are infected throughout the host.

For full details of the histological procedure refer to Chapter 5. For full details of the TEM procedure from glutaraldehyde-fixed material, refer also to Chapter 5. For full details of the TEM procedure from wax embedded tissues refer to Bojko et al. (2013).

9.3.5. Extraction, sequencing and molecular diagnostics

All potential hosts in the transmission experiments were assessed for microsporidian infection, as well as the homogenate that acted as infected feed, using the general MF1 (5'-CCGGAGAGGGAGCCTGAGA-3') MR1 (5'-GACGGGCGGTGTGTACAAA-3') primer set developed by Tourtip et al (2009) as used by Chapter 5. Infection by the microsporidian *C. ornata* was detected using a nested PCR approach, where the Mic18/19F (5'-ATAGAGGCGGTAGTAATGAGACGTA-3') and Mic18/19R (5'-TTTAACCATAAAATCTCACTC-3') primers developed by Grabner et al (2015) were used in a 50µl PCR mix for the second round after initial amplification by the MF1/MR1 primer set. The 50µl Go-Taq PCR reaction consisted of: 1.25U of Taq polymerase; 1µM of each primer; 0.25mM of each dNTP; 2.5 mM $MgCl_2$; and 2.5 µl of genome template or PCR product for each sample. T_c settings: 94˚C (5min); 94˚C (1 min); 58˚C (1min); 72˚C (1min); and finally, 72˚C (10min); steps 2, 3 and 4 were repeated 35 times.

Amplification of *Dikerogammarus haemobaphes bi-facies-like virus* (DhbflV) helicase gene was accomplished using a standard PCR protocol in 50µl quantities with the DHhelicaseF (5'-CGTGTGTTTAGGTACAAGAAC-3') and DHhelicaseR (5'-TAGAGAAGGTGGAAATGACTA-3') primer set. These primers were developed from the metagenomic data collected in Chapter 8 for this virus. The 50µl Go-Taq PCR reaction consisted of: 1.25U of Taq polymerase; 1µM of each primer; 0.25mM of each dNTP; 2.5 mM $MgCl_2$; and 2.5 µl of genome template for each sample. T_c settings included: 94˚C (5min); 94˚C (1 min); 52˚C (1min); 72˚C (1min); and finally 72˚C (10min); steps 2, 3 and 4 were repeated 35 times. Viral amplicons were produced at ~500bp.

In all cases, PCR amplicons were visualised on a 2% agarose gel alongside a hyperladder (100bp to 2000bp), or 1kb ladder (Promega), to diagnose infection by amplicon size. In *ad hoc* cases gel bands were excised and purified before being sent for forward and reverse sequencing via Eurofins sequencing barcode service (https://www.eurofinsgenomics.eu/en/custom-dna-sequencing.aspx).

9.3.6. Statistical analyses

Statistical analyses were conducted in R version 3.2.1 (R Core Team, 2013) through the Rstudio interface. Analysis of survival data employed the 'coxme' package developed by Therneau (2015a) and the 'survival' package developed by Therneau (2015b). Firstly a survival fit was created to describe survival variation in time to death between different groups. A Cox proportional hazards model was used to test the significance of different

factors (microsporidian infection, DhbflV infection, tank number) in determining differences in the time-to-death. Survivorship models contained the infection status of each individual as a fixed effect along with the food treatment as a random blocking effect.

Prior to analysis, continuous data collected from individuals (weight and length measurements) was log transformed to conform to normality based on a search for linearity using QQ-plots, and allowed the use of parametric statistics. Generalised linear models were used to compare count data (egg count, activity data) between infected and uninfected animals, and fitted with a quasi-Poisson error distribution to account for over-dispersion in all cases. The rest of the data was not normally distributed and was analysed using non-parametric statistics such as: Wilcoxon test (with continuity correction), Kruskal-Wallis test (KW), and Spearman's rank correlation; this included aggregation data.

Parasite and pathogen prevalence data comparisons were conducted using Pearson's chi squared test with Yates' continuity correction. Fisher's exact probability tests were applied to prevalence statistics for the animals involved in the transmission trial to determine the likelihood of microsporidian acquisition from experimental transmission.

10.4. Results

The results section is broken into four main sections: firstly, the histopathology noted for the symbionts observed; secondly, the results for the experimental assessment for activity in naturally infected hosts; thirdly, the results for the experimental assessment for aggregation in naturally infected hosts; and finally, the results for the transmission and survival assay for the type host and potential alternate hosts.

9.4.1. Histopathology and ultrastructure of novel pathogens

During the behavioural and transmission trials, several novel infections were observed alongside the previously described *C. ornata*. These include two novel viruses infecting the hepatopancreas and haemocytes, gregarines in the gut lumen and digenean trematodes encysted within the connective tissues around the gut and gonad. *Cucumispora ornata* was noted at 85.5% prevalence in the 282 specimens of *D. haemobaphes* collected for physiological and behavioural observations.

9.4.1.1. Dikerogammarus haemobaphes *Bacilliform Virus (DhBV)*

This is the first report of a viral infection in *D. haemobaphes*. The viral pathology noted during histological analysis revealed hypertrophic nuclei in the hepatopancreas of *D. haemobaphes* (Fig. 9.2a-b). The host chromatin was condensed to the margins of the nucleus (Fig. 9.2a) and the cytoplasm of cells was additionally condensed due to the hypertrophic nucleus. In some cases, a deep purple staining occlusion body was present (Fig. 9.2b). No immune responses such as melanisation of surrounding tissues or recruitment of granulocytes was observed in response to this infection. Infected individuals varied in the intensity of infection with some animals exhibiting only 1-2 infected nuclei and others with larger infections across the entire hepatopancreas. In all cases the infection was limited only to the nuclei of hepatopancreatocytes. Infection prevalence across the 282 sampled individuals was 77.7%. Individuals showed no external clinical signs of infection based on the observations made during this study before histological preservation.

Transmission electron microscopy of infected individuals revealed that infected nuclei were filled with a viroplasm that consisted of fully-formed and partially formed bacilliform virions, which were not in any crystalline order (Fig. 9.2c). Individual virions consisted of a rod-shaped electron-dense core and an enveloping membrane that maintains a close association to the core genetic material (Fig. 9.3, inset). The electron dense core measured approximately (n=30) 302 ± 13 nm in length and 55 ± 4 nm at its diameter. The outer membrane measured approximately 410 ± 25 nm in length and 98 ± 6 nm in width.

Based on viral morphology using electron microscopy, this study suggests it be referred to as '*Dikerogammarus haemobaphes* Bacilliform Virus' (*Dh*BV) until genetic data is available for a full taxonomic description.

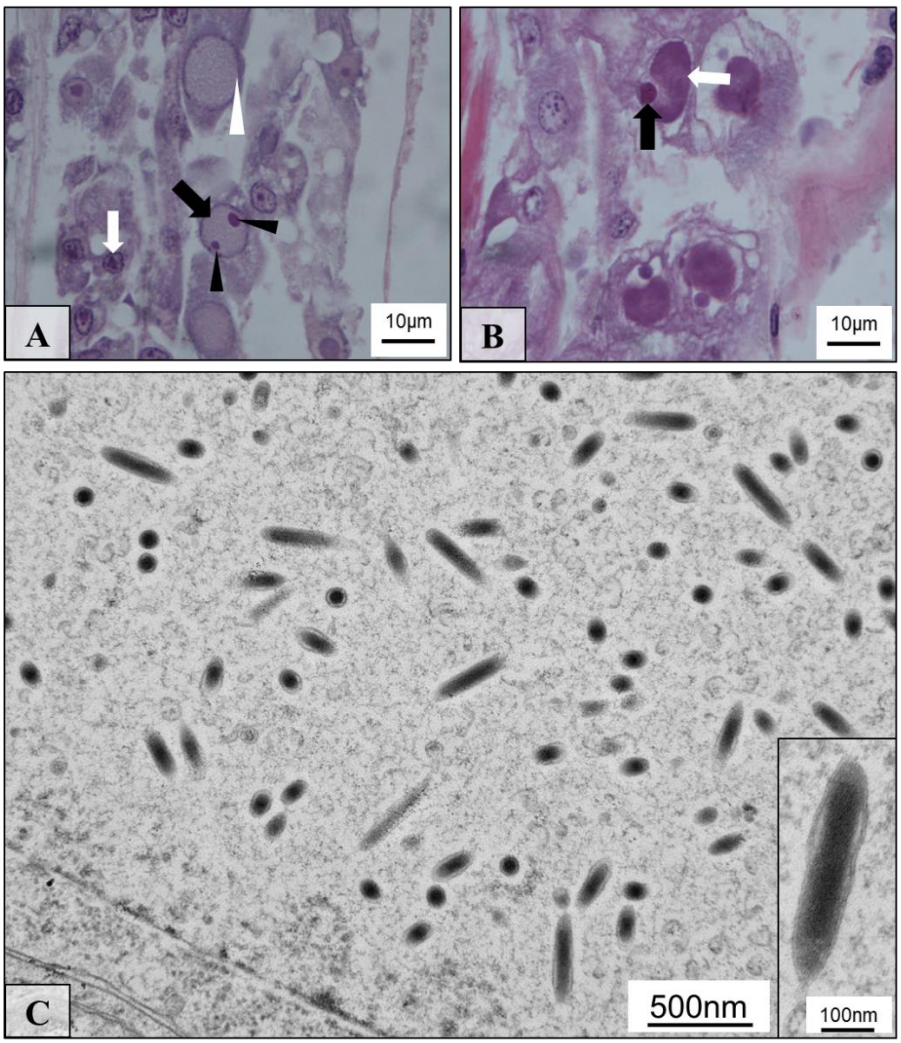


Figure 9.2: Histopathology and ultrastructure of DhBV. A) Early infections reveal a growing viroplasm (black triangles) within the nucleus of the hepatopancreatocytes (black arrow) and the host chromatin is marginated (white triangle). An uninfected nucleus is highlighted by a white arrow. B) Later stage infections are deep purple under H&E (white arrow) and are present with occlusion bodies (black arrow). TEM identified rod-shaped viruses in the nuclei, one of which is highlighted in greater detail in the inset.

9.4.1.2. Dikerogammarus haemobaphes *bi-faces-like Virus (DhbflV)*

Histology revealed the presence of a second viral pathology in the haemolymph (haemocytes/granulocytes), connective tissues and haematopoietic tissues around the carapace. Infected cells contained hypertrophic nuclei filled with a pink-purple staining viroplasm (Fig. 9.3a). This infection was noted in three individuals in the population of invasive *D. haemobaphes* from Carlton Brook in the UK. No immune responses were observed in relation to this virus and on all occasions infection intensity was pronounced with most haemocytes infected. Via TEM, cells could be diagnosed with a growing viroplasm consisting of a labyrinthine network of DNA and protein (Fig. 9.3b). In advanced infection, the viroplasm had arranged in to discrete virions (Fig. 9.3c); each with a pentagonal cross-section (Fig. 9.3d). Virions could be seen amongst complex

networks of membranes, proteins and nucleic acids (Fig. 9.3e). Individual virions are expected to have dsDNA due to their morphology. Each virion possessed a central, electron dense core measuring 52nm ± 6nm in width and 105nm ± 19nm in length, and was surrounded by a membrane measuring 111nm ± 9nm in width and 149nm ± 14nm in length. No genetic information is currently available for this virus. This virus has been termed: '*Dikerogammarus haemobaphes bi-faces-like Virus'* (DhbflV) until genetic information is available to place it correctly into current taxonomy.

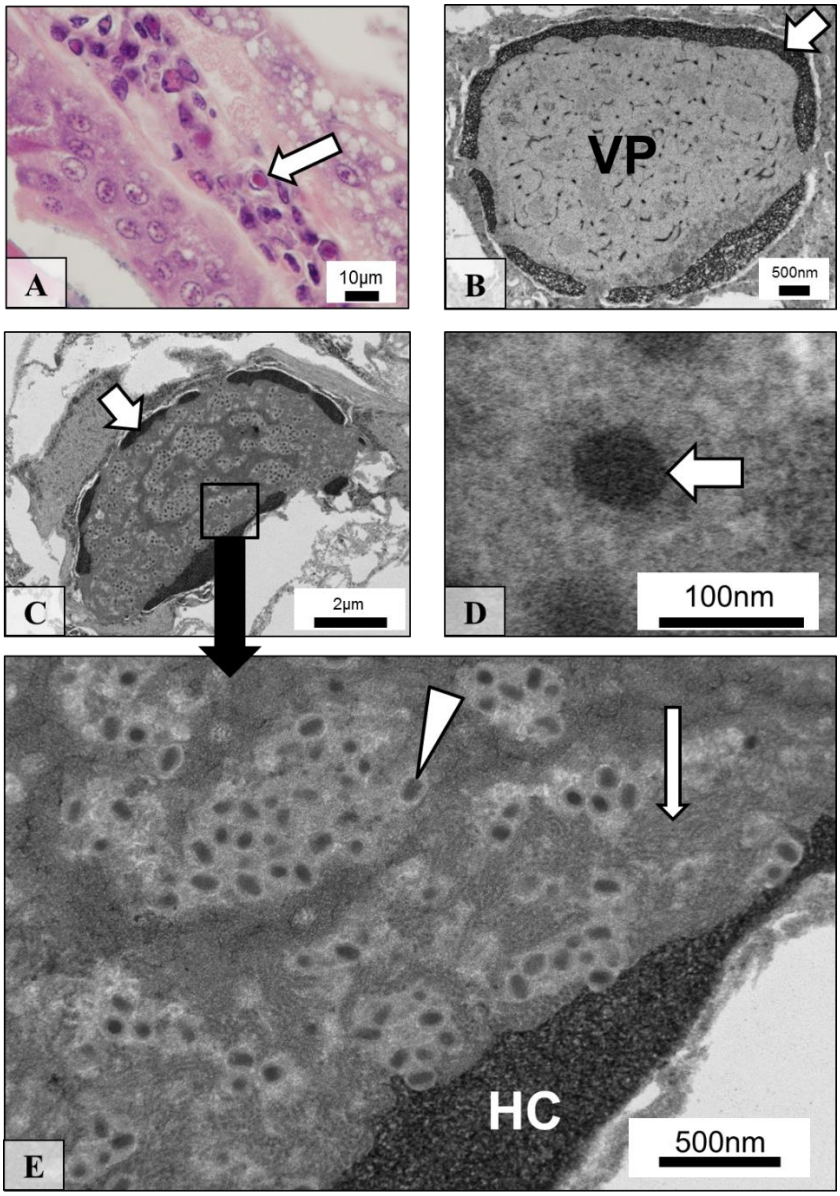


Figure 9.3: Histopathology and TEM of DhbflV. A) Haemocyte nuclei (white arrow) infected with the virus. B) TEM image of a growing viroplasm (VP) in a haemocyte nucleus (white arrow). C) A late stage nucleus (white arrow) with several virions. D) High magnification of a single virion core (white arrow) identifies it with a pentagonal cross-section. E) Higher magnification image of 'image C' identifies a labyrinthine network for viral assembly (white arrow), several virions (white triangle), and host chromatin (HC).

9.4.1.3. Apicomplexa and Digenea

Gregarine parasites (Apicomplexa) were noted in 51.8% of the 282 *D. haemobaphes* collected for assessment. The gregarines were often present in one of three life-stages: 1) intracellular stage, within the gut epithelia of the host (Fig. 9.4a-b); 2) in the gut lumen of the host (Fig. 9.4c); or undergoing syzygy in the hind-gut. In all cases of infection, no observable immune response was elicited by the presence of gregarines.

Digenean trematodes were present in a single individual from the 282 individuals (<1%). Digenea were observed to encyst within the connective tissues of their host, always present with an eosinophilic layer surrounding a central organism (Fig. 9.4d). In all cases the digeneans were not seen to elicit any immune response from the host.

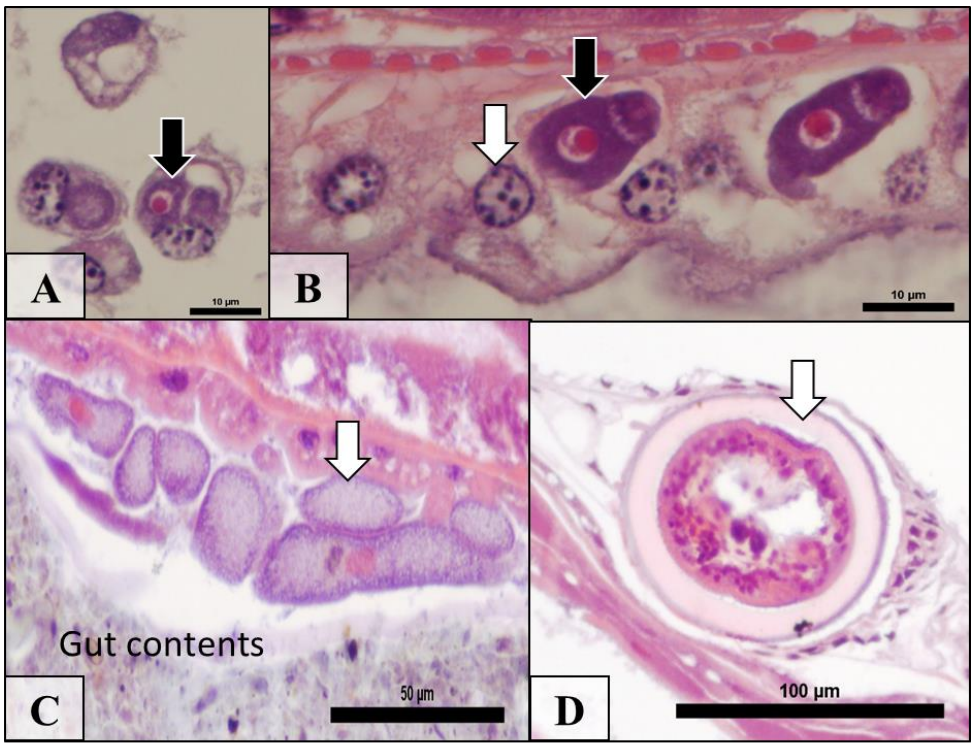


Figure 9.4: Gregarines and digeneans infecting *D. haemobaphes* from Carlton Brook. A) An intracellular life stage of gregarine development (black arrow). B) Gregarines (black arrow) enlarge and mature before emerging from the cells into the gut lumen. A host nucleus is identified by the white arrow. C) Gregarines (white arrow) align along the gut wall. D) A digenean cyst (white arrow) within the connective tissues of the host.

9.4.2. The effects of natural pathogen infection on host fitness

The physiological characteristics of sex, size, pairing status, and the presence and number of offspring, were measured for every *D. haemobaphes* (n=282) undergoing

behavioural/physiological assessment and analysed in combination with the parasites or pathogens the animal contained, as detected by histology.

The sex of the animal was recorded as either male, female or intersex, with the latter being rare at the Carlton Brook population (<1%) and so this category was removed from the sex analysis. The sex of the animal was not significantly associated with the presence or absence of *C. ornata* (Chi squared test, $X^2_{df=1}$ = 1.559, P = 0.212). The presence of *C. ornata* did not associate with either length (T-test, t= 1.021, df = 280, P = 0.308) or weight (T-test, t = 1.129, df = 280, P = 0.260). Animals that were originally in a pair did not reveal a higher or lower infection prevalence for *C. ornata* infected individuals (Chi squared test, $X^2_{df=1}$ = 0.233, P = 0.630). For females, gravidity was not associated with the presence of *C. ornata* (Chi squared test, $X^2_{df=1}$ = 3.315, P = 0.069). The size of the egg clutch was not associated with the presence or absence of microsporidia (quasi-Poisson GLM, dispersion parameter = 44.436, t value = 0.748, df = 109, P = 0.456), nor was it associated with the burden of any *C. ornata* infection level (quasi-Poisson GLM, Chi squared test on model, $X^2_{df=3}$, deviance = 4141.1, P = 0.063)

DhBV did not associate with one sex over the other (Chi squared test, $X^2_{df=1}$ = 0.000, P = 1.000), length (T-test, t = -1.238, df = 280, P = 0.217) or weight (T-test, t = -0.687, df = 280, P = 0.492). Previously paired animals did not exhibit a different rate of DhBV infection (Chi squared test, $X^2_{df=1}$ = <0.001, P = 0.996). The virus was not more prevalent in gravid females (Chi squared test, $X^2_{df=1}$ = 0.037, P = 0.847). DhBV infection prevalence did not appear to effect female egg clutch size (quasi-Poisson GLM, dispersion parameter = 45.719, t value = 0.263, df = 109, P = 0.793) and the burden of infection did not correlate with egg clutch size (quasi-Poisson GLM, dispersion parameter = 43.946, t value = -1.236, df = 109, P = 0.219).

Gregarines were more commonly associated with males than females (Chi squared test, $X^2_{df=1}$ = 4.297, P = 0.038). The length (T-test, t = -0.555, df = 280, P = 0.579) and weight (T-test, t = -0.896, df = 280, P = 0.371) of the host was not associated with the presence of gregarines. Previously paired individuals did not associate significantly with the presence of gregarines (Chi squared test, $X^2_{df=1}$ = 0.083, P = 0.773). Gravid females were not associated significantly with gregarine infection (Chi squared test, $X^2_{df=1}$ = 0.668, P = 0.414) and the clutch size of gravid females appeared not to be affected by the presence of gregarines (quasi-Poisson GLM, dispersion parameter = 43.708, t value = -1.345, df = 109, P = 0.181). The prevalence of Digenea and DhbflV was too low to conduct statistical assessment of correlation.

9.4.3. Activity assessment

9.4.3.1. Does physiology and morphology affect activity in D. haemobaphes*?*

Sex, clutch size and pair status all appear to be significant factors when assessing the activity of *D. haemobaphes*; where males are more active than females (quasi-Poisson GLM, dispersion parameter = 16.427, t-value = 3.663, df = 128, P<0.001), gravid females were not more active than females without young (quasi-Poisson GLM, dispersion parameter = 13.037, t-value = 2.241, df = 61, P = 0.029); increased activity correlates with increased size of the egg clutch (Spearman rank, rho = 0.327, S = 26725, P = 0.009) and animals not in a pair are more active (quasi-Poisson GLM, dispersion parameter = 17.030, t value = -2.787, df = 130, P = 0.006). Increasing weight (quasi-Poisson GLM, dispersion parameter = 18.696, t value = 1.604, df = 130, P = 0.111) and length (quasi-Poisson GLM, dispersion parameter = 18.579, t value = 1.809, df = 130, P = 0.073) did not significantly affect activity.

9.4.3.2. Effect of natural infection with C. ornata on the activity of D. haemobaphes

Histological screening revealed 241 individuals infected with microsporidia according to the pathological information provided for *C. ornata*, and 41 uninfected individuals. Infected individuals were split into one of 3 groups: low level infection (score = 1) (n=182); medium level infection (score = 2) (n=28); and high level infection (score = 3) (n=31), according to Figure 9.1.

Analysis revealed that the simple status of ‘infected’ or ‘uninfected’ was not associated with variation in the activity of the host (quasi-Poisson GLM, dispersion parameter = 18.666, t value = -0.240, df = 130, P = 0.810) (Fig. 9.5). In many cases (n = 182) animals were present with low level infections and showed a higher average activity in the behavioural assay (mean = 50.0 ± 2.2 line crosses) in comparison to uninfected individuals (mean = 46.1 ± 5.8 line crosses). Level 3 infection burden of microsporidian infection was shown to be a significant factor in the activity of the host (quasi-Poisson GLM, dispersion parameter = 15.999, t-value = -3.468, df = 130, P<0.001) (Fig. 9.5), with high level infections (score = 3) showing a significantly lower average activity score (mean = 20.0 ± 3.6).

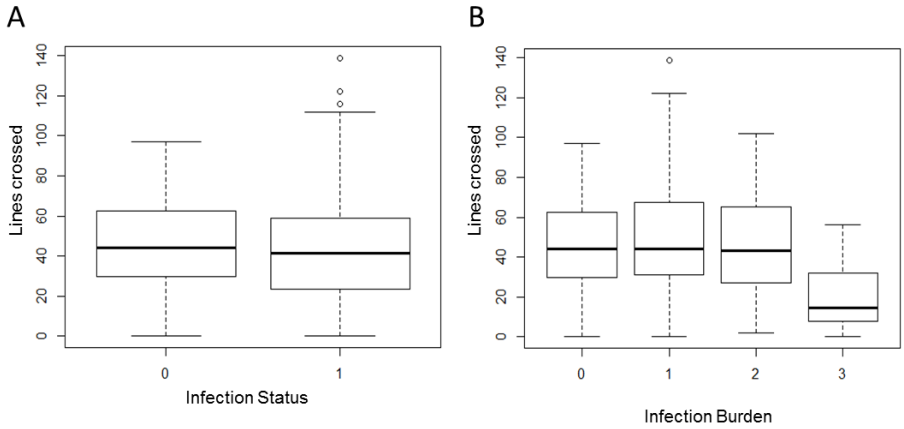


Figure 9.5: *Dikerogammarus haemobaphes* activity affected by *Cucumispora ornata* presence (1) or absence (0) (A), and against microsporidian burden (B) as according to Fig. 9.1.

9.4.3.3. Activity of Dh*BV infected individuals*

The presence or absence of infected nuclei in the hepatopancreas containing DhBV, was not associated with activity (quasi-Poisson GLM, dispersion parameter = 18.504, t value = 1.278, df = 130, P = 0.203) (Fig. 9.6). However, when burden (defined by the number of infected nuclei relative to the number of uninfected nuclei) was considered, there was a correlation between increased activity and higher viral burden (quasi-Poisson GLM, dispersion parameter = 17.802, t value = 2.147, df = 130, P = 0.034) (Fig. 9.6). However, because the presence of high level (level 3) microsporidian infections (noted in red on Fig. 9.6) have also been strongly correlated with lower host activity in this study, an interaction analysis was conducted, identifying a non-significant interaction which shows that the relationship between activity and DhBV infection intensity does not vary depending on microsporidian infection level (quasi-Poisson GLM, dispersion parameter = 15.143, t value = -1.618, df = 130, P = 0.108) (Fig. 9.6c).

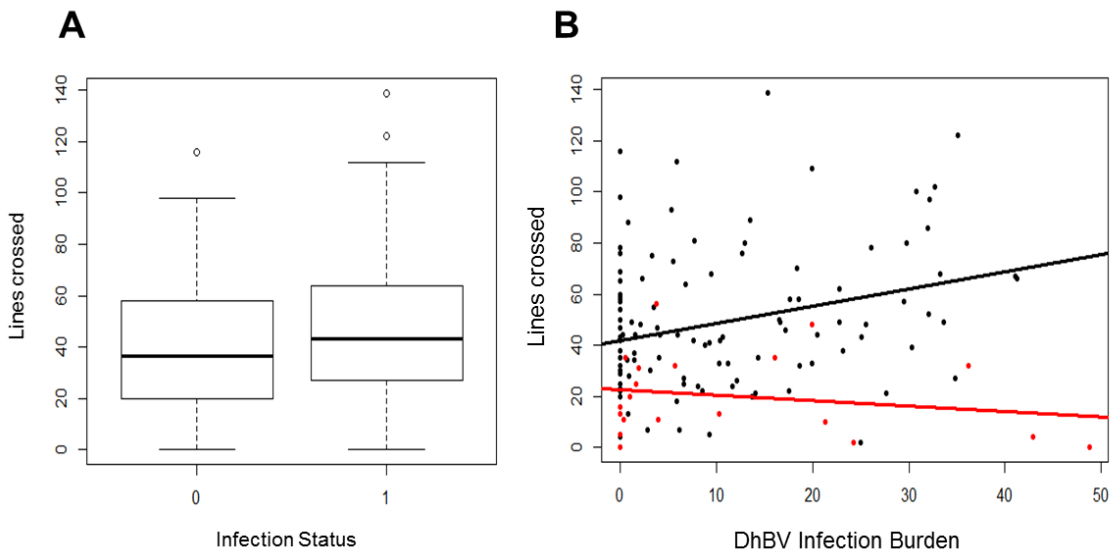


Figure 9.6: *Dikerogammarus haemobaphes* activity affected by DhBV presence (1) or absence (0) (A), and against viral burden (B). The scatter plot (B) identifies all data points, however those in red have a high microsporidian burden (level = 3). The black line identifies the increased activity observed by DhBV infected animals at various burdens of infection. The red line identifies the activity trend observed by those animals with DhBV infection, but also have a level 3 microsporidian infection.

Measurement	Estimate	Error	T value	P value
DhBV Burden	0.013	0.004	2.997	0.003
Microsporidian (level 3)	-0.628	0.250	-2.507	0.013
DhBV:Microsporidian (level 3)	-0.024	0.015	-1.618	0.108

Table 9.2: The interaction between DhBV burden and microsporidian level 3 infection.

9.4.3.4. Gregarine effect on activity

The presence or absence of gregarines was also analysed against the activity data, revealing that the presence of gregarines did not affect the activity of their host (quasi-Poisson GLM, dispersion parameter = 18.539, t value = 0.567, df = 130, P = 0.572) (Fig. 9.7). Due to the histology-oriented data collection method, accurate assessment of parasite burden could not be determined for gregarine infections as sections of the gut could not be standardised accurately.

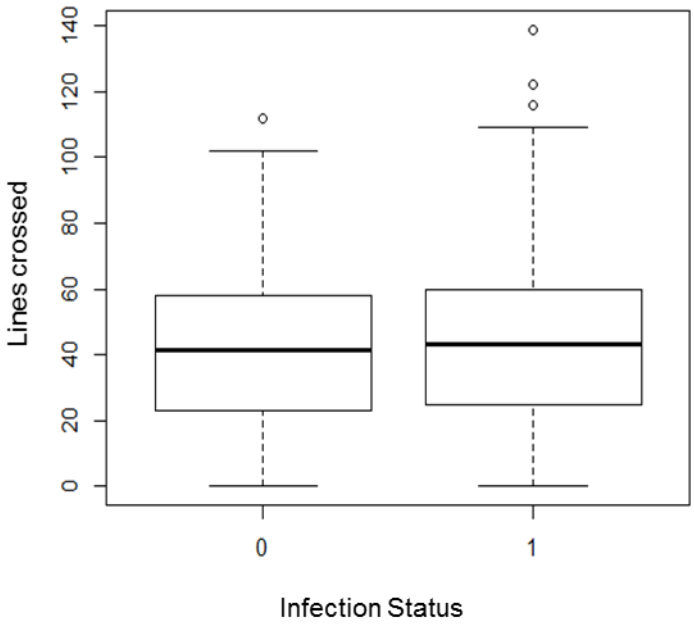


Figure 9.7: Dikerogammarus haemobaphes activity ('Lines crossed') affected by gregarine presence (1) or absence (0).

9.4.4. Aggregation assessment

Only male animals were used to measure behaviour in the aggregation assessment. The length (Spearman rank, rho = -0.147, S = 47774, P = 0.251), weight (Spearman rank, rho = -0.172, S = 48850, P = 0.177), or pair status (Wilcoxon test, W = 154.5, P = 0.818) of male individuals was found not to be significantly associated with amount of time in the social zone, where individuals had a choice between an empty shelter and a shelter containing four males.

The presence or absence of *C. ornata* did not associate with the amount of time spent in the social zone (Wilcoxon test, W = 283.5, P = 0.733) (Fig. 9.8), nor was a change noticed when the level of infection was considered (KW test, $X^2_{df=3}$ = 0.373, P = 0.946).

The presence or absence of DhBV did not significantly affect the amount of time spent in the social zone (Wilcoxon test, W = 456.5P = 0.119) (Fig. 9.9). When burden of infection was taken into account, no trend could be observed (Spearman rank, rho = -0.114, S = 46402, P = 0.375) (Fig. 9.10). The presence or absence of gregarines was also not associated with the amount of time spent in the social zone (Wilcoxon test, W = 509, P = 0.321) (Fig. 9.11).

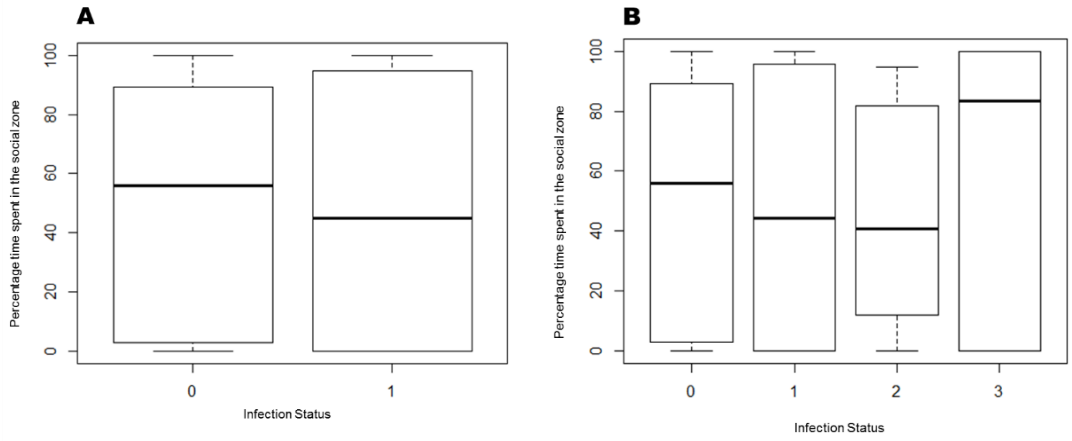


Figure 9.8: *Dikerogammarus haemobaphes* aggregation affected by *Cucumispora ornata* presence (1) or absence (0) (A), and against microsporidian burden (B) as according to Fig. 9.1. The aggregation proxy is the percentage of time spent in the social zone.

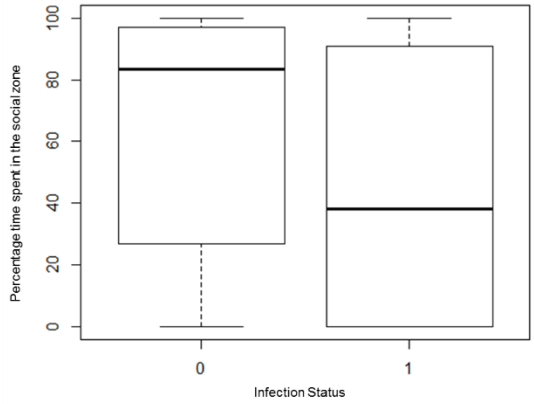


Figure 9.9: *Dikerogammarus haemobaphes* aggregation affected by DhBV presence (1) or absence (0). The aggregation proxy accounts for the percentage of time spent in the social zone.

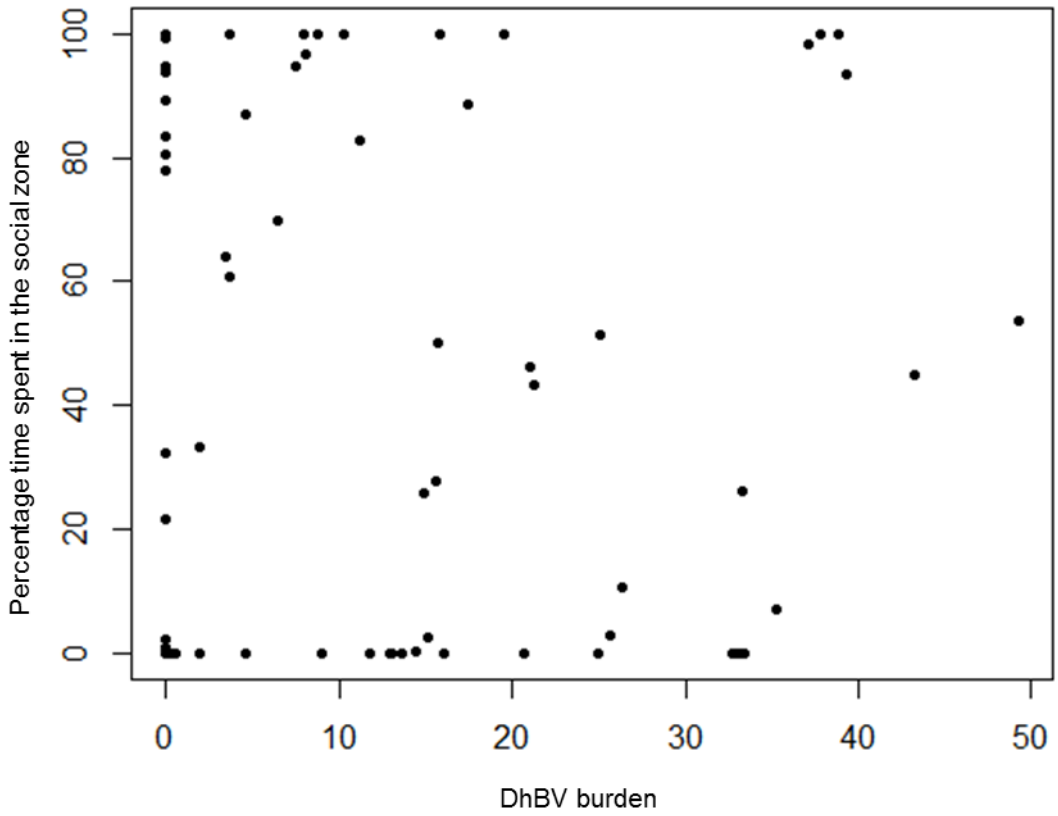


Figure 9.10: *Dikerogammarus haemobaphes* aggregation affected by DhBV burden. The aggregation proxy accounts for the amount of time spent in the social zone, which is expressed as a percentage.

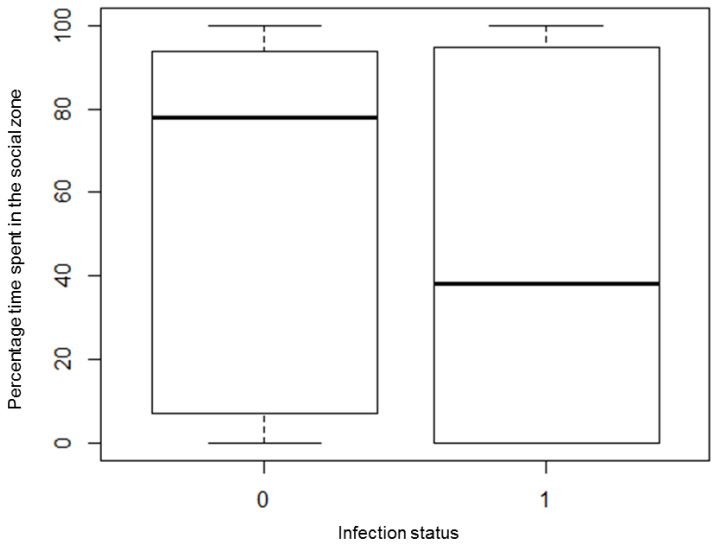


Figure 9.11: *Dikerogammarus haemobaphes* aggregation affected by gregarine presence (1) or absence (0). The aggregation proxy accounts for the percentage of time spent in the social zone.

9.4.5. Host range and impact upon host survival of demon shrimp pathogens

9.4.5.1. Alternate macroinvertebrate hosts of Cucumispora ornata

During the collection of *D. haemobaphes* and co-occurring *G. pulex* from Carlton Brook, several other aquatic invertebrates were also collected to screen for the presence of microsporidia and, specifically, *C. ornata*, using the same nested PCR approach. The general primers (MF1/MR1) provided four amplicons; two that were too weak to sequence, one that conformed to host (freshwater mussel) DNA (220bp) [*Sphaerium nucleus* (KC429383.1); 87% coverage; 96% identity; e-value = $1e^{-82}$] and one amplicon (884bp) from a likely novel microsporidian species, closest associating to *Encephalitozoon cuniculi* isolated from the kidney of a blue fox from China (KF169729) (99% coverage; 87% identity; e-value = 0.0) (Table 9.3). The specific primer set (Mic18/19) yielded five amplicons: two from freshwater mussels, one from a mosquito larvae, one from a beetle larva and one form a freshwater snail (Table 9.3). Use of specific PCR primers that amplify members of the genus *Cucumispora* (Grabner et al. 2015) gave five amplicons: one from a freshwater mussel; one from a freshwater snail; and one from a beetle larva. All of these amplicons shared 99-100% sequence identity, and 99-100% coverage, with *C. ornata*. The final two amplicons from the mosquito larvae and second freshwater mussel were not sequenced due to low concentration of product.

Taxonomy of the host	**n=**	**Infected**	**Nested 1st round**	**Nested 2nd round**
			MF1, MR1 (Tourtip et al. 2009)	Mic18/19F, Mic18/19R (Grabner et al. 2015)
Sphaeriidae	4	3	Host amplicon (~800bp)	*Cucumispora ornata* +ve (x2)
Coleopteran larvae 1	2	0	No amplification	No amplification
Coleopteran larvae 2	1	1	No amplification	*Cucumispora ornata* +ve
Trichoptera	1	0	No amplification	No amplification
Clitellata	4	0	No amplification	No amplification
Asellus aquaticus	2	1	Unconfirmed sequence	No amplification
Ephemeroptera	3	0	No amplification	No amplification
Tipulidae	2	0	No amplification	No amplification
Planorbis sp.	1	0	No amplification	No amplification
Lymnaea	4	1	No amplification	*Cucumispora ornata* +ve
Culicidae	1	1	No amplification	Unconfirmed positive
Crangonyx pseudogracillis	1	1	*Encephalitozoonidae* microsporidian	No amplification

Table 9.3: The macroinvertebrates collected alongside *D. haemobaphes* and *G. pulex* at the Carlton Brook site. Each specimen underwent DNA extraction and tested for the presence of *Cucumispora* via nested PCR.

9.4.5.2. Dikerogammarus haemobaphes *mortality in response to infection*

Individuals (n=30) sampled and fixed on-site at the same time as those collected for experimental studies were screened for *C. ornata* to obtain an indication of the wild prevalence of infection. After nested PCR diagnostics, a 0% (0/30) prevalence of *C. ornata* was confirmed, however prevalence of this microsporidian has been documented to be >70% in previous studies at this invasion site (Chapter 5); this may be a seasonal effect. PCR screening for individuals used in the experiment revealed a prevalence of 10.3% (3/29) for the animals used in the control group, and a prevalence of 22.2% (6/27) for the group fed with inoculum. A Fisher's exact probability test identified the likelihood of microsporidian acquisition from the inoculum as not significant (P = 0.220).Individuals that were positively diagnosed with *C. ornata* after the transmission trial via nested PCR showed higher mortality than uninfected individuals (Score (logrank) test, P<0.001) (Fig. 9.12).

Due to the availability of a PCR diagnostic for the haemocyte virus, DhbflV, it was possible to diagnose infection from the *D. haemobaphes* used in the transmission trial. The inoculum was PCR negative for this virus, so it is assumed that those *D. haemobaphes* positive for infection carried it into the laboratory. A Fisher's exact probability test identified the likelihood of viral acquisition from the inoculum as not significant (P = 0.283). Individuals that were PCR positive for DhbflV (9/56) showed higher mortality (Score (logrank) test, P<0.001) (Fig. 9.12). The prevalence for DhbflV was not tested for the animals fixed on site. *Dikerogammarus haemobaphes* were not fixed for histological analysis, limiting the detection of other pathogens and parasites to associate with mortality.

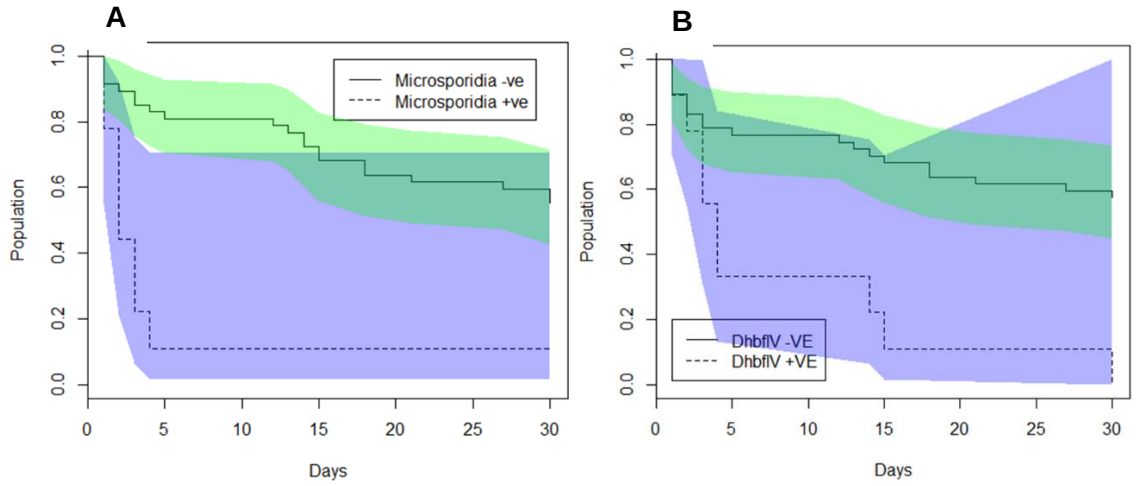


Figure 9.12: *Dikerogammarus haemobaphes* survival rate with *Cucumispora ornata* (A), where 9 individuals were microsporidian positive and 47 were microsporidian negative. *Dikerogammarus haemobaphes* survival rate with DhbflV (B) infections, where 9 individuals were PCR positive for infection and 47 were uninfected. In both cases the purple area represents the confidence interval (0.95) for microsporidian/virally infected individual's survival curve, and the green area represents the confidence interval (0.95) for the uninfected individuals.

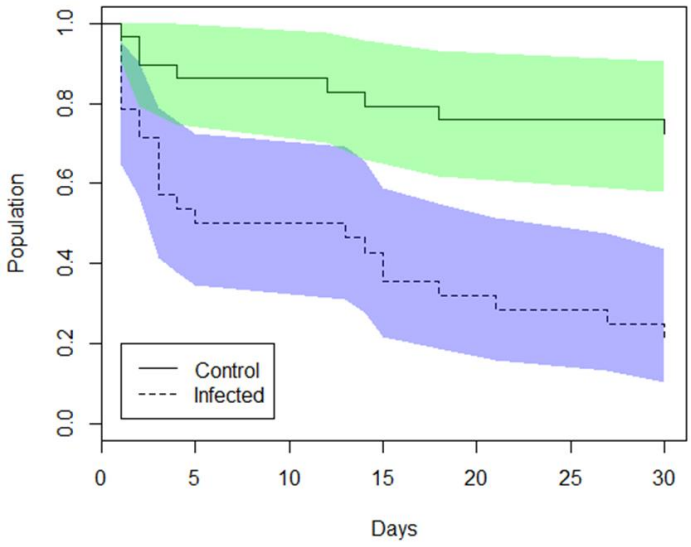


Figure 9.13: *Dikerogammarus haemobaphes* survival rate comparison between those animals in the control group (n=29) that were fed uninfected food pellets, and those animals in the exposure group ('infected') (n=27) that were fed with microsporidian inoculum. The purple area represents the confidence interval (0.95) for exposed individual's survival curve, and the green area represents the confidence interval (0.95) for the control group.

Dikerogammarus haemobaphes that were fed on carcass showed greater mortality than those in the control group, which were fed on food pellets (Score (logrank) test, P<0.001) (Fig. 9.13). The relative difference in mortality between all individual tanks was also significant (Score (logrank) test, P = 0.001).

9.4.5.3. Mortality in Dikerogammarus villosus *when fed on demon shrimp carcasses*

Individuals (n=30) sampled and fixed on-site at the same time as those collected for experimental studies were screened for *C. ornata* to obtain a wild prevalence. After nested PCR diagnostics, a 0% (0/30) prevalence of *C. ornata* was confirmed in the *D. villosus* population at Grafham Water. Based on the nested PCR diagnostic, no *D. villosus* that were used in the experiment became infected with *C. ornata* (0/57). Histological screening revealed one individual from the exposure group with a low-grade microsporidian infection, however this did not provide a positive PCR result in either the first or second round of the PCR diagnostic.

Assessment of whether the exposure group differed in mortality from the control group was not significant (score (logrank) test, P = 0.071) (Fig. 9.14), nor was the mortality difference between individual tanks (Score (logrank) test, P = 0.082).

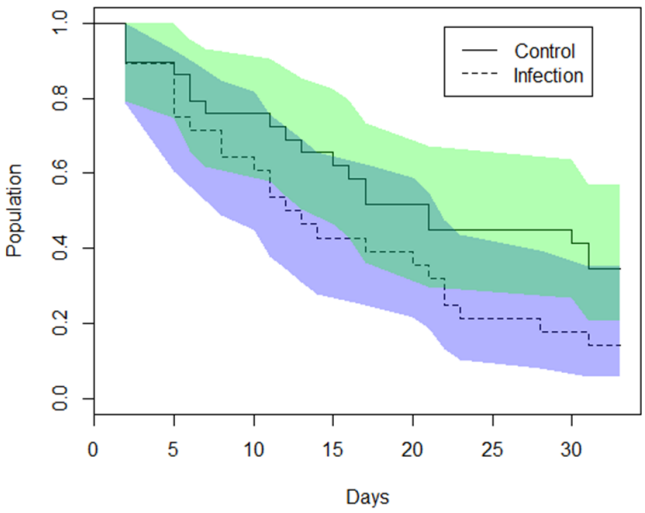


Figure 9.14: *Dikerogammarus villosus* survival rate comparison between those animals in the control group (n=29) that were fed uninfected food pellets, and those animals in the exposure group ('infected') (n=28) that were fed with microsporidian inoculum. The purple area represents the confidence interval (0.95) for exposed individual's survival curve, and the green area represents the confidence interval (0.95) for the control group.

9.4.5.4. Cucumispora ornata *in* Gammarus pulex *co-occurring at Carlton Brook*

One out of 17 *G. pulex* (5.9%) collected on-site at Carlton Brook was PCR positive for *C. ornata* confirming the presence of this microsporidian in wild native amphipod populations. *Gammarus pulex* in the laboratory trials showed a significant increase in mortality if positively diagnosed with *C. ornata* via nested PCR (4/19), relative to uninfected individuals (15/19) (Score (logrank) test, P = 0.042) (Fig. 9.15). The effect of being present in either the control (uninfected feed) or exposure group (infected feed) was not significantly associated with mortality (Score (logrank) test, P = 0.537) (Fig. 9.16). Histological screening of the remaining carcass identified one of the PCR positive animals with a visible microsporidian infection in the musculature. Fisher's exact probability test indicated a higher prevalence in the exposed group than the control group (P = 0.054), suggesting transmission from the infected feed.

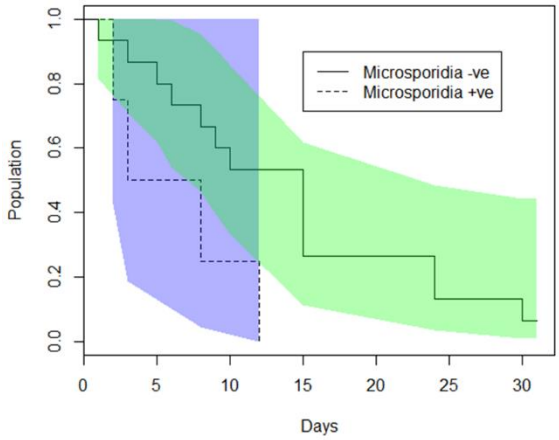


Figure 9.15: Gammarus pulex (from Carlton Brook) survival rate comparison between those animals with *Cucumispora ornata* infection (Microsporidia +ve) (n=4) and those without (Microsporidia -ve) (n=15). The purple area represents the confidence interval (0.95) for the microsporidian infected individual's survival curve, and the green area represents the confidence interval (0.95) for the uninfected individuals.

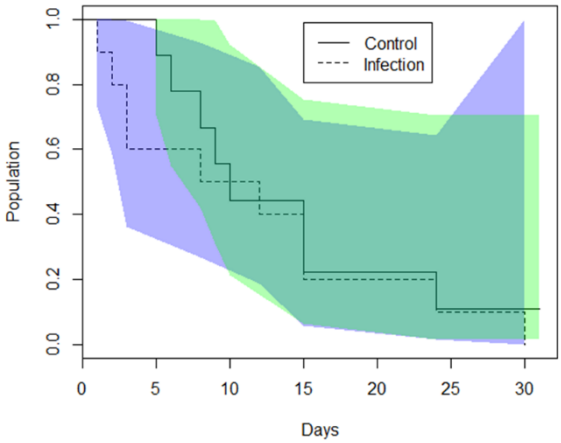


Figure 9.16: Gammarus pulex (from Carlton Brook) survival rate comparison between those animals in the control group (n=9) that were fed uninfected food pellets, and those animals in the exposure group ('infected') (n=10) that were fed with microsporidian inoculum. The purple area represents the confidence interval (0.95) for exposed individual's survival curve, and the green area represents the confidence interval (0.95) for the control group.

9.4.5.5. Cucumispora ornata *in* Gammarus pulex *from a naïve population*

Cucumispora ornata was not detected in the 30 *G. pulex* that were fixed on-site at Meanwood Park, Leeds, via nested PCR (0/30). Two individuals were PCR positive for *C. ornata* after mortality in the laboratory trial, both present in the 'infected' group and fed on infected material. No individuals were detected to be infected with *C. ornata* from the control group, however two were positive for unknown microsporidian species in the first round. Those animals positive for *C. ornata* infection (2/27) were associated with increased mortality relative to uninfected individuals (25/27) (Score (logrank) test, P = 0.033) (Fig. 9.17). Whether the animals were present in either laboratory trial (control or exposure) did not associate with mortality (Score (logrank) test, P = 0.511) (Fig. 9.18). Histological screening revealed one of the second-round PCR positive animals to have a microsporidian infection in the musculature. Fishers exact probability test revealed it was unlikely for the microsporidian to have been horizontally transmitted from the inoculum (P = 0.23).

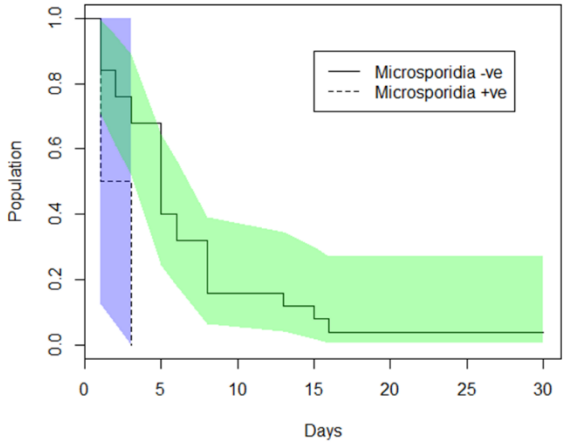


Figure 9.17: Gammarus pulex (from Meanwood Park) survival rate comparison between those animals with *Cucumispora ornata* infection (Microsporidia +ve) (n=2), and those without infection (Microsporidia -ve) (n=25). The purple area represents the confidence interval (0.95) for the microsporidian infected individual's survival curve, and the green area represents the confidence interval (0.95) for the uninfected individuals.

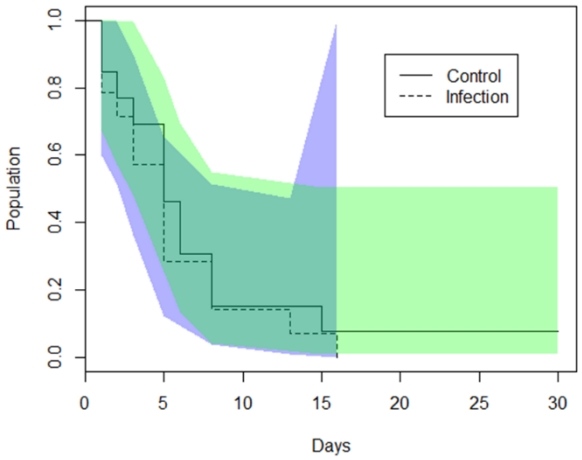


Figure 9.18: Gammarus pulex (from Meanwood Park) survival rate comparison between those animals in the control group (n=13) that were fed uninfected food pellets, and those animals in the exposure group ('infected') (n=14) that were fed with microsporidian inoculum. The purple area represents the confidence interval (0.95) for exposed individual's survival curve, and the green area represents the confidence interval (0.95) for the control group.

10.5. Discussion

This study aimed to explore the diversity and impacts of pathogens (including: viruses; gregarines; digeneans; and microsporidians) in non-native *D. haemobaphes* in the UK and to test the potential for pathogen transmission to other species. I show that *D. haemobaphes* are less active when infected with high burdens of the co-introduced microsporidian pathogen, *C. ornata*, but are potentially more active when infected with high burdens of DhBV infection. None of the parasites affect aggregation behaviours in their host.

Cucumispora ornata has been detected from *D. haemobaphes* invasive in Germany (Grabner et al. 2015) and Poland (NCBI), and has been confirmed to be present at the Carlton Brook site in the UK where it was initially described (Chapter 5). This microsporidian was detected via nested PCR in five novel hosts from Carlton Brook: a freshwater mussel; a beetle larva; a freshwater snail; a native amphipod (*G. pulex*) and a mosquito larvae. *Cucumispora ornata* was detected in the *G. pulex* population collected on-site at a prevalence of (1/17) 5.9% and experimental transmission increased this to (4/10) 40%. This identifies that the microsporidian is already present in several native species and constitutes a threat to wildlife. Transmission of *C. ornata* to naïve *G. pulex* occurred (14.3%) while transmission to invasive killer shrimp (*D. villosus*) did not. Mortality correlated with the presence of *C. ornata* infection in all cases, and these non-target effects (specifically the increased mortality of the keystone shredder *G. pulex*) likely mean that this parasite cannot be adapted as a control agent and is more likely a threat to wildlife.

9.5.1. *Cucumispora ornata*: 'wildlife threat' or 'control agent'?

Due to the increased research effort on the symbionts of the demon shrimp, it seems prudent to review those now known and provide a pathogen profile for this species in both its native and invasive range(s): a breakdown of this can be found in Table 9.4. An understanding of microbial diversity in this species provides insights into possible biocontrol development and further risk assessment for species that may be pathogenic to native hosts.

The microsporidian parasite, *C. ornata*, was identified to infect *G. pulex* from two UK sites and has been detected in one animal from the Carlton Brook environment. This is also the case for some insects and molluscs sampled on-site at Carlton Brook. It is yet to be determined whether the molluscs and insects are truly infected by *C. ornata* or if an environmental signal (eDNA contamination of the sample) is being detected. For

example, mussels are filter feeding species and microsporidian spores may concentrate within the animal through bioaccumulation (Willis et al. 2014). Histological screening of PCR positive tissue samples can often confirm infection and pathology and rule out false positives. Although unlikely, due to various negative controls supporting the statement, the use of a nested PCR approach is highly sensitive and there is some potential for contamination at the diagnostic stage that could result in false positives. The inoculum, although shown to be positive for *C. ornata* via nested PCR, was unlikely the source of parasite for the demon shrimp and *G. pulex* collected from Carlton Brook. Fishers exact probability test did state that transmission was likely from the inoculum to *G. pulex* collected from Meanwood Park, Leeds. This likely means that animals from Carlton brook carried *C. ornata* prior to being fed with inoculum.

The prevalence and seasonality of *C. ornata* differed greatly between the temporal samples, where those animals in the survival trials that were samples in August (2015) having a 0% (0/30) environmental prevalence of the parasite as determined by nested PCR, however those animals sampled in earlier months show a much greater prevalence, similar to that first reported in Chapter 5 from the 2014 screen of *D. haemobaphes* (>70% prevalence via histology). The temperature associated with seasonal conditions may explain why this microsporidians prevalence differs, however further study would be need to identify if temperature affects transmission. Alternatively, this difference in prevalence could perhaps indicate that histological screening was identifying a different microsporidian with similar pathology, perhaps a muscle infecting version of *D. berillonum*, a microsporidian also identified to infect *D. haemobaphes* in the UK (Green-Etxabe et al. 2015).

Survival analysis has shown that the detection of *C. ornata* in *G. pulex* is significantly associated with decreased survival rate. The analyses for this species included a low sample size due to difficulties in housing the population in the laboratory resulting in a higher than expected control mortality. Despite the low sample sizes used in this study, is seems that *C. ornata* could be devastating for *G. pulex* at the population level. The question of nutritional value must also be noted between the artificial food pellets and the homogenate demon shrimp tissues, which could have had an effect on host survival, however this is unlikely to have caused significant alterations to host mortality because the factor of food presence and tank was considered in the survival analysis. Cumulatively this suggests that *C. ornata* is likely a threat to native wildlife in the UK. The lack of detectable experimental transmission of *C. ornata* to invasive *D. villosus* from Grafham Water suggests that this microsporidian has no benefit as a control agent for this invader.

Cucumispora ornata has been shown to lower the activity of its type host at mid-high burden, and has been significantly associated with decreased survival rate, suggesting that this parasite limits its host's invasive capability, despite it being a potential threat to UK wildlife. Increased activity and survival have been associated with invasiveness, as has been determined for the red and grey squirrels across Europe and this likely has parallels with amphipod populations (Wauters et al. 2005). This decrease in activity and survival may explain why *D. haemobaphes* is considered a low-impact species in the UK (Bovy et al. 2014).

Parasite:	Species:	Location	Reference
Viruses	*Dikerogammarus haemobaphes* Bacilliform Virus	Carlton Brook, UK	This study; Chapter 8
	Dikerogammarus haemobaphes bi-facies-like Virus	Carlton Brook, UK	This study; Chapter 8
	Unidentified Circovirus	Carlton Brook, UK	Chapter 8
Bacteria	*Krokinobacter* sp.	Carlton Brook, UK	Chapter 8
	Thiothrix sp.	Carlton Brook, UK	Chapter 8
	Trachelomonas sp.	Carlton Brook, UK	Chapter 8
	Deefgea rivuli	Carlton Brook, UK	Chapter 8
Apicomplexa	*Cephaloidophora mucronata*	Danube Delta	Codreanu-Balcescu 1995
	Cephaloidophora similis	Danube Delta	Codreanu-Balcescu 1995
Oomycete	*Saprolegnia* sp.	Carlton Brook, UK	Chapter 8
Microsporidia	*Cucumispora* (=*Nosema*) *dikerogammari*	Goslawski Lake and Bug in Wyszków	Ovcharenko et al. 2009
	Thelohania brevilovum	Goslawski, Poland	Ovcharenko et al. 2009
	Dictyocoela mulleri	Goslawski, Poland	Ovcharenko et al. 2009
	Dictyocoela spp. ('Haplotype: 30-33')	Goslawski, Poland	Wilkinson et al. 2011
	Dictyocoela berillonum	Unknown/Wallingford Bridge and Bell Weir, UK	Wroblewski and Ovcharenko, Unpublished; Green-Etxabe et al. 2014; Chapter 8
	Cucumispora ornata	River Trent, UK	Chapter 5
Acanthocephala	*Acanthocephalus* (=*Pseudoechinirhynchus*) *clavula*	Danube Delta	Komarova et al. 1969
	Pomphorhynchus laevis	Volga River	Đikanovic et al. 2010
Cestoda	*Amphilina foliacea*	Caspian Sea	Bauer et al. 2002
	Bothriomonas fallax	Caspian Sea	Bauer et al. 2002
Nematoda	*Cystoopsis acipenseris*	Volga River, Russia	Bauer et al. 2002
Trematoda	*Nicolla skrjabini*	Danube Delta	Kirin et al. 2013
	Undetermined Digenean	Carlton Brook, UK	This study

Table 9.4: The parasites and pathogens that have been detected from *Dikerogammarus haemobaphes* from available literature and from this thesis.

9.5.2. The effect of viruses on the activity and survival of *D. haemobaphes*

This study has identified two newly discovered viruses, DhBV and DhbflV. *Dikerogammarus haemobaphes* Bacilliform Virus has been observed to infect the hepatopancreas of its host and is now the third virus isolated from the hepatopancreas

of an amphipod and is likely associated with the Nudiviridae (Bojko et al. 2013; Chapter 6). This virus does not yet have a PCR diagnosis method, restricting detection to either histology or TEM and leaving it without gene sequence information for adequate taxonomic description. This virus was found at high prevalence in the UK population of *D. haemobaphes* and was significantly associated with increased activity, relative to increased viral burden. This relationship suggests that DhBV may be increasing the invasive capabilities of its host by making it more active. For invasive species, the presence of beneficial viruses could provide a symbiotic relationship that increases invasiveness; a process that has been observed between invasive amphipods and their sex-distorting microsporidian pathogens (Slothouber-Galbreath et al. 2004). Studies using homopterans have found that viral infection can alter certain activities to increase viral transmission (Fereres and Moreno, 2009) and this study system may have parallels for crustacean viruses and their hosts. No behavioural assays involving hosts specifically infected with nudiviruses are available to corroborate these findings, but future studies could determine if this group of viruses are 'helpful' to the host instead of detrimental. Roossinck (2011) explores a variety of beneficial viruses in their review, such as: parvoviruses that stimulate the development of wings in aphids (conditional mutualism); polydnaviruses, which increase egg survival of parasitic wasps in their host (symbiogenic relationship); and pararetroviruses that protect plants against pathogenic viruses (symbiogenic relationship). Baculoviruses (relatives of Nudiviruses) have been shown to cause behavioural change in their host, causing them to move upward (phototactic response) so that upon decomposition the virions would increase their dispersal and increase their chance to infect further susceptible hosts (van Houte et al. 2014). Entomopathogenic fungi have also shown to have behavioural effects on their hosts, primarily by causing them to move higher within the canopy to spread fungal spores further – an activity increasing behavioural response (Gryganskyi et al. 2017). Whether DhBV infection in *D. haemobaphes* also reflects a phototactic response is unknown but should be tested in future assays, as should the mode of transmission of this virus, which could help to explain how it moves and whether increased activity increases the transmission of DhBV.

Dikerogammarus haemobaphes bi-faces-like virus is much rarer than DhBV, and has only been detected in hosts that have undergone behavioural or survival assays in the laboratory. This virus infects the haemocytes of the host, causing hypertrophy of the nucleus and likely reducing its host's immunological capabilities. Similar symptoms have been determined from PAV-1 infected Caribbean spiny lobsters (Sweet and Bateman, 2015). *Dikerogammarus haemobaphes bi-faces-like virus* was significantly associated with a decrease in survival rate, however the histological detection of the virus revealed

too few individuals to conduct adequate behavioural statistical analyses to correlate with activity or aggregation. The inoculum was PCR negative for this virus so assessment of experimental host range could not be conducted at this time. Manifestation of this virus indicates that infected *D. haemobaphes* were likely carrying the virus prior to collection and experimental trial, suggesting that stress may trigger infection. This data suggests that DhbfIV is now the most likely pathogen with the potential to be adapted as a control agent for the demon shrimp, although further work is needed to address the host range and behavioural change associated with DhbfIV infection.

9.5.3. Concluding remarks

Dikerogammarus haemobaphes is considered to be a low impact invader that has carried pathogens and parasites into its invasive range (Chapter 5; Green-Etxabe et al. 2015); a process that has also been noted for other non-native amphipod species (Chapter 6). The effects of pathogens and parasites on the *D. haemobaphes* population at Carlton Brook might explain the low direct impact of this host, however, some of these invasive pathogens are capable of infecting alternate hosts, such as the keystone shredder and native species, *G. pulex*; resulting in significant fitness costs. Hence we need a nuanced approach to monitoring risk through indirect trophic links that takes into account the entourage of invasive pathogens that impact both invaders and native species.

CHAPTER 10

General discussion and conclusions

The pathogens and parasites carried by invasive crustaceans have been shown to be diverse, ranging from viruses through to large metazoans (Bojko et al. 2013; Chapters 2-9). The relationships shared between an invader and its parasites can be complex by either benefiting or hindering the invader and adjusting its invasive potential (Simberloff et al. 2005; Dunn and Hatcher, 2015). Furthermore, the presence of some pathogens poses an invasion threat via their ability to infect, and induce mortality in native species. Alternatively, some pathogens may hold the potential to be used as biological control agents to regulate their invasive hosts' population size, activity and impact.

This thesis involved broad parasitological surveying of the invasive green crab, *Carcinus maenas*, along a northern Atlantic invasion pathway, and of invasive amphipods travelling through Europe towards the UK. Some of the pathogens and parasites observed during the screen were taxonomically identified using histology, electron microscopy, molecular diagnostics, genome sequencing, metagenomics and phylogenetics. The presence of a microsporidian pathogen, *Cucumispora ornata*, and several viruses, which have co-invaded the UK alongside the demon shrimp, *Dikerogammarus haemobaphes*, do appear to influence host survival and activity. *Cucumispora ornata* was found to infect non-target native species, revealing that despite controlling the population size and activity of the invasive demon shrimp host, it can transmit to native fauna. Hence it could affect both native and invasive amphipod populations. These findings illustrate that the impact of pathogens can be difficult to predict; a pathogen may exert population control on an invasive host, but a non-specialist parasite may also affect population dynamics of native hosts in the new range.

10.1. Invasive Crustacea and their pathogens

The global list of invasive aquatic invertebrates (IAIs) includes 1054 species, a large proportion of which (324) are invasive crustaceans (Chapter 1). Those 324 crustaceans have been associated with >529 different symbionts, many of which are not formally taxonomically identified and risk assessed and which are lacking studies into their host range, transmission and pathogenicity. The pathogens attributed to invasive crustaceans that pose the greatest threat as co-invaders, include: white-spot syndrome virus (Matorelli et al. 2010), *Vibrio cholera* (Martinelli-Filho et al. 2016), chytrid fungus

(McMahon et al. 2013), and crayfish plague (Tilmans et al. 2014), identified from previous studies. In this thesis *C. ornata* may now sit by the side of these invaders as a pathogen of both invasive and native species.

Species such as *Carcinus maenas* have undergone extensive pathogen profiling in both their invasive and native range; this species has been identified with a conservative 72 symbionts. To reiterate from Chapter 1: If each invasive crustacean has the potential to carry the same number of symbionts as *C. maenas*, the 324 invasive crustaceans have the potential to carry in excess of 23,328 taxonomically different symbionts. This estimate hints towards how little we know about invasive pathogen diversity (Roy et al. 2016).

The studies I include in this thesis have explored the diversity of pathogen groups in invasive and native *C. maenas*; detecting 19 separate symbionts (Chapter 2). Some are newly discovered and now taxonomically identified. *Parahepatospora carcini* is a microsporidian pathogen of *C. maenas*, infecting the hepatopancreas of the host. It was rare, present in only a single specimen from the Malagash site and may have possibilities to control the invasive populations, pending further research into host activity and survival assessment. *Neoparamoeba permaquidensis* and *Neoparamoeba peruans* were also identified from the *C. maenas* populations and have previously been associated with rapid mortality in salmon (Douglas-Helders et al. 2003; Feehan et al. 2013) and American lobster (Mullen et al. 2004; Mullen et al. 2005). Their presence in a high impact and wide spread invasive species may mean that these vulnerable aquaculture and fisheries species could come into contact with these deadly pathogens via spill-over from *C. maenas* populations. Additionally, a novel WSSV-like virus (RVCM/B-Virus) was identified from Canadian/Faroese *C. maenas* populations. If this virus shares virulence characteristics with WSSV (which causes high rates of mortality in shrimp aquaculture), it could reveal potential as a control agent for this invasive species. In addition, further knowledge of the *Nimaviridae* will help to understand the origins of WSSV. RVCM and B-virus now require taxonomic identification and risk assessment for both the invasive species and any vulnerable native species and fisheries/aquaculture.

The sampling method and diagnosis techniques used in Chapter 2 were aimed to be able to identify a wide range of symbionts that could be present alongside this species. Sampling with traps and along the shoreline allowed the capture of both adult and juvenile crabs but any size bias in trapping (Smith et al. 2004) has the potential to over or underestimate symbionts that are more common in different sized animals in trapped versus shoreline caught areas. Histology is a versatile detection method that enables detection of a broad range of symbiont species. However diagnostics is based on

screening of a single tissue slice. There is therefore a risk that some pathogens (in particular those present in low burden) may be missed. Nonetheless, sampling effort is consent between samples. This technique may also miss latent pathogens and others that do not necessarily result in an observable pathology in tissue section. This does open a debate as to how confident we can be that enemy release has occurred for *C. maenas* in this thesis. It is extremely difficult to be sure of enemy release, because proving the absence of a symbiont in this case would technically mean sampling the entire population. Despite this, the study conducted in Chapter 2 can serve as an initial look at pathogen diversity in these areas and can now be the start of developing molecular diagnostic tools, capable of high sensitivity diagnostics that could help to define whether enemy release has occurred along the invasion route of *C. maenas*, coupled with the use of power analyses based on the prevalence of symbionts observed in Chapter 2.

The broad scale screening of amphipods travelling through European invasion corridors, has also revealed a diversity of previously unknown pathogens, providing in-depth knowledge of pathogen profiling for some little studied amphipod species (Chapter 3). Two novel members of the *Cucumispora* are now taxonomically identified; one invasive in the UK alongside the demon shrimp (*C. ornata* in Chapter 5) and the second an invasion threat carried by *Gammarus roeselii* (*Cucumispora roeselii* in Chapter 6). Both of these hosts are non-native species that may be a high invasion risk as carriers of invasive pathogens (Bojko et al. 2017). My work herein has identified *C. ornata* to be capable of decreasing the survival of its type host and can also transmit to native species, also lowering their survival. These data identifies this microsporidian as a high risk to native amphipod species. This may be similar for *C. roeselii,* pending experimental analysis.

A novel RLO is taxonomically identified from *Gammarus fossarum*, native to Poland; and is taxonomically identified (Chapter 7). This is the first taxonomic characterisation of an RLO from an amphipod host and increases the range of known potential biocontrol agents for amphipod pests. The genomic work conducted on this new species has identified a range of virulence genes that suggest genetic engineering of host cells to accommodate bacterial pathogens, possibly resembling the pathways used by *Agrobacterium tumefaciens* to engineer plant cells. This discovery could lead to the use of *Aquarickettsiella* spp. to engineer crustacean cells. In addition to this interesting discovery, there is a possibility that such bacterial species could be used to regulate invasive populations through biocontrol, as have been used for insect pests in agriculture (Hajek et al. 2007; Lacey et al. 2015).

For bacterial pathogens to be assessed as possible biocontrol agents, rigorous testing would firstly be needed, perhaps following a similar format to that used in this thesis to explore the potential of *Cucumispora ornata* as a biocontrol agent (Chapter 9). Firstly, the pathological effects of the bacterial pathogen would need to be understood, including behavioural change and survival rates. Once the pathological effects are understood and characterised as usable within a biocontrol effort, transmission trials would then be needed to address the host range of the pathogen and to identify how it is capable of transmitting, and whether the transmission process is applicable to biocontrol. This would depend on whether the agent is transmissible horizontally or vertically; if horizontally transmitted it could be contained within a spray (commonly used in agriculture) or suspended in water and added directly to the water column. Growing cultures of pathogens (such as viruses and bacteria) that require specific hosts can be difficult if cell culture cannot be made, or enough animals housed to grow up the pathogenic agent to enough concentration for a spray to be developed. Rigorous assessment of these factors are crucial to avoid non-target effects on other potential hosts, which could become infected if susceptible (Lacey et al. 2015). If successful, the agent would need to be delivered to a population to cause an epizootic (high prevalence population infection) that would result in high levels of mortality, as has been observed for example for bacterial pathogens of the mole cricket, *Scapteriscus* sp. (Hudson et al. 2014). Specific methods of introducing agents (in this case an organism) to a population can involve a range of techniques, including but not limited to the use of pheromones to attract the target species to the control agent (Stebbing et al. 2003). With the new advent of molecular diagnostic techniques it has become easier to monitor how biocontrol agents are impacting organisms in an environment, and can help to understand the risks they pose (Gonzalez-Change et al. 2016).

The use of metagenomics in the field of invasive pathogen identification has been shown to be highly successful in identifying a range of different pathogen groups, in particular viral and bacterial species (Chapter 8). This technique has not been applied to identify and compare invasive pathogen profiles previously. Specific discoveries include the presence of a WSSV-like virus in *D. villosus* and the observation of several novel viruses in *D. haemobaphes*, which also have histological and ultrastructural data (Chapter 11). The use of this technique to identify species diversity carried by other invaders would be a worthwhile application of the tool, however its use in tandem with histology and electron microscopy forms a better way of understanding pathogens taxonomy and pathology. Data such as these for other invaders would help to fill in our knowledge gaps around

the invasive pathogens carried by invasive and non-native species: a crucial study focus outlined in recent reviews (Roy et al. 2016).

10.2. Progressing biological control for invasive crustaceans

To identify a biological control agent is a difficult process, requiring broad-scale screening of high numbers of specimens to detect the presence of parasites and pathogens that could lower the survival of their host. In this thesis, several potential biocontrol agents have been taxonomically identified: *P. carcini*; *C. ornata*; *C. roeselii*; and *Aquarickettsiella crustaci*.

The discovery of *P. carcini* in invasive shore crab populations in Canada likely reflects a parasite acquisition event due to the lack of detection in native populations (Bojko et al. 2016). Based on the pathology in the hepatopancreas it is assumed that this parasite would have an impact on the digestion processes in the crab that could affect its overall health status. Some high-profile diseases in aquaculture have been linked to related microsporidian species, such as *Enterocytozoon hepatopanaei*, which causes a hepatopancreatic disease in Crustacea and affects their survival (Tourtip et al. 2009). Examples like this suggest that *P. carcini* may have the potential to detrimentally impact its invasive host and be used as a control agent. Greater detail is now needed to better understand this parasite's transmission, host range and effect upon host survival and alteration to host behaviour.

The identification of two novel microsporidian pathogens (*C. roeselii* from the invasive amphipod *G. roeselii* and *C. ornata* from *D. haemobaphes*) increases the number of potential agents for amphipod control. Both show high levels of pathology in the musculature of the host. *Cucumispora ornata* lowers the activity and survival of its host (Chapter 9). However, despite the pathology suggesting this species can control the invasive host population size, some members of the *Cucumispora* group have been linked with a wide host range via field surveys for the parasite, and through laboratory experimentation (Bacela-Spychalska et al. 2014; Chapter 9). *Cucumispora ornata* can be transmitted from *D. haemobaphes* to the native keystone shredder *G. pulex* and infects, and reduces the survival of, this native amphipod species in the UK. This means *C. ornata* poses a threat as a wildlife pathogen and should not be applied as a biocontrol agent.

Bacteria have been utilised in the past as control agents (Hajek and Delalibera, 2010; Lacey et al. 2015). *Aquarickettsiella crustaci* causes a systemic intracellular pathology in the nerve tissue, musculature, haemocytes and gonad of its host, *G. fossarum*. If this

RLO is found to be host specific and to induce mortality or beneficial behavioural change, then it may be suitable as a possible control agent to avoid the environmental impact of its host, as described in section 10.1.

Viruses are also commonly used biocontrol agents (Hajek and Delalibera, 2010). DhbfIV causes a systemic pathology throughout the haemolymph and connective tissues and lowers the survival rate of infected *D. haemobaphes* (Chapters 8 and 10). The metagenomic study conducted in Chapter 8 has identified it as a relative of *Panulirus argus virus 1* (PaV-1), a virus from the Caribbean spiny lobster, *Panulirus argus*, specific to this host (Butler et al. 2008). For the fishery associated with *P. argus,* this is a negative aspect of the virus. However, if DhbfIV also has a restricted host range, then this pathogen could also have potential for biological control of the invasive *D. haemobaphes*. The identification of a similar virus (HLV) in *C. maenas* could lower host survival rate and could also feature as a possible control agent for this invasive crustacean, pending further studies to identify host range and survival rate.

The identification, risk assessment and potential implication of using biocontrol agents to regulate invasive crustaceans identifies potential for the use of this control method to help control current invasion issues. However, the application in practice, how this control method could be used, the logistics involved and how biocontrol can be applied in tandem with integrated pest management (IPM) all require consideration. Starting firstly with the application of a possible control agent, several factors must be accounted for, including: the mode of transmission would determine how to introduce the pathogen. If the pathogen can be horizontally transmitted into the population it may be possible to introduce it directly to the water column to be contracted by the aquatic invader. Alternatively the introduction of live infected animals may increase transmission of the potential control agent into the invasive population. Such techniques have been applied in agricultural practice, either by delivery through a spray or by providing infected material for consumption (Lacey et al. 2015).

The control method could have wide applications for aquatic environments, because movement of a waterborne control agents can be more rapid than those in terrestrial environments due to water currents (Wilkes et al. 2014). Direct application of a biocontrol agent could be difficult due to high water volumes, which may however require greater concentrations of control agent introduction relative to terrestrial systems, because of the size of rivers and lakes. Ocean dwelling invaders could be extremely difficult to control in this way due to rapid dispersal of the control agent into large amounts of open water. For both freshwater and marine systems, it may be more applicable to introduce control agents via a more specific method, possibly through the introduction of infected hosts to

initiate natural transmission of the control agent (Gumus et al. 2015), or by including a concentrated source of the agent which could be attractive to the target host, possibly via a baited trap spiked with pathogen or by a pheromone attraction method to an infection source – these techniques draw parallels with chemical control introduction methods (Stebbing et al. 2003). With the new advent of molecular diagnostic techniques it has become easier to track biocontrol agents and observe how they are impacting organisms in an environment (Gonzalez-Change et al. 2016). Knowledge of the number of infected specimens needed and/or the concentration of control agent needed would depend on the environment, predicted target population size and susceptibility to infection to advise the best methods of biocontrol agent introduction.

Although this thesis has specifically identified the potential for biocontrol to benefit invasive crustacean control, it is important to consider its application alongside other control methods in an integrated approach. The few examples of IPM for aquatic environments are outlined in Chapter 1, but despite the low number of documented aquatic cases, examples in terrestrial settings, are numerous and when controlling insects often include a biocontrol aspect. Integrated pest management can avoid rapid evolution of resistance through the application of several different control techniques in tandem and can prevent any one strain of target host from being resistant to all of the control methods, making it a desirable but often costly process (Hutchison et al. 2015; Naranjo et al. 2015). Combining physical, chemical, biological and autocidal control methods can help to rapidly reduce a population impact, possibly through mechanical removal of invaders (Hänfling et al. 2011), employing a specific chemical to reduce population size (Cecchinelli et al. 2012), and introducing a pathogen that could reduce survival and negatively alter host fecundity (Goddard et al. 2005). IPM could result in eradication of the invasive population after it has gotten a foothold in the environment, and allow the ecosystems present to recover without damaging them further by introducing generalised agents (such as chemical biocides).

10.3. A system for regulated screening of invasive crustaceans

Identifying pathogens acting as possible control agents and screening for wildlife disease are important factors that can help to better assess the impacts of invasive species. This thesis has followed a three-step process, involving: ‘broad-scale screening’; ‘invasive pathogen taxonomy’; and ‘invasive pathogen impact and control potential’ (Chapter 1: Fig. 7 and 8). This process includes the use of screening tools (histology, electron microscopy, molecular diagnostics and metagenomics) to determine the pathogen profile of the invasive population, and finally assess the symbionts behavioural impact, survival

impact and host range. Structuring the thesis in this way helps to understand the process of pathogen screening and discovery through to the collection of data required to accurately risk assess a co-invasive organism, and place it upon the scale of being an invasive pathogen or a potentially viable biological control agent.

Consideration of what an 'invasive pathogen' should be termed as, and how the symbionts carried by invasive species should be generally referend to, needs exploring further. This issue could be resolved by adapting a subjective scale for use by invasion biologists, which can be used to identify those symbionts travelling alongside invaders as either threats to the native ecology, or as species that represent little/no impact to the invaded community. This scale could factor in the host-behaviour change, alteration to host survival, pathological affects, host range and capability to infect native species, and whether the presence of a symbiont can increase the invasive capabilities of its host (Fig. 10.1).

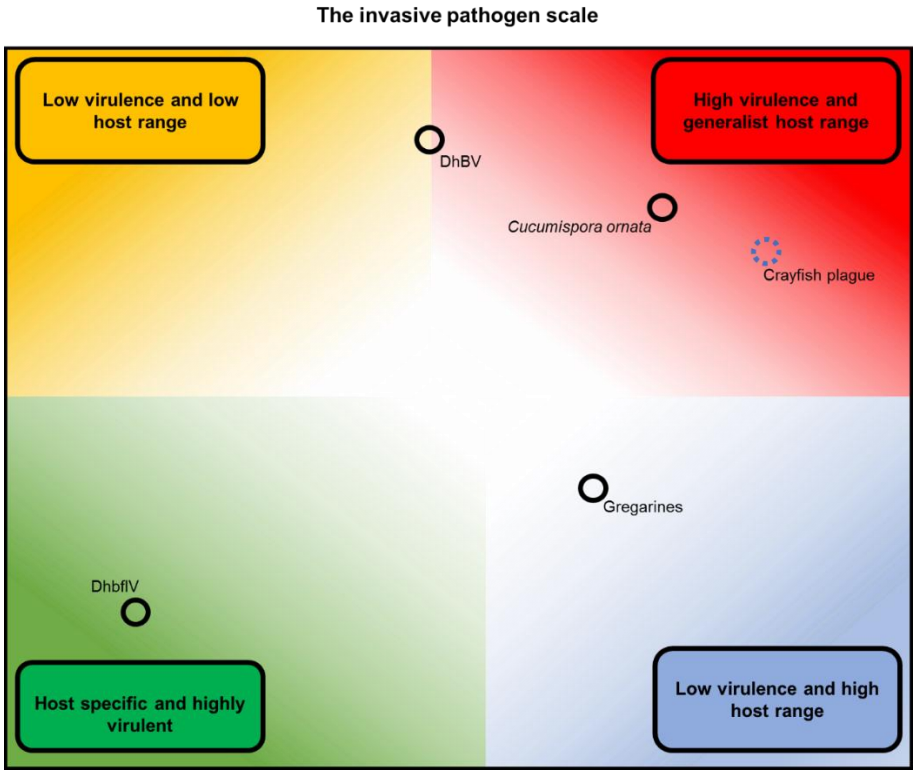


Figure 10.1: A representative scale accounting for how a co-invasive symbiont could affect invasive and native hosts in new environments. This can include acting as a possible biological control agent (green), acting as an invasive pathogen which can harm native wildlife (red), or having little impact upon its invasive host or surrounding environment (yellow/Blue). The pathogens carried by the demon shrimp are subjectively plotted onto the scale based on their affect upon their host and the surrounding environment (black circles). Also included is *Aphanomyces astaci* (Crayfish plague), a pathogen that impacts native species but has little pathological effects for its introductive invasive crayfish species' (blue broken circle). This scale can be applied to any pathogen group travelling with an invasive species, and could include the *C. maenas* data as a secondary example.

Using the demon shrimp invasion of the UK as one example, some of the parasites, pathogens and commensals carried into the UK have now been assessed for behavioural alteration and their capability to infect alternative species and reduce host survival. These include gregarines, *Dikerogammarus haemobaphes* Bacilliform Virus (DhBV), *Dikerogammarus haemobaphes* bi-faces-like Virus (DhbflV) and *Cucumispora ornata*. Using the subjective scale in Figure 10.1 to place each symbiont relative to the impacts it can have on invasive and native hosts, the scale can subjectively outline which symbionts benefit control, and which are invasive pathogens that could affect wildlife populations.

Those gregarines infecting *D. haemobaphes* have been shown to display a lack of pathology and immunological reactions by their presence in the gut and were found not to affect the behaviour (activity/aggregation) or physiology of their host. The effect of infection on host survival was not directly measured but similar gregarine infections have been suggested for this species, including *Cephaloidophora* sp., which has a general host range (Ovcharenko et al. 2009). The absence of pathology in the host tissue suggests limited impacts upon their host's survival, suggesting they are low risk to the invader but could infect native species due to their general host range.

DhBV has been found to cause pathology in the hepatopancreas and was associated with increased activity in its invasive host, which may provide an overall increase in its host's invasive capabilities. Increased activity means that this pathogen appears to be an accomplice to invasion and therefore sits between being a non-native species and an indirect threat to wildlife. On the scale this is represented as a low-virulence/low host range species with some overlap with being an 'invasive pathogen' by increasing host fitness.

DhbflV causes high levels of systemic pathology to its invasive host and has been associated with lower host survival rates (Chapter 9), defining it as a potential control agent. The collection of host range data for this virus may alter this subjective position on the scale, depending on if it is host specific or not.

Cucumispora ornata has been shown to cause high levels of systemic infection in its invasive host, lowering its host's activity and decreasing its host's survival rate. However, it can also infect native species (40% infection rate in experimental trial) and lower the survival of an alternate native host, *Gammarus pulex*. These features place it as an invasive pathogen and wildlife threat, which would not be adaptable as a biocontrol agent.

Using a symbiont example from an invasive crayfish study system, *Aphanomyces astaci* (crayfish plague) can infect and induce mortality in native, vulnerable crayfish species but causes a low level, asymptomatic infection in its invasive host, acting as an accomplice to invasion as well as infecting native species. This oomycete can therefore be placed on the scale as an invasive pathogen.

The addition of a quantitative scale to score the symbionts carried by invasive species could create a more robust method of identifying their level of threat to natural biodiversity, or their potential as control agents. Regulated screening efforts for invasive and non-native species are not formally documented in any current legislation (Chapter 1). Therefore, the development of a conceptual model to allow rapid collection and screening of invasive species entering the UK is of high importance. Such protocols could include an early warning system, by screening recent invaders to help prevent and avoid the introduction of harmful pathogens. Additionally, this could also help to identify novel species that could be used to possibly control their invasive host.

This thesis has demonstrated that a wide diversity of species can be recognised and taxonomically identified through collection, pathological screening using various tools and ending in publication of the data to aid policy. This process should also include the screening of native hosts to understand invasive pathogen epidemiology and employ analytical methods like: phylogenetics and bioinformatics, which can be used to understand the origin and phylogeny of invasive pathogens.

The general risk related to the symbionts carried by invasive and non-native species can be difficult to determine. The studies conducted in this thesis have shown that experimental systems (transmission assays; behavioural assays; survival assays) and analysis of pathology (histology; TEM; metagenomics), can help to determine the threats a co-invasive pathogen may pose to naïve ecosystems and their inhabitants. The methods described above constitute a good starting point for the risk analysis of any newly identified co-invasive symbionts. Representation of the relative threat posed by these species could be visualised using the scale designed in Figure 1, where the risks that co-invasive symbionts pose to invasion sites and their inhabitants and can be subjectively compared.

To conclude, I have taxonomically/morphologically identified several novel pathogens that could either threaten vulnerable native species or have the potential to be used as control agents for their invasive host. I determine that *C. ornata* is an invasive pathogen and that the further spread and invasion of its host, *D. haemobaphes*, should receive increased restriction using biosecurity and control mechanisms to prevent the spread of

this microsporidian. The haemocyte-infecting virus DhbfIV is the most likely pathogen to function as a possible biocontrol agent for *D. haemobaphes*, but requires further host-specificity testing. The mode of surveying crustaceans for pathogens outlined by this thesis provides proof and functionality upon the methods (histology, TEM, molecular diagnostics, metagenomics) of screening invasive species for invasive pathogen threats, and can additionally identify other symbionts that could be adapted into biological agents.

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Appendix

Appendix to Chapter 1

Appendix Table 1.1: A list of invasive aquatic invertebrates (IAIs) including 1054 species from around the globe accorind to the European Alien Species Database (EASIN), the European squatic invaders database (AquaNIS), and the Global Invasive Species Database (GISD).

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Abyla trigona	Cnidarian	Cnidarian	EU	Marine	Low/Unk	EASIN
Acantharctus posteli	Crustacean	Lobster	EU	Marine	Low/Unk	EASIN
Acanthaster planci	Echinoderm	Sea star	Global	Marine	High	GISD, EASIN
Acar plicata	Mollusc	Equivalve	EU	Marine	Low/Unk	EASIN
Acartia (Acanthacartia) fossae	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Acartia (Acanthacartia) tonsa	Crustacean	Copepod	EU	Marine	Low/Unk	AquaNIS
Acartia (Acartiura) omorii	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Acartia (Odontacartia) centrura	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Actaea savignii	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Actaeodes tomentosus	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Acteocina crithodes	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Acteocina mucronata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Actinocleidus oculatus	Eumetazoan	Eumetazoan	EU	Freshwater	Low/Unk	EASIN
Actinocleidus recurvatus	Eumetazoan	Eumetazoan	EU	Freshwater	Low/Unk	EASIN
Actumnus globulus	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Aedes aegypti	Insect	Mosquito	Global	Terrestrial and Freshwater	High	GISD
Aedes albopictus	Insect	Mosquito	Global	Terrestrial and Freshwater	High	GISD, EASIN
Aedes japonicus	Insect	Mosquito	EU	Terrestrial and Freshwater	High	EASIN
Aequorea conica	Cnidarian	Jellyfish	EU	Marine	Low/Unk	EASIN
Aequorea globosa	Cnidarian	Jellyfish	EU	Marine	Low/Unk	EASIN
Aetea anguina	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Aetea ligulata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Aetea longicollis	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Aetea sica	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Aetea truncata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Aeverrillia setigera	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Afrocardium richardi	Mollusc	Equivalve	EU	Marine	Low/Unk	EASIN
Aiptasia diaphana	Cnidarian	Anemone	EU	Marine	Low/Unk	AquaNIS
Aiptasia pulchella	Cnidarian	Anemone	EU	Marine	Low/Unk	EASIN
Alectryonella plicatula	Mollusc	Mollusc	EU	Marine	Low/Unk	EASIN
Aliculastrum cylindricum	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Alitta succinea	Annelid	Annelid	Global	Marine	Low/Unk	GISD, AquaNIS
Alkmaria romijni	Annelid	Annelid	EU	Marine	Low/Unk	AquaNIS
Allolepidapedon fistulariae	Platyhelminth	Trematode	EU	Marine	Low/Unk	EASIN
Alpheus audouini	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Alpheus inopinatus	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Alpheus migrans	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Alpheus rapacida	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Amathina tricarinata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Ameira divagans divagans	Crustacean	Maxillipod	EU	Marine	Low/Unk	AquaNIS, EASIN
Ametropus fragilis	Insect	Mayfly	EU	Freshwater	Low/Unk	EASIN
Ammothea hilgendorfi	Pantopod	Sea spider	EU	Marine	Low/Unk	AquaNIS, EASIN
Ampelisca cavicoxa	Crustacean	Amphipod	EU	Marine	Low/Unk	AquaNIS, EASIN
Ampelisca heterodactyla	Crustacean	Amphipod	EU	Marine	Low/Unk	AquaNIS, EASIN
Amphibalanus eburneus	Crustacean	Barnacle	EU	Marine	Low/Unk	AquaNIS, EASIN
Amphibalanus improvisus	Crustacean	Barnacle	EU	Marine	Low/Unk	AquaNIS
Amphibalanus reticulatus	Crustacean	Barnacle	EU	Marine	Low/Unk	AquaNIS
Amphibalanus variegatus	Crustacean	Barnacle	EU	Marine	Low/Unk	AquaNIS
Amphicorina pectinata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Amphioctopus aegina	Mollusc	Octopus	EU	Marine	Low/Unk	EASIN
Amphiodia (Amphispina) obtecta	Echinoderm	Brittle star	EU	Marine	Low/Unk	EASIN
Amphioplus (Lymanella) laevis	Echinoderm	Brittle star	EU	Marine	Low/Unk	EASIN
Amphogona pusilla	Cnidarian	Hydropolip	EU	Marine	Low/Unk	EASIN
Ampithoe bizseli	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Anadara broughtonii	Mollusc	Clam	EU	Marine	Low/Unk	EASIN
Anadara diluvii	Mollusc	Clam	EU	Marine	Low/Unk	AquaNIS
Anadara kagoshimensis	Mollusc	Clam	EU	Marine and Oligohaline	High	AquaNIS, EASIN
Anadara natalensis	Mollusc	Clam	EU	Marine	Low/Unk	EASIN
Anadara transversa	Mollusc	Clam	EU	Marine	High	EASIN
Anguillicola australiensis	Nematode	Nematode	EU	Freshwater, Marine and Oligohaline	Low/Unk	EASIN
Anguillicola novaezelandiae	Nematode	Nematode	EU	Freshwater and Marine	Low/Unk	EASIN
Anguillicoloides crassus	Nematode	Nematode	EU	Freshwater, Marine and Oligohaline	High	AquaNIS, EASIN

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Anilocra pilchardi	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Anoplodactylus californicus	Pantopod	Sea spider	EU	Marine	Low/Unk	EASIN
Anoplodactylus digitatus	Pantopod	Sea spider	EU	Marine	Low/Unk	EASIN
Antigona lamellaris	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Apanthura sandalensis	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Aphelochaeta marioni	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Apionsoma (Apionsoma) misakianum	Sipunculan	Sipunculan	EU	Marine	Low/Unk	EASIN
Apionsoma (Apionsoma) trichocephalus	Sipunculan	Sipunculan	EU	Marine	Low/Unk	EASIN
Aplysia dactylomela	Mollusc	Sea hare	EU	Marine	High	EASIN
Aquilonastra burtoni	Echinoderm	Sea star	EU	Marine	Low/Unk	EASIN
Arachnidium lacourti	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Arachnoidella protecta	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Arctapodema australis	Cnidarian	Cnidarian	EU	Marine	Low/Unk	EASIN
Arcuatula perfragilis	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Arcuatula senhousia	Mollusc	Bivalve	EU	Marine	High	EASIN
Argulus japonicus	Crustacean	Fish louse	EU	Freshwater	Low/Unk	EASIN
Aricidea hartmani	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Arietellus pavoninus	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Artemia franciscana	Crustacean	Brine shrimp	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Ashtoret lunaris	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Aspidosiphon (Akrikos) mexicanus	Aspidosiphonid	Aspidosiphonid	EU	Marine	Low/Unk	EASIN
Aspidosiphon (Aspidosiphon) elegans	Aspidosiphonid	Aspidosiphonid	EU	Marine	Low/Unk	EASIN
Astacus astacus	Crustacean	Crayfish	EU	Freshwater	High	EASIN
Astacus leptodactylus	Crustacean	Crayfish	EU	Freshwater	Low/Unk	EASIN
Asterias amurensis	Echinoderm	Sea star	Global	Marine	Low/Unk	GISD
Asterias rubens	Echinoderm	Sea star	EU	Marine	Low/Unk	EASIN
Atactodea striata	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Atergatis roseus	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Atyaephyra desmarestii	Crustacean	Shrimp	EU	Freshwater	Low/Unk	EASIN
Aulacomya atra	Mollusc	Mussel	EU	Marine	Low/Unk	EASIN
Austrominius modestus	Crustacean	Barnacle	EU	Marine and Oligohaline	Low/Unk	AquaNIS, EASIN
Autonoe spiniventris	Crustacean	Amphipod	EU	Freshwater	Low/Unk	AquaNIS
Baeolidia moebii	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Balanus amphitrite	Mollusc	Bivalve	EU	Marine	Low/Unk	AquaNIS
Balanus trigonus	Mollusc	Bivalve	EU	Marine	Low/Unk	AquaNIS, EASIN
Bankia fimbriatula	Mollusc	Bivalve	EU	Marine	Low/Unk	AquaNIS, EASIN
Barbronia weberi	Annelid	Leech	EU	Freshwater	Low/Unk	EASIN
Barentsia ramosa	Entoproctan	Entoproctan	EU	Marine	Low/Unk	EASIN
Batillaria attramentaria	Mollusc	Sea snail	Global	Marine	Low/Unk	GISD
Bdellocephala punctata	Platyhelminth	Flatworm	EU	Freshwater	Low/Unk	EASIN
Beania mirabilis	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Bedeva paivae	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS
Bellamya chinensis	Mollusc	Freshwater snail	Global	Freshwater	Low/Unk	GISD, AquaNIS, EASIN
Bemlos leptocheirus	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Beroe ovata	Cnidarian	Comb jellyfish	EU	Marine	Low/Unk	AquaNIS, EASIN
Biomphalaria glabrata	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Bispira polyomma	Annelid	Annelid	EU	Marine	Low/Unk	EASIN
Bithynia tentaculata	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Bivetiella cancellata	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS
Blackfordia virginica	Cnidarian	Jellyfish	EU	Marine and Oligohaline	High	AquaNIS, EASIN
Boccardia polybranchia	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Boccardia proboscidea	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Boccardia semibranchiata	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Boccardiella hamata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Boccardiella ligerica	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Boeckella triarticulata	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Boninia neotethydis	Platyhelminth	Flatworm	EU	Marine	Low/Unk	EASIN
Boonea bisuturalis	Mollusc	Sea snail	Global	Marine	Low/Unk	GISD
Borysthenia naticina	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Bostrycapulus odites	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Bothriocephalus acheilognathi	Platyhelminth	Tapeworm	EU	Freshwater	High	EASIN
Bothriocephalus gowkongensis	Platyhelminth	Tapeworm	EU	Freshwater	Low/Unk	EASIN
Bougainvillia macloviana	Cnidarian	Hydroid	EU	Marine	Low/Unk	AquaNIS
Bougainvillia muscus	Cnidarian	Hydroid	EU	Marine	Low/Unk	EASIN
Bougainvillia rugosa	Cnidarian	Hydroid	EU	Marine	Low/Unk	AquaNIS, EASIN
Bowerbankia gracillima	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Brachidontes exustus	Mollusc	Mussel	EU	Marine	Low/Unk	AquaNIS, EASIN
Brachidontes pharaonis	Mollusc	Mussel	EU	Marine	High	EASIN
Brachionus variabilis	Eumetazoan	Rotifer	EU	Freshwater	Low/Unk	EASIN
Branchiomma bairdi	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Branchiomma boholense	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Branchiomma luctuosum	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Branchiura sowerbyi	Annelid	Annelid	EU	Freshwater	Low/Unk	AquaNIS, EASIN
Brania arminii	Annelid	Annelid	EU	Marine	Low/Unk	AquaNIS
Bucephalus polymorphus	Platyhelminth	Flatworm	EU	Freshwater	Low/Unk	EASIN
Bugula avirostris	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Bugula dentata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Bugula fulva	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Bugula neritina	Bryozoan	Bryozoan	Global	Marine	High	GISD, AquaNIS, EASIN
Bugula simplex	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Bugula stolonifera	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Bugulina flabellata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Bulinus contortus	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	AquaNIS
Bulla arabica	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Bursatella leachii	Mollusc	Sea slug	EU	Marine	High	EASIN
Bythocaris cosmetops	Crustacean	Decapod	EU	Marine	Low/Unk	EASIN
Bythotrephes longimanus	Crustacean	Water flea	Global	Freshwater	Low/Unk	GISD, EASIN
Caecidotea communis	Crustacean	Isopod	EU	Freshwater	Low/Unk	EASIN
Calanipeda aquaedulcis	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Calanopia biloba	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Calanopia elliptica	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Calanopia media	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Calanopia minor	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Calappa hepatica	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Calappa pelii	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Caligus fugu	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Caligus pageti	Crustacean	Copepod	EU	Marine	Low/Unk	AquaNIS
Callinectes danae	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Callinectes exasperatus	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Callinectes sapidus	Crustacean	Crab	EU	Freshwater, Marine and Oligohaline	High	AquaNIS, EASIN
Callista florida	Mollusc	Clam	EU	Marin	Low/Unk	EASIN
Caloria indica	Mollusc	sea slug	EU	Marine	Low/Unk	EASIN
Calyptraea chinensis	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Cancer irroratus	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Caprella mutica	Crustacean	Shrimp	EU	Marine	High	AquaNIS, EASIN
Caprella scaura	Crustacean	Shrimp	EU	Marine	Low/Unk	AquaNIS, EASIN
Carcinus maenas	Crustacean	Crab	Global	Marine	High	GISD
Carijoa riisei	Cnidarian	Coral	Global	Marine	Low/Unk	GISD
Carupa tenuipes	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Caspiobdella fadejewi	Annelid	Leech	EU	Freshwater	Low/Unk	EASIN
Cassiopea andromeda	Cnidarian	Jellyfish	EU	Marine	Low/Unk	EASIN
Catenicella paradoxa	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Caulibugula zanzibarensis	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Cellana rota	Mollusc	Limpet	EU	Marine	Low/Unk	EASIN
Celleporaria aperta	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Celleporaria brunnea	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS, EASIN
Celleporella carolinensis	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Celtodoryx ciocalyptoides	Poriferan	Sponge	EU	Marine	Low/Unk	AquaNIS, EASIN
Centrocardita akabana	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Centropages furcatus	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Cerastoderma edule	Mollusc	Cockle	EU	Marine	Low/Unk	AquaNIS
Ceratonereis mirabilis	Annelid	Polychete worm	EU	Marnie	Low/Unk	EASIN
Ceratostoma inornatum	Mollusc	Sea snail	Global	Marine	Low/Unk	GISD
Cercaria sensifera	Platyhelminth	Trematode	EU	Marine	Low/Unk	EASIN
Cercopagis (Cercopagis) pengoi	Crustacean	Water flea	Global	Freshwater, Marine and Oligohaline	High	GISD, AquaNIS, EASIN
Cerithidium diplax	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cerithidium perparvulum	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cerithiopsis pulvis	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cerithiopsis tenthrenois	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cerithium columna	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cerithium egenum	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cerithium litteratum	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cerithium nesioticum	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cerithium scabridum	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Chaetogammarus warpachowskyi	Crustacean	Amphipod	EU	Freshwater, Marine and Oligohaline	Low/Unk	AquaNIS, EASIN
Chaetopleura (Chaetopleura) angulata	Mollusc	Chiton	EU	Marine	Low/Unk	AquaNIS, EASIN
Chalinula loosanoffi	Poriferan	Sponge	EU	Marine	Low/Unk	AquaNIS
Chama asperella	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Chama brassica	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Chama gryphoides	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS
Chama pacifica	Mollusc	Sea snail	EU	Marine	High	EASIN
Charybdis feriata	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Charybdis hellerii	Crustacean	Crab	Global	Marine	High	GISD, EASIN
Charybdis japonica	Crustacean	Crab	Global	Marine	High	GISD, EASIN

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Charybdis (Goniohellenus) longicollis	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Charybdis lucifera	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Chelicorophium curvispinum	Crustacean	Amphipod	EU	Freshwater and oligohaline	High	AquaNIS, EASIN
Chelicorophium robustum	Crustacean	Amphipod	EU	Freshwater	Low/Unk	AquaNIS, EASIN
Chelidonura fulvipunctata	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Cherax destructor	Crustacean	Crayfish	EU	Freshwater	High	EASIN
Chionoecetes opilio	Crustacean	Crab	EU	Marine	High	AquaNIS, EASIN
Chiton (Chiton) cumingsii	Mollusc	Chiton	EU	Marine	Low/Unk	EASIN
Chiton (Tegulaplax) hululensis	Mollusc	Chiton	EU	Marine	Low/Unk	EASIN
Chlamydotheca incisa	Crustacean	Shrimp	EU	Freshwater	Low/Unk	EASIN
Chorizopora brongniartii	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Choromytilus chorus	Mollusc	Mussel	EU	Marine	Low/Unk	EASIN
Chromodoris quadricolor	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Chrysallida fischeri	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Chrysallida maiae	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Chrysallida micronana	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Chthamalus proteus	Crustacean	Barnacle	Global	Marine	Low/Unk	GISD
Cinachyrella alloclada	Poriferan	Sponge	EU	Marine	Low/Unk	AquaNIS
Cingulina isseli	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Circe scripta	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Circenita callipyga	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Cirrholovenia tetranema	Cnidarian	Cnidarian	EU	Marine	Low/Unk	EASIN
Clavellisa ilishae	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Cleidodiscus monticelli	Platyhelminth	Platyhelminth	EU	Freshwater	Low/Unk	EASIN
Cleidodiscus pricei	Platyhelminth	Platyhelminth	EU	Freshwater	Low/Unk	EASIN
Cleidodiscus robustus	Platyhelminth	Platyhelminth	EU	Freshwater	Low/Unk	EASIN
Clementia papyracea	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Clinostomum complanatum	Platyhelminth	Trematode	EU	Freshwater	Low/Unk	EASIN
Clorida albolitura	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Clymenella torquata	Annelid	Bambou worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Clypeomorus bifasciata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Clytia hummelincki	Cnidarian	Hydroid	EU	Marine	Low/Unk	EASIN
Clytia linearis	Cnidarian	Hydroid	EU	Marine	Low/Unk	EASIN
Coleusia signata	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Conchoderma auritum	Crustacean	Barnacle	EU	Marine	Low/Unk	AquaNIS
Conomurex persicus	Mollusc	Conch	EU	Marine	Low/Unk	EASIN
Conus arenatus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Conus fumigatus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Conus inscriptus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Conus rattus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Coralliophila monodonta	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Corambe obscura	Mollusc	Nudibranch	EU	Marine	Low/Unk	AquaNIS, EASIN
Corbicula fluminalis	Mollusc	Bivalve	EU	Freshwater	High	AquaNIS, EASIN
Corbicula fluminea	Mollusc	Clam	EU	Freshwater	High	GISD, AquaNIS, EASIN
Cordylophora caspia	Cnidarian	Cnidarian	EU	Freshwater and oligohaline	Low/Unk	AquaNIS
Cornigerius maeoticus	Crustacean	Branchiopod	EU	Freshwater, Marine and Oligohaline	Low/Unk	AquaNIS, EASIN
Coryne eximia	Cnidarian	Hydroid	EU	Marine	Low/Unk	EASIN
Coscinasterias tenuispina	Echinoderm	Sea star	EU	Marine	Low/Unk	AquaNIS
Crangonyx pseudogracilis	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Craspedacusta sowerbii	Cnidarian	Jellyfish	EU	Freshwater	High	AquaNIS, EASIN
Crassostrea gigas	Mollusc	Oyster	EU	Marine	High	GISD, AquaNIS, EASIN
Crassostrea rivularis	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Crassostrea sikamea	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Crassostrea virginica	Mollusc	Oyster	EU	Marine	High	AquaNIS, EASIN
Crepidula fornicata	Mollusc	Sea snail	EU	Marine	High	GISD, AquaNIS, EASIN
Crepipatella dilatata	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Cristapseudes omercooperi	Crustacean	Kalliapseudid	EU	Marine	Low/Unk	EASIN
Crisularia serrata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Critomolgus actiniae	Crustacean	Maxillipod	EU	Marine	Low/Unk	AquaNIS
Cryptorchestia cavimana	Crustacean	Amphipod	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Cryptosoma cristatum	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Cryptosula pallasiana	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Cuapetes calmani	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Cucurbitula cymbium	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Cuthona perca	Mollusc	Nudibranch	EU	Marine	Low/Unk	EASIN
Cyclope neritea	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Cyclops kolensis	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Cyclops vicinus	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Cycloscala hyalina	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Cymothoa indica	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Cypretta turgida	Crustacean	Ostracod	EU	Freshwater	Low/Unk	EASIN

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Dactylogyrus anchoratus	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Dactylogyrus aristichthys	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Dactylogyrus hypophthalmichthys	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Dactylogyrus lamellatus	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Dactylogyrus nobilis	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Dactylogyrus suchengtaii	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Dactylogyrus vastator	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Dactylogyrus yinwenyingae	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Daira perlata	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Daphnia ambigua	Crustacean	Water flea	EU	Freshwater	Low/Unk	EASIN
Daphnia cristata	Crustacean	Water flea	EU	Freshwater	Low/Unk	EASIN
Daphnia longiremis	Crustacean	Water flea	EU	Freshwater	Low/Unk	EASIN
Daphnia lumholtzi	Crustacean	Water flea	Global	Freshwater	Low/Unk	GISD
Daphnia parvula	Crustacean	Water flea	EU	Freshwater	Low/Unk	EASIN
Delavalia inopinata	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Delavalia minuta	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Dendostrea cf. folium	Mollusc	Oyster	EU	Marine	High	EASIN
Dendostrea frons	Mollusc	Oyster	EU	Marine	Low/Unk	AquaNIS
Dendrocoelum romanodanubiale	Platyhelminth	Flatworm	EU	Freshwater	Low/Unk	EASIN
Dendrodoris fumata	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Desdemona ornata	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Diadema antillarum	Echinoderm	Sea urchin	EU	Marine	Low/Unk	AquaNIS
Diadema setosum	Echinoderm	Sea urchin	EU	Marine	Low/Unk	EASIN
Diadumene cincta	Cnidarian	Anemone	EU	Marine	Low/Unk	AquaNIS
Diadumene lineata	Cnidarian	Anemone	EU	Marine	Low/Unk	AquaNIS, EASIN
Diala semistriata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Diamysis bahirensis	Crustacean	Shrimp	EU	Marine	Low/Unk	AquaNIS, EASIN
Diaphanosoma chankensis	Crustacean	Brachiopod	EU	Freshwater	Low/Unk	EASIN
Dikerogammarus bispinosus	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Dikerogammarus haemobaphes	Crustacean	Amphipod	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Dikerogammarus villosus	Crustacean	Amphipod	EU	Freshwater and Oligohaline	High	AquaNIS, EASIN
Diodora funiculata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Diodora rueppellii	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Diopatra hupferiana	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Diopatra monroi	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Diphasia digitalis	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Diplodonta bogii	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Dipolydora quadrilobata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Dipolydora socialis	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Dipolydora tentaculata	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Disparalona hamata	Crustacean	Anomopodan	EU	Freshwater	Low/Unk	EASIN
Dispio magnus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Dispio uncinata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Divalinga arabica	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Dodecaceria capensis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Dolerocypris sinensis	Crustacean	Ostracod	EU	Freshwater	Low/Unk	EASIN
Dorippe quadridens	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Dorvillea similis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Dosinia erythraea	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Doxander vittatus	Mollusc	Conch	EU	Marine	Low/Unk	EASIN
Dreissena bugensis	Mollusc	Mussel	Global	Freshwater and Oligohaline	High	GISD, AquaNIS, EASIN
Dreissena polymorpha	Mollusc	Mussel	Global	Freshwater and Oligohaline	High	GISD, AquaNIS, EASIN
Dugesia tigrina	Platyhelminth	Platyhelminth	EU	Freshwater	Low/Unk	AquaNIS, EASIN
Dynamena quadridentata	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Dyspanopeus sayi	Crustacean	Mud crab	EU	Marine	Low/Unk	EASIN
Echinogammarus berilloni	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Echinogammarus (Chaetogammarus) ischnus	Crustacean	Amphipod	EU	Freshwater, Marine and Oligohaline	Low/Unk	AquaNIS, EASIN
Edwardsiella lineata	Cnidarian	Anemone	EU	Marine	Low/Unk	EASIN
Elamena mathoei	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Elasmopus pectenicrus	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Electra pilosa	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Electra tenella	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Electroma vexillum	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Elminius modestus	Crustacean	Barnacle	Global	Marine	Low/Unk	GISD
Elysia grandifolia	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Elysia tomentosa	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Emmericia patula	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Engina mendicaria	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Enhydrosoma vicinum	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Ensiculus cultellus	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Ensis directus	Mollusc	Clam	EU	Marine	High	AquaNIS, EASIN
Eocuma dimorphum	Crustacean	Cumacea	EU	Marine	Low/Unk	AquaNIS, EASIN
Eocuma rosae	Crustacean	Cumacea	EU	Marine	Low/Unk	EASIN

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Eocuma sarsii	Crustacean	Cumacea	EU	Marine	Low/Unk	EASIN
Ercolania viridis	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Ergalatax contracta	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Ergalatax junionae	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Ergasilus briani	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Ergasilus gibbus	Crustacean	Copepod	EU	Freshwater and Marine	Low/Unk	EASIN
Ergasilus sieboldi	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Erinaceusyllis serratosetosa	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Eriocheir sinensis	Crustacean	Crab	Global	Freshwater	High	GISD, AquaNIS, EASIN
Erosaria turdus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Erugosquilla massavensis	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Ervilia scaliola	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Escharina vulgaris	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS, EASIN
Ethminolia hemprichi	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Euchaeta concinna	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Eucheilota menoni	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	AquaNIS, EASIN
Eucheilota paradoxica	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Eucheilota ventricularis	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Eucidaris tribuloides	Echinoderm	Sea urchin	EU	Marine	Low/Unk	EASIN
Eucrate crenata	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Eudendrium capillare	Cnidarian	Cnidarian	EU	Marine	Low/Unk	EASIN
Eudendrium carneum	Cnidarian	Cnidarian	EU	Marine	Low/Unk	EASIN
Eudendrium merulum	Cnidarian	Cnidarian	EU	Marine	Low/Unk	EASIN
Eudendrium vaginatum	Cnidarian	Cnidarian	EU	Marine	Low/Unk	EASIN
Eudiaptomus gracilis	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Eudiplozoon nipponicum	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Eunapius carteri	Poriferan	Sponge	EU	Freshwater	Low/Unk	EASIN
Eunaticina papilla	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Eunice tubifex	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Euplana gracilis	Platyhelminth	Flatworm	EU	Marine	Low/Unk	AquaNIS, EASIN
Eurycarcinus integrifrons	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Eurytemora americana	Crustacean	Copepod	EU	Marine	Low/Unk	AquaNIS, EASIN
Eurytemora pacifica	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Eurytemora velox	Crustacean	Copepod	EU	freshwater	Low/Unk	EASIN
Eusarsiella zostericola	Crustacean	Ostrocod	EU	Marine	Low/Unk	AquaNIS, EASIN
Eusyllis kupfferi	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Evadne anonyx	Crustacean	Cladoceran	EU	Freshwater, Marine and Oligohaline	Low/Unk	AquaNIS, EASIN
Exogone (Exogone) breviantennata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Exogone africana	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Fabienna oligonema	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Fabriciola ghardaqa	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Fauveliopsis glabra	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Favorinus ghanensis	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Fenestrulina delicia	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Fenestrulina malusii	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Ferosagitta galerita	Annelid	Chaetognathan	EU	Marine	Low/Unk	EASIN
Ferrisia wautieri	Mollusc	Gastropod	EU	Freshwater, Marine and Oligohaline	Low/Unk	EASIN
Ferrissia fragilis	Mollusc	Limpet	EU	Freshwater	Low/Unk	EASIN
Ferrissia parallela	Mollusc	Limpet	EU	Freshwater	Low/Unk	EASIN
Ferrissia shimeki	Mollusc	Limpet	EU	Freshwater	Low/Unk	EASIN
Ficopomatus enigmaticus	Annelid	Tubeworm	Global	Marine and Oligohaline	High	GISD, AquaNIS, EASIN
Filellum serratum	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Finella pupoides	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Fistulobalanus albicostatus	Crustacean	Barnacle	EU	Marine	Low/Unk	EASIN
Fistulobalanus pallidus	Crustacean	Barnacle	EU	Marine	Low/Unk	EASIN
Flabellina rubrolineata	Mollusc	Nudibranch	EU	Marine	Low/Unk	EASIN
Fulvia (Fulvia) australis	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Fulvia fragilis	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Fusinus rostratus	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Fusinus verrucosus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Gafrarium savignyi	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Gammaropsis togoensis	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Gammarus pulex	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Gammarus roeselii	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Gammarus tigrinus	Crustacean	Amphipod	EU	Freshwater, Marine and Oligohaline	High	AquaNIS, EASIN
Gammarus (Echinogammarus) trichiatus	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Gammarus varsoviensis	Crustacean	Amphipod	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Garveia franciscana	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	AquaNIS, EASIN

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Geryonia proboscidalis	Cnidarian	Jellyfish	EU	Marine	Low/Unk	EASIN
Gemma gemma	Mollusc	Clam	Global	Marine	Low/Unk	GISD
Geukensia demissa	Mollusc	Mussel	Global	Marine	Low/Unk	GISD
Gibborissoia virgata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Gibbula adansoni	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Gibbula adriatica	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Gibbula albida	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Glabropilumnus laevis	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Glycera capitata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Glycera dayi	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Glycinde bonhourei	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Glycymeris arabica	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Glyphidohaptor plectocirra	Platyhelminth	Monogenean	EU	Marine	Low/Unk	EASIN
Gmelinoides fasciatus	Crustacean	Amphipod	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Godiva quadricolor	Mollusc	Nudibranch	EU	Marine	Low/Unk	EASIN
Goneplax rhomboides	Crustacean	Crab	EU	Marine	Low/Unk	AquaNIS
Goniadella gracilis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Goniobranchus annulatus	Mollusc	Nudibranch	EU	Marine	Low/Unk	EASIN
Gonioinfradens paucidentatus	Mollusc	Nudibranch	EU	Marine	Low/Unk	EASIN
Gonionemus vertens	Cnidarian	Jellyfish	EU	Marine	High	AquaNIS, EASIN
Gouldiopa consternans	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Grandidierella japonica	Crustacean	Amphipod	EU	Marine	Low/Unk	AquaNIS, EASIN
Grapsus granulosus	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Gyraulus chinensis	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Gyraulus parvus	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Gyrodactylus fairporti	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Gyrodactylus gasterostei	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Gyrodactylus mugili	Platyhelminth	Monogenean	EU	Marine	Low/Unk	EASIN
Gyrodactylus salaris	Platyhelminth	Monogenean	EU	Freshwater and Oligohaline	High	AquaNIS, EASIN
Gyrodactylus turnbuli	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Gyrodactylus zhukovi	Platyhelminth	Monogenean	EU	Marine	Low/Unk	EASIN
Halectinosoma abrau	Crustacean	Copepod	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Halgerda willeyi	Mollusc	Nudibranch	EU	Marine	Low/Unk	EASIN
Halimede tyche	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Haliotis discus	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS
Haliotis rugosa pustulata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Haliotis tuberculata	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Haliscera bigelowi	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Halitiara inflexa	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Hamimaera hamigera	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Haminoea cyanomarginata	Mollusc	Nudibranch	EU	Marine	Low/Unk	EASIN
Haminoea japonica	Mollusc	Nudibranch	EU	Marine	Low/Unk	AquaNIS, EASIN
Helisoma duryi	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Helobdella stagnalis	Annelid	Leech	EU	Freshwater	Low/Unk	EASIN
Hemicypris dentatomarginata	Crustacean	Ostracod	EU	Freshwater	Low/Unk	EASIN
Hemigrapsus penicillatus	Crustacean	Crab	EU	Marine	Low/Unk	AquaNIS
Hemigrapsus sanguineus	Crustacean	Crab	Global	Marine	High	GISD, AquaNIS, EASIN
Hemigrapsus takanoi	Crustacean	Crab	EU	Marine	High	AquaNIS, EASIN
Hemimysis anomala	Crustacean	Shrimp	EU	Freshwater and Oligohaline	High	AquaNIS, EASIN
Herbstia nitida	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Herrmannella duggani	Crustacean	Copepod	EU	Marine	Low/Unk	AquaNIS
Hesionides arenaria	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Hesionura serrata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Heterocope appendiculata	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Heterolaophonte hamondi	Crustacean	Copepod	EU	Marine	Low/Unk	AquaNIS
Heterosaccus dollfusi	Crustacean	Sacculinid	EU	Marine	Low/Unk	EASIN
Heterotentacula mirabilis	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Hexapleomera robusta	Crustacean	Tanaid	EU	Marine	Low/Unk	EASIN
Hexaplex (Trunculariopsis) trunculus	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Hiatella arctica	Mollusc	Clam	EU	Marine	Low/Unk	AquaNIS
Hiatula rosea	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Hippopodina feegeensis	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Hippopodina iririkiensis	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Hirudo medicinalis	Annelid	Leech	EU	Freshwater	Low/Unk	EASIN
Homarus americanus	Crustacean	Lobster	EU	Marine	High	AquaNIS, EASIN
Hyastenus hilgendorfi	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Hydroides albiceps	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Hydroides brachyacanthus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Hydroides dianthus	Annelid	Polychete worm	EU	Marine and Oligohaline	High	EASIN
Hydroides elegans	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Hydroides heterocerus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Hydroides homoceros	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Hydroides minax	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN

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Hydroides operculatus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Hyotissa hyotis	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Hyotissa inermis	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Hypania invalida	Annelid	Polychete worm	EU	Freshwater	Low/Unk	EASIN
Hypaniola kowalewskii	Annelid	Polychete worm	EU	Freshwater	Low/Unk	EASIN
Hypselodoris infucata	Mollusc	Nudibranch	EU	Marine	Low/Unk	EASIN
Ianiropsis tridens	Crustacean	Isopod	EU	Marine	Low/Unk	AquaNIS
Idotea metallica	Crustacean	Isopod	EU	Marine	Low/Unk	AquaNIS
Idyella pallidula	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Ilyanassa obsoleta	Mollusc	Mud snail	Global	Marine	Low/Unk	GISD
Imogine necopinata	Platyhelminth	Flatworm	EU	Marine	Low/Unk	AquaNIS
Incisocalliope aestuarius	Crustacean	Amphipod	EU	Marine	Low/Unk	AquaNIS, EASIN
Indothais lacera	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Indothais sacellum	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Iolaea neofelixoides	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Iphigenella shablensis	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Ischyrocerus commensalis	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Isochaetides michaelseni	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Isocypris beauchampi cicatricosa	Crustacean	Ostracod	EU	Freshwater	Low/Unk	EASIN
Isognomon radiatus	Mollusc	Oyster	EU	Marine	Low/Unk	AquaNIS, EASIN
Isolda pulchella	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Ixa monodi	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Jaera istri	Crustacean	Isopod	EU	Freshwater	Low/Unk	AquaNIS, EASIN
Jaera sarsi	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Janua (Dexiospira) marioni	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Jassa marmorata	Crustacean	Amphipod	EU	Marine	Low/Unk	AquaNIS, EASIN
Jasus lalandii	Crustacean	Lobster	EU	Marine	Low/Unk	AquaNIS
Jellyella tuberculata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Kantiella enigmatica	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Katamysis warpachowskyi	Crustacean	Shrimp	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Kellicottia bostoniensis	Eumetazoan	Rotifer	EU	Freshwater	Low/Unk	EASIN
Khawia sinensis	Platyhelminth	Cestode	EU	Freshwater	Low/Unk	EASIN
Kirchenpaueria halecioides	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	AquaNIS
Koinostylochus ostreophagus	Platyhelminth	Platyhelminth	EU	Marine	Low/Unk	EASIN
Labidocera detruncata	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Labidocera madurae	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Labidocera orsinii	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Labidocera pavo	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Laonice norgensis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Laonome calida	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Laonome elegans	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Laonome triangularis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Laternula anatina	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Latopilumnus malardi	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Lecithochirium magnicaudatum	Platyhelminth	Flatworm	EU	Marine	Low/Unk	EASIN
Leiochrides australis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Leodice antennata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Leonnates decipiens	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Leonnates indicus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Leonnates persicus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Lepidonotus tenuisetosus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Leptochela (Leptochela) aculeocaudata	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Leptochela (Leptochela) pugnax	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Lernaea cyprinacea	Annelid	Anchor worm	EU	Freshwater	High	EASIN
Lernanthropus callionymicola	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Leucotina natalensis	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Libinia dubia	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Licornia jolloisii	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Lienardia mighelsi	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Ligia italica	Crustacean	Isopod	EU	Marine	Low/Unk	AquaNIS
Ligia oceanica	Crustacean	Isopod	EU	Marine	Low/Unk	AquaNIS
Ligophorus kaohsianghsieni	Platyhelminth	Monogenean	EU	Marine	Low/Unk	EASIN
Limnodrilus cervix	Annelid	Tubificid worm	EU	Freshwater	Low/Unk	AquaNIS
Limnodrilus maumeensis	Annelid	Tubificid worm	EU	Freshwater	Low/Unk	EASIN
Limnomysis benedeni	Crustacean	Shrimp	EU	Freshwater and Oligohaline	High	AquaNIS, EASIN
Limnoperna fortunei	Mollusc	Mussel	Global	Marine	Low/Unk	GISD
Limnoperna securis	Mollusc	Mussel	EU	Marine	High	AquaNIS, EASIN
Limnoria quadripunctata	Crustacean	Isopod	EU	Marine	Low/Unk	AquaNIS
Limnoria tripunctata	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Limopsis multistriata	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Limulus polyphemus	Crustacean	Horseshoe crab	EU	Marine	Low/Unk	AquaNIS, EASIN
Linopherus canariensis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Lioberus ligneus	Mollusc	Mussel	EU	Marine	Low/Unk	EASIN
Lithoglyphus naticoides	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	AquaNIS, EASIN

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Lithophaga hanleyana	Mollusc	Mussel	EU	Marine	Low/Unk	EASIN
Littorina littorea	Mollusc	Sea snail	Global	Marine	Low/Unk	GISD
Littorina saxatilis	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Lophopodella carteri	Bryozoan	Bryozoan	EU	Freshwater	Low/Unk	EASIN
Lucifer hanseni	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Lumbrinerides neogesae	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Lumbrineris acutifrons	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Lumbrineris perkinsi	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Lumbrineris zatsepini	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Lymnaea cubensis	Mollusc	freshwater snail	EU	Freshwater	Low/Unk	EASIN
Lysidice collaris	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Lysmata kempi	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Macromedaeus voeltzkowi	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Macrophthalmus indicus	Crustacean	Decapod	EU	Marine	Low/Unk	EASIN
Macrorhynchia philippina	Cnidarian	Hydroid	EU	Marine	High	EASIN
Mactra lilacea	Mollusc	Equivalve	EU	Marine	Low/Unk	EASIN
Mactra olorina	Mollusc	Equivalve	EU	Marine	Low/Unk	EASIN
Maeotias marginata	Cnidarian	Jellyfish	EU	Marine	Low/Unk	AquaNIS, EASIN
Malleus regula	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Marenzelleria arctia	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Marenzelleria neglecta	Annelid	Polychete worm	EU	Marine	High	AquaNIS, EASIN
Marenzelleria viridis	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Margaritana margaritifera	Mollusc	Mussel	EU	Freshwater	Low/Unk	EASIN
Marginella glabella	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Marivagia stellata	Cnidarian	Jellyfish	EU	Marine	Low/Unk	EASIN
Marphysa sanguinea	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Marsupenaeus japonicus	Crustacean	Shrimp	EU	Marine	Low/Unk	AquaNIS
Marteilia refringens	Rhizarian	Rhizarian parasite	EU	Marine	Low/Unk	AquaNIS
Martesia striata	Mollusc	Bivalve	EU	Marine	Low/Unk	AquaNIS
Matuta victor	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Megabalanus coccopoma	Crustacean	Barnacle	EU	Marine	Low/Unk	AquaNIS, EASIN
Megabalanus tintinnabulum	Crustacean	Barnacle	EU	Marine	Low/Unk	AquaNIS, EASIN
Megalomma claparedei	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Melanoides tuberculatus	Mollusc	Freshwater snail	EU	Freshwater	HIGH	EASIN
Melibe viridis	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Melita nitida	Crustacean	Amphipod	EU	Marine	Low/Unk	AquaNIS, EASIN
Melithaea erythraea	Cnidarian	Coral	EU	Marine	Low/Unk	EASIN
Menaethius monoceros	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Menetus dilatatus	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Mercenaria mercenaria	Mollusc	Clam	EU	Marine	High	AquaNIS, EASIN
Metacalanus acutioperculum	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Metapenaeopsis aegyptia	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Metapenaeopsis mogiensis consobrina	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Metapenaeus affinis	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Metapenaeus monoceros	Crustacean	Shrimp	EU	Marine	High	EASIN
Metapenaeus stebbingi	Crustacean	Shrimp	EU	Marine	High	EASIN
Metasychis gotoi	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Metaxia bacillum	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Micippa thalia	Crustacean	Decapod	EU	Marine	Low/Unk	EASIN
Microphthalmus similis	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Microporella browni	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Microporella ciliata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Microporella genisii	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Microporella harmeri	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Micruropus possolskii	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Mimachlamys sanguinea	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Mitrapus oblongus	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Mitrella psilla	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Mitrocomium medusiferum	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Mizuhopecten yessoensis	Mollusc	Scallop	EU	Marine	Low/Unk	AquaNIS, EASIN
Mnemiopsis leidyi	Cnidarian	Jellyfish	Global	Marine and Oligohaline	High	GISD, AquaNIS, EASIN
Modiolus auriculatus	Mollusc	Mussel	EU	Marine	Low/Unk	EASIN
Moerisia carine	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Moerisia inkermanica	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	AquaNIS, EASIN
Moina affinis	Crustacean	Waterflea	EU	Freshwater	Low/Unk	EASIN
Moina weismanni	Crustacean	Waterflea	EU	Freshwater	Low/Unk	EASIN
Monilicaecum ventricosum	Platyhelminth	Trematode	EU	Marine	Low/Unk	EASIN
Monobothrium wageneri	Platyhelminth	Tapeworm	EU	Freshwater	Low/Unk	EASIN
Monocorophium acherusicum	Crustacean	Amphipod	EU	Freshwater and Marine	Low/Unk	AquaNIS
Monocorophium insidiosum	Crustacean	Amphipod	EU	Freshwater and Marine	Low/Unk	AquaNIS
Monocorophium sextonae	Crustacean	Amphipod	EU	Freshwater and Marine	Low/Unk	AquaNIS
Monocorophium uenoi	Crustacean	Amphipod	EU	Freshwater and Marine	Low/Unk	AquaNIS, EASIN
Monophorus perversus	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS

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Monotygma watsoni	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Muceddina multispinosa	Crustacean	Copepod	EU	Marine and Oligohaline	Low/Unk	AquaNIS
Murchisonella columna	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Murex (Murex) forskoehlii	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Murex brandardis	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS
Musculista senhousia	Mollusc	Mussel	Global	Marine	Low/Unk	GISD, AquaNIS
Musculium transversum	Mollusc	Bivalve	EU	Freshwater	Low/Unk	EASIN
Mya arenaria	Mollusc	Clam	Global	Freshwater, Marine and Oligohaline	High	GISD, AquaNIS, EASIN
Mycale (Carmia) micracanthoxea	Poriferan	Sponge	EU	Marine	Low/Unk	AquaNIS
Mycale (Carmia) senegalensis	Poriferan	Sponge	EU	Marine	Low/Unk	AquaNIS
Mycale grandis	Poriferan	Sponge	Global	Marine	Low/Unk	GISD
Myicola ostreae	Mollusc	Bivalve	EU	Marine	High	AquaNIS, EASIN
Mymarothecium viatorum	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Myra subgranulata	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Mysis relicta	Crustacean	Shrimp	EU	Freshwater	Low/Unk	EASIN
Mytilicola intestinalis	Annelid	Annelid	EU	Marine	Low/Unk	AquaNIS
Mytilicola orientalis	Annelid	Annelid	EU	Marine	High	AquaNIS, EASIN
Mytilopsis leucophaeata	Mollusc	Mussel	Global	Marine and Oligohaline	Low/Unk	GISD, AquaNIS, EASIN
Mytilopsis sallei	Mollusc	Mussel	Global	Marine	High	GISD, EASIN
Mytilus edulis	Mollusc	Mussel	EU	Marine	High	AquaNIS, EASIN
Mytilus galloprovincialis	Mollusc	Mussel	Global	Marine	Low/Unk	GISD
Myxobolus artus	Cnidarian	Myxozoan	EU	Freshwater	Low/Unk	EASIN
Naineris setosa	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Nanostrea fluctigera	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Nassa situla	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Nassarius arcularia plicatus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Nassarius concinnus	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS, EASIN
Nassarius mutabilis	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS
Nassarius stolatus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Neanthes agulhana	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Neanthes willeyi	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Necora puber	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Nemopsis bachei	Cnidarian	Jellyfish	EU	Marine	Low/Unk	AquaNIS, EASIN
Neodexiospira brasiliensis	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Neodexiospira steueri	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Neoergasilus japonicus	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Neomysis integer	Crustacean	Shrimp	EU	Marine and Oligohaline	Low/Unk	EASIN
Neopseudocapitella brasiliensis	Annelid	Annelid	EU	Marine	Low/Unk	EASIN
Nephasoma (Nephasoma) eremita	Sipunculan	Sipunculan	EU	Marine	Low/Unk	EASIN
Nephtys ciliata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Neptunea arthritica	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Nereis (Nereis) gilchristi	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Nereis jacksoni	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Nereis persica	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Nerita sanguinolenta	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Nikoides sibogae	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Nothobomolochus fradei	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Notocochlis gualteriana	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Notomastus aberans	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Notomastus mossambicus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Notopus dorsipes	Crustacean	crab	EU	Marine	Low/Unk	EASIN
Novafabricia infratorquata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Obesogammarus crassus	Crustacean	Amphipod	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Obesogammarus obesus	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Ocenebra erinaceus	Mollusc	Sea snail	EU	Marine	Low/Unk	AquaNIS
Ocenebra inornata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Ochetostoma erythrogrammon	Echiuran	Echiuran	EU	Marine	Low/Unk	EASIN
Ochlerotatus japonicus japonicus	Insect	Mosquito	Global	Terrestrial and Freshwater	Low/Unk	GISD
Octopus cyanea	Mollusc	Octopus	EU	Marine	Low/Unk	EASIN
Oculina patagonica	Cnidarian	Coral	EU	Marine	High	EASIN
Odontodactylus scyllarus	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Odostomia lorioli	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Oenone fulgida	Annelid	Bristle worm	EU	Marine	Low/Unk	EASIN
Ogyrides mjoebergi	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Oithona davisae	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Oithona plumifera	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Oithona setigera	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Olindias singularis	Cnidarian	Jellyfish	EU	Marine	Low/Unk	EASIN
Onchocerca gutturosa	Nematode	Nematode	EU	Terrestrial and Freshwater	Low/Unk	EASIN
Onchocleidus dispar	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Onisimus sextoni	Crustacean	Amphipod	EU	Marine	Low/Unk	AquaNIS
Ophiactis macrolepidota	Echinoderm	Brittle star	EU	Marine	Low/Unk	EASIN
Ophiactis savignyi	Echinoderm	Brittle star	EU	Marine	Low/Unk	EASIN
Ophiocoma scolopendrina	Echinoderm	Brittle star	EU	Marine	Low/Unk	EASIN
Ophryotrocha diadema	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Ophryotrocha japonica	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Orchestia cavimana	Crustacean	Amphipod	EU	Marine	Low/Unk	AquaNIS
Orconectes immunis	Crustacean	Crayfish	EU	Freshwater	Low/Unk	EASIN
Orconectes limosus	Crustacean	Crayfish	EU	Freshwater	High	AquaNIS, EASIN
Orconectes rusticus	Crustacean	Crayfish	Global	Freshwater	Low/Unk	GISD, EASIN
Orconectes virilis	Crustacean	Crayfish	Global	Freshwater	High	GISD, AquaNIS, EASIN
Oscilla galilae	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Oscilla jocosa	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Ostrea angasi	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Ostrea chilensis	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Ostrea denselamellosa	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Ostrea edulis	Mollusc	Oyster	Global	Marine	Low/Unk	GISD
Ostrea equestris	Mollusc	Oyster	EU	Marine	Low/Unk	AquaNIS, EASIN
Ostrea puelchana	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Owenia borealis	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Oxynoe viridis	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Pachycordyle navis	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	AquaNIS, EASIN
Pacifastacus leniusculus	Crustacean	Crayfish	Global	Freshwater	High	GISD, EASIN
Pacificincola perforata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Palaemon elegans	Crustacean	Shrimp	EU	Marine	Low/Unk	AquaNIS
Palaemon macrodactylus	Crustacean	Shrimp	EU	Marine and Oligohaline	High	AquaNIS, EASIN
Palaemonella rotumana	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Palmadusta lentiginosa	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Palola valida	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Panulirus guttatus	Crustacean	Lobster	EU	Marine	Low/Unk	AquaNIS
Panulirus ornatus	Crustacean	Lobster	EU	Marine	Low/Unk	EASIN
Paphia textile	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Paracalanus indicus	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Paracaprella pusilla	Crustacean	Shrimp	EU	Marine	Low/Unk	AquaNIS, EASIN
Paracartia grani	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Paracerceis sculpta	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Paracytaeis octona	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Paradella dianae	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Paradiplozoon marinae	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Paradyte crinoidicola	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Paraehlersia weissmanniodes	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Paraergasilus longidigitus	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Paralaeospira malardi	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Paraleucilla magna	Poriferan	Sponge	EU	Marine	Low/Unk	AquaNIS, EASIN
Paralithodes camtschaticus	Crustacean	Crab	EU	Marine	High	AquaNIS, EASIN
Paramphiascella vararensis	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Paramysis (Mesomysis) intermedia	Crustacean	Shrimp	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Paramysis (Serrapalpisis) lacustris	Crustacean	Shrimp	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Paramysis baeri	Crustacean	Shrimp	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Paramysis ullskyi	Crustacean	Shrimp	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Paranais botniensis	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS
Paranais frici	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Paranthura japonica	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Paraonides nordica	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Parasmittina egyptiaca	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Parasmittina protecta	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS, EASIN
Parasmittina serruloides	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Parasmittina spondylicola	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Paratenuisentis ambiguus	Acanthocephalan	Eoacanthocephalan	EU	Freshwater	Low/Unk	AquaNIS, EASIN
Parvocalanus crassirostris	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Parvocalanus elegans	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Parvocalanus latus	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Patelloida saccharina	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Pectinatella magnifica	Bryozoan	Bryozoan	EU	Freshwater	Low/Unk	EASIN
Pellucidhaptor pricei	Platyhelminth	Platyhelminth	EU	Freshwater	Low/Unk	EASIN
Penaeus aztecus	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Penaeus hathor	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Penaeus japonicus	Crustacean	Shrimp	EU	Marine	High	EASIN
Penaeus merguiensis	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Penaeus semisulcatus	Crustacean	Shrimp	EU	Marine	High	EASIN
Penaeus subtilis	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Penilia avirostris	Crustacean	Water flea	EU	Marine	Low/Unk	AquaNIS

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Percnon gibbesi	Crustacean	Crab	EU	Marine	High	AquaNIS, EASIN
Perinereis aibuhitensis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Perinereis nuntia	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Perkinsyllis augeneri	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Perna perna	Mollusc	Mussel	Global	Marine	High	GISD
Perna viridis	Mollusc	Mussel	Global	Marine	High	GISD
Petricola fabagella	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Petricolaria pholadiformis	Mollusc	Clam	EU	Marine	High	AquaNIS, EASIN
Phagocata woodworthi	Platyhelminth	Platyhelminth	EU	Freshwater	Low/Unk	EASIN
Phascolion (Isomya) convestitum	Sipunculan	Sipunculan	EU	Marine	Low/Unk	EASIN
Phascolosoma (Phascolosoma) scolops	Sipunculan	Sipunculan	EU	Marine	Low/Unk	EASIN
Philinopsis speciosa	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Photis lamellifera	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Phyllodoce longifrons	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Phyllorhiza punctata	Cnidarian	Jellyfish	Global	Marine	High	GISD, EASIN
Physella acuta	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Physella gyrina	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Physella heterostropha	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Physella integra	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Pileolaria berkeleyana	Annelid	Polychete worm	EU	Marine	High	EASIN
Pileolaria militaris	Annelid	Polychete worm	EU	Marine	High	AquaNIS
Pilumnoides inglei	Crustacean	Crab	EU	Marine	Low/Unk	AquaNIS, EASIN
Pilumnopeus vauquelini	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Pilumnus minutus	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Pilumnus spinifer	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Pinctada imbricata radiata	Mollusc	Oyster	EU	Marine	High	AquaNIS, EASIN
Pinctada margaritifera	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Piscicola haranti	Annelid	Annelid	EU	Freshwater	Low/Unk	EASIN
Pisione guanche	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Pista unibranchia	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Plagusia squamosa	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Planaxis savignyi	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Planorbarius corneus	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Planostrea pestigris	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Platorchestia platensis	Crustacean	Amphipod	EU	Terrestrial and Marine	High	AquaNIS, EASIN
Platyscelus armatus	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Pleurobranchus forskalii	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Plicatula plicata	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Plocamopherus ocellatus	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Plocamopherus tilesii	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Podarkeopsis capensis	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Pollia dorbignyi	Mollusc	Whelk	EU	Marine	Low/Unk	AquaNIS
Pollicipes pollicipes	Crustacean	Barnacle	EU	Marine	Low/Unk	AquaNIS
Polycera hedgpethi	Mollusc	Opisthobranch	EU	Marine	Low/Unk	EASIN
Polycerella emertoni	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Polycirrus twisti	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Polydora colonia	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Polydora cornuta	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Polydora hoplura	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Polypodium hydriforme	Cnidarian	Cnidarian parasite	EU	Freshwater	High	EASIN
Pomacea canaliculata	Mollusc	Freshwater snail	Global	Freshwater	Low/Unk	GISD
Pomacea insularum	Mollusc	Freshwater snail	Global	Freshwater	Low/Unk	GISD
Pontogammarus aestuarius	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Pontogammarus robustoides	Crustacean	Amphipod	EU	Freshwater and Oligohaline	High	AquaNIS, EASIN
Porcellidium ovatum	Crustacean	Copepod	EU	Marine	Low/Unk	AquaNIS
Porcelloides tenuicaudus	Crustacean	Crab	EU	Marine	High	EASIN
Portunus (Portunus) segnis	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Potamocorbula amurensis	Mollusc	Clam	Global	Marine	Low/Unk	GISD
Potamopyrgus antipodarum	Mollusc	Mud snail	Global	Freshwater, Marine and Oligohaline	Low/Unk	GISD, AquaNIS, EASIN
Potamothrix bavaricus	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Potamothrix bedoti	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Potamothrix heuscheri	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Potamothrix moldaviensis	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	AquaNIS, EASIN
Potamothrix vejdovsky	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Potamothrix vejdovskyi	Annelid	Annelid	EU	Marine	Low/Unk	AquaNIS, EASIN
Prionospio aucklandica	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Prionospio depauperata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Prionospio paucipinnulata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Prionospio pulchra	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Prionospio pygmaeus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Prionospio saccifera	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Prionospio sexoculata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Proameira simplex	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Proasellus coxalis	Crustacean	Isopod	EU	Freshwater	Low/Unk	EASIN
Proasellus meridianus	Crustacean	Isopod	EU	Freshwater	Low/Unk	EASIN
Procambarus acutus	Crustacean	Crayfish	EU	Freshwater	Low/Unk	EASIN
Procambarus clarkii	Crustacean	Crayfish	Global	Freshwater	High	GISD, EASIN
Procambarus fallax f. virginalis	Crustacean	Crayfish	EU	Freshwater	Low/Unk	AquaNIS
Proceraea cornuta	Annelid	Annelid	EU	Marine	Low/Unk	AquaNIS, EASIN
Prosphaerosyllis longipapillata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Proteocephalus osculatus	Platyhelminth	Platyhelminth	EU	Freshwater	Low/Unk	EASIN
Protoreaster nodosus	Echinoderm	Sea star	EU	Marine	Low/Unk	EASIN
Psammoryctides moravicus	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Psammotreta praerupta	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Pseudobacciger harengulae	Platyhelminth	Digenean	EU	Marine	High	AquaNIS, EASIN
Pseudochama corbierei	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Pseudocuma (Stenocuma) graciloides	Crustacean	Copepod	EU	Marine	Low/Unk	AquaNIS, EASIN
Pseudocuma cercaroides	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Pseudodactylogyrus anguillae	Platyhelminth	Monogenean	EU	Freshwater, Marine and Oligohaline	High	AquaNIS, EASIN
Pseudodactylogyrus bini	Platyhelminth	Monogenean	EU	Freshwater, Marine and Oligohaline	High	AquaNIS, EASIN
Pseudodiaptomus inopinus	Crustacean	Copepod	Global	Marine	Low/Unk	GISD
Pseudodiaptomus marinus	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Pseudominolia nedyma	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Pseudomyicola spinosus	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Pseudonereis anomala	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Pseudopolydora paucibranchiata	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Pseudorhaphitoma iodolabiata	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Pseudostylochus ostreophagus	Platyhelminth	Platyhelminth	EU	Marine	Low/Unk	AquaNIS
Pseudosuccinea columella	Mollusc	Freshwaer snail	EU	Freshwater	Low/Unk	EASIN
Psiloteredo megotara	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS
Pteria hirundo	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Pteropurpura (Ocinebrellus) inornata	Mollusc	Oyster drill	EU	Marine	Low/Unk	AquaNIS
Ptilohyale littoralis	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Puellina innominata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Purpuradusta gracilis notata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Pyrgulina pirinthella	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Pyrunculus fourierii	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Rangia cuneata	Mollusc	Clam	Global	Marine	Low/Unk	GISD, AquaNIS, EASIN
Rapana venosa	Mollusc	Whelk	Global	Marine	High	GISD, AquaNIS, EASIN
Reptadeonella violacea	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Retusa desgenettii	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Rhabdosoma whitei	Crustacean	Amphipod	EU	Marine	Low/Unk	EASIN
Rhinoclavis kochi	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Rhinoclavis sinensis	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Rhithropanopeus harrisii	Crustacean	Crab	Global	Marine and Oligohaline	High	GISD, AquaNIS, EASIN
Rhizogeton nudus	Cnidarian	Cnidarian	EU	Marine	Low/Unk	AquaNIS
Rhopilema nomadica	Cnidarian	Jellyfish	EU	Marine	High	EASIN
Rhynchozoon larreyi	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Rimapenaeus similis	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Rissoina ambigua	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Rissoina bertholleti	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Rissoina spirata	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Robertgurneya rostrata	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Ruditapes decussatus	Mollusc	Bivalve	EU	Marine	Low/Unk	AquaNIS
Ruditapes philippinarum	Mollusc	Bivalve	EU	Marine	Low/Unk	AquaNIS
Sabella spallanzanii	Annelid	Polychete worm	Global	Marine	Low/Unk	GISD, AquaNIS, EASIN
Saccostrea cucullata	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Saccostrea glomerata	Mollusc	Oyster	EU	Marine	Low/Unk	EASIN
Saduria entomon	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Sanguinicola inermis	Platyhelminth	Blood fluke	EU	Freshwater	Low/Unk	EASIN
Saron marmoratus	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Sarsamphiascus tenuiremis	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Scherocumella gurneyi	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Schizoporella errata	Bryozoan	Bryozoan	Global	Marine	Low/Unk	GISD, AquaNIS, EASIN
Schizoporella japonica	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Schizoporella pungens	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Schizoporella unicornis	Bryozoan	Bryozoan	Global	Marine	Low/Unk	GISD, AquaNIS

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Schizoretepora hassi	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Scolecithrix sp.	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Scolelepis korsuni	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Scolionema suvaense	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Scorpiodinipora costulata	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Scottolana longipes	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Scruparia ambigua	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Scrupocellaria bertholetti	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Scyllarus caparti	Crustacean	Lobster	EU	Marine	Low/Unk	EASIN
Semisalsa dalmatica	Mollusc	Gastropod	EU	Freshwater	Low/Unk	EASIN
Sepia pharaonis	Mollusc	Cuttlefish	EU	Marine	Low/Unk	EASIN
Sepioteuthis lessoniana	Mollusc	Squid	EU	Marine	Low/Unk	EASIN
Septifer cumingii	Mollusc	Mussel	EU	Marine	Low/Unk	EASIN
Sertularia marginata	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Sertularia tongensis	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Sigambra parva	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Sigambra tentaculata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Simocephalus hejlongjiangensis	Crustacean	Water flea	EU	Freshwater	Low/Unk	EASIN
Sinanodonta woodiana	Mollusc	Clam	EU	Freshwater	High	EASIN
Sinelobus stanfordi	Crustacean	Tanaid	EU	Marine	Low/Unk	AquaNIS
Siphonaria crenata	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Siphonaria pectinata	Mollusc	Gastropod	EU	Marine	Low/Unk	EASIN
Sirpus monodi	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Skistodiaptomus pallidus	Crustacean	Copepod	EU	Freshwater	Low/Unk	AquaNIS, EASIN
Smaragdia souverbiana	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Smittina nitidissima	Bryozoan	Bryozoan	EU	Marine	Low/Unk	EASIN
Smittoidea prolifica	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS, EASIN
Solenocera crassicornis	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Sphaerocoryne bedoti	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Sphaeroma quoianum (=S. quoyanum)	Crustacean	Isopod	Global	Marine	Low/Unk	GISD
Sphaeroma serratum	Crustacean	Isopod	EU	Marine	Low/Unk	AquaNIS
Sphaeroma walkeri	Crustacean	Isopod	EU	Marine	Low/Unk	EASIN
Sphaerozius nitidus	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Sphenia rueppelli	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Spiophanes algidus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Spirobranchus kraussii	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Spirobranchus tetraceros	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Spirorbis marioni	Annelid	Polychete worm	EU	Marine	High	EASIN
Spisula solidissima	Mollusc	Clam	EU	Marine	Low/Unk	AquaNIS
Spondylus nicobaricus	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Spondylus spinosus	Mollusc	Bivalve	EU	Marine	High	EASIN
Sternaspis scutata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Sternodromia spinirostris	Crustacean	Decapod	EU	Marine	Low/Unk	EASIN
Stomatella impertusa	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Stomolophus meleagris	Cnidarian	Jellyfish	EU	Marine	Low/Unk	EASIN
Strandesia spinulosa	Crustacean	Ostracod	EU	Freshwater	Low/Unk	EASIN
Streblosoma comatus	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Streblospio benedicti	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Streblospio gynobranchiata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Stygobromus ambulans	Crustacean	Amphipod	EU	Freshwater	Low/Unk	EASIN
Stylarioides grubei	Annelid	Polychete worm	EU	Marine	Low/Unk	
Stylochus flevensis	Platyhelminth	Flatworm	EU	Marine	Low/Unk	AquaNIS
Sulculeolaria turgida	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	
Sycon scaldiense	Poriferan	Sponge	EU	Marine	Low/Unk	AquaNIS
Syllis bella	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Syllis hyllebergi	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Syllis pectinans	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Synaptula reciprocans	Echinoderm	Sea cucumber	EU	Marine	Low/Unk	EASIN
Synidotea laevidorsalis	Crustacean	Isopod	EU	Marine and Oligohaline	Low/Unk	EASIN
Synidotea laticauda	Crustacean	Isopod	EU	Marine	Low/Unk	AquaNIS, EASIN
Syphonota geographica	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Syrnola cinctella	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Syrnola fasciata	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Syrnola lendix	Mollusc	Sea slug	EU	Marine	Low/Unk	EASIN
Taeniacanthus lagocephali	Crustacean	Copepod	EU	Marine	Low/Unk	AquaNIS, EASIN
Tanycypris pellucida	Crustacean	Ostracod	EU	Freshwater	Low/Unk	EASIN
Tegillarca granosa	Mollusc	Cockle	EU	Marine	Low/Unk	EASIN
Tellina compressa	Mollusc	Bivalve	EU	Marine	Low/Unk	AquaNIS
Tellina flacca	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Tellina valtonis	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Telmatogeton japonicus	Insect	Midge	EU	Terrestrial and Marine	High	AquaNIS, EASIN
Terebella lapidaria	Annelid	Polychete worm	EU	Marine	Low/Unk	AquaNIS, EASIN
Teredo bartschi	Mollusc	Bivalve	EU	Marine	Low/Unk	AquaNIS, EASIN
Teredo navalis	Mollusc	Clam	EU	Marine	Low/Unk	AquaNIS
Teredothyra dominicensis	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Tessepora atlanticum	Crustacean	Isopod	EU	Marine	Low/Unk	AquaNIS

Species	Taxon	Organism Type	Database range	Environment	Impact	Reference database
Tetraclita squamosa rufotinta	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Tetrancistrum polymorphum	Platyhelminth	Monogenean	EU	Marine	Low/Unk	EASIN
Tetrancistrum strophosolenus	Platyhelminth	Monogenean	EU	Marine	Low/Unk	EASIN
Tetrancistrum suezicum	Platyhelminth	Monogenean	EU	Marine	Low/Unk	EASIN
Tetrorchis erythrogaster	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Thalamita gloriensis	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Thalamita indistincta	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Theodoxus danubialis	Mollusc	Freshwaer snail	EU	Freshwater	Low/Unk	EASIN
Theodoxus fluviatilis	Mollusc	Freshwaer snail	EU	Freshwater	Low/Unk	EASIN
Theodoxus transversalis	Mollusc	Freshwaer snail	EU	Freshwater	Low/Unk	EASIN
Theora lubrica	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Tiaropsis multicirrata	Cnidarian	Jellyfish	EU	Marine	Low/Unk	EASIN
Timarete caribous	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Timarete dasylophius	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Timarete punctata	Annelid	Polychete worm	EU	Marine	Low/Unk	EASIN
Timoclea marica	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Tonicia atrata	Mollusc	Chiton	EU	Marine	Low/Unk	EASIN
Tracheliastes maculatus	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Tracheliastes polycolpus	Crustacean	Copepod	EU	Freshwater	Low/Unk	EASIN
Trachysalambria palaestinensis	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Trapezium oblongum	Mollusc	Bivalve	EU	Marine	Low/Unk	EASIN
Tremoctopus gracilis	Mollusc	Octopus	EU	Marine	Low/Unk	EASIN
Tricellaria inopinata	Bryozoan	Bryozoan	EU	Marine	High	AquaNIS, EASIN
Trichydra pudica	Cnidarian	Hydrozoan	EU	Marine	Low/Unk	EASIN
Triconia hawii	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Triconia minuta	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Triconia rufa	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Triconia umerus	Crustacean	Copepod	EU	Marine	Low/Unk	EASIN
Trivirostra triticum	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Trochus erithreus	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Tubastraea coccinea	Cnidarian	Coral	Global	Marine	Low/Unk	GISD
Tubifex newaensis	Annelid	Annelid	EU	Freshwater and Oligohaline	Low/Unk	EASIN
Tubificoides heterochaetus	Annelid	Annelid	EU	Marine	Low/Unk	AquaNIS
Tubificoides pseudogaster	Annelid	Annelid	EU	Marine	Low/Unk	AquaNIS, EASIN
Tulleariocaris neglecta	Crustacean	Shrimp	EU	Marine	Low/Unk	AquaNIS
Turbonilla edgarii	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Unio mancus	Mollusc	Mussel	EU	Freshwater	Low/Unk	EASIN
Urnatella gracilis	Bryozoan	Bryozoan	EU	Freshwater	Low/Unk	EASIN
Urocaridella pulchella	Crustacean	Shrimp	EU	Marine	Low/Unk	EASIN
Urocleidus dispar	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Urocleidus principalis	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Urocleidus similis	Platyhelminth	Monogenean	EU	Freshwater	Low/Unk	EASIN
Urosalpinx cinerea	Mollusc	Sea snail	Global	Marine	High	GISD, AquaNIS, EASIN
Venerupis philippinarum	Mollusc	Clam	EU	Marine	High	EASIN
Ventomnestia girardi	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Vexillum (Pusia) depexum	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Victorella pavida	Bryozoan	Bryozoan	EU	Marine and Oligohaline	Low/Unk	AquaNIS
Viviparus acerosus	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Viviparus viviparus	Mollusc	Freshwater snail	EU	Freshwater	Low/Unk	EASIN
Voorwindia tiberiana	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Watersipora subtorquata	Bryozoan	Bryozoan	Global	Marine	Low/Unk	GISD, AquaNIS
Wlassicsia pannonica	Crustacean	Branchiopod	EU	Freshwater	Low/Unk	EASIN
Xanthias lamarckii	Crustacean	Crab	EU	Marine	Low/Unk	EASIN
Xironogiton instabilis	Annelid	Annelid	EU	Freshwater	Low/Unk	EASIN
Zafra savignyi	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Zafra selasphora	Mollusc	Sea snail	EU	Marine	Low/Unk	EASIN
Zoobotryon verticillatum	Bryozoan	Bryozoan	EU	Marine	Low/Unk	AquaNIS
Zygochlamys patagonica	Mollusc	Scallop	EU	Marine	Low/Unk	EASIN

Appendix Table 1.2: Global database for invasive species (GISD), detailing priority invasive aquatic invertebrates (IAIs) across the globe, by country.

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
Afghanistan	*none*	-
Albania	*Aedes albopictus*	Insect
Algeria	*none*	-
Andorra	*none*	-
Angola	*none*	-
Antigua and Barbuda	*Aedes aegypti*	Insect
Argentina	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Bugula neritina	Bryozoan
	Corbicula fluminea	Clam
	Ficopomatus enigmaticus	Annelid
	Limnoperna fortunei	Mussel
	Alitta succinea	Annelid
Armenia	*none*	-
Aruba	*Aedes aegypti*	Insect
	Tubastraea coccinea	Coral
Australia	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Alitta succinea	Annelid
	Asterias amurensis	Sea star
	Bugula neritina	Bryozoan
	Carcinus maenas	Crab
	Crassostrea gigas	Oyster
	Musculista senhousia	Mussel
	Mya arenaria	Clam
	Mytilopsis sallei	Mussel
	Mytilus galloprovincialis	Mussel
	Ostrea edulis	Oyster
	Perna viridis	Mussel
	Phyllorhiza punctata	Jellyfish
	Potamopyrgus antipodarum	Mud snail
	Sabella spallanzanii	Annelid
	Schizoporella errata	Bryozoan
	Schizoporella unicornis	Bryozoan
	Watersipora subtorquata	Bryozoan
	Acanthaster planci	Sea Star
	Ceratostoma inornatum	Sea snail
	Mycale grandis	Sponge
	Tubastraea coccinea	Coral
Austria	*Dreissena polymorpha*	Mussel
	Eriocheir sinensis	Crab
	Pacifastacus leniusculus	Crayfish
	Potamopyrgus antipodarum	Mud snail
Azerbaijan	*Mnemiopsis leidyi*	Comb jellyfish
Bahamas, The	*Aedes aegypti*	Insect
	Tubastraea coccinea	Coral
Bahrain	*none*	-
Bangladesh	*none*	-
Barbados	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
Belarus	*Dreissena polymorpha*	Mussel
	Potamopyrgus antipodarum	Mud snail
Belgium	*Aedes albopictus*	Insect
	Bugula neritina	Bryozoan
	Corbicula fluminea	Clam
	Crassostrea gigas	Oyster
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mytilopsis leucophaeata	Mussel
	Ochlerotatus japonicus japonicus	Insect
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Rangia cuneata	Clam
	Schizoporella unicornis	Bryozoan
Belize	*Aedes aegypti*	Insect
	Procambarus clarkii	Crayfish
	Tubastraea coccinea	Coral
Benin	*none*	-
Bhutan	*none*	-
Bolivia	*Aedes aegypti*	Insect
	Aedes albopictus	Insect

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
Bosnia and Herzegovina	*Aedes albopictus*	Insect
Botswana	*none*	-
Brazil	*Aedes aegypti*	Insect
	Bugula neritina	Bryozoan
	Charybdis hellerii	Crab
	Daphnia lumholtzi	Water flea
	Limnoperna fortunei	Mussel
	Mytilopsis leucophaeata	Mussel
	Phyllorhiza punctata	Jellyfish
	Procambarus clarkii	Crayfish
	Schizoporella errata	Bryozoan
	Schizoporella unicornis	Bryozoan
	Tubastraea coccinea	Coral
	Alitta succinea	Annelid
	Watersipora subtorquata	Bryozoan
Brunei	*none*	-
Bulgaria	*Mnemiopsis leidyi*	Comb jellyfish
	Rhithropanopeus harrisii	Mud crab
Burkina Faso	*none*	-
Burma (Myanmar)	*Aedes aegypti*	Insect
	Tubastraea coccinea	Coral
Burundi	*none*	-
Cambodia	*Aedes aegypti*	Insect
	Pomacea canaliculata	Freshwater snail
Cameroon	*Aedes albopictus*	Insect
Canada	*Batillaria attramentaria*	Sea snail
	Bellamya chinensis	Freshwater snail
	Bythotrephes longimanus	Water flea
	Carcinus maenas	Crab
	Ceratostoma inornatum	Sea snail
	Crassostrea gigas	Oyster
	Daphnia lumholtzi	Water flea
	Dreissena bugensis	Mussel
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Ilyanassa obsoleta	Mud snail
	Littorina littorea	Sea snail
	Musculista senhousia	Mussel
	Mya arenaria	Clam
	Mytilus galloprovincialis	Mussel
	Ochlerotatus japonicus japonicus	Insect
	Orconectes rusticus	Crayfish
	Orconectes virilis	Crayfish
	Ostrea edulis	Oyster
	Potamopyrgus antipodarum	Mud snail
	Schizoporella unicornis	Bryozoan
	Urosalpinx cinerea	Sea snail
	Alitta succinea	Annelid
	Boonea bisuturalis	Sea snail
Cape Verde	*Tubastraea coccinea*	Coral
	Watersipora subtorquata	Bryozoan
Central African Republic	*none*	-
Chad	*none*	-
Chile	*Aedes albopictus*	Insect
	Bugula neritina	Bryozoan
	Crassostrea gigas	Oyster
China	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Bugula neritina	Bryozoan
	Crassostrea gigas	Oyster
	Musculista senhousia	Mussel
	Pomacea canaliculata	Freshwater snail
	Procambarus clarkii	Crayfish
	Schizoporella errata	Bryozoan
	Sphaeroma quoianum (=S. quoyanum)	Isopod
Colombia	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Charybdis hellerii	Crab
	Alitta succinea	Annelid
	Tubastraea coccinea	Coral
Comoros	*none*	-
Congo, Democratic Republic of the	*none*	-
Congo, Republic of the	*none*	-
Costa Rica	*Aedes aegypti*	Insect

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
	Aedes albopictus	Insect
	Procambarus clarkii	Crayfish
	Tubastraea coccinea	Coral
	Acanthaster planci	Sea Star
Cote d'Ivoire	*none*	-
Croatia	*Aedes albopictus*	Insect
	Dreissena polymorpha	Mussel
	Hemigrapsus sanguineus	Crab
Cuba	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Charybdis hellerii	Crab
	Tubastraea coccinea	Coral
Curacao	*none*	-
Cyprus	*Charybdis hellerii*	Crab
	Crassostrea gigas	Oyster
	Procambarus clarkii	Crayfish
Czech Republic	*Dreissena polymorpha*	Mussel
	Eriocheir sinensis	Crab
	Potamopyrgus antipodarum	Mud snail
Denmark	*Alitta succinea*	Annelid
	Crassostrea gigas	Oyster
	Crepidula fornicata	Sea snail
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
	Rhithropanopeus harrisii	Mud crab
Dijibouti	*Tubastraea coccinea*	Coral
Dominica	*Aedes aegypti*	Insect
	Tubastraea coccinea	Coral
Dominican Republic	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Procambarus clarkii	Crayfish
	Tubastraea coccinea	Coral
East Timor (Timor-Leste)	*Aedes aegypti*	Insect
Ecuador	*Aedes aegypti*	Insect
	Bugula neritina	Bryozoan
	Procambarus clarkii	Crayfish
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
Egypt	*Bugula neritina*	Bryozoan
	Charybdis hellerii	Crab
	Musculista senhousia	Mussel
	Procambarus clarkii	Crayfish
	Schizoporella errata	Bryozoan
	Acanthaster planci	Sea Star
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
El Salvador	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
Equatorial Guinea	*Aedes albopictus*	Insect
Eritrea	*none*	-
Estonia	*Cercopagis pengoi*	Water flea
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
Ethiopia	*none*	-
Fiji	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Mytilopsis sallei	Mussel
	Ostrea edulis	Oyster
	Acanthaster planci	Sea Star
Finland	*Cercopagis pengoi*	Water flea
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
	Mytilopsis leucophaeata	Mussel
	Pacifastacus leniusculus	Crayfish
	Potamopyrgus antipodarum	Mud snail
France	*Aedes albopictus*	Insect
	Bugula neritina	Bryozoan

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
	Ceratostoma inornatum	Sea snail
	Corbicula fluminea	Clam
	Crassostrea gigas	Oyster
	Crepidula fornicata	Sea snail
	Dreissena polymorpha	Mussel
	Elminius modestus	Barnacle
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Hemigrapsus sanguineus	Crab
	Musculista senhousia	Mussel
	Mya arenaria	Clam
	Mytilopsis leucophaeata	Mussel
	Orconectes rusticus	Crayfish
	Pacifastacus leniusculus	Crayfish
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Rapana venosa	Whelk
	Rhithropanopeus harrisii	Mud crab
	Schizoporella unicornis	Bryozoan
	Watersipora subtorquata	Bryozoan
Gabon	*Aedes albopictus*	Insect
Gambia, The	*none*	-
Georgia	*Mnemiopsis leidyi*	Comb jellyfish
	Procambarus clarkii	Crayfish
Germany	*Bugula neritina*	Bryozoan
	Cercopagis pengoi	Water flea
	Crassostrea gigas	Oyster
	Dreissena bugensis	Mussel
	Dreissena polymorpha	Mussel
	Elminius modestus	Barnacle
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Mya arenaria	Clam
	Mytilopsis leucophaeata	Mussel
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Rhithropanopeus harrisii	Mud crab
	Schizoporella errata	Bryozoan
	Alitta succinea	Annelid
Ghana	*none*	-
Greece	*Aedes albopictus*	Insect
	Crassostrea gigas	Oyster
	Mnemiopsis leidyi	Comb jellyfish
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
	Schizoporella unicornis	Bryozoan
	Alitta succinea	Annelid
Grenada	*Aedes aegypti*	Insect
Guatemala	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
Guinea	*none*	-
Guinea-Bissau	*none*	-
Guyana	*Aedes aegypti*	Insect
Haiti, Republic of	*Aedes aegypti*	Insect
Holy See	*none*	-
Honduras	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Tubastraea coccinea	Coral
Hong Kong	*Mytilopsis sallei*	Mussel
	Mytilus galloprovincialis	Mussel
	Pomacea canaliculata	Freshwater snail
	Tubastraea coccinea	Coral
Hungary	*none*	-
Iceland	*Dreissena polymorpha*	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
India	*Aedes aegypti*	Insect
	Bugula neritina	Bryozoan
	Mytilopsis sallei	Mussel
	Acanthaster planci	Sea Star
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
Indonesia	*Aedes aegypti*	Insect
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
	Acanthaster planci	Sea Star
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
Iran	*Eriocheir sinensis*	Crab
	Mnemiopsis leidyi	Comb jellyfish
	Alitta succinea	Annelid
Iraq	*Potamopyrgus antipodarum*	Mud snail
Ireland	*Dreissena polymorpha*	Mussel
	Elminius modestus	Barnacle
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Mytilus galloprovincialis	Mussel
	Schizoporella unicornis	Bryozoan
Israel	*Aedes albopictus*	Insect
	Bugula neritina	Bryozoan
	Charybdis hellerii	Crab
	Musculista senhousia	Mussel
	Ostrea edulis	Oyster
	Pomacea insularum	Freshwater snail
	Procambarus clarkii	Crayfish
	Schizoporella errata	Bryozoan
Italy	*Crepidula fornicata*	Sea snail
	Dreissena polymorpha	Mussel
	Elminius modestus	Barnacle
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Musculista senhousia	Mussel
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Rhithropanopeus harrisii	Mud crab
	Alitta succinea	Annelid
	Bugula neritina	Bryozoan
Jamaica	*Perna viridis*	Mussel
	Tubastraea coccinea	Coral
Japan	*Bugula neritina*	Bryozoan
	Carcinus maenas	Crab
	Corbicula fluminea	Clam
	Elminius modestus	Barnacle
	Ficopomatus enigmaticus	Annelid
	Mytilopsis sallei	Mussel
	Mytilus galloprovincialis	Mussel
	Ostrea edulis	Oyster
	Pacifastacus leniusculus	Crayfish
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Rhithropanopeus harrisii	Mud crab
	Acanthaster planci	Sea Star
	Alitta succinea	Annelid
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
Jordan	*none*	-
Kazakhstan	*Mnemiopsis leidyi*	Comb jellyfish
Kenya	*Procambarus clarkii*	Crayfish
	Tubastraea coccinea	Coral
Kiribati	*Tubastraea coccinea*	Coral
Korea, North	*Bugula neritina*	Bryozoan
	Mytilus galloprovincialis	Mussel
Korea, South	*Bugula neritina*	Bryozoan
	Crassostrea gigas	Oyster
	Mytilus galloprovincialis	Mussel
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Tubastraea coccinea	Coral
Kuwait	*Tubastraea coccinea*	Coral
Kyrgyzstan	*none*	-
Laos	*none*	-
Latvia	*Cercopagis pengoi*	Water flea
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
Lebanon	*Aedes albopictus*	Insect

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
	Charybdis hellerii	Crab
	Potamopyrgus antipodarum	Mud snail
Lesotho	*none*	-
Liberia	*none*	-
Libya	*none*	-
Liechtenstein	*none*	-
Lithuania	*Cercopagis pengoi*	Water flea
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
	Rhithropanopeus harrisii	Mud crab
Luxembourg	*none*	-
Macau	*none*	-
Macedonia	*none*	-
Madagascar	*Aedes albopictus*	Insect
	Musculista senhousia	Mussel
	Acanthaster planci	Sea Star
	Tubastraea coccinea	Coral
Malawi	*none*	-
Malaysia	*Aedes aegypti*	Insect
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Acanthaster planci	Sea Star
	Mycale grandis	Sponge
	Tubastraea coccinea	Coral
Maldives	*Acanthaster planci*	Sea Star
	Tubastraea coccinea	Coral
Mali	*none*	-
Malta	*Crassostrea gigas*	Oyster
Marshall Islands	*Tubastraea coccinea*	Coral
	Acanthaster planci	Sea Star
Mauritania	*none*	-
Mauritius	*Ostrea edulis*	Oyster
	Acanthaster planci	Sea Star
	Tubastraea coccinea	Coral
Mexico	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Bugula neritina	Bryozoan
	Geukensia demissa	Mussel
	Musculista senhousia	Mussel
	Mycale grandis	Sponge
	Mytilus galloprovincialis	Mussel
	Perna perna	Mussel
	Procambarus clarkii	Crayfish
	Boonea bisuturalis	Sea snail
	Mytilopsis sallei	Mussel
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
Micronesia	*Chthamalus proteus*	Barnacle
	Pomacea canaliculata	Freshwater snail
	Schizoporella errata	Bryozoan
	Tubastraea coccinea	Coral
	Acanthaster planci	Sea Star
Moldova	*none*	-
Monaco	*none*	-
Mongolia	*none*	-
Montenegro	*Aedes albopictus*	Insect
Morocco	*Crassostrea gigas*	Oyster
Mozambique	*Tubastraea coccinea*	Coral
Namibia	*Mytilus galloprovincialis*	Mussel
	Ostrea edulis	Oyster
Nauru	*none*	-
Nepal	*none*	-
Netherlands	*Aedes albopictus*	Insect
	Bellamya chinensis	Freshwater snail
	Bugula neritina	Bryozoan
	Crassostrea gigas	Oyster
	Crepidula fornicata	Sea snail
	Dreissena bugensis	Mussel
	Dreissena polymorpha	Mussel
	Elminius modestus	Barnacle
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Hemigrapsus sanguineus	Crab

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
	Mytilopsis leucophaeata	Mussel
	Mytilus galloprovincialis	Mussel
	Orconectes virilis	Crayfish
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Rhithropanopeus harrisii	Mud crab
	Urosalpinx cinerea	Sea snail
Netherlands Antilles	*Aedes aegypti*	Insect
	Tubastraea coccinea	Coral
New Zealand	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Bugula neritina	Bryozoan
	Charybdis japonica	Crab
	Crassostrea gigas	Oyster
	Ficopomatus enigmaticus	Annelid
	Musculista senhousia	Mussel
	Ochlerotatus japonicus japonicus	Insect
	Ostrea edulis	Oyster
	Sabella spallanzanii	Annelid
	Schizoporella errata	Bryozoan
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
	Acanthaster planci	Sea Star
Nicaragua	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
Niger	*none*	-
Nigeria	*Aedes albopictus*	Insect
Norway	*Crassostrea gigas*	Oyster
	Crepidula fornicata	Sea snail
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
Oman	*Acanthaster planci*	Sea Star
	Tubastraea coccinea	Coral
Pakistan	*Aedes aegypti*	Insect
Palau	*Acanthaster planci*	Sea Star
Palestinian Territories	*none*	-
Panama	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Bugula neritina	Bryozoan
	Corbicula fluminea	Clam
	Rhithropanopeus harrisii	Mud crab
	Acanthaster planci	Sea Star
	Tubastraea coccinea	Coral
Papua New Guinea	*Aedes aegypti*	Insect
	Pomacea canaliculata	Freshwater snail
	Acanthaster planci	Sea Star
Paraguay	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Limnoperna fortunei	Mussel
Peru	*Aedes aegypti*	Insect
Philippines	*Aedes aegypti*	Insect
	Bugula neritina	Bryozoan
	Phyllorhiza punctata	Jellyfish
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Procambarus clarkii	Crayfish
	Acanthaster planci	Sea Star
	Tubastraea coccinea	Coral
Poland	*Cercopagis pengoi*	Water flea
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
	Rhithropanopeus harrisii	Mud crab
Portugal	*Crassostrea gigas*	Oyster
	Elminius modestus	Barnacle
	Eriocheir sinensis	Crab
	Procambarus clarkii	Crayfish
	Rhithropanopeus harrisii	Mud crab
Qatar	*none*	-
Romania	*Cercopagis pengoi*	Water flea
	Dreissena bugensis	Mussel
	Eriocheir sinensis	Crab

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
	Mnemiopsis leidyi	Comb jellyfish
	Potamopyrgus antipodarum	Mud snail
	Rhithropanopeus harrisii	Mud crab
Russia	*Mnemiopsis leidyi*	Comb jellyfish
	Mytilopsis leucophaeata	Mussel
	Bellamya chinensis	Freshwater snail
	Corbicula fluminea	Clam
	Cercopagis pengoi	Water flea
	Dreissena bugensis	Mussel
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
	Potamopyrgus antipodarum	Mud snail
Rwanda	*none*	-
Saint Kitts and Nevis	*Aedes aegypti*	Insect
Saint Lucia	*Aedes aegypti*	Insect
Saint Vincent and the Grenadines	*Aedes aegypti*	Insect
Samoa	*Aedes aegypti*	Insect
	Acanthaster planci	Sea Star
San Marino	*none*	-
Sao Tome and Principe	*none*	-
Saudi Arabia	*Acanthaster planci*	Sea Star
	Tubastraea coccinea	Coral
Senegal	*none*	-
Serbia	*Aedes albopictus*	Insect
	Eriocheir sinensis	Crab
Seychelles	*Tubastraea coccinea*	Coral
Sierra Leone	*none*	-
Singapore	*Aedes aegypti*	Insect
	Mytilopsis sallei	Mussel
	Pomacea canaliculata	Freshwater snail
	Tubastraea coccinea	Coral
Sint Maarten	*none*	-
Slovakia	*Potamopyrgus antipodarum*	Mud snail
Slovenia	*Aedes albopictus*	Insect
	Dreissena polymorpha	Mussel
	Musculista senhousia	Mussel
	Potamopyrgus antipodarum	Mud snail
Solomon Islands	*Aedes aegypti*	Insect
Somalia	*none*	-
South Africa	*Aedes albopictus*	Insect
	Carcinus maenas	Crab
	Crassostrea gigas	Oyster
	Elminius modestus	Barnacle
	Ficopomatus enigmaticus	Annelid
	Mytilus galloprovincialis	Mussel
	Ostrea edulis	Oyster
	Procambarus clarkii	Crayfish
	Acanthaster planci	Sea Star
	Watersipora subtorquata	Bryozoan
South Sudan	*none*	-
Spain	*Aedes albopictus*	Insect
	Bugula neritina	Bryozoan
	Crassostrea gigas	Oyster
	Crepidula fornicata	Sea snail
	Dreissena polymorpha	Mussel
	Elminius modestus	Barnacle
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Mya arenaria	Clam
	Mytilopsis leucophaeata	Mussel
	Pomacea insularum	Freshwater snail
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
Sri Lanka	*Aedes aegypti*	Insect
	Pomacea canaliculata	Freshwater snail
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
Sudan	*Procambarus clarkii*	Crayfish
	Acanthaster planci	Sea Star
Suriname	*Aedes aegypti*	Insect
Swaziland	*none*	-
Sweden	*Cercopagis pengoi*	Water flea
	Crepidula fornicata	Sea snail

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Mya arenaria	Clam
	Orconectes virilis	Crayfish
	Pacifastacus leniusculus	Crayfish
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Alitta succinea	Annelid
Switzerland	*Aedes albopictus*	Insect
	Dreissena polymorpha	Mussel
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
Syria	*Mnemiopsis leidyi*	Comb jellyfish
	Aedes albopictus	Insect
	Charybdis hellerii	Crab
Taiwan	*Aedes albopictus*	Insect
	Mytilopsis sallei	Mussel
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Procambarus clarkii	Crayfish
	Tubastraea coccinea	Coral
Tajikistan	*none*	-
Tanzania	*Musculista senhousia*	Mussel
	Tubastraea coccinea	Coral
Thailand	*Aedes aegypti*	Insect
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Acanthaster planci	Sea Star
	Aedes albopictus	Insect
	Tubastraea coccinea	Coral
Togo	*none*	-
Tonga	*Aedes aegypti*	Insect
	Ostrea edulis	Oyster
Trinidad and Tobago	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Perna viridis	Mussel
Tunisia	*Crassostrea gigas*	Oyster
Turkey	*Bugula neritina*	Bryozoan
	Cercopagis pengoi	Water flea
	Charybdis hellerii	Crab
	Mnemiopsis leidyi	Comb jellyfish
	Potamopyrgus antipodarum	Mud snail
Turkmenistan	*Mnemiopsis leidyi*	Comb jellyfish
Tuvalu	*Aedes aegypti*	Insect
Uganda	*Procambarus clarkii*	Crayfish
Ukraine	*Alitta succinea*	Annelid
	Cercopagis pengoi	Water flea
	Dreissena bugensis	Mussel
	Eriocheir sinensis	Crab
	Mnemiopsis leidyi	Comb jellyfish
	Mytilopsis leucophaeata	Mussel
	Potamopyrgus antipodarum	Mud snail
United Arab Emirates	*none*	-
United Kingdom	*Bugula neritina*	Bryozoan
	Crassostrea gigas	Oyster
	Crepidula fornicata	Sea snail
	Daphnia lumholtzi	Water flea
	Dreissena polymorpha	Mussel
	Elminius modestus	Barnacle
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Mya arenaria	Clam
	Mytilopsis leucophaeata	Mussel
	Mytilus galloprovincialis	Mussel
	Orconectes virilis	Crayfish
	Pacifastacus leniusculus	Crayfish
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Rhithropanopeus harrisii	Mud crab
	Schizoporella errata	Bryozoan
	Schizoporella unicornis	Bryozoan
	Urosalpinx cinerea	Sea snail
	Watersipora subtorquata	Bryozoan
	Alitta succinea	Annelid
United States of America	*Perna viridis*	Mussel

Country/Area	Aquatic/Semi-aquatic Invertebrate Invader	Organism type
	Acanthaster planci	Sea star
	Aedes aegypti	Insect
	Aedes albopictus	Insect
	Alitta succinea	Annelid
	Batillaria attramentaria	Sea snail
	Bellamya chinensis	Freshwater snail
	Boonea bisuturalis	Sea snail
	Bugula neritina	Bryozoan
	Bythotrephes longimanus	Water flea
	Carcinus maenas	Crab
	Carijoa riisei	Coral
	Ceratostoma inornatum	Sea snail
	Cercopagis pengoi	Water flea
	Charybdis helleri	Crab
	Chthamalus proteus	Barnacle
	Corbicula fluminea	Clam
	Crassostrea gigas	Oyster
	Crepidula fornicata	Sea snail
	Daphnia lumholtzi	Water flea
	Dreissena bugensis	Mussel
	Dreissena polymorpha	Mussel
	Eriocheir sinensis	Crab
	Ficopomatus enigmaticus	Annelid
	Gemma gemma	Clam
	Geukensia demissa	Mussel
	Hemigrapsus sanguineus	Crab
	Ilyanassa obsoleta	Mud snail
	Littorina littorea	Sea snail
	Musculista senhousia	Mussel
	Mya arenaria	Clam
	Mycale grandis	Sponge
	Mytilopsis leucophaeata	Mussel
	Mytilus galloprovincialis	Mussel
	Orconectes rusticus	Crayfish
	Orconectes virilis	Crayfish
	Ostrea edulis	Oyster
	Perna perna	Mussel
	Phyllorhiza punctata	Jellyfish
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Potamocorbula amurensis	Clam
	Potamopyrgus antipodarum	Mud snail
	Procambarus clarkii	Crayfish
	Pseudodiaptomus inopinus	Copepod
	Schizoporella errata	Bryozoan
Uruguay	*Aedes aegypti*	Insect
	Ficopomatus enigmaticus	Annelid
	Limnoperna fortunei	Mussel
	Rapana venosa	Whelk
	Alitta succinea	Annelid
Uzbekistan	*none*	-
Vanuatu	*Aedes aegypti*	Insect
	Crassostrea gigas	Oyster
	Schizoporella errata	Bryozoan
	Acanthaster planci	Sea Star
Venezuela	*Aedes aegypti*	Insect
	Aedes albopictus	Insect
	Charybdis hellerii	Crab
	Geukensia demissa	Mussel
	Perna viridis	Mussel
	Procambarus clarkii	Crayfish
	Tubastraea coccinea	Coral
	Watersipora subtorquata	Bryozoan
Vietnam	*Aedes aegypti*	Insect
	Pomacea canaliculata	Freshwater snail
	Pomacea insularum	Freshwater snail
	Tubastraea coccinea	Coral
Yemen	*none*	-
Zambia	*Procambarus clarkii*	Crayfish
Zimbabwe	*none*	-

Appendix Table 1.3: The symbionts associated with the invasive crustaceans, including any known taxonomic information about themselves and their host.

Host Species	**Organism Type**	Pathogen or disease	**Pathogen Type**	**Reference**
Acantharctus posteli	Lobster	*None*	-	-
Acartia (Acanthacartia) fossae	Copepod	*None*	-	-
Acartia (Acanthacartia) tonsa	Copepod	*Epistylus sp.*	Ciliate protozoan	Turner et al. 1979
		Zoothamnium intermedium	Epibiont	Utz, 2008
		Bacterial infection	Bacteria	Turner et al. 1979
		Probopyrus pandalicola	Isopod	Beck, 1979
		Acartia tonsa copepod circo-like virus	Virus	Dunlap et al. 2013
Acartia (Acartiura) omorii	Copepod	*None*	-	-
Acartia (Odontacartia) centrura	Copepod	*None*	-	-
Actaea savignii	Crab	*None*	-	-
Actaeodes tomentosus	Crab	*None*	-	-
Actumnus globulus	Crab	*None*	-	-
Alpheus audouini	Shrimp	*None*	-	-
Alpheus inopinatus	Shrimp	*None*	-	-
Alpheus migrans	Shrimp	*None*	-	-
Alpheus rapacida	Shrimp	*None*	-	-
Ameira divagans	Maxillipod	*None*	-	-
Ampelisca cavicoxa	Amphipod	*None*	-	-
Ampelisca heterodactyla	Amphipod	*None*	-	-
Amphibalanus eburneus	Barnacle	*None*	-	-
Amphibalanus improvisus	Barnacle	*None*	-	-
Amphibalanus reticulatus	Barnacle	*None*	-	-
Amphibalanus variegatus	Barnacle	*None*	-	-
Ampithoe bizseli	Amphipod	*None*	-	-
Anilocra pilchardi	Ectoparasitic Isopod	*None*	-	-
Apanthura sandalensis	Ectoparasitic Isopod	*None*	-	-
Argulus japonicus	Ectoparasitic Fish louse	*None*	-	-
Arietellus pavoninus	Copepod	*None*	-	-
Artemia franciscana	Brine shrimp	*Vibrio harveyi*	Bacterial	Defoirdt et al. 2006
		Vibrio campbellii	Bacterial	Defoirdt et al. 2006
		Vibrio parahaemolyticus	Bacterial	Defoirdt et al. 2006
		Vibrio anguillarum	Bacterial	Defoirdt et al. 2005
		Aeromonas hydrophila	Bacterial	Defoirdt et al. 2005
		White Spot Syndrome Virus	Virus	Li et al. 2003
		Flamingolepis liguloides	Cestode	Georgiev et al. 2007
		Flamingolepis flamingo	Cestode	Georgiev et al. 2007
		Gynandrotaenia stammeri	Cestode	Georgiev et al. 2007
		Wardium stellorae	Cestode	Georgiev et al. 2007
		Confluaria podicipina	Cestode	Georgiev et al. 2007
		Anomotaenia tringae	Cestode	Georgiev et al. 2007
		Anomotaenia microphallos	Cestode	Georgiev et al. 2007
		Eurycestus avoceti	Cestode	Georgiev et al. 2007
		Fimbriarioides tadornae	Cestode	Georgiev et al. 2007
		unidentified hymenolepidid species	Cestode	Georgiev et al. 2007
		Nosema artemiae	Microsporidian	Ovcharenko and Wita, 2005
		Anostracospora rigaudi	Microsporidian	Rode et al. 2013b
		Enterocytospora artemiae	Microsporidian	Rode et al. 2013b
		Cryptosporidium parvum	Protozoan	Mendez-Hermida et al. 2006
		Giardia intestinalis	Protozoan	Mendez-Hermida et al. 2006
		Necrotizing hepatopancreatitis bacteria (NHPB)	Bacteria	Avila-Villa et al. 2011
Ashtoret lunaris	Crab	*None*	-	-
Astacus astacus	Crayfish	*Astacus astacus Bacilliform Virus*	Virus	Edgerton et al. 1996
		Aphanomyces astaci (variable strains)	Fungus	Vennerström et al. 1998
		Infectious pancreatic necrosis virus (IPNV)	Virus	Halder and Ahne, 1988
		Psorospermium haeckeli	Mesomycetozoan	Cerenius et al. 1991
		Thelohania contejeani	Microsporidian	Mario and Salvidio, 2000
		Unspecified nematode parasite	Nematode	Ljungberg and Monne, 1968
		Trichosporon beigelii	Fungus	Söderhäll et al. 1993
		WSSV (experimental infection)	Virus	Baumgartner et al. 2009
Astacus leptodactylus	Crayfish	*Saprolegnia parasitica*	Fungus	Söderhäll et al. 1991
		WSSV (experimental infection)	Virus	Corbel et al. 2001
		Aphanomyces astaci	Fungus	Rahe and Soylu, 1989
		Thelohania contejeani	Microsporidian	Quilter, 1976
		Psorospermium haeckeli	Mesomycetozoan	Vranckx and Durliat, 1981

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Listeria monocytogenes	Bacteria	Khamesipour et al. 2013
		Aeromonas hydrophila (experimental infection)	Bacteria	SamCookiyaei et al. 2012
		Branchiobdella pentodonta	Protist	Subchev et al. 2007
		Branchiobdella parasitia	Protist	
		Branchiobdella hexodonta	Protist	
		Histricosoma chappuisi	Protist	
		Tetrahymena pyriformis	Protist	NekuieFard et al. 2015
		Epistylis chrysemidis	Protist	
		Vorticella similis	Protist	
		Cothurnia sieboldii	Protist	
		Pyxicola annulata	Protist	
		Chilodonella spp.	Protist	
		Zoothamnium intermedium	Protist	
		Opercularia articulate	Protist	
		Podophrya fixa	Protist	
		Epistylus niagarae	Protist	Harlioglu, 1999
		Acremonium sp.	Fungus	Diler and Bolat, 2001
		Astacotrema tuberculatum	Trematode	Wu, 1938
Atergatis roseus	Crab	*None*	-	-
Atyaephyra desmarestii	Shrimp	*Solenophrya polypoides*	Ciliated protist	Fernandez-Leborans and Tato-Porto, 2000
		Hydrophrya miyashitai	Ciliated protist	
		Spelaeophrya lacustris	Ciliated protist	
		Spathocyathus caridina	Ciliated protist	
		Acineta karamani	Ciliated protist	
Austrominius modestus	Barnacle	*Echinostephilla patellae*	Trematode	Prinz et al. 2009
		Parorchis acanthus	Trematode	
		Renicola roscovita	Trematode	Goedknegt et al. 2015
Autonoe spiniventris	Amphipod	*None*	-	-
Bemlos leptocheirus	Amphipod	*None*	-	-
Boeckella triarticulata	Copepod	*Tuzetia boeckella*	Microsporidian	Milner and Meyer, 1982
		Epistylis daphniae	Epizotic ciliate	Xu and Burns, 1991
		Microcystis aeruginosa	Algae	Boon et al. 1994
Bythocaris cosmetops	Decapod	*None*	-	-
Bythotrephes longimanus	Water flea	*Undetermined "brood parasite infection"*	Unknown	Kim et al. 2014
Caecidotea communis	Isopod	*Fessisentis friedi*	Acanthocephalan	Muzzall, 1978
		Acanthocephalus tahlequahensis	Acanthocephalan	Hernandez and Sukhdeo, 2008
		Acanthocephalus parksidei	Acanthocephalan	Amin et al. 1980
		Allocreadium lobatum	Digenean	Muzzall, 1981
Calanipeda aquaedulcis	Copepod	*None*	-	-
Calanopia biloba	Copepod	*None*	-	-
Calanopia elliptica	Copepod	*None*	-	-
Calanopia media	Copepod	*None*	-	-
Calanopia minor	Copepod	*None*	-	-
Calappa hepatica	Crab	*Sacculina pilosa*	Barnacle	Chan et al. 2004
		Loxothylacus setaceus	Barnacle	
Calappa pelii	Crab	*None*	-	-
Caligus fugu	Copepod	*None*	-	-
Caligus pageti	Copepod	*None*	-	-
Callinectes danae	Crab	*Loxothylacus texanus*	Barnacle	Christmas, 1969
		Chelonibia patula	Barnacle	Negreiros-Fransozo et al. 2015
		Balanus venustus	Barnacle	
		Octolasmis lowei	Barnacle	Mantelatto et al. 2003
		Carcinonemertes carcinophila imminuta	Nemertean	
		Myzobdella platensis	Leech	Zara et al. 2009
		WSSV	Virus	Costa et al. 2012
Callinectes exasperatus	Crab	*None*	-	-
Callinectes sapidus	Crab	*Hematodinium sp.*	Dinoflagellate	Messick and Shields, 2000
		Baculo-B virus	Virus	Messick, 1998
		RLV-RhVA	Virus	
		RLM	Virus	
		Strandlike	Virus	
		Microsporidia	Microsporidian	
		Mesanophrys chesapeakensis	Ciliophoran	
		Lagenophrys callinectes	Ciliophoran	
		Epistylis sp.	Ciliophoran	
		Unidentified gregarine	Apicomplexan	
		Unidentified metacercariae	Trematode	
		Urosporidium crescens	Haplosporidian	
		Carcinonemertes carcinophila	Nemertean	
		WSSV	Virus	Corbel et al. 2001
		Vibrio spp.	Bacteria	Yalcinkaya et al. 2003
		Baculo-A	Virus	Bonami and Zhang, 2011
		RLV	Virus	
		Shell disease	Unknown	Noga et al. 2000

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		YHV	Virus	Ma et al. 2009
		Hematodinium perezi	Dinoflagellate	Rogers et al. 2015
		Ameson michaelis	Microsporidian	
		Paramoeba perniciosa	Amoeba	Stentiford, 2008
Cancer irroratus	Crab	*Gafkya homori*	Bacteria	Cornick and Stewart, 1968a
		Vibrio spp.	Bacteria	Stentiford, 2008
		Chlamydiales spp.	Bacteria	
		Paramoeba pernicosa	Amoeba	
		Digenea	Trematodes	
		Acanthocephalans	Helminths	
		Choniosphaera cancrorum	Copepod	
		Shell disease	Unknown	Mancusco, 2014
		Chitinoclastic bacteria	Bacteria	Wang, 2011
		Hematodinium spp.	Dinoflagellate	Hoppes, 2011
		Mesanophrys spp.	Ciliophoran	Morado, 2011
Caprella mutica	Shrimp	*None*	-	-
Caprella scaura	Shrimp	*None*	-	-
Carcinus maenas	Crab	First Virus?	Virus	Vago, 1966
		Undetermined virus of the Y-organ	Virus	Chassard-Bouchard et al. 1976, Bonami 1976
		CmBV	Virus	Bonami 1976; Johnson, 1983; Stentiford and Feist, 2005
		Haemocytopenic disease (Virus 'Bang')	Virus	Johnson, 1983; Bang 1971, Bang 1974, Hoover 1977 (PhD), Hoover and Bang 1976, 1978; Sinderman 1990
		B1 Virus	Virus	Bazin et al. 1974; Bonami, 1976
		RV-CM	Virus	Johnson, 1988
		Unidentified bacterial infection	Bacteria	Spindler-Barth 1976
		Black necrotic disease	Unknown	Perkins, 1967; Comely & Ansell, 1989
		Milky Disease (various bacteria)	Bacterial	Eddy et al. 2007
		Arudinula sp.	Unknown	Léger & Duboscq, 1905
		Abelspora portucalensis	Microsporidian	Azevedo, 1987
		Ameson pulvis (=Nosema pulvis)	Microsporidian	Sprague & Couch, 1971
		Thelohania maenadis	Microsporidian	Sprague & Couch, 1971
		Nematopsis portunidarum	Apicomplexan	Sprague & Couch, 1971
		'Myxosporidia sp.'	Myxosporan	Cuénot, 1895
		Nosema spelotremae (in Microphallus similis)	Hyperparasite	Sprague & Couch, 1971
		Nadelspora carcini	Microsporidian	Stentiford et al. 2013
		Parahepatospora canadia	Microsporidian	Bojko et al. In Press
		Hematodinium perezi	Dinoflagellate	Hamilton et al., 2007, 2009, 2010; Stentiford & Feist, 2005
		Haplosporidium littoralis	Haplosporidian	Stentiford et al. 2004; Stentiford et al. 2013
		Anophrys maggii	Ciliate	Couch, 1983
		Foettingeria sp.	Ciliate	Chatton & Lwoff, 1935
		Folliculina viridis	Ciliate	Sprague & Couch, 1971
		Gymnodinioides inkystans	Ciliate	Sprague & Couch, 1971
		Phtorophrya insidiosa	Ciliate	Sprague & Couch, 1971
		Synophrya hypertrophica	Ciliate	Sprague & Couch, 1971
		Zoothamnium hydrobiae	Ciliate	Crothers, 1968
		Aggregata eberthi	Apicomplexan	Vivier et al. 1970
		Fecampia erythrocephala	Helminth	Bourdon, 1965; Kuris et al., 2002
		Cercaria emasculans	Trematode	James, 1969
		Distomum sp.	Digenean	von Linstow, 1878
		Maritrema subdolum	Parasitic fluke	Deblock et al. 1961
		Levinseniella carcinidis	Trematode	Rankin, 1939
		Megalophallus carcini	Trematode	Prévot & Deblock, 1970
		Maritrema portucalensis	Parasitic fluke	Pina et al. 2011
		Microphallus bittii	Trematode	Prévot, 1973
		Microphallus primas	Trematode	Deblock & Tran Van Ky, 1966
		Microphallus similis	Trematode	Stunkard, 1956; Deblock & Tran Van Ky, 1966
		Renicola (=Cercaria) roscovita	Trematode	James, 1969
		Calliobothrium ventricillatum	Cestode	Monticelli, 1890
		Eutetrarhynchus ruficollis	Cestode	Vivares, 1971

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Tetraphyllidean larvae	Cestode	Vivares, 1971
		Ascarophis morrhuae	Nematode	Sudhaus, 1974
		Enoplus communis	Nematode	Sudhaus, 1974
		Filaria sp.	Nematode	von Linstow, 1878
		Monhystera disjuncta	Nematode	Sudhaus, 1974
		Proleptus robustus	Nematode	Vaullegeard, 1896
		Proleptus obtusus	Nematode	Hall, 1929
		Viscosia glabra	Nematode	Sudhaus, 1974
		Carcinonemertes carcinophila	Nemertean	Vivares 1971, MBA, 1957
		Profilcollis (=Polymorphus) botulus	Acanthocephalan	Liat & Pike, 1980
		Janua pagenstecheri	Polychaete worm	Crothers, 1966
		Pomatoceros triqueter	Polychaete worm	Crothers, 1968
		Spirorbis tridentatus	Polychaete worm	Crothers, 1966
		Alcyonidium sp.	Bryozoan	Richard, 1899
		Electra pilosa	Bryozoan	Macintosh, 1865
		Triticella korenii	Bryozoan	Duerden, 1893
		Balanus balanus	Barnacle	Hartnoll, 1963a
		Balanus crenatus	Barnacle	Richard 1899; Heath, 1976
		Chelonibia patula	Barnacle	Richard, 1899
		Chirona hameri	Barnacle	Richard, 1899
		Elminius modestus	Barnacle	Crothers, 1966
		Sacculina carcini	Barnacle	Boschma 1955
		Veruca stroemia	Barnacle	Richard, 1899
		Heterolaophonte stromi	Crustacean	Scott, 1902
		Portunion maenadis	Crustacean	Bourdon, 1963
		Priapion fraissei	Crustacean	Goudswaard, 1985; Choy, 1987
		Mytilus edulis	Mussel	Giard & Bonnier, 1887
		Ascidiella scabra	Tunicate	Crothers, 1966
		Botrylloides leachi	Tunicate	Crothers, 1966
		Botryllus schlosseri	Tunicate	Crothers, 1966
		Molgula manhattensis	Tunicate	Crothers, 1966
Carupa tenuipes	Crab	*None*	-	-
Centropages furcatus	Copepod	*Vibrio cholerae*	Bacteria	Rawlings, 2005
Cercopagis pengoi	Water flea	*None*	-	-
Chaetogammarus warpachowskyi	Amphipod	*None*	-	-
Charybdis feriata	Crab	*WSSV*	Virus	Flegel, 1997
		Benedenia spp.	Metazoan	Parado-Estepa et al. 2002
		Ectoparasites (Various)	Various	
		16 species of Fungi (unspecified)	Fungi	Ghaware and Jadhao, 2015
		5 species of bacteria (unspecified)	Bacteria	
		Sacculina serenei	Barnacle	Boschma, 1954
Charybdis hellerii	Crab	*Sacculina spp.*	Barnacle	Elumalai et al. 2014
Charybdis japonica	Crab	Serpulid polychaete worms	Polychaete	Miller et al. 2006
		Ascaridoid nematode	nematode	
		Trematode metacercaria	trematode	
		Balanomorph barnacles	Crustacea	
		Vibrio alginolyticus	Bacteria	Xu et al. 2013
		Sacculina lata	Rhizocephalan	Chan, 2004
		Halocrusticida okinawaensis	fungi	Yasunobu, 2001
		Vibrio paraheamolyticus	Bacteria	Wang et al. 2010
Charybdis (Goniohellenus) longicollis	Crab	*Heterosaccus dollfusi*	Rhizocephalan	Innocenti and Galil, 2011
Charybdis lucifera	Crab	*WSSV*	Virus	Otta et al. 1999
		Sacculina spp.	Rhizocephala	Elumalai et al. 2014
Chelicorophium curvispinum	Amphipod	*Pomphorhynchus sp.*	Acanthocephala	Van Riel et al. 2003
Chelicorophium robustum	Amphipod	*None*	-	-
Cherax destructor	Crayfish	*WSSV*	Virus	Edgerton, 2004
		Parvo-like Virus	Virus	Edgerton and Webb, 1997
		Thelohania montirivulorum	Microsporidian	Moodie et al. 2003a
		Thelohania parastaci	Microsporidian	Moodie et al. 2003b
		Vairimorpha cheracis	Microsporidian	Moodie et al. 2003c
		Parasitic nematodes	Nemtaode	Herbert, 1987
		C. destructor Bacilliform Virus	Virus	Edgerton, 1996
		Austramphilina elongata	Platyhelminth	Rohde and Watson, 1989
Chionoecetes opilio	Crab	*Hematodinium sp.*	Dinoflagellate	Taylor and Kahn, 1995
		Aerococcus viridans	Bacteria	Cornick and Stewart, 1975
		Trichomaris invadans	Ascomycete	Hibbits et al. 1981
		Heamocytic Bacilliform Virus	Virus	Kon et al. 2011
		Milky Disease	Bacteria	
		Fungal encrusting	Fungi	Hyning and Scarborough, 1973
		Vasichona opiliophila	Ciliate	Taylor et al. 1995

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Marine leeches	Leech	Meyer and Kahn, 1979
		Halocrusticida okinwaensis	Fungi	Yasunobu, 2001
Chlamydotheca incisa	Shrimp	*None*	-	-
Chthamalus proteus	Barnacle	*None*	-	-
Clavellisa ilishae	Copepod	*None*	-	-
Clorida albolitura	Shrimp	*None*	-	-
Coleusia signata	Crab	*None*	-	-
Conchoderma auritum	Barnacle (whale ectoparasite)	*None*	-	-
Cornigerius maeoticus	Branchiopod	*None*	-	-
Crangonyx pseudogracilis	Amphipod	*Fibrillanosema crangonycis*	Microsporidian	Johanna et al. 2004
		4 x Microsporidium sp.	Microsporidian	Galbreath et al. 2010
Cristapseudes omercooperi	Kalliapseudid	*None*	-	-
Critomolgus actiniae	Copepod	*None*	-	-
Cryptorchestia cavimana	Amphipod	*None*	-	-
Cryptosoma cristatum	Crab	*None*	-	-
Cuapetes calmani	Shrimp	*None*	-	-
Cyclops kolensis	Copepod	*Schistocephalus solidus*	Tapeworm	Franz and Kurtz, 2002
		Proteocephalus longicollis	Cestode	Scholz, 1999
		Proteocephalus percae		
		Proteocephalus thymalli		
Cyclops vicinus	Copepod	*Bothriocephalus claviceps*	Helminth	Nie and Kennedy, 1993
		Anguillicola crassus	Nematode	Kennedy and Fitch, 1990
		Ligula intestinalis	Cestode	Loot et al. 2006
Cymothoa indica	Isopod	*None*	-	-
Cypretta turgida	Ostracod	*None*	-	-
Daira perlata	Crab	*None*	-	-
Daphnia ambigua	Water flea	*None*	-	-
Daphnia cristata	Water flea	*None*	-	-
Daphnia longiremis	Water flea	*None*	-	-
Daphnia lumholtzi	Water flea	*None*	-	-
Daphnia parvula	Water flea	*Tanaorhamphus longirostris*	Acanthocephalan	Hubschman, 1983
Delavalia inopinata	Copepod	*None*	-	-
Delavalia minuta	Copepod	*None*	-	-
Diamysis bahirensis	Shrimp	*None*	-	-
Diaphanosoma chankensis	Brachiopod	*None*	-	-
Dikerogammarus bispinosus	Amphipod	*None*	-	-
Dikerogammarus haemobaphes	Amphipod	*Nicolla skrjabini*	Trematode	Kirin et al. 2013
		Cystoopsis acipenseris	Nematode	Bauer et al. 2002
		Bothriomonas fallax	Cestode	
		Amphilina foliacea	Cestode	
		Pomphorhynchus laevis	Acanthocephalan	Đikanovic et al. 2010
		Acanthocephalus (=Pseudoechinirhynchus) clavula	Acanthocephalan	Komarova et al. 1969
		Cucumispora ornata	Microsporidian	Bojko et al. 2015
		Cucumispora (=Nosema) dikerogammari	Microsporidia	Ovcharenko et al. 2010
		Thelohania brevilovum		
		Dictyocoela mulleri		
		Dictyocoela spp. ('Haplotype: 30-33')	Microsporidia	Wilkinson et al. 2011
		Dictyocoela berillonum	Microsporidian	Green-Etxabe et al. 2014
		Cephaloidophora similis	Gregarine	Codreanu-Balcescu, 1995
		Cephaloidophora mucronata		
Dikerogammarus villosus	Amphipod	*Plagioporus skrjabini*	Trematodes	Review by: Rewicz et al. 2014
		Unidentified trematode		
		Pomphorhynchus tereticollis	Acanthocephalan	
		Cephaloidophora spp.	Gregarines	
		Uradiophora spp.		
		Cucumispora dikerogammari	Microsporidia	
		Nosema granulosis		
		Dictyocoela muelleri		
		Dictyocoela berillonum		
		Dictyocoela roeselum		
		Unidentified bacteria	Bacteria	
		Dikerogammarus villosus Bacilliform Virus	Virus	
		Unidentified nematode	Nematode	Bojko et al. 2013
		Unidentified ciliated protists	Protist	
		Unidentified isopod	Crustacean	
		Unidentified commensal worms	Helminth	
Disparalona hamata	Anomopodan	*None*	-	-
Dolerocypris sinensis	Ostracod	*None*	-	-
Dorippe quadridens	Crab	*None*	-	-

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
Dyspanopeus sayi	Crab	*Loxothylacus panopei*	Rhizocephalan	Hines et al. 1997
		Nematopsis legeri	Gregarine	Lindsey et al. 2006
		Cancricepon choprae	Isopod	Boyko and Williams, 2004
		Hematodinium-like	Fungi	Small, 2012
Echinogammarus berilloni	Amphipod	*Dictyocoela spp.*	Microsporidia	Wilkinson et al. 2011
		Polymorphus minutus	Acanthocephalan	Jacquin et al. 2014
		Cephaloidophora echinogammari	Gregarine	Goodrich, 1949
		Coitocaecum angusticolle	Digenea	Lefebvre and Poulin, 2005
		Nicolla gallica		
		Pleurogenoides medians		
		Theodoxia fluviatilis	Digenea	Fischthal and Kuntz, 1963
Echinogammarus (Chaetogammarus) ischnus	Amphipod	Oomycete	Oomycete	Van Rensburg, 2010
Echinogammarus trichiatus	Amphipod	*Dictyocoela berillonum*	Microsporidian	Garbner et al. 2015
Elamena mathoei	Crab	*None*	-	-
Elasmopus pectenicrus	Amphipod	*None*	-	-
Elminius modestus	Barnacle	*Hemioniscus balani*	Isopod	Crisp and Davies, 1955
Enhydrosoma vicinum	Copepod	*None*	-	-
Eocuma dimorphum	Cumacea	*None*	-	-
Eocuma rosae	Cumacea	*None*	-	-
Eocuma sarsii	Cumacea	*None*	-	-
Ergasilus briani	Parasitic Copepod	*None*	-	-
Ergasilus gibbus	Parasitic Copepod	*None*	-	-
Ergasilus sieboldi	Copepod	*None*	-	-
Eriocheir sinensis	Crab	Rickettsia-like organism	Bacteria	Wang and Gu, 2002
		Virus-like particles	Virus	
		Microsporidian-like protozoan	Microsporidia	
		Paragonimus westemanii	Lung fluke	Cohen and Carlton, 1997
		Reovirus	Virus	Zhang et al. 2004
		Hepatospora (= Endoreticulatus) eriocheir	Microsporidian	Stentiford et al. 2011
		Spiroplasma eriocheiris	Bacteria	Wang et al. 2004
		Roni-like virus	Virus	Zhang and Bonami, 2007
		Aphanomyces astaci	Fungi	Schrimpf et al. 2014
		Aeromonas hydrophila	Bacteria	Guo et al. 2011
		Listonella anguillarum	Bacteria	Zhang et al. 2010
		Micrococcus luteus	Bacteria	
		Intestinal bacteria	Bacteria	Li et al. 2007
		Citrobacter freundii	Bacteria	Chen et al. 2006
		Picornavirus	Virus	Lu et al. 1999
		Vibrio anguillarum	Bacteria	Sui et al. 2012
		Polyascus gregarius	Rhizocephalan	Li et al. 2011
		Herpes-like virus	Virus	Shengli et al. 1995
		WSSV	Virus	Ding et al. 2015
Erugosquilla massavensis	Shrimp	*None*	-	-
Euchaeta concinna	Copepod	*None*	-	-
Eucrate crenata	Crab	*None*	-	-
Eudiaptomus gracilis	Copepod	*Diphyllobothrium latum*	Cestode	Klekowski and Guttowa, 1968
		Diphyllobothrium norvegicum	Cestode	Halvorsen, 1966
		Aphanomyces sp.	Fungi	Miao and Nauwerck, 1999
		Chytrids	Fungi	Kagami et al. 2011
		Triaenophorus nodulosus	Cestode	Guttowa, 1968
		Proteocephalus torulosus	Cestode	Scholz, 1993
		Ligula intestinalis	Cestode	Glazunova and Polunina, 2009
		Diphyllobothrium dendriticum	Cestode	Wicht et al. 2008
		Triaenophorus crassus	Cestode	Pulkkinen et al. 1999
Eurycarcinus integrifrons	Crab	*None*	-	-
Eurytemora americana	Copepod	*None*	-	-
Eurytemora pacifica	Copepod	*None*	-	-
Eurytemora velox	Copepod	*None*	-	-
Eusarsiella zostericola	Ostrocod	*None*	-	-
Evadne anonyx	Cladoceran	*None*	-	-
Fistulobalanus albicostatus	Barnacle	*None*	-	-
Fistulobalanus pallidus	Barnacle	*None*	-	-
Gammaropsis togoensis	Amphipod	*Anilorca pilchardi*	Isopod	Souissi et al. 2010
Gammarus pulex	Amphipod	*Pomphorhynchus laevis*	Acanthocephalan	Bakker et al. 1997
		Polymorphus minutus	Acanthocephalan	Bauer et al. 2005
		Echinorhynchus truttae	Acanthocephalan	Fielding et al. 2003
		Cyathocephalus truncatus	Cestode	Franceschi et al. 2007
		Dictyocoela duebenum	Microsporidia	Garbner et al. 2015
		Dictyocoela mulleri		
		Microsporidium sp. G		
		Microsporidium sp. I		
		Microsporidium sp. RR2		
		Microsporidium sp. 515		

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Microsporidium sp. 505		
		Microsporidium sp. BPAR3		
		Microsporidium sp. RR1		
Gammarus roeselii	Amphipod	*Polymorphus minutus*	Acanthocephalan	Médoc et al. 2006
		Pomphorhynchus tereticollis	Acanthocephalan	Špakulová, et al. 2011
		Pomphorhynchus laevis	Acanthocephalan	Bauer et al. 2000
		Dictyocoela muelleri	Microsporidian	Haine et al. 2004
		Dictyocoela roeseleum	Microsporidian	
		Nosema granulosis	Microsporidian	
		Microsporidium sp. G	Microsporidian	Garbner et al. 2015
		Microsporidium sp. 505	Microsporidian	
		Microsporidium sp. nov. RR2	Microsporidian	
		Microsporidium sp. nov. RR1	Microsporidian	
Gammarus tigrinus	Amphipod	*Paratenuisentis ambiguus*	Acanthocephalan	Gollash and Zander, 1995
		Maritrema subdolum	Trematode	Rolbiecki and Normant, 2005
		Dictyocoela duebenum	Microsporidia	Terry et al. 2004
		Dictyocoela berillonum		
Gammarus varsoviensis	Amphipod	*None*	-	-
Glabropilumnus laevis	Crab	*None*	-	-
Gmelinoides fasciatus	Amphipod	*Dictyocoela sp.*	Microsporidia	Wilkinson et al. 2011
		6 unspecificied microsporidian SSU sequences		Kumenkova et al. 2008
		Dictyocoela duebenum		
		Nicolla skrjabini	Trematode	Tyutin et al. 2013
Goneplax rhomboides	Crab	*Triticella flava*	Bryozoan	Fernandez-Leborans, 2003
		Zoothamnium sp. (hyperepibiont)	Protist	
		Cothurnia sp. (hyperepibiont)		
		Corynophrya sp. (hyperepibiont)		
Grandidierella japonica	Amphipod	*None*	-	-
Grapsus granulosus	Crab	*None*	-	-
Halectinosoma abrau	Copepod	*None*	-	-
Halimede tyche	Crab	*None*	-	-
Hamimaera hamigera	Amphipod	*None*	-	-
Hemicypris dentatomarginata	Ostracod	*None*	-	-
Hemigrapsus penicillatus	Crab	*Enteromyces callianassae*	Eccrinales	McDermott, 2011
		Levinseniella conicostoma	Trematode	
		Maritrema longiforme		
		Maritrema setoenensis		
		Microphalloides japonicus		
		Probolocoryphe asadai		
		Spelotrema macrorchis		
		Sacculina sp.	Rhizocephalan	
Hemigrapsus sanguineus	Crab	*Unidentified microsporidian parasite*	Microsporidia	McDermott, 2011
		Maritrema jebuensis	Trematode	
		Maritrema setoenensis		
		Microphalloides japonicus		
		Probolocoryphe asadai		
		Spelotrema capellae		
		Unidentified larval nematode	Nematode	
		Polyascus polygenea	Rhizocephala	
		Sacculina nigra		
		Sacculina senta		
Hemigrapsus takanoi	Crab	*Himasthla elongata*	Trematode	Welsh et al. 2014
		Renicola roscovita		Goedknegt et al. 2015
Hemimysis anomala	Shrimp	*None*	-	-
Herbstia nitida	Crab	*None*	-	-
Herrmannella duggani	Copepod	*None*	-	-
Heterocope appendiculata	Copepod	*Acineta euhaetae*	Suctorian	Samchyshyna, 2008
		Diphyllobothrium norvegicum	Cestode	Halvorsen, 1966
		Proteocephalus torulosus		Sysoev et al. 1994
Heterolaophonte hamondi	Copepod	*None*	-	-
Heterosaccus dollfusi	Rhizocephalan	*None*	-	-
Hexapleomera robusta	Tanaidacean	*None*	-	-
Homarus americanus	Lobster	*Gaffkya homari*	Bacteria	Cornick and Stewart, 1968b
		Anophryoides haemophila	Ciliated protist	Cawthorn et al. 1996
		Lagenidium callinectes	Fungi	Gill-Turnes and Fenical, 1992
		Various epibiotic bacteria	Bacteria	
		Fusarium sp.	Fungi	Lightner and Fontaine, 1975
		Vibrio sp. BML 79-078	Bacteria	Bowser et al. 1981
		Vibrio anguillarum		

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Protozoan parasite	Protist	Russell et al. 2000
		Aerococcus viridans	Bacteria	Johnson et al. 1981
		Vibrio fluvialis	Bacteria	Beale et al. 2008
		Ascarophis sp.	Nematode	Boghen, 1978
		Flagellate	Protist	
		Histriobdella homari	Annelid	
		Porospora gigantea	Gregarine	
		Paramoeba sp.	Amoeba	Mullen et al. 2004
		Polymorphus botulus	Acanthocephalan	Brattey and Campbell, 1986
		Hysterothylacium sp.	Nematode	
		Stichocotyle nephropsis	Trematode	
		Hyphomicrobiumindicum indicum	Bacteria	Cawthorn, 2011
		Leucothrix mucor		
		Haliphthoros mildfordensis	Oomycete	
		Neoparamoeba pemaquidensis	Amoeba	
		WSSV	Virus	Clark et al. 2013
		170 bacterial taxa via pyrosequencing	Bacteria	Meres et al. 2012
		Necrotizing hepatopancreatitis	Bacteria	Shield et al. 2012
		Idiopathic blindness		
		Nicothoe astaci	Copepod	Davies et al. 2015
		Arcobacter sp.	Bacteria	Welsh et al. 2011
		Aspergillus awamori	Fungi	Karthikeyan et al. 2015
		Nectonema agile	Helminth	Schmidt-Rhaesa et al. 2013
Hyastenus hilgendorfi	Crab	*None*	-	-
Ianiropsis tridens	Isopod	*None*	-	-
Idotea metallica	Isopod	*None*	-	-
Idyella pallidula	Copepod	*None*	-	-
Incisocalliope aestuarius	Amphipod	*None*	-	-
Iphigenella shablensis	Amphipod	*None*	-	-
Ischyrocerus commensalis	Amphipod	*None*	-	-
Isocypris beauchampi cicatricosa	Ostracod	*None*	-	-
Ixa monodi	Crab	*None*	-	-
Jaera istri	Isopod	*None*	-	-
Jaera sarsi	Isopod	*None*	-	-
Jassa marmorata	Amphipod	*None*	-	-
Jasus lalandii	Lobster	*None*	-	-
Katamysis warpachowskyi	Shrimp	*None*	-	-
Labidocera detruncata	Copepod	*None*	-	-
Labidocera madurae	Copepod	*None*	-	-
Labidocera orsinii	Copepod	*None*	-	-
Labidocera pavo	Copepod	*None*	-	-
Latopilumnus malardi	Crab	*None*	-	-
Leptochela aculeocaudata	Shrimp	*Echinobothrium reesae*	Cestode	Ramadevi and Rao, 1974
Leptochela pugnax	Shrimp	*None*	-	-
Lernanthropus callionymicola	Copepod	*Obruspora papernae*	Microsporidian	Diamant et al. 2014
Libinia dubia	Crab	*Nosema sp.*	Microsporidian	Walker and Hinsch, 1972
		Lagenidium callinectes	Fungus	Bland and Amerson, 1974
		Hematodinium sp.	Dinoflagellate	Sheppard et al. 2003
		Frenzlina olivia	Gregarine	Watson, 1916
Ligia italica	Isopod	*Asellaria ligiae*	Fungus	Valle, 2006
Ligia oceanica	Isopod	*Maritrema linguilla*	Digenea	Benjamin and James, 1987
		Wolbachia sp.	Bacterial	Cordaux et al. 2001
Limnomysis benedeni	Shrimp	*None*	-	-
Limnoria quadripunctata	Isopod	*Mirofolliculina limnoriae*	Protist	Fernandez-Leborans, 2009
Limnoria tripunctata	Isopod	*Mirofolliculina limnoriae*	Protist	Fernandez-Leborans, 2009
		Alacrinella limnoriae	Fungus	Manier, 1961
		Gut Bacteria	Bacteria	Harris, 1993
		Vibrio proteolyticus	Bacteria	Gonzales et a. 2003
		Lobochona prorates	Protist	Mohr et al. 1963
Limulus polyphemus	Horseshoe crab	*"Bacterial disease"*	Bacterial	Bang, 1956
Lucifer hanseni	Shrimp	*None*	-	-
Lysmata kempi	Shrimp	*None*	-	-
Macromedaeus voeltzkowi	Crab	*None*	-	-
Macrophthalmus indicus	Decapod	*None*	-	-
Marsupenaeus japonicas (AKA *Penaeus japonicus*)	Shrimp	*WSSV*	Virus	Inouye et al. 1994
		Vibrio parahemolyticus	Bacteria	Zong et al. 2008
		Vibrio nigripulchritudo	Bacteria	Tahara et al. 2005
		Mourilyan virus	Virus	Sellars et al. 2005
		Vibrio zhuhaiensis	Bacteria	Jin et al. 2013
		Baculoviral mid-gut gland necrosis virus (BMNV)	Virus	Takahashi et al. 1996
		Vibrio penaeicida	Bacteria	Ishimaru et al. 1995
		Hepatopancreatic parvo-like virus (HPV)	Virus	Spann et al. 1997
		IPN-like virus	Virus	Bovo et al. 1984

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Infectious hypodermal and hematopoietic necrosis virus (IHHN)	Virus	Lightner et al. 1983
		Aeromonas spp.	Bacteria	Yasuda and Kitao, 1980
		Vibrio spp.		
		Pseudomonas spp.		
		Flavobacterium spp.		
		Staphylococcus spp.		
		Unknown bacterial species		
		Vibrio alginolyticus	Bacteria	Lee et al. 1996
		Fusarium solani	Fungus	Bian and Egusa, 1981
		Fusarium moniliforme	Fungus	Rhoobunjongde et al. 1991
		Unknown microsporidian	Microsporidian	Hudson et al. 2001
		Fusarium oxysporum	Fungus	Souheil et al. 1999
		Mollicute-like organism	Bacterial	Choi et al. 1996
Matuta victor	Crab	*None*	-	-
Megabalanus coccopoma	Barnacle	*None*	-	-
Megabalanus tintinnabulum	Barnacle	*Cephaloidophora communis*	Gregarine	Lacombe et al. 2002
Melita nitida	Amphipod	*None*	-	-
Menaethius monoceros	Crab	*Tylokepon biturus*	Isopod	An, 2009
		Sacculina calva	Sacculinid	Boschma, 1950
Metacalanus acutioperculum	Copepod	*None*	-	-
Metapenaeopsis aegyptia	Shrimp	*None*	-	-
Metapenaeopsis mogiensis consobrina	Shrimp	*None*	-	-
Metapenaeus affinis	Shrimp	Yellow Head Virus	Virus	Longyant et al. 2006
		Hepatopancreatic parvovirus	Virus	Manjanaik et al. 2005
		WSSV	Virus	Joseph et al. 2015
		Cotton shrimp disease	Microsporidia	Jose, 2000
		Bacterial disease	Bacteria	Rao and Soni, 1988
		Ciliated protists	Protoza	
		Perezia affinis	Microsporidia	
		Vibrio paraheamolyticus	Bacteria	Chakraborty et al. 2008
Metapenaeus monoceros	Shrimp	*WSSV*	Virus	Hossain et al. 2001
		Monodon baculovirus	Virus	Manivannan et al. 2004
		Orbione sp.	Isopod	An et al. 2013
				Printrakoonand Purivirojkul, 2012
		Protozoa	Protozoa	Deepa, 1997
		Perezia nelsoni	Microsporidia	Boyko, 2012
Metapenaeus stebbingi	Shrimp	*None*	-	-
Micippa thalia	Decapod	*None*	-	-
Micruropus possolskii	Amphipod	*None*	-	-
Mitrapus oblongus	Copepod	*None*	-	-
Moina affinis	Waterflea	*Bunodera spp.*	Trematode	Cannon, 1971
Moina weismanni	Waterflea	*None*	-	-
Monocorophium acherusicum	Amphipod	*None*	-	-
Monocorophium insidiosum	Amphipod	*None*	-	-
Monocorophium sextonae	Amphipod	*None*	-	-
Monocorophium uenoi	Amphipod	*None*	-	-
Muceddina multispinosa	Copepod	*None*	-	-
Myra subgranulata	Crab	*None*	-	-
Mysis relicta	Shrimp	*Cyanthocephalus truncatus*	trematode	Amin, 1978
		Acanthocephalan species	Acanthocephala	Wolff, 1984
		Echinorhynchus leidyi	Acanthocephala	Prychitko and Nero, 1983
		Various protozoan epibionts	Protozoa	Fernandez-Leborans, 2004
		Cystidicola cristivomeri	Nematode	Black and Lankester, 1980
Necora puber	Crab	*Hematodinium sp.*	Dinoflagellate	Stentiford et al. 2003
		Yeast-like organism	Yeast	
		Polymorphus botulus	Acanthocephalan	Nickol et al. 1999
		Protozoan epibionts	Protozoa	Fernandez-Leborans and Gabilondo, 2008
Neoergasilus japonicus	Copepod	*None*	-	-
Neomysis integer	Shrimp	*None*	-	-
Nikoides sibogae	Shrimp	*None*	-	-
Nothobomolochus fradei	Copepod	*None*	-	-
Notopus dorsipes	crab	*None*	-	-
Obesogammarus crassus	Amphipod	*Pleistophora muelleri*	Microsporidia	Ovcharenko and Yemeliyanova, 2009
		Nosema pontogammari		
		Cephaloidophora sp.	Gregarine	
		Uradiophora ramosa		
Obesogammarus obesus	Amphipod	*None*	-	-
Odontodactylus scyllarus	Shrimp	*None*	-	-
Ogyrides mjoebergi	Shrimp	*None*	-	-
Oithona davisae	Copepod	*None*	-	-
Oithona plumifera	Copepod	*Blastodinium oviforme*	Protozoa	Skovgaard and Saiz, 2006

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Paradinium spp.	Protozoa	Skovgaard and Daugbjerg, 2008
		Vibrio cholarae	Bacteria	Lizárraga-Partida et al. 2009
		Blastodinium oviforme	Dinoflagellate	Skovgaard and Salomonsen, 2009
Oithona setigera	Copepod	*None*	-	-
Onisimus sextoni	Amphipod	*None*	-	-
Orchestia cavimana	Amphipod	*Dictyocoela cavimanum*	Microsporidia	Terry et al. 2004
Orconectes immunis	Crayfish	*Aphanomyces astaci*	Oomycete	Schrimpf et al. 2013
		Psorospermium sp.	Mesomycetozoan	Hentonen et al. 1994
Orconectes limosus	Crayfish	*Aphanomyces astaci*	Oomycete	Kozubíková et al. 2011
		WSSV	Virus	Corbel et al. 2001
		Psorospermium orconectis	Mesomycetozoan	Hentonen et al. 1994
		Psorospermium haeckeli		Vogt and Rug, 1995
		Epistylis niagarae	Ciliated protozoa	Fernandez-Leborans and Tato-Porto, 2000
		Cothurnia curva		
		Cothurnia variabilis		
		Cyclodonta staphylinus		
		Branchiobdella hexodonta	Annelid	Ďuris et al. 2006
Orconectes rusticus	Crayfish	*Microphallus sp.*	Trematode	Sargent et al. 2014
		Psorospermium sp.	Mesomycetozoan	Henttonen et al. 1994
		Crepidostomum cornutum	Trematode	Corey, 1988
		4 Branchiobdellidan worms	Annelida	Duris et al. 2006
		Dreissena polymorpha	Mussel	
		Argulus cf. foliaceus	Crustacean	
		Plumatella repens	Bryozoan	
		Aphanomyces astaci	Oomycete	Svoboda et al. 2017
Orconectes virilis	Crayfish	*Batrachochytrium dendrobatidis*	Fungus	McMahon et al. 2013
		Thelohania contejeani	Microsporidian	Graham and France, 1986
		WSSV	Virus	Davidson et al. 2010
		Spiroplama penaei	Bacteria	
		H. bacteriophora	Nematode	
		H. marelatus	Nematode	
		Microphallus sp.	Trematode	Sargent et al. 2014
		Psorospermium sp.	Mesomycetozoan	Henttonen et al. 1994
		Aphanomyces astaci	Oomycete	Svoboda et al. 2017
Pacifastacus leniusculus	Crayfish	*WSSV*	Virus	Liu et al. 2006
		Aeromonas hydrophila	Bacteria	Jiravanichpaisal et al. 2009
		Aphanomyces astaci	Oomycete	Persson et al. 1987
		Thelohania contejeani	Microsporidian	Dunn et al. 2009
		Fusarium solani	Fungus	Chinain and Vey, 1988
		Pacifastacus leniusculus bacilliform virus	Virus	Longshaw et al. 2011
		Psorospermium sp.	Mesomycetozoan	
Palaemon elegans	Shrimp	Infectious Pancreatic Necrosis Virus (IPNV)	Virus	Mortensen, 1993
		Bay of Piran shrimp virus (BPSV)	Virus	Vogt, 1996
		Hepatopancreatic brush border lysis (HBL)	Bacteria	Vogt, 1992
		Rickettsiae	Bacteria	Vogt and Strus, 1998
		Palaemon B-cell Reo-like virus (PBRV)	Virus	
		Aggregata octopiana	Apicomplexa	Arias et al. 1998
Palaemon macrodactylus	Shrimp	*Lagenidium callinectes*	Fungi	Fisher, 1983
		WSSV	Virus	Matorelli et al. 2010
		Infectious hypodermal and haematopoietic necrosis virus	Virus	
Palaemonella rotumana	Shrimp	*Metaphrixus intutus*	Bopyrid	Bruce, 1986
Panulirus guttatus	Lobster	*None*	-	-
Panulirus ornatus	Lobster	*WSSV*	Virus	Musthaq et al. 2006
		Vibrio owensii	Bacteria	Goulden et al. 2012
		Vibrio harveyi	Bacteria	Bourne et al. 2006
		Microsporidian sp.	Microsporidia	Kiryu et al. 2009
		Various microbial commensals in culture	Various	Bourne et al. 2004
		Fusarium sp.	Fungus	Nha et al. 2009
Paracalanus indicus	Copepod	*Atelodinium sp.*	Dinoflagellate	Kimmerer and McKinnon, 1990
Paracaprella pusilla	Shrimp	*None*	-	-
Paracartia grani	Copepod	*Marteilia refringens*	Protist	Audemard et al. 2002
Paracerceis sculpta	Isopod	*None*	-	-
Paradella dianae	Isopod	*None*	-	-
Paraergasilus longidigitus	Copepod	*None*	-	-
Paralithodes camtschaticus	Crab	*Ciliates*	Protozoa	Jansen et al. 1998
		Flagellates	Protozoa	
		Turbellaria	Helminth	
		Nemertea (2 spp.)	Helminth	
		Hirudinea	Helminth	

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Acanthocephala	Helminth	
		Ischyrocercus commensalis	Amphipod	
		Tisbe sp.	Copepod	
		Mytilus edulis	Mussel	
		Johanssonia arctica	Leech	Falk-Peterson et al. 2011
		Hematodinium sp.	Dinoflagellate	Ryazanova et al. 2010
		Fouling community (various)	Various	Dvoretsky and Dvoretsky, 2009
		Herpes-Like virus	Virus	Ryazanova et al. 2015
		Thelohania/Ameson	Microsporidia	Ryazanova and Eliseikina, 2010
		Notosmobdella cyclostoma	Leech	Zara et al. 2009
Paramphiascella vararensis	Copepod	*None*	-	-
Paramysis (Mesomysis) intermedia	Shrimp	*None*	-	-
Paramysis (Serrapalpisis) lacustris	Shrimp	*None*	-	-
Paramysis baeri	Shrimp	*None*	-	-
Paramysis ullskyi	Shrimp	*None*	-	-
Paranthura japonica	Isopod	*None*	-	-
Parvocalanus crassirostris	Copepod	*None*	-	-
Parvocalanus elegans	Copepod	*None*	-	-
Parvocalanus latus	Copepod	*None*	-	-
Penaeus aztecus	Shrimp	*IHHN Virus*	Virus	Bray et al. 1994
		WSSV	Virus	Lightner et al. 1998
		Yellow head virus	Virus	
		Taura symdrome	Virus	Overstreet et al. 1997
		Cestdoe larvae	Cestode	Kruse, 1959
		Fusarium sp.	Fungus	Solangi and Lightner, 1976
		Baculovirus penaei	Virus	Momoyama and sano, 1989
		Tuzetia weidneri	Microsporidia	Tourtip et al. 2009
		Vibrio sp.	Bacteria	Anderson et al. 1987
		Prochristianella penaei	Cestode	Ragen and Aldrich, 1972
Penaeus hathor	Shrimp	*None*	-	-
Penaeus merguiensis	Shrimp	*WSSV*	Virus	Wang et al. 2002
		Epipenaeon ingens	Bopyrid	Owens, 1983
		Hepatopancreatic parvo-like virus (PmergDNV)	Virus	Roubal et al. 1989
		Baculovirus	Virus	Doubrovsky et al. 1988
		Various bacteria flora	Bacteria	Oxley et al. 2002
		Microsporidian sp.	Fungi	Enriques et al. 1980
		Gill-associated virus	Virus	Spann et al. 2000
		Polypocephalus sp.	Cestode	Owens, 1985
		Spawner isolated mortality virus	Virus	Owen et al. 2003
		IHHNV	Virus	Krabsetsve et al. 2004
		Mourilyan virus	Virus	Cowley et al. 2005
Penaeus semisulcatus	Shrimp	*Epipenaeon ingens*	Bopyrid	Somers and Kirkwood, 1991
		Epipenaeon elegans	Bopyrid	Abu-Hakima, 1984
		WSSV	Virus	Venegas et al. 2000
		YHV	Virus	
		Fusarium sp.	Fungi	Colorni, 1989a
		Sporozoan infection	Microsporidia	Thomas, 1976
		HPV	Virus	Manjanaik et al. 2005
		IHHN	Virus	Colorni, 1989b
		Bacterial necrosis	Bacteria	Tareen, 1982
		Vibrio sp.	Bacteria	
		Filamentous Bacteria	Bacteria	
		Shell disease	Unknown	
		Lagenidium sp.	Fungi	
		Various protozoa	Protist	
		BMNV	Virus	Coman and Crocos, 2003
		Ameson sp.	Microsporidia	Owens and Glazebrook, 1988
		Thelohania sp.	Microsporidia	
Penaeus subtilis	Shrimp	*WSSV*	Virus	Vijayan et al. 2005
		IHHNV	Virus	Coelho et al. 2009
		Baculovirus	Virus	LeBlanc et al. 1991
Penilia avirostris	Water flea	*Hyphochyrium peniliae*	Fungus	Porter. 1986
		Vibrio cholerae	Bacteria	Martinelli-Filho et al. 2016
Percnon gibbesi	Crab	*None*	-	-
Photis lamellifera	Amphipod	*None*	-	-
Pilumnoides inglei	Crab	*None*	-	-
Pilumnopeus vauquelini	Crab	*None*	-	-
Pilumnus minutus	Crab	*None*	-	-
Pilumnus spinifer	Crab	*Aggregata sp.*	Gregarine	Vivares, 1970
Plagusia squamosa	Crab	*None*	-	-
Platorchestia platensis	Amphipod	*Levinseniella carteretensis*	Trematode	Bousfield and Heard, 1986
Platyscelus armatus	Amphipod	*None*	-	-
Pollicipes pollicipes	Barnacle	*None*	-	-

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
Pontogammarus aestuarius	Amphipod	*None*	-	-
Pontogammarus robustoides	Amphipod	*Dictyocoela sp.*	Microsporidia	Wilkinson et al. 2011
		Nosema sp.	Microsporidia	Ovcharenko and Yemeliyanova, 2009
		Cephaloidophora mucronata	Gregarine	Ovcharenko et al. 2009
		Uradiophora ramosa	Gregarine	
		Thelohania sp.	Microsporidia	
Porcellidium ovatum	Copepod	*None*	-	-
Porcelloides tenuicaudus	Crab	*None*	-	-
Portunus segnis	Crab	*Heterosaccus dollfusi*	Barnacle	Innocenti and Galil, 2011
Proameira simplex	Copepod	*None*	-	-
Proasellus coxalis	Isopod	*Acanthocephalus sp.*	Acanthocephalan	Contoli et al. 1967
		Asellaria gramenei	Fungi	Valle, 2006
		Maritrema feliui	Trematode	Tkach, 1998
Proasellus meridianus	Isopod	*Asellaria gramenei*	Trichomycete	Valle, 2006
Procambarus acutus	Crayfish	*Alloglossoides caridicola*	Trematode	Lumsden et al. 1999
		Alloglossidium dolandi	Trematode	Turner, 2007
		Aphanomyces astaci	Oomycete	Tilmans et al. 2014
		Annelids	Anndelid	Miller, 1981
Procambarus clarkii	Crayfish	*Sprioplasma*	Bacteria	Wang et al. 2005
		WSSV	Virus	Jha et al. 2006
		Aphanomyces astaci	Oomycete	Diegues-Uribeondo and Soderhall, 1993
		Psorospermium sp.	Mesomycetozoan	Henttonen et al. 1997
		Three Commensal Protozoa	Protozoa	Vogelbein and Thune, 1988
		Digenea	Trematode	Longshaw et al. 2012
		Aeromonas hydrophila	Bacteria	Dong et al. 2011
Procambarus fallax f. virginalis	Crayfish	*Aphanomyces astaci*	Oomycete	Keller et al. 2014
		Psorospermium sp.	Mesomycetozoan	Henttonen et al. 1994
		Coccidian RLO	Bacteria	Longshaw et al. 2012
		Aeromonas sobria	Bacteria	
		Citrobacter freundii	Bacteria	
		Grimontia hollisae	Bacteria	
		Pasteurella multocida	Bacteria	
		Ciliated protists	Protozoa	
		Unspecified Ostracod	Ostracod	
		Unspecified mites	Mite	
Pseudocuma (Stenocuma) graciloides	Copepod	*None*	-	-
Pseudocuma cercaroides	Copepod	*None*	-	-
Pseudodiaptomus inopinus	Copepod	*None*	-	-
Pseudodiaptomus marinus	Copepod	*None*	-	-
Pseudomyicola spinosus	Copepod	*Mid-gut bacteria*	Bacteria	Yoshikoshi and Ko, 1991
Ptilohyale littoralis	Amphipod	*None*	-	-
Rhabdosoma whitei	Amphipod	*None*	-	-
Rhithropanopeus harrisii	Crab	*Cancricepon choprae*	Isopod	Markham, 1975
		Loxothylacus panopei	Parasitic barnacle	Boschma, 1972
		Potential vector of: *Dermocystidium marinum*	Fungus	Hoese, 1962
		Haplosporidium (= *Minchinia*) *cadomensis*	Haplosporidian	Marchand and Sprauge, 1979
		Haplosporidium sp.	Haplosporidian	Rosenfield et al. 1969
Rimapenaeus similis	Shrimp	*None*	-	-
Robertgurneya rostrata	Copepod	*None*	-	-
Saduria entomon	Isopod	*Cryptococcus laurentii*	Yeast	Hryniewiecka-Szyfter and Babula, 1997
		Mesanophrys	Protozoa	Hryniewiecka-Szyfter et al. 2001
Saron marmoratus	Shrimp	*Bopyrella saronae*	Bopyrid	Bourdon and Bruce, 1979
Sarsamphiascus tenuiremis	Copepod	*None*	-	-
Scherocumella gurneyi	Copepod	*None*	-	-
Scolecithrix sp.	Copepod	*Blastodinium galatheanum*	Dinoflagellate	Skovgaard and Salomonsen, 2009
Scottolana longipes	Copepod	*None*	-	-
Scyllarus caparti	Lobster	*None*	-	-
Simocephalus hejlongjiangensis	Water flea	*None*	-	-
Sinelobus stanfordi	Tanaid	*None*	-	-
Sirpus monodi	Crab	*None*	-	-
Skistodiaptomus pallidus	Copepod	*Bothriocephalus acheilognathi*	Tapeworm	Marcogliese and Esch, 1989
Solenocera crassicornis	Shrimp	*Various bacteria*	Bacteria	Prasad et al. 1989
		WSSV	Virus	Pradeep et al. 2012
Sphaeroma quoianum	Isopod	*None*	-	-
Sphaeroma serratum	Isopod	*Palavascia sphaeromae*	Trichomycete	Manier, 1978
		Vorticella minima	Protist	Naidenova and Mordvinova, 1985
		Vorticella sphaeroma		
		Vorticella lima		
		Zoothamnium alternans		

Host Species	Organism Type	Pathogen or disease	Pathogen Type	Reference
		Zoothamnium sphaeroma		
		Zoothamnium perejaslawzeva		
		Cothurnia achtiari		
		Delamurea loricata		
		Delamurea maeatica		
		Tanriella lomi		
		Aceneta tuberosa		
Sphaeroma walkeri	Isopod	*Lagenophrys cochinensis*	Protist	Fernandez-Leborans, 2009
Sphaerozius nitidus	Crab	*None*	-	-
Sternodromia spinirostris	Decapod	*None*	-	-
Strandesia spinulosa	Ostracod	*Neoechinorhynchus cylindratus*	Acanthocephalan	Eure, 1976
Stygobromus ambulans	Amphipod	*None*	-	-
Synidotea laevidorsalis	Isopod	*None*	-	-
Synidotea laticauda	Isopod	*None*	-	-
Taeniacanthus lagocephali	Copepod	*None*	-	-
Tanycypris pellucida	Ostracod	*None*	-	-
Tessepora atlanticum	Isopod	*None*	-	-
Tetraclita squamosa rufotinta	Copepod	*None*	-	-
Thalamita gloriensis	Crab	*None*	-	-
Thalamita indistincta	Crab	*None*	-	-
Tracheliastes maculatus	Parasitic Copepod	*None*	-	-
Tracheliastes polycolpus	Parasitic Copepod	*None*	-	-
Trachysalambria palaestinensis	Shrimp	*None*	-	-
Triconia hawii	Copepod	*None*	-	-
Triconia minuta	Copepod	*None*	-	-
Triconia rufa	Copepod	*None*	-	-
Triconia umerus	Copepod	*None*	-	-
Tulеariocaris neglecta	Shrimp	*None*	-	-
Urocaridella pulchella	Shrimp	*None*	-	-
Wlassicsia pannonica	Branchiopod	*None*	-	-
Xanthias lamarckii	Crab	*None*	-	-

Appendix to Chapter 7

Appendix Table 7.1: Clostest similarity, and scores, for genes belonging to *Aquarickettsiella crustaci*.

A. crustaci (PROKKA)	Subject Sequence ID	Subject Name	Sequence similarity	Alignment length	Mismatched bases	Gaps	e-value	bitscore
1	gi|966509820|ref|WP_058526411.1|	hypothetical protein [Legionella erythra]	43.4	341	179	4	8.00E-86	276
2	gi|966415125|ref|WP_058458410.1|	P-type conjugative transfer protein VirB9 [Fluoribacter bozemanae]	49.58	236	111	4	2.00E-73	236
3	gi|966477512|ref|WP_058508245.1|	hypothetical protein [Legionella quinlivanii]	41.38	232	132	3	8.00E-55	188
4	gi|966415123|ref|WP_058458408.1|	Legionella vir-like protein LvhB6 [Fluoribacter bozemanae]	40.22	358	206	4	6.00E-88	281
5	gi|966442368|ref|WP_058482630.1|	hypothetical protein [Legionella spiritensis]	38.71	124	70	2	4.00E-18	85.1
6	gi|966400663|ref|WP_058444258.1|	helix-turn-helix transcriptional regulator [Legionella feeleii]	37.5	104	61	1	2.00E-11	66.6
7	gi|698848203|emb|CEG62203.1|	exported protein of unknown function [Tatlockia micdadei]	38.46	39	23	1	1.2	33.9
8	gi|966442367|ref|WP_058482629.1|	hypothetical protein [Legionella spiritensis]	50.21	235	117	0	1.00E-70	228
9	gi|489728678|ref|WP_003632794.1|	hypothetical protein [Legionella longbeachae]	44.71	823	450	4	0	741
10	gi|1003856556|ref|WP_061468067.1|	hypothetical protein [Legionella pneumophila]	43.62	94	52	1	3.00E-18	83.6
11	gi|966509827|ref|WP_058526418.1|	hypothetical protein [Legionella erythra]	42.67	75	39	1	4.00E-07	54.3
12	gi|499260817|ref|WP_010958357.1|	hypothetical protein [Coxiella burnetii]	59.57	282	112	2	2.00E-112	338
13	gi|644964296|ref|WP_025385051.1|	hypothetical protein [Legionella oakridgensis]	63.19	163	60	0	4.00E-72	227
14	gi|769981819|ref|WP_045097803.1|	hypothetical protein [Legionella fallonii]	72.15	219	60	1	2.00E-113	337
15	gi|769981818|ref|WP_045097802.1|	MULTISPECIES: hypothetical protein [Legionella]	60.95	210	79	2	6.00E-90	275
16	gi|492905054|ref|WP_006035460.1|	hypothetical protein [Rickettsiella grylli]	56.31	206	89	1	6.00E-75	237
17	gi|498284818|ref|WP_010598974.1|	hypothetical protein [Diplorickettsia massiliensis]	74.34	339	84	2	0	529
18	gi|498284817|ref|WP_010598973.1|	hypothetical protein [Diplorickettsia massiliensis]	49.89	435	190	7	3.00E-120	369
19	gi|966442380|ref|WP_058482642.1|	conjugal transfer protein TraD [Legionella spiritensis]	54.02	87	40	0	1.00E-23	97.1
20	gi|1006638066|ref|WP_061818919.1|	hypothetical protein [Legionella pneumophila]	55.88	68	27	2	7.00E-10	60.1
21	gi|1011913874|ref|WP_062727088.1|	Ti-type conjugative transfer relaxase TraA [Legionella pneumophila]	46.95	475	243	5	2.00E-143	446
22	gi|406939893|gb|EKD72822.1|	hypothetical protein ACD_45C00578G09 [uncultured bacterium]	29.1	134	83	5	0.059	42.7
23	gi|1010983068|ref|WP_061941777.1|	hypothetical protein [Collimonas pratensis]	53.92	204	79	2	4.00E-70	226
24	gi|406937722|gb|EKD71097.1|	hypothetical protein ACD_46C00272G02 [uncultured bacterium]	59.19	223	90	1	3.00E-88	272
25	gi|1028824319|ref|WP_064005173.1|	hypothetical protein [Piscirickettsiaceae bacterium NZ-RLO]	41.57	89	52	0	3.00E-14	80.1
26	gi|500791719|ref|WP_011997223.1|	response regulator [Coxiella burnetii]	37.9	124	75	1	1.00E-18	86.7
27	gi|159121699|gb|EDP47037.1|	hypothetical protein RICGR_0037 [Rickettsiella grylli]	92.86	56	4	0	9.00E-28	105
28	gi|492904680|ref|WP_006035086.1|	tryptophan/tyrosine permease [Rickettsiella grylli]	81.39	403	75	0	0	595
29	gi|492904781|ref|WP_006035187.1|	(Fe-S)-cluster assembly protein [Rickettsiella grylli]	62.99	127	46	1	5.00E-50	167
30	gi|750333118|ref|WP_040615037.1|	hypothetical protein [Rickettsiella grylli]	94.38	89	5	0	1.00E-52	171
31	gi|492904600|ref|WP_006035006.1|	hypothetical protein [Rickettsiella grylli]	68.81	295	89	2	9.00E-146	425
32	gi|492905113|ref|WP_006035519.1|	peptidase C69 [Rickettsiella grylli]	74.77	444	111	1	0	702

A. crustaci (PROKKA)	Subject Sequence ID	Subject Name	Sequence similarity	Alignment length	Mismatched bases	Gaps	e-value	bitscore
33	gi|492905392|ref|WP_006035798.1|	rhodanese domain protein [Rickettsiella grylli]	81.43	140	26	0	1.00E-77	239
34	gi|494080950|ref|WP_007022990.1|	glutaredoxin 3 [Neptuniibacter caesariensis]	64.63	82	29	0	2.00E-30	114
35	gi|492904526|ref|WP_006034932.1|	preprotein translocase subunit SecB [Rickettsiella grylli]	77.07	157	35	1	4.00E-83	254
36	gi|492904870|ref|WP_006035276.1|	dephospho-CoA kinase [Rickettsiella grylli]	59.21	228	90	1	9.00E-90	276
37	gi|492905103|ref|WP_006035509.1|	hypothetical protein [Rickettsiella grylli]	56.83	586	224	9	0	650
38	gi|498283656|ref|WP_010597812.1|	outer membrane protein TolC [Diplorickettsia massiliensis]	59.37	443	171	3	0	535
39	gi|492904702|ref|WP_006035108.1|	ADP-ribose pyrophosphatase [Rickettsiella grylli]	67.48	206	67	0	5.00E-95	288
40	gi|492904551|ref|WP_006034957.1|	DNA topoisomerase IV subunit B [Rickettsiella grylli]	86.35	630	83	3	0	1134
41	gi|492904599|ref|WP_006035005.1|	SAM-dependent methyltransferase [Rickettsiella grylli]	73.06	219	59	0	3.00E-115	340
43	gi|492904778|ref|WP_006035184.1|	carbonate dehydratase [Rickettsiella grylli]	78.22	202	44	0	9.00E-118	345
44	gi|492905380|ref|WP_006035786.1|	iron-sulfur cluster-binding protein [Rickettsiella grylli]	59.33	209	84	1	2.00E-81	254
45	gi|492905551|ref|WP_006035957.1|	methionine--tRNA ligase [Rickettsiella grylli]	73.41	549	146	0	0	877
46	gi|492904584|ref|WP_006034990.1|	sodium:proton antiporter [Rickettsiella grylli]	75.91	274	65	1	2.00E-150	434
47	gi|492905018|ref|WP_006035424.1|	deoxycytidine triphosphate deaminase [Rickettsiella grylli]	90.37	187	18	0	1.00E-122	357
48	gi|492905425|ref|WP_006035831.1|	tryptophan--tRNA ligase [Rickettsiella grylli]	80.33	361	71	0	0	618
49	gi|492905487|ref|WP_006035893.1|	phosphoenolpyruvate carboxykinase (ATP) [Rickettsiella grylli]	78.78	523	110	1	0	878
50	gi|406936432|gb|EKD70154.1|	Pyrroline-5-carboxylate reductase [uncultured bacterium]	53.87	271	123	2	1.00E-92	287
51	gi|492904839|ref|WP_006035245.1|	mannose-1-phosphate guanyltransferase [Rickettsiella grylli]	76	225	53	1	3.00E-120	353
52	gi|492904458|ref|WP_006034864.1|	aminoglycoside phosphotransferase [Rickettsiella grylli]	70.5	339	98	1	1.00E-175	503
53	gi|492904255|ref|WP_006034661.1|	4-hydroxy-tetrahydrodipicolinate synthase [Rickettsiella grylli]	71.43	294	80	1	9.00E-155	447
54	gi|750333121|ref|WP_040615040.1|	hypothetical protein [Rickettsiella grylli]	60.27	73	28	1	3.00E-18	82.4
56	gi|492904389|ref|WP_006034795.1|	2'-5' RNA ligase [Rickettsiella grylli]	92.23	193	15	0	2.00E-125	364
57	gi|750333123|ref|WP_040615042.1|	cytochrome ubiquinol oxidase subunit I [Rickettsiella grylli]	83.04	460	78	0	0	801
58	gi|492905541|ref|WP_006035947.1|	ubiquinol oxidase subunit II, cyanide insensitive [Rickettsiella grylli]	81.82	330	60	0	0	547
59	gi|492904622|ref|WP_006035028.1|	hypothetical protein [Rickettsiella grylli]	31.07	441	268	10	3.00E-38	155
60	gi|492905152|ref|WP_006035558.1|	peptide deformylase [Rickettsiella grylli]	88.62	167	19	0	5.00E-103	305
61	gi|492904912|ref|WP_006035318.1|	methionyl-tRNA formyltransferase [Rickettsiella grylli]	82.86	315	53	1	0	546
62	gi|492905311|ref|WP_006035717.1|	16S rRNA (cytosine(967)-C(5))-methyltransferase [Rickettsiella grylli]	64.37	435	154	1	0	570
63	gi|498283606|ref|WP_010597762.1|	hypothetical protein [Diplorickettsia massiliensis]	40.71	140	74	3	2.00E-25	108
64	gi|498283605|ref|WP_010597761.1|	hypothetical protein [Diplorickettsia massiliensis]	38.26	264	159	1	4.00E-49	177
65	gi|492904634|ref|WP_006035040.1|	arginine--tRNA ligase [Rickettsiella grylli]	76.36	588	137	2	0	949
66	gi|492905562|ref|WP_006035968.1|	hypothetical protein [Rickettsiella grylli]	53.78	225	98	5	6.00E-67	218
67	gi|492904803|ref|WP_006035209.1|	ATP-dependent protease subunit HslV [Rickettsiella grylli]	95.68	185	8	0	6.00E-124	360
68	gi|159120412|gb|EDP45750.1|	heat shock protein HslVU, ATPase subunit HslU [Rickettsiella grylli]	84.94	498	74	1	0	850
69	gi|492905256|ref|WP_006035662.1|	hypothetical protein [Rickettsiella grylli]	66.37	113	37	1	1.00E-48	163
70	gi|492904320|ref|WP_006034726.1|	tyrosine--tRNA ligase [Rickettsiella grylli]	80.5	400	78	0	0	681

A. crustaci (PROKKA)	Subject Sequence ID	Subject Name	Sequence similarity	Alignment length	Mismatched bases	Gaps	e-value	bitscore
71	gi|492905166|ref|WP_006035572.1|	rRNA (cytidine-2'-O-)-methyltransferase [Rickettsiella grylli]	72.5	280	76	1	2.00E-139	407
72	gi|492904559|ref|WP_006034965.1|	amino acid permease [Rickettsiella grylli]	86.31	453	62	0	0	758
73	gi|750333126|ref|WP_040615045.1|	hypothetical protein [Rickettsiella grylli]	80.08	1009	188	5	0	1558
74	gi|492905087|ref|WP_006035493.1|	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase [Rickettsiella grylli]	70.59	357	105	0	0	531
75	gi|492905072|ref|WP_006035478.1|	periplasmic protein [Rickettsiella grylli]	51.54	813	380	9	0	801
76	gi|159120398|gb|EDP45736.1|	outer membrane protein [Rickettsiella grylli]	65.28	576	196	3	0	766
77	gi|545360178|ref|WP_021615961.1|	hypothetical protein [Aggregatibacter sp. oral taxon 458]	30.38	79	50	2	0.29	40
78	gi|498283574|ref|WP_010597730.1|	hypothetical protein [Diplorickettsia massiliensis]	42.86	84	48	0	3.00E-11	66.6
79	gi|915327257|ref|WP_050763945.1|	D-alanyl-D-alanine carboxypeptidase [Rickettsiella grylli]	80.3	396	78	0	0	676
80	gi|492905411|ref|WP_006035817.1|	glycerol acyltransferase [Rickettsiella grylli]	71.48	298	84	1	3.00E-153	443
81	gi|492905552|ref|WP_006035958.1|	hydroxymethylbilane synthase [Rickettsiella grylli]	71.66	307	87	0	6.00E-152	441
82	gi|492904831|ref|WP_006035237.1|	endonuclease III [Rickettsiella grylli]	78.67	211	45	0	8.00E-112	331
83	gi|492905367|ref|WP_006035773.1|	peptidase, family S24 [Rickettsiella grylli]	86.12	209	29	0	7.00E-131	380
85	gi|492904429|ref|WP_006034835.1|	30S ribosomal protein S15 [Rickettsiella grylli]	87.06	85	11	0	2.00E-44	149
86	gi|750333380|ref|WP_040615299.1|	polyribonucleotide nucleotidyltransferase [Rickettsiella grylli]	86.42	707	94	2	0	1221
88	gi|492904424|ref|WP_006034830.1|	dihydroorotate dehydrogenase [Rickettsiella grylli]	66.85	356	116	2	6.00E-167	483
89	gi|750333382|ref|WP_040615301.1|	carbamoyl phosphate synthase small subunit [Rickettsiella grylli]	79.49	351	71	1	0	589
90	gi|750333132|ref|WP_040615051.1|	carbamoyl phosphate synthase large subunit [Rickettsiella grylli]	85.03	1062	159	0	0	1834
91	gi|750333134|ref|WP_040615053.1|	aspartate carbamoyltransferase [Rickettsiella grylli]	76.43	297	70	0	9.00E-157	453
92	gi|492904592|ref|WP_006034998.1|	aspartate carbamoyltransferase regulatory subunit [Rickettsiella grylli]	74.34	152	39	0	2.00E-75	234
93	gi|492905124|ref|WP_006035530.1|	dihydroorotase [Rickettsiella grylli]	77.7	408	91	0	0	658
94	gi|492904823|ref|WP_006035229.1|	HemY protein [Rickettsiella grylli]	66.32	291	98	0	3.00E-130	385
95	gi|492905267|ref|WP_006035673.1|	hypothetical protein [Rickettsiella grylli]	48.29	350	170	4	7.00E-86	275
96	gi|492904635|ref|WP_006035041.1|	uroporphyrinogen III methyltransferase [Rickettsiella grylli]	59.23	260	105	1	3.00E-93	288
97	gi|492905584|ref|WP_006035990.1|	phosphoglycerate kinase [Rickettsiella grylli]	71.61	391	111	0	0	544
98	gi|492905002|ref|WP_006035408.1|	pyruvate kinase [Rickettsiella grylli]	84.45	476	74	0	0	810
99	gi|492905448|ref|WP_006035854.1|	transcriptional repressor [Rickettsiella grylli]	84.89	139	21	0	4.00E-82	250
100	gi|492904862|ref|WP_006035268.1|	outer membrane protein assembly factor BamE [Rickettsiella grylli]	71.11	90	26	0	7.00E-42	144
101	gi|759381182|ref|WP_043107695.1|	RnfH family protein [endosymbiont of unidentified scaly snail isolate Monju]	52.17	92	44	0	2.00E-26	105
102	gi|492905426|ref|WP_006035832.1|	ubiquinone-binding protein [Rickettsiella grylli]	76.39	144	34	0	2.00E-76	236
103	gi|492904245|ref|WP_006034651.1|	SsrA-binding protein [Rickettsiella grylli]	83.97	156	25	0	1.00E-93	280
105	gi|492905447|ref|WP_006035853.1|	glycine cleavage system regulatory protein [Rickettsiella grylli]	80.92	173	31	1	3.00E-100	298
106	gi|492904974|ref|WP_006035380.1|	peroxiredoxin [Rickettsiella grylli]	79.87	154	31	0	1.00E-84	258
107	gi|492904363|ref|WP_006034769.1|	AI-2E family transporter [Rickettsiella grylli]	85.47	358	52	0	0	601
108	gi|492905119|ref|WP_006035525.1|	GMP synthetase [Rickettsiella grylli]	86.23	523	72	0	0	933

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109	gi|492904666|ref|WP_006035072.1|	IMP dehydrogenase [Rickettsiella grylli]	83.26	484	80	1	0	828
110	gi|498283509|ref|WP_010597665.1|	hypothetical protein [Diplorickettsia massiliensis]	71.56	218	60	1	9.00E-116	342
111	gi|498283508|ref|WP_010597664.1|	hypothetical protein [Diplorickettsia massiliensis]	56.33	158	69	0	2.00E-60	196
112	gi|492904543|ref|WP_006034949.1|	glycerophosphodiester phosphodiesterase [Rickettsiella grylli]	73.83	256	67	0	5.00E-139	405
113	gi|492904802|ref|WP_006035208.1|	nucleoside-diphosphate kinase [Rickettsiella grylli]	74.1	139	36	0	9.00E-69	216
114	gi|492904365|ref|WP_006034771.1|	bifunctional tRNA (adenosine(37)-C2)-methyltransferase TrmG/ribosomal RNA large subunit methyltransferase RlmN [Rickettsiella grylli]	76.08	372	82	1	0	600
115	gi|492904674|ref|WP_006035080.1|	type IV pilus biogenesis/stability protein PilW [Rickettsiella grylli]	71.32	265	70	3	1.00E-132	388
116	gi|492905145|ref|WP_006035551.1|	histidine--tRNA ligase [Rickettsiella grylli]	74.24	427	109	1	0	652
117	gi|492904339|ref|WP_006034745.1|	hypothetical protein [Rickettsiella grylli]	59.42	207	82	1	8.00E-75	236
118	gi|492904855|ref|WP_006035261.1|	outer membrane protein assembly factor BamB [Rickettsiella grylli]	69.17	386	118	1	0	572
119	gi|750333137|ref|WP_040615056.1|	ribosome biogenesis GTPase Der [Rickettsiella grylli]	76.39	449	104	2	0	668
120	gi|492905443|ref|WP_006035849.1|	DNA adenine methylase [Rickettsiella grylli]	72.93	266	72	0	5.00E-140	407
121	gi|492905287|ref|WP_006035693.1|	hypothetical protein [Rickettsiella grylli]	47.04	625	306	9	0	554
122	gi|492904655|ref|WP_006035061.1|	hypothetical protein [Rickettsiella grylli]	61.38	246	93	2	3.00E-97	298
123	gi|492905055|ref|WP_006035461.1|	type 11 methyltransferase [Rickettsiella grylli]	65.24	187	63	1	8.00E-80	248
124	gi|159120323|gb|EDP45661.1|	histidinol-phosphate aminotransferase [Rickettsiella grylli]	64.01	339	121	1	1.00E-141	419
125	gi|492904430|ref|WP_006034836.1|	type III pantothenate kinase [Rickettsiella grylli]	81.08	259	49	0	5.00E-144	417
126	gi|915327261|ref|WP_050763949.1|	hypothetical protein [Rickettsiella grylli]	58.74	223	92	0	2.00E-91	282
127	gi|492905171|ref|WP_006035577.1|	siderophore biosynthesis protein [Rickettsiella grylli]	76.35	630	143	6	0	985
128	gi|492905306|ref|WP_006035712.1|	MFS transporter [Rickettsiella grylli]	63.76	378	135	1	2.00E-164	479
133	gi|492905032|ref|WP_006035438.1|	acyl-[ACP]--phospholipid O-acyltransferase [Rickettsiella grylli]	80.93	1143	217	1	0	1895
134	gi|492904249|ref|WP_006034655.1|	ATPase AAA [Rickettsiella grylli]	77.25	422	96	0	0	699
135	gi|492905196|ref|WP_006035602.1|	ribosomal protein S6 modification protein [Rickettsiella grylli]	94.54	293	16	0	0	568
136	gi|492905444|ref|WP_006035850.1|	ribosomal protein S6 modification protein [Rickettsiella grylli]	78.38	148	32	0	3.00E-79	243
137	gi|159121512|gb|EDP46850.1|	stringent starvation protein B [Rickettsiella grylli]	84.62	130	19	1	1.00E-74	230
138	gi|492904629|ref|WP_006035035.1|	stringent starvation protein A [Rickettsiella grylli]	84.65	215	33	0	1.00E-132	384
139	gi|492905260|ref|WP_006035666.1|	ubiquinol--cytochrome c reductase cytochrome c1 subunit [Rickettsiella grylli]	60.94	233	83	2	3.00E-95	292
140	gi|915327339|ref|WP_050764027.1|	cytochrome b [Rickettsiella grylli]	71.53	404	113	1	0	570
141	gi|492904343|ref|WP_006034749.1|	ubiquinol-cytochrome c reductase iron-sulfur subunit [Rickettsiella grylli]	69.95	193	56	2	4.00E-95	287
142	gi|492904946|ref|WP_006035352.1|	30S ribosomal protein S9 [Rickettsiella grylli]	85.42	144	21	0	4.00E-71	222
143	gi|492904657|ref|WP_006035063.1|	50S ribosomal protein L13 [Rickettsiella grylli]	82.07	145	26	0	1.00E-80	246
144	gi|492905472|ref|WP_006035878.1|	delta-aminolevulinic acid dehydratase [Rickettsiella grylli]	79.57	328	67	0	0	562
146	gi|159121430|gb|EDP46768.1|	trigger factor [Rickettsiella grylli]	67.05	431	141	1	0	590
147	gi|492904658|ref|WP_006035064.1|	ATP-dependent Clp protease proteolytic subunit [Rickettsiella grylli]	91.86	221	17	1	2.00E-139	402
148	gi|492904593|ref|WP_006034999.1|	ATP-dependent Clp protease ATP-binding subunit ClpX [Rickettsiella grylli]	95.22	439	21	0	0	855

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149	gi|492905034|ref|WP_006035440.1|	endopeptidase La [Rickettsiella grylli]	88.31	830	90	4	0	1487
150	gi|492905578|ref|WP_006035984.1|	transcriptional regulator [Rickettsiella grylli]	75.82	91	22	0	6.00E-42	144
153	gi|492904518|ref|WP_006034924.1|	peptidyl-prolyl cis-trans isomerase [Rickettsiella grylli]	55.31	490	211	5	7.00E-179	524
154	gi|492904892|ref|WP_006035298.1|	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase [Rickettsiella grylli]	67.26	226	73	1	9.00E-107	320
155	gi|671582934|ref|WP_031560268.1|	DNA ligase (NAD(+)) LigA [Ruminococcus flavefaciens]	44.74	38	21	0	2.2	37
156	gi|492904460|ref|WP_006034866.1|	3'(2'),5'-bisphosphate nucleotidase CysQ [Rickettsiella grylli]	65.4	263	90	1	3.00E-121	359
157	gi|159120766|gb|EDP46104.1|	malate dehydrogenase [Rickettsiella grylli]	78.48	330	71	0	0	531
158	gi|492904297|ref|WP_006034703.1|	DNA translocase FtsK [Rickettsiella grylli]	79.33	774	148	4	0	1137
159	gi|492905235|ref|WP_006035641.1|	thioredoxin-disulfide reductase [Rickettsiella grylli]	76.11	314	74	1	4.00E-174	498
160	gi|492905500|ref|WP_006035906.1|	ABC transporter [Rickettsiella grylli]	78.26	230	46	2	4.00E-130	380
161	gi|492904914|ref|WP_006035320.1|	DNA starvation/stationary phase protection protein [Rickettsiella grylli]	85.53	159	23	0	5.00E-96	287
162	gi|492905246|ref|WP_006035652.1|	RNA-binding protein [Rickettsiella grylli]	82.01	139	19	1	5.00E-56	183
163	gi|492904407|ref|WP_006034813.1|	amidophosphoribosyltransferase [Rickettsiella grylli]	67.08	243	78	2	6.00E-111	331
164	gi|492904494|ref|WP_006034900.1|	glutamine--fructose-6-phosphate aminotransferase [Rickettsiella grylli]	75.93	615	141	4	0	940
165	gi|492905081|ref|WP_006035487.1|	phosphoglucosamine mutase [Rickettsiella grylli]	77.25	444	100	1	0	699
166	gi|159120370|gb|EDP45708.1|	ATP-dependent metallopeptidase HflB [Rickettsiella grylli]	92.36	641	47	1	0	1212
167	gi|492905006|ref|WP_006035412.1|	23S rRNA methyltransferase [Rickettsiella grylli]	76.56	209	48	1	6.00E-113	333
168	gi|492905520|ref|WP_006035926.1|	MFS transporter [Rickettsiella grylli]	84.14	435	69	0	0	761
169	gi|492904929|ref|WP_006035335.1|	MFS transporter [Rickettsiella grylli]	83.14	439	73	1	0	759
171	gi|750333714|ref|WP_040615633.1|	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase [Rickettsiella grylli]	71.25	160	46	0	7.00E-74	230
172	gi|492904763|ref|WP_006035169.1|	hypothetical protein [Rickettsiella grylli]	81.03	195	34	1	5.00E-114	338
173	gi|492905042|ref|WP_006035448.1|	crossover junction endodeoxyribonuclease RuvA [Rickettsiella grylli]	73.38	139	37	0	3.00E-70	220
174	gi|159120685|gb|EDP46023.1|	integral membrane protein MviN [Rickettsiella grylli]	80.94	509	97	0	0	842
175	gi|492905176|ref|WP_006035582.1|	bifunctional riboflavin kinase/FMN adenylyltransferase [Rickettsiella grylli]	69.38	307	94	0	4.00E-155	449
176	gi|492904380|ref|WP_006034786.1|	hypothetical protein [Rickettsiella grylli]	39.94	313	148	8	1.00E-51	196
176	gi|492904380|ref|WP_006034786.1|	hypothetical protein [Rickettsiella grylli]	33.21	265	159	7	2.00E-30	134
177	gi|492905332|ref|WP_006035738.1|	ferredoxin--NADP(+) reductase [Rickettsiella grylli]	80.97	247	47	0	8.00E-144	415
178	gi|159120961|gb|EDP46299.1|	6,7-dimethyl-8-ribityllumazine synthase [Rickettsiella grylli]	70.73	164	43	1	4.00E-78	241
179	gi|492904552|ref|WP_006034958.1|	bifunctional 3,4-dihydroxy-2-butanone 4-phosphate synthase/GTP cyclohydrolase II [Rickettsiella grylli]	83.08	396	67	0	0	698
180	gi|492905025|ref|WP_006035431.1|	bifunctional diaminohydroxyphosphoribosylaminopyrimidine deaminase/5-amino-6-(5-phosphoribosylamino)uracil reductase [Rickettsiella grylli]	64.44	360	128	0	1.00E-167	485
181	gi|492904408|ref|WP_006034814.1|	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase [Rickettsiella grylli]	72.95	451	121	1	0	676
182	gi|492905523|ref|WP_006035929.1|	6-phosphofructokinase [Rickettsiella grylli]	79	419	88	0	0	692
183	gi|492904931|ref|WP_006035337.1|	hypothetical protein [Rickettsiella grylli]	83.71	221	36	0	6.00E-136	393
184	gi|492904317|ref|WP_006034723.1|	4'-phosphopantetheinyl transferase [Rickettsiella grylli]	52.79	233	108	2	6.00E-75	239

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185	gi|492904463|ref|WP_006034869.1|	type IV pilus assembly protein TapB [Rickettsiella grylli]	66.2	568	188	2	0	738
186	gi|492905115|ref|WP_006035521.1|	pilus assembly protein PilC [Rickettsiella grylli]	64.85	367	128	1	5.00E-161	469
187	gi|159120410|gb|EDP45748.1|	bacterial Peptidase A24 N- domain family [Rickettsiella grylli]	61.13	265	98	2	2.00E-105	320
188	gi|492905110|ref|WP_006035516.1|	glycerol-3-phosphate dehydrogenase [Rickettsiella grylli]	77.61	326	73	0	0	528
189	gi|159120950|gb|EDP46288.1|	putative aconitate hydratase [Rickettsiella grylli]	84.6	643	98	1	0	1136
190	gi|492905504|ref|WP_006035910.1|	disulfide bond formation protein DsbB [Rickettsiella grylli]	83.63	171	28	0	5.00E-84	257
191	gi|492904746|ref|WP_006035152.1|	hypothetical protein [Rickettsiella grylli]	70.62	194	57	0	6.00E-82	254
192	gi|492904888|ref|WP_006035294.1|	microcin C7 self-immunity protein [Rickettsiella grylli]	71.75	308	84	1	3.00E-153	445
193	gi|492904277|ref|WP_006034683.1|	DNA gyrase subunit B [Rickettsiella grylli]	86.28	853	111	3	0	1493
194	gi|492904663|ref|WP_006035069.1|	alanine--tRNA ligase [Rickettsiella grylli]	74.66	872	220	1	0	1371
195	gi|492905510|ref|WP_006035916.1|	aspartate kinase [Rickettsiella grylli]	81.82	407	74	0	0	644
196	gi|492904358|ref|WP_006034764.1|	carbon storage regulator [Rickettsiella grylli]	89.86	69	7	0	3.00E-35	125
200	gi|962280680|gb|KTD64499.1|	transposase (IS652) [Legionella spiritensis]	80.22	91	18	0	3.00E-47	158
201	gi|492904548|ref|WP_006034954.1|	hypothetical protein [Rickettsiella grylli]	28.87	672	370	26	1.00E-47	189
202	gi|492904248|ref|WP_006034654.1|	type IV prepilin TapA [Rickettsiella grylli]	83.22	149	25	0	6.00E-77	237
203	gi|492905215|ref|WP_006035621.1|	isoleucine--tRNA ligase [Rickettsiella grylli]	76.64	946	220	1	0	1568
204	gi|750333396|ref|WP_040615315.1|	signal peptidase II [Rickettsiella grylli]	77.5	160	35	1	8.00E-82	251
205	gi|492904788|ref|WP_006035194.1|	transporter [Rickettsiella grylli]	73.63	455	120	0	0	639
206	gi|492905379|ref|WP_006035785.1|	conjugal transfer protein TrbN [Rickettsiella grylli]	71.32	136	38	1	1.00E-60	195
207	gi|159120725|gb|EDP46063.1|	lipopolysaccharide heptosyltransferase I [Rickettsiella grylli]	57.23	325	137	1	3.00E-132	392
208	gi|492905245|ref|WP_006035651.1|	primosomal protein N' [Rickettsiella grylli]	75.37	678	161	2	0	1047
209	gi|492904438|ref|WP_006034844.1|	L-serine ammonia-lyase [Rickettsiella grylli]	74.35	464	118	1	0	723
210	gi|159121111|gb|EDP46449.1|	CDP-diacylglycerol--serine O-phosphatidyltransferase [Rickettsiella grylli]	86.23	247	34	0	2.00E-151	437
211	gi|492905556|ref|WP_006035962.1|	DNA mismatch repair protein MutS [Rickettsiella grylli]	73.94	871	218	5	0	1320
212	gi|492904809|ref|WP_006035215.1|	dihydroneopterin aldolase [Rickettsiella grylli]	55.37	121	54	0	1.00E-40	142
213	gi|498283633|ref|WP_010597789.1|	hypothetical protein [Diplorickettsia massiliensis]	52.41	145	69	0	7.00E-51	171
214	gi|492905309|ref|WP_006035715.1|	hydroxyacylglutathione hydrolase [Rickettsiella grylli]	82.56	258	44	1	5.00E-155	444
215	gi|492904580|ref|WP_006034986.1|	acyl-CoA thioesterase [Rickettsiella grylli]	83.75	160	26	0	1.00E-93	281
216	gi|492904366|ref|WP_006034772.1|	phosphatidylserine decarboxylase [Rickettsiella grylli]	71.94	278	78	0	3.00E-146	424
217	gi|492904527|ref|WP_006034933.1|	hypothetical protein [Rickettsiella grylli]	62.34	640	231	8	0	795
218	gi|492905114|ref|WP_006035520.1|	hypothetical protein [Rickettsiella grylli]	42.65	490	269	4	4.00E-120	386
218	gi|492905114|ref|WP_006035520.1|	hypothetical protein [Rickettsiella grylli]	50.96	104	46	2	3.00E-19	102
219	gi|492905404|ref|WP_006035810.1|	tRNA nucleotidyltransferase [Rickettsiella grylli]	73.74	396	103	1	0	601
220	gi|492904607|ref|WP_006035013.1|	amino acid dehydrogenase [Rickettsiella grylli]	82.71	347	59	1	0	592
221	gi|492905546|ref|WP_006035952.1|	pyruvate dehydrogenase (acetyl-transferring) E1 component subunit alpha [Rickettsiella grylli]	75.28	356	88	0	0	557
222	gi|492904829|ref|WP_006035235.1|	2-oxoisovalerate dehydrogenase subunit beta [Rickettsiella grylli]	85.58	326	47	0	0	586

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223	gi|492905048|ref|WP_006035454.1|	dihydrolipoamide acyltransferase [Rickettsiella grylli]	69.92	389	110	3	0	539
224	gi|492904309|ref|WP_006034715.1|	16S rRNA (adenine(1518)-N(6)/adenine(1519)-N(6))-dimethyltransferase [Rickettsiella grylli]	61.54	52	20	0	5.00E-14	73.2
225	gi|492904309|ref|WP_006034715.1|	16S rRNA (adenine(1518)-N(6)/adenine(1519)-N(6))-dimethyltransferase [Rickettsiella grylli]	72.36	199	55	0	4.00E-101	306
226	gi|492904995|ref|WP_006035401.1|	CsbD family protein [Rickettsiella grylli]	73.91	69	18	0	5.00E-29	109
227	gi|492904614|ref|WP_006035020.1|	peptidylprolyl isomerase [Rickettsiella grylli]	73.62	254	61	3	7.00E-126	370
228	gi|492905233|ref|WP_006035639.1|	tRNA uridine(34) 5-carboxymethylaminomethyl synthesis enzyme MnmG [Rickettsiella grylli]	82.45	621	109	0	0	1062
229	gi|492904398|ref|WP_006034804.1|	transcription-repair coupling factor [Rickettsiella grylli]	75.89	1149	276	1	0	1808
230	gi|492904651|ref|WP_006035057.1|	hypothetical protein [Rickettsiella grylli]	42.7	363	189	10	2.00E-75	249
231	gi|492905096|ref|WP_006035502.1|	chaperone SurA (Peptidyl-prolyl cis-trans isomerase surA) (PPIase surA) (Rotamase surA) [Rickettsiella grylli]	66.05	433	144	2	0	580
232	gi|492905232|ref|WP_006035638.1|	organic solvent tolerance protein [Rickettsiella grylli]	73.39	838	216	3	0	1283
233	gi|492904448|ref|WP_006034854.1|	hypothetical protein [Rickettsiella grylli]	61.11	126	48	1	9.00E-49	164
234	gi|492905377|ref|WP_006035783.1|	ribulose-phosphate 3-epimerase [Rickettsiella grylli]	72.27	220	60	1	8.00E-112	332
235	gi|492904641|ref|WP_006035047.1|	molecular chaperone DjlA [Rickettsiella grylli]	82.72	272	46	1	1.00E-160	460
236	gi|492905610|ref|WP_006036016.1|	3-deoxy-D-manno-octulosonic acid transferase [Rickettsiella grylli]	69.27	423	128	1	0	582
237	gi|492905450|ref|WP_006035856.1|	riboflavin synthase subunit alpha [Rickettsiella grylli]	66.82	217	72	0	4.00E-108	322
238	gi|492905056|ref|WP_006035462.1|	phosphoglycolate phosphatase [Rickettsiella grylli]	70.45	220	65	0	1.00E-110	329
239	gi|492905217|ref|WP_006035623.1|	hypothetical protein [Rickettsiella grylli]	41.27	315	169	7	3.00E-69	231
240	gi|737485920|ref|WP_035465661.1|	peptidyl-prolyl cis-trans isomerase [Alicyclobacillus pomorum]	27.66	94	60	3	4.5	36.2
241	gi|552355101|gb|ERW14001.1|	deoxyribodipyrimidine photolyase [Pseudomonas aeruginosa BWHPSA021]	52.22	473	215	5	9.00E-169	496
242	gi|492905285|ref|WP_006035691.1|	hypothetical protein [Rickettsiella grylli]	69.57	23	7	0	0.087	37
243	gi|702630640|ref|WP_033227240.1|	hypothetical protein [Diplorickettsia massiliensis]	84.13	63	9	1	9.00E-29	109
244	gi|159121703|gb|EDP47041.1|	conserved hypothetical protein [Rickettsiella grylli]	96.77	31	1	0	5.00E-11	63.2
245	gi|493409788|ref|WP_006365775.1|	twitching motility protein PilT [Chlorobium ferrooxidans]	41.98	131	75	1	8.00E-23	97.8
246	gi|492904336|ref|WP_006034742.1|	hypothetical protein [Rickettsiella grylli]	47.77	404	196	8	4.00E-105	330
247	gi|492904942|ref|WP_006035348.1|	16S rRNA methyltransferase G [Rickettsiella grylli]	67.92	212	68	0	2.00E-105	315
248	gi|159120421|gb|EDP45759.1|	dihydrodipicolinate reductase [Rickettsiella grylli]	69.14	243	75	0	5.00E-119	352
249	gi|1028823927|ref|WP_064004781.1|	hypothetical protein, partial [Piscirickettsiaceae bacterium NZ-RLO]	38.79	281	165	3	3.00E-63	213
250	gi|492904439|ref|WP_006034845.1|	aminopeptidase N [Rickettsiella grylli]	70.78	876	254	2	0	1306
251	gi|492905095|ref|WP_006035501.1|	transporter [Rickettsiella grylli]	70	290	87	0	3.00E-132	390
252	gi|750333154|ref|WP_040615073.1|	RND transporter [Rickettsiella grylli]	73.05	501	133	1	0	725
253	gi|750333416|ref|WP_040615335.1|	MexH family multidrug efflux RND transporter periplasmic adaptor subunit [Rickettsiella grylli]	74.46	372	95	0	0	562
254	gi|492905263|ref|WP_006035669.1|	acriflavine resistance protein B [Rickettsiella grylli]	84.89	1026	154	1	0	1745
255	gi|915327369|ref|WP_050764057.1|	endonuclease [Rickettsiella grylli]	78.12	160	35	0	2.00E-89	271
256	gi|498283874|ref|WP_010598030.1|	hypothetical protein [Diplorickettsia massiliensis]	58.7	92	38	0	2.00E-29	115
257	gi|159121542|gb|EDP46880.1|	guanylate kinase [Rickettsiella grylli]	82.44	205	36	0	1.00E-123	361

A. crustaci (PROKKA)	Subject Sequence ID	Subject Name	Sequence similarity	Alignment length	Mismatched bases	Gaps	e-value	bitscore
258	gi|159120920|gb|EDP46258.1|	conserved hypothetical protein [Rickettsiella grylli]	69.44	288	88	0	9.00E-137	400
259	gi|492905588|ref|WP_006035994.1|	ribonuclease PH [Rickettsiella grylli]	74.58	236	58	1	2.00E-123	363
260	gi|492905566|ref|WP_006035972.1|	hypothetical protein [Rickettsiella grylli]	46.79	265	134	4	5.00E-60	218
261	gi|492905566|ref|WP_006035972.1|	hypothetical protein [Rickettsiella grylli]	55.62	1922	809	18	0	2065
262	gi|528216635|gb|EPY20041.1|	glutamate dehydrogenase [Strigomonas culicis]	65.52	29	8	1	3.4	35
263	gi|492904941|ref|WP_006035347.1|	amino acid permease [Rickettsiella grylli]	79.91	453	91	0	0	709
264	gi|492905238|ref|WP_006035644.1|	UDP-N-acetylenolpyruvoylglucosamine reductase [Rickettsiella grylli]	72.41	290	80	0	2.00E-152	441
265	gi|492904347|ref|WP_006034753.1|	UDP-N-acetylmuramate--L-alanine ligase [Rickettsiella grylli]	81.16	467	88	0	0	741
266	gi|492904434|ref|WP_006034840.1|	cell division protein FtsW [Rickettsiella grylli]	88.3	376	44	0	0	657
267	gi|492905419|ref|WP_006035825.1|	UDP-N-acetylmuramoylalanine--D-glutamate ligase [Rickettsiella grylli]	68.71	441	138	0	0	638
268	gi|492904668|ref|WP_006035074.1|	tRNA 2-thiouridine(34) synthase MnmA [Rickettsiella grylli]	72.98	359	97	0	0	551
269	gi|492905601|ref|WP_006036007.1|	SCO family protein [Rickettsiella grylli]	60.47	215	76	5	7.00E-85	263
270	gi|492904667|ref|WP_006035073.1|	protoheme IX farnesyltransferase [Rickettsiella grylli]	75.8	281	68	0	1.00E-142	416
271	gi|159120684|gb|EDP46022.1|	hypothetical protein RICGR_0247 [Rickettsiella grylli]	23.22	422	253	17	0.12	45.4
272	gi|504465619|ref|WP_014652721.1|	beta-galactosidase [Paenibacillus mucilaginosus]	30	80	49	3	4	35.8
273	gi|159121097|gb|EDP46435.1|	cytochrome oxidase assembly protein [Rickettsiella grylli]	61.86	333	127	0	3.00E-109	334
274	gi|492905195|ref|WP_006035601.1|	hypothetical protein [Rickettsiella grylli]	39.55	177	100	2	6.00E-29	117
275	gi|750333160|ref|WP_040615079.1|	hypothetical protein [Rickettsiella grylli]	51.87	241	115	1	6.00E-80	253
276	gi|492904711|ref|WP_006035117.1|	cytochrome c oxidase subunit III [Rickettsiella grylli]	60.07	288	114	1	4.00E-106	323
277	gi|492905142|ref|WP_006035548.1|	cytochrome c oxidase assembly protein [Rickettsiella grylli]	73.37	184	49	0	3.00E-90	275
278	gi|492904874|ref|WP_006035280.1|	cytochrome c oxidase subunit I [Rickettsiella grylli]	91.27	527	46	0	0	984
279	gi|492904306|ref|WP_006034712.1|	cytochrome c oxidase subunit II [Rickettsiella grylli]	79.1	268	56	0	8.00E-157	450
280	gi|492904952|ref|WP_006035358.1|	cytochrome c [Rickettsiella grylli]	72.11	502	137	2	0	768
281	gi|492905401|ref|WP_006035807.1|	threonylcarbamoyl-AMP synthase [Rickettsiella grylli]	54.87	308	138	1	1.00E-111	339
282	gi|492905281|ref|WP_006035687.1|	disulfide bond formation protein DsbB [Rickettsiella grylli]	74.74	194	49	0	1.00E-95	290
283	gi|492905376|ref|WP_006035782.1|	transcription termination factor Rho [Rickettsiella grylli]	93.06	418	29	0	0	791
284	gi|492904817|ref|WP_006035223.1|	thiol reductase thioredoxin [Rickettsiella grylli]	72.73	110	29	1	4.00E-50	167
285	gi|492905062|ref|WP_006035468.1|	hypoxanthine-guanine phosphoribosyltransferase [Rickettsiella grylli]	84.57	188	29	0	3.00E-115	338
286	gi|915477358|ref|WP_050816891.1|	beta-hexosaminidase [Diplorickettsia massiliensis]	62.43	338	126	1	1.00E-145	427
288	gi|492904986|ref|WP_006035392.1|	tRNA preQ1(34) S-adenosylmethionine ribosyltransferase-isomerase QueA [Rickettsiella grylli]	71.14	350	99	2	0	518
289	gi|159120855|gb|EDP46193.1|	preprotein translocase, YajC subunit [Rickettsiella grylli]	82.88	111	18	1	1.00E-57	185
290	gi|492905399|ref|WP_006035805.1|	preprotein translocase subunit SecD [Rickettsiella grylli]	81.83	622	110	2	0	983
291	gi|492904645|ref|WP_006035051.1|	preprotein translocase subunit SecF [Rickettsiella grylli]	85.86	304	41	2	1.00E-176	503
292	gi|492905430|ref|WP_006035836.1|	inositol monophosphatase [Rickettsiella grylli]	86.04	265	37	0	1.00E-167	478
293	gi|492904594|ref|WP_0060350.1|	RNA methyltransferase [Rickettsiella grylli]	69.17	240	69	2	8.00E-114	338

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294	gi|492905118|ref|WP_006035524.1|	tRNA-guanine(34) transglycosylase [Rickettsiella grylli]	80.73	384	73	1	0	660
295	gi|594556907|gb|EXU80930.1|	membrane protein [Comamonas aquatica DA1877]	54.55	55	25	0	8.00E-09	56.6
296	gi|492904370|ref|WP_006034776.1|	3-deoxy-manno-octulosonate cytidylyltransferase [Rickettsiella grylli]	68.06	263	84	0	2.00E-124	368
297	gi|492905163|ref|WP_006035569.1|	phosphoglycerate mutase [Rickettsiella grylli]	58.96	212	87	0	4.00E-90	276
298	gi|492905210|ref|WP_006035616.1|	D-alanyl-D-alanine dipeptidase (D-Ala-D-Aladipeptidase) (Vancomycin B-type resistance protein VanX) [Rickettsiella grylli]	63.76	218	78	1	3.00E-97	295
299	gi|492905275|ref|WP_006035681.1|	catalase HPII [Rickettsiella grylli]	70.07	695	202	3	0	1028
301	gi|915327267|ref|WP_050763955.1|	hypothetical protein [Rickettsiella grylli]	57.33	75	28	1	3.00E-19	90.1
302	gi|951583253|ref|WP_057896905.1|	glutamyl-tRNA amidotransferase [Lactobacillus oeni]	33.93	56	35	1	1.2	34.7
303	gi|915327321|ref|WP_050764009.1|	hypothetical protein [Rickettsiella grylli]	55.68	273	117	1	8.00E-93	289
304	gi|492905586|ref|WP_006035992.1|	hypothetical protein [Rickettsiella grylli]	23.83	214	118	7	6.00E-04	51.6
305	gi|492905497|ref|WP_006035903.1|	RNA polymerase sigma factor RpoD [Rickettsiella grylli]	85.25	651	82	4	0	1103
306	gi|492904724|ref|WP_006035130.1|	folate synthesis bifunctional protein [Rickettsiella grylli]	71.14	447	128	1	0	664
307	gi|492904349|ref|WP_006034755.1|	glycine dehydrogenase [Rickettsiella grylli]	81.93	487	83	1	0	790
308	gi|492904969|ref|WP_006035375.1|	glycine dehydrogenase [Rickettsiella grylli]	76.33	452	107	0	0	744
309	gi|498283350|ref|WP_010597506.1|	glycine cleavage system protein H [Diplorickettsia massiliensis]	65.57	122	42	0	7.00E-52	172
310	gi|492905385|ref|WP_006035791.1|	glycine cleavage system protein T [Rickettsiella grylli]	74.52	361	92	0	0	575
311	gi|492904598|ref|WP_006035004.1|	chromosome partitioning protein ParB [Rickettsiella grylli]	78.47	288	61	1	5.00E-153	442
312	gi|159121713|gb|EDP47051.1|	sporulation initiation inhibitor protein soj [Rickettsiella grylli]	79.09	287	59	1	5.00E-158	454
313	gi|492904964|ref|WP_006035370.1|	ABC transporter substrate-binding protein [Rickettsiella grylli]	62.41	290	107	2	9.00E-124	368
314	gi|492904344|ref|WP_006034750.1|	zinc ABC transporter permease [Rickettsiella grylli]	83.09	272	44	1	5.00E-152	438
315	gi|159121306|gb|EDP46644.1|	ABC Mn2+/Zn2+ transporter, inner membrane subunit [Rickettsiella grylli]	80.95	273	52	0	2.00E-149	431
316	gi|492904377|ref|WP_006034783.1|	ribonucleotide-diphosphate reductase subunit beta [Rickettsiella grylli]	92.48	359	26	1	0	696
317	gi|492905388|ref|WP_006035794.1|	ribonucleotide-diphosphate reductase subunit alpha [Rickettsiella grylli]	86.95	950	120	3	0	1731
318	gi|492904583|ref|WP_006034989.1|	phosphomannomutase [Rickettsiella grylli]	79.96	464	92	1	0	759
319	gi|492904577|ref|WP_006034983.1|	exodeoxyribonuclease III [Rickettsiella grylli]	75.4	252	62	0	7.00E-142	410
320	gi|492905445|ref|WP_006035851.1|	competence protein CinA [Rickettsiella grylli]	68.9	164	50	1	9.00E-66	210
321	gi|492905557|ref|WP_006035963.1|	translation initiation factor IF-1 [Rickettsiella grylli]	89.02	82	9	0	4.00E-46	154
322	gi|492904620|ref|WP_006035026.1|	ATP-dependent Clp protease ATP-binding subunit ClpA [Rickettsiella grylli]	92.09	771	59	2	0	1444
323	gi|492904794|ref|WP_006035200.1|	isocitrate dehydrogenase (NADP(+)) [Rickettsiella grylli]	83.1	426	72	0	0	753
324	gi|667638953|ref|XP_007603795.1|	hypothetical protein VICG_00342 [Vittaforma corneae ATCC 50505]	28.1	121	75	3	4.7	38.9
325	gi|492905592|ref|WP_006035998.1|	hypothetical protein [Rickettsiella grylli]	28.29	205	114	9	0.002	50.4
326	gi|492905251|ref|WP_006035657.1|	peptidase M50 [Rickettsiella grylli]	89	209	23	0	1.00E-108	323
327	gi|492904648|ref|WP_006035054.1|	chromosome segregation protein ScpA [Rickettsiella grylli]	69.03	268	80	1	1.00E-122	363
328	gi|492905583|ref|WP_006035989.1|	SDR family oxidoreductase [Rickettsiella grylli]	68.55	248	78	0	2.00E-126	371
329	gi|492905017|ref|WP_006035423.1|	purine-nucleoside phosphorylase [Rickettsiella grylli]	75.85	265	64	0	5.00E-143	416

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330	gi|492905414|ref|WP_006035820.1|	Fe(2+)-trafficking protein [Rickettsiella grylli]	81.93	83	15	0	1.00E-42	145
331	gi|492904799|ref|WP_006035205.1|	A/G-specific adenine glycosylase [Rickettsiella grylli]	66.19	352	118	1	2.00E-164	476
332	gi|492904555|ref|WP_006034961.1|	AsmA family [Rickettsiella grylli]	58.82	561	227	4	0	662
333	gi|492905329|ref|WP_006035735.1|	hypothetical protein [Rickettsiella grylli]	77.78	108	24	0	2.00E-57	185
334	gi|159120483|gb|EDP45821.1|	conserved hypothetical protein [Rickettsiella grylli]	60.86	304	119	0	1.00E-133	395
335	gi|492905127|ref|WP_006035533.1|	hypothetical protein [Rickettsiella grylli]	78.49	186	40	0	3.00E-104	310
336	gi|492905284|ref|WP_006035690.1|	MFS transporter [Rickettsiella grylli]	67.96	412	129	1	0	559
337	gi|915327284|ref|WP_050763972.1|	tRNA dimethylallyltransferase [Rickettsiella grylli]	67.91	296	94	1	4.00E-142	415
338	gi|492904615|ref|WP_006035021.1|	DNA mismatch repair protein MutL [Rickettsiella grylli]	66.4	631	182	7	0	790
339	gi|492904515|ref|WP_006034921.1|	GtrA family protein [Rickettsiella grylli]	77.05	353	81	0	0	550
340	gi|492904820|ref|WP_006035226.1|	tRNA threonylcarbamoyladenosine biosynthesis protein TsaE [Rickettsiella grylli]	54.67	150	68	0	8.00E-55	182
341	gi|492905403|ref|WP_006035809.1|	energy-dependent translational throttle protein EttA [Rickettsiella grylli]	83.12	545	92	0	0	941
342	gi|492905609|ref|WP_006036015.1|	serine hydroxymethyltransferase [Rickettsiella grylli]	78.47	418	90	0	0	700
343	gi|492904253|ref|WP_006034659.1|	transcriptional regulator NrdR [Rickettsiella grylli]	87.95	166	20	0	5.00E-102	302
344	gi|492905107|ref|WP_006035513.1|	N utilization substance protein B [Rickettsiella grylli]	69.59	148	45	0	3.00E-65	207
345	gi|492905185|ref|WP_006035591.1|	thiamine-phosphate kinase [Rickettsiella grylli]	67.18	323	106	0	8.00E-151	439
346	gi|492904966|ref|WP_006035372.1|	phosphatidylglycerophosphatase A [Rickettsiella grylli]	83.12	154	26	0	5.00E-87	264
347	gi|492905014|ref|WP_006035420.1|	23S rRNA (pseudouridine(1915)-N(3))-methyltransferase RlmH [Rickettsiella grylli]	72.44	156	43	0	9.00E-75	232
348	gi|492904595|ref|WP_006035001.1|	ribosome silencing factor RsfS [Rickettsiella grylli]	80.91	110	20	1	2.00E-58	187
349	gi|492905189|ref|WP_006035595.1|	nicotinate-nicotinamide nucleotide adenylyltransferase [Rickettsiella grylli]	65.38	208	72	0	4.00E-88	270
350	gi|492904755|ref|WP_006035161.1|	DNA polymerase III subunit delta [Rickettsiella grylli]	61.19	335	129	1	5.00E-142	419
351	gi|159120820|gb|EDP46158.1|	B transmembrane [Rickettsiella grylli]	54.65	172	75	2	1.00E-54	183
352	gi|492905346|ref|WP_006035752.1|	leucine--tRNA ligase [Rickettsiella grylli]	77.15	836	186	4	0	1329
353	gi|492905493|ref|WP_006035899.1|	apolipoprotein N-acyltransferase [Rickettsiella grylli]	69.9	505	149	1	0	730
354	gi|159120374|gb|EDP45712.1|	probable protease SohB [Rickettsiella grylli]	76.52	328	77	0	0	516
355	gi|492904777|ref|WP_006035183.1|	heme ABC exporter, ATP-binding protein CcmA [Rickettsiella grylli]	62.38	210	79	0	3.00E-73	233
356	gi|492904816|ref|WP_006035222.1|	heme exporter protein B [Rickettsiella grylli]	65.71	210	72	0	2.00E-87	270
357	gi|492904690|ref|WP_006035096.1|	heme ABC transporter permease [Rickettsiella grylli]	72.8	239	65	0	1.00E-119	354
358	gi|492905312|ref|WP_006035718.1|	hypothetical protein [Rickettsiella grylli]	27.27	264	157	8	9.00E-13	79
359	gi|492904426|ref|WP_006034832.1|	3-deoxy-8-phosphooctulonate synthase [Rickettsiella grylli]	81.59	277	51	0	3.00E-168	479
360	gi|492904482|ref|WP_006034888.1|	phosphopyruvate hydratase [Rickettsiella grylli]	78.29	433	94	0	0	685
361	gi|492905327|ref|WP_006035733.1|	cell division protein FtsB [Rickettsiella grylli]	67.01	97	31	1	1.00E-39	138
362	gi|492904731|ref|WP_006035137.1|	hypothetical protein [Rickettsiella grylli]	66.8	244	79	2	2.00E-117	347
363	gi|518046335|ref|WP_019216543.1|	helix-turn-helix transcriptional regulator [Legionella tunisiensis]	38.3	94	58	0	1.00E-15	78.6
364	gi|492904897|ref|WP_006035303.1|	response regulator [Rickettsiella grylli]	58.54	164	65	2	6.00E-62	200
365	gi|492904902|ref|WP_006035308.1|	lipoprotein releasing system, ATP-binding protein [Rickettsiella grylli]	77.38	221	50	0	6.00E-120	353

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366	gi|492904864|ref|WP_006035270.1|	lipoprotein-releasing system protein LolC [Rickettsiella grylli]	81.53	417	77	0	0	702
367	gi|492904472|ref|WP_006034878.1|	enoyl-ACP reductase [Rickettsiella grylli]	82.96	270	46	0	2.00E-164	469
368	gi|915327373|ref|WP_050764061.1|	uridine kinase [Rickettsiella grylli]	88.64	220	25	0	2.00E-139	402
370	gi|406915587|gb|EKD54655.1|	hypothetical protein ACD_60C060G0023 [uncultured bacterium]	25.56	446	325	4	2.00E-36	151
371	gi|494088207|ref|WP_007029042.1|	twin-arginine translocation pathway signal protein [Amycolatopsis decaplanina]	47.61	397	207	1	2.00E-138	414
372	gi|703484077|ref|WP_033436703.1|	hypothetical protein [Saccharothrix sp. NRRL B-16314]	40.28	422	246	4	3.00E-115	357
373	gi|494088211|ref|WP_007029046.1|	NAD-dependent epimerase [Amycolatopsis decaplanina]	52.16	324	151	2	1.00E-119	360
374	gi|946815952|gb|KRG22569.1|	Multidrug resistance protein MdtM [Coxiellaceae bacterium HT99]	39.4	368	212	3	2.00E-86	279
375	gi|966402194|ref|WP_058445789.1|	hypothetical protein [Legionella feeleii]	34.02	244	155	1	7.00E-40	152
377	gi|492904631|ref|WP_006035037.1|	c-type cytochrome biogenesis protein CcmF [Rickettsiella grylli]	66.67	600	199	1	0	826
378	gi|750333182|ref|WP_040615101.1|	hypothetical protein [Rickettsiella grylli]	64.6	161	56	1	5.00E-68	218
379	gi|492904446|ref|WP_006034852.1|	cytochrome c-type biogenesis protein CcmH [Rickettsiella grylli]	63.64	110	37	1	5.00E-39	140
380	gi|498284527|ref|WP_010598683.1|	4'-phosphopantetheinyl transferase [Diplorickettsia massiliensis]	76.27	177	37	1	2.00E-89	275
382	gi|499590553|ref|WP_011271315.1|	4a-hydroxytetrahydrobiopterin dehydratase [Rickettsia felis]	64.52	93	33	0	1.00E-37	134
383	gi|503701028|ref|WP_013935104.1|	hypothetical protein [Simkania negevensis]	22.52	373	254	12	0.002	51.6
384	gi|505085|ref|WP_015187187.1|	hypothetical protein [Gloeocapsa sp. PCC 7428]	32.65	49	33	0	0.029	40.8
385	gi|962233384|gb|KTD17932.1|	glutamate rich protein GrpB [Legionella jordanis]	35.67	443	276	4	3.00E-94	304
386	gi|1041905663|ref|WP_065239994.1|	peptide synthetase [Legionella maceachernii]	32.4	287	193	1	1.00E-46	187
387	gi|692233611|ref|WP_032113978.1|	hypothetical protein [Candidatus Paracaedibacter symbiosus]	41.01	217	115	5	4.00E-38	154
387	gi|692233611|ref|WP_032113978.1|	hypothetical protein [Candidatus Paracaedibacter symbiosus]	34.86	218	131	4	1.00E-33	141
388	gi|751309940|ref|WP_041018004.1|	MFS transporter [Criblamydia sequanensis]	32.78	418	246	8	4.00E-45	172
389	gi|757197246|ref|WP_042739907.1|	hypothetical protein [Staphylococcus gallinarum]	30.49	364	247	3	5.00E-39	154
390	gi|406915038|gb|EKD54165.1|	hypothetical protein ACD_60C00119G0011 [uncultured bacterium]	57.05	312	134	0	1.00E-128	382
391	gi|1004814385|gb|KYC40344.1|	non-ribosomal peptide synthetase [Scytonema hofmannii PCC 7110]	30.43	1055	681	22	4.00E-145	489
391	gi|1004814385|gb|KYC40344.1|	non-ribosomal peptide synthetase [Scytonema hofmannii PCC 7110]	34.98	586	357	12	1.00E-98	355
392	gi|374712055|gb|AEZ64585.1|	short-chain dehydrogenase/reductase SDR [Streptomyces chromofuscus]	37.87	169	103	2	8.00E-32	128
393	gi|160334169|gb|ABX24493.1|	putative hydroxylase [Streptomyces cacaoi subsp. asoensis]	30.81	172	117	1	2.00E-24	105
394	gi|966427975|ref|WP_058470471.1|	phenylalanine 4-monooxygenase [Legionella jordanis]	43.82	251	139	1	8.00E-69	226
395	gi|818394475|gb|KKQ73675.1|	dihydroorotate dehydrogenase PyrD [Candidatus Woesebacteria bacterium GW2011_GWB1_38_5b]	61.99	171	64	1	2.00E-72	237
396	gi|779878290|ref|WP_045359890.1|	hypothetical protein [[Enterobacter] aerogenes]	39.09	417	235	7	1.00E-93	301
397	gi|757197251|ref|WP_042739909.1|	radical SAM protein [Staphylococcus gallinarum]	52.06	436	203	5	3.00E-156	462
398	gi|740679195|ref|WP_038464484.1|	hypothetical protein [Candidatus Paracaedibacter acanthamoebae]	45.54	527	283	2	1.00E-164	491
399	gi|663375239|ref|WP_030371615.1|	tRNA pseudouridine synthase D [Streptomyces rimosus]	34.63	335	213	3	2.00E-66	225
400	gi|335387315|gb|AEH57248.1|	putative tyrosine/serine phosphatase NikL-like protein [Prochloron didemni P3-Solomon]	34.72	193	124	1	2.00E-28	119
401	gi|942692888|ref|WP_055397565.1|	oxidoreductase [Acidovorax sp. SD340]	32.88	222	142	5	1.00E-28	118
402	gi|938927900|ref|WP_054709834.1|	topology modulation protein [Bacillus sp. JCM 19041]	35	180	103	3	7.00E-27	111

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403	gi|915860769|ref|WP_050915586.1|	phosphoanhydride phosphorylase [Yersinia enterocolitica]	61.49	444	163	5	0	574
404	gi|749010525|ref|WP_040069782.1|	hypothetical protein [Pseudomonas batumici]	47.62	168	85	2	2.00E-43	154
405	gi|406938341|gb|EKD71595.1|	hypothetical protein ACD_46C00151G02 [uncultured bacterium]	42.65	68	39	0	3.00E-08	58.5
406	gi|749010523|ref|WP_040069780.1|	hypothetical protein [Pseudomonas batumici]	58.88	197	81	0	4.00E-80	251
407	gi|938273222|gb|KPQ08317.1|	Pyridine nucleotide-disulfide oxidoreductase [Rhodobacteraceae bacterium HLUCCA12]	45.92	392	209	3	3.00E-129	390
408	gi|763182102|ref|WP_044061188.1|	hypothetical protein [Pseudomonas aeruginosa]	42.15	121	69	1	8.00E-21	96.3
409	gi|489415663|ref|WP_003321498.1|	N-acetyltransferase GCN5 [Bacillus alcalophilus]	32.54	169	95	7	1.00E-11	70.1
410	gi|749010525|ref|WP_040069782.1|	hypothetical protein [Pseudomonas batumici]	45.83	168	88	2	1.00E-40	147
411	gi|156529194|gb|ABU74279.1|	hypothetical protein VIBHAR_06388 [Vibrio campbellii ATCC BAA-1116]	43.75	336	184	4	4.00E-97	303
412	gi|406938364|gb|EKD71611.1|	hypothetical protein ACD_46C00144G01 [uncultured bacterium]	50.51	198	98	0	9.00E-72	229
413	gi|737769950|ref|WP_035737972.1|	hypothetical protein, partial [Francisella philomiragia]	43.56	388	205	6	4.00E-93	304
414	gi|505211886|ref|WP_015398988.1|	type IV secretion protein VblB2 [Bartonella vinsonii]	37.97	79	48	1	2.00E-08	58.2
415	gi|390189910|emb|CCD32144.1|	Plasmid conjugal transfer protein, TrbD/VirB3 [Methylocystis sp. SC2]	37.36	91	56	1	5.00E-09	59.3
416	gi|970541478|ref|WP_058808312.1|	MULTISPECIES: type VI secretion protein [Sphingopyxis]	37.93	783	464	10	0	563
417	gi|518048131|ref|WP_019218339.1|	hypothetical protein [Legionella tunisiensis]	28.02	232	136	8	2.00E-12	73.9
418	gi|518455702|ref|WP_019625909.1|	hypothetical protein [Thioalkalivibrio sp. ALJT]	53.12	32	15	0	0.47	36.6
419	gi|494046167|ref|WP_006988285.1|	hypothetical protein [Gillisia limnaea]	27.08	96	60	3	0.028	42.7
420	gi|518048128|ref|WP_019218336.1|	hypothetical protein [Legionella tunisiensis]	30.75	322	200	9	1.00E-27	121
421	gi|966475325|ref|WP_058506086.1|	hypothetical protein [Legionella nautarum]	32.57	218	144	3	1.00E-25	111
422	gi|498284829|ref|WP_010598985.1|	type IV secretion system protein VirB9 [Diplorickettsia massiliensis]	83.67	98	15	1	2.00E-50	171
423	gi|652971093|ref|WP_027223957.1|	hypothetical protein [Legionella pneumophila]	40.23	343	189	5	5.00E-65	222
424	gi|570550699|gb|ETO91955.1|	P-type DNA transfer ATPase VirB11 [Candidatus Xenolissoclinum pacificiensis L6]	46.63	326	164	5	6.00E-93	291
425	gi|519069421|ref|WP_020225296.1|	DNA-binding response regulator [Holdemania massiliensis]	40.87	115	60	3	4.00E-14	76.6
427	gi|769983727|ref|WP_045099709.1|	helix-turn-helix transcriptional regulator [Tatlockia micdadei]	43.62	94	53	0	3.00E-16	80.1
428	gi|910160496|ref|WP_0509369.1|	site-specific DNA-methyltransferase [Candidatus Glomeribacter gigasporarum]	62.68	276	103	0	6.00E-125	372
429	gi|492904776|ref|WP_006035182.1|	hypothetical protein [Rickettsiella grylli]	52.1	167	79	1	3.00E-56	189
430	gi|492905120|ref|WP_006035526.1|	hypothetical protein [Rickettsiella grylli]	80.09	221	40	1	6.00E-109	331
431	gi|492904509|ref|WP_006034915.1|	hypothetical protein [Rickettsiella grylli]	97.55	204	5	0	6.00E-145	416
432	gi|492904608|ref|WP_006035014.1|	DNA repair protein RadA [Rickettsiella grylli]	79.48	463	92	1	0	705
433	gi|492904712|ref|WP_006035118.1|	D-glycero-beta-D-manno-heptose-1,7-bisphosphate 7-phosphatase [Rickettsiella grylli]	67.38	187	61	0	3.00E-86	264
434	gi|492905461|ref|WP_006035867.1|	hypothetical protein [Rickettsiella grylli]	45.21	73	37	2	7.00E-07	55.1
435	gi|750333184|ref|WP_040615103.1|	hypothetical protein [Rickettsiella grylli]	57.61	394	163	1	8.00E-166	483
436	gi|492904879|ref|WP_006035285.1|	NAD-dependent malic enzyme [Rickettsiella grylli]	74.51	565	142	1	0	867
437	gi|492905590|ref|WP_006035996.1|	ubiquinone biosynthesis hydroxylase UbiH/UbiF/VisC/COQ6 [Rickettsiella grylli]	61.61	422	158	4	1.00E-165	485
438	gi|492904800|ref|WP_006035206.1|	Xaa-Pro aminopeptidase [Rickettsiella grylli]	65.59	433	146	1	0	592
439	gi|492905071|ref|WP_006035477.1|	hypothetical protein [Rickettsiella grylli]	85.42	192	28	0	4.00E-109	323

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440	gi|498284320|ref|WP_010598476.1|	hypothetical protein [Diplorickettsia massiliensis]	64.8	196	61	1	3.00E-84	259
441	gi|915327330|ref|WP_050764018.1|	hypothetical protein [Rickettsiella grylli]	51.46	103	50	0	2.00E-32	122
442	gi|492905254|ref|WP_006035660.1|	5-formyltetrahydrofolate cyclo-ligase [Rickettsiella grylli]	59.69	191	77	0	2.00E-76	240
443	gi|654774540|ref|WP_028229017.1|	toxin [Paraburkholderia ferrariae]	28.23	124	73	4	1.5	43.9
444	gi|492904650|ref|WP_006035056.1|	hypothetical protein [Rickettsiella grylli]	50.37	135	64	3	6.00E-38	137
445	gi|492905129|ref|WP_006035535.1|	alanine racemase [Rickettsiella grylli]	70.65	368	104	2	0	536
446	gi|492905499|ref|WP_006035905.1|	replicative DNA helicase [Rickettsiella grylli]	93.61	454	29	0	0	879
447	gi|492904886|ref|WP_006035292.1|	50S ribosomal protein L9 [Rickettsiella grylli]	80	150	30	0	5.00E-74	230
448	gi|492905226|ref|WP_006035632.1|	hypothetical protein [Rickettsiella grylli]	72.22	288	80	0	4.00E-126	374
449	gi|657659739|ref|WP_029463594.1|	30S ribosomal protein S18 [Diplorickettsia massiliensis]	93.59	78	5	0	2.00E-46	154
450	gi|492905099|ref|WP_006035505.1|	30S ribosomal protein S6 [Rickettsiella grylli]	76.15	130	29	1	7.00E-67	210
451	gi|492904314|ref|WP_006034720.1|	octaprenyl-diphosphate synthase [Rickettsiella grylli]	70.19	322	96	0	3.00E-165	476
452	gi|492904616|ref|WP_006035022.1|	hypothetical protein [Rickettsiella grylli]	51.19	168	74	5	2.00E-38	146
453	gi|492904616|ref|WP_006035022.1|	hypothetical protein [Rickettsiella grylli]	44.44	135	61	2	1.00E-21	99.4
454	gi|9305991|ref|WP_054111041.1|	hypothetical protein [Brevundimonas sp. AAP58]	41.98	162	90	1	6.00E-42	149
456	gi|492905400|ref|WP_006035806.1|	integrase [Rickettsiella grylli]	66.17	334	110	3	4.00E-148	433
457	gi|492904672|ref|WP_006035078.1|	hypothetical protein [Rickettsiella grylli]	88.89	36	4	0	4.00E-14	70.9
458	gi|498283463|ref|WP_010597619.1|	hypothetical protein [Diplorickettsia massiliensis]	82.73	220	38	0	2.00E-119	362
459	gi|498283465|ref|WP_010597621.1|	hypothetical protein [Diplorickettsia massiliensis]	67.02	191	62	1	2.00E-78	244
460	gi|498283466|ref|WP_010597622.1|	hypothetical protein [Diplorickettsia massiliensis]	65.52	87	30	0	5.00E-31	117
461	gi|498283467|ref|WP_010597623.1|	hypothetical protein [Diplorickettsia massiliensis]	87.8	295	34	1	0	549
462	gi|902510153|ref|WP_049600395.1|	hypothetical protein [Yersinia nurmii]	38.31	308	154	12	4.00E-50	179
463	gi|896647676|ref|WP_049526957.1|	hypothetical protein [Yersinia enterocolitica]	40.12	162	89	5	1.00E-31	123
464	gi|498283423|ref|WP_010597579.1|	hypothetical protein [Diplorickettsia massiliensis]	70.95	148	43	0	1.00E-72	229
465	gi|498284627|ref|WP_010598783.1|	hypothetical protein [Diplorickettsia massiliensis]	36.59	82	51	1	7.00E-08	55.5
466	gi|498283474|ref|WP_010597630.1|	hypothetical protein [Diplorickettsia massiliensis]	86.44	295	39	1	0	542
467	gi|498283476|ref|WP_010597632.1|	hypothetical protein [Diplorickettsia massiliensis]	77.05	61	14	0	1.00E-24	98.2
468	gi|657659770|ref|WP_029463625.1|	hypothetical protein [Diplorickettsia massiliensis]	72.99	137	37	0	4.00E-60	194
469	gi|498283479|ref|WP_010597635.1|	hypothetical protein [Diplorickettsia massiliensis]	58.87	124	50	1	2.00E-47	160
471	gi|723577924|ref|XP_010309118.1|	PREDICTED: cyclic AMP-responsive element-binding protein 3-like, partial [Balearica regulorum gibbericeps]	43.18	44	25	0	0.47	37.7
472	gi|492904571|ref|WP_006034977.1|	hypothetical protein [Rickettsiella grylli]	75	112	28	0	1.00E-52	174
474	gi|492905478|ref|WP_006035884.1|	hypothetical protein [Rickettsiella grylli]	34.16	281	150	5	6.00E-36	140
475	gi|966460167|ref|WP_058492597.1|	MerR family transcriptional regulator [Legionella worsleiensis]	52.08	96	44	2	2.00E-23	97.4
476	gi|492905400|ref|WP_006035806.1|	integrase [Rickettsiella grylli]	76.92	91	21	0	3.00E-45	160
477	gi|492904257|ref|WP_006034663.1|	carboxyl-terminal processing protease [Rickettsiella grylli]	72.34	423	113	2	0	630

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478	gi|159120972|gb|EDP46310.1|	2,3-bisphosphoglycerate-independent phosphoglycerate mutase [Rickettsiella grylli]	71.32	516	148	0	0	775
479	gi|159121679|gb|EDP47017.1|	putative probable multidrug resistance protein NorM (Multidrug-effluxtransporter) [Rickettsiella grylli]	74.11	448	116	0	0	656
480	gi|492904601|ref|WP_006035007.1|	prolipoprotein diacylglyceryl transferase [Rickettsiella grylli]	79.92	259	52	0	1.00E-149	431
481	gi|492904846|ref|WP_006035252.1|	hypothetical protein [Rickettsiella grylli]	60.71	448	175	1	1.00E-159	474
482	gi|492905427|ref|WP_006035833.1|	rare lipoprotein A [Rickettsiella grylli]	70.73	287	74	4	1.00E-131	388
483	gi|492904333|ref|WP_006034739.1|	lytic murein transglycosylase B [Rickettsiella grylli]	73.37	338	90	0	3.00E-171	492
484	gi|159121035|gb|EDP46373.1|	rod shape-determining protein RodA [Rickettsiella grylli]	82.31	373	66	0	0	577
485	gi|492905553|ref|WP_006035959.1|	LysM domain-containing protein [Rickettsiella grylli]	68.85	321	98	2	8.00E-157	455
486	gi|492904625|ref|WP_006035031.1|	sporulation protein [Rickettsiella grylli]	86.89	267	35	0	2.00E-170	484
487	gi|492905416|ref|WP_006035822.1|	integration host factor [Rickettsiella grylli]	94.02	117	7	0	8.00E-69	215
488	gi|492904469|ref|WP_006034875.1|	AFG1-family ATPase [Rickettsiella grylli]	61	341	129	3	5.00E-125	375
489	gi|492905227|ref|WP_006035633.1|	hypothetical protein [Rickettsiella grylli]	68.37	215	68	0	2.00E-103	310
490	gi|492904280|ref|WP_006034686.1|	ABC transporter [Rickettsiella grylli]	87.54	305	38	0	0	551
491	gi|492904948|ref|WP_006035354.1|	ABC transporter permease [Rickettsiella grylli]	80.16	257	51	0	9.00E-144	416
492	gi|492904544|ref|WP_006034950.1|	ferrochelatase [Rickettsiella grylli]	58.92	314	129	0	2.00E-132	392
493	gi|778251813|gb|KJR41878.1|	hypothetical protein MCHI_002255 [Candidatus Magnetoovum chiemensis]	35.14	185	88	6	1.00E-16	84
494	gi|492905170|ref|WP_006035576.1|	membrane protein [Rickettsiella grylli]	79.77	440	82	2	0	703
495	gi|492904565|ref|WP_006034971.1|	hypothetical protein [Rickettsiella grylli]	22.52	515	336	19	8.00E-07	63.9
496	gi|492905029|ref|WP_006035435.1|	hypothetical protein [Rickettsiella grylli]	33.17	416	235	13	1.00E-49	195
497	gi|750333198|ref|WP_040615117.1|	endonuclease [Rickettsiella grylli]	69.08	207	64	0	6.00E-96	291
498	gi|492905603|ref|WP_006036009.1|	hypothetical protein [Rickettsiella grylli]	76.19	105	25	0	1.00E-52	172
499	gi|492904432|ref|WP_006034838.1|	adenylate cyclase [Rickettsiella grylli]	71.23	212	59	1	8.00E-100	301
500	gi|159121535|gb|EDP46873.1|	conserved hypothetical protein [Rickettsiella grylli]	55.17	58	26	0	2.00E-13	68.6
501	gi|492904554|ref|WP_006034960.1|	RNA polymerase factor sigma-32 [Rickettsiella grylli]	82.93	287	49	0	2.00E-171	489
502	gi|492905372|ref|WP_006035778.1|	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase [Rickettsiella grylli]	77.97	404	89	0	0	672
503	gi|498284346|ref|WP_010598502.1|	peptidoglycan-binding domain 1 protein [Diplorickettsia massiliensis]	51.65	393	171	3	1.00E-140	420
504	gi|406940764|gb|EKD73433.1|	Transposase IS4 [uncultured bacterium]	67.11	76	25	0	1.00E-30	115
505	gi|938082948|gb|KPP78078.1|	unconventional myosin-Vc-like [Scleropages formosus]	25	164	104	4	0.28	42.7
506	gi|492904980|ref|WP_006035386.1|	hypothetical protein [Rickettsiella grylli]	52.03	123	58	1	6.00E-39	139
507	gi|492905355|ref|WP_006035761.1|	single-stranded-DNA-specific exonuclease RecJ [Rickettsiella grylli]	72.35	575	156	3	0	810
508	gi|492904743|ref|WP_006035149.1|	hypothetical protein [Rickettsiella grylli]	36.59	82	48	2	0.003	42.7
509	gi|492905509|ref|WP_006035915.1|	tRNA dihydrouridine synthase DusA [Rickettsiella grylli]	71.52	316	88	2	1.00E-158	459
510	gi|159120963|gb|EDP46301.1|	conserved hypothetical protein [Rickettsiella grylli]	52.7	74	35	0	2.00E-18	82.8
511	gi|492905028|ref|WP_006035434.1|	ferrous iron transporter B [Rickettsiella grylli]	70.56	754	217	3	0	1093
512	gi|915327294|ref|WP_050763982.1|	ferrous iron transport protein A [Rickettsiella grylli]	75.32	77	19	0	8.00E-33	120

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513	gi|492904409|ref|WP_006034815.1|	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase [Rickettsiella grylli]	72.62	493	134	1	0	740
514	gi|780110932|ref|XP_011676476.1|	PREDICTED: serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit A-like, partial [Strongylocentrotus purpuratus]	31.18	680	406	16	3.00E-90	319
514	gi|780110932|ref|XP_011676476.1|	PREDICTED: serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit A-like, partial [Strongylocentrotus purpuratus]	31.32	645	418	12	6.00E-90	318
514	gi|780110932|ref|XP_011676476.1|	PREDICTED: serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit A-like, partial [Strongylocentrotus purpuratus]	29.89	746	482	19	1.00E-82	298
514	gi|780110932|ref|XP_011676476.1|	PREDICTED: serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit A-like, partial [Strongylocentrotus purpuratus]	31.86	543	352	10	2.00E-69	259
514	gi|780110932|ref|XP_011676476.1|	PREDICTED: serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit A-like, partial [Strongylocentrotus purpuratus]	30.58	399	261	9	6.00E-40	170
514	gi|780110932|ref|XP_011676476.1|	PREDICTED: serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit A-like, partial [Strongylocentrotus purpuratus]	27.99	268	180	5	2.00E-15	92
516	gi|159121571|gb|EDP46909.1|	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase (UDP-MurNAc-pentapeptide synthetase) (D-alanyl-D-alanine-adding enzyme) [Rickettsiella grylli]	62.39	444	166	1	0	541
517	gi|492905003|ref|WP_006035409.1|	phospho-N-acetylmuramoyl-pentapeptide-transferase [Rickettsiella grylli]	88.89	360	40	0	0	631
518	gi|492905116|ref|WP_006035522.1|	hypothetical protein [Rickettsiella grylli]	77.46	213	48	0	7.00E-114	337
519	gi|740385944|ref|WP_038220508.1|	hypothetical protein [Xenorhabdus nematophila]	29.77	1085	653	33	2.00E-108	400
520	gi|543941776|ref|WP_021032746.1|	integrase, partial [Pseudoalteromonas rubra]	72.19	169	47	0	4.00E-84	261
521	gi|406979037|gb|EKE00893.1|	hypothetical protein ACD_21C00256G05 [uncultured bacterium]	61.7	282	101	3	4.00E-117	353
522	gi|492905050|ref|WP_006035456.1|	hypothetical protein [Rickettsiella grylli]	90.7	86	8	0	3.00E-42	144
523	gi|492904250|ref|WP_006034656.1|	lcmS [Rickettsiella grylli]	82.14	112	19	1	3.00E-62	197
524	gi|492904242|ref|WP_006034648.1|	bifunctional proline dehydrogenase/L-glutamate gamma-semialdehyde dehydrogenase [Rickettsiella grylli]	75.79	1045	253	0	0	1657
525	gi|492904992|ref|WP_006035398.1|	sodium:hydrogen antiporter [Rickettsiella grylli]	94.1	390	23	0	0	704
526	gi|492904328|ref|WP_006034734.1|	hypothetical protein [Rickettsiella grylli]	80.16	247	49	0	2.00E-134	391
527	gi|492904782|ref|WP_006035188.1|	pyruvate dehydrogenase (acetyl-transferring), homodimeric type [Rickettsiella grylli]	85.02	888	133	0	0	1609
528	gi|159121655|gb|EDP46993.1|	dihydrolipoyllysine-residue acetyltransferase component of pyruvatedehydrogenase complex (E2) (Dihydrolipoamideacetyltransferase component of pyruvate dehydrogenase complex) [Rickettsiella grylli]	69.5	436	128	3	0	614
529	gi|492905417|ref|WP_006035823.1|	dihydrolipoyl dehydrogenase [Rickettsiella grylli]	82.09	469	83	1	0	759
530	gi|640595450|ref|WP_025024165.1|	arginine:ornithine antiporter [Lactobacillus nodensis]	27.7	148	94	3	1.3	41.2
531	gi|492904709|ref|WP_006035115.1|	ATP-dependent DNA helicase RecG [Rickettsiella grylli]	72.26	721	198	2	0	1007
532	gi|159120465|gb|EDP45803.1|	acetyl-CoA carboxylase, biotin carboxyl carrier protein [Rickettsiella grylli]	56.46	147	61	1	7.00E-50	168
533	gi|492905352|ref|WP_006035758.1|	acetyl-CoA carboxylase biotin carboxylase subunit [Rickettsiella grylli]	90.99	444	40	0	0	820
534	gi|159121109|gb|EDP46447.1|	ribosomal protein L11 methyltransferase [Rickettsiella grylli]	55.1	294	132	0	2.00E-115	347
535	gi|492904422|ref|WP_006034828.1|	glutamyl-tRNA reductase [Rickettsiella grylli]	69.31	404	123	1	0	580
536	gi|907678006|ref|XP_013105759.1|	PREDICTED: facilitated trehalose transporter Tret1 [Stomoxys calcitrans]	32.08	106	63	3	2.1	40.4
538	gi|492904623|ref|WP_006035029.1|	ABC transporter [Rickettsiella grylli]	72.25	173	46	2	4.00E-82	254
539	gi|492905455|ref|WP_006035861.1|	ABC transporter substrate-binding protein [Rickettsiella grylli]	76.6	265	62	0	2.00E-146	423

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540	gi|492904764|ref|WP_006035170.1|	iron ABC transporter ATP-binding protein [Rickettsiella grylli]	78.16	261	57	0	5.00E-147	424
541	gi|492904923|ref|WP_006035329.1|	ABC transporter permease [Rickettsiella grylli]	75.6	377	90	2	0	545
542	gi|750333214|ref|WP_040615133.1|	hypothetical protein [Rickettsiella grylli]	86.92	107	14	0	7.00E-61	193
543	gi|492905395|ref|WP_006035801.1|	peptide chain release factor 1 [Rickettsiella grylli]	84.4	359	56	0	0	615
544	gi|492904425|ref|WP_006034831.1|	hypothetical protein [Rickettsiella grylli]	86.92	107	14	0	7.00E-22	94
545	gi|492904677|ref|WP_006035083.1|	protein-(glutamine-N5) methyltransferase, release factor-specific [Rickettsiella grylli]	66.79	280	93	0	1.00E-127	377
546	gi|159120921|gb|EDP46259.1|	suppressor protein DksA [Rickettsiella grylli]	75.88	311	57	5	7.00E-131	388
547	gi|492905587|ref|WP_006035993.1|	nicotinate phosphoribosyltransferase [Rickettsiella grylli]	79.71	478	96	1	0	786
549	gi|492904359|ref|WP_006034765.1|	nicotinamidase [Rickettsiella grylli]	85.78	204	29	0	5.00E-128	372
550	gi|492905146|ref|WP_006035552.1|	EF-P lysine aminoacylase GenX [Rickettsiella grylli]	71.17	326	93	1	3.00E-165	476
551	gi|492905159|ref|WP_006035565.1|	Dot/Icm secretion system ATPase DotB [Rickettsiella grylli]	86.29	372	49	2	0	660
552	gi|492904624|ref|WP_006035030.1|	type IV secretion system protein DotC [Rickettsiella grylli]	77.47	253	57	0	7.00E-147	426
553	gi|492904959|ref|WP_006035365.1|	lipoprotein DotD [Rickettsiella grylli]	72.67	161	43	1	7.00E-78	241
554	gi|492904395|ref|WP_006034801.1|	methyltransferase [Rickettsiella grylli]	64.17	187	67	0	4.00E-81	251
555	gi|333470584|gb|AEF33829.1|	signal recognition particle-receptor alpha subunit [Candidatus Rickettsiella isopodorum]	78.18	330	69	1	3.00E-172	494
556	gi|492904928|ref|WP_006035334.1|	rubredoxin [Rickettsiella grylli]	87.5	56	7	0	2.00E-29	110
557	gi|492904915|ref|WP_006035321.1|	membrane protein [Rickettsiella grylli]	67.15	137	45	0	1.00E-59	193
558	gi|492905153|ref|WP_006035559.1|	coproporphyrinogen III oxidase [Rickettsiella grylli]	73.86	306	74	4	4.00E-162	466
559	gi|518973378|ref|WP_020129253.1|	transcriptional regulator [Streptomyces sp. 303MFCol5.2]	40.48	42	25	0	4.8	35
560	gi|1011036369|ref|WP_061992493.1|	integrase [Flammeovirgaceae bacterium 311]	61.57	229	88	0	7.00E-101	308
561	gi|492905341|ref|WP_006035747.1|	integrase [Rickettsiella grylli]	80.58	412	79	1	0	683
562	gi|492904531|ref|WP_006034937.1|	hypothetical protein [Rickettsiella grylli]	38.37	490	268	6	2.00E-95	310
563	gi|492905505|ref|WP_006035911.1|	hypothetical protein [Rickettsiella grylli]	39.46	484	245	12	8.00E-89	293
564	gi|492904453|ref|WP_006034859.1|	glutamine amidotransferase subunit PdxT [Rickettsiella grylli]	65.76	184	63	0	4.00E-79	246
565	gi|492905016|ref|WP_006035422.1|	pyridoxal biosynthesis lyase PdxS [Rickettsiella grylli]	84.59	279	43	0	2.00E-172	491
566	gi|492904353|ref|WP_006034759.1|	RNA helicase [Rickettsiella grylli]	66.09	404	135	2	0	535
567	gi|492905456|ref|WP_006035862.1|	inverse autotransporter beta-barrel domain-containing protein [Rickettsiella grylli]	45.7	582	285	11	1.00E-150	461
568	gi|916312048|ref|WP_051047094.1|	hypothetical protein [Nocardia asiatica]	45.76	59	31	1	0.001	43.5
569	gi|962264413|gb|KTD48464.1|	integrase [Legionella rubrilucens]	60.22	357	141	1	2.00E-154	452
570	gi|159121287|gb|EDP46625.1|	putative DNA repair endonuclease [Rickettsiella grylli]	73.53	68	18	0	7.00E-30	113
571	gi|492905478|ref|WP_006035884.1|	hypothetical protein [Rickettsiella grylli]	68.09	282	89	1	2.00E-133	392
572	gi|492904873|ref|WP_006035279.1|	hypothetical protein [Rickettsiella grylli]	57.27	337	107	4	9.00E-125	374
573	gi|492904776|ref|WP_006035182.1|	hypothetical protein [Rickettsiella grylli]	69.94	173	52	0	3.00E-88	270
574	gi|492904274|ref|WP_006034680.1|	hypothetical protein [Rickettsiella grylli]	69.57	23	7	0	0.2	36.6
575	gi|492905516|ref|WP_006035922.1|	hypothetical protein [Rickettsiella grylli]	78.79	66	14	0	3.00E-30	112
576	gi|406942276|gb|EKD74548.1|	hypothetical protein ACD_44C00406G01 [uncultured bacterium]	61.54	78	30	0	1.00E-26	104

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577	gi|763835022|gb|KJB95474.1|	twitching motility protein PilT [Skermanella aerolata KACC 11604]	60	135	54	0	4.00E-47	160
578	gi|492905012|ref|WP_006035418.1|	transcriptional regulator [Rickettsiella grylli]	88.35	103	8	1	2.00E-56	181
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	43.98	1462	735	37	0	769
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	41.94	1414	727	37	0	707
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	41.49	1451	757	37	0	691
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	41.85	1424	760	31	0	680
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	42.06	1417	745	38	0	676
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	41.29	1463	773	39	0	676
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	41.09	1436	775	32	0	654
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	40.77	1403	765	33	0	647
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	40.93	1422	744	37	0	643
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	40.18	1426	774	34	0	642
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	40.47	1433	776	40	0	639
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	40.03	1399	748	34	0	622
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	40.32	1302	706	28	6.00E-171	582
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	41.6	1053	560	26	2.00E-151	525
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	39.77	767	398	25	2.00E-78	298
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	41.18	527	280	12	6.00E-72	278
579	gi|918641325|ref|WP_052526970.1|	hypothetical protein [Kineosporia aurantiaca]	40.91	264	141	8	1.00E-25	127
580	gi|492905526|ref|WP_006035932.1|	50S ribosomal protein L21 [Rickettsiella grylli]	73.83	107	23	2	4.00E-48	160
581	gi|492905044|ref|WP_006035450.1|	50S ribosomal protein L27 [Rickettsiella grylli]	91.57	83	7	0	2.00E-47	158
582	gi|492904402|ref|WP_006034808.1|	GTPase ObgE [Rickettsiella grylli]	80.54	334	65	0	3.00E-175	502
583	gi|492905496|ref|WP_006035902.1|	integration host factor subunit beta [Rickettsiella grylli]	88.17	93	11	0	6.00E-53	172
584	gi|492904896|ref|WP_006035302.1|	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase [Rickettsiella grylli]	78.65	192	41	0	2.00E-104	311
585	gi|492905155|ref|WP_006035561.1|	DnaA regulatory inactivator Hda [Rickettsiella grylli]	78.35	231	50	0	3.00E-130	379
586	gi|492904360|ref|WP_006034766.1|	NAD(P)H quinone oxidoreductase [Rickettsiella grylli]	85.64	195	28	0	1.00E-120	352
587	gi|492904950|ref|WP_006035356.1|	30S ribosomal protein S2 [Rickettsiella grylli]	83.77	265	40	2	7.00E-159	455
588	gi|492904327|ref|WP_006034733.1|	elongation factor Ts [Rickettsiella grylli]	70.71	297	86	1	5.00E-146	425
589	gi|492905134|ref|WP_006035540.1|	UMP kinase [Rickettsiella grylli]	77.31	238	54	0	1.00E-132	386
590	gi|492904573|ref|WP_006034979.1|	ribosome recycling factor [Rickettsiella grylli]	86.02	186	25	1	2.00E-109	323
591	gi|492904716|ref|WP_006035122.1|	di-trans,poly-cis-decaprenylcistransferase [Rickettsiella grylli]	78.4	250	54	0	2.00E-141	410
592	gi|492905486|ref|WP_006035892.1|	phosphatidate cytidylyltransferase [Rickettsiella grylli]	69.5	259	79	0	8.00E-111	333
593	gi|492904985|ref|WP_006035391.1|	1-deoxy-D-xylulose-5-phosphate reductoisomerase [Rickettsiella grylli]	77.61	393	88	0	0	631
594	gi|492904420|ref|WP_006034826.1|	outer membrane protein assembly factor BamA [Rickettsiella grylli]	74.07	783	199	1	0	1188
595	gi|492905544|ref|WP_006035950.1|	outer membrane protein [Rickettsiella grylli]	70.24	168	50	0	9.00E-81	249
596	gi|492904774|ref|WP_006035180.1|	UDP-3-O-(3-hydroxymyristoyl)glucosamine N-acyltransferase [Rickettsiella grylli]	75.37	341	84	0	0	524

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597	gi|492904938|ref|WP_006035344.1|	beta-hydroxyacyl-ACP dehydratase [Rickettsiella grylli]	88.51	148	16	1	4.00E-88	266
598	gi|750333218|ref|WP_040615137.1|	acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase [Rickettsiella grylli]	84.05	257	41	0	6.00E-159	454
599	gi|492904627|ref|WP_006035033.1|	lipid-A-disaccharide synthase [Rickettsiella grylli]	69.71	383	116	0	0	547
600	gi|492904987|ref|WP_006035393.1|	ribonuclease HII [Rickettsiella grylli]	73.4	188	50	0	4.00E-97	292
601	gi|750672007|ref|WP_040947928.1|	hypothetical protein [Coxiella burnetii]	27.64	275	172	8	4.00E-09	68.2
603	gi|492905611|ref|WP_006036017.1|	D-alanine--D-alanine ligase A [Rickettsiella grylli]	63.93	366	127	2	2.00E-166	483
604	gi|660515783|ref|YP_009046742.1|	hypothetical protein IIV31_128L [Armadillidium vulgare iridescent virus]	28.23	928	467	36	3.00E-74	273
605	gi|492905476|ref|WP_006035882.1|	hypothetical protein [Rickettsiella grylli]	62.21	217	82	0	2.00E-91	281
606	gi|492905013|ref|WP_006035419.1|	NADH:ubiquinone oxidoreductase subunit A [Rickettsiella grylli]	83.9	118	19	0	1.00E-62	198
607	gi|492904581|ref|WP_006034987.1|	NADH dehydrogenase subunit B [Rickettsiella grylli]	94.34	159	9	0	4.00E-108	317
608	gi|492905225|ref|WP_006035631.1|	NADH dehydrogenase subunit C [Rickettsiella grylli]	79.13	230	48	0	1.00E-132	385
609	gi|492904273|ref|WP_006034679.1|	NADH dehydrogenase subunit D [Rickettsiella grylli]	93.53	417	27	0	0	821
610	gi|492904745|ref|WP_006035151.1|	NADH dehydrogenase subunit E [Rickettsiella grylli]	74.56	169	42	1	2.00E-86	263
611	gi|492905187|ref|WP_006035593.1|	NADH-quinone oxidoreductase subunit F [Rickettsiella grylli]	87.56	426	53	0	0	781
612	gi|492904602|ref|WP_006035008.1|	NADH-quinone oxidoreductase subunit G [Rickettsiella grylli]	70.05	798	229	3	0	1146
613	gi|492905524|ref|WP_006035930.1|	NADH-quinone oxidoreductase subunit H [Rickettsiella grylli]	87.1	341	44	0	0	580
614	gi|492905564|ref|WP_006035970.1|	NADH-quinone oxidoreductase subunit I [Rickettsiella grylli]	93.33	165	11	0	1.00E-109	322
615	gi|492904951|ref|WP_006035357.1|	NADH-quinone oxidoreductase [Rickettsiella grylli]	70.26	195	58	0	1.00E-82	256
616	gi|492904496|ref|WP_006034902.1|	NADH-quinone oxidoreductase subunit K [Rickettsiella grylli]	87.13	101	13	0	3.00E-45	153
617	gi|492905132|ref|WP_006035538.1|	NADH-quinone oxidoreductase subunit L [Rickettsiella grylli]	75.89	643	148	4	0	955
618	gi|492904790|ref|WP_006035196.1|	NADH-quinone oxidoreductase subunit M [Rickettsiella grylli]	85.07	509	76	0	0	891
619	gi|492905303|ref|WP_006035709.1|	NADH-quinone oxidoreductase subunit N [Rickettsiella grylli]	77.78	486	108	0	0	711
620	gi|492904970|ref|WP_006035376.1|	BON domain-containing protein [Rickettsiella grylli]	80.53	190	37	0	1.00E-105	314
621	gi|750333220|ref|WP_040615139.1|	aminotransferase [Rickettsiella grylli]	85.89	397	55	1	0	715
622	gi|915327306|ref|WP_050763994.1|	peptide chain release factor 2 [Rickettsiella grylli]	80.62	320	62	0	0	533
623	gi|159120572|gb|EDP45910.1|	lysyl-tRNA synthetase [Rickettsiella grylli]	76.15	499	118	1	0	794
624	gi|492904486|ref|WP_006034892.1|	50S ribosomal protein L33 [Rickettsiella grylli]	94	50	3	0	2.00E-23	94
625	gi|159121237|gb|EDP46575.1|	conserved domain protein [Rickettsiella grylli]	76.92	78	18	0	1.00E-35	127
626	gi|492904361|ref|WP_006034767.1|	hypothetical protein [Rickettsiella grylli]	80.36	224	44	0	4.00E-131	381
627	gi|492904968|ref|WP_006035374.1|	EVE domain-containing protein [Rickettsiella grylli]	72.48	149	40	1	1.00E-72	228
628	gi|492905582|ref|WP_006035988.1|	proline--tRNA ligase [Rickettsiella grylli]	72.31	567	156	1	0	852
629	gi|492905517|ref|WP_006035923.1|	type I antifreeze protein [Rickettsiella grylli]	53.98	113	39	3	5.00E-30	115
630	gi|492904880|ref|WP_006035286.1|	aspartate--tRNA ligase [Rickettsiella grylli]	77.63	590	132	0	0	967
631	gi|492905299|ref|WP_006035705.1|	hypothetical protein [Rickettsiella grylli]	48.3	265	119	5	6.00E-58	197
632	gi|498283938|ref|WP_010598094.1|	hypothetical protein [Diplorickettsia massiliensis]	74.79	238	60	0	2.00E-127	373

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633	gi|492904932|ref|WP_006035338.1|	crossover junction endodeoxyribonuclease RuvC [Rickettsiella grylli]	72.43	185	48	2	2.00E-75	236
634	gi|492904325|ref|WP_006034731.1|	Holliday junction ATP-dependent DNA helicase RuvA [Rickettsiella grylli]	70.94	203	52	2	7.00E-98	295
635	gi|228013288|gb|ACP49049.1|	Ankyrin [Sulfolobus islandicus Y.N.15.51]	34.55	165	96	2	7.00E-16	84.7
635	gi|228013288|gb|ACP49049.1|	Ankyrin [Sulfolobus islandicus Y.N.15.51]	33.33	162	96	2	2.00E-13	77.8
635	gi|228013288|gb|ACP49049.1|	Ankyrin [Sulfolobus islandicus Y.N.15.51]	32.87	143	84	2	8.00E-10	67.8
635	gi|228013288|gb|ACP49049.1|	Ankyrin [Sulfolobus islandicus Y.N.15.51]	39.39	66	40	0	5.00E-04	50.8
636	gi|492905373|ref|WP_006035779.1|	Holliday junction DNA helicase RuvB [Rickettsiella grylli]	87.46	351	44	0	0	619
637	gi|492905393|ref|WP_006035799.1|	protein TolQ [Rickettsiella grylli]	79.4	233	48	0	7.00E-133	386
638	gi|492905489|ref|WP_006035895.1|	protein TolR [Rickettsiella grylli]	68.87	151	44	2	4.00E-64	205
639	gi|915327308|ref|WP_050763996.1|	protein TolA [Rickettsiella grylli]	55.33	291	111	7	3.00E-88	276
640	gi|492905198|ref|WP_006035604.1|	MFS transporter [Rickettsiella grylli]	77.23	426	95	1	0	608
641	gi|406938524|gb|EKD71739.1|	Cytochrome b561 transmembrane protein [uncultured bacterium]	60.57	175	69	0	3.00E-67	215
642	gi|492905203|ref|WP_006035609.1|	Tol-Pal system beta propeller repeat protein TolB [Rickettsiella grylli]	69.84	451	136	0	0	657
643	gi|492904903|ref|WP_006035309.1|	peptidoglycan-associated lipoprotein [Rickettsiella grylli]	67.86	168	46	3	2.00E-76	239
644	gi|492905051|ref|WP_006035457.1|	tol-pal system protein YbgF [Rickettsiella grylli]	56.18	340	113	7	1.00E-106	327
645	gi|492905363|ref|WP_006035769.1|	tRNA pseudouridine(38,39,40) synthase TruA [Rickettsiella grylli]	66.02	259	88	0	3.00E-123	364
646	gi|492904930|ref|WP_006035336.1|	putrescine/spermidine ABC transporter ATP-binding protein [Rickettsiella grylli]	85.87	361	50	1	0	635
647	gi|492904564|ref|WP_006034970.1|	spermidine/putrescine ABC transporter permease [Rickettsiella grylli]	81.6	288	53	0	1.00E-164	471
648	gi|492905192|ref|WP_006035598.1|	spermidine/putrescine ABC transporter permease PotC [Rickettsiella grylli]	85.83	254	36	0	6.00E-148	427
649	gi|492905567|ref|WP_006035973.1|	spermidine/putrescine ABC transporter substrate-binding protein [Rickettsiella grylli]	75.87	344	82	1	0	561
650	gi|492904784|ref|WP_006035190.1|	acetyl-CoA carboxylase subunit beta [Rickettsiella grylli]	83.5	297	49	0	0	521
651	gi|492905378|ref|WP_006035784.1|	FolC bifunctional protein [Rickettsiella grylli]	66.59	413	137	1	0	573
652	gi|492905364|ref|WP_006035770.1|	sporulation domain protein [Rickettsiella grylli]	55.77	156	63	1	2.00E-52	176
653	gi|492904729|ref|WP_006035135.1|	orotidine 5'-phosphate decarboxylase [Rickettsiella grylli]	66.67	261	87	0	1.00E-125	370
654	gi|492904830|ref|WP_006035236.1|	cytidylate kinase [Rickettsiella grylli]	64.83	236	78	3	9.00E-94	287
655	gi|492905453|ref|WP_006035859.1|	30S ribosomal protein S1 [Rickettsiella grylli]	89.21	519	56	0	0	942
655	gi|492905453|ref|WP_006035859.1|	30S ribosomal protein S1 [Rickettsiella grylli]	31.22	362	230	8	1.00E-43	173
656	gi|492905368|ref|WP_006035774.1|	membrane protein [Rickettsiella grylli]	82.29	96	17	0	3.00E-48	160
657	gi|492904757|ref|WP_006035163.1|	hypothetical protein [Rickettsiella grylli]	79.3	372	77	0	0	587
658	gi|966466426|ref|WP_058497752.1|	ABC transporter ATP-binding protein [Legionella gratiana]	60.42	518	205	0	0	642
659	gi|492904456|ref|WP_006034862.1|	hypothetical protein [Rickettsiella grylli]	46.31	529	266	6	8.00E-145	453
660	gi|966395171|ref|WP_058440583.1|	hypothetical protein [Legionella brunensis]	44.58	323	169	3	1.00E-81	263
661	gi|727286736|ref|WP_033744642.1|	molybdopterin-guanine dinucleotide biosynthesis protein MobA [Helicobacter pylori]	25.77	194	118	8	1	43.1
662	gi|890832011|ref|WP_048901581.1|	cell division inhibitor, NAD(P)-binding protein [Candidatus Hamiltonella defensa]	66	300	101	1	4.00E-142	416
663	gi|498283519|ref|WP_010597675.1|	hypothetical protein [Diplorickettsia massiliensis]	82.14	224	40	0	6.00E-127	370
664	gi|498283518|ref|WP_010597674.1|	TspO and MBR-like protein [Diplorickettsia massiliensis]	78.21	156	34	0	2.00E-80	247

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665	gi|517522885|ref|WP_018693093.1|	hypothetical protein [Algicola sagamiensis]	35.45	347	205	8	2.00E-55	202
666	gi|406941937|gb|EKD74294.1|	hypothetical protein ACD_45C06G02 [uncultured bacterium]	60.15	271	108	0	2.00E-109	330
667	gi|492905222|ref|WP_006035628.1|	hypothetical protein [Rickettsiella grylli]	39.97	603	317	11	1.00E-115	374
668	gi|492904433|ref|WP_006034839.1|	hypothetical protein [Rickettsiella grylli]	63.27	275	100	1	3.00E-121	360
669	gi|492904654|ref|WP_006035060.1|	response regulator [Rickettsiella grylli]	62.41	133	47	1	6.00E-50	169
670	gi|657659699|ref|WP_029463554.1|	methionine ABC transporter ATP-binding protein [Diplorickettsia massiliensis]	59.94	347	139	0	9.00E-137	405
671	gi|769979903|ref|WP_045095888.1|	methionine ABC transporter permease [Legionella fallonii]	59.26	216	82	2	1.00E-79	250
672	gi|492171274|ref|WP_005769431.1|	membrane protein [Coxiella burnetii]	54.75	263	119	0	9.00E-98	300
673	gi|492904844|ref|WP_006035250.1|	GTP cyclohydrolase I FolE [Rickettsiella grylli]	79.78	178	36	0	3.00E-100	299
674	gi|492905382|ref|WP_006035788.1|	glycosyl transferase family 39 [Rickettsiella grylli]	73.29	483	129	0	0	684
675	gi|505487224|ref|WP_015671870.1|	aspartyl/asparaginyl beta-hydroxylase-like dioxygenase [Serratia marcescens]	75.33	300	74	0	2.00E-173	494
676	gi|492904461|ref|WP_006034867.1|	adenosine/AMP deaminase [Rickettsiella grylli]	60.45	493	193	2	0	623
677	gi|549047107|emb|CCX13606.1|	Similar to Calcium-binding protein 39; acc. no. Q9Y376 [Pyronema omphalodes CBS 100304]	31.88	69	36	1	3.1	36.6
678	gi|492905037|ref|WP_006035443.1|	hypothetical protein [Rickettsiella grylli]	75.97	258	62	0	2.00E-141	410
679	gi|492905406|ref|WP_006035812.1|	DNA polymerase III subunit delta' [Rickettsiella grylli]	61.92	323	121	2	3.00E-128	382
680	gi|492904617|ref|WP_006035023.1|	dTMP kinase [Rickettsiella grylli]	81.22	213	40	0	7.00E-123	360
681	gi|973269723|gb|KUL34713.1|	acetyltransferase [Streptomyces sp. NRRL F-4489]	38.18	55	33	1	1.7	37
682	gi|1028824284|ref|WP_064005138.1|	hypothetical protein [Piscirickettsiaceae bacterium NZ-RLO]	42.12	292	155	7	8.00E-57	215
683	gi|492905466|ref|WP_006035872.1|	aminodeoxychorismate lyase [Rickettsiella grylli]	64.75	366	126	1	4.00E-171	494
684	gi|159121041|gb|EDP46379.1|	3-oxoacyl-[acyl-carrier-protein] synthase 2 [Rickettsiella grylli]	90.57	424	40	0	0	800
685	gi|492904406|ref|WP_006034812.1|	acyl carrier protein [Rickettsiella grylli]	96.05	76	3	0	3.00E-41	142
686	gi|492905173|ref|WP_006035579.1|	beta-ketoacyl-ACP reductase [Rickettsiella grylli]	75.92	245	59	0	2.00E-132	386
687	gi|492904550|ref|WP_006034956.1|	malonyl CoA-acyl carrier protein transacylase [Rickettsiella grylli]	77.27	308	70	0	1.00E-175	501
688	gi|492904649|ref|WP_006035055.1|	3-oxoacyl-ACP synthase [Rickettsiella grylli]	83.91	317	50	1	0	541
689	gi|492905482|ref|WP_006035888.1|	phosphate acyltransferase [Rickettsiella grylli]	88.12	345	41	0	0	622
690	gi|498282885|ref|WP_010597041.1|	50S ribosomal protein L32 [Diplorickettsia massiliensis]	86.21	58	8	0	9.00E-28	105
691	gi|492904988|ref|WP_006035394.1|	ferredoxin [Rickettsiella grylli]	75.29	85	21	0	5.00E-38	133
692	gi|492904984|ref|WP_006035390.1|	pantetheine-phosphate adenylyltransferase [Rickettsiella grylli]	76.58	158	37	0	2.00E-83	255
693	gi|492904355|ref|WP_006034761.1|	4-hydroxybenzoate octaprenyltransferase [Rickettsiella grylli]	62.63	281	105	0	1.00E-122	365
694	gi|492904798|ref|WP_006035204.1|	outer membrane protein [Rickettsiella grylli]	74.86	175	44	0	3.00E-90	275
695	gi|492905598|ref|WP_006036004.1|	hypothetical protein [Rickettsiella grylli]	57.67	215	88	2	1.00E-78	246
696	gi|492905442|ref|WP_006035848.1|	OmpA/MotB domain protein [Rickettsiella grylli]	58.94	207	66	4	1.00E-71	228
697	gi|492904468|ref|WP_006034874.1|	hypothetical protein [Rickettsiella grylli]	55.9	229	74	6	2.00E-69	224
698	gi|492904514|ref|WP_006034920.1|	outer membrane protein OmpA [Rickettsiella grylli]	57.71	201	77	3	2.00E-79	248
699	gi|492905008|ref|WP_006035414.1|	excinuclease ABC subunit A [Rickettsiella grylli]	83.8	957	153	2	0	1627
700	gi|515076667|ref|WP_016706465.1|	hypothetical protein [Pseudoalteromonas haloplanktis]	38.98	59	35	1	0.055	38.5

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701	gi|492904806|ref|WP_006035212.1|	single-stranded DNA-binding protein [Rickettsiella grylli]	81.01	158	21	3	1.00E-80	247
702	gi|492905082|ref|WP_006035488.1|	transporter [Rickettsiella grylli]	72.48	109	30	0	3.00E-49	164
703	gi|750333239|ref|WP_040615158.1|	inverse autotransporter beta-barrel domain-containing protein [Rickettsiella grylli]	50.4	625	279	13	0	543
704	gi|492905456|ref|WP_006035862.1|	inverse autotransporter beta-barrel domain-containing protein [Rickettsiella grylli]	46.5	628	266	16	8.00E-161	488
705	gi|492905569|ref|WP_006035975.1|	murein transglycosylase [Rickettsiella grylli]	68.56	617	192	2	0	845
706	gi|492904818|ref|WP_006035224.1|	hypothetical protein [Rickettsiella grylli]	86.18	398	55	0	0	711
707	gi|492905428|ref|WP_006035834.1|	DUF378 domain-containing protein [Rickettsiella grylli]	87.67	73	9	0	2.00E-37	131
708	gi|492904640|ref|WP_006035046.1|	universal stress protein UspA [Rickettsiella grylli]	86.39	147	20	0	2.00E-86	261
710	gi|518973378|ref|WP_020129253.1|	transcriptional regulator [Streptomyces sp. 303MFCol5.2]	40.48	42	25	0	5	35
711	gi|492904491|ref|WP_006034897.1|	integration host factor subunit alpha [Rickettsiella grylli]	76.19	84	20	0	2.00E-34	125
712	gi|492905228|ref|WP_006035634.1|	phenylalanine--tRNA ligase subunit beta [Rickettsiella grylli]	60.86	792	307	2	0	996
713	gi|492904244|ref|WP_006034650.1|	phenylalanine--tRNA ligase subunit alpha [Rickettsiella grylli]	80.06	341	66	1	0	570
714	gi|517435158|ref|WP_018606056.1|	hypothetical protein [Uliginosibacterium gangwonense]	35.4	113	67	3	5.00E-11	65.5
715	gi|492905035|ref|WP_006035441.1|	hypothetical protein [Rickettsiella grylli]	91.94	62	5	0	5.00E-31	114
716	gi|492904613|ref|WP_006035019.1|	tRNA threonylcarbamoyladenosine biosynthesis protein TsaB [Rickettsiella grylli]	64.07	231	81	2	1.00E-96	294
717	gi|518057623|ref|WP_019227831.1|	DNA-binding response regulator [Sedimentibacter sp. B4]	27.95	161	94	7	0.56	41.2
718	gi|524659825|emb|CDD71955.1|	putative endoribonuclease L-PSP [Sutterella sp. CAG:397]	40.35	57	32	1	1.1	38.9
719	gi|159120559|gb|EDP45897.1|	ferredoxin [Rickettsiella grylli]	85.98	107	15	0	6.00E-59	188
720	gi|492904945|ref|WP_006035351.1|	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase [Rickettsiella grylli]	82.42	182	32	0	6.00E-103	307
721	gi|492904476|ref|WP_006034882.1|	excinuclease ABC subunit C [Rickettsiella grylli]	71.03	604	175	0	0	890
722	gi|750333234|ref|WP_040615153.1|	hypothetical protein [Rickettsiella grylli]	62	100	34	2	3.00E-34	125
723	gi|492904925|ref|WP_006035331.1|	DNA-binding response regulator [Rickettsiella grylli]	94.06	219	13	0	2.00E-146	420
725	gi|492904352|ref|WP_006034758.1|	tRNA-specific adenosine deaminase [Rickettsiella grylli]	62.84	148	53	1	9.00E-61	197
726	gi|492904957|ref|WP_006035363.1|	hypothetical protein [Rickettsiella grylli]	75.64	78	18	1	1.00E-33	122
727	gi|492904400|ref|WP_006034806.1|	23S rRNA (guanosine(2251)-2'-O)-methyltransferase RlmB [Rickettsiella grylli]	57.69	260	102	2	6.00E-99	302
728	gi|743942488|ref|XP_011015738.1|	PREDICTED: uncharacterized protein LOC105119307 isoform X3 [Populus euphratica]	23.3	176	112	5	1.7	41.2
729	gi|492904999|ref|WP_006035405.1|	ribonuclease R [Rickettsiella grylli]	83.77	727	118	0	0	1281
730	gi|492905165|ref|WP_006035571.1|	16S rRNA (uracil(1498)-N(3))-methyltransferase [Rickettsiella grylli]	61.98	242	91	1	2.00E-104	315
731	gi|492904481|ref|WP_006034887.1|	outer membrane lipoprotein LolB [Rickettsiella grylli]	53.96	202	93	0	8.00E-74	234
733	gi|492904291|ref|WP_006034697.1|	ribose-phosphate pyrophosphokinase [Rickettsiella grylli]	88.33	317	37	0	0	584
734	gi|492905231|ref|WP_006035637.1|	50S ribosomal protein L25/general stress protein Ctc [Rickettsiella grylli]	79.57	235	47	1	7.00E-130	379
735	gi|492904508|ref|WP_006034914.1|	aminoacyl-tRNA hydrolase [Rickettsiella grylli]	64.62	195	69	0	2.00E-85	263
736	gi|492905106|ref|WP_006035512.1|	GTP-binding protein YchF [Rickettsiella grylli]	76.31	363	86	0	0	577
737	gi|750333169|ref|WP_040615088.1|	hypothetical protein [Rickettsiella grylli]	37.99	229	130	2	1.00E-41	167
738	gi|492904824|ref|WP_006035230.1|	hypothetical protein [Rickettsiella grylli]	33.68	576	347	14	2.00E-69	246
739	gi|498282989|ref|WP_010597145.1|	pyridoxal-5'-phosphate-dependent protein [Diplorickettsia massiliensis]	77.12	319	73	0	0	521

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740	gi|492905369|ref|WP_006035775.1|	succinate--CoA ligase subunit alpha [Rickettsiella grylli]	88.93	289	32	0	0	521
741	gi|492904891|ref|WP_006035297.1|	succinate--CoA ligase subunit beta [Rickettsiella grylli]	84.36	390	61	0	0	672
742	gi|492905470|ref|WP_006035876.1|	dihydrolipoamide succinyltransferase [Rickettsiella grylli]	77.8	410	84	5	0	630
743	gi|492905108|ref|WP_006035514.1|	2-oxoglutarate dehydrogenase subunit E1 [Rickettsiella grylli]	79.41	923	188	1	0	1551
744	gi|492905216|ref|WP_006035622.1|	succinate dehydrogenase iron-sulfur subunit [Rickettsiella grylli]	85.78	232	33	0	3.00E-149	427
745	gi|492904419|ref|WP_006034825.1|	succinate dehydrogenase flavoprotein subunit [Rickettsiella grylli]	88.27	588	69	0	0	1082
746	gi|492905477|ref|WP_006035883.1|	succinate dehydrogenase, hydrophobic membrane anchor protein [Rickettsiella grylli]	70.94	117	34	0	1.00E-53	176
747	gi|492904908|ref|WP_006035314.1|	succinate dehydrogenase, cytochrome b556 subunit [Rickettsiella grylli]	62.6	123	46	0	3.00E-39	139
748	gi|492904877|ref|WP_006035283.1|	RAP domain family [Rickettsiella grylli]	38.31	462	278	5	2.00E-87	306
748	gi|492904877|ref|WP_006035283.1|	RAP domain family [Rickettsiella grylli]	38.62	334	195	4	6.00E-54	209
748	gi|492904877|ref|WP_006035283.1|	RAP domain family [Rickettsiella grylli]	36.36	308	193	3	2.00E-46	187
748	gi|492904877|ref|WP_006035283.1|	RAP domain family [Rickettsiella grylli]	34.58	321	205	3	7.00E-45	183
748	gi|492904877|ref|WP_006035283.1|	RAP domain family [Rickettsiella grylli]	36.9	271	170	1	4.00E-44	181
748	gi|492904877|ref|WP_006035283.1|	RAP domain family [Rickettsiella grylli]	32.81	320	210	3	4.00E-43	177
748	gi|492904877|ref|WP_006035283.1|	RAP domain family [Rickettsiella grylli]	33.94	327	210	4	2.00E-41	172
749	gi|492905502|ref|WP_006035908.1|	23S rRNA pseudouridylate synthase B [Rickettsiella grylli]	68.44	244	77	0	6.00E-116	345
750	gi|493925039|ref|WP_006869866.1|	alkyl sulfatase [Legionella drancourtii]	61.81	631	240	1	0	850
751	gi|492904653|ref|WP_006035059.1|	SMC-Scp complex subunit ScpB [Rickettsiella grylli]	76.51	166	38	1	3.00E-84	259
752	gi|492904267|ref|WP_006034673.1|	hydroxyethylthiazole kinase [Rickettsiella grylli]	63.1	271	99	1	8.00E-116	347
753	gi|492904807|ref|WP_006035213.1|	thiamine phosphate synthase [Rickettsiella grylli]	55.61	205	91	0	1.00E-74	236
754	gi|492904502|ref|WP_006034908.1|	hydroxymethylpyrimidine/phosphomethylpyrimidine kinase [Rickettsiella grylli]	70.48	271	79	1	2.00E-129	381
755	gi|492905160|ref|WP_006035566.1|	thiaminase II [Rickettsiella grylli]	58.33	216	88	1	2.00E-84	261
756	gi|492904753|ref|WP_006035159.1|	hypothetical protein [Rickettsiella grylli]	37.96	893	477	16	4.00E-161	521
756	gi|492904753|ref|WP_006035159.1|	hypothetical protein [Rickettsiella grylli]	25.8	628	377	13	1.00E-38	167
757	gi|492905345|ref|WP_006035751.1|	TonB-dependent receptor [Rickettsiella grylli]	68.42	114	36	0	2.00E-47	160
758	gi|492904735|ref|WP_006035141.1|	hypothetical protein [Rickettsiella grylli]	55.45	880	386	5	0	964
759	gi|492904867|ref|WP_006035273.1|	hypothetical protein [Rickettsiella grylli]	39.03	515	299	8	5.00E-116	367
760	gi|915327325|ref|WP_050764013.1|	hypothetical protein [Rickettsiella grylli]	56.11	1121	479	9	0	1215
761	gi|492904396|ref|WP_006034802.1|	alkaline phosphatase, DedA family [Rickettsiella grylli]	74.71	174	44	0	1.00E-75	236
762	gi|492905335|ref|WP_006035741.1|	hypothetical protein [Rickettsiella grylli]	79.35	92	19	0	1.00E-45	154
763	gi|492904475|ref|WP_006034881.1|	prevent-host-death family protein [Rickettsiella grylli]	84.52	84	13	0	1.00E-43	147
764	gi|492904810|ref|WP_006035216.1|	endopeptidase IV [Rickettsiella grylli]	75.16	306	71	2	2.00E-159	459
765	gi|492904512|ref|WP_006034918.1|	MFS transporter [Rickettsiella grylli]	66.27	504	169	1	0	662
767	gi|492904793|ref|WP_006035199.1|	cysteine--tRNA ligase [Rickettsiella grylli]	72.01	468	126	2	0	722
768	gi|492905575|ref|WP_006035981.1|	glutamate--tRNA ligase [Rickettsiella grylli]	69.96	466	140	0	0	676
769	gi|492905280|ref|WP_006035686.1|	UDP-2,3-diacylglucosamine diphosphatase [Rickettsiella grylli]	55.79	242	106	1	2.00E-88	274

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770	gi|406940116|gb|EKD72964.1|	LysR protein, partial [uncultured bacterium]	72.54	244	67	0	1.00E-125	371
771	gi|966395839|ref|WP_058440930.1|	alkyl hydroperoxide reductase [Legionella brunensis]	74.43	176	45	0	5.00E-96	288
772	gi|515946782|ref|WP_017377365.1|	hypothetical protein [Piscirickettsia salmonis]	56.9	174	75	0	3.00E-64	207
773	gi|492904381|ref|WP_006034787.1|	colicin V production protein CvpA [Rickettsiella grylli]	80	170	34	0	4.00E-90	273
774	gi|492904981|ref|WP_006035387.1|	orotate phosphoribosyltransferase [Rickettsiella grylli]	68.6	172	54	0	6.00E-79	245
775	gi|492905579|ref|WP_006035985.1|	DNA gyrase subunit A [Rickettsiella grylli]	87.41	858	101	1	0	1504
776	gi|492904791|ref|WP_006035197.1|	hypothetical protein [Rickettsiella grylli]	42.72	103	44	4	7.00E-09	60.1
777	gi|492905397|ref|WP_006035803.1|	ribonuclease E (RNase E) [Rickettsiella grylli]	63.54	790	255	15	0	929
778	gi|492904558|ref|WP_006034964.1|	acid phosphatase, HAD superfamily protein [Rickettsiella grylli]	66.12	242	80	2	5.00E-115	343
779	gi|498283417|ref|WP_010597573.1|	hypothetical protein [Diplorickettsia massiliensis]	65.67	67	23	0	5.00E-22	93.2
781	gi|492904292|ref|WP_006034698.1|	glutamate--tRNA ligase [Rickettsiella grylli]	73.9	456	119	0	0	694
782	gi|492905049|ref|WP_006035455.1|	threonylcarbamoyl-AMP synthase [Rickettsiella grylli]	78.37	208	45	0	7.00E-114	336
783	gi|492904337|ref|WP_006034743.1|	septation protein A [Rickettsiella grylli]	81.01	179	34	0	6.00E-100	298
784	gi|498283028|ref|WP_010597184.1|	BolA family transcriptional regulator [Diplorickettsia massiliensis]	64.37	87	31	0	5.00E-36	128
785	gi|492904546|ref|WP_006034952.1|	hypothetical protein [Rickettsiella grylli]	39.78	651	336	14	1.00E-132	415
786	gi|492905292|ref|WP_006035698.1|	hypothetical protein [Rickettsiella grylli]	86.39	999	136	0	0	1823
787	gi|492904303|ref|WP_006034709.1|	hypothetical protein [Rickettsiella grylli]	72.38	181	50	0	5.00E-94	284
788	gi|159120854|gb|EDP46192.1|	IcmD protein [Rickettsiella grylli]	89.08	119	12	1	3.00E-63	201
789	gi|492905383|ref|WP_006035789.1|	hypothetical protein [Rickettsiella grylli]	73.57	140	37	0	3.00E-49	166
790	gi|492904741|ref|WP_006035147.1|	hypothetical protein [Rickettsiella grylli]	74.63	205	51	1	2.00E-106	318
791	gi|492905253|ref|WP_006035659.1|	hypothetical protein [Rickettsiella grylli]	53.97	239	108	2	2.00E-75	240
792	gi|492904504|ref|WP_006034910.1|	IcmE protein [Rickettsiella grylli]	58.93	728	220	9	0	803
793	gi|492905133|ref|WP_006035539.1|	IcmK [Rickettsiella grylli]	75.7	321	68	2	6.00E-157	454
794	gi|492904305|ref|WP_006034711.1|	type IV secretion system protein IcmL [Rickettsiella grylli]	84.91	212	32	0	1.00E-132	384
795	gi|492904895|ref|WP_006035301.1|	hypothetical protein [Rickettsiella grylli]	60.56	71	28	0	1.00E-23	96.3
796	gi|498283039|ref|WP_010597195.1|	OmpA/MotB domain-containing protein [Diplorickettsia massiliensis]	38.55	166	92	4	5.00E-24	103
797	gi|492905291|ref|WP_006035697.1|	phosphoesterase [Rickettsiella grylli]	86.62	777	100	3	0	1384
798	gi|492904842|ref|WP_006035248.1|	hypothetical protein [Rickettsiella grylli]	76.01	371	88	1	0	594
799	gi|157429090|gb|ABV56609.1|	type IVa secretion system component IcmQ [Rickettsiella melolonthae]	75.54	184	45	0	6.00E-96	289
800	gi|492905151|ref|WP_006035557.1|	hypothetical protein [Rickettsiella grylli]	43.33	60	32	2	0.11	37.7
801	gi|492904539|ref|WP_006034945.1|	hypothetical protein [Rickettsiella grylli]	61.17	394	151	1	1.00E-172	500
802	gi|492904972|ref|WP_006035378.1|	pteridine reductase [Rickettsiella grylli]	73.71	251	66	0	1.00E-135	395
803	gi|492904748|ref|WP_006035154.1|	SUF system Fe-S cluster assembly regulator [Rickettsiella grylli]	73.24	142	38	0	3.00E-65	208
804	gi|492905038|ref|WP_006035444.1|	Fe-S cluster assembly protein SufB [Rickettsiella grylli]	87.5	480	60	0	0	892
805	gi|492904936|ref|WP_006035342.1|	ABC transporter ATP-binding protein [Rickettsiella grylli]	82.26	248	44	0	1.00E-146	424
806	gi|492905204|ref|WP_006035610.1|	Fe-S cluster assembly protein SufD [Rickettsiella grylli]	58.43	433	171	6	2.00E-166	488

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807	gi|492904241|ref|WP_006034647.1|	cysteine desulfurase [Rickettsiella grylli]	80.19	414	82	0	0	696
808	gi|492905356|ref|WP_006035762.1|	iron-sulfur cluster assembly scaffold protein [Rickettsiella grylli]	73.51	151	40	0	2.00E-76	237
809	gi|492904442|ref|WP_006034848.1|	SUF system Fe-S cluster assembly protein [Rickettsiella grylli]	68.47	111	32	1	3.00E-47	159
810	gi|498284853|ref|WP_010599009.1|	hypothetical protein [Diplorickettsia massiliensis]	58.2	122	50	1	3.00E-36	140
811	gi|492905181|ref|WP_006035587.1|	NAD(P)H-hydrate dehydratase [Rickettsiella grylli]	67.04	270	88	1	7.00E-111	333
812	gi|800983852|ref|WP_046010127.1|	short-chain dehydrogenase [Oleispira antarctica]	64.77	264	93	0	3.00E-120	357
813	gi|492904574|ref|WP_006034980.1|	glutathione synthase [Rickettsiella grylli]	67.95	312	100	0	6.00E-154	446
814	gi|492905340|ref|WP_006035746.1|	glutamate--cysteine ligase [Rickettsiella grylli]	76.38	436	103	0	0	687
815	gi|492904979|ref|WP_006035385.1|	amino acid transporter [Rickettsiella grylli]	86.66	652	87	0	0	1110
816	gi|492904378|ref|WP_006034784.1|	hypothetical protein [Rickettsiella grylli]	59.6	151	60	1	1.00E-44	155
817	gi|492905577|ref|WP_006035983.1|	GTPase Era [Rickettsiella grylli]	70.34	290	86	0	3.00E-144	420
818	gi|492904484|ref|WP_006034890.1|	ribonuclease III [Rickettsiella grylli]	87.89	223	27	0	3.00E-142	410
819	gi|492905068|ref|WP_006035474.1|	S26 family signal peptidase [Rickettsiella grylli]	76.74	258	60	0	1.00E-146	423
820	gi|492905139|ref|WP_006035545.1|	elongation factor 4 [Rickettsiella grylli]	89.28	597	64	0	0	1073
821	gi|492904536|ref|WP_006034942.1|	carboxylesterase [Rickettsiella grylli]	88.34	223	26	0	1.00E-145	418
822	gi|492905501|ref|WP_006035907.1|	diaminopimelate decarboxylase [Rickettsiella grylli]	66.59	413	137	1	0	568
823	gi|492904935|ref|WP_006035341.1|	diaminopimelate epimerase [Rickettsiella grylli]	80.14	277	54	1	3.00E-167	477
824	gi|492905538|ref|WP_006035944.1|	class II fumarate hydratase [Rickettsiella grylli]	84.65	469	72	0	0	831
825	gi|492904983|ref|WP_006035389.1|	EF-P beta-lysylation protein EpmB [Rickettsiella grylli]	68.83	324	101	0	8.00E-161	465
826	gi|492905456|ref|WP_006035862.1|	inverse autotransporter beta-barrel domain-containing protein [Rickettsiella grylli]	47.62	609	271	13	3.00E-170	512
827	gi|492905290|ref|WP_006035696.1|	inverse autotransporter beta-barrel domain-containing protein [Rickettsiella grylli]	48.33	598	278	9	4.00E-167	503
828	gi|159120951|gb|EDP46289.1|	peptidoglycan synthetase FtsI (Peptidoglycanglycosyltransferase 3) (Penicillin-binding protein 3) (PBP-3) [Rickettsiella grylli]	78.35	559	120	1	0	894
829	gi|492904696|ref|WP_006035102.1|	hypothetical protein [Rickettsiella grylli]	78.57	112	23	1	2.00E-53	175
830	gi|492905061|ref|WP_006035467.1|	16S rRNA (cytosine(1402)-N(4))-methyltransferase [Rickettsiella grylli]	74.6	311	78	1	2.00E-165	476
831	gi|492904459|ref|WP_006034865.1|	division/cell wall cluster transcriptional repressor MraZ [Rickettsiella grylli]	78.21	156	29	1	2.00E-78	241
832	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	28.12	256	169	7	5.00E-13	81.6
832	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	27.01	274	157	10	4.00E-11	75.5
832	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	25.39	256	176	8	8.00E-07	62
832	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	26.89	264	176	9	2.00E-06	60.8
832	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	23.85	239	170	6	9.00E-06	58.9
832	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	23.47	294	200	8	2.00E-04	54.3
833	gi|492904315|ref|WP_006034721.1|	anhydro-N-acetylmuramic acid kinase [Rickettsiella grylli]	72.24	371	103	0	0	565
834	gi|492904919|ref|WP_006035325.1|	iron-sulfur cluster insertion protein ErpA [Rickettsiella grylli]	65.67	134	39	3	2.00E-52	174
835	gi|750333241|ref|WP_040615160.1|	hypothetical protein [Rickettsiella grylli]	72.86	140	38	0	2.00E-69	218
836	gi|492905519|ref|WP_006035925.1|	hypothetical protein [Rickettsiella grylli]	65.85	82	26	1	3.00E-25	100

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837	gi|492904689|ref|WP_006035095.1|	hypothetical protein [Rickettsiella grylli]	77.53	178	39	1	2.00E-96	290
838	gi|492905283|ref|WP_006035689.1|	cytochrome C biogenesis protein CcmE [Rickettsiella grylli]	68.22	129	41	0	7.00E-55	180
839	gi|492904815|ref|WP_006035221.1|	guanosine monophosphate reductase [Rickettsiella grylli]	84.7	353	54	0	0	630
840	gi|492905449|ref|WP_006035855.1|	DNA polymerase I [Rickettsiella grylli]	77.31	899	203	1	0	1420
841	gi|492905471|ref|WP_006035877.1|	RNA-binding protein Hfq [Rickettsiella grylli]	90.22	92	9	0	4.00E-53	172
842	gi|492904857|ref|WP_006035263.1|	GTPase HflX [Rickettsiella grylli]	67.44	43	13	1	1.00E-07	57.8
843	gi|492904284|ref|WP_006034690.1|	protease modulator HflK [Rickettsiella grylli]	53.67	395	174	4	5.00E-141	419
844	gi|492905052|ref|WP_006035458.1|	protease modulator HflC [Rickettsiella grylli]	46.79	280	144	2	7.00E-79	254
845	gi|492905271|ref|WP_006035677.1|	adenylosuccinate synthase [Rickettsiella grylli]	76.64	428	100	0	0	691
846	gi|406916013|gb|EKD55049.1|	putative thiamine pyrophosphate enzyme [uncultured bacterium]	69.75	605	171	3	0	900
847	gi|406916015|gb|EKD55051.1|	hypothetical protein ACD_60C028G0048 [uncultured bacterium]	73.65	334	88	0	2.00E-176	505
848	gi|406916016|gb|EKD55052.1|	hypothetical protein ACD_60C028G0049 [uncultured bacterium]	67.62	281	91	0	8.00E-136	399
849	gi|754818628|ref|WP_042181150.1|	dolichol monophosphate mannose synthase [Paenibacillus sp. FSL R7-0331]	59.22	309	126	0	2.00E-140	412
850	gi|918238331|ref|WP_052369368.1|	hypothetical protein [Planktothrix agardhii]	49.68	314	148	4	5.00E-100	309
851	gi|754788706|ref|WP_042152402.1|	UDP-glucuronate decarboxylase [Planktothrix agardhii]	61.78	348	132	1	2.00E-156	456
852	gi|675587636|gb|KFN39581.1|	polysaccharide biosynthesis protein GtrA [Sulfuricurvum sp. MLSB]	44.64	112	62	0	2.00E-26	107
853	gi|962199672|gb|KTC84672.1|	cell wall biosynthesis regulatory pyridoxal phosphate-dependent protein [Legionella drozanskii LLAP-1]	71.46	403	115	0	0	637
854	gi|302582830|gb|ADL56841.1|	CDP-glucose 4,6-dehydratase [Gallionella capsiferriformans ES-2]	55.56	351	149	2	1.00E-149	439
855	gi|406916012|gb|EKD55048.1|	hypothetical protein ACD_60C028G0045 [uncultured bacterium]	68.75	272	80	1	2.00E-140	408
856	gi|1027687332|ref|WP_063625095.1|	hypothetical protein [Paraburkholderia mimosarum]	41.1	584	335	7	1.00E-145	452
857	gi|492904260|ref|WP_006034666.1|	glycosyl transferase family 1 [Rickettsiella grylli]	54.57	372	169	0	2.00E-143	424
858	gi|492905101|ref|WP_006035507.1|	mannose-1-phosphate guanylyltransferase/mannose-6-phosphate isomerase [Rickettsiella grylli]	56.43	498	212	3	0	591
859	gi|159120778|gb|EDP46116.1|	mannosyltransferase B [Rickettsiella grylli]	64.14	382	133	3	1.00E-175	507
860	gi|492904541|ref|WP_006034947.1|	GDP-mannose 4,6-dehydratase [Rickettsiella grylli]	80.67	326	63	0	0	564
861	gi|499692611|ref|WP_011373345.1|	methyltransferase FkbM [Sulfurimonas denitrificans]	63.22	87	32	0	2.00E-31	124
862	gi|492904324|ref|WP_006034730.1|	methyltransferase FkbM [Rickettsiella grylli]	50	138	66	1	4.00E-40	147
863	gi|492905092|ref|WP_006035498.1|	glycosyl transferase group 1 family protein [Rickettsiella grylli]	51.93	882	368	15	0	843
864	gi|159121215|gb|EDP46553.1|	hypothetical protein RICGR_0933 [Rickettsiella grylli]	47.33	131	65	1	1.00E-30	126
865	gi|498283116|ref|WP_010597272.1|	sugar ABC transporter ATP-binding protein [Diplorickettsia massiliensis]	68.55	248	78	0	7.00E-121	357
866	gi|492905481|ref|WP_006035887.1|	ABC transporter [Rickettsiella grylli]	62.69	268	100	0	2.00E-114	343
867	gi|492904374|ref|WP_006034780.1|	CTP synthetase [Rickettsiella grylli]	90.98	543	49	0	0	1018
868	gi|492905053|ref|WP_006035459.1|	DUF2063 domain-containing protein [Rickettsiella grylli]	57.92	259	109	0	3.00E-104	316
869	gi|492904905|ref|WP_006035311.1|	hypothetical protein [Rickettsiella grylli]	81.95	277	50	0	7.00E-172	489
871	gi|492904296|ref|WP_006034702.1|	undecaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase [Rickettsiella grylli]	68.01	347	110	1	5.00E-153	447

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872	gi|750333251|ref|WP_040615170.1|	lipid A export permease/ATP-binding protein MsbA [Rickettsiella grylli]	82.65	582	100	1	0	974
873	gi|750333253|ref|WP_040615172.1|	protease TldD [Rickettsiella grylli]	82.37	482	85	0	0	806
874	gi|492905462|ref|WP_006035868.1|	hypothetical protein [Rickettsiella grylli]	44	150	75	3	7.00E-32	125
875	gi|492904863|ref|WP_006035269.1|	DUF3971 domain-containing protein [Rickettsiella grylli]	58.95	989	403	3	0	1177
876	gi|492905387|ref|WP_006035793.1|	glycosyl transferase family 2 [Rickettsiella grylli]	67.04	270	89	0	1.00E-131	386
877	gi|492905313|ref|WP_006035719.1|	O-Antigen Polymerase family [Rickettsiella grylli]	67.34	395	129	0	1.00E-172	501
878	gi|492904605|ref|WP_006035011.1|	LPS biosynthesis protein [Rickettsiella grylli]	71.2	250	71	1	2.00E-126	371
879	gi|492905576|ref|WP_006035982.1|	LPS heptosyltransferase III [Rickettsiella grylli]	68.75	352	109	1	0	525
880	gi|492905073|ref|WP_006035479.1|	hypothetical protein [Rickettsiella grylli]	88.41	69	8	0	2.00E-37	130
881	gi|492905255|ref|WP_006035661.1|	hypothetical protein [Rickettsiella grylli]	65.06	83	29	0	1.00E-30	114
882	gi|492905438|ref|WP_006035844.1|	rod shape-determining protein MreD [Rickettsiella grylli]	72.05	161	45	0	1.00E-75	235
883	gi|492904694|ref|WP_006035100.1|	rod shape-determining protein MreC [Rickettsiella grylli]	77.51	249	56	0	2.00E-135	395
884	gi|492904262|ref|WP_006034668.1|	rod shape-determining protein [Rickettsiella grylli]	96.24	346	13	0	0	667
885	gi|492905220|ref|WP_006035626.1|	asparaginyl/glutamyl-tRNA amidotransferase subunit C [Rickettsiella grylli]	67.37	95	31	0	2.00E-36	130
886	gi|750333613|ref|WP_040615532.1|	aspartyl/glutamyl-tRNA amidotransferase subunit A [Rickettsiella grylli]	83.02	483	82	0	0	806
887	gi|492905446|ref|WP_006035852.1|	aspartyl/glutamyl-tRNA amidotransferase subunit B [Rickettsiella grylli]	77.89	493	106	1	0	798
888	gi|492904780|ref|WP_006035186.1|	tRNA (N6-isopentenyl adenosine(37)-C2)-methylthiotransferase MiaB [Rickettsiella grylli]	83.98	437	70	0	0	766
889	gi|492905547|ref|WP_006035953.1|	ATP-binding protein [Rickettsiella grylli]	87.65	324	39	1	0	592
890	gi|492905247|ref|WP_006035653.1|	16S rRNA maturation RNase YbeY [Rickettsiella grylli]	67.52	157	51	0	2.00E-70	221
891	gi|492904545|ref|WP_006034951.1|	magnesium transporter [Rickettsiella grylli]	76.49	285	65	2	9.00E-153	441
892	gi|492904664|ref|WP_006035070.1|	NAD-dependent succinate-semialdehyde dehydrogenase [Rickettsiella grylli]	73.59	462	122	0	0	719
893	gi|492905168|ref|WP_006035574.1|	deoxyuridine 5'-triphosphate nucleotidohydrolase [Rickettsiella grylli]	78.15	151	33	0	6.00E-79	243
894	gi|492904570|ref|WP_006034976.1|	hypothetical protein [Rickettsiella grylli]	84.34	83	13	0	4.00E-20	87.8
895	gi|492905015|ref|WP_006035421.1|	chromosome segregation protein SMC [Rickettsiella grylli]	64.12	1176	421	1	0	1429
896	gi|492904513|ref|WP_006034919.1|	putative cell division protein ZipA [Rickettsiella grylli]	61.93	218	78	3	1.00E-88	273
897	gi|492905147|ref|WP_006035553.1|	DNA ligase (NAD(+)) LigA [Rickettsiella grylli]	73.29	674	180	0	0	1009
898	gi|492905484|ref|WP_006035890.1|	DNA-binding response regulator [Rickettsiella grylli]	86.61	224	29	1	2.00E-136	394
899	gi|492905130|ref|WP_006035536.1|	two-component sensor histidine kinase [Rickettsiella grylli]	72.44	468	128	1	0	685
901	gi|492904533|ref|WP_006034939.1|	long-chain-fatty-acid--CoA ligase [Rickettsiella grylli]	68.6	551	172	1	0	799
902	gi|492904671|ref|WP_006035077.1|	septum site-determining protein MinC [Rickettsiella grylli]	78.99	238	48	1	7.00E-131	382
903	gi|492905452|ref|WP_006035858.1|	peptide chain release factor 3 [Rickettsiella grylli]	79.36	528	109	0	0	893
905	gi|492904768|ref|WP_006035174.1|	DNA polymerase III subunit gamma/tau [Rickettsiella grylli]	73.45	531	127	5	0	746
906	gi|492904404|ref|WP_006034810.1|	hypothetical protein [Rickettsiella grylli]	77.06	109	25	0	9.00E-51	168
907	gi|492905608|ref|WP_006036014.1|	recombination protein RecR [Rickettsiella grylli]	81.82	198	36	0	2.00E-117	345
909	gi|492904699|ref|WP_006035105.1|	50S ribosomal protein L20 [Rickettsiella grylli]	89.83	118	12	0	1.00E-65	206
910	gi|492904767|ref|WP_006035173.1|	50S ribosomal protein L35 [Rickettsiella grylli]	84.38	64	10	0	7.00E-30	111

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911	gi|492905545|ref|WP_006035951.1|	translation initiation factor IF-3 [Rickettsiella grylli]	90.3	165	16	0	1.00E-101	303
913	gi|492905040|ref|WP_006035446.1|	excinuclease ABC subunit B [Rickettsiella grylli]	84.9	669	101	0	0	1180
914	gi|492905202|ref|WP_006035608.1|	aspartate aminotransferase [Rickettsiella grylli]	77.1	393	90	0	0	636
915	gi|492904450|ref|WP_006034856.1|	MFS transporter [Rickettsiella grylli]	83.81	420	67	1	0	670
916	gi|498284565|ref|WP_010598721.1|	50S ribosomal protein L31 [Diplorickettsia massiliensis]	72.29	83	23	0	4.00E-39	137
917	gi|492904364|ref|WP_006034770.1|	acyloxyacyl hydrolase [Rickettsiella grylli]	67.25	171	54	1	1.00E-78	246
918	gi|492905084|ref|WP_006035490.1|	DNA topoisomerase IV subunit A [Rickettsiella grylli]	79.95	733	147	0	0	1226
919	gi|492904853|ref|WP_006035259.1|	membrane protein [Rickettsiella grylli]	78.74	301	64	0	4.00E-168	482
920	gi|820795809|ref|WP_046757343.1|	kynureninase [Kordia jejudonensis]	44.58	424	219	6	5.00E-124	379
921	gi|1010984200|ref|WP_061942838.1|	arylformamidase [Collimonas pratensis]	43.56	202	105	4	4.00E-41	150
922	gi|962186445|gb|KTC71589.1|	tyrosine-specific transport protein [Legionella birminghamensis]	43.4	394	213	5	6.00E-79	261
923	gi|499845761|ref|WP_011526495.1|	tryptophan synthase subunit alpha [Lawsonia intracellularis]	53.91	256	118	0	1.00E-92	286
924	gi|499845762|ref|WP_011526496.1|	tryptophan synthase subunit beta [Lawsonia intracellularis]	71.98	389	109	0	0	578
925	gi|499845763|ref|WP_011526497.1|	phosphoribosylanthranilate isomerase [Lawsonia intracellularis]	54.74	190	79	3	3.00E-57	191
926	gi|499845764|ref|WP_011526498.1|	indole-3-glycerol-phosphate synthase [Lawsonia intracellularis]	53.57	224	104	0	2.00E-76	244
927	gi|499845765|ref|WP_011526499.1|	anthranilate phosphoribosyltransferase [Lawsonia intracellularis]	45.9	329	173	2	3.00E-86	275
928	gi|123469483|ref|XP_001317953.1|	espin [Trichomonas vaginalis G3]	36.33	245	148	3	2.00E-38	154
928	gi|123469483|ref|XP_001317953.1|	espin [Trichomonas vaginalis G3]	38.29	222	129	3	6.00E-35	144
928	gi|123469483|ref|XP_001317953.1|	espin [Trichomonas vaginalis G3]	31.48	216	107	2	4.00E-24	112
928	gi|123469483|ref|XP_001317953.1|	espin [Trichomonas vaginalis G3]	37.93	116	69	1	3.00E-15	87
928	gi|123469483|ref|XP_001317953.1|	espin [Trichomonas vaginalis G3]	41.18	85	50	0	1.00E-10	73.2
929	gi|492904752|ref|WP_006035158.1|	thiol:disulfide interchange protein DsbD (Protein-disulfide reductase) (Disulfide reductase) (C-type cytochromebiogenesis protein cycZ) (Inner membrane copper tolerance protein) [Rickettsiella grylli]	70.19	530	151	3	0	774
930	gi|492905413|ref|WP_006035819.1|	Fis family transcriptional regulator [Rickettsiella grylli]	98.96	96	1	0	4.00E-60	190
932	gi|123398905|ref|XP_001301368.1|	ankyrin repeat protein [Trichomonas vaginalis G3]	43.16	190	90	5	1.00E-27	120
932	gi|123398905|ref|XP_001301368.1|	ankyrin repeat protein [Trichomonas vaginalis G3]	41.11	180	89	4	1.00E-27	120
932	gi|123398905|ref|XP_001301368.1|	ankyrin repeat protein [Trichomonas vaginalis G3]	39.04	187	89	5	2.00E-22	105
932	gi|123398905|ref|XP_001301368.1|	ankyrin repeat protein [Trichomonas vaginalis G3]	40.7	172	84	6	1.00E-21	103
933	gi|492905125|ref|WP_006035531.1|	oligopeptide transporter, OPT family [Rickettsiella grylli]	70.86	659	185	5	0	885
934	gi|492904316|ref|WP_006034722.1|	serine--tRNA ligase [Rickettsiella grylli]	79.95	424	85	0	0	718
935	gi|492905321|ref|WP_006035727.1|	bifunctional methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase [Rickettsiella grylli]	77.39	283	64	0	3.00E-153	442
936	gi|492904937|ref|WP_006035343.1|	peptidase M17 [Rickettsiella grylli]	71.05	456	130	2	0	687
937	gi|492904431|ref|WP_006034837.1|	alanine dehydrogenase [Rickettsiella grylli]	81.72	372	68	0	0	613
938	gi|498283422|ref|WP_010597578.1|	hypothetical protein [Diplorickettsia massiliensis]	38.67	181	100	6	9.00E-25	109
939	gi|492904345|ref|WP_006034751.1|	DNA primase [Rickettsiella grylli]	68.84	584	181	1	0	840

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940	gi|159121587|gb|EDP46925.1|	GatB/Yqey domain protein [Rickettsiella grylli]	73.15	149	40	0	1.00E-67	214
941	gi|492904885|ref|WP_006035291.1|	30S ribosomal protein S21 [Rickettsiella grylli]	94.67	75	4	0	1.00E-40	139
942	gi|492904561|ref|WP_006034967.1|	tRNA N6-adenosine(37)-threonylcarbamoyltransferase complex transferase subunit TsaD [Rickettsiella grylli]	79.26	352	72	1	0	580
943	gi|498284309|ref|WP_010598465.1|	hypothetical protein [Diplorickettsia massiliensis]	34.29	105	61	4	0.001	53.1
943	gi|498284309|ref|WP_010598465.1|	hypothetical protein [Diplorickettsia massiliensis]	24.53	212	110	7	6.4	41.2
944	gi|492904646|ref|WP_006035052.1|	acyl-phosphate glycerol 3-phosphate acyltransferase [Rickettsiella grylli]	70.16	191	57	0	7.00E-90	274
945	gi|492904850|ref|WP_006035256.1|	oligoribonuclease [Rickettsiella grylli]	87.29	181	23	0	5.00E-113	332
946	gi|498284304|ref|WP_010598460.1|	elongation factor P [Diplorickettsia massiliensis]	79.26	188	39	0	4.00E-109	322
948	gi|492904642|ref|WP_006035048.1|	hypothetical protein [Rickettsiella grylli]	85.71	42	4	2	3.00E-10	60.8
949	gi|492905412|ref|WP_006035818.1|	tRNA pseudouridine(55) synthase TruB [Rickettsiella grylli]	73.46	309	81	1	2.00E-159	459
950	gi|492905182|ref|WP_006035588.1|	ribosome-binding factor A [Rickettsiella grylli]	71.88	128	35	1	6.00E-54	177
951	gi|492905354|ref|WP_006035760.1|	translation initiation factor IF-2 [Rickettsiella grylli]	82.77	824	127	5	0	1369
952	gi|492904335|ref|WP_006034741.1|	transcription termination/antitermination protein NusA [Rickettsiella grylli]	85.88	517	68	3	0	874
953	gi|492904351|ref|WP_006034757.1|	ribosome maturation factor [Rickettsiella grylli]	71.24	153	44	0	4.00E-76	236
955	gi|492904890|ref|WP_006035296.1|	ankyrin repeat domain protein [Rickettsiella grylli]	70.78	462	134	1	0	648
956	gi|492905534|ref|WP_006035940.1|	hypothetical protein [Rickettsiella grylli]	50.3	165	75	4	2.00E-40	145
957	gi|492904751|ref|WP_006035157.1|	aspartate-semialdehyde dehydrogenase [Rickettsiella grylli]	76.85	337	78	0	0	538
958	gi|159121687|gb|EDP47025.1|	protein-(glutamine-N5) methyltransferase, ribosomal protein L3-specific [Rickettsiella grylli]	72.44	312	85	1	5.00E-162	467
959	gi|492904882|ref|WP_006035288.1|	Hpt domain protein [Rickettsiella grylli]	50.43	115	57	0	9.00E-31	117
960	gi|657659862|ref|WP_029463717.1|	50S ribosomal protein L17 [Diplorickettsia massiliensis]	79.34	121	25	0	5.00E-64	202
961	gi|492905300|ref|WP_006035706.1|	DNA-directed RNA polymerase subunit alpha [Rickettsiella grylli]	88.76	347	38	1	0	630
962	gi|492904524|ref|WP_006034930.1|	30S ribosomal protein S4 [Rickettsiella grylli]	88.83	206	23	0	3.00E-133	385
963	gi|159121169|gb|EDP46507.1|	ribosomal protein S11 [Rickettsiella grylli]	89.26	149	14	1	1.00E-92	277
964	gi|492904279|ref|WP_006034685.1|	30S ribosomal protein S13 [Rickettsiella grylli]	90.76	119	11	0	2.00E-69	216
965	gi|492905122|ref|WP_006035528.1|	preprotein translocase subunit SecY [Rickettsiella grylli]	92.26	439	32	1	0	822
966	gi|492905555|ref|WP_006035961.1|	50S ribosomal protein L15 [Rickettsiella grylli]	72.6	146	36	2	3.00E-64	205
967	gi|498284277|ref|WP_010598433.1|	50S ribosomal protein L30 [Diplorickettsia massiliensis]	73.77	61	16	0	5.00E-23	93.6
968	gi|492904922|ref|WP_006035328.1|	30S ribosomal protein S5 [Rickettsiella grylli]	96.41	167	6	0	1.00E-109	322
969	gi|492905086|ref|WP_006035492.1|	50S ribosomal protein L18 [Rickettsiella grylli]	84.17	120	19	0	2.00E-66	209
970	gi|498284274|ref|WP_010598430.1|	50S ribosomal protein L6 [Diplorickettsia massiliensis]	75	176	44	0	2.00E-90	273
971	gi|492905596|ref|WP_006036002.1|	30S ribosomal protein S8 [Rickettsiella grylli]	81.68	131	24	0	2.00E-74	229
972	gi|492904283|ref|WP_006034689.1|	30S ribosomal protein S14 [Rickettsiella grylli]	92.08	101	8	0	5.00E-60	191
973	gi|492905295|ref|WP_006035701.1|	50S ribosomal protein L5 [Rickettsiella grylli]	88.33	180	21	0	5.00E-116	339
974	gi|498284269|ref|WP_010598425.1|	50S ribosomal protein L24 [Diplorickettsia massiliensis]	75.47	106	26	0	2.00E-48	161
975	gi|492904638|ref|WP_006035044.1|	50S ribosomal protein L14 [Rickettsiella grylli]	92.62	122	9	0	1.00E-72	224

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976	gi|492905431|ref|WP_006035837.1|	30S ribosomal protein S17 [Rickettsiella grylli]	74.23	97	25	0	5.00E-44	149
977	gi|657659858|ref|WP_029463713.1|	50S ribosomal protein L29 [Diplorickettsia massiliensis]	63.08	65	24	0	1.00E-21	90.1
978	gi|492905468|ref|WP_006035874.1|	50S ribosomal protein L16 [Rickettsiella grylli]	96.35	137	5	0	1.00E-79	243
979	gi|492904982|ref|WP_006035388.1|	30S ribosomal protein S3 [Rickettsiella grylli]	84.29	261	34	3	7.00E-153	439
980	gi|492904340|ref|WP_006034746.1|	50S ribosomal protein L22 [Rickettsiella grylli]	90.43	115	11	0	5.00E-70	217
981	gi|492904717|ref|WP_006035123.1|	30S ribosomal protein S19 [Rickettsiella grylli]	86.6	97	13	0	3.00E-56	181
982	gi|492905563|ref|WP_006035969.1|	50S ribosomal protein L2 [Rickettsiella grylli]	89.09	275	30	0	5.00E-169	481
983	gi|498284259|ref|WP_010598415.1|	50S ribosomal protein L23 [Diplorickettsia massiliensis]	71.15	104	30	0	1.00E-45	154
984	gi|492904852|ref|WP_006035258.1|	50S ribosomal protein L4 [Rickettsiella grylli]	78.54	205	44	0	2.00E-116	342
985	gi|492905282|ref|WP_006035688.1|	50S ribosomal protein L3 [Rickettsiella grylli]	80.18	222	44	0	2.00E-130	379
986	gi|492904490|ref|WP_006034896.1|	30S ribosomal protein S10 [Rickettsiella grylli]	88.98	118	6	1	3.00E-64	202
987	gi|492904312|ref|WP_006034718.1|	elongation factor Tu [Rickettsiella grylli]	94.5	400	22	0	0	783
988	gi|492905274|ref|WP_006035680.1|	elongation factor G [Rickettsiella grylli]	91.89	703	57	0	0	1348
989	gi|492904881|ref|WP_006035287.1|	30S ribosomal protein S7 [Rickettsiella grylli]	85.95	185	14	2	6.00E-105	311
990	gi|492905506|ref|WP_006035912.1|	30S ribosomal protein S12 [Rickettsiella grylli]	96.8	125	4	0	4.00E-80	243
991	gi|750333266|ref|WP_040615185.1|	hypothetical protein [Rickettsiella grylli]	38.19	940	497	21	1.00E-164	520
992	gi|159120583|gb|EDP45921.1|	DNA-directed RNA polymerase, beta' subunit [Rickettsiella grylli]	92.86	1485	96	4	0	2819
993	gi|492905257|ref|WP_006035663.1|	DNA-directed RNA polymerase subunit beta [Rickettsiella grylli]	92.23	1377	107	0	0	2620
994	gi|492904285|ref|WP_006034691.1|	50S ribosomal protein L7/L12 [Rickettsiella grylli]	79.84	129	24	2	8.00E-45	154
995	gi|492905066|ref|WP_006035472.1|	50S ribosomal protein L10 [Rickettsiella grylli]	85.31	177	26	0	8.00E-102	303
996	gi|492904910|ref|WP_006035316.1|	50S ribosomal protein L1 [Rickettsiella grylli]	82.89	228	39	0	3.00E-125	367
997	gi|492905405|ref|WP_006035811.1|	50S ribosomal protein L11 [Rickettsiella grylli]	88.73	142	16	0	6.00E-89	267
998	gi|492904626|ref|WP_006035032.1|	transcription termination/antitermination protein NusG [Rickettsiella grylli]	83.26	215	34	1	5.00E-121	354
999	gi|492905460|ref|WP_006035866.1|	preprotein translocase subunit SecE [Rickettsiella grylli]	72.12	104	29	0	3.00E-45	154
1004	gi|159121345|gb|EDP46683.1|	putative membrane protein [Rickettsiella grylli]	82.74	197	34	0	4.00E-96	290
1005	gi|159120741|gb|EDP46079.1|	ornithine--oxo-acid transaminase [Rickettsiella grylli]	81.2	415	76	2	0	672
1006	gi|492904786|ref|WP_006035192.1|	sodium:proton antiporter [Rickettsiella grylli]	86.19	724	100	0	0	1213
1007	gi|915327328|ref|WP_050764016.1|	polynucleotide adenylyltransferase PcnB [Rickettsiella grylli]	73.7	403	97	2	0	607
1008	gi|492905230|ref|WP_006035636.1|	glucose-6-phosphate isomerase [Rickettsiella grylli]	63.4	530	190	4	0	677
1009	gi|805452839|ref|WP_046106607.1|	twitching motility protein PilT [Devosia geojensis]	68.6	121	38	0	1.00E-53	176
1010	gi|493510999|ref|WP_006465343.1|	CopG family transcriptional regulator [Herbaspirillum frisingense]	57.14	70	30	0	1.00E-21	90.9
1011	gi|492904447|ref|WP_006034853.1|	lysine decarboxylase [Rickettsiella grylli]	86.01	286	39	1	8.00E-179	508
1012	gi|492904766|ref|WP_006035172.1|	hypothetical protein [Rickettsiella grylli]	29.7	734	387	26	2.00E-55	221
1013	gi|492905549|ref|WP_006035955.1|	hypothetical protein [Rickettsiella grylli]	30.53	380	229	11	2.00E-22	107
1014	gi|492904665|ref|WP_006035071.1|	hypothetical protein [Rickettsiella grylli]	46.58	161	76	2	8.00E-37	136
1015	gi|492905389|ref|WP_006035795.1|	type IV secretion system protein DotA [Rickettsiella grylli]	66.54	795	250	7	0	1068

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1016	gi|492904977|ref|WP_006035383.1|	hypothetical protein [Rickettsiella grylli]	62.42	149	56	0	2.00E-57	187
1017	gi|492904872|ref|WP_006035278.1|	hypothetical protein [Rickettsiella grylli]	82.93	123	21	0	1.00E-64	205
1018	gi|492905140|ref|WP_006035546.1|	hypothetical protein [Rickettsiella grylli]	41.3	184	85	4	6.00E-26	108
1019	gi|750333274|ref|WP_040615193.1|	hypothetical protein [Rickettsiella grylli]	64.02	328	115	2	7.00E-135	400
1020	gi|492904710|ref|WP_006035116.1|	1-deoxy-D-xylulose-5-phosphate synthase [Rickettsiella grylli]	81.43	630	111	2	0	1066
1021	gi|492905304|ref|WP_006035710.1|	preprotein translocase subunit SecA [Rickettsiella grylli]	85.1	906	125	2	0	1606
1022	gi|492904898|ref|WP_006035304.1|	type I methionyl aminopeptidase [Rickettsiella grylli]	86.05	258	36	0	5.00E-169	480
1023	gi|498283207|ref|WP_010597363.1|	multidrug ABC transporter [Diplorickettsia massiliensis]	56.74	178	76	1	3.00E-67	220
1024	gi|406980397|gb|EKE020.1|	acriflavin resistance plasma membrane protein [uncultured bacterium]	49.56	1013	497	8	0	976
1025	gi|492905074|ref|WP_006035480.1|	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase [Rickettsiella grylli]	73.06	271	73	0	5.00E-136	397
1026	gi|492905342|ref|WP_006035748.1|	hypothetical protein [Rickettsiella grylli]	70.51	156	43	2	8.00E-73	228
1028	gi|492904557|ref|WP_006034963.1|	preprotein translocase subunit SecG [Rickettsiella grylli]	65.35	127	33	2	2.00E-40	142
1029	gi|492905344|ref|WP_006035750.1|	triose-phosphate isomerase [Rickettsiella grylli]	71.37	241	69	0	7.00E-119	352
1030	gi|1012711928|ref|WP_062816431.1|	glycosyltransferase [Alcanivorax sp. NBRC 102024]	25.56	180	121	4	0.4	42.4
1031	gi|1004620112|gb|AMP46292.1|	alpha-11 giardin [Giardia muris]	33.33	54	32	1	0.5	38.9
1033	gi|492904740|ref|WP_006035146.1|	NAD kinase [Rickettsiella grylli]	79.12	297	60	1	6.00E-170	485
1034	gi|492905123|ref|WP_006035529.1|	nucleotide exchange factor GrpE [Rickettsiella grylli]	61.47	218	79	1	1.00E-82	257
1035	gi|159120428|gb|EDP45766.1|	chaperone protein DnaK [Rickettsiella grylli]	79.55	660	118	4	0	1051
1036	gi|492904978|ref|WP_006035384.1|	molecular chaperone DnaJ [Rickettsiella grylli]	80.99	384	64	2	0	643
1037	gi|159120586|gb|EDP45924.1|	transcription elongation factor GreA [Rickettsiella grylli]	84.18	158	25	0	4.00E-91	274
1038	gi|492905156|ref|WP_006035562.1|	thymidylate synthase [Rickettsiella grylli]	76.52	264	62	0	6.00E-152	437
1039	gi|492904704|ref|WP_006035110.1|	UDP-glucose 6-dehydrogenase [Rickettsiella grylli]	79.55	440	90	0	0	738
1040	gi|750333660|ref|WP_040615579.1|	UTP--glucose-1-phosphate uridylyltransferase [Rickettsiella grylli]	81.31	289	54	0	1.00E-170	487
1041	gi|492905375|ref|WP_006035781.1|	lytic transglycosylase [Rickettsiella grylli]	73.26	430	103	6	0	622
1042	gi|492904841|ref|WP_006035247.1|	methyltransferase [Rickettsiella grylli]	70.42	240	67	3	8.00E-109	325
1043	gi|492904393|ref|WP_006034799.1|	ribonuclease HI [Rickettsiella grylli]	85.71	147	21	0	8.00E-88	265
1044	gi|492905229|ref|WP_006035635.1|	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase [Rickettsiella grylli]	95.25	316	14	1	0	593
1045	gi|492904455|ref|WP_006034861.1|	cell division protein FtsZ [Rickettsiella grylli]	87.47	391	48	1	0	604
1046	gi|492905004|ref|WP_006035410.1|	cell division protein FtsA [Rickettsiella grylli]	92.89	408	28	1	0	764
1047	gi|492904587|ref|WP_006034993.1|	polypeptide-transport-associated, FtsQ-type [Rickettsiella grylli]	71.04	259	74	1	2.00E-131	385
1048	gi|492904884|ref|WP_006035290.1|	DNA polymerase III subunit alpha [Rickettsiella grylli]	76.67	1170	264	4	0	1853
1049	gi|492905488|ref|WP_006035894.1|	hybrid sensor histidine kinase/response regulator [Rickettsiella grylli]	58.79	825	316	8	0	911
1050	gi|492905315|ref|WP_006035721.1|	AMP-binding protein [Rickettsiella grylli]	40.35	2104	1128	51	0	1377
1051	gi|492904686|ref|WP_006035092.1|	NAD-glutamate dehydrogenase [Rickettsiella grylli]	85.94	1615	226	1	0	2887
1052	gi|492904487|ref|WP_006034893.1|	bifunctional 3-demethylubiquinone 3-O-methyltransferase/2-octaprenyl-6-hydroxy phenol methylase [Rickettsiella grylli]	65.38	234	81	0	1.00E-111	333

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1053	gi|492905223|ref|WP_006035629.1|	phosphoglycolate phosphatase, bacterial [Rickettsiella grylli]	66.36	220	74	0	2.00E-102	309
1054	gi|498284158|ref|WP_010598314.1|	hypothetical protein [Diplorickettsia massiliensis]	27.34	139	86	3	0.45	41.6
1055	gi|492905490|ref|WP_006035896.1|	acyl-CoA thioesterase [Rickettsiella grylli]	78.12	128	28	0	1.00E-57	187
1056	gi|498284409|ref|WP_010598565.1|	cell division topological specificity factor MinE [Diplorickettsia massiliensis]	83.91	87	14	0	1.00E-44	150
1057	gi|492904963|ref|WP_006035369.1|	septum site-determining protein MinD [Rickettsiella grylli]	93.07	274	19	0	0	516
1058	gi|492904386|ref|WP_006034792.1|	DNA repair protein RecO [Rickettsiella grylli]	77.31	238	54	0	1.00E-121	358
1059	gi|492904586|ref|WP_006034992.1|	membrane protein [Rickettsiella grylli]	61.25	160	62	0	4.00E-50	170
1060	gi|492905045|ref|WP_006035451.1|	MFS transporter [Rickettsiella grylli]	75.36	414	100	1	0	612
1061	gi|350287179|gb|EGZ68426.1|	hypothetical protein NEUTE2DRAFT_73536, partial [Neurospora tetrasperma FGSC 2509]	37.74	53	32	1	6.6	32.7
1062	gi|1064455|gb|KXJ41737.1|	co-chaperone GroES [Methylothermaceae bacteria B42]	72.34	94	26	0	4.00E-37	132
1063	gi|492905149|ref|WP_006035555.1|	molecular chaperone GroEL [Rickettsiella grylli]	88.93	533	59	0	0	952
1064	gi|492905554|ref|WP_006035960.1|	zinc metalloprotease HtpX [Rickettsiella grylli]	86.8	303	36	2	0	529
1065	gi|966510299|ref|WP_058526890.1|	crotonase [Legionella erythra]	54.75	652	284	8	0	730
1066	gi|406915440|gb|EKD54523.1|	hypothetical protein ACD_60C075G02 [uncultured bacterium]	64.14	435	155	1	0	581
1067	gi|406915441|gb|EKD54524.1|	hypothetical protein ACD_60C075G03 [uncultured bacterium]	55.1	735	325	2	0	845
1068	gi|159120666|gb|EDP46004.1|	hypothetical protein RICGR_1155 [Rickettsiella grylli]	47.06	153	79	2	1.00E-37	138
1069	gi|492905024|ref|WP_006035430.1|	hypothetical protein [Rickettsiella grylli]	57.3	281	120	0	3.00E-109	330
1070	gi|492904334|ref|WP_006034740.1|	type 4 fimbrial biogenesis protein PilV [Rickettsiella grylli]	45.76	118	64	0	2.00E-24	101
1071	gi|492905441|ref|WP_006035847.1|	leucyl aminopeptidase [Rickettsiella grylli]	73.84	497	127	2	0	753
1072	gi|492904676|ref|WP_006035082.1|	LPS export ABC transporter permease LptF [Rickettsiella grylli]	75.34	373	92	0	1.00E-170	493
1073	gi|492905513|ref|WP_006035919.1|	LPS export ABC transporter permease LptG [Rickettsiella grylli]	74.93	355	89	0	0	574
1074	gi|492904924|ref|WP_006035330.1|	NAD+ synthase [Rickettsiella grylli]	69.83	537	161	1	0	777
1075	gi|492905241|ref|WP_006035647.1|	competence protein ComL [Rickettsiella grylli]	78.48	237	51	0	3.00E-133	388
1076	gi|492904734|ref|WP_006035140.1|	hypothetical protein [Rickettsiella grylli]	92.96	71	5	0	6.00E-25	99.4
1077	gi|492905098|ref|WP_006035504.1|	23S rRNA pseudouridine synthase D [Rickettsiella grylli]	77.88	321	70	1	2.00E-179	512
1078	gi|492904440|ref|WP_006034846.1|	hypothetical protein [Rickettsiella grylli]	63.67	245	86	2	2.00E-109	328
1079	gi|927397051|ref|XP_013944371.1|	hypothetical protein TRIATDRAFT_161191 [Trichoderma atroviride IMI 206040]	30.43	69	48	0	3.9	35.8
1080	gi|492905351|ref|WP_006035757.1|	membrane protein [Rickettsiella grylli]	82.65	392	68	0	0	669
1081	gi|492905294|ref|WP_006035700.1|	cytochrome c biogenesis protein [Rickettsiella grylli]	71.33	143	39	2	1.00E-60	195
1082	gi|492904785|ref|WP_006035191.1|	signal recognition particle protein [Rickettsiella grylli]	81.82	451	82	0	0	768
1083	gi|159120807|gb|EDP46145.1|	ribosomal protein S16 [Rickettsiella grylli]	65.56	90	27	2	5.00E-32	119
1084	gi|159121460|gb|EDP46798.1|	16S rRNA processing protein RimM [Rickettsiella grylli]	63.58	173	58	2	8.00E-73	229
1085	gi|492904507|ref|WP_006034913.1|	tRNA (guanosine(37)-N1)-methyltransferase TrmD [Rickettsiella grylli]	75.81	248	60	0	1.00E-135	394
1086	gi|492905186|ref|WP_006035592.1|	50S ribosomal protein L19 [Rickettsiella grylli]	79.51	122	25	0	3.00E-63	201
1087	gi|492904421|ref|WP_006034827.1|	methylated-dna--protein-cysteine methyltransferase (6-o-methylguanine-dna methyltransferase) (mgmt) (o-6-methylguanine-dna-alkyltransferase) [Rickettsiella grylli]	62.42	149	56	0	2.00E-59	193

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1088	gi|492905026|ref|WP_006035432.1|	competence protein ComEC [Rickettsiella grylli]	63.17	782	281	2	0	999
1090	gi|492905135|ref|WP_006035541.1|	inorganic phosphate transporter [Rickettsiella grylli]	88.62	334	38	0	0	562
1091	gi|159120495|gb|EDP45833.1|	succinyl-diaminopimelate desuccinylase [Rickettsiella grylli]	71.88	377	105	1	0	569
1092	gi|492904958|ref|WP_006035364.1|	hypothetical protein [Rickettsiella grylli]	79.11	225	47	0	8.00E-129	375
1093	gi|492905530|ref|WP_006035936.1|	hypothetical protein [Rickettsiella grylli]	71.32	129	32	3	1.00E-46	159
1094	gi|492905358|ref|WP_006035764.1|	citrate (Si)-synthase [Rickettsiella grylli]	87.27	440	56	0	0	807
1095	gi|159121196|gb|EDP46534.1|	ribosomal large subunit pseudouridine synthase C [Rickettsiella grylli]	74.11	309	79	1	1.00E-161	466
1096	gi|492904718|ref|WP_006035124.1|	adenylate kinase [Rickettsiella grylli]	75.11	221	55	0	2.00E-119	351
1097	gi|750333676|ref|WP_040615595.1|	3'-5' exonuclease [Rickettsiella grylli]	76.45	259	59	2	5.00E-147	424
1098	gi|492905326|ref|WP_006035732.1|	23S rRNA (uracil(1939)-C(5))-methyltransferase [Rickettsiella grylli]	72.13	445	121	2	0	679
1099	gi|492904532|ref|WP_006034938.1|	D-alanyl-D-alanine carboxypeptidase [Rickettsiella grylli]	80.17	479	95	0	0	802
1100	gi|492904762|ref|WP_006035168.1|	GTP pyrophosphokinase [Rickettsiella grylli]	85.48	737	106	1	0	1315
1101	gi|492905289|ref|WP_006035695.1|	exodeoxyribonuclease VII large subunit [Rickettsiella grylli]	76.32	397	94	0	0	623
1102	gi|492905595|ref|WP_006036001.1|	DNA topoisomerase I [Rickettsiella grylli]	87.6	774	94	2	0	1418
1103	gi|492904775|ref|WP_006035181.1|	DNA processing protein DprA [Rickettsiella grylli]	61.27	408	134	3	2.00E-166	484
1104	gi|492904739|ref|WP_006035145.1|	inorganic pyrophosphatase [Rickettsiella grylli]	84.44	180	28	0	1.00E-110	326
1105	gi|492905338|ref|WP_006035744.1|	histidine triad nucleotide-binding protein [Rickettsiella grylli]	72.57	113	31	0	9.00E-57	183
1106	gi|492904761|ref|WP_006035167.1|	hypothetical protein [Rickettsiella grylli]	66.07	168	57	0	7.00E-78	243
1107	gi|492904489|ref|WP_006034895.1|	DNA polymerase III subunit chi [Rickettsiella grylli]	58.9	146	58	1	8.00E-54	178
1108	gi|159120498|gb|EDP45836.1|	valyl-tRNA synthetase [Rickettsiella grylli]	73.26	920	243	2	0	1411
1109	gi|953250421|emb|CUS38951.1|	Sensory response regulator with diguanylate cyclase domain [Candidatus Nitrospira nitrosa]	26.32	95	70	0	2.5	37.4
1110	gi|492904994|ref|WP_006035400.1|	DNA polymerase III subunit epsilon [Rickettsiella grylli]	71.18	229	65	1	3.00E-110	329
1111	gi|492904801|ref|WP_006035207.1|	Na+/H+ antiporter NhaA [Rickettsiella grylli]	71.65	381	106	2	2.00E-179	517
1112	gi|966516370|ref|WP_058532864.1|	hypothetical protein [Legionella sp. LH-SWC]	24.83	145	96	7	1.3	40.8
1113	gi|449541787|gb|EMD32769.1|	hypothetical protein CERSUDRAFT_108595 [Gelatoporia subvermispora B]	36.07	61	35	2	1.5	37
1114	gi|492904688|ref|WP_006035094.1|	uroporphyrinogen decarboxylase [Rickettsiella grylli]	74.01	354	89	3	0	554
1115	gi|492905308|ref|WP_006035714.1|	FUSC family protein [Rickettsiella grylli]	67.51	357	114	1	8.00E-170	490
1116	gi|492905209|ref|WP_006035615.1|	putative fimbrial assembly protein PilQ [Rickettsiella grylli]	57.6	434	175	5	2.00E-166	489
1117	gi|492905457|ref|WP_006035863.1|	hypothetical protein [Rickettsiella grylli]	28.14	295	190	9	5.00E-15	83.2
1118	gi|159121124|gb|EDP46462.1|	hypothetical protein RICGR_1207 [Rickettsiella grylli]	31.61	174	114	4	7.00E-12	70.5
1119	gi|492904575|ref|WP_006034981.1|	hypothetical protein [Rickettsiella grylli]	46.69	317	154	6	8.00E-80	258
1120	gi|492905224|ref|WP_006035630.1|	peptidase [Rickettsiella grylli]	84.94	810	117	2	0	1421
1121	gi|492904754|ref|WP_006035160.1|	thioredoxin [Rickettsiella grylli]	68.75	144	44	1	3.00E-66	209
1122	gi|492905348|ref|WP_006035754.1|	iron ABC transporter ATP-binding protein [Rickettsiella grylli]	73.55	242	61	1	2.00E-121	358
1123	gi|492905436|ref|WP_006035842.1|	ABC transporter permease [Rickettsiella grylli]	59.3	285	111	1	6.00E-102	312
1124	gi|492904670|ref|WP_006035076.1|	putative thiamine biosynthesis protein [Rickettsiella grylli]	65.27	311	107	1	8.00E-147	428

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1125	gi|492904843|ref|WP_006035249.1|	DNA-dependent helicase II [Rickettsiella grylli]	79.83	719	143	1	0	1220
1126	gi|492905097|ref|WP_006035503.1|	Smr protein/MutS2 [Rickettsiella grylli]	55.31	179	75	3	5.00E-56	187
1127	gi|159120402|gb|EDP45740.1|	LppC [Rickettsiella grylli]	61.99	371	135	5	8.00E-152	446
1128	gi|159121211|gb|EDP46549.1|	conserved hypothetical protein [Rickettsiella grylli]	61.24	129	47	1	1.00E-47	161
1129	gi|492904367|ref|WP_006034773.1|	phosphoheptose isomerase [Rickettsiella grylli]	89.18	194	21	0	2.00E-121	354
1130	gi|492904488|ref|WP_006034894.1|	glycine cleavage system protein T [Rickettsiella grylli]	56.03	307	129	3	2.00E-107	327
1131	gi|492905605|ref|WP_006036011.1|	hypothetical protein [Rickettsiella grylli]	60.14	138	49	3	8.00E-45	155
1132	gi|492904286|ref|WP_006034692.1|	MFS transporter [Rickettsiella grylli]	68.94	425	130	1	0	572
1134	gi|492904765|ref|WP_006035171.1|	pyridoxal kinase [Rickettsiella grylli]	68.64	287	88	1	4.00E-143	416
1135	gi|938981834|ref|WP_054759641.1|	MULTISPECIES: heme exporter protein CcmD [Methylomonas]	41.3	46	25	1	0.007	40.4
1136	gi|492904516|ref|WP_006034922.1|	tetraacyldisaccharide 4'-kinase [Rickettsiella grylli]	74.47	329	84	0	0	516
1137	gi|492905178|ref|WP_006035584.1|	NAD-dependent dehydratase [Rickettsiella grylli]	77.81	338	73	1	0	555
1138	gi|492904522|ref|WP_006034928.1|	putative gnat family acetyltransferase [Rickettsiella grylli]	63.07	241	86	2	2.00E-103	312
1139	gi|492904747|ref|WP_006035153.1|	4-deoxy-4-formamido-L-arabinose-phosphoundecaprenol deformylase [Rickettsiella grylli]	74.17	302	78	0	2.00E-167	479
1140	gi|492905371|ref|WP_006035777.1|	UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate aminotransferase [Rickettsiella grylli]	78.66	314	67	0	0	532
1141	gi|492904939|ref|WP_006035345.1|	dolichyl-phosphate-mannose--protein mannosyltransferase [Rickettsiella grylli]	66.32	576	191	3	0	764
1142	gi|492905418|ref|WP_006035824.1|	isoprenoid biosynthesis protein ElbB [Rickettsiella grylli]	76.71	219	51	0	4.00E-117	345
1143	gi|492904467|ref|WP_006034873.1|	tRNA (guanosine(46)-N7)-methyltransferase TrmB [Rickettsiella grylli]	72.07	222	60	1	3.00E-110	328
1144	gi|492905190|ref|WP_006035596.1|	YggW family oxidoreductase [Rickettsiella grylli]	71.5	379	108	0	0	573
1145	gi|966517405|ref|WP_058533899.1|	ATP-dependent DNA ligase [Legionella sp. LH-SWC]	64.29	84	30	0	1.00E-27	116
1146	gi|962216239|gb|KTD01005.1|	DNA ligase D [Fluoribacter gormanii]	63.93	122	44	0	6.00E-52	174
1147	gi|492904384|ref|WP_006034790.1|	Ku protein [Rickettsiella grylli]	72.59	259	71	0	4.00E-138	403
1148	gi|492904548|ref|WP_006034954.1|	hypothetical protein [Rickettsiella grylli]	36.23	461	266	14	3.00E-59	224
1148	gi|492904548|ref|WP_006034954.1|	hypothetical protein [Rickettsiella grylli]	28.72	282	189	6	2.00E-23	116
1149	gi|498284804|ref|WP_010598960.1|	hypothetical protein [Diplorickettsia massiliensis]	27.48	393	255	12	4.00E-34	145
1150	gi|966518855|ref|WP_058535349.1|	Ti-type conjugative transfer relaxase TraA [Legionella sp. LH-SWC]	31.98	516	295	11	7.00E-65	239
1151	gi|492904433|ref|WP_006034839.1|	hypothetical protein [Rickettsiella grylli]	62.55	275	102	1	1.00E-121	362
1152	gi|731151801|emb|CEK10351.1|	putative phosphoesterase [Legionella hackeliae]	52.32	409	185	8	4.00E-146	435
1153	gi|159120590|gb|EDP45928.1|	hypothetical protein RICGR_1333 [Rickettsiella grylli]	72	75	20	1	6.00E-25	108
1154	gi|966416618|ref|WP_058459903.1|	hypothetical protein [Fluoribacter bozemanae]	67.34	199	65	0	4.00E-97	297
1155	gi|736317050|ref|WP_034344066.1|	GNAT family N-acetyltransferase [Deinococcus misasensis]	37.66	154	88	3	2.00E-25	107
1156	gi|159120874|gb|EDP46212.1|	hypothetical protein RICGR_1337 [Rickettsiella grylli]	43.13	473	242	8	5.00E-117	367
1157	gi|498284571|ref|WP_010598727.1|	hypothetical protein [Diplorickettsia massiliensis]	23.98	417	281	13	7.00E-09	69.3
1158	gi|498284571|ref|WP_010598727.1|	hypothetical protein [Diplorickettsia massiliensis]	22.88	389	269	11	9.00E-10	72
1159	gi|159120874|gb|EDP46212.1|	hypothetical protein RICGR_1337 [Rickettsiella grylli]	22.65	490	336	17	1.00E-18	99.8

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1161	gi|159120711|gb|EDP46049.1|	sensory box sensor histidine kinase/response regulator [Rickettsiella grylli]	53.45	653	289	10	0	657
1162	gi|931357221|gb|KPJ49596.1|	hypothetical protein AMJ38_03085 [Dehalococcoidia bacterium DG_22]	55.81	344	151	1	2.00E-145	427
1163	gi|951144612|ref|WP_057625430.1|	MFS transporter [Coxiellaceae bacterium CC99]	40.17	346	203	3	3.00E-75	249
1164	gi|492904812|ref|WP_006035218.1|	response regulator [Rickettsiella grylli]	48.08	52	24	1	7.00E-04	45.1
1165	gi|492904894|ref|WP_006035300.1|	hypothetical protein [Rickettsiella grylli]	53.29	152	66	2	4.00E-41	149
1166	gi|498283234|ref|WP_010597390.1|	response regulator [Diplorickettsia massiliensis]	45.24	126	69	0	8.00E-27	111
1167	gi|492173614|ref|WP_005770124.1|	hypothetical protein [Coxiella burnetii]	45.19	208	101	4	1.00E-46	165
1168	gi|492172610|ref|WP_005770121.1|	hypothetical protein [Coxiella burnetii]	39.36	94	57	0	1.00E-19	87.4
1169	gi|755600525|ref|WP_042527328.1|	membrane protein [Coxiella burnetii]	44.07	236	128	1	1.00E-65	216
1170	gi|492172608|ref|WP_005770119.1|	membrane protein [Coxiella burnetii]	46.67	240	126	2	1.00E-64	214
1171	gi|522064027|ref|WP_020575236.1|	hypothetical protein [Actinopolymorpha alba]	29.31	331	197	11	1.00E-38	150
1172	gi|492904500|ref|WP_006034906.1|	ankrd17 protein [Rickettsiella grylli]	30.89	463	283	10	2.00E-46	178
1173	gi|737940848|ref|WP_035905229.1|	phenazine biosynthesis protein PhzF family [Knoellia subterranea]	57.69	26	11	0	0.18	38.1
1174	gi|750333183|ref|WP_040615102.1|	hypothetical protein [Rickettsiella grylli]	46.88	32	17	0	4.9	32.3
1175	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	34.68	496	321	2	5.00E-78	284
1175	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	34.09	443	288	3	1.00E-61	235
1175	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	32.31	294	199	0	1.00E-39	169
1175	gi|657659787|ref|WP_029463642.1|	hypothetical protein [Diplorickettsia massiliensis]	29.61	304	213	1	5.00E-28	132
1176	gi|492904548|ref|WP_006034954.1|	hypothetical protein [Rickettsiella grylli]	29.9	204	139	3	8.00E-11	75.9
1176	gi|492904548|ref|WP_006034954.1|	hypothetical protein [Rickettsiella grylli]	26.67	345	214	17	5.00E-06	60.5
1177	gi|498284788|ref|WP_010598944.1|	hybrid sensor histidine kinase/response regulator [Diplorickettsia massiliensis]	48.5	367	176	3	9.00E-108	337
1178	gi|498284850|ref|WP_010599006.1|	hypothetical protein [Diplorickettsia massiliensis]	53.26	291	132	4	8.00E-99	305
1179	gi|966402265|ref|WP_058445860.1|	MFS transporter [Legionella feeleii]	31.43	175	116	2	2.00E-14	81.3
1180	gi|492904388|ref|WP_006034794.1|	hypothetical protein [Rickettsiella grylli]	54.7	287	111	3	6.00E-98	303
1181	gi|492904826|ref|WP_006035232.1|	peptide-methionine (S)-S-oxide reductase [Rickettsiella grylli]	74.4	293	75	0	8.00E-158	454
1182	gi|159121344|gb|EDP46682.1|	peroxiredoxin-2 [Rickettsiella grylli]	88.59	184	21	0	8.00E-119	347
1183	gi|492904705|ref|WP_006035111.1|	geranyltranstransferase (Farnesyl-diphosphate synthase)(FPP synthase) [Rickettsiella grylli]	57.49	287	115	4	8.00E-111	335
1184	gi|492904443|ref|WP_006034849.1|	exodeoxyribonuclease VII small subunit [Rickettsiella grylli]	67.06	85	28	0	3.00E-33	121
1185	gi|492905248|ref|WP_006035654.1|	peptidase M16 [Rickettsiella grylli]	78.4	449	97	0	0	731
1186	gi|492904269|ref|WP_006034675.1|	peptidase M16 [Rickettsiella grylli]	63.07	436	161	0	0	567
1187	gi|492905046|ref|WP_006035452.1|	hypothetical protein [Rickettsiella grylli]	48.21	251	129	1	2.00E-63	233
1188	gi|492905046|ref|WP_006035452.1|	hypothetical protein [Rickettsiella grylli]	30.95	84	57	1	3.4	36.2
1189	gi|492904572|ref|WP_006034978.1|	aspartate aminotransferase family protein [Rickettsiella grylli]	77.55	432	95	2	0	663
1190	gi|492904562|ref|WP_006034968.1|	penicillin-binding protein 2 [Rickettsiella grylli]	78.74	668	138	2	0	1080
1191	gi|498283716|ref|WP_010597872.1|	30S ribosomal protein S20 [Diplorickettsia massiliensis]	79.79	94	19	0	7.00E-45	152
1192	gi|492904307|ref|WP_006034713.1|	hypothetical protein [Rickettsiella grylli]	57.04	284	121	1	1.00E-109	332

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1193	gi|492905036|ref|WP_006035442.1|	small-conductance mechanosensitive channel [Rickettsiella grylli]	64.84	364	125	1	3.00E-175	506
1194	gi|492904814|ref|WP_006035220.1|	2-nonaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase [Rickettsiella grylli]	66.82	214	69	1	4.00E-97	294
1195	gi|492905535|ref|WP_006035941.1|	protease [Rickettsiella grylli]	82.58	419	72	1	0	664
1196	gi|159121643|gb|EDP46981.1|	tRNA(Ile)-lysidine synthase (tRNA(Ile)-lysidinesynthetase) (tRNA(Ile)-2-lysyl-cytidine synthase) [Rickettsiella grylli]	59.37	443	176	4	0	532
1197	gi|492904900|ref|WP_006035306.1|	nicotinamide mononucleotide transporter PnuC [Rickettsiella grylli]	63.96	197	69	1	5.00E-67	216
1198	gi|492905201|ref|WP_006035607.1|	acetyl-CoA carboxylase carboxyltransferase subunit alpha [Rickettsiella grylli]	81.27	315	59	0	0	516
1199	gi|492904797|ref|WP_006035203.1|	hypothetical protein [Rickettsiella grylli]	81.63	98	18	0	5.00E-45	152
1200	gi|492905529|ref|WP_006035935.1|	heat-shock protein [Rickettsiella grylli]	79.56	137	25	2	2.00E-71	224
1201	gi|492904962|ref|WP_006035368.1|	lipid A biosynthesis acyltransferase [Rickettsiella grylli]	74.83	302	75	1	2.00E-165	474
1202	gi|492905337|ref|WP_006035743.1|	tryptophan/tyrosine permease [Rickettsiella grylli]	68.34	398	126	0	7.00E-170	494
1203	gi|492904926|ref|WP_006035332.1|	tryptophan/tyrosine permease [Rickettsiella grylli]	70.05	394	117	1	4.00E-170	494
1204	gi|492905089|ref|WP_006035495.1|	transketolase [Rickettsiella grylli]	79.1	665	139	0	0	1137
1205	gi|492905560|ref|WP_006035966.1|	type I glyceraldehyde-3-phosphate dehydrogenase [Rickettsiella grylli]	80.36	336	66	0	0	565
1206	gi|492905262|ref|WP_006035668.1|	DNA-directed RNA polymerase subunit omega [Rickettsiella grylli]	81.01	79	14	1	3.00E-37	131
1207	gi|750333321|ref|WP_040615240.1|	RelA/SpoT family protein [Rickettsiella grylli]	85.69	706	100	1	0	1238
1208	gi|750333323|ref|WP_040615242.1|	pantoate--beta-alanine ligase [Rickettsiella grylli]	69.44	252	76	1	8.00E-129	378
1209	gi|492905301|ref|WP_006035707.1|	3-methyl-2-oxobutanoate hydroxymethyltransferase [Rickettsiella grylli]	80.08	261	52	0	5.00E-148	427
1210	gi|159120356|gb|EDP45694.1|	phosphopantothenoylcysteine decarboxylase/phosphopantothenate--cysteine ligase [Rickettsiella grylli]	73.92	395	102	1	0	618
1211	gi|492905518|ref|WP_006035924.1|	hypothetical protein [Rickettsiella grylli]	65.69	510	159	7	0	662
1212	gi|492904452|ref|WP_006034858.1|	hypothetical protein [Rickettsiella grylli]	77.5	240	53	1	4.00E-101	306
1213	gi|492904288|ref|WP_006034694.1|	hypothetical protein [Rickettsiella grylli]	67.93	474	138	3	0	652
1214	gi|492904288|ref|WP_006034694.1|	hypothetical protein [Rickettsiella grylli]	67.23	473	152	3	0	652
1215	gi|492905258|ref|WP_006035664.1|	monothiol glutaredoxin, Grx4 family [Rickettsiella grylli]	68.22	107	34	0	3.00E-50	166
1216	gi|492904498|ref|WP_006034904.1|	superoxide dismutase [Rickettsiella grylli]	75.65	193	47	0	3.00E-107	318
1217	gi|492905424|ref|WP_006035830.1|	acetylornithine aminotransferase [Rickettsiella grylli]	80.2	394	78	0	0	674
1218	gi|492904454|ref|WP_006034860.1|	cystathionine beta-lyase [Rickettsiella grylli]	77.55	383	86	0	0	645
1219	gi|1040105268|ref|WP_065089499.1|	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase [Acidihalobacter prosperus]	73.36	244	65	0	6.00E-133	392
1220	gi|492904832|ref|WP_006035238.1|	molecular chaperone HtpG [Rickettsiella grylli]	72.52	644	170	5	0	940
1221	gi|492905093|ref|WP_006035499.1|	bifunctional D-altronate/D-mannonate dehydratase [Rickettsiella grylli]	88.34	403	45	2	0	736
1222	gi|492904246|ref|WP_006034652.1|	short-chain dehydrogenase [Rickettsiella grylli]	80.08	261	52	0	1.00E-157	451
1223	gi|492905211|ref|WP_006035617.1|	MFS transporter [Rickettsiella grylli]	78.22	473	102	1	0	743
1224	gi|492905459|ref|WP_006035865.1|	gluconolaconase [Rickettsiella grylli]	76.22	286	67	1	1.00E-166	476
1225	gi|498283684|ref|WP_010597840.1|	galactose mutarotase [Diplorickettsia massiliensis]	63.64	352	124	4	2.00E-158	461
1226	gi|492904869|ref|WP_006035275.1|	2-dehydro-3-deoxygluconokinase (2-keto-3-deoxygluconokinase) (3-deoxy-2-oxo-D-gluconate kinase) (KDG kinase) [Rickettsiella grylli]	67.75	307	98	1	4.00E-153	444

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1227	gi|492905323|ref|WP_006035729.1|	khg/kdpg aldolase [Rickettsiella grylli]	67.63	207	67	0	3.00E-100	301
1228	gi|159120808|gb|EDP46146.1|	tena/thi-4 family [Rickettsiella grylli]	79.42	243	50	0	2.00E-143	414
1229	gi|750333350|ref|WP_040615269.1|	UDP-N-acetylglucosamine 1-carboxyvinyltransferase [Rickettsiella grylli]	94.27	419	24	0	0	811
1230	gi|492904591|ref|WP_006034997.1|	sulfate transporter/antisigma-factor antagonist STAS [Rickettsiella grylli]	68.75	96	29	1	1.00E-36	131
1231	gi|492904944|ref|WP_006035350.1|	toluene tolerance protein Ttg2D [Rickettsiella grylli]	71.78	202	54	2	4.00E-99	298
1232	gi|159120430|gb|EDP45768.1|	ABC-type transport system involved in resistance to organic solvents periplasmic component [Rickettsiella grylli]	81.41	156	29	0	2.00E-87	265
1233	gi|159120992|gb|EDP46330.1|	toluene tolerance protein Ttg2B [Rickettsiella grylli]	85.11	262	38	1	2.00E-155	446
1234	gi|492905359|ref|WP_006035765.1|	ABC transporter ATP-binding protein [Rickettsiella grylli]	80.92	262	50	0	4.00E-152	437
1235	gi|492904691|ref|WP_006035097.1|	thiol:disulfide interchange protein DsbA [Rickettsiella grylli]	80.53	226	43	1	1.00E-132	386
1236	gi|492904304|ref|WP_006034710.1|	hypothetical protein [Rickettsiella grylli]	61.54	65	25	0	2.00E-23	95.1
1237	gi|492905105|ref|WP_006035511.1|	ribose-5-phosphate isomerase [Rickettsiella grylli]	76.61	218	51	0	7.00E-119	350
1238	gi|492905179|ref|WP_006035585.1|	adenosylhomocysteinase [Rickettsiella grylli]	88.81	438	49	0	0	810
1239	gi|492904568|ref|WP_006034974.1|	methionine adenosyltransferase [Rickettsiella grylli]	89.62	395	40	1	0	744
1240	gi|492904805|ref|WP_006035211.1|	MFS transporter [Rickettsiella grylli]	82.94	428	72	1	0	714
1241	gi|492905536|ref|WP_006035942.1|	MFS transporter [Rickettsiella grylli]	75.29	433	107	0	0	597
1242	gi|492905039|ref|WP_006035445.1|	thymidine kinase [Rickettsiella grylli]	72.92	192	51	1	3.00E-97	293
1243	gi|492905199|ref|WP_006035605.1|	thioredoxin family protein [Rickettsiella grylli]	74.59	185	46	1	4.00E-97	291
1244	gi|159121456|gb|EDP46794.1|	hypothetical protein RICGR_1430 [Rickettsiella grylli]	28.9	346	211	10	7.00E-20	105
1245	gi|492904728|ref|WP_006035134.1|	hypothetical protein [Rickettsiella grylli]	42.86	91	51	1	4.00E-11	77.4
1246	gi|492905331|ref|WP_006035737.1|	sulfur transfer protein TusE [Rickettsiella grylli]	77.48	111	25	0	1.00E-59	190
1247	gi|492904271|ref|WP_006034677.1|	BAX inhibitor protein [Rickettsiella grylli]	89.73	224	23	0	4.00E-134	389
1248	gi|492905057|ref|WP_006035463.1|	glutamate racemase [Rickettsiella grylli]	81.41	269	49	1	9.00E-157	450
1249	gi|492905088|ref|WP_006035494.1|	hypothetical protein [Rickettsiella grylli]	82.55	235	41	0	1.00E-113	340
1250	gi|492904435|ref|WP_006034841.1|	cobalt transporter [Rickettsiella grylli]	75.08	297	74	0	3.00E-153	443
1251	gi|492905370|ref|WP_006035776.1|	outer membrane lipoprotein carrier protein LolA [Rickettsiella grylli]	59.22	206	83	1	1.00E-77	244
1252	gi|492905270|ref|WP_006035676.1|	dethiobiotin synthase [Rickettsiella grylli]	58.85	226	90	1	5.00E-90	277
1253	gi|492904477|ref|WP_006034883.1|	malonyl-[acyl-carrier protein] O-methyltransferase BioC [Rickettsiella grylli]	70.98	286	83	0	8.00E-141	411
1254	gi|492904612|ref|WP_006035018.1|	8-amino-7-oxononanoate synthase [Rickettsiella grylli]	65.62	384	132	0	9.00E-175	505
1255	gi|492904973|ref|WP_006035379.1|	biotin synthase BioB [Rickettsiella grylli]	77.85	325	72	0	0	520
1256	gi|492904808|ref|WP_006035214.1|	integral membrane protein [Rickettsiella grylli]	60.64	282	111	0	3.00E-108	329
1257	gi|492904669|ref|WP_006035075.1|	adenosylmethionine--8-amino-7-oxononanoate aminotransferase BioA [Rickettsiella grylli]	78.31	438	95	0	0	722
1258	gi|492905599|ref|WP_006036005.1|	hypothetical protein [Rickettsiella grylli]	68.97	174	53	1	1.00E-82	254
1259	gi|492905158|ref|WP_006035564.1|	RNA polymerase sigma factor RpoS [Rickettsiella grylli]	89.12	331	35	1	0	595
1260	gi|159121492|gb|EDP46830.1|	membrane protein, DedA family [Rickettsiella grylli]	79.01	181	38	0	2.00E-94	286
1261	gi|492904610|ref|WP_006035016.1|	5'/3'-nucleotidase SurE [Rickettsiella grylli]	88.19	254	30	0	8.00E-167	474

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1262	gi|492905533|ref|WP_006035939.1|	hypothetical protein [Rickettsiella grylli]	81.9	105	19	0	2.00E-40	141
1263	gi|492904375|ref|WP_006034781.1|	Tfp pilus assembly protein FimT [Rickettsiella grylli]	53.81	197	89	2	3.00E-68	219
1264	gi|159121053|gb|EDP46391.1|	phage SPO1 DNA polymerase domain protein [Rickettsiella grylli]	72.27	238	65	1	3.00E-124	365
1265	gi|492904956|ref|WP_006035362.1|	hypothetical protein [Rickettsiella grylli]	61	100	31	2	1.00E-32	121
1266	gi|492905574|ref|WP_006035980.1|	octanoyltransferase [Rickettsiella grylli]	73	200	54	0	2.00E-102	307
1267	gi|492904833|ref|WP_006035239.1|	lipoyl synthase [Rickettsiella grylli]	83.76	314	51	0	0	553
1268	gi|492905458|ref|WP_006035864.1|	membrane protein [Rickettsiella grylli]	71.23	664	191	0	0	944
1269	gi|492904971|ref|WP_006035377.1|	agmatinase [Rickettsiella grylli]	80.69	290	56	0	5.00E-172	491
1270	gi|492904390|ref|WP_006034796.1|	deoxyhypusine synthase [Rickettsiella grylli]	83.57	347	57	0	0	613
1271	gi|492905065|ref|WP_006035471.1|	ornithine decarboxylase [Rickettsiella grylli]	82.28	395	70	0	0	692
1272	gi|492904270|ref|WP_006034676.1|	bis(5'-nucleosyl)-tetraphosphatase (symmetrical) [Rickettsiella grylli]	72.56	266	73	0	2.00E-143	416
1273	gi|492905094|ref|WP_006035500.1|	hypothetical protein [Rickettsiella grylli]	60.33	421	165	2	4.00E-179	519
1274	gi|492904301|ref|WP_006034707.1|	zinc-finger domain-containing protein [Rickettsiella grylli]	70.31	64	19	0	1.00E-26	102
1275	gi|492905548|ref|WP_006035954.1|	lipopolysaccharide heptosyltransferase II [Rickettsiella grylli]	62.97	343	126	1	3.00E-158	459
1276	gi|159120852|gb|EDP46190.1|	tRNA modification GTPase TrmE [Rickettsiella grylli]	69.11	463	142	1	0	650
1277	gi|492905435|ref|WP_006035841.1|	membrane protein insertase YidC [Rickettsiella grylli]	77.55	548	113	3	0	884
1278	gi|498284734|ref|WP_010598890.1|	membrane protein insertion efficiency factor YidD [Diplorickettsia massiliensis]	53.66	82	38	0	2.00E-25	101
1279	gi|492904758|ref|WP_006035164.1|	chromosomal replication initiation protein DnaA [Rickettsiella grylli]	93.78	450	27	1	0	848
1280	gi|492905374|ref|WP_006035780.1|	DNA polymerase III subunit beta [Rickettsiella grylli]	85.14	370	55	0	0	649
1281	gi|492904918|ref|WP_006035324.1|	DNA recombination protein RecF [Rickettsiella grylli]	70.28	360	104	1	6.00E-171	493
1282	gi|492905522|ref|WP_006035928.1|	QacE family quaternary ammonium compound efflux SMR transporter [Rickettsiella grylli]	74.77	107	27	0	9.00E-47	157
1283	gi|492904383|ref|WP_006034789.1|	sulfurtransferase [Rickettsiella grylli]	70.17	238	71	0	5.00E-109	327
1284	gi|492904727|ref|WP_006035133.1|	hypothetical protein [Rickettsiella grylli]	27.32	721	427	21	7.00E-38	160
1285	gi|492905328|ref|WP_006035734.1|	hypothetical protein [Rickettsiella grylli]	39.78	93	43	6	0.98	37.7
1286	gi|514395342|ref|WP_016556205.1|	heat-shock protein Hsp20 [Rhizobium grahamii]	31.52	92	56	4	2.4	36.2
1288	gi|518973378|ref|WP_020129253.1|	transcriptional regulator [Streptomyces sp. 303MFCol5.2]	40.48	42	25	0	7.7	35
1289	gi|492904560|ref|WP_006034966.1|	biotin--[acetyl-CoA-carboxylase] ligase [Rickettsiella grylli]	56.79	324	137	3	7.00E-119	358
1290	gi|492905075|ref|WP_006035481.1|	Fis family transcriptional regulator [Rickettsiella grylli]	74.1	498	129	0	0	743
1291	gi|492904321|ref|WP_006034727.1|	hypothetical protein [Rickettsiella grylli]	80.46	87	17	0	5.00E-41	141
1292	gi|492905136|ref|WP_006035542.1|	Uma3 [Rickettsiella grylli]	72.15	517	144	0	0	769
1293	gi|492904700|ref|WP_006035106.1|	cyclopropane-fatty-acyl-phospholipid synthase [Rickettsiella grylli]	78.48	381	82	0	0	645
1294	gi|492904822|ref|WP_006035228.1|	RNA pyrophosphohydrolase [Rickettsiella grylli]	85.47	179	26	0	3.00E-106	314
1295	gi|492905594|ref|WP_0060360.1|	phosphoenolpyruvate--protein phosphotransferase [Rickettsiella grylli]	85.62	758	107	2	0	1338
1296	gi|492904949|ref|WP_006035355.1|	oxidoreductase FAD-binding [Rickettsiella grylli]	64.43	447	157	2	0	584
1297	gi|492904342|ref|WP_006034748.1|	oligopeptidase A [Rickettsiella grylli]	76.08	669	159	1	0	1081
1298	gi|492904412|ref|WP_006034818.1|	regulatory protein RecX [Rickettsiella grylli]	57.34	143	61	0	2.00E-48	165

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1299	gi|492905183|ref|WP_006035589.1|	DNA recombination/repair protein RecA [Rickettsiella grylli]	87.43	350	44	0	0	627
1300	gi|492904576|ref|WP_006034982.1|	bifunctional heptose 7-phosphate kinase/heptose 1-phosphate adenyltransferase [Rickettsiella grylli]	74	477	124	0	0	731
1301	gi|492905343|ref|WP_006035749.1|	ADP-L-glycero-D-mannoheptose-6-epimerase [Rickettsiella grylli]	74.05	316	82	0	3.00E-179	511
1302	gi|492905302|ref|WP_006035708.1|	competence protein ComEA [Rickettsiella grylli]	58.93	112	40	3	9.00E-29	112
1303	gi|492904693|ref|WP_006035099.1|	cytochrome c5 [Rickettsiella grylli]	63.91	133	47	1	3.00E-55	182
1304	gi|492905463|ref|WP_006035869.1|	fructose-bisphosphate aldolase [Rickettsiella grylli]	83.82	346	56	0	0	612
1305	gi|159121100|gb|EDP46438.1|	putative ATP synthase I chain [Rickettsiella grylli]	54.01	137	59	3	3.00E-36	132
1306	gi|492905011|ref|WP_006035417.1|	F0F1 ATP synthase subunit A [Rickettsiella grylli]	88.85	269	30	0	7.00E-173	491
1307	gi|492904465|ref|WP_006034871.1|	F0F1 ATP synthase subunit C [Rickettsiella grylli]	99.01	101	1	0	3.00E-60	191
1308	gi|492905286|ref|WP_006035692.1|	F0F1 ATP synthase subunit B [Rickettsiella grylli]	84.62	156	24	0	2.00E-86	262
1309	gi|492904673|ref|WP_006035079.1|	ATP synthase F1, delta subunit [Rickettsiella grylli]	67.42	178	58	0	8.00E-81	249
1310	gi|492904372|ref|WP_006034778.1|	ATP synthase subunit alpha [Rickettsiella grylli]	90.27	514	50	0	0	957
1311	gi|492904975|ref|WP_006035381.1|	F0F1 ATP synthase subunit gamma [Rickettsiella grylli]	87.41	286	36	0	0	531
1312	gi|159121001|gb|EDP46339.1|	ATP synthase F1, beta subunit [Rickettsiella grylli]	93.51	462	30	0	0	879
1313	gi|492905479|ref|WP_006035885.1|	F0F1 ATP synthase subunit epsilon [Rickettsiella grylli]	83.22	143	24	0	1.00E-78	241
1314	gi|492904464|ref|WP_006034870.1|	UDP-N-acetylglucosamine diphosphorylase/glucosamine-1-phosphate N-acetyltransferase [Rickettsiella grylli]	80.35	453	89	0	0	754
1315	gi|916264925|ref|WP_050999971.1|	nucleoside transporter [Cardinium endosymbiont of Encarsia pergandiella]	59.67	243	96	1	7.00E-101	306
1316	gi|492904695|ref|WP_006035101.1|	hypothetical protein [Rickettsiella grylli]	68.21	151	48	0	8.00E-72	224
1317	gi|159120442|gb|EDP45780.1|	glutamyl-tRNA(Gln) amidotransferase subunit A (Glu-ADTsubunit A) [Rickettsiella grylli]	72.08	462	129	0	0	695
1318	gi|406915841|gb|EKD54886.1|	Superoxide dismutase [Cu-Zn] [uncultured bacterium]	57.06	163	68	2	3.00E-58	192
1319	gi|750333793|ref|WP_040615712.1|	LysR family transcriptional regulator [Rickettsiella grylli]	84.14	290	46	0	3.00E-177	503
1320	gi|492905565|ref|WP_006035971.1|	short-chain dehydrogenase/reductase SDR [Rickettsiella grylli]	65.97	238	81	0	1.00E-109	328
1321	gi|966513398|ref|WP_058529952.1|	hypothetical protein [Legionella londiniensis]	63.64	99	34	2	6.00E-36	129
1322	gi|962235308|gb|KTD19811.1|	hypothetical protein Llon_1983 [Legionella londiniensis]	67.95	78	25	0	5.00E-27	105
1323	gi|492904792|ref|WP_006035198.1|	aconitate hydratase B [Rickettsiella grylli]	81.41	850	156	1	0	1474
1324	gi|488760806|ref|WP_002684017.1|	YggS family pyridoxal phosphate enzyme [Beggiatoa alba]	50.66	229	110	2	1.00E-73	236
1325	gi|492904990|ref|WP_006035396.1|	glycine--tRNA ligase [Rickettsiella grylli]	83.37	457	76	0	0	824
1326	gi|492904392|ref|WP_006034798.1|	GTP-binding protein [Rickettsiella grylli]	89.88	603	61	0	0	1118
1327	gi|492905511|ref|WP_006035917.1|	hypothetical protein [Rickettsiella grylli]	77.97	177	39	0	3.00E-96	290
1328	gi|492904265|ref|WP_006034671.1|	bifunctional demethylmenaquinone methyltransferase/2-methoxy-6-polyprenyl-1,4-benzoquinol methylase [Rickettsiella grylli]	75.82	244	59	0	2.00E-136	397
1329	gi|750333337|ref|WP_040615256.1|	hypothetical protein [Rickettsiella grylli]	64.62	195	68	1	3.00E-83	257
1330	gi|492904825|ref|WP_006035231.1|	ubiquinone biosynthesis regulatory protein kinase UbiB [Rickettsiella grylli]	76.31	553	128	3	0	871
1331	gi|492905408|ref|WP_006035814.1|	hypothetical protein [Rickettsiella grylli]	48.53	68	34	1	2.00E-08	55.8
1332	gi|492904618|ref|WP_006035024.1|	response regulator [Rickettsiella grylli]	64.6	113	40	0	7.00E-47	159
1333	gi|492904519|ref|WP_006034925.1|	hypothetical protein [Rickettsiella grylli]	45.27	243	112	4	9.00E-54	186

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1334	gi|492905559|ref|WP_006035965.1|	4-hydroxy-3-methylbut-2-enyl diphosphate reductase [Rickettsiella grylli]	81.27	315	59	0	0	545
1335	gi|654937938|ref|WP_028388186.1|	aquaporin [Legionella fairfieldensis]	66.96	230	76	0	4.00E-100	303
1336	gi|492905266|ref|WP_006035672.1|	prepilin-type N-terminal cleavage/methylation domain-containing protein [Rickettsiella grylli]	70.16	124	36	1	8.00E-53	174
1337	gi|492904495|ref|WP_006034901.1|	peptidase S49 [Rickettsiella grylli]	83.65	318	52	0	1.00E-178	509
1338	gi|492904399|ref|WP_006034805.1|	ATP-dependent chaperone ClpB [Rickettsiella grylli]	87.49	863	107	1	0	1551
1339	gi|492905001|ref|WP_006035407.1|	adenylosuccinate lyase [Rickettsiella grylli]	75.16	455	113	0	0	720
1340	gi|492904252|ref|WP_006034658.1|	ribosomal subunit interface protein [Rickettsiella grylli]	84.68	111	17	0	4.00E-59	189
1341	gi|492905278|ref|WP_006035684.1|	ABC transporter ATP-binding protein [Rickettsiella grylli]	88.8	241	27	0	6.00E-154	440
1342	gi|492904278|ref|WP_006034684.1|	lipopolysaccharide transport periplasmic protein LptA [Rickettsiella grylli]	60.34	174	61	2	2.00E-63	205
1343	gi|492905009|ref|WP_006035415.1|	LPS export ABC transporter periplasmic protein LptC [Rickettsiella grylli]	61.17	188	71	2	2.00E-65	211
1344	gi|492904387|ref|WP_006034793.1|	arabinose-5-phosphate isomerase [Rickettsiella grylli]	82.3	322	56	1	0	541
1345	gi|492904834|ref|WP_006035240.1|	nitrate ABC transporter ATP-binding protein [Rickettsiella grylli]	90.62	437	41	0	0	817
1346	gi|492905602|ref|WP_006036008.1|	sulfonate ABC transporter permease [Rickettsiella grylli]	83.22	578	96	1	0	942
1347	gi|492904675|ref|WP_006035081.1|	oligopeptide transporter, OPT family [Rickettsiella grylli]	84.34	664	102	2	0	1113
1348	gi|492905137|ref|WP_006035543.1|	YihA family ribosome biogenesis GTP-binding protein [Rickettsiella grylli]	68.69	198	62	0	4.00E-95	287
1349	gi|159120409|gb|EDP45747.1|	cytoChrome c, class I [Rickettsiella grylli]	59.05	210	82	2	1.00E-82	256
1350	gi|492905469|ref|WP_006035875.1|	methyltransferase domain family [Rickettsiella grylli]	61.81	576	218	1	0	719
1351	gi|492904706|ref|WP_006035112.1|	phosphohistidine phosphatase [Rickettsiella grylli]	56.1	164	70	2	2.00E-57	189
1352	gi|492905128|ref|WP_006035534.1|	DNA-binding protein [Rickettsiella grylli]	87.62	105	13	0	2.00E-63	199
1353	gi|492904849|ref|WP_006035255.1|	exodeoxyribonuclease III [Rickettsiella grylli]	73.95	261	68	0	4.00E-143	415
1354	gi|492904405|ref|WP_006034811.1|	cation transporter [Rickettsiella grylli]	71.93	374	105	0	0	528
1355	gi|499908804|ref|WP_011589538.1|	MULTISPECIES: hypothetical protein [Alcanivorax]	54.67	75	34	0	7.00E-25	100
1356	gi|500425286|ref|WP_011930179.1|	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase [Calyptogena okutanii thioautotrophic gill symbiont]	35.59	59	38	0	4.00E-05	50.1
1357	gi|750333225|ref|WP_040615144.1|	hypothetical protein [Rickettsiella grylli]	28.93	159	92	4	2.00E-06	57
1358	gi|159120874|gb|EDP46212.1|	hypothetical protein RICGR_1337 [Rickettsiella grylli]	29.46	370	223	13	3.00E-33	142
1359	gi|915327277|ref|WP_050763965.1|	hypothetical protein [Rickettsiella grylli]	53.03	66	27	1	1.00E-12	68.9
1360	gi|406903354|gb|EKD45461.1|	hypothetical protein ACD_69C00281G05 [uncultured bacterium]	69	100	30	1	8.00E-41	142
1361	gi|654939163|ref|WP_028389364.1|	addiction module killer protein [Legionella fairfieldensis]	52.78	108	51	0	1.00E-32	121
1362	gi|702630640|ref|WP_033227240.1|	hypothetical protein [Diplorickettsia massiliensis]	49.06	53	27	0	1.00E-07	53.5
1363	gi|485817245|ref|WP_001436423.1|	plasmid partition protein ParG [Escherichia coli]	44	50	28	0	0.017	39.7
1364	gi|748801321|ref|WP_040048681.1|	hypothetical protein [Burkholderia sp. MR1]	38.37	86	49	1	2.00E-11	65.9
1365	gi|492905285|ref|WP_006035691.1|	hypothetical protein [Rickettsiella grylli]	42.03	69	40	0	1.00E-04	47.4
1366	gi|739708259|ref|WP_037562237.1|	hypothetical protein [Spirochaeta sp. JC202]	36.92	65	40	1	0.096	38.5
1367	gi|668344470|emb|CDW93302.1|	conserved hypothetical protein [Thiomonas sp. CB2]	40.38	52	31	0	3.00E-05	47.8
1368	gi|492905285|ref|WP_006035691.1|	hypothetical protein [Rickettsiella grylli]	76.92	78	18	0	6.00E-35	126

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1369	gi|498283443|ref|WP_010597599.1|	hypothetical protein [Diplorickettsia massiliensis]	49.33	150	74	1	3.00E-39	142
1370	gi|498283445|ref|WP_010597601.1|	hypothetical protein [Diplorickettsia massiliensis]	73.95	261	65	2	7.00E-128	387
1371	gi|702630651|ref|WP_033227243.1|	hypothetical protein [Diplorickettsia massiliensis]	54.76	42	19	0	3.00E-04	45.8
1372	gi|498283462|ref|WP_010597618.1|	hypothetical protein [Diplorickettsia massiliensis]	64.17	187	65	2	7.00E-71	229
1373	gi|498283885|ref|WP_010598041.1|	hypothetical protein [Diplorickettsia massiliensis]	65.74	108	37	0	5.00E-44	152
1374	gi|498283460|ref|WP_010597616.1|	hypothetical protein [Diplorickettsia massiliensis]	64.58	528	156	2	0	691
1375	gi|498283459|ref|WP_010597615.1|	hypothetical protein [Diplorickettsia massiliensis]	66.1	236	76	2	6.00E-86	274
1376	gi|498283457|ref|WP_010597613.1|	hypothetical protein [Diplorickettsia massiliensis]	67.37	803	247	4	0	1131
1377	gi|498283456|ref|WP_010597612.1|	tail collar domain protein [Diplorickettsia massiliensis]	66.37	342	90	2	4.00E-152	446
1378	gi|498283453|ref|WP_010597609.1|	hypothetical protein [Diplorickettsia massiliensis]	83.03	271	46	0	9.00E-169	489
1379	gi|941954218|ref|WP_055247749.1|	sensor domain-containing diguanylate cyclase [Xanthomonas sp. Mitacek01]	50	30	15	0	4	35
1380	gi|910349561|ref|XP_013178810.1|	PREDICTED: uncharacterized protein LOC106125934 [Papilio xuthus]	58.94	246	100	1	1.00E-104	317
1381	gi|338216718|gb|EGP02725.1|	helicase family protein [Pasteurella multocida subsp. multocida str. Anand1_goat]	32.58	89	57	2	0.45	42.4
1382	gi|498283234|ref|WP_010597390.1|	response regulator [Diplorickettsia massiliensis]	41.67	180	98	3	1.00E-35	136
1383	gi|754877144|ref|WP_042237191.1|	transcriptional regulator [Legionella pneumophila]	51.52	99	48	0	8.00E-31	117
1384	gi|493733799|ref|WP_006683031.1|	hypothetical protein [Candidatus Glomeribacter gigasporarum]	69.47	95	29	0	3.00E-38	135
1385	gi|1003854967|ref|WP_061468058.1|	hypothetical protein [Legionella pneumophila]	39.38	612	338	9	3.00E-131	412
1386	gi|769984314|ref|WP_045100296.1|	P-type DNA transfer ATPase VirB11 [Tatlockia micdadei]	57.45	329	136	2	7.00E-135	400
1387	gi|750333225|ref|WP_040615144.1|	hypothetical protein [Rickettsiella grylli]	41.61	560	278	8	1.00E-111	355
1388	gi|750333225|ref|WP_040615144.1|	hypothetical protein [Rickettsiella grylli]	35.14	333	176	7	2.00E-33	141
1390	gi|492905046|ref|WP_006035452.1|	hypothetical protein [Rickettsiella grylli]	39.74	78	47	0	2.00E-04	49.7
1391	gi|492905046|ref|WP_006035452.1|	hypothetical protein [Rickettsiella grylli]	35.14	589	364	8	8.00E-78	279
1392	gi|780187026|ref|XP_011662837.1|	PREDICTED: uncharacterized protein LOC105437667 [Strongylocentrotus purpuratus]	45.13	113	62	0	9.00E-24	103
1393	gi|492904993|ref|WP_006035399.1|	transposase [Rickettsiella grylli]	98.96	96	1	0	4.00E-60	191
1394	gi|750333225|ref|WP_040615144.1|	hypothetical protein [Rickettsiella grylli]	43.03	244	94	4	2.00E-41	158

Appendix Table 7.2: Predicted mitochondrial and nuclear genes of the host, *Gammarus fossarum* and their closest similarity hits.

See Appendix Files, Chapter 7 for:

Nuclear genes of *Gammarus fossarum*:							
Assembly Number	PREDICTED: host genes (*G. fossarum*)	Subject Sequence ID	Subject Name	Sequence similarity	Sequence coverage	e-value	BLAST method
35	18S rRNA gene	JF966133	Gammarus fossarum voucher SLOCHN119 18S ribosomal RNA gene, partial sequence	99%	100%	0	N
35	28S rRNA gene	EF582955	Gammarus fossarum voucher 649 28S ribosomal RNA gene, partial sequence	100%	100%	0	N
1400	Lysyl oxidase	XP_018017478	PREDICTED: lysyl oxidase homolog 2-like isoform X1 [Hyalella azteca]	86%	84%	6e-44	X
355	Hypothetical/Transposase	XP_015438005	PREDICTED: uncharacterized protein LOC107193120 [Dufourea novaeangliae]	59%	77%	3e-97	X
3906	Superoxide dismutase	AGH30393	mMn-SOD [Procambarus clarkii]	91%	92%	2e-27	X
4184	MOB-like protein	XP_018018118	PREDICTED: MOB-like protein phocein [Hyalella azteca]	100%	98%	1e-25	X
10769	CAD-Protein	XP_018023058	PREDICTED: LOW QUALITY PROTEIN: CAD protein-like [Hyalella azteca]	91%	97%	6e-29	X
3822	Hypothetical	WP_042958545	hypothetical protein [Moraxella catarrhalis]	48%	55%	1e-06	X
4217	JNK-interacting protein	XP_018024606	JNK-interacting protein 3-like [Hyalella azteca]	89%	65%	2e-30	X
48	Histone 2B	XP_018011448	PREDICTED: histone H2B [Hyalella azteca]	99%	99%	3e-64	X
9134	Protein Kinase	XP_018014697	PREDICTED: serine/threonine-protein kinase PAK 3-like [Hyalella azteca]	96%	57%	3e-28	X
8600	Amyloid B	XP_018017990	PREDICTED: uncharacterized protein LOC108674539 isoform X2 [Hyalella azteca]	98%	100%	2e-25	X
Mitochondrial genes of *Gammarus foaasrum*:							
25	NADH-quinone oxidoreductase subunit H	YP_009339291	NADH dehydrogenase subunit 1 [Eulimnogammarus cyaneus]	63%	94%	9e-121	X
25	Cytochrome b/c1	YP_006234453	CYTB gene product [Gammarus duebeni]	70%	96%	1e-149	X
25	hypothetical protein	YP_006234452	ND6 gene product [Gammarus duebeni]	49%	93%	2e-17	X
25	NADH-ubiquinone/plastoquinone oxidoreductase chain 4L	YP_006234451	ND4L gene product [Gammarus duebeni]	55%	98%	2e-12	X
25	NADH-quinone oxidoreductase subunit M	YP_006234450	ND4 gene product [Gammarus duebeni]	62%	93%	4e-147	X
25	NADH-quinone oxidoreductase subunit L	YP_009339286	NADH dehydrogenase subunit 5 [Eulimnogammarus cyaneus]	54%	98%	1e-159	X
25	hypothetical protein	YP_006234448	ND3 gene product [Gammarus duebeni]	68%	57%	2e-17	X
25	Cytochrome c oxidase subunit 3	YP_009339284	cytochrome c oxidase subunit III [Eulimnogammarus cyaneus]	74%	99%	3e-115	X
25	ATP synthase subunit a	YP_006234446	ATP6 gene product [Gammarus duebeni]	67%	80%	4e-74	X
25	Cytochrome c oxidase subunit 2 precursor	YP_006234444	COX2 gene product [Gammarus duebeni]	73%	92%	2e-112	X
25	Cytochrome c oxidase subunit 1	YP_006234443	COX1 gene product [Gammarus duebeni]	82%	98%	0	X
25	NADH-quinone oxidoreductase subunit N	YP_009118052	NADH dehydrogenase subunit 2 [Brachyuropus grewingkii]	57%	90%	3e-58	X

File 7.1: Metaxa2 results for the forward raw MiSeq reads

File 7.2: Metaxa2 results for the reverse raw MiSeq reads

Appendix to Chapter 8

Due to the large amount of sequence similarity data, the tables and files are located separately on an accompanying disk (see below for details).

Table 8.1: *Bacterial SSU sequence data for Dikerogammarus haemobaphes assembled reads*

Table 8.2: *Eukaryotic SSU sequence data for D. haemobaphes assembled reads*

Table 8.3: *Bacterial SSU sequence data for D. haemobaphes raw reads*

Table 8.4: *Eukaryotic SSU sequence data for D. haemobaphes raw reads*

Table 8.5: *Mitochondrial SSU sequence data for D. haemobaphes raw reads*

Table 8.6: *Bacterial SSU sequence data for D. villosus raw reads*

Table 8.7: *Eukaryotic and Mitochondrial SSU sequence data for D. villosus raw reads*

Table 8.8: *Dikerogammarus haemobaphes Bacilliform Virus gene annotation*

Table 8.9: *Dikerogammarus haemobaphes bi-faces-like virus gene annotation*

Table 8.10: *Nimaviridae annotated genes*

Table 8.11: *Nimaviridae gene function*

Table 8.12: *Dikerogammarus villosus Bacilliform Virus gene annotation*

Table 8.13: *Dikerogammarus villosus Bacilliform Virus gene function*

Table 8.14: *Dikerogammarus haemobaphes nuclear and mitochondrial genes*

Table 8.15: *Dikerogammarus villosus nuclear and mitochondrial genes*

File 8.1: *Proteins associating to Peinibacillus* from *D. haemobaphes*

File 8.2: *Proteins associating to 'gill symbiotic bacteria' from D. haemobaphes*

File 8.3: *Proteins associating to Opisthokonta from D. haemobaphes*

File 8.4: *Proteins associating to Acrasiomycetes from D. haemobaphes*

File 8.5: *Proteins associating to Amoebozoa from D. haemobaphes*

File 8.6: *Proteins associating to Microsporidia from D. haemobaphes*

File 8.7: *Proteins associating to Fungi from D. haemobaphes*

File 8.8: *Proteins associating to Rhabditida from D. haemobaphes*

File 8.9: *Proteins associating to Burkholderia from D. villosus*

File 8.10: *Proteins associating to Rickettsialles from D. villosus*

File 8.11: *Proteins associating to protists from D. villosus*

File 8.12: *Proteins associating to Fungi from D. villosus*