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Challenges in using *Chara contr*aria A.Braun ex Kützing oospores as taxonomic markers

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INTRODUCTION & AIM

Chara contraria A.Braun ex Kützing is an endangered charophyte species in Serbia, widely recognized for its highly variable thallus morphology, which often hinders precise and reliable identification, leading to misidentification. Oospores, resistant reproductive structures, had already been used as promising taxonomic markers for the delineation of several charophyte species in Serbia. However, the oospores of this highly variable species have not been examined in Serbia before. In this study, we aimed to describe *C. contraria* oospores and to explore their usefulness as taxonomic parameters.

METHOD

A total number of 537 *C. contraria* oosporangia containing mature oospores were collected from the individuals from various localities in Serbia. The oosporangia were left in 30% hydrogen peroxide for up to 10 minutes, after which the spiral cells and coronula were removed, and the oospores were prepared for further analysis. First, visual inspection was performed, based on the general appearance of the oospores and the appearance of their apical and basal poles. The oospores were photographed and a standard set of quantitative characteristics was measured. Analyzed oospore specimens were subjected to K-means clustering, an unsupervised machine learning procedure, to determine the potential existence of homogeneous subgroups within them and further investigate their variability.

RESULTS & DISCUSSION

The oospores of *C. contraria* ranged in color from dark brown to black and showed considerable variability in their general appearance. K-means cluster analysis further confirmed the observed

qualitative variability, by identifying three clusters. The parameters that contributed most to the separation into clusters were oospore height, length, and the isopolarity index.



Figure 1. Considerable variability in the general appearance of *C. contraria* oospores. Scale bar: 200 μm.

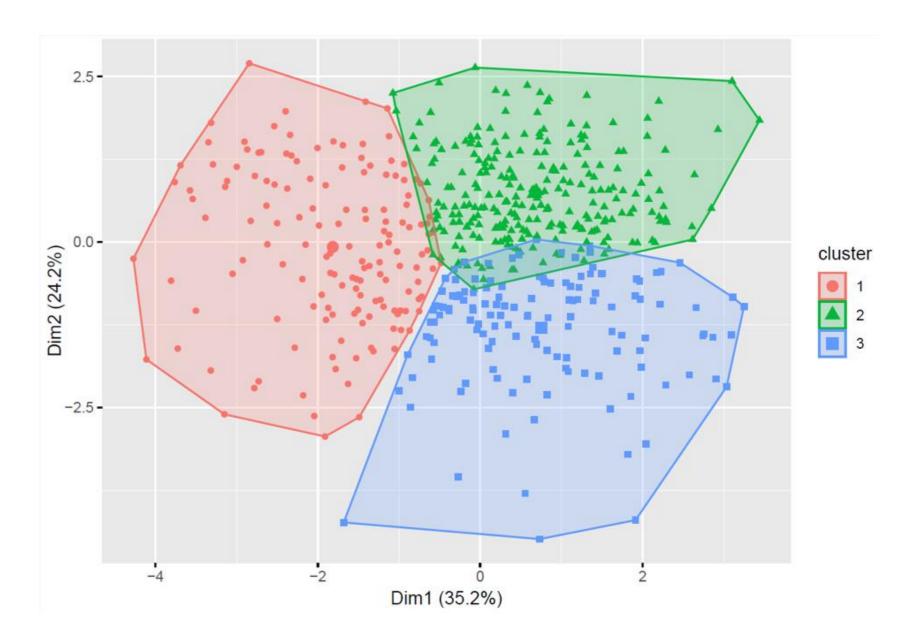


Figure 2. K-means clustering of *C. contraria* oospores.

CONCLUSION

Our results suggest that the application of *C. contraria* oospores as taxonomic parameters is limited by the pronounced variability of both qualitative and quantitative characteristics.

FUTURE WORK

Integrating molecular and morphological approaches is recommended to enhance the reliability of taxonomic assessments.