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Cover
Ranunculus peltatus Schrank (pond water-crowfoot), a freshwater hydrophyte species observed in lentic waters of Portugal, South-Western Europe; Francisca C. Aguiar.

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Developing eDNA-based methods for exploring charophyte algae diversity in lakes - Oral presentation

Over the last two decades, eDNA metabarcoding has rapidly evolved into a cost-effective and time-efficient methodology, widely used in areas such as biodiversity assessment, biomonitoring, invasive species control, and the discovery of rare, cryptic and unknown species. This has boosted a great number of new studies and the development of new methods. Still, eDNA studies on aquatic plants lag behind those on animals, as it is much more difficult to find suitable eDNA markers, but a considerable number have recently emerged. Yet, an important group of aquatic plants, the charophytes, remains neglected. Charophyte algae play a vital role in aquatic ecosystems and contribute to a better ecological status of the ecosystems. Due to their sensitivity to water quality, they have long been used for ecological status assessment of lakes. However, their diversity is often underestimated as they have great morphological plasticity and sometimes short life cycles. Within the BIOLAWEB project, we investigated charophyte algae diversity in lakes using both eDNA metabarcoding and a traditional morphological approach. Samples were collected in two saline and two freshwater lakes in Serbia, in three seasons. rbcL gene marker was used for metabarcoding and Oxford Nanopore technology for sequencing. Bioinformatic analyses were conducted using the cloud-based metagenomic platform CZID, using NCBI as a database. The aligned sequences were checked manually and the consensus sequences were then aligned with confirmed sequences from the Boldv4 database. The morphological approach revealed the presence of charophytes in three out of four lakes. Two lakes had only one charophyte species, both belonging to the genus *Chara*, while the third lake had six species belonging to three genera, *Chara*, *Nitella* and *Nitellopsis*. The eDNA metabarcoding approach detected the presence of charophytes in all four lakes, all belonging to the genus *Chara*. However, due to the high similarity of the selected rbcL barcode, the *Chara* sequences could only be assigned to two broad charophyte species-complexes (*Chara hispida* and *Chara vulgaris* complex). This study is the first time eDNA metabarcoding has been successfully used to detect charophytes in lakes. To provide species-level identification and identify representatives of all genera, further fine-tuning of the method is required, particularly in generating primers that better discriminate charophyte diversity.

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