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to

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discipline of biological sciences

**Examination of the of the PhD thesis of Adrien QUILES entitled “Evolutionary histories of symbioses between microsporidia and their amphipod hosts: contribution of studying tow hosts over their geographic ranges”**

The thesis was conducted in the framework of a joint PhD between the University of Burgundy (Joint research unit Biogeosciences UMR 6282) and the University of Lodz (Department of Invertebrate Zoology) under the supervision of Dr. Thierry RIGAUD and the co-supervision of Dr. Rémi WATTIER and Dr. Karolina BACELA.

The focus of the thesis is the evolutionary consequences of host-parasite interactions using amphipods and their obligate intracellular parasites, the microsporidia. The question of the evolution of symbiotic associations ranging from parasitism to mutualism, is a major open question. It is indeed recognized that symbiotic relationships play an essential role in the evolution of species, so that a host and its symbionts are now considered as a single evolutionary entity called a holobiont. It is in this context that Adrien Quiles' work has been carried out by trying to understand what are factors structuring at the phylogenetic level the diversity of microsporidia infecting two gammarids, *G. roeselli* and *G. balcanicus*, over their geographical range. The choice of this model system is relevant and judicious because i) amphipods from the *Gammarus* genus are highly diversified exhibiting high level of cryptic species, some of them being invasive, ii) they are recognized to play key roles in aquatic systems and iii) they are often used as models of host-parasite interactions. The phylogenetic relationships of the two gammarids was previously established by a Polish-French consortium using a large sampling scheme and published by Mamos *et al.* (*Molecular Ecology* 2016, **25**(3) 795-810, DOI: 10.1111/mec.13499) and Grabowski *et al.* (2017 *PeerJ* **5**, e3016, DOI: 10.7717/peerj.3016). The candidate has greatly benefitted from access to this excellent data set which was completed by his own contribution to sampling.

The thesis of Adrien QUILES consists of five chapters. The first one, “General background”, put the thesis in context, introducing the symbioses and their evolutionary consequences and the amphipods-microsporidia systems. The core part of the thesis are presented in the following chapters. The chapter I is a study published in *Parasite & Vectors* (2019, DOI: 10.1186/s13071-019-3571-z, IF=3.03) entitled “Microsporidian infections in the species complex *Gammarus roeselli* (Amphipoda) over its geographical range: evidence for host-parasite co-diversification and recent host shifts”. The chapter II explores the host-parasite association between *Gammarus balcanicus* species complex and the diversity of its microsporidian parasites. The chapter III focusses on the peculiar case of *Nosema granulosis* infecting *G. balcanicus* and *G. roeselli*. Finally, in chapter IV “General conclusion and perspectives”, results of the work are put in context and directions for future research are given.

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The form of the manuscript is a dissertation by articles including one already published study (A. Quiles being the first author) in the chapter I, and two preprints constituting the chapters II and III (unfortunately, no information is provided on the journals targeted). In order to avoid potential pitfalls of such a format, Adrien Quiles took the precaution of introducing the subject in a large first chapter (General background) so that the reading of the manuscript remains pleasant and allows to realize the mastery of the subject and the work done by the author. Methodological aspects are sufficiently developed in the publication or in the preprints to satisfy the reader's curiosity. The manuscript is well written and illustrated with a selected iconography.

The "General background" chapter is a presentation of the concepts involved in understanding the evolution of symbiotic interactions. This chapter is rich and accompanied by the appropriate literature but perhaps deserves to be more precise on certain points. In particular, in the "Symbioses and their evolutionary consequences" section, the theoretical expectations of the evolution of symbiotic associations with vertical (VT) or horizontal transmission (HT) would have merited longer developments. Indeed, these expectations are presented on page 8 dealing with the different transmission strategies of microsporidia but without further discussion: what are the evolutionary implications of these strategies and why? The following sections introduce the amphipods-microsporidia associations. These sections nicely present the subject of the thesis by introducing the problem of the diversity of gammarids and microsporidia. These sections are illustrated with figures from the literature. However, some of these figures should have been more explicit. For example, Figure 5 is not very clear and another presentation could have been chosen. Similarly, figures 6, 8 and 10 representing the phylogenetic relationships of microsporidia and gammarids could have been better discussed and synthesized in the text. In particular, the classification of the microsporidia based on the origin of the host is clearly not pertinent as this classification is paraphyletic. The phylogenetic tree of Gammaridae (Fig. 8) from Hou et al. (2014) should also be discussed as many nodes of the trees are not well supported. However, these illustrations help to understand the complexity of the diversity of both the parasites and the hosts. Adrien Quiles' essential contribution to this introduction is the collection from the literature and sequence databases of all records of the presence of microsporidia infecting amphipod species (Table 1). Although these results are rarely discussed in the text, they are a very good source of information for the topic. This chapter ends with a description of the objectives of the thesis. The objectives are clearly presented and the work plan is well introduced.

Chapters I and II are very similar in their objectives as they aimed at identify and determine the phylogenetic relationships of microsporidian parasites of the gammarids *G. roeselli* (chapter I) and *G. balcanicus* (chapter II). Therefore the methodologies are identical since they use the same genetic marker (the small subunit rRNA gene). It is unfortunate that these chapters were not preceded by a short introduction of the studies. In particular, we would like a justification for the choice of the marker. We understood that the amplification of SSU rDNA was quite difficult and we wonder if an alternative strategy might have been possible. Indeed, it is well known that ITS (Internal Transcribed Spacer), located between structural ribosomal RNAs, is especially useful for elucidating relationships among closely related species, constituting one of the common genetic markers used for Microsporidia typing and more generally for fungi. As such, information from the genetic markers used to identify microsporidia could have ideally complemented the Table 1. The sequencing strategy should also be discussed: i) direct sequencing of PCR products results in the sequence of the dominant haplotype that masks the possible variations and ii) why using the forward primer alone for sequencing? Therefore one other concern is the definition of the haplogroups: as the resulting sequences (seeing alignments in supplementary material) are highly variables in lengths and in some parts does not overlap, it is not clear if the strategy for defining haplogroups is conservative or not.

Chapter I consists of the article published in 2019 in *Parasites & Vectors*. The main result is that the evolutionary history of the *G. roeselli*-microsporidia associations is depending of the transmission pattern of the parasites: a co-cladogenesis for vertically transmitted parasites (*Nosema granulosis* and *Dictyocoela*



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*roeselum*) where multiple host shifts suggest recent acquisition of the other microsporidia (*D. muelleri*, *Cucumispora* sp. and some other rare parasites). The results presented in chapter II show that the infection in *G. balcanicus* is more diverse than in *G. roeselli*. However the pattern seems similar with an ancient co-diversification of the vertically transmitted parasites *N. granulosis*, *D. roeselum* and *D. muelleri* and a more “patchy” distribution for the other microsporidia. A short conclusion comparing the results obtained from the two hosts studied would have been useful. Here is some minor comments for Chapter II: the Figure 3 (p. 83) is difficult to read on the paper copy of the thesis (it is better on the numeric document) due to the blue background of some labels. The prevalence disequilibrium results (see Table 1, p. 99) should be taken with precaution as the power of the Fisher exact test fall to *ca.* 30% for *D. muelleri* and *D. roeselum* and to 3% for *D. duebenum*.

Chapter III focus on *N. granulosis* infection across *G. roeselli* and *G. balcanicus* by using an alternative genetic marker, the DNA-directed RNA Polymerase II largest subunit (*rpb1* gene). The use of this coding gene allow a finer resolution of the phylogeny of *N. granulosis* even if some haplogroups previously determined with the SSU rDNA maker were not amplified. The results could perhaps have been better exploited by using the phylogenetic networks and split tree methods (e.g. D. H. Huson and D. Bryant 2006 Application of Phylogenetic Networks in Evolutionary Studies, *Mol. Biol. Evol.*, **23**(2):254-267, DOI: 10.1093/molbev/msj030) allowing the identification of reticulate evolution. In summary, the results are in favour of an origin of the association at the very beginning of the differentiation of the *Gammarus* genus, followed by two evolutionary trajectories of the parasite: one clade of vertically transmitted *N. granulosis* exhibiting low genetic diversity, and the other clade of horizontally transmitted parasites retaining more genetic diversity. This result, if confirmed, is very interesting as it could confirm theoretical expectations.

The general discussion includes the major results and convincingly discusses the contradictory results obtained during the thesis. Several perspectives are presented to corroborate or discuss the results and arguments from recent literature support this point.

In conclusion, at the end of a consistent work carried out on a complex model, Mr Adrien Quiles brings original and important results supported by one publication and probable further valorisations. He has done an impressive amount of work and demonstrated a strong competence in the analysis of host-parasite interactions. Therefore the thesis meets the requirement for doctoral dissertations according to Polish and French regulations. **Taking all these elements into account, I give a favourable opinion for admission of Mr. Adrien Quiles to further stages of the doctoral dissertation with the view of obtaining the degree of Doctor within the framework of a joint PhD between the University of Burgundy and the University of Lodz.**

