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## Variations in infection levels and parasite-induced mortality among sympatric cryptic lineages of native amphipods and a congeneric invasive species: Are native hosts always losing?

Matthias Galipaud<sup>a</sup>, Loïc Bollache<sup>b</sup>, Clément Lagrue<sup>c,\*</sup><sup>a</sup> Department of Evolutionary Biology, Bielefeld University, Konsequenz 45, 33615 Bielefeld, Germany<sup>b</sup> UMR 6249 Chrono-environment, Université Bourgogne Franche-Comté, 6 Boulevard Gabriel, 21000 Dijon, France<sup>c</sup> Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

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### ABSTRACT

Shared parasites can strongly influence the outcome of competition between congeneric, sympatric hosts, and thus host population dynamics. Parasite-mediated competition is commonly hypothesized as an important factor in biological invasion success; invasive species often experience lower infection levels and/or parasite-induced mortality than native congeneric hosts. However, variation in infection levels among sympatric hosts can be due to contrasting abilities to avoid infection or different parasite-induced mortality rates following infection. Low parasite infection levels in a specific host can be due to either factor but have drastically different implications in interaction outcomes between sympatric hosts.

We assessed acanthocephalan infection levels (prevalence and abundance) among cryptic molecular taxonomic units (MOTU) of the native *G. pulex*/*G. fossarum* species complex from multiple populations where they occur in sympatry. We concomitantly estimated the same parameters in the invasive *Gammarus roeselii* commonly found in sympatry with *G. pulex*/*G. fossarum* MOTUs. We then tested for potential differences in parasite-induced mortality among these alternative hosts. As expected, the invasive *G. roeselii* showed relatively low infection level and was not subject to parasite-induced mortality. We also found that both acanthocephalan infection levels and parasite-induced mortality varied greatly among cryptic MOTUs of the native amphipods. Contrary to expectations, some native MOTUs displayed levels of resistance to their local parasites similar to those observed in the invasive *G. roeselii*. Overall, cryptic diversity in native amphipods coupled with high levels of variability in infection levels and parasite-induced mortality documented here may strongly influence inter-MOTU interactions and native population dynamics as well as invasion success and population dynamics of the congeneric invasive *G. roeselii*.

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### 1. Introduction

Parasitism is an important biotic determinant of animal population dynamics and community structure (Hudson and Greenman, 1998). Parasites can influence host numbers within a population by increasing mortality rate (Goater and Ward, 1982; Rousset et al., 1996). For parasites with complex life cycles, intermediate host mortality attributable to parasites can occur through reduction in host survival due to the pathological consequences of parasitic infection, or through host manipulation increasing predation on

infected hosts (Thomas et al., 1995; Latham and Poulin, 2002; Parker et al., 2003; Hansen and Poulin, 2005; Benesh and Valtonen, 2007; Violante-González et al., 2016). However, host species often differ in their susceptibility to infection and/or parasite-induced mortality (Sánchez et al., 2012). For example, invasive species generally suffer less from parasitism than native species (Dunn and Dick, 1998; Torchin et al., 2003; Genner et al., 2008). Native parasites are often less effective at infecting invasive hosts due to the lack of co-evolutionary history between the novel hosts and local parasites (Ebert, 1994; Kaltz and Shykoff, 1998; Emblidge Fromme and Dybdahl, 2006; Genner et al., 2008). Host-parasites interactions proceed in a co-evolutionary context; both species must continually adapt to each other (May and

\* Corresponding author.

E-mail address: [clement.lagrue@gmail.com](mailto:clement.lagrue@gmail.com) (C. Lagrue).

Anderson, 1983; van Baalen, 1998; Dieckmann et al., 2002; Vale and Little, 2009). Parasites are generally considered to stay ahead of host defenses during co-evolution and are often expected to be adapted to their local hosts (Kaltz and Shykoff, 1998; Morgan et al., 2005). Even if invasive hosts are congeneric species taxonomically close to native hosts, parasites may be so closely adapted to their local host species that they are unable to infect or induce pathogenic effects in the invader (Cornet et al., 2010; Westram et al., 2011a). Local parasites that are able to infect novel, invasive hosts may still reach lower infection levels and pathogenic effects in native than invasive host species (Lagrué et al., 2016). In turn, local, native hosts are also continuously mounting counter-adaptations specifically targeting their local parasites and may be better at dealing with native parasites than a naïve, invasive host (Lagrué et al., 2016). Overall, native and invasive hosts can vary wildly in their vulnerability to infection and local parasites in their host specificity and pathogenic effects (Redón et al., 2015).

Generally, two closely related and sympatric host species infected by the same parasite can exhibit strongly contrasted vulnerability to infection and/or parasite-induced mortality (Thomas et al., 1995; Rousset et al., 1996). Although evidence for parasite-induced host mortality from field data remains difficult to obtain (Anderson and Gordon, 1982), multiple studies have inferred parasite-induced host mortality from distribution of parasite abundances within host populations (Gordon and Rau, 1982; Thomas et al., 1995; Rousset et al., 1996; Médoc et al., 2006). But the direct links between parasite prevalence or abundance and host mortality can be difficult to determine (Benesh, 2011). Differences in parasite prevalence and/or abundance among sympatric host species can be due to one host being more efficient at avoiding the parasite and/or resisting infection following encounter; i.e. lower infection levels result from higher resistance to the parasite. Alternatively or concomitantly, contrasting parasite-induced mortality between co-occurring host species can in turn induce widely different parasite prevalence and/or abundance between hosts. Hosts in which survival is not affected by the infection will tend to accumulate parasites, thus displaying higher prevalence and/or abundance levels than hosts with high parasite-induced mortality, even if their encounter rates and vulnerability to infection are similar. Assessing which of these potential factors affect apparent infection levels among alternative host species in wild host populations remains extremely difficult in the field however.

In France, the native *Gammarus fossarum* and *G. pulex* are often found co-existing with the invasive *Gammarus roeseli*, an established Balkan species (Bauer et al., 2000). These species are also hosts to a variety of acanthocephalan parasites (Bauer et al., 2000, 2005; Westram et al., 2011a). Previous studies have shown that acanthocephalan parasites can induce various behavioural, phenotypic, and physiologic changes in *G. fossarum* and *G. pulex*. These include altered reaction to light (Bauer et al., 2000, 2005; Cézilly et al., 2000; Perrot-Minnot, 2004; Tain et al., 2006), impaired antipredator behaviour (Kaldonski et al., 2007; Dianne et al., 2014), partial castration (Bollache et al., 2002), and lower immune activity (Rigaud and Moret, 2003). No such effects were apparent, or were significantly lower, in the invasive *G. roeseli* (Bauer et al., 2000, 2005; Rigaud and Moret, 2003). Contrasting host manipulation in native compared to invasive amphipod hosts reflected a difference in the ability of the acanthocephalan *Pomphorhynchus laevis* to alter brain serotonergic activity of the two host species (Tain et al., 2007). As a result, predation rates on acanthocephalan-infected *G. fossarum/G. pulex* were significantly higher than on infected individuals of the invasive *G. roeseli* (Lagrué et al., 2007; Tain et al., 2007). Consequently, mean parasite abundance relative to host size displayed the characteristic bell-shaped curve supporting a higher mortality in acanthocephalan-infected

*G. fossarum/G. pulex* individuals compared to uninfected amphipods (Lagrué et al., 2007). Here, parasite-induced host mortality likely resulted from increased predation on infected amphipods induced by host manipulation (Lafferty, 1999; Outreman et al., 2007; Violante-González et al., 2016). Again, no such pattern was observed in *G. roeseli*; mean parasite abundance simply increased with amphipod host size, indicating an accumulation of acanthocephalan parasites over time by invasive hosts with no apparent induced mortality (Lagrué et al., 2007). Furthermore, although acanthocephalan prevalence is higher in the invasive *G. roeseli* than in the native *G. fossarum/G. pulex* in the field, laboratory controlled infections showed that native amphipods were more susceptible to infection by local acanthocephalan (Lagrué et al., 2007; Bauer and Rigaud, 2015). Overall, differences in vulnerability to infection, acanthocephalan prevalence and size distributions of infected individuals suggest a differential parasite-induced mortality between *G. fossarum/G. pulex* and the invasive *G. roeseli*. Such pattern of enemy release is commonly observed in a biological invasion context and provides a competitive advantage to the invader (Clay, 2003; Cornet et al., 2010; Arundell et al., 2015). Differential susceptibility to infection by local parasites and parasite-induced mortality are often seen as key factors, among others, for the ability of *G. roeseli* to invade, establish and co-exist with local populations of the native *G. fossarum/G. pulex* (Lagrué et al., 2011).

*Gammarus pulex* and *Gammarus fossarum* are widely distributed “morphological” species and are often considered to be single, independent functional taxonomic units (Karaman and Pinkster, 1977; Müller, 1998). However, extensive use of DNA barcoding have recently allowed the detection of high degrees of cryptic diversity (i.e. genetically diverging lineages that differ little or not at all in their morphology) across a variety of taxa (Hebert et al., 2003; Pfenninger and Schwenk, 2007; Fontaneto et al., 2009; Trontelj and Fišer, 2009). Amphipods are no exception with a number of cases of morphologically cryptic lineages reported (Hogg et al., 1998, 2006; Witt and Hebert, 2000; Witt et al., 2006; Lefébure et al., 2007; Sutherland et al., 2010; Grabowski et al., 2012). Several recent studies showed extensive genetic divergence within *G. fossarum* and *G. pulex* (Müller, 1998, 2000; Westram et al., 2011b; Lagrué et al., 2014; Weiss et al., 2014; Weiss and Leese, 2016). Indeed, Lagrué et al. (2014) showed that, in Eastern France, *G. fossarum* and *G. pulex* form a complex of cryptic lineages, or molecular operational taxonomic units (i.e. MOTU; Blaxter et al., 2005), that are morphologically undistinguishable and often co-occur in sympatric populations. Whether these cryptic MOTUs display differential vulnerability to shared parasites remains unclear. For instance, the respective vulnerability to parasites of each *G. fossarum/G. pulex* lineage compared to that of the sympatric invasive *G. roeseli* is completely unknown. Many of the studies cited above were conducted on amphipod populations that have proved, in hindsight, to be made of several sympatric MOTUs of *G. fossarum/G. pulex* (Bauer et al., 2000, 2005; Lagrué et al., 2007, 2014), thus preventing reliable comparisons of parasitism between native and invasive species (Bauer and Rigaud, 2015).

Morphologically cryptic lineages of amphipod hosts may be physiologically, behaviourally and/or ecologically different and thus not be cryptic to parasites (Cothran et al., 2013a, b). In our study system, whether acanthocephalan parasites are generalists and able to infect any available native amphipod host or are highly specific to particular host MOTUs is yet to be assessed. However, the occurrence of sympatric but genetically divergent native host lineages, coupled with the presence of the invasive *G. roeseli*, has the potential to affect acanthocephalan infection levels, host-parasite interactions and ultimately host-host competition among lineages and/or species (Gandon, 2004; Rigaud et al., 2010; Westram et al., 2011a). In such cases, contrasting parasite-induced mortality

among sympatric host species or MOTUs may result in differences in their relative competitiveness (Kennedy et al., 1978; Rauque et al., 2003; Lively, 2010).

Here, we investigated potential differences in acanthocephalan infection levels (prevalence and abundance; see Bush et al., 1997 for terminology) among the invasive *G. roeseli* and cryptic MOTUs of the native *G. pulex*/*G. fossarum* species complex from multiple populations/sampling sites where they occur in sympatry. In populations containing two or more *G. pulex*/*G. fossarum* MOTUs, we assessed the effects of host cryptic diversity on apparent infection patterns and potential differences in infection susceptibility and parasite-induced mortality among sympatric MOTUs. In amphipod populations where it was found, we also tested whether the invading *G. roeseli* was less vulnerable to infection by local acanthocephalan parasites and, when infected, less subject to parasite-induced mortality. We hypothesized that the invader should be less vulnerable than native amphipod MOTUs, both in terms of infection levels (prevalence and abundance) and pathological effects (parasite-induced mortality). We also predicted variations among MOTUs in parasitism and predicted that cryptic diversity would influence apparent acanthocephalan infection levels and pathological effects documented in the field. Finally, we discussed how parasites may influence inter-MOTU competition as much as interspecific competition among native and invasive amphipod host species.

## 2. Materials and methods

A previous study revealed high cryptic diversity among and within populations of the native *G. fossarum*/*G. pulex* species complex in Burgundy, France (Lagrue et al., 2014). Multiple divergent molecular operational taxonomic units (i.e. MOTU; Blaxter et al., 2005) were found and populations/sampling sites often contained several MOTUs occurring in sympatry. For the present study, we collected amphipods from 15 sampling sites/ rivers containing two or more divergent MOTUs of *G. fossarum*/*G. pulex* or at least one *G. fossarum*/*G. pulex* MOTU and *G. roeseli* in sympatry (Table S1), and where acanthocephalan parasites also occurred.

### 2.1. Field sampling

Amphipods from the *Gammarus fossarum*/*Gammarus pulex* (hereafter referred to as Gf and Gp) species complexes were collected using kick nets from 15 different rivers (Table S1) in Autumn 2010 (From October to December). Amphipods were collected on only one occasion in each river to avoid potential temporal variation effects on our data. When present in the river, invasive *Gammarus roeseli* (Gr) amphipods were also collected. All potential habitats present at any given site were sampled. Amphipods collected were pooled in a large container from which between 250 and a 1000 were haphazardly sampled and preserved individually in 1.5 mL Eppendorf tubes filled with 100% ethanol. Remaining amphipods were then released. Back in the laboratory, ethanol was renewed in each tube and samples kept at  $-20^{\circ}\text{C}$  until needed for measurements, genetic identification and dissections.

### 2.2. Laboratory processing

All amphipods were sexed and measured using height of the fourth coxal plate as a proxy for body size (Bollache et al., 2000). Amphipods were then dissected under a dissecting microscope using fine forceps and all acanthocephalan parasites found were identified and counted. Amphipods from sampling sites containing *Gammarus roeseli* and MOTU(s) of *G. fossarum*/*G. pulex* were first sorted morphologically as *Gammarus roeseli* is easily identified by

its dorsal spines (Bollache et al., 2006). Furthermore, as a relatively recent invader, *G. roeseli* differs little genetically among populations and all individuals collected during our study can be considered as a single Gr MOTU (Moret et al., 2007). Individuals of *G. fossarum*/*G. pulex* were genetically identified using a PCR-RFLP method (Levy et al., 2002; Pfeiffer et al., 2004) and assigned to their respective Molecular Operational Taxonomic Unit (MOTU): Gf-I, Gf-II, Gf-III, Gf-VI, Gf-VII or Gp-D as determined in Lagrue et al., 2014. These MOTUs show COI genetic divergences ranging from about 3% up to 26% (Fig. 1; Lagrue et al., 2014).

Amphipod DNA was extracted from one to three pereopods (i.e. 'walking legs' in amphipod crustaceans), depending on individual size, following methods described in Lagrue et al., 2014. A total of 7261 individuals were amplified for the 5' part of the mtDNA cytochrome *c* oxidase subunit I (COI) using universal primers (LCO1490 and HCO2198; Folmer et al., 1994) and then assigned to their respective MOTU using RFLP. Restriction endonucleases specific to a single MOTU were identified from the 565bp long sequence of the COI previously obtained by sequencing using the Cleaver Software (Jarman, 2006; for details on DNA extraction, PCR and sequencing, see Lagrue et al., 2014). The PCR amplified DNA products were then digested overnight using the appropriate restriction endonuclease(s) (Table S2), following manufacturer's instructions (New England Biolabs). Resulting fragments were separated by gel electrophoresis in a 2% agarose gel. Restriction enzyme profiles were visualized with ethidium bromide on a UV bench and used to assign each individual amphipod to its respective MOTU.

### 2.3. Infection level estimations

Parasite prevalence and abundance were calculated for each MOTU at each sampling site as the proportion of infected individuals (%) and the mean number of parasites per amphipod host, respectively (Bush et al., 1997). Dissections recovered acanthocephalan cystacanths belonging to three different species occurring at varying infection levels: *Pomphorhynchus laevis*, *Pomphorhynchus tereticollis* and *Polymorphus minutus*. *Pomphorhynchus laevis* and *P. tereticollis* use fish as definitive hosts while *P. minutus* is a bird parasite (Holmes and Bethel, 1972; Kennedy, 2006; Jacquin et al., 2014). Prevalence and abundance were thus estimated for each parasite species separately but overall prevalence and abundance were also calculated with all three parasite species grouped together.

### 2.4. Statistical analyses

We tested our hypotheses using four different statistical models. With the first two models we tested for potential differences in parasite prevalences and abundances among MOTUs using mixed

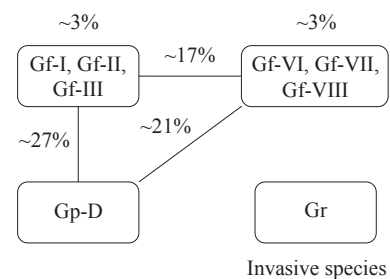


Fig. 1. Genetic divergence levels (%) among MOTUs of the *G. fossarum*/*G. pulex* species complex found in our sampling sites/ rivers. *Gammarus roeseli* was identified morphologically rather than genetically.

effect general linear models with a logit and a log link function, respectively. In these models, the sampling site (i.e. river) was considered as a random effect to account for potential differences in parasite prevalence or abundance among rivers. Given that amphipods were frequently infected by parasites of more than one species simultaneously, amphipod mortality induced by parasites could not be assessed independently for each parasite species. We therefore performed the subsequent models with no regards to parasite species, investigating overall parasitism effect on infection avoidance and parasite-induced mortality among MOTUs. Using a third model, we then analyzed parasite abundance as a function of amphipod body size, taken as a proxy for amphipod age. We modeled parasite abundance using a general mixed effect model with a Poisson distribution and a log link function. We considered amphipod MOTU, body size and the interaction between the two variables as fixed effects and the sampled river as a random effect. Under the hypothesis that parasites induce mortality in their host, parasite abundance is first expected to increase with amphipod's age/size before decreasing due to the death of heavily infected hosts. Accordingly, we considered the effect of amphipod body size on parasite abundance as second degree polynomial of the form  $\text{abundance} \sim \beta_2 * \text{size}^2 + \beta_1 * \text{size} + \text{intercept}$ , rather than linear. A significant effect of the interaction between amphipod MOTU and body size would suggest that parasite induced mortality differs significantly among MOTUs. This model was performed using the R package "lme4" (Bates et al., 2015) and subsequent post hoc analyses were performed using the R package "multcomp" (Hothorn et al., 2008). The data contained an excess of uninfected individuals, as indicated by the excess of zeros in the distribution of parasite abundance across MOTUs. In the context of our study, amphipods can be uninfected for two different reasons: (1) they have never encountered any parasite by chance or as a result of strategic avoidance, or (2) they have encountered parasites but resisted their infection. Statistically, the first source of zero is called false zero in contrast to true zeros representing genuine exposure to the phenomenon at the origin of the count process. These two sources of zeros reflect two quite different mechanisms by which individuals avoid being infected. With a fourth analysis, we finally modeled parasite abundance using a zero-inflated mixture model to investigate in further detail the mechanisms of infection avoidance. This was performed using the R package "pscl" (Zeileis et al., 2008) and the function "zeroinfl". This function allows accounting simultaneously for predictor variables affecting parasite abundance (which is assumed to follow a negative binomial distribution with a log link function) and for predictor variables affecting the probability that the data contains an excess of zeros (i.e. the probability of false zeros, which are modeled as a binomial process with a logit link function). We therefore considered the effect of amphipod MOTU, the polynomial effect of amphipod body size and their interaction on parasite abundance and the effect of amphipod MOTU only on the probability of false zero. In the context of our study, a significant effect of the amphipod MOTU on the probability of false zeros would suggest a significant difference among MOTUs in the mechanisms by which amphipods avoid being infected. For model convergence purposes and to ease interpretations of polynomial fit parameter estimates, we scaled amphipod body size by mean-centering prior to every performed analysis. We also used orthogonal polynomials in models including a polynomial effect of amphipod body size to avoid problems related to multicollinearity.

### 3. Results

Overall prevalences and abundances varied significantly among MOTUs (prevalence: likelihood ratio test,  $\chi^2 = 403.8$ ,  $df = 7$ ,  $P < 0.001$ ; abundance: likelihood ratio test,  $\chi^2 = 885.2$ ,  $df = 7$ ,

$P < 0.001$ , Figs. 2 and 3). The invasive *G. roeseli* was significantly less parasitized by local acanthocephalans than native amphipod MOTUs Gf-II, Gf-III, Gf-VI and Gf-VIII but showed prevalences similar to those of Gf-I and Gf-VII MOTUs. *Gammarus roeseli* was even significantly more parasitized than Gp-D amphipods (Fig. 2). Overall, differences in acanthocephalan prevalence among MOTUs did not seem to depend on their level of genetic divergence, as illustrated by the fact that closely related Gf-III and Gf-I or Gf-VI and Gf-VII MOTUs harboured substantially different acanthocephalan prevalences (Figs. 1 and 2).

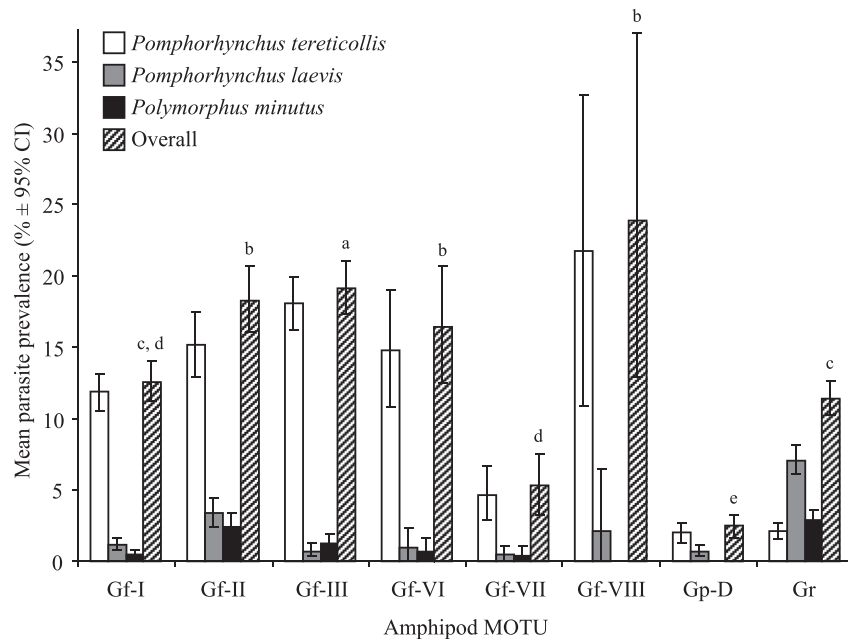
Parasite-induced mortality varied among MOTUs as suggested by the significant effect of the interaction between amphipod MOTU and the polynomial parameters of amphipod body size on parasite abundance (likelihood ratio test,  $\chi^2 = 105.8$ ,  $df = 14$ ,  $P < 0.001$ , Fig. 4). Two main patterns of parasite abundance as a function of amphipod body size (i.e. amphipod age) were found. Individuals from Gf-I, Gf-II, Gf-VI, Gf-VII and Gp-D MOTUs showed a bell-shaped relationship between parasite abundance and host body size, with amphipods of intermediate size, and thus age, being more heavily infected than smaller/younger or larger/older ones (Table 1, Fig. 4). Contrastingly, individuals from native Gf-III and Gf-VIII MOTUs, and the invasive *G. roeseli* showed increasing parasite abundance with increasing body size (i.e. host age; Table 1, Fig. 4). This dichotomy is also well reflected when interpreting parameter estimates  $\beta_1$  and  $\beta_2$  for the polynomial effect of amphipod body size on parasite abundance. In general,  $\beta_1$  is to be interpreted as the rate of change in abundance for mean amphipod body size (i.e. mean age). In other words, positive  $\beta_1$  values indicate that from mean amphipod body size (i.e. age) upward, parasite abundance is still expected to increase, whereas it is expected to decrease for negative  $\beta_1$  values.  $\beta_2$  reflects steepness and curvature of the fitted curve. Positive and negative  $\beta_2$  values reflect convex and concave curvatures respectively. Greater absolute  $\beta_2$  values indicate steeper curves. Native MOTUs Gf-III, Gf-VIII and the invasive *G. roeseli* showed  $\beta_1$  values significantly greater than 0 and greater than  $\beta_1$  values of Gf-I, Gf-II, Gf-VI, Gf-VII and Gp-D MOTUs (albeit not significant for the Gf-III/Gf-VII, Gf-VIII/Gf-VII and Gf-VIII/Gp-D pairs), indicating a steady increase in parasitic abundance with amphipod size across the entire size range (Table 1). Compared to Gf-I and Gf-VI MOTUs in which larger individuals tended to be uninfected, Gf-III, Gf-VIII MOTUs and the invasive *G. roeseli* amphipods also showed lower absolute  $\beta_2$  values (down to  $\beta_2 \approx 0$  for Gr) indicating moderate or no decrease in parasitic abundance in larger amphipods (Table 1, Fig. 4).

Including the effects of amphipod MOTU on the probability of false zeros in the zero-inflated mixture model significantly increased the fit of the model, suggesting that the mechanisms responsible for infection avoidance vary among MOTUs (likelihood ratio test,  $\chi^2 = 58.86$ ,  $df = 26$ ,  $P < 0.001$ , Table 2). Native MOTUs Gf-I, Gf-II, Gf-III and Gp-D showed rather high estimated probabilities of false zeros (albeit not always significantly different from zero, Table 2), suggesting that uninfected individuals in these MOTUs did not actually encounter parasites rather than resisted infection post-encounter. On the contrary, Gf-VI, Gf-VII, Gf-VIII, and the invasive *G. roeseli* had rather low probabilities of false zero, suggesting that uninfected individuals encountered parasites but resisted their infection (Table 2).

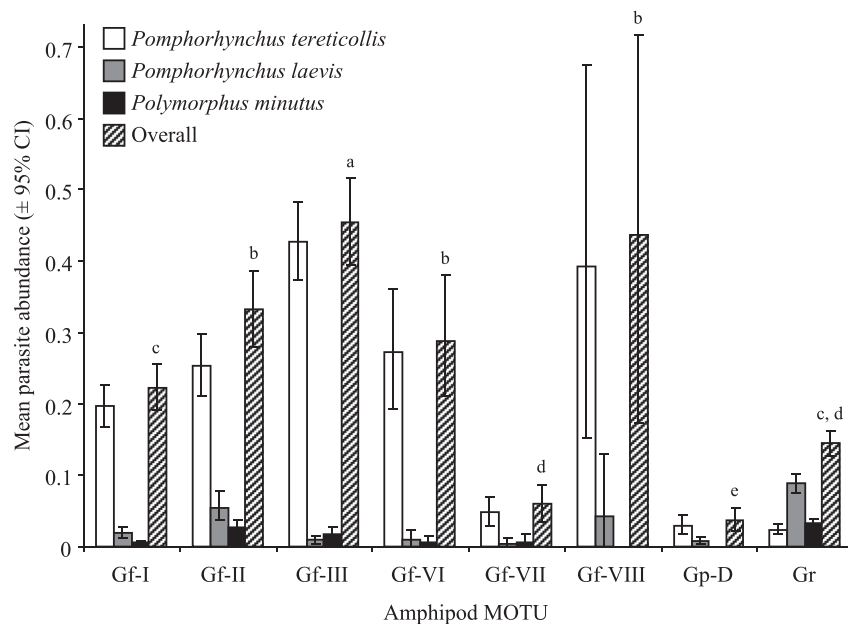
### 4. Discussion

Despite high genetic divergence values among MOTUs, diagnostic morphological features classically used to distinguish *G. fossarum* from *G. pulex* do not allow reliable discrimination among sympatric MOTUs in the amphipod populations used here (Lagrué et al., 2014). As a result, *G. fossarum*/*G. pulex* populations





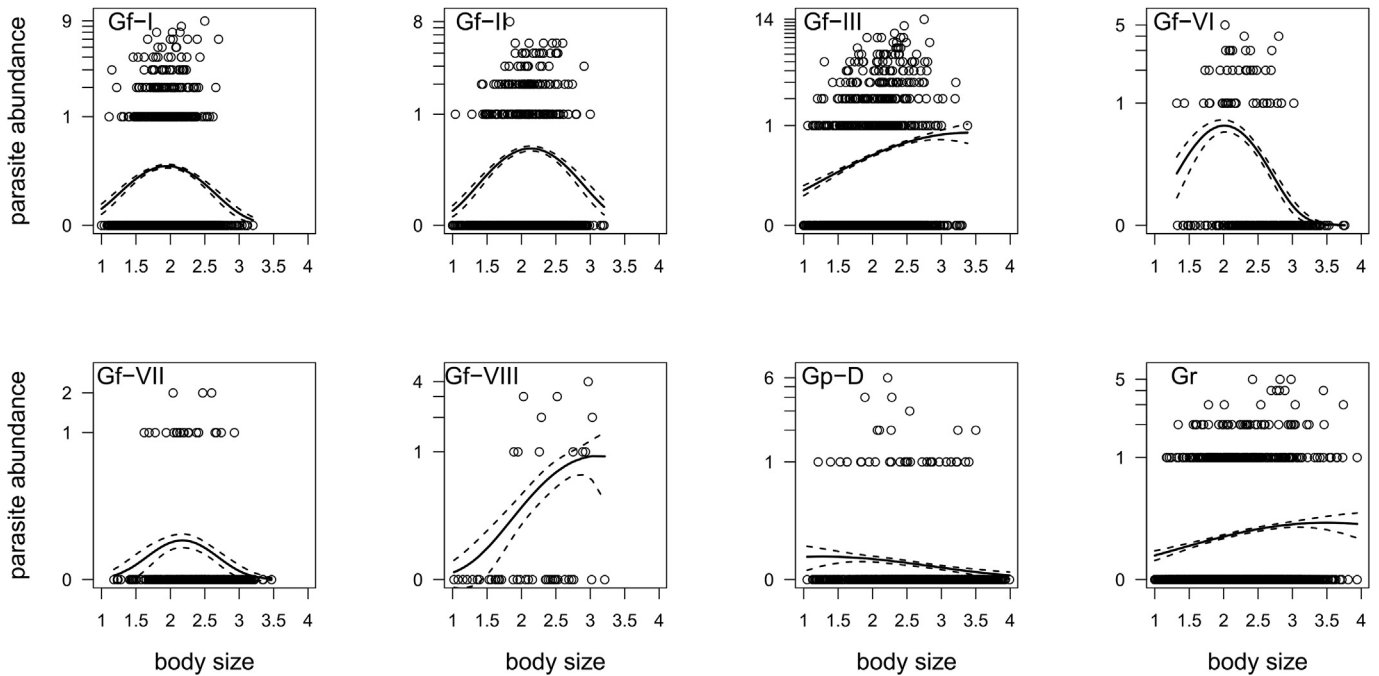
**Fig. 2.** Mean parasite prevalences (proportion of infected individuals in %) among amphipod populations/sampling sites and their bootstrapped 95% confidence intervals in the different MOTUs sampled and for the three acanthocephalan species, separately and overall (all three parasites grouped). Overall prevalences in MOTUs assigned different letters are significantly different at the 0.05 level.



**Fig. 3.** Mean parasite abundances (mean number of acanthocephalan larvae per individual host) among amphipod populations/sampling sites and their bootstrapped 95% confidence intervals in the different MOTUs sampled and for the three acanthocephalan species, separately and overall (all three parasites grouped). Overall abundances in MOTUs assigned different letters are significantly different at the 0.05 level.

have long been considered as a single functional unit when it comes to assessing parasite infection levels, parasite-induced mortality and other life history traits, and to comparing them to the invasive *G. roeseli* (Bauer et al., 2000; Rigaud and Moret, 2003; Bollache et al., 2006; Lagrue et al., 2007, 2011; Tain et al., 2007; Kaldonski et al., 2008). However, we showed in a recent study that two thirds of amphipod populations surveyed contained two or three sympatric *G. fossarum*/*G. pulex* MOTUs (Lagrue et al., 2014).

Furthermore, many sampling sites also contain the invasive *G. roeseli*. It is often expected that invasive species will be less infected than native hosts by local parasites, thus providing the invasive with a competitive advantage and higher invasion success (Clay, 2003; Torchin et al., 2003; Colautti et al., 2004; Prenter et al., 2004; MacNeil and Dick, 2011). However, such a pattern was not clear from parasite prevalences. We found that acanthocephalan prevalences varied greatly among native MOTUs of the *G. fossarum*/



**Fig. 4.** Parasite abundance as a function of amphipod body size (used as a proxy for age) in each of the 8 amphipod MOTUs. The polynomial effect of body size on parasite abundance is modeled with a general mixed effect linear model with a Poisson distribution and a log link function. The y axis is in log scale for representation purposes. Body size is rescaled to initial values in the graph for representation purposes. Predicted curves are represented in plain black lines with their standard errors in dotted lines.

**Table 1**  
Parameter estimates for the polynomial effect of amphipod host body size on parasite abundance among MOTUs. P-values presented next to  $\beta_1$  and  $\beta_2$  estimates test their significant difference from zero. P-values from pairwise comparisons of parameter estimates among MOTUs are also provided in the right part of the table; differences in  $\beta_1$  and  $\beta_2$  estimates are presented below and above the matrix diagonal, respectively. Significant differences are indicated in bold.

MOTU	Parameters					Multiple comparisons									
	Int (S.E.)	$\beta_1$ size (S.E.)	P-value	$\beta_2$ size <sup>2</sup> (S.E.)	P-value	Gf-I	Gf-II	Gf-III	Gf-VI	Gf-VII	Gf-VIII	Gp-D	Gr		
Gf-I	-1.71 (0.24)	-0.17 (0.13)	<0.001	-0.4 (0.11)	<0.001		0.99	<0.001	0.06	0.94	<b>0.01</b>	0.71	<0.001		
Gf-II	-0.31 (0.26)	-0.04 (0.1)	0.07	-0.51 (0.1)	<0.001	0.96		<0.01	0.09	0.99	<b>0.04</b>	0.99	<0.001		
Gf-III	-1.21 (0.24)	0.34 (0.05)	<0.001	-0.19 (0.05)	<0.001	0.06	<b>0.03</b>		<0.001	0.06	0.61	<b>0.03</b>	0.91		
Gf-VI	0.23 (0.3)	-0.51 (0.18)	<0.001	-0.47 (0.19)	<b>0.01</b>	0.99	1	0.65		0.13	<0.001	<b>0.02</b>	<0.001		
Gf-VII	-2.31 (0.34)	0.07 (0.34)	0.61	-0.61 (0.38)	0.11	0.08	0.99	<0.01	0.98		0.08	1	<b>0.01</b>		
Gf-VIII	-0.09 (0.41)	0.92 (0.37)	<0.001	-0.26 (0.28)	<0.001	0.81	0.65	0.99	0.94	0.07		0.10	0.82		
Gp-D	-4.00 (0.29)	-0.15 (0.16)	0.30	0.02 (0.09)	0.82	<0.01	<0.001	0.14	0.14	<0.001	0.55		<0.01		
Gr	-2.00 (0.24)	0.35 (0.06)	<0.001	-0.01 (0.04)	0.84	<0.001	<0.001	<b>0.02</b>	0.08	<0.001	0.36	0.99			

Footnote: Int = when Int is transformed to logit, it represents mean parasite abundance for each MOTU and for amphipod host mean body size in each MOTU.

**Table 2**  
Probability of false zeros among MOTUs (i.e. the probability that amphipod individuals are uninfected because they did not actually encountered the parasite rather than they resisted infection) and their respective P-values to test their difference from zero. P-values from pairwise comparisons of probability of false zero among MOTUs are also provided in the right part of the table. Significant differences are in indicated in bold.

MOTU	Parameters			Multiple comparisons									
	Int (S.E.)	Probability of false zero	P-value	Gf-I	Gf-II	Gf-III	Gf-VI	Gf-VII	Gf-VIII	Gp-D			
Gf-I	0.14 (0.41)	0.54	0.73										
Gf-II	-0.64 (0.65)	0.34	0.32	<b>0.04</b>									
Gf-III	-0.02 (0.41)	0.49	0.94	0.41	0.09								
Gf-VI	-1.62 (1.69)	0.17	0.34	0.23	0.47	0.28							
Gf-VII	-2.37 (7.51)	0.08	0.75	0.80	0.85	0.74	0.92						
Gf-VIII	-13.8 (1324)	0	0.99	0.83	0.85	0.81	0.88	0.95					
Gp-D	1.90 (0.39)	0.87	<0.001	<0.001	<0.001	<0.001	<b>0.02</b>	0.56	0.99				
Gr	-2.52 (3.45)	0.07	0.46	0.39	0.51	0.43	0.74	0.98	0.99	0.15			

*G. pulex* species complex. Furthermore, the invasive *G. roeseli* displayed local parasite prevalences varying from significantly lower to comparable, up to higher than prevalences recorded in sympatric, native MOTUs. There was also no clear link between genetic

divergence among MOTUs and acanthocephalan prevalence; i.e. the level of genetic divergence between two MOTUs did not predict, in any way, potential differences in infection levels by larval acanthocephalans.

Alternatively or concomitantly, invasive hosts may also be less susceptible than native congeners to local parasite-induced mortality (Hudson and Greenman, 1998; Roche et al., 2010; Blackburn and Ewen, 2016). Accordingly, we found that the invasive *G. roeseli* amphipod was not affected by parasite-induced mortality (i.e. acanthocephalan parasite abundance increased with host size; Fig. 4) while many native *G. fossarum*/*G. pulex* MOTUs showed the bell-shaped curve of parasite abundance across host size, a pattern indicative of parasite-induced mortality in hosts infected with larval helminths (Crofton, 1971; Amin et al., 1980; Bratney, 1986; Thomas et al., 1995; Brown et al., 2001, 2003; Latham and Poulin, 2002; Outreman et al., 2007; Bates et al., 2010; Heins et al., 2010). Furthermore, our results suggest that *G. roeseli* is also able to resist infection upon encounter with acanthocephalan parasites better than most native *G. fossarum*/*G. pulex* MOTUs. Interestingly however, two cryptic MOTUs (Gf-III and Gf-VIII) of the native species did not exhibit parasite-induced mortality either. Although variable infection levels among cryptic lineages have been reported previously in *G. fossarum* (Westram et al., 2011a), this is the first time that such pattern is documented among sympatric cryptic MOTUs and invasive species concomitantly. Like *G. roeseli*, Gf-VIII uninfected individuals seemed to have resisted infection upon parasite encounter rather than avoided parasites (Table 2). The pattern is slightly different in Gf-III uninfected individuals which showed rather high probability of false zeros (Table 2). This suggests that when encountering a parasite, Gf-III individual do not resist infection, but do not suffer increased mortality from being infected either. These patterns contrast with the common assumption that native host species are generally more susceptible to infection and more affected by local parasites than congeneric invasive species (Clay, 2003; Torchin et al., 2003).

Contrastingly, Gf-I, Gf-II, Gf-VI, Gf-VII and Gp-D amphipods did seem to suffer from parasite induced mortality; only uninfected individuals were able to survive to old age (i.e. large size), as indicated by the pattern of decreasing parasite abundance with age/size (Fig. 4). Also, in Gf-I, Gf-II and Gp-D, uninfected individuals are more likely to have avoided infection by chance rather than by being resistant to it. This pattern is particularly striking in Gp-D individuals which seem to efficiently avoid parasites and are therefore rarely infected. However, when they do acquire parasites, they suffer from parasite-induced mortality. Although acanthocephalan prevalence and mean parasite abundance varied greatly among these MOTUs, similar patterns of relationship between parasite abundance and host size or probability of false zero suggest similar vulnerability to acanthocephalan parasites. Generally, when amphipod individuals of native MOTUs Gf-I, Gf-II, and Gp-D encounter the parasite, they get infected and die from it; only individuals that do not encounter the parasite reach a large size/old age. Overall, acanthocephalan parasite infection levels and mortality effects on amphipod hosts varied greatly among native MOTUs and may thus be providing some, like Gf-III and Gf-VIII, with a competitive advantage in inter-MOTU interactions or better resistance to invasion by *G. roeseli*. *Gammarus roeseli* may also have a parasite-induced competitive advantage against most native MOTUs, potentially providing the invader with a competitive edge against native, local congeneric.

The exact causes of the differences in host parasite-induced mortality among sympatric amphipod hosts documented here remain unclear however. The respective roles of increased predation on infected hosts due to host-manipulation by acanthocephalan and/or direct pathogenic effects of the infection, and potential inter-MOTU variations in their respective effects, are yet to be tested (Benesh, 2011). Additional experiments testing for the exact causes of mortality are thus needed but should also account for other factors influencing potential variability in predation-linked

mortality rates among cryptic MOTUs and invasive species. This for instance includes microhabitat segregation among MOTUs, body size differences and/or variable behavioural and morphological defences (Bollache et al., 2000, 2006; Kaldonski et al., 2008; Galipaud et al., 2015b). Furthermore, the “snapshot” nature of our sampling did not allow for testing the temporal stability of the infection patterns observed or the mechanisms involved, or even the stability of co-occurrence of MOTUs in amphipod populations. In sympatric MOTU populations, parasites may promote stable coexistence of cryptic MOTUs or eventually drive all but one to extinction through competitive advantage to the less affected MOTU (Chesson, 2000). However, we cannot draw conclusion on the potential effects of parasites on long term co-occurrence of sympatric MOTUs from our results. Still, results suggest that some morphologically cryptic MOTUs may possess behavioural and/or immunological mechanisms preventing and/or reducing their vulnerability to acanthocephalan parasites while others are highly vulnerable. Cryptic diversity should thus be considered as an additional layer of complexity and accounted for when assessing infection patterns in host populations as sympatric, cryptic lineages of the same morphological species may display highly contrasting patterns of vulnerability to infection and parasite-induced mortality. High genetic divergence among amphipod MOTUs, and the occurrence of populations composed of sympatric MOTUs, may have profound ecological and/or evolutionary implications on observed infections patterns or host-parasite co-evolution and local adaptation (Cothran et al., 2013a, b). It is becoming obvious that amphipod species present high levels of cryptic diversity (Müller, 2000; Sutherland et al., 2010; Westram et al., 2011b; Cothran et al., 2013a; Lagrue et al., 2014; Weiss et al., 2014). Evidence also suggests that different MOTUs of *G. fossarum* have variable sensitivities to contaminants (Feckler et al., 2012). Here, we show that vulnerability to macroparasites may also be highly variable and not directly linked to genetic divergence levels.

In conclusion, our data showed contrasting levels of infection by acanthocephalan parasites among *G. fossarum*/*G. pulex* MOTUs, but also compared to the invasive *G. roeseli*. Highly variable parasite prevalence and abundance among sympatric MOTUs and between native and invasive amphipods may be due to a combination of factors including MOTU-specific vulnerability to infection and/or parasite induced-mortality. Overall these results, and that of other recent studies (Lagrue et al., 2014; Galipaud et al., 2015a, b), indicate not only that cryptic MOTUs of the native *G. fossarum*/*G. pulex* complex are commonly found in sympatry, but also that they can display widely different vulnerability to local parasites. Although the mechanistic causes of such differences are still unclear, they potentially have large effects in the outcome of inter-MOTUs competition and co-occurrence (Chesson, 2000). Furthermore, our results show that parasite effects on interactions between native and invasive amphipods is likely MOTU specific. Cryptic diversity thus adds an extra layer of complexity in intra and interspecific interactions with potentially deep implications in invasion dynamics that cannot, and should not be ignored.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ijppaw.2017.04.005>.

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