Enemy release and genetic founder effects in invasive killer shrimp populations of Great Britain

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Abstract

The predatory “killer shrimp” *Dikerogammarus villosus* invaded Britain from mainland Europe in 2010. Originating in the Ponto-Caspian region, this invader has caused significant degradation of European freshwater ecosystems by predating and competitively excluding native invertebrate species. In contrast to continental Europe, in which invasions occurred through the migration of large numbers of individuals along rivers and canals, the invasion of Great Britain must have involved long distance dispersal across the sea. This makes the loss of genetic diversity and of debilitating parasites more likely. Analysis of nuclear microsatellite loci and mitochondrial DNA sequences of *D. villosus* samples from the four known populations in Britain reveal loss of rare alleles, in comparison to reference populations from the west coast of continental Europe. Screening of the British *D. villosus* populations by PCR detected no microsporidian parasites, in contrast with continental populations of *D. villosus* and native amphipod populations, most of which are infected with microsporidia. These findings suggest that the initial colonisation of Great Britain and subsequent long distance dispersal within Britain were associated with genetic founder effects and enemy release due to loss of parasites. Such effects are also likely to occur during future long-distance dispersal events of *D. villosus* to Ireland or North America.

Keywords: *Dikerogammarus villosus*; Great Britain; microsporidia; genetics; enemy release

41 Introduction

42

43 The last century has witnessed many invasions of aquatic ecosystems by alien invasive
44 species. Freshwater ecosystems in particular, have been extensively disrupted by human
45 activities, rendering them more susceptible to invasion. Simultaneously, the naturally high
46 potential for the dispersal of species through freshwater ecosystems has been enhanced by
47 human activities such as the construction of canals and the transport of ballast water by ships.
48 As a result, alien invasive species are considered to be the third most important cause of
49 decline in aquatic ecosystems (Sala et al. 2000).

50 Crustaceans are arguably the most important and successful taxonomic group of
51 aquatic invaders, making up 53% of alien invasive species in European freshwater
52 ecosystems (Karatayev et al. 2009; Hanfling et al. 2011). Among crustacean invaders,
53 amphipods play a major role, particularly in European and North American aquatic
54 ecosystems. In terms of numbers, native amphipods are frequently dominant or sub-dominant
55 in freshwater and aquatic ecosystems (Vainola et al. 2008), where they play a major role in
56 nutrient cycling through their shredding activities (Piscart et al. 2011). Alien invasive
57 amphipods tend to differ from natives in specific ways. They typically mature earlier,
58 produce larger broods and have more generations per year than native species (Grabowski et
59 al. 2007). They also tend to be more tolerant of human disturbance and more generalist in
60 their diet and habitat preferences (Grabowski et al. 2007). At least ten alien amphipod
61 invaders of European and American freshwater ecosystems originated in the Ponto-Caspian
62 region (bij de Vaate et al. 2002), where high levels of environmental variability and
63 instability appear to have preadapted amphipod species to invade disturbed ecosystems
64 (Grabowski et al. 2007). In many localities, invasive amphipods, such as *Dikerogammarus*
65 *villosus* have displaced native amphipod species at a local level (Dick and Platvoet 2000),

66 with a potential reduction in the efficiency of nutrient cycling and additional disruptive
67 effects due to the integration of invasive amphipods into food webs at various trophic levels
68 (van Riel et al. 2006). Although native species tend not to be displaced at a regional scale,
69 due to their occupancy of privileged microhabitats (Piscart et al. 2010), there is concern that
70 each destabilising invasion of an aquatic ecosystem makes it more vulnerable to subsequent
71 invasions, with the risk of eventual invasional meltdown (Ricciardi 2001).

72 The role of genetic diversity in the establishment and persistence of invasive species
73 remains somewhat ambiguous. If range extensions occur through dispersal of a small number
74 of propagules, then the resulting founder effect may diminish the genetic diversity of the
75 invader (Sax et al. 2005). In theory, this could limit the invader's ability to adapt to new
76 habitats and to resist new or existing natural enemies. However, genetic drift associated with
77 invasion founder effects can also unlock the adaptive potential of genetic variation that is
78 masked by dominance or epistasis in the native range, allowing rapid adaptation by invasive
79 species (Lee 2002). Reductions in genetic diversity at neutral marker loci have been
80 documented in the invasive amphipods *Crangonyx pseudogracilis* (Slothouber Galbreath et
81 al. 2010), *Echinogammarus ischnus* (Cristescu et al. 2004) and *Gammarus tigrinus* (Kelly et
82 al. 2006), all highly successful transcontinental invaders. However, where progagule pressure
83 is high, as in cases of recurrent invasions or those involving large numbers of individuals,
84 genetic diversity within the invaded range may be as high, or even higher than in the native
85 range. Admixture of populations from different North American sources has meant that some
86 invasive *G. tigrinus* populations in Europe have higher genetic diversity than any single
87 North American source population (Kelly et al. 2006).

88 Founder effects during invasion can also reduce the diversity of parasites carried by
89 invasive species. Stochastic parasite loss is most likely to occur where the number of host
90 propagules is small and the parasite prevalence is low. Where a parasite reduces host

resilience, adverse conditions during transport may also remove infected hosts and susceptible host genotypes, increasing the likelihood that the parasite will be lost. In theory, such parasite loss might enhance the productivity of the invasive species, and hence its likelihood of successful establishment (a case of enemy release (Torchin et al. 2003)). However, the likelihood of this depends upon the nature of the parasites concerned and on their mechanism of transmission (Hatcher and Dunn 2011). For example, the diversity of microsporidian parasites showed no significant reduction between source and invasive populations of the amphipod *C. pseudogracilis* (Slothouber Galbreath et al. 2010). In this case, the parasites were avirulent and vertically transmitted, passed predominantly from mothers to offspring and hence less affected by host population density or harsh transport conditions than would have been the case with more virulent, horizontally transmitted parasite species.

Invasive species may also acquire new parasites within their extended range, potentially increasing parasite diversity, reducing the fitness of the invader, and acting as reservoirs for parasite spillback to native hosts (Dunn et al. 2012). Genetic bottlenecks may increase the susceptibility of invasive species to novel parasites by impairing diversity-based mechanisms of resistance and reducing the ability to evolve resistance to new parasites (Colautti et al. 2004; Hatcher and Dunn 2011). Conversely, parasites carried by invasive species may infect native species with which they come into contact (Strauss et al. 2012), as occurred in the case of the oomycete pathogen *Aphanomyces astaci*, cause of 'crayfish plague' which was transmitted from the invasive North American crayfish to native crayfish, in Great Britain and other parts of Europe (Holdich and Reeve 1991; Holdich et al. 2009).

The killer shrimp *D. villosus* is one of the most damaging amphipod invaders of European aquatic ecosystems (DAISIE 2009). It is common for amphipod species to compete and prey upon one another simultaneously (Dick and Platvoet 1996), a phenomenon known

as intraguild predation (Polis et al. 1989). The large size and aggressive behaviour of *D. villosus* create an asymmetry to such interactions in which both native species and other invaders can be displaced and driven locally extinct (Dick and Platvoet 2000). The generalist predatory behaviour of *D. villosus* also impacts other aquatic invertebrates and places this invertebrate in competition with predatory fish (MacNeil et al. 2010). Unusually, the colonisation history of this Ponto-Caspian species in Europe is well-documented (Wattier et al. 2007). *D. villosus* is now well-established in major European river basins including the Danube, Vistula, Elbe, Oder, Rhine, Rhone, Seine and Loire, and has recently colonised Great Britain, occurring at four separate sites in southern England and Wales (Bojko et al. 2013; MacNeil et al. 2010).

No losses of genetic diversity or parasites were noted during the invasion of the Rhine, Rhone, Seine and Loire basins of mainland Europe by *D. villosus* (Wattier et al. 2007). This is presumably due to high propagule pressure as *D. villosus* invaded in successive waves, involving high numbers of individuals, along the courses of rivers and canals. However, some evidence supports the acquisition of new parasites during the expansion of *D. villosus*, since the microsporidia *Nosema granulosis*, *Dictyocoela muelleri* and *Dictyocoela berillonum* were not discovered in *D. villosus* within its native range but did occur within certain invasive populations in Europe (Wattier et al. 2007). These, and other microsporidia also infect native British amphipods (Table 1), presenting a risk of transfer to invading *D. villosus*, even if it escapes its former parasitic enemies during transport to Great Britain. If *D. villosus* has carried Ponto-Caspian parasites, such as *Cucumispora dikerogammari* to Britain, then these may pose a risk to native fauna. Laboratory studies indicate that *C. dikerogammari* can infect *Gammarus pulex*, a species native to Great Britain, although infected *G. pulex* have not yet been discovered in natural European populations (Bacela-Spychalska et al. 2012).

The recent colonisation of Great Britain involved transport across the English Channel or North Sea, perhaps in ballast water or carried on fishing or watersports equipment. Although the precise mechanism of transportation remains unknown (MacNeil et al. 2010), it is likely to have involved significantly fewer individuals than previous European invasions and may have imposed harsher conditions during transit. The fact that, following the invasion of Europe's west coast, sixteen years passed before *D. villosus* became established in Great Britain suggests that this was a low-probability event, perhaps more similar to the transatlantic voyages of *G. tigrinus*, *C. pseudogracilis* and *E. ischnus* than to the previous march of *D. villosus* along the rivers and canals of Eurasia. Losses of genetic diversity, similar to those of the former three species may therefore be expected in British populations of *D. villosus*. Given that microsporidian parasites occur at low prevalence in putative source populations, the loss of these parasites during an invasion bottleneck is also likely. By studying the effects of the invasion of Great Britain on the population genetics and parasite diversity of *D. villosus*, it is therefore possible to gain a general insight into the impact of long-distance dispersal events upon the viability and adaptability of this damaging invasive species. This is particularly important, given fears that *D. villosus* will, in future, be carried across the Irish Sea to Ireland and the Isle of Man, and across the Atlantic to North America (Dick et al. 2002; Casellato et al. 2007).

Recent histological analysis detected no evidence of infection with microsporidian parasites in two of the four known British populations of *D. villosus* (Cardiff Bay and Barton Broad) (Bojko et al. 2013). A single microsporidian infection was discovered in a very large sample of *D. villosus* (N=1937) from a third British population (Grafham Water) but this parasite bore little resemblance to microsporidia known from the native range of *D. villosus*, suggesting that it may have been acquired locally (Bojko et al. 2013). Microsporidian infections of amphipods can involve low numbers of parasites and such light infections may

be overlooked during histological analysis. Furthermore, morphological examination of microsporidia by light or electron microscopy can be inadequate for species identification. In contrast, PCR screening can detect microsporidia even at very low burden while DNA sequencing can be used to accurately identify microsporidian isolates and assign them reliably to taxonomic groups (Hogg et al. 2002).

The hypothesis that the invasion of Britain has produced a genetic founder effect and release from microsporidian infection in *D. villosus* was tested by surveying the four known British populations of *D. villosus* for microsporidian parasites by PCR screening and also assessing their genetic diversity at mitochondrial and nuclear microsatellite loci. In order to establish levels of microsporidian infection and genetic diversity in putative source populations, two reference populations from the Siene and Rhine catchments on the Western coast of continental Europe were also screened using the same methods. Where native or invasive amphipod species co-occurred with *D. villosus*, these were also screened for microsporidian parasites in order to detect possible parasite transmission between invasive and native species.

Methods

Sample collection and preparation

During summer 2011, adult *D. villosus* were collected from the four known invasive populations in Great Britain; Grafham Water (52°18’05”N, 0°19’14”W), Cardiff Bay (51°27’35”N, 3°10’03”W), Eglwys Nunydd (51°32’58”N, 3°44’22”W) and Barton Broad (52°44’19”N, 1°29’45”E). All four of these sites are artificial freshwater lakes, and all are used for water sports and recreational fishing. Barton Broad was created in the 13th Century CE by peat digging and subsequently flooded, while Eglwys Nunydd and Grafham Water

were created as artificial reservoirs in the 1920s and 1960s respectively. The freshwater lake at Cardiff Bay was created most recently, in 1999, by the construction of the Cardiff Bay Barrage. Cardiff Bay, Barton Broad and Grafham Water have been colonised by the zebra mussel *Dreissena polymorpha*, another Ponto-Caspian invader which can provide a habitat for *D. villosus* (MacNeil et al. 2010).

Additional *D. villosus* samples were collected from populations within putative source drainages on the west coast of Europe; from Nogent-sur-Marne, Seine drainage, France (48°49'55"N, 2°29'39"E) and from the Gouwzee at Monnickendam, Rhine drainage, Netherlands (52°26'22"N, 5°02'05"E). Samples were collected by turning stones in shallow water, sweeping beneath stones with a hand net and removing individuals from the underside of stones by hand. Other amphipod species discovered at sites near to *D. villosus* habitat were collected in the same way and identified using appropriate keys (Lincoln 1979; Karaman and Pinkster 1977). Following collection, amphipods were placed immediately into absolute ethanol.

Individual amphipods of all species were dissected under a light microscope. The hard exoskeleton was discarded and all remaining soft tissue was used for DNA extraction. Each dissection was performed in a separate disposable dish and all dissection implements were sterilised by dipping into ethanol and flaming in a Bunsen burner between dissections. DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions and quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific). The identity of species other than *D. villosus* was confirmed by amplifying a fragment of the mitochondrial Cytochrome Oxidase gene (*COI*) using the barcoding primers HCO2198 and LCO1490 (Folmer et al. 1994) from a single individual of each species. These primers did not work for a species collected from the River Seine at Rouen and identified morphologically as *Gammarus zaddachi*, so an alternative set of COI primers was designed,

based on published *G. zaddachi* sequences (GsCOI_F1: GTTAGGAGCTTGGTCTAGTG; GsCOI_R1: AAATAGGGTCTCCTCCACC). For both primer sets, PCR was performed on a Primus thermal cycler (MWG Biotech) using 30 cycles with an annealing temperature of 50°C and an extension time of 1 minute. The resulting PCR products were sequenced using an ABI Prism 3100 Genetic Analyser and compared to sequences on the Genbank database using NCBI's BLAST tool.

Parasite diversity

All samples were screened for microsporidian parasites by PCR, using the general microsporidian 16S rDNA primers V1F and 530R, which have shown to amplify DNA from all of the microsporidia known to be associated with *D. villosus* (*C. dikerogammari*, *Di. muelleri*, *D. berillonum* and *N. granulosis*) reliably, along with many other microsporidian parasites of amphipods (Wattier et al. 2007). Amplification was performed with Invitrogen Taq polymerase on a Primus thermal cycler, using 30 cycles with an annealing temperature of 50°C and an extension time of one minute. Samples were run on a 1% agarose gel, stained with SYBR® Safe and viewed using a UV transilluminator. Where a band of the expected size (400-450 bp) was obtained, a longer fragment (1300-1400 bp) was amplified, using the primers V1F and 1492R (Hogg et al. 2002) (annealing temperature: 50°C, extension time: 1 minute), and sequenced. For each sequence, a sequence similarity search of the NCBI databases was conducted using BLAST. For each population, parasite prevalence of each microsporidian species was calculated as a proportion of individuals infected and a 95% confidence interval for prevalence was calculated, based on the binomial distribution.

The 16S rRNA gene of *Dictyocoela* contains a hypervariable region, allowing isolates to be assigned to specific haplotypes, some of which are associated with particular host species (Wilkinson et al. 2011). In order to infer their most likely origin, *Dictyocoela*

sequences obtained from *D. villosus* and co-occurring amphipod species were aligned with other published *Dictyocoela* sequences using ClustalW (Thompson et al. 1994), implemented in Bioedit 7.2.5 (Hall 1999) and corrected manually. Positions that could not be aligned unambiguously were excluded from subsequent analysis. A phylogenetic tree was constructed using Bayesian inference in MrBayes (Huelsenbeck and Ronquist 2001). A maximum likelihood test of 24 different nucleotide substitution models, implemented in Mega 6 (Tamura et al. 2013) indicated that the general time reversible model, with gamma-distributed rate variation and a proportion of invariant sites (GTR+I+G) provided a good fit the data according to the Akaike Information Criterion (corrected) and so this model was used. A tree search was conducted over 1,000,000 generations, sampling every 100 generations, with a burn in of 2500 generations.

From native amphipods in the River Seine, France and Monnickendam, Netherlands, 16S rDNA sequences were obtained that did not produce exact matches to any published microsporidian sequences. These were aligned, as described previously, with published microsporidian 16S rDNA sequences from the major microsporidian taxonomic groups, including other amphipod parasites, and, as before, 24 models of nucleotide substitution were tested against each alignment. Phylogenetic trees were constructed using Bayesian inference in order to provide an indication of their phylogenetic affiliations. In each case, a general time reversible model of evolution was used, with gamma-distributed rate variation and a proportion of invariant sites, and a search was conducted over 100,000 generations, sampling every 100 generations, with a burn in of 250 generations.

Population genetics

All *D. villosus* individuals were genotyped using species-specific microsatellite markers (Wattier et al. 2006) with forward primers fluorescently-labelled as follows: DikF(6FAM),

DikQ(VIC), DikS(PET). All primers were combined in a single multiplex PCR reaction. Amplification was carried out using a QIAGEN Multiplex PCR Kit (QIAGEN, CA-USA) in a final volume of 10µl, containing 5µl of Multiplex Kit Buffer 2X and 2.5µg of genomic DNA. 35 PCR cycles were used with an annealing temperature of 59°C and an extension time of 45 seconds. Products were then run alongside a GS500LIZ size standard in an ABI3730xl Genetic Analyzer (Applied Biosystems) and alleles were scored using GENEMAPPER4.0 (Applied Biosystems).

Each population was also screened for single nucleotide polymorphisms in the mitochondrial gene for cytochrome c oxidase subunit 1 (COI). An 538 bp fragment of COI was amplified from each individual using the primers DvCO1F1 (AGTGTAATTATTCGGTCGGA) and DvCO1R1 (CGATCTGTCAAGAGTATCGT), designed on the basis of a *D. villosus* sequence deposited in Genbank (AY529048). Amplification was performed with Invitrogen Taq polymerase, using 30 cycles with an annealing temperature of 50°C and an extension time of one minute. PCR products were sequenced using an ABI Prism 3100 Genetic Analyser and aligned using ClustalW (Thompson et al. 1994), implemented in Bioedit 7.2.5 (Hall 1999).

Heterozygosity and allelic diversity at the three microsatellite loci within each *D. villosus* population were estimated using Excel Microsatellite Toolkit (Park 2001). Pairwise F_{ST} values were calculated and subjected to a permutation test of significance with 9999 permutations using GenAlEx 6.5 (Peakall and Smouse 2012). Measures of pairwise F_{ST} can be affected by the presence of null alleles. Frequencies of null alleles at the three *D. villosus* microsatellite loci were estimated using the expectation algorithm of Dempster et al. (1977), implemented in the programme FreeNA (Chapuis and Estoup 2007). Where null alleles were detected, pairwise measures of F_{ST} were recalculated, using the *ENA* method of Chapuis and Estoup (2007). An AMOVA was performed in Arlequin 3.5 to assess the proportions of

genetic variance falling within populations, between populations and between regions and these were tested for significance using a permutation test with 10100 permutations. Initially, the Seine drainage, the Rhine drainage and Great Britain were considered as different regions. However, given the high degree of genetic similarity revealed between the Seine and Rhine populations by the F_{ST} analysis, these were then placed within a single region for comparison with the British populations. A matrix of geographic distances between the British *D. villosus* populations was calculated using Geographic Distance Matrix Generator and used to perform a Mantel test of isolation by distance, implemented in Arlequin 3.5, against the matrix of pairwise F_{ST} values, with a significance test using 1000 permutations.

Invasion bottlenecks are expected to result in the loss of rare alleles. Rare alleles were identified in the putative source populations of Monnickendam and Nogent-sur-Marne and their presence or absence was noted in the British populations. In this case, an allele is defined as rare if it occurs at a frequency of less than 0.1 in the putative source population, following Luikart et al. (1998) and Wattier et al. (2007).

Results

Parasite diversity

PCR with general microsporidian SSU rDNA primers revealed no evidence of microsporidian infection in any British population of *D. villosus* (Table 2). Of the mainland European populations, the sample from Nogent-sur-Marne contained a single individual infected with *C. dikerogammari*, while the sample from the Gouwzee at Monnickendam contained individuals infected with *C. dikerogammari* and *Dictyocoela* spp., all at low prevalence. At Monnickendam, populations of a small amphipod were discovered, occupying microhabitats separate from those of *D. villosus*. This was identified as *Echinogammarus*

trichiatus, another Ponto-Caspian invader, which now occurs across Europe and has previously been recorded in the Gouwzee (Boets et al. 2012). DNA barcoding confirmed the identity of this species, producing a sequence (Genbank: KM024679) identical to that of an *E. trichiatus* individual collected from the Danube Delta (Genbank: AY529051). Screening of *E. trichiatus* by PCR, using general microsporidian SSU rDNA primers, revealed infection with *Dictyocoela berillonum* at high prevalence (Table 2). No amphipods other than *D. villosus* were discovered at Nogent-sur-Marne, but amphipods identified as *Gammarus zaddachi* by morphology and DNA barcoding (Genbank: KM024680) were discovered 156 km downstream at Rouen, where *D. villosus* was not found. Screening of *G. zaddachi* by PCR revealed infection with a microsporidian parasite at a relatively high prevalence (Table 2). The SSU rDNA sequence of this parasite was not similar to those of any parasite obtained from *D. villosus*.

Phylogenetic analysis of small subunit ribosomal DNA sequences (Figure 1) placed two *Dictyocoela* isolates from *D. villosus* in a clade containing isolates from various native and invasive amphipods, including isolates described as *D. duebenum* and *D. muelleri*. Sequences obtained from these isolates (Genbank accession KJ019842-KJ019843) were extremely similar to that of an isolate obtained from *Dikerogammarus haemobaphes* from Poland (Wilkinson et al. 2011). Isolates with very similar sequences have also been obtained from the native European species *Gammarus duebeni*, from Ireland and the Baltic Sea. The remaining *Dictycoela* isolate from *D. villosus* (Genbank accession KJ019844) was placed in a clade containing an isolate described as *D. berillonum*. All of the *Dictyocoela* isolates obtained from *E. trichiatus* produced sequences identical to this one. These sequences differed by only a single base pair from a sequence obtained from an isolate from *Pontogammarus robustoides*, another Ponto-Caspian invader (Wilkinson et al. 2011).

An additional microsporidian sequence from *E. trichiatus* (Genbank accession KJ019845) did not match any sequences deposited in Genbank to date. Phylogenetic analysis of this sequence (Supplementary information) placed it as a close sister to parasites of the Baikalian endemic amphipod *Dorogostaiskia parasitica* and of a North American population of the amphipod *Corophium volutator*. These occur within a wider clade of microsporidia containing parasites of various aquatic hosts, including amphipods, insects, oligochaetes and bryozoa. Microsporidian SSU rDNA sequences obtained from the native amphipod *G. zaddachi* from the River Seine (Genbank accession KJ019846-KJ019850) did not match any sequences deposited in Genbank to date. Phylogenetic analysis of these sequences (Supplementary information) placed them in a well-defined clade consisting predominantly of parasites of fish and crustaceans but also containing *Enterocytozoon bieneusi*, a parasite of mammals.

Population genetics

All four British *D. villosus* populations lack alleles which are present at low frequency in the continental reference populations (Table 3). The Eglwys Nunydd population exhibits particularly strong evidence for allelic loss, lacking two alleles at locus DikF (248 and 250) and one allele at locus DikQ (123), all three of which are present in the other British and continental samples. No microsatellite alleles are present in both continental populations and absent from all British populations. One single nucleotide polymorphism was detected, at position 421 of the mitochondrial COI sequence of *D. villosus* (Genbank accession KJ019851- KJ019852). This polymorphism occurs only in the Eglwys Nunydd sample, but the additional allele (421A) dominates there, occurring at a frequency of 0.64.

Permutation tests of pairwise F_{ST} (Table 4) indicate significant genetic isolation (P<0.05) between most populations, the only exception being between Cardiff Bay and

Nogent-sur-Marne. It is notable that samples from Eglwys Nunydd and Barton Broad show consistently high values of F_{ST} when compared with all other populations. Estimation of the frequencies of null alleles, based on deviation from Hardy-Weinberg proportions, suggest the presence of null alleles at moderate frequency ($0.05 \leq r < 0.20$) at locus DikF in most populations and at locus DikQ in the Cardiff Bay population only. However, recalculation of F_{ST} values using the *ENA* correction produced no qualitative changes in the significance of the results (Table 4). An AMOVA indicated that, while a significant amount of genetic variance occurred among populations within Great Britain, there was no discernable partitioning of genetic variance between Great Britain and mainland Europe (Table 5). A Mantel test identified no significant isolation by distance among *D. villosus* populations within Great Britain (Rxy=0.000, P>0.10) .

Discussion

Population genetic analysis of four British *D. villosus* populations provides limited support for bottlenecks arising from founder effects during the invasion of Britain. All four British populations lack certain rare alleles present in the mainland populations, another indication of a genetic bottleneck (Luikart et al. 1998). Interestingly, different alleles are missing from different British populations while certain alleles present in the British populations of Cardiff Bay and Eglwys Nunydd were not detected in the reference populations. Allele frequencies within the Eglwys Nunydd population are very divergent from the other three British populations, with the loss of several rare alleles at the three microsatellite loci and dominance of a mitochondrial COI haplotype not detected in samples from other British or continental populations. These suggest either a different source or significant genetic drift within this population, consistent with a strong founder effect.

The British samples show significant population differentiation from one another, as measured by F_{ST}. Given the disjunct distribution of the British populations it is therefore possible that they represent several independent colonisations of Britain, either from different sources or from the same mainland source. Alternatively, a single colonisation of Britain may have been followed by several subsequent introductions from the original invasive population to other British localities, each associated with a founder effect. However, in this case, significant isolation by distance between the British populations would be expected. A Mantel test comparing matrices of genetic and geographic distances provides no support for this hypothesis.

Given the prevalence of microsporidia, particularly *C. dikerogammari*, among invasive *D. villosus* populations in mainland Europe (Wattier et al. 2007), the absence of microsporidian parasites from British *D. villosus* samples suggests enemy release. The most likely cause for this apparent loss of parasites would be a population bottleneck, coupled with stressful transport conditions, during passage to Great Britain over the English Channel or North Sea. Although *C. dikerogammari* appears to be vertically transmitted (Ovcharenko et al. 2010) and avirulent in the early stages of infection (Bacela-Spychalska et al. 2012), it does reduce the survival of its host and shows density dependence, making it potentially susceptible to extinction during a host bottleneck. *Dictyocoela* species show high levels of vertical transmission and some strains or species appear to be avirulent (Ironside et al. 2003; Terry et al. 2004). Coupled with the ability of at least some strains to feminise male hosts (Ironside et al. 2003), potentially increasing the rate of host population increase on arrival (Hatcher and Dunn 2011), these attributes appear to make *Dictyocoela* a good candidate for survival during transport. However, the low prevalence of *Dictyocoela* in European populations of *D. villosus* makes it vulnerable to stochastic loss during a founder event.

Within the two mainland European populations, prevalence of *C. dikerogammari* is not significantly higher than when previously measured in 2002 (Wattier et al. 2007). This contradicts Wattier et al's (2007) hypothesis that microsporidian parasite prevalence tends to increase with time since colonisation, and suggests that differences in prevalence may show a geographical or ecological pattern instead. For example, Bacela-Spychalska et al. (2012) suggest that prevalence of *C. dikerogammari* may be influenced by host population density.

Although *C. dikerogammari* can infect hosts of the genus *Gammarus* in the laboratory (Bacela-Spychalska et al. 2012), it was not detected in samples of the native gammarid *G. zaddachi*. This is unsurprising, given the low prevalence of this parasite in its typical host and the fact that *D. villosus* occurred in different locations to the native species, limiting opportunities for direct contact. A *Dictyocoela* parasite belonging to the *D. berillonum* clade was found in both *D. villosus* and co-occurring *E. trichiatus*, raising the possibility of transmission between these hosts. Although *D. berillonum* was not detected in a survey of *D. villosus* in its native Ponto-Caspian range (Wattier et al. 2007), it has been discovered previously at high prevalence in an invasive population of the Ponto-Caspian amphipod *P. robustoides* in Latvia (Wilkinson et al. 2011). The discovery of *D. berillonum* in three invasive Ponto-Caspian host, strengthens the hypothesis that this parasite also occurs in the native Ponto-Caspian range of *D. villosus*. *D. berillonum* also infects a range of native European amphipods and was detected in native European amphipods of Great Britain prior to the arrival of *D. villosus* (Table 1) indicating that it is a European native rather than a Ponto-Caspian invader.

A *Dictyocoela* parasite belonging to the *D. duebenum*/*muelleri* clade was also found in the Monnickendam population of *D. villosus*. *Dictyocoela* parasites of this clade have been detected over a wide geographical and species range in Eurasia. They are widespread and abundant in native European freshwater gammarids and have also been detected in endemic

amphipods of Siberia's Lake Baikal (Wilkinson et al. 2011). Although this *Dictyocoela* parasite was also absent from samples of *D. villosus* within its native range (Wattier et al. 2007), similar parasites have been found infecting *D. haemobaphes*, a close congener of *D. villosus*, in invasive populations in Europe (Wilkinson et al. 2011). Interestingly, one *Dictyocoela* isolate obtained from *D. villosus* in Monnickendam had an identical 16S rDNA sequence to an isolate obtained from *D. haemobaphes* in Poland. It is therefore possible that this parasite strain originated in the Ponto-Caspian region but was missed by Wattier et al.'s (2007) survey. Alternatively, both *D. villosus* and *D. haemobaphes* may have acquired the parasite after invading Europe. The latter hypothesis is supported by the occurrence of genetically similar *Dictyocoela* strains in the native European amphipod *G. duebeni* in Ireland (Wilkinson et al. 2011), which has not yet been reached by Ponto-Caspian amphipods.

Unlike the two *Dictyocoela* species, the microsporidian species discovered in the Loire population of *G. zaddachi* and the Monnickendam population of *E. trichiatus* do not appear to have made the transition to *D. villosus* yet. The parasite found in *G. zaddachi* has not been reported from other surveys of European amphipods, so possibly its host range is restricted to *G. zaddachi*, or more broadly to members of the *G. zaddachi* species group (also including *G. locusta*, *G. salinus* and *G. oceanicus*), which have not been extensively surveyed. The *E. trichiatus* parasite is genetically similar to parasites of the Baikalian amphipod *Dorogostaiskia parasitica* and the North Atlantic amphipod *Corophium volutator*. Although they have not been discovered previously in European or Ponto-Caspian amphipods, microsporidia of this type clearly have a wide geographical and species range. They may therefore have the potential to infect *D. villosus*.

In conclusion, genetic and parasitological evidence suggests that the recent invasion of Great Britain by *D. villosus* was accompanied by losses of genetic diversity and of

parasites. Genetic population structure among the four British populations suggests either multiple separate introductions to Britain or to repeated founder effects during translocations within Britain. The apparent escape of *D. villosus* from its native parasites may facilitate the spread of this invader within Britain. The apparent absence of the exotic pathogen *C. dikerogammari* also means that this parasite is not yet a threat to native amphipods. However, repeated invasions of Britain by *D. villosus* are likely to increase the genetic diversity of existing populations as well as increasing opportunities for emerging infectious diseases such as *C. dikerogammari* to infect native amphipods. Furthermore, several microsporidian parasites of native British amphipods appear capable of infecting *D. villosus*. Although the outcome of such host switches is difficult to predict, it is possible that spillback of native parasites from the invasive species may lead to higher prevalence in the native species (Hatcher et al 2012).

In order to limit the genetic diversity of *D. villosus* and prevent the introduction of invasive parasites, subsequent introductions of *D. villosus* to Great Britain should be avoided, even if attempts to eradicate existing populations fail. These considerations are also important at a global scale. *D. villosus* populations formed by single, long-distance colonisation events may be hampered by a lack of genetic diversity and are also unlikely to carry virulent pathogens such as *C. dikerogammari*. However, repeated introductions will enhance genetic diversity and may eventually result in the spread of pathogens, so measures to restrict long-distance dispersal should be maintained, even after *D. villosus* has become established.

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658 Figure Legends

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660 Figure 1: Bayesian phylogenetic tree of *Dictyocoela* isolates collected from native and

661 invasive amphipods in Europe and Siberia. Isolates from *D. villosus* and *E. trichiatus* are

662 shown in bold.

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