

Review of a PhD dissertation by MsC Michala Tůmová entitled

"The relationships among functional traits of tardigrades (Tardigrada), their diet, and environmental conditions"

The PhD dissertation focuses on the ecology, feeding behaviour, and functional groups of tardigrades – a poorly studied group of ubiquitous microscopic invertebrates. Although the Tardigrada phylum is known to inhabit diverse ecosystems, its role is recognized to a small extent, and each attempt towards understanding its function in the ecosystem is highly appreciated. I have had the great pleasure of reading the PhD thesis of Michala Tůmová, who presented novel data generated by using time-consuming observations and well-designed experiments submitted as original papers and a manuscript.

The dissertation is 140 pages long, divided into *Introduction*, three *chapters*, *thesis summary*, and *curriculum vitae*. Additionally, before the *Introduction* there are sections such as *annotations*, *declarations* and *acknowledgments* included in the thesis. One of the chapters has been prepared as a manuscript which is ready for submission, whereas two others have already been published as articles in prestigious scientific journals. The fact that other colleagues have already evaluated the papers, makes the role of a reviewer even more difficult. But I wholeheartedly appreciate the efforts of MsC Michala Tůmová in preparing the manuscripts. It means that the PhD candidate is familiar with the process of a manuscript preparation and review, which allows her to be one step ahead with respect to academic and scientific experience.

I. Introduction

The introduction consists of a robust background of the PhD study and some fundamental facts concerning the ecology of tardigrades. The author of the thesis introduces the reader to some basic information about tardigrades: their relationship to environmental factors, the phenomenon of cryptobiosis, the role of tardigrades in soil food webs, their diet, functional traits, and species diversity. The most significant papers have been cited, both the old and the most recent ones, which indicates that the PhD candidate is familiar with both the “old-school” and the contemporary literature. Although the introduction sounds reasonable, I would replace some sections and intuitively begin with the position of tardigrades in the tree of

life, followed by their general morphology, systematics, habitats, and species diversity, and then continue with the description of their role.

Despite the fact that introduction provides a great deal of important information, it lacks some basic information concerning the biomass or density of tardigrades in ecosystems, which is crucial in assessing their role. It is worth mentioning that biomass might be a functional trait.

On page 12, the author describes the reasoning behind using functional traits in the studies on tardigrade ecology instead of species diversity. A similar statement has been provided in the annotation "*The goal of the thesis was to show that it is possible to study tardigrade ecology even without species identification*". I am convinced that particular approaches correspond to specific scientific questions. Nobody claims that species identification is necessary for studies on the ecology of invertebrates. Marine, freshwater, and soil ecologists often used trophic guilds or biomass as ecological predictors, so I find this explanation satisfactory.

On page 6, the author claims that "*the knowledge about their typical abundance in various habitats is usually based on one or two local studies*". As a matter of fact, there are dozens of good studies on the spatial distribution and diversity of tardigrades in a large scale e.g., Dastych 1985 - Spitsbergen, Dastych 1988 - Poland, Kaczmarek et al. 2011 - Costa Rica. Finally, the Introduction lacks information about the second order of tardigrades - mainly Heterotardigrada.

These few minor comments, however, have no impact on a general, positive impression of the Introduction.

II. Chapter I

The first chapter of the thesis has been published in the prestigious *Zoological Journal of the Linnean Society*. Definitely this part of the thesis is a milestone in studies on tardigrade feeding behaviour. The author used laboratory experiments with 18 potential food sources for three tardigrade species representing different feeding modes (herbivorous, omnivorous, and carnivorous). Moreover, the author compared feeding modes of adult tardigrades and juveniles, then compared their survivability under different food regimes. What is substantial in the study, is that the author stated that gut content analysis may be misleading as a method of studying tardigrade feeding habits. The paper is novel, well-designed, and provides original data which required a long, time-consuming observation of living animals.

The authors used cyanobacteria and eukaryotic algae, bryophytes, fungi, protozoans, and metazoans including nematodes, and rotifers and other tardigrades as a potential food for

tardigrades. The analysis revealed that tardigrades considered to be herbivorous or carnivorous have a much more complex diet and feed on available food sources. However, tardigrade *Milnesium inceptum* can only lay eggs on animal prey. Moreover, the observation of tardigrades revealed that they differ among themselves with respect to the method of consumption, for example the swallowing versus sucking of prey.

It is a great shame that the author did not use the representatives of tardigrades with flexible buccopharyngeal apparatus like the genera *Pilatobius* or *Diphascon* which have been suggested to be microbivorous (by Hallas & Yeates 1972). It would fully explain how tardigrades (Eutardigrada in particular) with different morphology of buccal apparatus select and then consume food.

I have some concerns about testing fecundity. Even though animals laid eggs, it doesn't mean the offspring will hatch. I have personal experience with *Dactylobiotus* species which laid tens of eggs but without proper food, eggs were without embryos.

I am glad that the author showed that tardigrades may feed on cyanobacteria. The cyanobacteria genus *Phormidium* drew my special attention. This taxon creates thick mats when compared to other cyanobacteria, and it is known to produce EPS (extracellular polymeric substances) that can impede consumption. Indeed, *Phormidium* may produce cyanotoxins, but the concentration during the experiment would have been too low to affect tardigrades. The EPS is the most reasonable explanation.

The author observed that tardigrades used in the experiment did not consume mosses. However, there have been three species of tardigrades used and, as it has been written by author of the thesis, other scientists revealed strong trophic links between bryophytes and other tardigrades. Mosses and hepatics offer a broad spectrum of associated organisms as a food for tardigrades. Moreover, some recent papers revealed a strong need of heterotardigrades for bryophytes. This topic definitely requires more attention.

Finally, although a sample size of each treatment seems to be low (10 specimens per treatment), I am perfectly aware how time-consuming and hard the observation of tardigrades is. Therefore, I am fully convinced that the results provide valuable contribution and a corner stone for future experiments on the diet of water bears.

III. Chapter II

The second chapter is the unpublished manuscript entitled "*Species and trophic group compositions are determined by soil physical properties in the polar habitats*". The author conducted a very important study, highly valuable not only for understanding soil tardigrades

but also microinvertebrate communities in Arctic soils – the latter has only been studied at basic level. The PhD candidate investigated the difference in soil tardigrade communities of five polar habitats (which covers almost all potential soil ecosystems for tardigrades in Svalbard) using tardigrade diversity and trophic groups composition. The results showed that habitats differ with respect to tardigrade species and a trophic group composition as well as overall abundance, species richness, and diversity.

The analysis of the trophic group composition showed that early successional stages (glacier forelands) are represented only by herbivores, whereas the carnivores and omnivores were present only in the habitats with long development time (dry and wet tundra), which is in accordance with the paper of Zmudczyńska et al. (2017) who found that the complexity of a trophic network increases along with the age of tundra.

I really appreciate the efforts of only collecting a soil layer from each sample as well as the measurements of soil's physico-chemical properties. What is more, the author used body size and confirmed that small pores in the soil can host smaller tardigrades. Additionally, the author showed that hydration capacity of substratum is a more important factor than the actual moisture, which is of great significance as polar terrestrial ecologists have often used SWC (soil water content) as an ecological predictor. Finally, owing to this piece of this work, Arctic soil biologists can gain some knowledge about microinvertebrates in soil and potential factors shaping their assemblages.

Due to the fact that chapter II has not been published yet, I would like to draw attention to a few issues that could be resolved during the next stages of correction or at least incorporated after reviewing the manuscript.

- I would be happy to find the explanation of how abiotic factors may determine the role of tardigrades in a food web. I believe the determination of the role is much more complicated and requires a specific analysis of animals' feeding modes and behaviour.
- Why is a bird cliff fellfield an unstable habitat? Unstable with respect to what? A dynamic glacier foreland seems to be much more unstable than mature tundra under cliffs.
- In the introduction the author referred to Jennings (1977) in terms of seabird colonies in Svalbard. Indeed, Jennings was working on ornithogenic soils but in Antarctica, close to penguin colonies.

- It is worth to emphasize that samples were collected on two opposite sides of Billefjorden, which differ in history and geology. One analysis showing that geology does not influence tardigrade diversity would be a good point to the discussion since Dastyh (1985, 1988) suggested that geological setting strongly influences tardigrade assemblages.
- I cannot see the description of a bird cliff. What bird species inhabit the cliff? It is important because for instance *Alle alle* and *Risa triadactyla* feed on different food and produce different guano (acidic versus alkalic) which influences both tundra cryptogams and invertebrate assemblages.
- I am sceptical as to using Bermann funnels for tardigrade extraction. Such a method provides information about alive and active specimens only. The eggs and animals in dormant stages remain in the samples. It is especially dangerous with respect to dry soils, mostly from crust and forefields.
- 20 samples per habitat is a relatively small sample size when compared to other papers. I would be careful with strong statements like, for example, the presence of exclusively herbivorous species in a forefield.
- The author claimed they have identified species according to Degma et al. (2021), which is a checklist. I believe the author used specific keys, revisions and redescriptions.
- How has the author defined stoniness and bulk density? What is more, in table 2 the PhD candidate provided differences in a mineral particle size. It is highly unlikely for small stones around of 0.5-1 cm in size to be absent from the forefield samples. Did the author remove them?
- Table 3. after the species name the author and the year of description should be provided.
- Taking into account that the author extracted only 167 individuals of tardigrades and 77% of them have been found in tundra habitats, I believe all the analyses should be treated with caution. I would rather focus on the comparison of tardigrade species and their numbers to previous studies (forefields, birdcliffs), or alternatively calculate diversity indices like Chao.
- I was surprised to learn that the author did not measure pH, which can shape tardigrades in Arctic terrestrial ecosystems. Both geology and birds are responsible for shaping pH conditions in soils, which then impacts invertebrates. It should be included somewhere in the discussion.

I would be grateful if PhD candidate could explain all these issues.

The appendix deserves a positive comment. The candidate has carefully described trophic groups and species found in the samples. Such descriptions are of high importance and yet, they are missing from modern papers.

IV. Chapter III

The last chapter has been published as an article entitled "*Buccal tube dimensions and prey preferences in predatory tardigrades*" in a specialist and prestigious journal *Applied Soil Ecology*.

Again, the PhD candidate has shown a novel approach to using functional traits of tardigrades in ecological studies. Due to the fact that tardigrades are important consumers in soil ecosystems, for example they feed on nematodes and soil algae, the knowledge on their feeding preferences is crucial in understanding ecological networks, formation, functioning and evolution of soil ecosystems. Moreover, the understanding of how soil communities are controlled by predators and grazers is crucial in the era of global changes of soil ecosystems and maintaining them in a sustainable condition.

Therefore, the PhD candidate tested if the preferences towards prey can be better predicted by species identity of a predator or by selected functional traits, i.e., body size, buccal tube length, buccal tube width, and buccal tube width/length ratio. The candidate used nine predatory tardigrade species including various genera in manipulative laboratory conditions for the preferences for three types of microfauna prey. Additionally, the author tested if the preference for a specific prey type can be predicted by predator species identity or body and buccal tube dimensions. The buccal tube length was revealed as the best predictor of tardigrades' prey preference. It is a very interesting finding. Until now, the width of the buccal tube was usually regarded as significant in the division of trophic groups (carnivorous versus omnivorous). The PhD candidate suggested a novel approach that can be applied in a more detailed division of tardigrades into trophic groups. This chapter has already been reviewed by specialists, so I can only provide a few comments about minor mistakes. In table 1, the style of the list of tardigrade species is inconsistent. Sometimes the author uses et al. between the author of species and a year, sometimes et al., and sometimes omits a dot or a comma. A proper, commonly adopted punctuation is a dot and a comma.

The discussion about cannibalism is truly appealing, and I believe it is an excellent direction to take in future studies.

V. Summary of academic and scientific achievements

Regarding the *Curriculum vitae*, the PhD candidate is a co-author of papers related to functional traits of invertebrates as well as the ecology of Arctic terrestrial environments. All the papers have been published in approved scientific journals. According to Scopus, the total number of citations amounts to 80. The candidate took part in international conferences, has been working in a few projects related to tardigrades and/or soil ecosystems functioning. Moreover, the candidate took part in scientific expeditions to Ladakh and Arctic. In the course of her academic career, she supervised high school and bachelor students as well as taught soil zoology, soil biology, botany and zoology. In my opinion, all these achievements and the candidate's practical experience make her fully equipped to get academic background for future work at a university or another academic or scientific centre.

The PhD candidate is a highly motivated person with a good ability of planning and conducting experiments. In my opinion, Michala Tůmová is an independent scientist. **After my evaluation of the PhD dissertation, I would like to express that the thesis meets all the standards of a PhD thesis, and I definitely recommend it to be defended.**

Santa Caterina di Valfurva, 01.08.2022.

