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A DNA (meta)barcoding approach to tackle marine benthic biodiversity

Macrobenthos is recognized as a good biological indicator to measure changes in marine ecosystems. However, biodiversity assessments require accurate species identifications, which are commonly based on morphological features. DNA barcoding (species) and metabarcoding (communities) may provide a fast alternative. We developed a DNA metabarcoding method using Illumina MiSeq technology. Various barcoding primers were checked against publicly available sequences to select the most optimal barcode region and primer sequences for the macrobenthos species present in our study area. Next, amplicon sequencing was executed using barcoding primers designed for the 18S target region. DNA extracts of individual species, and of pooled samples in which tissues or DNA extracts of different species were mixed, were amplified using this method. This setup allowed us to check the effectiveness of the primers to detect species in single or mixed samples, and to investigate the relationship between read counts per species and the proportion of species in mixed samples. Based on the 18S target region, 39 of the 50 macrobenthos species were detected. For some species (e.g. *Nephtys* sp.) this setup will not allow us to discriminate between species of the same genus. As species of the order Amphipoda were not detected, an additional target region (COI) was included. COI amplicons of individual species were Sanger sequenced in anticipation of our COI metabarcoding results. This setup allowed us to evaluate which DNA barcode provides the best taxonomic resolution for the collected macrobenthos species. First results of the COI barcoding approach revealed an advanced taxonomic resolution for species of the order Amphipoda. The 18S and COI barcode sequences were added to our DNA reference library.

Keywords: DNA (meta)barcoding; macrobenthos; amplicon sequencing; 18S; COI