

Review of the PhD thesis of Jana Martinů

The core of the PhD thesis of Jana Martinů consists of a set of 4 publications on host-parasite coevolution using rodent and bird hosts with louse and *Eimeria* parasites as models. These publications are introduced by an overview of the field followed by a summary of the main results of the 4 publications and a short concluding paragraph. The 4 publications are all published in peer-reviewed journals, 3 in leading journals in the field of parasitology and evolutionary ecology and the 4th technical paper in *Folia Parasitologica*. Jana is the lead author of three of the papers and the 5th author of the last paper in agreement with her contribution clearly stated in the thesis.

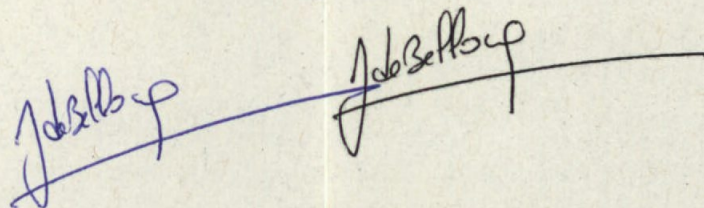
Strict host parasite co-phylogeny would require infinitely strong barriers to the movement of parasites between host taxa. Even if such movements are very rare, over evolutionary timescales incongruences between host and parasite phylogenies will accumulate. When data are scarce these may not be detected, but recent improvements in the resolution of genetic data mean more are now being discovered. The circumstances in which parasites can successfully host switch are of interest and are likely influenced by factors such as the geographic distribution of hosts and parasites and the characteristics, ecology and life history traits of both parasite and host. The candidate explores several of these factors at both macro- and micro-evolutionary scales using diverse host-parasite models.

In this framework, the candidate has characterised and developed 16 microsatellite markers for the louse model *Polyplax serrata* in rodents (paper 1). She used these microsats together with a mitochondrial sequencing to perform population genetic analyses of a large sample of *Polyplax serrata* collected from many localities across Europe and investigate the pattern of genetic diversity in different subtaxa of this louse species which are associated with different host spectra. She also tested Nadler's prediction stating that specific parasites will show higher genetic structure and lower genetic diversity than generalist ones. She then used another louse model, the chewing lice from the genus *Menacanthus* parasitizing birds to first investigate their evolutionary history and then the effect of geographical distribution by focusing on two species differing in their host spectrum. Finally she investigated the evolutionary history of *Eimeria* parasites in European rodents (*Apodemus*, *Microtus* and *Myodes*). The sampling size and the genetic data generated in this thesis for the three studies are remarkable: Paper 2: 2 352 *Apodemus* mice collected across 14 European countries; about 430 lice and 300 hosts genotyped for mt and microsatellite markers; Paper 3: *Menacanthus* lice collected from 29 localities in 12 countries; 168 sequences of COI gene and 151 sequences of EF1 α . Paper 4: More than 2000 rodents collected and more than 200 sequences of COI gene used in the study. Another interesting aspect is the investigation of host-parasite evolution at both macro- and micro-evolutionary scales, which implied that the candidate had to use varied population genetic analysis tools, from phylogenetic reconstructions (Bayesian and Maximum likelihood based) to population structure estimation (varied population genetics parameters, clustering algorithms). For each study, the state of the art and goals are clearly described and the data adequately analysed and interpreted. One

reservation is the use of NCPA (Nested Clade Phylogeographic Analysis) in paper 3. Since this method has been shown to make incorrect (false positive) inferences about population history in 75% of cases, why use it when the haplotype network already allows the same story to be suggested. Finally there is a remarkable result in paper 2 with the occurrence of a secondary contact of the parasite lineage S of *Polyplax serrata* in central Europe, detectable with mitochondrial sequences, but not with the parasite nuclear markers and neither with host mitochondrial and nuclear markers. This is to my knowledge quite unique and an exciting model for further studies.

Contrary to the high quality of the published papers, the 17 pages of the thesis introducing them lacks clarity. It is not always easy to follow the logic in some of the paragraphs from sections 1.1 and 1.2 and several notions/concepts are not very clearly explained (e.g. host specificity, intimacy, ecological fitting hypothesis). The Koop et al 2014 hypothesis of 'hosts as islands' is not new ("limited contact between host's individuals create a barrier restricting gene flow between infra-populations of their parasites. As a result populations of the parasites were fragmented to a greater degree than those of their hosts and more prone to the effect of genetic drift") and this paper just tests one of the predictions presented in Nadler (1995) that the candidate introduces in her previous paragraph. The rest of this section on 'Model organisms' and 'Aims' are clear. The 'Summary of results' section describes the main findings of each paper and a final discussion very succinctly proposes some future directions to explore for each of the host-parasite models. This 17 pages' section suffers from English formulation and spelling mistakes. Finally, since all the papers have been published for some time (last year or even before), an additional paragraph in this section could have discussed some of the results of the papers further in light of newly available studies (I am thinking here about recent studies on the house mouse hybrid zone and their parasites *Syphacia* and *Eimeria*). Fortunately, this lack offers me the opportunity for questions below.

In conclusion, this thesis is an original contribution to our understanding of the evolution and genetic diversity of parasites and brings new pieces to the puzzle of host-parasite co-evolution. I enjoyed very much reading this work and my comments above and the questions that I have for the candidate do not challenge the underlying scientific value of the thesis. I thus recommend the PhD thesis of Jana Martinů to be defended.

A handwritten signature in blue ink, reading 'Joelle Gouy de Bellocq', is written over a horizontal line. The signature is stylized and appears to be written with a pen or marker.

Joelle Gouy de Bellocq, PhD
Institute of Vertebrate Biology
Research Facility Studenec
The Czech Academy of Sciences
Studenec 122, 675 02 Konesin

Questions

Paper 2 *Polyplax serrata* in *Apodemus*

1- Generally the mt cyt b marker is used for rodent phylogeography. This would have allowed you to supplement your dataset with data from Genbank from additional geographic areas (including the data used to infer the phylogeographic histories of *A. sylvaticus* and *A. flavicollis*). Why was the D-loop region chosen instead?

2-The observed geographic structure of the *Polyplax serrata* S mt clades is remarkable because it is consistent with a secondary contact of louse taxa, yet no similar geographic structure is detected with the louse nuclear markers, nor with the host's mt or nuclear markers.

In contrast, for the house mouse model and its parasite *Syphacia obvelata* (nematode) (Gouy de Bellocq et al. 2018), a similar geographic structure is seen across all data compartments: parasite mt and nuclear, host mt and nuclear, as would be expected after secondary contact of hosts carrying diverged intimate parasites. The two cases are in sharp contrast despite similar host divergence times (long term isolation in refugia of 0.4 to 0.6 My mentioned in the paper for *A. flavicollis* – house mouse subspecies separation is about ~0.5 My) and both involving intimate parasites.

Why do not the microsats in the case of *Polyplax* show any genetic structure? Could that come from the way they were characterised and selected? Indeed you used a pool of individuals from the N and S louse lineages during the microsat development and not Swest and Seast lineages only...? Do the two parasites (*Polyplax* and *Syphacia*) present different evolutionary rates that will explain why the pattern is visible for *Syphacia* and not for *Polyplax* if we assume the divergence time and the re-contact time of the host taxa are comparable for the two systems?

Paper 3. *Menacanthus* chewing lice in birds

1-Haplotype 39 found in Cisticolidae in Senegal is quite divergent from the rest of f *Menacanthus eurysternus* clade. Could that be a different species?

Paper 4. *Eimeria* in rodents

1- Methodological question: What is the percentage of co-infection in the samples (estimated by microscopic observation and after observation of the sequence chromatograms)? How many different species of *Eimeria* can be simultaneously found in a sample? I think this is an important detail to provide in the paper because the methodology used with direct sequencing of PCR products may be prone to generate chimeric dataset for individuals with co-infections, the sequence from one marker belonging to a given *Eimeria* species and the sequence for the other marker from a different *Eimeria* species.

2- A recent paper currently available in BioRxiv (Jarquin-Diaz et al 2019) and citing your paper questioned the lack of resolution of established markers (the ones used in your paper) and showed that a multilocus genotyping approach allows a host species level resolution for one *Eimeria* species *E. falciformis* but not for *E. vermiformis* in the house mouse. On the figures of this paper, both house mouse *Eimeria* species cluster with *Eimeria apionodes* from Arvicolinae and *Apodemus* – so there is clearly some overlapping of the datasets but which is not easy to compare for somebody not familiar with *Eimeria*. Do the results of this paper affect some of the conclusions of your study?

Minor comments

In papers 1 and 2, the designation of the bank voles is incorrect. The correct taxonomic name is *Myodes glareolus*. Note that the name is correctly used in paper 4.

Paper 2:

“*Clethrionomys*” (*Myodes*) *glareolus* is not mentioned in your Materials and Methods but 5 individuals are mentioned on the map and some individuals are mentioned in Table 1 and Supplementary Table S1.

12S tRNA → 12 S rRNA (p28)

P28.

“For the hosts, *Apodemus sylvaticus* and *A. flavicollis* phylogenies were rooted with three individuals of the other species (three of *A. sylvaticus* with *A. flavicollis* and vice versa).” There are only 2 sequences in the phylogenetic tree of *A. flavicollis* and 1 in the phylogenetic tree of *A. sylvaticus*!.

P34. Paragraph 3.3 Figure S13 and S14 have been interchanged.

The supporting information Document S1 is not available on the website of the journal.

Paper 3

(Abstract) It is suggested that frequent **host switching** maintains gene flow between *M. eurysternus* populations on unrelated hosts in local populations.

I think “host switching “ is not appropriate in this sentence and should be replaced by “transmission between unrelated sympatric hosts”

The “host switching” process implies that the parasite will subsequently adapt to the new hosts. It seems here that it is just sharing of hosts.

References:

Goüy de Bellocq J., Ribas A., Bryja J., Piálek J. & Baird S.J. (2018). Holobiont suture zones: Parasite evidence across the European house mouse hybrid zone. *Molecular Ecology*, 27, 5214-5227.

Víctor Hugo Jarquín-Díaz, Alice Balard, Anna Mácová, Jenny Jost, Tabea Roth von Szepesbéla, Karin Berktold, Steffen Tank, Jana Kvičerová, Emanuel Heitlinger. Generalist *Eimeria* species in rodents: multilocus analyses indicate inadequate resolution of established markers. 2019. bioRxiv 690487; doi: <https://doi.org/10.1101/690487>

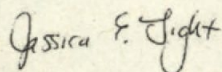
1 October 2019

To Whom It May Concern,

I have read Ms. Jana Martinu's dissertation with interest. Her dissertation is, in my opinion, suitable to be defended. I think Jana did a lot of work, certainly enough to justify receiving a PhD. The four main publications resulting from her dissertation research are scientifically sound and well written, and I think these manuscripts contribute significantly to the literature on host parasite associations. I think she could make some corrections to her introduction and summary pages, mostly little grammatical changes and clarifying some unclear sentences/sections. I have included my comments as track-changes on her dissertation, which I have sent to her advisor. Below, I include some broader questions for Jana to consider as part of her defense.

- 1) *Polyplax serrata* is a widespread louse species in general, parasitizing many host species across a large geographic area. Could this widespread host and geographic distribution affect your results (manuscript 2)? If yes, how?
- 2) For manuscript 2: What effect might homozygote excess (heterozygote deficiency) have in population genetic studies? (this question refers to manuscript 2)
- 3) What other explanations might there be for the discrepancy between host evolutionary history (postglacial recolonization) and parasite evolutionary history (beyond differences in symbionts)? Could the lice be responding to something about the hosts that is not observable using mtDNA or microsatellite data? (this question refers to manuscript 2)
- 4) After analyzing your 3 parasites groups, what do you think of Fahrenholz's Rule? Do you think this Rule may apply more for a particular type of parasite or host? Along the same lines, after completing your research, how important do you think cospeciation is in determining host-parasite associations?

Again, I think Jana has done a fine job with her dissertation research, and the work is suitable for defense. Many thanks for allowing me to review her research.



Jessica E. Light
Associate Professor and Curator of Mammals
Department of Wildlife and Fisheries Sciences
Texas A&M University

October 1, 2019

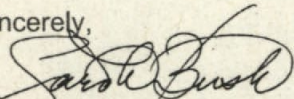
To Whom It May Concern,

I reviewed the dissertation of Jana Martinu: "Host specificity, genetic variability and genealogy in populations of model parasite species." The work presented is a solid contribution to the field. Moreover, it contributes to an area of biological science that is in great need of active research. I am especially impressed that so many of the chapters have already been published in peer-reviewed journals. I recommend without reservation that Jana Martinu proceed with a defense of this research.

Although, I cannot be at the defense in person, I would like to pose the following questions to Ms. Martinu for her consideration:

1. Regarding Nadler's hypothesis: are there any cases of a non-specific parasite having less genetic diversity than a specific parasite?
2. How does "intimacy" relate to co-phylogenetic congruence? Is it possible for a parasite to have an intimate interaction with their host but not exhibit co-phylogenetic congruence? Conversely, is it possible to have co-phylogenetic congruence without an intimate interaction?
3. I find the "oscillation theory" intriguing. If you were to rigorously test this hypothesis, how would you design the study? (Imagine that you could sample any organisms, and money was unlimited).
4. The data clearly show that *Menecanthus* spp. as a group do not follow the pattern of cospeciation with hosts that is often (but not always) seen among other parasitic lice. You mention that this makes the genus *Menecanthus* an ideal system with which to understand the evolution of host specificity. Other than what you have already done, what do you envision we can learn by more research in this system?

Sincerely,



Sarah E. Bush, Ph.D.

President, International Society of Phthirapterists
Associate Professor
School of Biological Sciences