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**Unidad Mérida**
**Departamento de Recursos del Mar**

**Review of the PhD thesis "Composition and structure of the larval trematode communities in model freshwater pulmonate gastropods in euthropic environments in Central Europe"**

*Comments of*: **Victor Manuel Vidal Martínez**

*Author*: **Miroslava Soldánová**

*Date*: **May 20 2010**

**General comments**

Larval trematode communities in snails represent a useful model for studying the potential influence of competition as a structuring factor and the relative importance of bottom-up ecological processes. The thesis presented by Mgr. Soldánová not only provides evidence of the role of these bottom up processes in structuring infracommunities through competitive interactions, but also generates the novel implication that these processes are affecting the composition and structure of the component communities. The thesis is a coherent collection of papers published in well-recognized scientific journals and well written manuscripts.

Methods used are adequate and they allow testing the hypotheses in an appropriate way. The use of null models in paper III was especially interesting. However, I was wondering whether the author collected and fixed snails infected to undertake histological studies. I think this technique would be useful to document the competitive interactions suggested in paper III. What I mean is that all the interpretations were based on the number of infected snails, but there was not direct proof of the competitive interaction among these larval trematodes in the snails. Additionally for future work, I would recommend noy only "going up" but also "going down" and consider the role of spatial structure as a source of variability in this system. Since geographical distance can be measured from pond to pond, I would expect that ponds that are nearer to each other would present higher faunal similarity than those further away.

I found several minor errors especially misspellings that are indicated in the electronic version of the thesis that I have sent back to Mgr. Soldánová. In addition, I raised a number of questions (as sticky notes in the pdf file) that the author should address.

**Specific comments**

1. **General introduction, page 4.** You are talking about nested spatial scales. What spatial scales? Please explain.
2. **Page 7.** "only study system..,,,. What about the studies on interspecific competition in the intestines of birds like *Aythya affinis* or fishes such as eels (e.g. Bush and Holmes, 1986,

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Kennedy, 1994; Vidal-Martinez and Kennedy, 1998)? I do not think this is the only system where competition has been tested. Make reference to pertinent papers please.

3. **Aim and objectives. Page 27.** "To provide novel data on.... „ This objective seems obvious to me. Probably, something like: "to determine patterns of...." would be useful.
4. **Paper I, page 34.** "Only snails of the sexually mature cohorts were sampled.....„ This way of sampling would produce bias, since part of the population has not been sampled. How do you justify that?
5. **Paper I, page 34.** " samples of < 20 snails were excluded from analyses. „ Why? I think this could produce bias with respect to the spatial distribution of the trematodes. How do you justify this?
6. **Paper I, page 39.** " However, with only 2% of the variance in similarity accounted for by inter-pond distance.... „. This is the reason I feel you need "going down„ in addition to "going up„. See my general comments.
7. **Paper II, page 64.** " and the significantly higher rates of subordinate species.... „ Maybe the eggs of the digeneans are being eaten in pond V by fishes (like carps) and they do not reach the snails. What do you think?
8. **Summary (Paper III part), and Paper III page 90.** This is interesting. In fact, competition happens at infra-community level because at that level, the individuals can find each other in the same host. At other hierarchical levels such as component community, other processes such a heterogeneity in transmission could be occurring but not necessarily produced by competition. My point here is that I think it is necessary to undertake histological studies to document the competitive interactions suggested in paper III. This is because all the interpretations in this paper were based on the differences in number of infected snails, but there was not direct proof of the competitive interaction among trematode species in the snails. The main question to answer at this point would be: Are the larval trematodes in the same microhabitat (= organ) in the snail or in different ones? And if they are in the same microhabitat, do they interact? In intestinal helminth communities of birds and fishes, there is a clear gradient of resources in the intestine starting from the pilorous to the rectum. What about in the snails?
9. **Paper III, page 101.** "*D. spathaceum* induces changes in *L. stagnalis* with immature defense system, thus infecting only young snails„.But, the young ones were removed from the analysis (see my point 4 and page 34). How do you explain the absence of young ones if they are important?
10. **Paper V, page 143.** "The overall probability of infection was strongly dependent on snail size and.... „ If this is so, why did you remove the small snails from your analysis (chapter I)?

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**Conclusion**

The thesis requires minor revision, with several changes all of them indicated in the pdf file but minor in their importance. Mgr. Soldánová has demostrated the ability to undertake independent scientific research and to publish her results in high quality scientific journals. Therefore, from my point of view the quality of her thesis fulfills the requirements for the obtention of a PhD.

Sincerely,

Victor Manuel Vidal Martínez, PhD.

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22.05.2011

**Report on the Ph.D. thesis of Miroslava Soldánová, entitled "Composition and structure of larval trematode communities in model freshwater pulmunate gastropods in eutrophic environments in central Europe"**

The thesis Ms. Soldánová handed in represents a comprehensive study on communities of digenean trematodes in their freshwater snail first intermediate hosts in man-made ponds in the Czech Rebublic and a system of dammed lakes in Germany. The general introduction is a detailed synopsis of the field of the ecology of trematode-snail interactions, taking into account the relevant literature, and clearly leads up to the topic and the chosen approach in this thesis.

The five papers of the thesis appear as a logical chain of studies, all dealing with ecological, epidemiological and population-dynamic aspects of the chosen system, but in a successive order of levels: **paper I** starts with a comparison of general structures in digenean communities of different ponds, based on an extremely impressive dataset of the prevalence of 14 trematode species in >6,000 *Lymnaea stagnalis* snails, sampled repeatedly in five localities during more than 2 yrs. The different flukes were assigned to core, secondary and satellite species, but most interestingly, the status of the most prevalent species varied not only between the different localities but also over time/season. Three plausible (not mutually exclusive) hypotheses for the different patterns in community structure were discussed, namely either species-specific colonization capabilities, including the strategy of snail infection (free-swimming miracidium *vs.* ingested egg), environmental differences (pond size, pond management) resulting in different efficiencies of the transmission strategies to the various second intermediate hosts and the final host, and finally, competition within snail hosts.

Consequently, **paper II** deals with the dynamics of colonization of *L. stagnalis* snails, using particularly the findings of mark-release-recapture sampling in the previously mentioned dataset. By analysing the individual infection history of almost 1,500 snails, the authors determined the extraordinary high yearly colonization rate, but also extinction of infections and replacement events of the different trematode species by others. This way, they could evaluate a hierarchy of digenean species with high and low colonizing potential and competitive exclusion of species, which could also be related to characteristics of the different ponds.

The special role of interspecific competition was the topic of **paper III**, where especially the species composition in the 280 double and triple infected snails within the mark-recapture dataset were analysed in relation to environmental characteristics of 7 ponds. The expected community structure under an assumed random distribution differed significantly from the actually observed lower number of multiple infections. On the species level, only 19 instead of the possible 91 combinations of double infections occurred. Analysis of shifts in the prevalence of the different combinations revealed a dominance hierarchy, with echinostomes (development via rediae) and (surprisingly) *Diplostomum pseudospathaceum* (sporocysts) being all highly competitive, dominant over the rediae-forming *Opisthioglyphe* and *Plagiorchis*, which again had higher competitive abilities in interactions with 3 sporocyst –forming strigeids. All these findings revealed not only strong interspecific competition within the host snail, the outcome of these interactions differed also significantly between the different habitats/ponds, indicating environmental influence on competitive exclusion.

**Paper IV** represents a kind of zoom in into the trematode fauna of *L. stagnalis* and *Planobarius corneus* of two ponds, where the infection dynamics were correlated with age-distribution of the snails in three samplings within one year. Diversity and similarity of these regional trematode communities of both hosts were analysed in relation to a larger geographical scale, using data from the literature.

Finally, in **paper V** trematode communities were investigated in a different type of freshwater system, a series of four man-made reservoirs of the river Ruhr in Germany, which differed not only in several ecological aspects from the Bohemian carp ponds, but due to another additional predominant snail (*Radix auricularia*) also in the prevalent digenean species.

**Specific comments & Questions:**

1. The majority of studies referred to in the introduction were done in a marine/coastal environment. How comparable is a continental system of man-made carp ponds with the situation in salt marshes and intertidal zones?

2. Findings in paper I and IV indicated that the trematode communities varied significantly over time/season within ponds. Could these patterns also be an indication of host-parasite coevolution (Red-Queen dynamics)?

3. In paper II, 4.1% of the snails apparently lost their infection. What could be the reasons why the snails stopped shedding cercariae?

4. Although developing via sporocysts, in the dominance hierarchy of paper III surprisingly *D. pseudospathaceum* was on the same level with several echinostomes. In another study (Rauch et al. 2005), individual *L. stagnalis* were found to be infected with up to 9 different clones of this eyefluke. How likely is it that *D. pseudospathaceum*, instead of competing successfully with echinostomes, is just more prevalent and repeatedly re-infects snails harboring already an echinostome infection?

5. Besides competitive exclusion by direct interactions among different trematode species within the snail host, what other mechanisms might lead to a lower number of double-infections than expected by random effects?

6. Did the size of the respective ponds relate to the recapture-rate? And to the proportion of double infections? And were the population sizes of *L. stagnalis* calculated on the basis of the recapture-rates?

7. Size distribution of snails in paper IV differed between two ponds. Would you expect this to be related with the prevalence and species-composition of the trematodes?

8. Any idea why in *P. corneus* there were more trematode species found infecting mammals, but in *L. stagnalis* more bird parasites?

**General evaluation:**

The thesis of Miroslava Soldánová is remarkable in several respects. Like most ecological studies on community level, the topic is very complex. But especially in cryptic species or species combinations like the intramolluscan stages of trematodes within a complex environment, defining hypothesis and designing sufficient data collection protocol accordingly is already challenging. Here the candidate did a very good job, which was only possible with a thorough study of all the relevant literature and the theoretical background, but also requires in-depth knowledge of the biology of the study organisms themselves. And not at least, the logistics of the sampling and determining the isolated cercariae from several thousands of snails is a really impressive piece of work. Furthermore, for detecting patterns and verifying her hypothesis with the complex datasets, she successfully applied sophisticated statistical methods of community ecology. The outcome of the analysis are well described, and due to her broad knowledge of the topic, the results are discussed with several aspects and implications, maybe in some cases a bit too detailed; for the sake of clarity the discussion sections could have been slightly more focussed, especially in papers I and III.

I regard her work as a valuable contribution to our knowledge of parasite ecology; in particular her findings on frequency and dynamics of multi-species trematode infections in snails are intriguing and may have implications also on applied aspects of parasitology, e.g. for the biological control of snail-transmitted diseases, like Schistosomiasis.

As a result of this excellent work, she presents five chapters, of which three are already published or in press, and I have no doubts that the remaining two manuscripts will be published in good international parasitological journals as well.

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**Conclusion:**

With this thesis, Miroslava Soldánová has proven herself as an excellent scientist with a broad knowledge in ecological parasitology and trematode biology, talented in organizing field research, with an impressive expertise in statistical analysis of large and complex datasets and successful in scientific communication.

I am convinced that she deserves to defend her PhD thesis and I am looking forward to discussing some of the specific critical question listed above on that occasion.

Yours sincerely,

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**Composition and structure of larval trematode communities in model freshwater pulmonate gastropods in eutrophic environments in Central Europe**

Ph.D. Thesis by Miroslava Soldánová, University of South Bohemia, České Budějovice

Review by Markéta Ondračková, IVB Brno

Submitted Ph.D. thesis represents a comprehensive set of mutually linked studies that significantly advances our understanding of the patterns in structure, composition and variability of trematode communities in the first intermediate host, freshwater pulmonate snails, at both infracommunity and component community scales. The thesis focuses on the relationship between parasite community structure and interspecific interactions and the assessment of the contribution of particular factors (e.g. habitat size and disturbance, host age, trematode recruitment dynamics, competition) on community organisation in freshwater snail-trematode systems. The thesis comprises three published (or in press) papers in SCI journals (Parasitology, Parasitology Research, Parasites & Vectors) and two submitted manuscripts. Miroslava Soldánová is a first author on four of the five papers and a second author on one paper. Despite the papers represent the results of a wider working group, M. Soldánová's participation in particular studies on study design, fieldwork, parasite screening and identification, statistical analysis and manuscript drafting appears to be substantial.

The presented papers fit together well and are logically connected and the composition of the Ph.D. thesis is well balanced and successful. Both the content and formal aspects of the thesis are also adequate. A general introduction summarising the current knowledge on trematode life cycles with snails as intermediate hosts, an ecological overview of trematode communities in snail hosts and on utilisation of larval trematodes as bioindicators appropriately interconnects the five manuscripts comprising the major part of the thesis. The manuscripts are nicely prefaced by a chapter entitled "This study", where the author explains the history and progress of particular studies. A similarly good chapter, "Concluding remarks and future prospects", highlights the importance of the results presented and brings the thesis to an end. Unfortunately, I missed a wider chapter including a kind of general discussion. Not all theses submitted as a set of publications are as logically connected as this one was. The use of a variety of statistical methods leads to results covering a range of points of view, which clearly offers an opportunity for an overall summary and discussion. This chapter could easily follow the format of the introduction chapter "This study" and, similarly, answer the specific questions raised in a consecutive manner.

The author collected a high-quality set of data from the Czech Republic and Germany over the 2006-2009 sampling period, and the number of samples analysed demonstrates the author's diligence. Even thought some studies share the major part of the samples, these studies answered different questions. The data were analysed using advanced statistical methods with a comparative approach; however, the results and, consequently, their interpretation may have been, in some cases, significantly affected through the use of inappropriate methodology leading to misguided interpretation (see bellow in Comments).

Having said this, the presented study meets all the requirements for a Ph.D. thesis and a successful defence. It contains original scientific results and documents the author's ability to meet all the scientific challenges faced by ecologically oriented biologists, including also the

obtaining of financial support for a research stay abroad. Therefore, I fully recommend the Ph.D. thesis of Miroslava Soldánová to the defence panel.

Unfortunately, I cannot be personally present during the Ph.D. defence, for which I apologise. I have, however, provided a list of selected comments and questions bellow this review.

Brno, 18. 5. 2011 Mgr. Markéta Ondračková, PhD.

Comments:

1) Methodological comments: Statistical analyses regarding the "pond effect" (at least in Papers I and Paper II) are encumbered with the error of temporal autocorrelation (repeated sampling of one site). In such cases, the results may potentially suffer from an artificially magnified effect of a particular factor (e.g. pond size, hibernation) and, therefore, be imprecise and/or misleading. Instead of using GLM methods, mixed models with random effects representing repeated sampling events of the same habitats, would be more appropriate.
   In Paper II (p. 61), the results of ANCOVA on sample-based estimates of colonisation rates do not correspond (i.e. the values of F, d.f. and p).
2) Formal comments: Reading through all papers, there are some inaccuracies in formulation, e.g. the current larval trematode richness in *L. stagnalis* is 24 spp. in Paper I, but 23 spp. in paper IV.
   The sentence in the Discussion of Paper II (p. 66): „Goater et al. (1989) reported losses of infection in 27 overwintered recaptured snails..." is very misleading. They found losses of infection in 10 of 31 recaptured snails, and this is definitely not clear from the text.
   The text in Abstract of Paper III (p. 83) does not precisely correspond to the results. In the Abstract: "Seven top dominant species ....dominated over other trematodes possessing **only** sporocysts in their life cycle." However, one redial species *M. anceps* appears to be subordinate, being denoted in the Discussion as "weak non-predatory type of redial species" (p. 97).
   Interestingly, the number of sampling sites differed between papers, although the data originate from the same sampling season and region (Papers I-III). I'm only curious as to why the number of sites consecutively increases from Paper I to Paper III, and why not all sampling sites, being situated in the same region of South Bohemia, were used in all studies.
3) As far as I am aware, it is not usual for citations to appear in the Results section. In Paper III, I would prefer that the results originating from the different studies appear in the Discussion and not the Results section (p. 90, 92-93). Similarly, the first paragraph of the Dominance hierarchy chapter (p. 91) should be in the Methods.
4) In Annotation/General introduction, the author considers 2 nested scales of community organisation, but in the Concluding remarks, she mentions three scales. Does this study show the data of two or three scales?

Questions:

1) Multiple infections and their consequent interactions represent for me the most interesting part of the thesis. Their low occurrence is connected predominantly with parasite

competition. Could it be possible, however, that the relatively rare occurrence of multiple infections in nature reflects the increased mortality of such hosts, either by increased stress or selective predation? Have such cases, previously noted for other intermediate host groups, been observed in aquatic snails?

2) Was the presence of the trematode metacercariae also documented during the survey of pulmonate snails (i.e. the 781 snails examined as a control in order to compare the number of released cercariae and real numbers in snails)? If yes, was there any relationship between their occurrence and presence of parasites in the redia/sporocyst stage? Previous studies by some members of the research team showed the relatively common occurrence of metacercariae in pulmonate snail hosts. Could it be expected that, for example, hosts infected by metacercariae are more vulnerable to the next infection, e.g. by species which actively penetrate the host?

3) As shown in the Discussion of Paper II, the life span of freshwater intra-molluscan stages of trematodes, in contrast to marine species, is shorter and loss of infection has been described in both field and laboratory studies. What is known about the life span of particular parasite species in *L. stagnalis* found in this study? Is it comparable among all species? If not, could this trait affect, for example, the colonisation rate (e.g. short lived species will colonise their hosts more rapidly than long lived species)? Could the variance in life span influence the results of dominance/hierarchy of particular parasite species and, eventually, parasite loss?

4) It would be expected that time of colonisation is dependent on the presence of a definitive host at the sampling site. Were the potential definitive host species of all trematodes analysed in this study present over the whole year?
Does maximum colonisation time in Table 3 (336 days, *P. elegans* from Pond Z) mean that the snail was repeatedly recaptured without sign of infection up to day 336, or that this snail was recaptured after 336 days? Was the possibility of a potentially different time of snail recapture considered in analysis?

5) In the Paper V, the authors tested for the effect of pH: „... pH was also identified as an important factor with a negative effect on the probability of infection" (p. 146). At which level does pH affect the probability of mollusc intermediate host infection? Could it be presence of the definitive host, miracidial activity, egg survival, or other?

6) What is the meaning of the sentence: „*L. stagnalis*, *R. auricularia* and *P. corneus* represent the most suitable trematode intermediate hosts with a Palaearctic distribution"? Does it mean that these three species are the most parasitised by trematode parasites among freshwater snails in the Palaearctic? Could the infection of particular snail host species not to be dependent on the specific locality/region and presence of potential definitive hosts, as was e.g. shown in Paper V, where species richness in *L. stagnalis* in German lakes was relatively low compared to ponds in the Czech Republic, and conversely the species richness in *R. auricularia* in Germany was higher than in eutrophic ponds in South Bohemia, as published by Faltýnková 2005?

7) What might be the reason for evolutionarily „different roles of lymnaeid and planorbid hosts in trematode transmission"? Could this be applied to these groups generally or only to the three species examined?