**Referee report on the PhD thesis of Ana Ticha "Changes in nutrient availability imprinted in long-term diatom succession in lakes"**

by Enikő Magyari (Professor in Earth Sciences, Department of Environmental and Landscape Geography, Eötvös Loránd University, 1117 Budapest, Hungary)

Long-term changes in the primary production of lowland and mountain lakes in the context of ongoing climate change and intensification of both aerial and runoff derived nutrient enrichment gives particular importance to the topic studied by Ana Ticha using paleolimnological methods on Bohemian upland and lowland lakes.

Mastering herself in diatom analysis, she worked as an important member of a lively and excellent research team that worked out many scientific papers in the last couple of years on the Holocene paleoenvironmental and paleolimnological change using the Bohemian Massif glacial lake sediments.

The thesis consists of a collation of three scientific papers, one first authored published paper, one co-authored published paper, and one first authored, but yet unpublished paper. Her contribution to these scientific works is clearly indicated in Page 3.

Overall, my impression is that these three papers demonstrate the rapid progress of mastering herself in the numerical techniques that by the third paper led to quantitative diatom based inferences of past pH and available P changes in Lake Prášilské jezero, Bohemian Massif, Czech Republic.

The merits of the works:

My general impression is that the candidate knows the relevant scientific publications and uses them adequately.

In her interpretation of the supporting proxies she understands and uses particularly well the chemical record, takes into account terrestrial vegetation changes and invokes the results of cladoceran and chironomid analyses. This shows that she understands aquatic ecosystem functioning very well.

The number of records analysed by the candidate in scope of her PhD is reasonable (Lake Komorany: 32 samples, Lake Prášilské jezero: about 30-45 samples).

She applied consciously and consistently diatom concentration and influx markers: divinylbenzene microspheres that greatly improved the conclusions to be drawn on diatom production vs environmental change.

She also consistently applied selective counting of diatom valves to avoid under-representation of rare diatom species.

The introduction chapter is very well written, for me it gave an overview on the trophic changes, climate-determinism and soil-change related questions well.

There are only a few weaknesses of this excellent work, what I have to mention. I missed photo-documentation of the diatom work in the thesis. In the supplement, I would have expected a more detailed photo documentation of the sites studied (thesis format allows for it). I found that the results section of chapter 4 is still a bit messy, particularly the description of the results is difficult to follow. Since basic diagrams were published in the supplement, reading of the thesis was sometimes cumbersome. Furthermore, I expected that the thesis introduction will advance the scientific literature on diatom paleolimnology in the Czech Republic, I missed very much a historical overview here.

I had several specific comments and questions to the thesis, of which I list below the most important comments and highlight in bold my questions:

***1.4.3.1 Direct and indirect records, Page 11:*** I think you should elaborate this water column P availability  and sedimentary P concentration better. Many dimictic lakes are characterised by anoxic P-release that distort strongly this relationship and in dystrophic lakes you often get a reversed relationship between sedimentary P and diatom inferred available : (see e. g. Magyari et al. 2009, Lake St Anne, diatom-based P reconstruction and P conc. in the sediment). They can behave antagonistically.

Page 21: some information on current base rock would be essential here with chemical composition.

Fig. 8 on Page 38: **If the chronology is correct then we may say that the Younger Dryas (YD) remains invisible in the Lake Komorany pale-records, and LOI increase date the YD onset. How do you explain this?**

Page 39: I think the low accumulation rate in this shallow lake probably preclude the separation of the YD even if 1 mm resolution sampling is applied. YD often shows up as extended ice cover period in lakes that shifts the pH into negative direction, but this is associated often with dominant benthic taxa. **Quantitative pH reconstruction would have been important here. Why was it not attempted here?**

**What you observe here, the decrease in trophic level at the onset of the Holocene must be, in my opinion, connected to the expansion of conifer dominated forests and pH decrease in the lake that led towards brownification and dystrophy. Why do you not invoke in your interpretation this possibility?**

Page 40: **Not clear from this section what was the dominant canopy around Komorany at the Holocene onset. From the diagram it is likely that *Pinus* and *Betula* were the main canopy trees, and you should have expressed these pollen curves as influx curves to get some information on the population size changes, as both are notorious pollen producers. Can you show us this relationship?**

Page 40: „*The recorded gradual development of terrestrial vegetation documents a very limited climatic forcing of the studied lowland environment at the LG/H transition transferring to a less pronounced response of the lacustrine ecosystem* „ This is probably a false inference given the lack of pollen influx data. Your species spectrum remained the same, but your LOI values indeed show that the landscape became more forested, soil development started and it was likely in the podzol soil direction.

Page 42: „*A more probable connection of P. kawraiskyi to low temperatures rather than low nutrient level supports our interpretation of there being relatively high nutrient levels since the LG.* „ In this respect, please note that nutrient concentration was high in several lowland and even mid-altitude lakes in SE Europe during the last glacial termination (e.g. Lake Balaton, Lake St. Ana; Cserny & Bodor, 2000; Buczkó et al., 2022, Magyari et al., 2022). This all suggests weak soil formation, less chemical weathering, but intense loess accumulation with Ca, Fe, and to some extent P entering the lakes via aerial transport as well in this period. In addition, soil liquids were not yet low pH. See also Schaller et al (2019). **Overall, my question is how glacial conditions at Lake Komorany influenced the chemical composition of runoff water and the inflowing stream water in your opinion?**

Page 43 last section: **Please, explain how your diatom and chironomid assemblages and the chemical composition of the sediment of Lake Komorany reflect seasonal anoxia during the Late Glacial and its disappearance at the onset of the Holocene. What would have been the effect of a pH decrease on the chironomid fauna at the onset of the Holocene?**

Page 46 top section: Here again I miss the consideration of the pH change and it consequence on P availability. No mention of the pre-industrial exploitation water chemistry, and pH.

Chapter 3 Page Page 53: „*The ratio of chrysophycean stomatocyst total abundance and diatom total abundance (C:D; chrysophycean:diatom ratio) was used as a proxy for water mixing (Werner and Smol, 2005).*" **In this chapter C-D ratio is used as water mixing indicator. Please, explain why you considered water mixing as the main driver of C-D ratio change**? The literature is very wide, and depending on the study region, C:D ratio seems to be used on an increasingly versatile way, but looking through the relevant literature, it is still considered to be mainly driven by water level change and the duration of winter ice cover. **Under what circumstances can water mixing be the mains driver of C:D ratio change?** A justification is not provided in this chapter, not to mention that from the same lake you use this ratio on a modified way ($C.D_{per}$) to infer a thermal stratification imprint.

Page 66: **Could you please inform us about the current oxygen availability profile of Lake Prasilske? You use the diatom, cladoceran and chironomid fauna here to argue for decreased oxygen availability with intensified browning of the lake since 6400 cal BP. Please, list chemical indicators of oxygen depletion at this point in the sediment. Can water-depth increase alone not explain the change in the aquatic biota? How intensified browning show up in your diatom flora at his point?**

Page 75: **The application of C:D ratio should be discussed at the viva**, because different literatures use this differently. It was used as a productivity proxy in the past, with higher Chrysophycean cyst ratio in case of nutrient shortage, you mentioned it in the previous chapter as a proxy of turbidity in the water column, and in this chapter it is used as thermal stratification imprint. **Please, show us some examples where C:D ratio was used to infer change in thermal stratification!**

Page 81: I miss here a description of the pH range of the applied training sets. Neither a mention of the taxonomical problems (new nomenclature) is provided here.

Page 82: bit messy description of all proxies in one zone that is defined by the diatom assemblages mainly; pity that the ecological indicator value of the guilds is not explained further up. ***"Bottom***

***substrate species*": this ecological group is difficult to imagine. What do you mean on bottom substrate group? What is the difference between well-oxygenated bottom substrate group and this? Is this the profundal group?**

Page 82: **Please, explain the ecological indicator value of the following diatom guilds: low-profile, high-profile, motile!**

Page 90: If I get it right, you suggest that even though planktonic diatoms and chrysophytes became more abundant, their overall influx decreased suggesting a less productive lake. **Could you please, show the euplanktonic influx values in the discussion to support this inference? Why do you have more plankton in a brownified acidic water? Please, explain!** (I could not find a total diatom influx curve from the lake, and it seems that euplanktonic diatom influx increased considerably in this zone, it replaced tychoplanktonic and periphytic species. This overall suggest to me that in the water column available nutrient level increased. This is against your interpretation, please explain why?)

Page 96: **Although you admit the large standard error of the reconstruction, would you please introduce us the di-pH and di-P reconstruction statistics (RMSE, R, ML). The SE values are very large in both record, could you please explain us what is causing this large error range?**

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