Review by an official opponent
of the dissertation for the degree of Candidate of Biological Sciences
of SU JIAHUI
«Structural and functional characteristics of testate amoeba assemblages in terrestrial habitats of the Northern Eurasia»
Specialty 1.5.15. Ecology (Biological sciences)

The dissertation by Su Jiahui employs diverse methodologies to characterize the communities of testate amoebae across a wide range of sphagnum bogs and drained habitats in northern Eurasia. In the view of most ecologists, the subject of the dissertation, communities of the testate amoebae, primarily serve as sensitive indicators of past climatic conditions. This underpins a certain one-sidedness in many studies, which tend to concentrate on the responses of testate amoebae to abiotic factors, chiefly moisture regime and groundwater level. In contrast, the present dissertation predominantly addresses the ecological roles of testate amoebae, their adaptive strategies, and the functional niches of species, thereby improving the interpretation of testate amoebae responses to environmental change. This defines the novelty and relevance of the work.

Another novel and actual component of the work is the use of functional traits for both the classification of testate amoebae communities and the assessment of their relationships with the environment and biotic surroundings. In this regard, the dissertation aligns with contemporary developments in soil ecology that have been implemented across a wide range of taxonomic groups. However, for the community of testate amoebae, such a scale and level of detail appear to be undertaken for the first time. A new classification system, developed based on 18 traits, offers an alternative, or rather an addition or supplement, to traditional morphology-based approaches. Perhaps even more important, it provides a basis for a mechanistic understanding of microbial ecology, as it is translated into morphological and functional adaptations.

A high degree of scientific novelty is reflected in the defended statements, which are well substantiated by the study results. The dissertation's conclusions combine more specific observations (e.g., Conclusion 1) with broader, fundamental propositions, such as Conclusion 4. Yet all of them are directly corroborated by the materials presented in the work.

The practical significance of the study, despite its focus on immediate ecological relationships, appears to lie largely in the application of testate amoebae as indicators of past environmental conditions. The trait-based approach for historical reconstructions is still at an early stage, and the presented work undoubtedly makes a significant contribution to the development of this approach.

The dissertation is organized in a traditional manner and is divided into introductory sections, a literature review, materials and methods, three chapters presenting research results, and a conclusion. Noteworthy, the chapters containing results are based on publications in high-impact international journals. In most of these publications, Su Jiahui is the lead author, which further confirms the relevance and high quality of the dissertation.

The overall length of the dissertation is 157 pages, and the bibliography contains 196 entries. The work is written in clear, accessible language with only a minimal number of typographical errors. The abstract fully reflects the content of the dissertation.

Remarks on the work. Against the background of the generally favorable impression produced by the dissertation, I deem it necessary to discuss several points that arose questions or elicited objections.

First, in my view the dissertation (especially given its specialization in Ecology) lacks explicit working hypotheses. Undoubtedly, biological adaptations and ecological patterns are addressed, but primarily in the form of

descriptions and observations. The presence of clearly stated and well-grounded working hypotheses would not only help to structure the work more coherently and foster better integration of its parts, but also to highlight various aspects of its novelty.

The study employs a very developed and sophisticated statistical apparatus, which, however, at times appears detached from the underlying biological phenomena. In addition, the figure captions are sometimes insufficiently detailed, compelling the reader to infer the statistical or methodological meaning behind the presented results. For example, in Figures 23, 24, 25, and especially 26A and 27B, statements such as "only significant effects with $p < 0.05$ are shown" are used, yet in some cases the slope is not significantly different from zero. What exactly constitutes a significant influence of longitude or precipitation on species richness? Similarly, the assessment of statistical significance for differences in Figure 22E and some other panels seems rather liberal.

In summary, while the statistical analyses are strong, their alignment with explicit biological questions and hypotheses should be enhanced. It would also benefit from more precise figure annotations to avoid ambiguity in the interpretation of results.

Literature Review. The literature review covers the key questions, yet in many parts, such as sections 1.2, 1.3.4, 1.3.5, 1.4.1, and several others, it remains too general and detached from the core subject of the study; testate amoebae are not even mentioned. Furthermore, the dissertation is aimed at readers well familiar with the fundamental principles of ecology who do not require examples such as "For example, in arid ecosystems, where water is scarce, microbial communities are dominated by drought-tolerant taxa…" (p. 37). In some cases, trivial information could obviously be replaced with more interesting and up-to-date examples drawn from the biology of testate amoebae, especially given the extensive literature available.

Deviation from the main subject of research sometimes leads to strange statements. I was surprised to learn that "ants, fungi, and parasites tend to exhibit higher species richness at elevated latitudes … (Vasconcelos et al., 2018; Větrovský et al., 2019; Johnson, Haas, 2021)" (p. 22). In the case of ants and parasites, this statement largely does not hold true, as the cited references describe local situations and specific cases. Regarding fungi, more recent reviews also do not support this claim (Niskanen et al. 2023). I cannot help but agree with the author's own remark on a related issue: "some research has targeted specific habitats or regions, which could introduce biases and lead to incomplete knowledge regarding the true diversity and distribution" (p. 23).

Most important, given the computational nature of the dissertation, there is a conspicuous lack of discussion in the literature review of key metrics of functional diversity used in the study, and on the statistical approaches used in general. For example, the null-model metrics such as SES.MPD are not covered in the literature review. This omission is particularly annoying because SES.MPD underpins a central conclusion (Conclusion 4) of the study, and a more thorough treatment would enrich the reader's understanding of the analytical framework and its implications.

The main criticism about the **Materials and Methods** chapter is the lack of many details in the description of the study design. For instance, I did not understand what "All data" means in Fig. 1 and why is there almost no information in the text on the samples from Kamchatka, Karelia and the Baltic Sea area, although there is probably a lot of data from these locations. Data on these points are completely missing in section 2.1.3 with a description of climatic conditions and various latitude and longitude gradients. A complete and detailed table describing all the analyzed samples should be provided, as is done in section 5.2, but not in other sections.

Despite being tagged “crucial”, words on page 47, at the end of the section, are pretty vague. Who did the fieldwork is clear, but what did the "all data" provided by Yuri Mazei include? Which numerical data on the structure of testate amoeba communities were obtained from published works, and which were obtained anew (if any)?

Methodological issues include some problems with the terminology. The work is replete with phrases like “In contrast, mineral soils, which tend to have lower moisture levels and organic matter content...”. Mineral soil is constantly mentioned as opposed to sphagnum bog habitats, including in the context of "nutrient-poor mineral soils" (p. 39). However, in the methods we learn that samples of "mineral soil" included leaf litter near tree butts, litter under tree crowns, inter-crown spaces, and sites dominated by mosses, *Sphagnum* sp., and *Cladonia* sp." (p. 46). Where there is a place for the actual mineral soil remains unclear. Perhaps it was necessary to look for other definitions for well-drained habitats.

Results. Figures 7 to 12 (Chapter 3) seemed of little use, since they illustrate correlations of features with abstract axes constructed based on these same features. Undoubtedly, such correlations should take place. However, such exercises are more technical in nature, unlike a very informative Table 3, which clearly summarizes the clustering results and the features of functional groups.

Strangely, the conclusion to Chapter 3 discusses the preferred habitats of different functional groups. The topic is undoubtedly important and interesting, but nothing is said about it in the chapter itself.

The general tone of Chapters 3 and especially 4 sometimes seems overly optimistic (“Compared to taxonomic diversity, functional diversity and traits hold greater ecological significance ... offering a deeper understanding of ecosystem function and resilience"). There is no doubt that direct measurement of morphological and physiological features provides information about ecological interactions. However, the taxonomic system is also a way of

dividing the continuum of life into units according to a certain set of characteristics. In this narrow sense, the only fundamental difference is the discreteness and continuity of the two systems, but with a sufficiently high number of species in the community, it becomes insignificant.

The already accumulated (and very extensive) experience of using the trait approach in various fields of ecology does not give grounds to say that it is always more productive than the old "taxonomic diversity" approach. In any case the trait (or functional) approach deserves healthy criticism and discussion, which I did not find in this work.

Meanwhile, the dissertation provides sufficient grounds for such a discussion – for example, the taxonomic approach in section 4.1.2 suggests much more significant differences between habitat types and regions than the "functional" approach in section 4.2.1. As for functional traits and the community-weighted means (CWMs) of functional traits as the dependent variable in the section 4.2.2, they produce a pattern close to the taxonomic approach. Moreover, formal statistical rules in this case likely require adjustments for multiple comparisons, which do not seem to have been made. At least, this is not reflected in the captions of figures and tables.

Ultimately, the last Chapter 5 of the Results did pretty well with the traditional taxonomy.

These critical remarks should be the subject of discussion and do not diminish the significance of the dissertation research. The dissertation of Su Jiahui meets the requirements and standards established by the Lomonosov Moscow State University for candidate dissertations. The content of the dissertation corresponds to specialty 1.5.15. Ecology (Biological sciences), and adheres to the criteria defined in clauses 2.1-2.5 of the Regulations on Awarding Academic Degrees at the Lomonosov Moscow State University. The dissertation research was completed and formatted in accordance with the requirements of the Regulations on the Council for the Defense of Dissertations for the degrees

of Candidate of Sciences and Doctor of Sciences of Lomonosov Moscow State University.

Thus, the applicant Su Jiahui deserves to be awarded the degree of Candidate of Biological Sciences, specialty 1.5.15. Ecology (Biological sciences).

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