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Dear Committee members,

it was a great pleasure for me to read and assess the PhD thesis of Michala Tůmová. The thesis entitled "The relationships among functional traits of tardigrades (Tardigrada), their diet, and environmental conditions" comprises on 133 pages an Introduction, 3 chapters and a Thesis Summary. The three chapters are written in the form of scientific articles, two of which have already been published in high-ranking, peer-reviewed scientific journals: Chapter 1 in 2020 in Zoological Journal of the Linnean Society (impact factor 2020: 3.3), and chapter 3 in 2022 in Applied Soil Ecology (impact score 2021: 5.3). Chapter 2 seems to be prepared for submission or is even already submitted. Michala is first author in the two papers and the manuscript.

According to her own statements, Michala had a 70% share of the work in two of the three papers (she performed the extensive laboratory experiments together with a co-author), and 50% in the third, in which the demanding taxonomic identification of the tardigrades was entirely her responsibility, I will come back to that). The analysis of the results, the statistical evaluation and the leading part of the writing was in all three papers by Michala.

Michala's thesis deals with soil-inhabiting tardigrades. This is already much appreciated, as soils are highly important ecosystems because of the many functions and services soils provide for us. And soils are highly understudied in terms of tardigrades. Most studies of terrestrial tardigrades so far consider moss habitats. With her investigations, Michala was able to contribute substantially to our understanding of the soil food web structure and of the relations of soil tardigrades to their biotic and abiotic environments.

From the **formal point of view**, the introduction and thesis summary, as well as the three chapters are well structured and written in a scientifically correct manner. The contexts are easy to understand and her conclusions are straightforward to follow. Only, in the introduction, I missed a citation in some (few) places, e.g. page 6 last paragraph and page 7 first paragraph. Also, once a publication is not correctly cited: Jönsson et al. 2007 on page 4,

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first citation without et al. But these are minor details only mentioned as there were no bigger issues.

The **Introduction** provides an excellent overview of the state of the art in tardigrade feeding ecology and all major publications in the field. It discusses our knowledge of influencing environmental factors, food sources, feeding behavior, species and functional diversity, and considers how traits can help infer unknown feeding relationships. The introduction leads very well to the topics and research questions in the three chapters.

In the subchapter on cryptobiosis, several statements were too general in my view, for example: "in case of unfavorable conditions, tardigrades can dispose of the water and in less than 10 minutes enter the state of cryptobiosis". Not all tardigrade species are able to enter cryptobiosis and if they can, not all of the specimens survive. It has been observed that even populations of the same species do not have the same ability. *What would you say is the trigger that one population has the potential for anhydrobiosis and another population of the same species does not?*

Michala states that one of the ecological significances of tardigrades in soils is their "ability to regulate nematodes". I would again say that this is too general and also a bit misleading. *What tardigrade feeding types would be able to regulate nematodes? Is there any study that has really proven that tardigrades could regulate nematodes in the field? Is it our desire to regulate all nematodes in the soil? What do you know about so-called pest nematodes and would tardigrades be able to feed on these?*

Chapter 1 – this is a published paper on laboratory experiments where 3 tardigrade species (a carnivore, a herbivore and an omnivore) were placed on 18 different food sources. Michala tested 10 adults and 10 juveniles of each species with each food source in order to determine if they switch from one food source to another while growing adult. Thus, an impressive total of 1080 animals were studied in individual approaches with one food source each. Their guts were checked after 12 hours whether they were filled or empty. She found that many more food sources were ingested than expected from their feeding types, e.g. tardigrades of all feeding types ingested cyanobacteria, algae and fungi *Saccharomyces* (yeast, first record as food of tardigrades). None of the tardigrades fed on moss and none on protists (*Euplotes*). *What mouth structures allow tardigrades to feed on all sorts of food items, even those that are bigger than their mouth cavity? Michala, you did not test bacteria. Wouldn't this have been a possible food source as well?*

Michala also performed long-term experiments (30-40 days) in order to see if tardigrades can live and reproduce on these diets (algae, fungi or rotifers). Herbivorous *Hypsibius exemplaris* lived and reproduced on algae and fungi, omnivorous *Paramacrobiotus fairbanksi* on all three food types and carnivorous *Milnesium* only on rotifers. Together the long- and short-term observations demonstrated that tardigrades may ingest much more than what they digest, which makes gut analyses questionable. *What would you say is the role of learning in tardigrade feeding behavior? Would you say it might be easier for the tardigrades to forage and feed on a food source that they got used to before the experiments on their culture plates, e.g. rotifers for *Milnesium* and *Paramacrobiotus*, *Chlorella* and *Chlorococcum* for *Hypsibius* and *Paramacrobiotus*?*

Chapter 2 – this is a yet not published manuscript. Michala compared functional and species composition of tardigrade communities and their significance with respect to different successional stages in a field study in the Arctic (Svalbard). I agree with Michala: Species identification is very difficult with tardigrades, there are few characters: claws, bucco-pharyngeal apparatus, in some groups cuticle and eggs. There are few specialists who feel confident to do species identification. It's all the more noteworthy that Michala has worked her way into it. I highly appreciate that. *Where did you learn tardigrade identification?*

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I assume that the climate changes a lot in Svalbard. *What date and season were the samples taken? How long are the soils frozen during the year?* p.71 Michala explains lack of omnivores and carnivores by low abundance of prey. *Are there data of other soil animal groups = rotifers and nematodes from the same samples?* It would be interesting to correlate them.

I particularly like the supplements with the description of the feeding types, which clearly explains how the morphological differences were used in the present work to group species into the 4 feeding types. I much appreciate as well the listing of the species with their identification characteristics and the indication of observed differences from the original description. Both are highly valuable in assessing the work done and help in the discussion and comparison of similarly oriented field studies.

Chapter 3 – this is a published paper on food choice lab experiments with 9 species presented with 3 food sources simultaneously: tardigrades, nematodes, rotifers. Michala calculated the number of prey consumed in 12 hours per tardigrade and evaluated the relationship between preferred diet and various measures of the bucco-pharyngeal apparatus. So far there are only few food choice experiments with tardigrades and thus the results of this study are new and valuable. The authors found that the majority of tardigrades preferred nematode prey over tardigrades and discussed this with different behavior and locomotion of the different prey groups, which in general makes a lot of sense to me. The authors argue that the artificial undulation behavior exhibited by nematodes when they are outside a 3-dim. pore structure, helps them escape when attacked by tardigrades. I think that this is the case, only if the prey density is high, then this behavior confuses the predator. At low prey densities, as in the present work, another consequence of nematode wiggling should actually become important: Wiggling increases the probability of contact between predator and prey and thus decreases search time and increases feeding rate. Thus, it may be a factor that actually explains the preference for nematodes. *What do we know about tardigrade sense organs, with which they experience their environment and find their prey?*

I especially liked the **Thesis Summary**, because it nicely sums up the merit of the three studies, convincingly assesses their significance. And it provides perspectives on investigations and methods that must now follow to answer existing and emerging questions. For example, manipulative experiments under more natural conditions with more than two levels of food web.

In summary, I believe that Michala Tůmová has made a substantial contribution to our understanding of tardigrade communities in soil habitats, of the role of tardigrades in the soil food web and she helped translating morphological traits into functional traits. The quality of the thesis is great and the author clearly demonstrated her ability to defend her research to other scientists and produce sound publications. She proved knowledge on relevant literature, building experimental designs, data analyses and discussion of the results obtained in a larger context. I have really enjoyed to read it and recommend this thesis to be accepted for the award of PhD.

Görlitz, 29 July 2022

