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Rapid Communication

Microsporidia are coming: *Cucumispora ornata* and *Dictyocoela berillonum* invade Northern Britain

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Abstract

Biological invasions are a driving force for biodiversity decline, worldwide. These dynamic systems often include the transference of symbiotic or pathogenic organisms that display their own threat to local fauna. Alternatively, parasites introduced during an invasion can help to control the invasive host population and limit ecological damage. To understand invasion systems that include parasites, it is important to record the presence of invasive parasites as they travel to novel locations. In this study, we screen non-native *Dikerogammarus haemobaphes* located in Boroughbridge (United Kingdom) for microsporidian parasites, using a PCR diagnostic. We found a prevalence of 9.1% for two microsporidian pathogens: *Cucumispora ornata* and *Dictyocoela berillonum*. Genetic data for the two parasites and accompanying haplotype maps are used to determine potential origin and relatedness. Our hapmap for *C. ornata* indicates two haplotypes of this parasite in the UK, sharing similarities with isolates from Poland and Germany. For *D. berillonum*, our data concur with recent findings that this parasite does not appear to have high relative genetic variability and shares similarities with isolates across the EU and from multiple hosts. These microsporidian pathogens have a wide host range and pose a risk to surrounding native crustacean fauna. We report the presence of these two parasites in their most northern range and consider their likely origin and connectivity with other isolates across the UK, Europe, and Russia.

Key words: parasite, pathogen, transmission, disease, translocation**Introduction**

Invasive species can transfer, transmit and harbour symbiotic or parasitic organisms (Roy et al. 2017). Despite the risks associated with the introduction of parasitic species, invasive and non-native hosts remain a less-explored phenomenon (Bojko et al. 2020; Chinchio et al. 2020). For example, crustacean invaders harbour a diversity of parasitic groups (viruses, bacteria, protozoa, microsporidians, Fungi, and Metazoa), but only 32% of crustacean invaders have one or more symbiotic records (Bojko et al. 2020). This lack of data is due, in part, to limited studies that record parasites in invasive species, resulting in a lack of detailed information regarding symbiont diversity and invasion history.

One crustacean invader, the demon shrimp (*Dikerogammarus haemobaphes* Eichwald, 1841), originates from the Ponto-Caspian region and has recently become established at multiple sites across England, since its first detection in the River Severn in 2012 (Johns et al. 2018). The invasion history of *D. haemobaphes* involves multiple pathways across Europe and has been well described via dedicated genetic studies (Bij de Vaate et al. 2002; Jądzewska et al. 2020). A high degree of interconnectivity within the English canal network appears to be a facilitator of spread for this invader (Johns et al. 2019). The most northerly record to date, within the UK, is the Ripon Canal, which branches from the River Ure (Johns et al. 2018). The introduction of parasites via this invader is considered a risk to UK freshwater species (Bojko et al. 2019), and as the invader continues its trajectory north, parasites may follow.

The parasites of this invader include a mininucleovirus (Subramaniam et al. 2020), a nudivirus (Allain et al. 2020), multiple bacterial symbionts, microsporidian parasites, an Oomycete, and multiple metazoan groups (reviewed by Bojko et al. 2019). Of particular interest are the microsporidian pathogens of this host, several of which continue to be discovered within its invasive range across Europe (Quiles et al. 2020, 2021). Two microsporidians have been identified from UK populations of this host: *Cucumispora ornata* Bojko, Dunn, Stebbing, Ross, Kerr and Stentiford 2015 (Bojko et al. 2015) and *Dictyocoela berillonum* Terry, Smith, Sharpe, Rigaud, Littlewood, Ironside, Rollinson, Bouchon, MacNeil, Dick and Dunn 2004 (*D. berillonum sensu lato* according to Bacela-Spychalska et al. 2018) (Green-Etxabe et al. 2015; Bacela-Spychalska et al. 2018). The pathological impact of *D. berillonum* on *D. haemobaphes* remains undetermined; however, its transmission is likely to be vertical (Bacela-Spychalska et al. 2018). Better understood is the impact of *C. ornata*, which reduces activity and increases mortality in the invasive host population, likely resulting in population control, but also presenting a pathogenic risk to native *Gammarus pulex* (Linnaeus, 1758) (Bojko et al. 2019).

Herein, we use molecular diagnostic methods to reveal that the continued invasion of the *D. haemobaphes* population is co-transporting both *C. ornata* and *D. berillonum* as it invades the Northern territories of the United Kingdom.

Materials and methods

DNA extraction, PCR and sequencing

Dikerogammarus haemobaphes (n = 11) were sampled using nets from the River Ure, Boroughbridge, United Kingdom (54.097492; -1.394724) in April 2021. A low number of specimens were obtained from the field site, this may be due to the recent establishment of the demon shrimp in this area. Animals were morphologically identified (for species specific morphological

characteristics see Müller et al. 2002) and fixed in 98% ethanol before undergoing DNA extraction. DNA extraction was carried out using the Wizard Genomic DNA purification kit (Promega). PCR was performed on all the specimens, using the MF1 (5'-CCG GAGAGGGAGCCTGAGA-3') and MR1 (5'-GACGGGCGGTGTGTACAAA-3') primer protocol detailed in Bojko et al. (2015). These primers, initially designed by Tourtip et al. (2009), amplify a variable region of the microsporidian SSU rRNA gene. PCR products were run on a 1% gel (130 V/60 min) and amplicons were excised and purified using the QIAEX II Gel Extraction Kit (Qiagen). Sanger sequencing was performed at Eurofins Genomics, UK.

Haplotype networks

To generate the haplotype networks, *C. ornata* (n = 9) sequences and *D. berillonum* (n = 26) sequences were obtained from NCBI, to be used alongside the isolates sequenced in this study (Boroughbridge *C. ornata*: MZ734489, Boroughbridge *D. berillonum*: MZ734491). The sequences were trimmed in CLC (*C. ornata*: 516 bp, *D. berillonum*: 795 bp) (<https://digitalinsights.qiagen.com>) and aligned using MAFFT (XSEDE v7.402) via the CIPRES science gateway (Miller et al. 2011). Minimum spanning networks were generated in PopArt v1.7.2 using default parameters (Leigh and Bryant 2015).

Results and discussion

The effect of an invasive parasite on an ecosystem can be as harmful as their invasive host (Prenter et al. 2004). The continued global spread of invasive crustaceans poses the risk of co-transporting symbiotic or pathogenic organisms (Bojko et al. 2020). Using a molecular diagnostic technique, we report the presence of two microsporidian parasites in the new location of Boroughbridge, UK; the most northern extent that these parasites have been reported (Table 1). Finding these parasites in this population suggests that this invader maintains host-parasite relationships as it moves into new territories and has not lost microsporidian parasites through enemy release.

Cucumispora ornata moving northward

Cucumispora ornata is a Clade V microsporidian parasite related to *C. dikerogammari* Ovcharenko, Bacela-Spychalska, Wilkinson, Ironside, Rigaud, Wattier 2010 and *C. roeseli* Bojko, Bacela-Spychalska, Stebbing, Dunn, Grabowski, Rachalewski, Stentiford 2017 and was first described in 2015 from UK populations of *D. haemobaphes* (Bojko et al. 2015). Since its initial discovery, multiple studies have conducted further screening for this species, determining that related isolates are spread throughout central Europe and that this pathogen is likely to have originated outside of the UK

Table 1. Location, host, and associated accession number of *Cucumispora ornata* and *Dictyocoela berillonum* isolates that have been sequenced and identified to date. The accession numbers associate with the data presented in Figure 1. Accession numbers marked with an asterisk (*) were not included in subsequent haplotype analysis due to short sequence length.

Parasite	Location	Hosts	Accessions	References
<i>Cucumispora ornata</i>	Germany	<i>Gammarus roeselii</i> , <i>Dikerogammarus haemobaphes</i> , <i>Gammarus pulex</i>	KR871366-69	Quiles et al. 2019; Grabner et al. 2015
	Austria	<i>Gammarus roeselii</i>	MK719282-84*	Quiles et al. 2019
	Romania	<i>Gammarus roeselii</i> , <i>Gammarus balcanicus</i>	MT949307-9*	Quiles et al. 2019
	France	<i>Gammarus roeselii</i>	Unassigned	Quiles et al. 2019
	Poland	<i>Gammarus roeselii</i>	KP699690	Quiles et al. 2019
	UK	<i>Dikerogammarus haemobaphes</i>	KR190602 MZ734489	Bojko et al. 2015; This study
		<i>Gammarus chevreuxi</i>	AJ438962	Terry et al. 2004
	Bulgaria	<i>Gammarus balcanicus</i>	MT949306*	Quiles et al. 2021
Russia	<i>Dorogostaiskia parasitica</i> , <i>Pallasea cancellus</i>	FJ756112, KM977846	Madyarova et al. 2015	
<i>Dictyocoela berillonum</i>	UK	<i>Echinogammarus berilloni</i> , <i>Dikerogammarus haemobaphes</i> , <i>Echinogammarus marinus</i> , <i>Gammarus duebeni duebeni</i> , <i>Gammarus tigrinus</i>	AJ438957, KM486059, JQ673481, MZ734491	Terry et al. 2004; Green-Etxabe et al. 2015; Yang et al. 2011; This study
	Poland	<i>Gammarus roeselii</i> , <i>Dikerogammarus haemobaphes</i> , <i>Dikerogammarus villosus</i> , <i>Pontogammarus robustoides</i> , <i>Chaetogammarus ischnus</i>	MK719346*, MG773248-50, MG7732553-54, KF830272, KR349184, KR349181, KM657354, MG773259-60, MG773256, MG773252	Quiles et al. 2019; Bacela-Spychalska et al. 2018
	Belgium	<i>Dikerogammarus villosus</i>	EF119216	Wattier et al. 2007
	Netherlands	<i>Chaetogammarus trichiatus</i>	MG773246-47	Bacela-Spychalska et al. 2018
	Ukraine	<i>Pontogammarus robustoides</i> , <i>Dikerogammarus villosus</i>	MG773251, MG773257-58	Bacela-Spychalska et al. 2018
	Germany	<i>Chaetogammarus trichiatus</i> , <i>Dikerogammarus haemobaphes</i> , <i>Echinogammarus triachtus</i>	MG773255, KR871364-65	Bacela-Spychalska et al. 2018; Grabner et al. 2015
	Bulgaria	<i>Gammarus balcanicus</i>	MT932331-32*	Quiles et al. 2021
	Macedonia	<i>Gammarus balcanicus</i>	MT932330*	Quiles et al. 2021

(Bojko et al. 2019; Quiles et al. 2019, 2020, 2021). This study identified a single specimen positive for *C. ornata* at Boroughbridge, UK, now considered the most northerly extent of this parasites invasive range. The 908bp SSU sequence was 100% similar to “*Microsporidium* sp. Dhae17W” (KP699690; cov. = 100%, ID = 100%), a *C. ornata* isolate from Poland. Other UK isolates of this species showed variable sequence similarity to the new isolate (KR190602: cov = 100%, ID = 99.81%; AJ438962: cov = 100%, ID = 98.84%).

The isolate sequenced as part of this study appears more closely related to an isolate from Poland than to other UK isolates, potentially hinting that several introductions of this parasite may have taken place. Figure 1 details the co-existence of 2 haplotypes of *C. ornata* in the UK, the isolate sequenced in this study belongs to a haplotype found in individuals in the UK, Germany, and Poland. Two distinct haplotypes appear in both England and Germany, according to our haplotype network (Figure 1). Further, two Russian isolates of this parasite also form distinct groups within the hapmap and may be the closest genetic representatives of the native parasite.

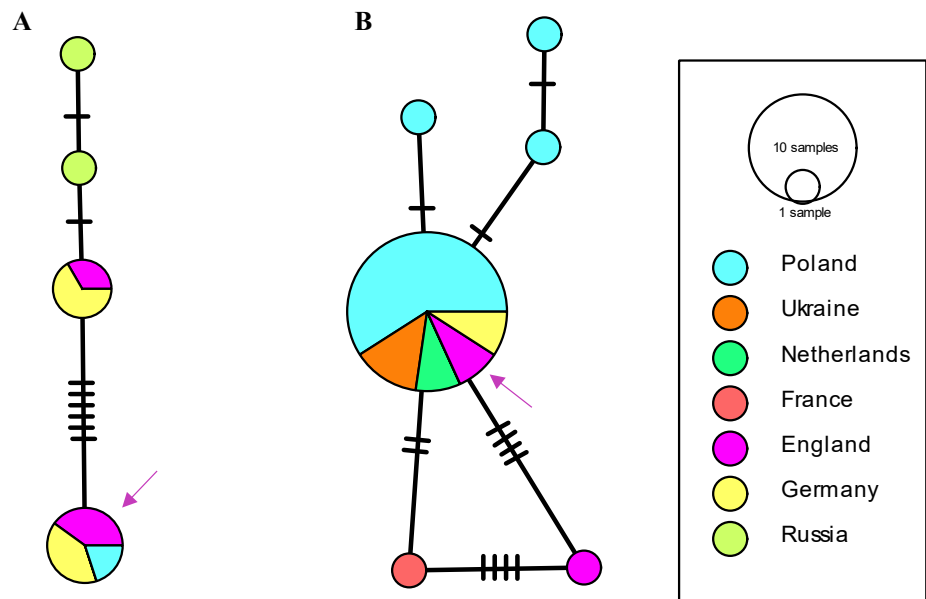


Figure 1. Haplotype networks inferred using the Minimum Spanning Network method in PopArt for two microsporidian parasites. A) Sequence data for *Cucumispora ornata* (n = 10). B) Sequence data for *Dictyocoela berillonum* (n = 27). Isolates sequenced in this study are highlighted with an arrow. The size of each circle is proportional to the frequency of the haplotype. The colour indicates the location where the sequence was obtained. The hatch marks present along the lines indicate the relative number of differences in the nucleotide sequences between the adjoining specimens.

A limited number of *C. ornata* sequences makes it difficult to compare populations across the invasive range; however, further diagnostic applications for invasive and native demon shrimp populations may help to gain greater clarity on the spread of the parasite. It is important to maintain surveillance for this parasite, since it can cause mortality in the native keystone species, *G. pulex* (Bojko et al. 2019) and to continue exploring dynamics relating to enemy release (Hatcher et al. 2015).

Dictyocoela berillonum moving northward

Dictyocoela berillonum is a vertically transmitted microsporidian parasite first observed from several amphipod species from Northern Europe in 2004 (Terry et al. 2004). The *Dictyocoela* genus was then ratified in 2018 (Bacela-Spychalska et al. 2018). The broad geographic distribution of this microsporidian species across Europe has been revealed via PCR of amphipod hosts (Table 1). This study identified the presence of *D. berillonum* in a single specimen sampled from Boroughbridge (UK). A 926bp SSU sequence of a *D. berillonum* isolate from *D. haemobaphes* in Boroughbridge, showed closest similarity to “*D. berillonum* isolate B13” (MG773257; cov. = 100%, ID = 99.68%), which parasitises *Dikerogammarus villosus* (Sowinsky, 1894) in Ukraine.

Haplotype networks generated using *D. berillonum* isolates as part of this study show a high degree of genetic homogeneity across Europe, with one common haplotype central to rarer genotypes (Figure 1). Larger scale phylogeographic studies of this microsporidian suggest that there is

low haplotype diversity across several amphipod species, meaning that its host range is broad and includes several susceptible genera (Bacela-Spychalska et al. 2018). One haplotype has been frequently identified as the most common, potentially indicating a highly successful co-invasion event between this parasite and its associated Ponto-Caspian hosts (Grabner et al. 2015).

The pathological impact of *D. berillonum* on *D. haemobaphes* remains largely undetermined; however, *D. berillonum* was found to be near ubiquitous in some UK populations of demon shrimp, causing a high prevalence of intersexuality in populations in the UK (Green-Etxabe et al. 2015). Although the underlying developmental effect of this parasite causes a physiological change to its host, the parasite does not appear to have impeded its distribution across the UK (Green-Etxabe et al. 2015). Our study has now shown that the *D. haemobaphes* population continues to spread throughout the UK, carrying parasites alongside of it.

Continued monitoring of invasive pathogens

Invasive parasites can be as harmful as their invasive host, presenting a risk to native species and ecosystem functioning (Prenter et al. 2004; Roy et al. 2017). Our findings identify two microsporidian parasites in North Yorkshire, which may have the capacity to spill into populations of native hosts (Bojko et al. 2019). Tracking the movement of emerging invasive diseases, alongside their invasive host counterparts, is important to help elucidate the role of parasites during biological invasions (Dunn 2009). Previous literature has suggested that *C. ornata* may control population size and health in new populations and detection of the continued success of this parasite in new, northern, populations of the UK suggest that it may readily invade along with its host. The mode by which this parasite persists within the population requires experimental examination; however, it seems likely that it may have a biological trait that helps it to maintain its prevalence in the host population, or may readily co-invade through hydrologically connected populations, or populations linked by anthropogenic introductions.

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Authors' contribution

AB and JB contributed to the design and implementation of the research. AB and JB collected animal specimens. AB performed the laboratory work and analysis of the results. AB and JB drafted and finalised the text.

Ethics and permits

Ethical clearance was obtained from Teesside University Life Sciences Research Ethics Committee (Ethics approval number: 2020 Nov 1793 Burgess). All research pertaining to this article did not require any research permits.

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