

## NEXT-GENERATION SEQUENCING (NGS) METHODS IN ENVIRONMENTAL BARCODING – A PROGRESS REPORT ON BIOMONITORING OF BENTHIC RIVER DIATOMS.

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DNA barcoding is a tool that uses a short, standard portion of DNA to identify organisms based on a shared database of DNA sequences. In diatoms, a consensus on an appropriate DNA barcode has not yet been reached, and several markers are still in discussion (e.g. nuclear 18S rRNA, 28S rRNA, 5.8S rRNA + ITS2, mitochondrial *cox1*, plastid *rbcL*). But recent findings in the protist community are favouring the V4 region on the 18S rRNA gene at least as a pre-barcode for biodiversity assessments.

DNA barcoding is especially interesting in regard to high throughput methodology (e.g. NGS) used in the analysis of environmental samples. Therefore we have established a standard procedure for DNA barcoding in diatoms to develop a standardised identification tool to serve routine water quality assessments using the 18S V4 region (Zimmermann et al. 2011; [http://www.gbif.de/botany/barcoding\\_3](http://www.gbif.de/botany/barcoding_3)).

Here we demonstrate the feasibility of using the V4 barcode in a 454-pyrosequencing approach for species-level analysis of benthic freshwater diatoms commonly used for biomonitoring. Although considerable effort is still needed until NGS tools will be commonly applied to identify species from environmental samples, our results indicate the potential of an environmental barcoding approach for biomonitoring programs.

To optimise environmental barcoding with the V4 marker for water quality assessment we chose the river system Neisse/Oder (Czech Republic/Germany) as study area. The diatom flora of the Neisse/Oder is well known and is representative for a typical central European river system. This enables us to compare the findings of the NGS as well as the morphological analysis of the same samples to evaluate the accuracy of the DNA barcoding approach.

This applied part needs as a fundament a liable reference database, which is established at the BGBM, which will provide the basis for a DNA based identification system. To build the reference database DNA barcodes generated via Sanger sequencing of specific diatom taxa (cultivated in unialgal cultures from the environmental samples) are linked to light-/scanning electron microscope-photographed vouchers simultaneously. This database contains up to now sequences of 550 diatom strains. It is constantly updated and will be freely accessible through *AlgaTerra* ([www.algaterra.org](http://www.algaterra.org)).