Peer Community In Infections

Gammarid is not equal gammarid for acanthocephalan parasites

Daniel Grabner *based on peer reviews by 2 anonymous reviewers*

Alexandre Bauer, Lucie Develay Nguyen, Sébastien Motreuil, Maria Teixeira, Nelly Debrosse, Thierry Rigaud (2024) Differences in specificity, development time and virulence between two acanthocephalan parasites, infecting two cryptic species of *Gammarus fossarum*. HAL, ver. 2, peer-reviewed and recommended by Peer Community in Infections. https://hal.science/hal-04455823

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The question on the role of different alternative hosts in the life cycle of acanthocephalan parasites has not been fully resolved to date. There is some information on the use of fish hosts in the genus Pomphorhynchus (Perrot-Minnot et al. 2019). It is known that acanthocephalans of the genus *Pomphorhynchus* can infect a number of different amphipod species (e.g. Bauer et al. 2000; Cornet et al. 2010; Dezfuli et al. 1999) but it is not clear if some host species might be more "advantageous" for the parasite, or if the parasite is more virulent to some host species than to others. Bauer et al. (2024) investigated different well characterized cryptic lineages of *Gammarus fossarum* (Weiss et al. 2013) for their susceptibility for two *Pomphorhynchus* sp. The results show that there is a difference in susceptibility to acanthocephalans between different linages of *G. fossarum*. Additionally, a parasite species specific difference was detected: the difference in susceptibility was more pronounced for *P. tereticollis* than for *P. laevis*. *P. tereticollis* was less virulent and developed slower than *P. laevis* (in *G. fossarum*).

Besides the improved understanding of the biology of acanthocephalan parasites, this study clearly points out that we have to be careful with putting the "generalist" label on parasites simply due to the number of alternative host species we find them in. Instead, we should always have in mind that some of these hosts might be less suitable for the parasite than others when comparing quantitative data on the infection success.

I highly appreciate the experimental approach taken that allows more profound conclusions than evaluations of field data. Experiments and analyses have been conducted well. I think this paper is significantly enhancing our knowledge on the specificity for the intermediate host. I find it highly remarkable that this was even found among different host lineages.

References:

Bauer, A., Trouve, S., Gregoire, A., Bollache, L., Cezilly, F. (2000) Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on the behaviour of native and invader gammarid species. International Journal for Parasitology, 30(14), 1453-1457. https://doi.org/10.1016/s0020-7519(00)00138-7

Bauer, A., Develay Nguyen, L., Motreuil, S., Teixeira, M., Debrosse, N., Rigaud, T. (2024) Experimental infections reveal differences in specificity, development time and virulence between the acanthocephalan parasite *Pomphorhynchus tereticollis* and its sympatric counterpart *P. laevis*, in two cryptic species of *Gammarus fossarum*. HAL, Ver. 2, Peer-Reviewed and Recommended by Peer Community in Infections, hal-04455823. https://hal.science/hal-04455823

Cornet, S., Sorci, G., Moret, Y. (2010) Biological invasion and parasitism: invaders do not suffer from physiological alterations of the acanthocephalan *Pomphorhynchus laevis*. Parasitology, 137(1), 137-147. https://doi.org/10.1017/S0031182009991077

Dezfuli, B.S., Rossetti, E., Bellettato, C.M., Maynard, B.J. (1999) *Pomphorhynchus laevis* in its intermediate host *Echinogammarus stammeri* in the River Brenta, Italy. Journal of Helminthology, 73(2), 95-102. https://doi.org/10.1017/S0022149X00700277

Perrot-Minnot, M.J., Guyonnet, E., Bollache, L., Lagrue, C. (2019) Differential patterns of definitive host use by two fish acanthocephalans occurring in sympatry: *Pomphorhynchus laevis* and *Pomphorhynchus tereticollis*. International Journal for Parasitology: Parasites and Wildlife, 8, 135-144. https://doi.org/10.1016/j.ijppaw.2019.01.007

Weiss, M., Macher, J.N., Seefeldt, M.A., Leese, F. (2013) Molecular evidence for further overlooked species within the *Gammarus fossarum* complex (Crustacea: Amphipoda). Hydrobiologia, 721(1), 165-184. https://doi.org/10.1007/s10750-013-1658-7

Reviews

Evaluation round #1

DOI or URL of the preprint: https://hal.science/hal-04455823
Version of the preprint: 1

Authors' reply, 13 June 2024

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Decision by Daniel Grabner, posted 12 April 2024, validated 12 April 2024

Based on the reviewer comments, I suggest a major revision

Dear authors,

generally, I believe this is a nice, interesting and relevant study. Nevertheless, the reviewers raised several important points that should be considered before I can recommend the article.

Specifically, you should:

- consider the specific points addressed by the reviewers
- carefully revise the language
- consider using GLMs for analysis of the data (optional)
- streamline the title

My main concern is that the presentation of the results (table 1) is really confusing. It is not clear what is meant by the controls (with 0 uninfected for G. pulex???) Probably, it is a good idea to remove G. pulex due to the low number of individuals. Also, I suggest removing the "Suzon" population of G. pulex from the table and report the quality test only in methods. But you should have some sort of threshold for this to make any sense (e.g. a minimum of 60% infection with the control population must be achieved).

After thorough revision, the paper might be recommended.

Best regards,

Daniel Grabner

Reviewed by anonymous reviewer 1, 11 April 2024

MS-hal-04455823 Experimental infections reveal differences in specificity, development time and virulence between the acanthocephalan parasite *Pomphorhynchus tereticollis* and its sympatric counterpart *P. laevis*, in two cryptic species of *Gammarus fossarum*

This ms represent a valuable research work addressing the host specificity, development and virulence of two acanthocephalan species with respect to their intermediate host. In my opinion the outcome of the paper fills the knowledge gapes in this area, which might contribute to the understanding of distribution patterns of different Pomphorhynchus species in different aquatic habitats. However, I have some remarks which need clarification before the ms could be published.

General comments

The title appears to me very long and elaborated. It is like a short abstract that summarizes almost the whole output of the research, which makes it not catchy at all.

In my opinion the results/aspects related to the infection with *G. pulex* should be omitted throughout the entire ms. The authors stated that they was used them "…as control for parasite "quality"…" (see Line 118), however the number of exposed and infected individuals was too low in order to make any conclusions based on comparisons.

In MM part it is not very clear how the infection of gammarids was proceed. There are no details how many individuals in total were taken for the infection experiment. The authors provide in table 1 as well as figures 2 and 3 the numbers, however, it is not clear how many of them was used as control group (this is relevant for the evaluation of the virulence/mortality). The sample size of control animals seem to me very low, on the one hand and on the other hand, the higher number of early deaths during the *P. tereticollis* exposure exceeded the number of the ones that survived (uninfected group). Were the early deaths also considered in the calculations of the survival rates of control group? According to the data in table 1 it is visible that the early death rates were much higher (3 to 4 times higher) in both controls and exposed groups during the infection experiment with *P. tereticollis*. Was it due to maintenance of gammarids (stress)? The authors assumed that this was not linked to the confrontation of gammarids with the parasite's eggs (lines 434-437), however such differences in early mortality could make the comparisons of survival rates between both acanthocephalan species questionable. Therefore, here is more clarification needed instead providing only a personal assumption!

It is also not clear, whether the individuals of Gf2 and Gf6 lineages were pre-sorted prior the start of infection experiment or at the end, when the gammarids were sacrificed. I guess that it was done at the end, as cutting of locomotor appendages would induce additional stress to the animals. This is also true for the identification of both acanthocephalan species. Here I assume that it was done molecularly prior the collection of the eggs based on extracted DNA of the adults, but the authors stated that they also identified them at acanthella stage (lines 206-207). Please, clarify in MM part at which stage the identification was done!

The molecular method that was used to distinguish between both acanthocephalan species is based on the size of PCR products, however, it is not applicable for proper identification of other close related *Pomphorhynchus* species like *P. bosniacus*, as recently published by Reier et al. 2019. In this regard, did the authors also sequenced a larger sample size using the procedure of Reier et al. in order to exclude any

incidental infections with *P. bosniacus*, which might also exhibit completely different host specificity and thus blur the results?

Another issue that deserves clarification is related to the egg suspensions that were used for the infection. In lines 151-152 the authors stated that batches with at least 50% mature eggs were used. How this percentage was determined/quantified? Additionally, inconsistent percentage of mature eggs in the suspensions (variation between 50% and 100 %) could potentially bias the infection success, in my opinion. My question here is: did the authors quantified also the upper range (%) of mature eggs? If yes, please provide it.

Specific comments:

In addition of the comments listed above, in my opinion, this ms needs in depth linguistic improvement. Even if I'm not a native English speaker, I've detected various wording/phrasing issues as well as wrong prepositions and partly improperly used grammar. As there are many of them, I'll not address every single one. Therefore, I recommend a proof reading by a native speaker, if it is possible.

Further, the genus names should be consistently abbreviated once they were introduced (e.g. *P. laevis, P. tereticollis, G. pulex, G. fossarum*).

I also recommend to combine figures 5 and 6 as well as figures 7 and 8. In this case there won't be a confusing overlap in the ranges of x-axis and different resolutions (e.g. figures 5&6), whereas the line for the infected group will start at day 40 and 60 for *P. laevis* and *P. tereticollis*, respectively.

In my opinion, the tables (e.g. 2, 3 and 4) could be taken out of the main script and placed in the supplementary data.

Based on comments above I recommend a major revision with a focus on language/text improvement/editing.

Reviewed by anonymous reviewer 2, 15 March 2024

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