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ORIGINAL PAPER

Differential infection of exotic and native freshwater amphipods by a parasitic water mold in the St. Lawrence River

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Abstract A parasitic water mold (oomycete) of unknown origin was discovered infecting live native and exotic freshwater gammarid amphipods (*Gammarus fasciatus* and *Echinogammarus ischnus*, respectively) in the upper St. Lawrence River. Infections were associated with rapid die-offs of natural populations of amphipods, especially the exotic *E. ischnus*. Analysis of sequences of three different segments of the rRNA gene cluster indicated that the parasite was in the Saprolegniaceae family, and is related to other crustacean-associated Saprolegniaceae. Specific primers were designed based on the SSU rRNA gene and utilized for semi-quantitative analysis of parasite presence in live and dead amphipods. In laboratory experiments, infection prevalence was higher in *E. ischnus* individuals than in native amphipods. In addition, dead *E. ischnus* individuals exhibited more intense infections than *G. fasciatus* individuals. In contrast to the Great Lakes where *E. ischnus* has replaced *G. fasciatus* at many locations, the native species remains abundant in the St. Lawrence River more than a decade after invasion by *E. ischnus* in the late 1990s. We hypothesize that the parasite is facilitating the co-existence of the two amphipod species by reducing the abundance of *E. ischnus* in environments in which it might otherwise be dominant.

Keywords Biotic resistance · Parasite spillback · Parasite spillover · *Leptolegnia* · *Saprolegnia* · Crustacean hosts

Introduction

The role that parasites play in species invasions has been relatively unexplored (Torchin et al. 2002). However, existing evidence suggests that parasites can facilitate invasions and exacerbate their impacts (Prenter et al. 2004). This occurs when an introduced host transmits its parasites to more susceptible native hosts (parasite "spillover"; Diéguez-Uribeondo and Söderhäll 1993; Tompkins et al. 2003; Torchin et al. 2002), or when introduced species are less susceptible than native species to host-specific endemic parasites (Dunn and Dick 1998; MacNeil et al. 2003a; Settle and Wilson 1990) leading to parasite "spillback" (Kelly et al. 2009). Host-switching appears to be common among parasites, but current empirical evidence is dominated by cases demonstrating its detrimental consequences (as opposed to potential positive impacts) for native biodiversity (Kelly et al. 2009; Kozubikova et al. 2008; Tompkins et al. 2003).

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Rarely have parasites been found to impede an invasion (Dunn et al. 2009; Prenter et al. 2004), perhaps because such events are more likely to be overlooked. Yet, when introduced species are more susceptible than native species to resident parasites, this differential susceptibility may result in a reduction in the abundance or per capita impact of the introduced species; for example, intraguild predation by the invasive *Gammarus pulex* on the native *G. duebeni celticus* is lowered when the invader is infected by the acanthocephalan parasite *Echinorynchus truttae* (MacNeil et al. 2003b). The invasion success of an introduced species may be limited by the presence of predators (deRivera et al. 2005; Hunt and Yamada 2003). It is conceivable that intense local parasitism could similarly repel an introduced species, or prevent it from dominating a community (Prenter et al. 2004). However, if the parasite cannot successfully transmit from the invasive host, this host would act as a sink for the parasite.

Amphipod crustaceans host a wide range of parasites, and their susceptibility to infection by a particular parasite can differ greatly among species (Dunn and Dick 1998; Johnson 1985, 1986a, b; MacNeil et al. 2003a, b). Oomycetes (water molds) are parasites known to cause high levels of mortality in many groups of aquatic organisms (Barron 2004; Cerenius et al. 1988; Ramaiah 2006; Wolinska et al. 2008). Much attention has been given to their impact on economically valuable species such as salmonids, crayfish, shrimp and oysters (Gouda and Moharram 2009; Phillips et al. 2008; Unestam 1973), but their effects on amphipods are poorly known (but see Kiziewicz and Nalepa 2008; Phillips et al. 2008; Unestam 1973), despite the important functional role that amphipods serve in freshwater communities, e.g., as grazers or prey for higher trophic levels (MacNeil et al. 1997; Boisclair and Leggett 1989).

The Ponto-Caspian amphipod *Echinogammarus ischnus* has invaded benthic communities throughout the Great Lakes—St. Lawrence River system, and has replaced the confamilial native *Gammarus fasciatus* as the dominant amphipod in rocky nearshore areas of Lake Erie and Lake Ontario (Dermott et al. 1998). Surprisingly, even though it has been present in the region for more than 10 years since its initial discovery in 1998, *E. ischnus* still rarely dominates amphipod communities on rocks in the upper St. Lawrence River (which drains the Great Lakes) despite favourable physicochemical conditions (Kestrup and Ricciardi 2009; Palmer and Ricciardi 2004).

In the spring of 2009, we observed evidence of mass die-offs of amphipods, predominantly the exotic *E. ischnus*, at some sites in the river. Live amphipods collected from the river to be used in behavioural experiments suffered a similar die-off in the laboratory soon after arrival; again, the exotic amphipod seemed to be disproportionately affected. Both amphipod species showed signs of infection by an unidentified oomycete. Following these observations, we aimed to determine (1) the phylogenetic placement of the parasite, (2) whether *E. ischnus* is more susceptible to the oomycete than the native *G. fasciatus*, and (3) whether the infection intensity is higher in *E. ischnus* than *G. fasciatus*.

Methods

Collection and field observation

On May 7–8, 17–18, and 30–31, 2009, live adult *E. ischnus* and *G. fasciatus* were collected at a site with a bottom substrate of cobble and sand, located on the south shore of the St. Lawrence River near Montreal (Chateauguay West, 45°21.80 N, 73°47.20 W). Water temperature was measured with an electronic meter. Amphipods were collected using a kick net and by removing rocks and cobble, and then brought to the laboratory where they were sorted by species. They were kept in aerated aquaria (2 aquaria/species) with filtered (11 μm filter paper) source water and catfish pellets ad libitum at 12°C. Those amphipods collected on May 7–8 and 17–18 appeared to be healthy at the time of collection, but after a week in the lab many individuals showed signs of infection. The majority of *E. ischnus* obtained on May 30–31 also had visible signs of infection at the time of collection. To examine the parasite at higher resolution, small pieces of the carapaces of dead animals with attached hyphae were placed on a microscope slide and stained with methylene blue, and examined under a binocular microscope at 400× magnification.

Laboratory infection experiment

To estimate the frequency of infection in live and newly dead animals and the intensity of infection in

individual amphipods, we collected live, apparently uninfected animals on June 9–10, 2009. The animals were obtained from a site 2 km downstream of the initial site (Chateauguay East, 45°22.52 N, 73°46.55 W). This additional site was chosen because amphipods were found in extremely low abundances at the former site. In the laboratory, 200 individuals of each species were placed in 20 replicate aerated aquaria (10 *E. ischnus* + 10 *G. fasciatus* per aquarium) with 2 L of unfiltered water from Chateauguay West. This is where the parasite was initially found, and we hypothesized that the parasite would still be present in the water from this region. Aquaria also contained cobble and catfish pellets ad libitum. These densities of amphipods were chosen to minimize the risk and intensity of scavenging on dead individuals, which occurs frequently among both species (Kestrup and Ricciardi 2009). The aquaria were checked twice daily for 7 days. Dead individuals were removed and stored in 70% ethanol (hereafter referred to as "dead"). After 7 days, the remaining animals (hereafter referred to as "live") were preserved in 70% ethanol. Data on mortality (measured as proportion dead individuals per species per aquarium) were arcsine square root transformed (Sokal and Rohlf 2001) prior to analysis using a paired *t*-test in Systat 12.

Identification of parasite

In order to phylogenetically describe the oomycete parasite and to design molecular tools for sample analysis, clone libraries were derived from live and dead infected *E. ischnus*. To ensure that the parasite was the same in both host species, we also generated a clone library from dead *G. fasciatus*.

DNA extraction

Ethanol-preserved amphipods were utilized for DNA extraction. For nine of the replicate aquaria, DNA was extracted from each of the individual amphipods, both dead and alive (i.e., 20 total DNA extractions per replicate except in the case of cannibalized animals, which were not available for extraction). Extractions were performed using the Mo Bio PowerSoil DNA Isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA). Bead tube contents were decanted into a clean 2.0 mL microcentrifuge tube and a single preserved animal was transferred to each empty bead tube using sterile forceps. A small volume of bead tube liquid was added back to the tube and a sterile microcentrifuge tube pestle (Bel-Art Products, Pequannock, NJ) was used for homogenization of each animal before beads and residual liquid were returned to the bead tube. The remaining procedure followed the manufacturer's instructions. DNA was quantified using a Nanodrop ND-1000 (Thermoscientific, Wilmington, DE).

Clone libraries

Two SSU rRNA gene clone libraries were established using the oligonucleotide primers (5′-ACCTGGT TGATCCTGCCAG-3′, 5′-TGATCCTTCYGCAGGT TCAC-3′) complementary to regions near the 5′ and 3′ ends of SSU rRNA genes (Moon-van der Staay et al. 2000). The primer pair yielded amplicons between 1,500 and 2,000 bp in length. One library was established with DNA extracted from live *E. ischnus* collected in the field. Another library was generated from *E. ischnus* that died due to infection in the laboratory. Finally, a third library was generated from *G. fasciatus* that died due to infection in the laboratory. The total volume of each PCR mixture was 20 μL and contained (final concentrations): 1× GoTaq Flexi Buffer (Promega Corporation, Madison, WI), 1.5 mM $MgCl_2$, 200 μM of each deoxynucleoside triphosphate (Promega), 500 nM of each primer, and 1 unit of GoTaq (Promega). Additional clone libraries were established from dead *E. ischnus* DNA for the LSU rRNA gene and for the ITS using the protocol described by White et al. (1990). Fresh PCR products were cloned into the TOPO vector pCR 2.1 (TA Cloning Kit, Invitrogen) according to the manufacturer's instructions. Transformants from each of the clone libraries were selected and assessed for the presence of the expected gene insert (screened) using PCRs containing vector-targeted primers M13F and M13R (Zhou et al. 1997). However, due to lower biomass of parasite in the *G. fasciatus* individuals (see Results), clone libraries made from the universal SSU rRNA gene yielded only host sequences. Thus, Oomycete-targeted SSU rRNA gene primers (Oom 106F 5′-CTT GAT AGT ACC TTA CTA CTT GGA TAA-3′ and Oom1720R 5′-GCA AAC GAA TCA ACG GTC-3′) designed to amplify a 1,614 bp fragment were utilized to generate a parasite clone library from this host species.

Sequence and phylogenetic analysis

To obtain sequence information of cloned SSU rRNA gene fragments, PCR products from M13 primer amplification of 28 representative *E. ischnus* clones and 30 representative *G. fasciatus* clones were sequenced by Nevada Genomics Center (University of Nevada, Reno, NV). Only products of the expected size (between 1,500 and 2,000 bp) were sequenced. SSU rRNA gene-targeted primers described above as well as internal primers (EK-555F and EK-1269R; Lopez-Garcia et al. 2001 for universal SSU rRNA gene fragments; Oom721F 5′-GAG TTG GTG GTT GGG TAG-3′ for oomycete-specific SSU rRNA gene fragments) were used for sequencing in order to achieve complete coverage of the nearly full-length sequence. LSU- and ITS-specific primers were utilized for sequencing those two regions (White et al. 1990). Sequence fragments were assembled using Geneious Pro (http://www.geneious.com; Biomatters Ltd, Auckland, New Zealand). The phylogenetic relationship of the consensus sequence from oomycete-like sequence fragments were inferred using the Neighbor-Joining method in MEGA4 (Saitou and Nei 1987; Tamura et al. 2007). Fifteen oomycete SSU rRNA gene sequences, nineteen LSU sequences, and 24 ITS sequences from the NCBI non-redundant database were aligned using Clustal W (MEGA4; http://www.megasoftware.net/). Included in the alignment were sequences of related Saprolegniaceae with *Phytophthora undulata* as the outgroup. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are reported (Felsenstein 1985). The evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura et al. 2004). All missing data and positions containing gaps were eliminated from the dataset (MEGA4; Complete deletion option).

Design of species-specific primers and Saprolegnia-targeted PCR

Using the alignment described above, areas of SSU rRNA gene sequence similarity were highlighted and primers 161Fwd (5′-GTCAAATACCCAACTGCTTG-3′) and 854Rev (5′-CTATTAATCATTACCTCGGTGTGC-3′) were selected manually to target areas of highest sequence variation. The expected amplicon from primers 161Fwd and 854Rev is 693 bp in length. Primer sequences were analyzed using BLAST (Altschul et al. 1990) to test for specificity. PCRs were carried out using the same cycling conditions and reagent concentrations as listed above.

Analysis of infection prevalence and intensity

The intensity of infection in individual amphipods was assessed in a subset of the replicates from the lab infection experiment. These analyses also allowed us to molecularly verify infection status of individuals from the experimental aquaria (see "Laboratory infection experiment" section above). We initially analyzed 5 randomly chosen replicates selected using a random number generator. These all contained at least three of the four possible categories of individuals (live *E. ischnus*, dead *E. ischnus*, and live *G. fasciatus*), but three of the replicates did not contain any dead *G. fasciatus* and the fourth contained only a single dead *G. fasciatus*. This prevented us from obtaining reliable information on infection intensity in dead *G. fasciatus*. Therefore, we non-randomly chose 4 additional replicates with higher numbers of dead *G. fasciatus*. DNA templates were added to PCRs at 5, 0.5, and 0.05 ng μL^{-1} concentrations. PCR results were evaluated via visual detection of fragments on ethidium bromide-stained agarose gels.

The three concentrations of template DNA were used to produce three detection limits of the parasite and, thus, semi-quantitative results. The intensity of infection was regarded as high (level 3) if the parasite was detected using low concentrations of DNA (0.05 ng μL^{-1}), intermediate (level 2) the parasite was detected using 0.5 ng μL^{-1} of DNA, and low (level 1) if the parasite was detected using the highest concentrations of DNA (5 ng μL^{-1}). If no parasite was detected using the highest concentrations of DNA, the infection level was designated as 0, although lack of detection does not guarantee that an individual is parasite free. We also calculated an overall measure of infection prevalence, considering all individuals in levels 1–3 as infected and those with no parasite detected (level 0) as uninfected. Data on proportion infected were arcsin square root transformed (Sokal and Rohlf 2001) prior to analysis with a Mann–Whitney *U* test in Systat 12. Differences in intensity of infection were also tested using Mann–Whitney *U*-tests.

Results

Collection

Individuals of *E. ischnus* that were collected on May 7–8 (Temp: 11.4°C) and kept in two aquaria in the lab suffered 100% mortality 7–9 days after collection. The native amphipod *G. fasciatus* appeared unaffected. The pattern was repeated with apparently healthy animals (i.e., active and without spots or hyphae) collected on May 17–18 (Temp: 12.0°C). However, during the same time period, mortality was very low among animals that were kept isolated in individual trays immediately after collection (A. Derry, Department of Biology, McGill University, personal communication), possibly because this procedure eliminated the risk of rapid cross-infection among individuals.

Crustaceans infected by water molds are distinguished by dark spots on the cuticle, resulting from melanin being laid down by the host on the surface of the parasitic hyphae as a defence (Phillips et al. 2008; Söderhäll et al. 1991; Söderhäll et al. 1988). On May 30–31 (Temp: 14.4°C), virtually all *E. ischnus* collected in the field had visible signs of infection: animals appeared pale with dark spots on the carapace (Fig. 1). Collected *G. fasciatus* appeared healthy but closer examination revealed that many individuals had microscopic dark spots on the carapace. In the lab, *E. ischnus* were placed into individual trays but suffered high mortality nonetheless, with half the animals dead following 1 day and 95% dead after 3 days. In addition to the presence of melanin in the carapace of both alive and newly dead individuals (Fig. 1a, b), many animals showed signs of paralysis (lethargy, resting on their dorsum, unresponsive to handling) and died shortly after. On one occasion, hyphae were observed growing out from the legs of a lethargic *E. ischnus*; otherwise, emergent hyphae growing out from the body were observed only in dead individuals (Fig. 1c, d). Examination under a microscope revealed that the hyphae were coenocytic (Fig. 1c).

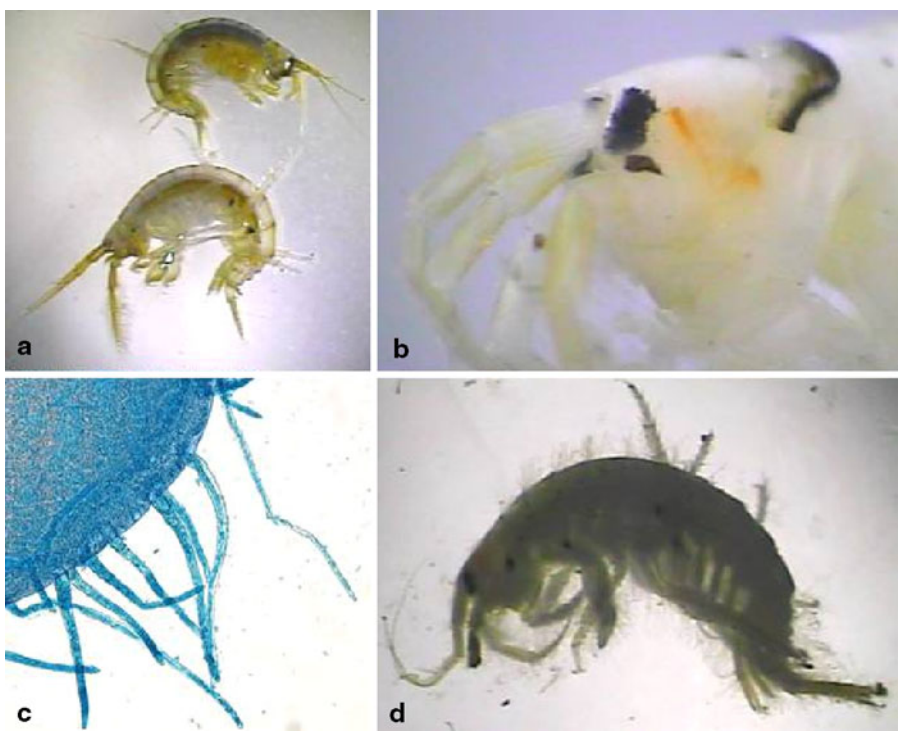

Fig. 1 Infected live female (*top*) and male (*bottom*) *E. ischnus* with melanized spots (**a**), newly dead *E. ischnus* (**b**), hyphae growing out from a gill (**c**), and a dead *E. ischnus* overgrown with hyphae (**d**)

Laboratory infection experiment

After 7 days of incubation in the laboratory, mortality was significantly higher among the exotic *E. ischnus* than among *G. fasciatus* ($t_{19} = 6.5$, $P < 0.001$). The mortality of *E. ischnus* (52.3% of 197 individuals) was threefold higher than that of *G. fasciatus* (16.0% of 194 individuals). Four *E. ischnus* and 5 *G. fasciatus* were missing due to predation, cannibalism or scavenging.

Identification of parasite

Clone library sequences verified that the parasite was an oomycete and that it fell into the Saprolegniaceae family: 28 out of 30 screened SSU rRNA gene clones resulting from the dead *E. ischnus* were sequenced. Of the final 28 sequences recovered from infected *E. ischnus*, eight clones corresponded to an oomycete while 20 sequences reflected the presence of host DNA (sequences from the family Gammaridae). The eight Saprolegniaceae sequences were the only clone sequences obtained that corresponded to a potential parasite. Oomycete clone sequences were identical and assembled together into a single 1,741 bp contig with a consensus sequence that, according to BLAST analysis, corresponded to sequences in the family Saprolegniaceae. Identical sequences were recovered from infected *G. fasciatus* clone libraries. All of the eighteen screened SSU rRNA gene clones resulting from live *E. ischnus* were sequenced. No oomycete- or fungal-related sequences were detected in the live *E. ischnus* library. All live *E. ischnus* sequences corresponded to amphipods in the family Gammaridae. LSU and ITS clone libraries from dead *E. ischnus* had similar results to the dead *E. ischnus* SSU clone library, with sequences corresponding to either amphipods or oomycetes in the family Saprolegniaceae.

BLAST analysis revealed that the parasite's nearly complete SSU rRNA gene sequence was 99% similar (1709/1722) to the closest cultured representative, *Leptolegnia caudata* (Dick et al. 1999; Fig. 2a). The unknown parasite SSU rRNA gene sequence was more closely related (sequence identity of 99% [1714/1722]) to uncultured clone sequences *Saprolegnia* sp. SAP1 (Wolinska et al. 2009; Fig. 2a).

Since previous authors have submitted different genes for different organisms, additional genes were utilized to pinpoint the appropriate phylogenetic placement for this oomycete. The partial sequence of its LSU gene was found to be nearly identical to the abbreviated *Saprolegnia* sp. WM 3 sequence (sequence identity of 99% [674/675]) (Wolinska et al. 2008; Fig. 2b) and also closely related to *Leptolegnia caudata* (sequence identity of 96% [680/705]). The final sequence that we analyzed for phylogeny of the unknown parasite was the ITS sequence. The ITS sequence, like the LSU gene sequence, was most closely related to *Saprolegnia* sp. WM 3 (sequence identity of 99% [655/656]) as well as Saprolegniaceae sp. VI03839 (sequence identity of 99% [711/712]) but the ITS sequence for *L. caudata* was not available for analysis (Fig. 2c). Phylogenetic trees generated using the Neighbor Joining method revealed similar clustering for each of the analyzed sequences, with a distinct cluster formed by the unknown parasite, previously published *Saprolegnia* sp. WM 3, SAP1, and SAP3, and *Leptolegnia* spp. (Fig. 2a–c).

Analysis of infection prevalence and intensity

Infection prevalence was higher in *E. ischnus* than in *G. fasciatus* in the 5 randomly selected replicate aquaria (Mann–Whitney $U = 25.0$, $P = 0.009$; Fig. 3) when analyzing live and dead individuals together. When looking at infection intensity, there was no significant difference between the two host species in live individuals in the randomly selected replicates (Mann–Whitney $U = 18.0$, $P = 0.20$; Fig. 4a). Among the dead individuals, the parasite was detected in all 19 *E. ischnus*, and in 4 out of 5 *G. fasciatus*. The number of dead *G. fasciatus* in these randomly chosen samples was too low to allow for a statistical test of differences in infection intensity between the two species.

To better compare infection frequency and intensity in dead individuals, we non-randomly chose samples that contained 3–5 dead *G. fasciatus*. As with the randomly selected replicates, infection prevalence was higher in *E. ischnus* in the non-randomly selected replicates (Mann–Whitney $U = 16.0$, $P = 0.02$; Fig. 3). Infection intensity was substantially higher in dead *E. ischnus* than in dead *G. fasciatus* (Mann–Whitney $U = 16.0$, $P = 0.02$; Fig. 4b).

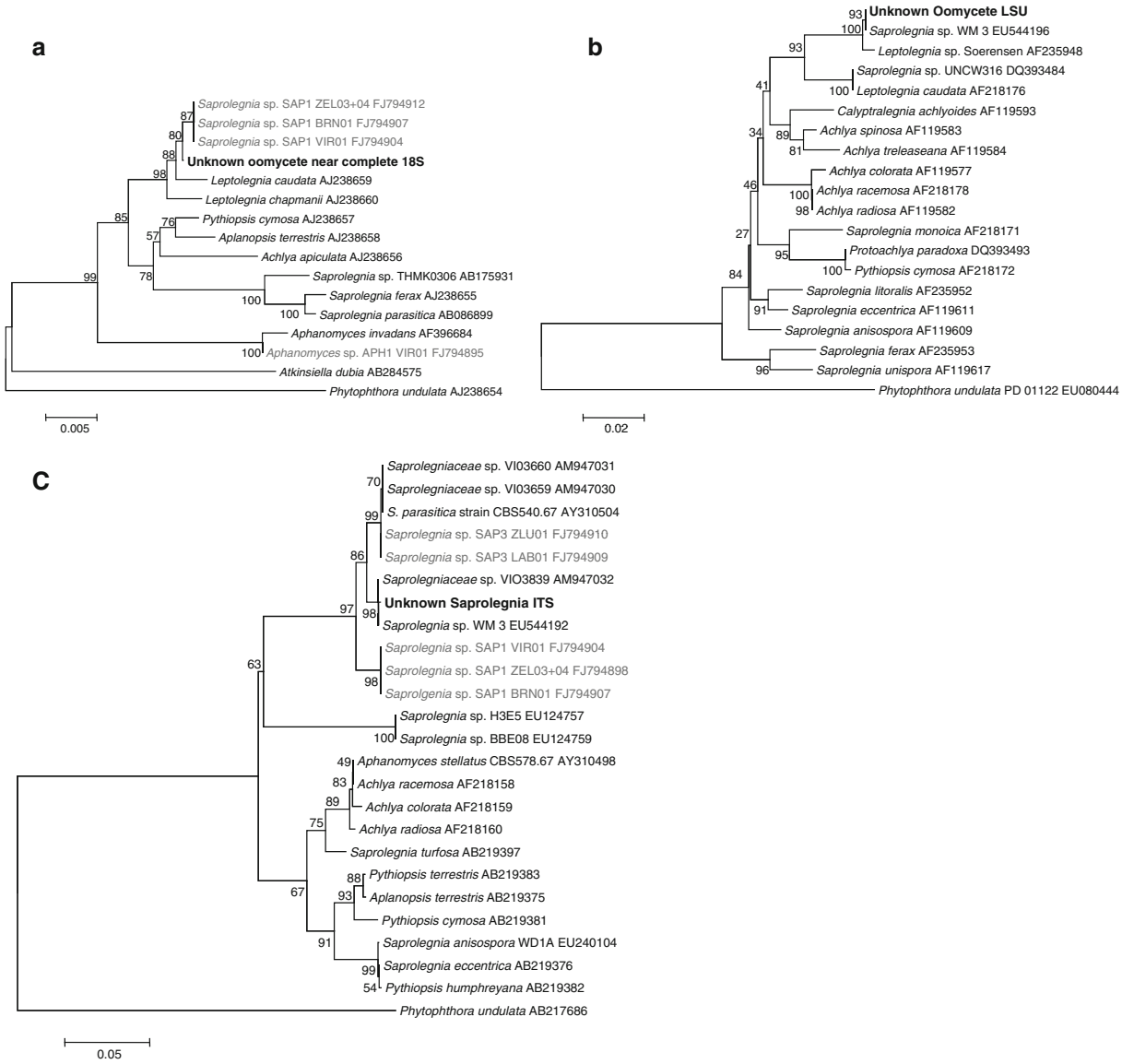

Fig. 2 Phylogenetic trees based on **a** the SSU rRNA gene, **b** the large subunit rRNA (LSU), and **c** the rRNA internal transgenic spacer region (ITS) of the unknown oomycete parasite and several other oomycetes from the NCBI GenBank database (accession number given). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site, as indicated by the *scale bar*. *Numbers above* and *below* the nodes are bootstrap values for 500 replicates. *Gray font* indicates clone sequences of uncultured representatives

Discussion

In this study, we documented infections of amphipods by a parasitic oomycete, and showed that infection prevalence and intensity were higher in exotic *E. ischnus* amphipods than in native *G. fasciatus*. Overall, our results suggest that the parasite may be preventing the replacement of the native species in the St. Lawrence River.

The biogeographic origin of this oomycete is unknown. Based on sequence analysis, it is closely related to recently described *Daphnia* parasites from North American (Michigan and Illinois) ponds and European lakes (Wolinska et al. 2008, 2009). In

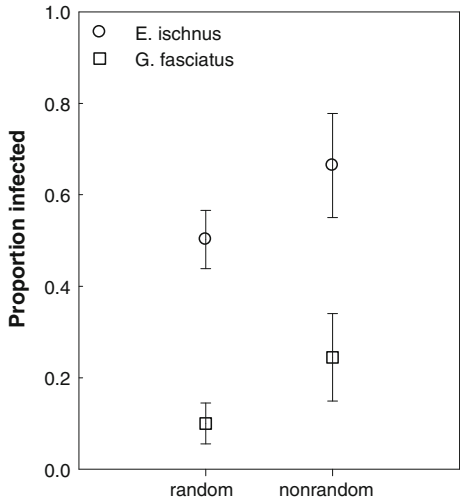

Fig. 3 Prevalence of infection in *E. ischnus* and *G. fasciatus* individuals. Data are presented for randomly selected and non-randomly selected replicates (including both live and dead individuals) for the two host species. Infection prevalence was significantly greater in *E. ischnus* than in *G. fasciatus* in both randomly selected (Mann–Whitney $U = 25.0$, $P = 0.009$) and non-randomly selected (Mann–Whitney $U = 16.0$, $P = 0.02$) replicates. Points represent means ±1 standard error

addition, closely related strains have been described in Norway as parasites of the crayfish *Aphanomyces astaci* (*Saprolegnia* sp. VIO3839; Vrålstad et al. 2009; Fig. 2c). While many of the recently described strains have been attributed to the genus *Saprolegnia*, *Leptolegnia* may be a more appropriate genus classification according to the phylogenetic analyses presented here and that of SSU rRNA genes by Dick et al. (1999). Sequence analysis alone does not justify a novel species designation for this oomycete, nor can we determine whether the species is native or introduced to the St. Lawrence River. Infection experiments to determine host specificity are needed to describe ecotype and to justify species designations. Future experiments along these lines will be facilitated by the ease of culturing this parasite: we have successfully cultured it on several different agars (including corn meal agar, Sabouraud dextrose agar and potato dextrose agar; M. A. Duffy et al., unpubl. data).

Regardless of its exact species identity, it is clear that this parasite has the potential to strongly influence the ecological interactions between the exotic and native amphipods. The parasite was detected more frequently in *E. ischnus* than in *G. fasciatus* (both live and dead). Further, dead *E. ischnus* were more heavily infected than live and dead *G. fasciatus* (Fig. 4), even though live *E. ischnus* had similar infection intensities to live

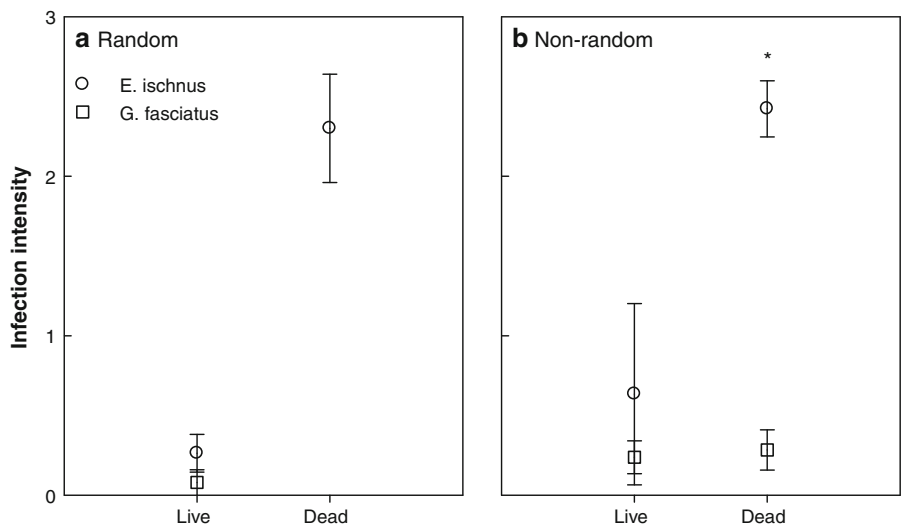

Fig. 4 Infection intensity (as determined by semi-quantitative PCR) in live and dead individuals of *E. ischnus* and *G. fasciatus*. Data are presented for **a** randomly selected experimental replicates and **b** replicates that were selected non-randomly (to ensure high numbers of dead *G. fasciatus*). The *asterisk* indicates that infection intensity of dead *E. ischnus* in the non-random sample was significantly greater than the infection intensity of dead *G. fasciatus* (Mann–Whitney $U = 16.0$, $P = 0.02$). Points represent means ±1 standard error. Only one of the random replicates contained more than one dead *G. fasciatus*, preventing us from obtaining an accurate measure of infection intensity

G. fasciatus. These data indicate that the largest reservoir of parasite is dead *E. ischnus*. Thus, the presence of *E. ischnus* may substantially increase the parasite load of the total system, and the parasite may drive apparent competition between the native and exotic species (Holt and Lawton 1994). Apparent competition may explain why *G. fasciatus* remains the dominant amphipod in the upper St. Lawrence River, even though *E. ischnus* has been present in the river for more than 10 years and has replaced *G. fasciatus* as the dominant amphipod in many areas of Lake Erie and Lake Ontario (Dermott et al. 1998). *E. ischnus* is more susceptible to infection by the parasite and dead individuals harbor substantially more intense infections; together, these may lead to a positive feedback that maintains *E. ischnus* at low densities. Previously, it had been difficult to explain why *E. ischnus* had not replaced *G. fasciatus* in the upper St. Lawrence River despite favourable physicochemical conditions (Kestrup and Ricciardi 2009; Palmer and Ricciardi 2004).

We did not compare the mortality of infected and uninfected native and exotic amphipods, although this would be informative. *E. ischnus* individuals carrying the parasite died within days, but uninfected individuals could survive for weeks in the laboratory, indicating that the parasite strongly affects mortality. In addition, since parasitic water molds are also saprophytic, the death of the host does not eliminate the risk of transmitting the parasite to healthy individuals. Treating newly collected individuals in a bath of 15 ppm Malachite green in filtered source water for 15 min and thereafter keeping them in individual containers with filtered source water reduces the risk of cross-infection between animals kept in the laboratory.

An interesting focus for future study is the effect of changing water temperatures on the infectivity and virulence of the parasite, as well as on the outcome of apparent competition between the two species. Water molds generally grow faster in warmer temperatures (Nechwatal and Mendgen 2006; Wolinska et al. 2008), as has been observed for this amphipod parasite (M. A. Duffy et al. unpubl. data). However, several studies have found that oomycete-induced mortality is higher at lower temperatures (Bly et al. 1993; Leano et al. 1999; Quiniou et al. 1998; Ruthig 2008; Sagvik et al. 2008), and this has been ascribed to reduced immunosuppression of host organisms and higher levels of zoospore production. Given the strongly seasonal nature of the upper St. Lawrence River (Rondeau 1993), temperature-driven changes in infectivity or virulence may lead to seasonally differences in the outcomes of the parasite-host interaction for both amphipod species.

In conclusion, the parasite detected in St. Lawrence River amphipods may be maintaining the coexistence of *E. ischnus* and *G. fasciatus* by reducing the invader's abundance and impact in physicochemical conditions in which it might otherwise be dominant (Kestrup and Ricciardi 2009). To more fully understand the role of this parasite in mediating the outcome of interactions between native and exotic amphipods, future studies documenting the host range of this parasite and the effects of temperature on parasite infectivity and virulence must be conducted.

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