

Projektledare Maria Kahlert
Projekttitel <i>Streckkodning av sötvattenorganismer för förbättrade biodiversitetsbedömningar</i> Barcoding of freshwater taxa for improved assessment of biodiversity (FRESHBAR)
Diarienummer NV 802-0171-18

WP1 - Kiselalger/Diatoms

In accordance with the WP1 aims, we performed in parallel activities to (1) supplement reference databases with Swedish benthic diatom species, and to (2) establish diatom metabarcoding for freshwater monitoring and assessment in Sweden.

1a. Genomförda aktiviteter/performed activities

- 312 diatom monoclonal diatom cultures (strains) were established using single-cell isolation, of which 258 strains were successfully sequenced with Sanger technique for barcodes rbcL and 18S. A selection of strains were sent alive for long-term storage to DCG Diatoms Collection i Ghent, Belgien and to the UTEX Culture Collection of Algae, USA.
- The morphological identification of the strains is ongoing (SEM pictures taken during a guest visit at BGBM, Berlin, LM slides prepared at SLU). Vouchering of the strains at BGBM has been discussed and prepared.
- The development of long-read sequencing (PacBio) for diatoms was started by guest researcher Demetrio Mora in cooperation with UMBLA (SLU Metabarcoding Laboratory)
- Establishing diatom metabarcoding at SLU was started with UMBLA, now continued at IVMs new molecular laboratory (cooperation with HaV projects 1928-2020 & 1152-21)

1b. Preliminary results & conclusions

- The 258 sequences were preliminary identified to represent 50 species. Identification is challenging and validation will require cooperation with other taxonomic experts.
- A long-read sequencing protocol was successfully established
- Diatom collections could only accept few strains due to capacity limits, and cryopreservation did not work out for many strains, thus long-term preservation of most strains not possible.

2. Project plan

- WP1 follows the project plan as closely as Covid allowed, except that diatom identification & vouchering is delayed due to Covid-closed labs. Furthermore could the long-read sequencing protocol not be tested, and the planned test for other markers could not be performed.

3. Project deliveries

- [FRESHBAR hemsida](#)
- Project meetings (8 under 2020), presentations at HaV 2019 & 2020 (eDNA conference, utförmöte, joint project meetings)
- In general, most planned communication meetings had to be cancelled due to Covid

4. Future time plan

- For WP1, diatom identification, vouchering & long-term sequencing is planned to continue in 2023. Sequences, taxa names and metadata will be incorporated into BGBM, and Diat.barcode, in the end of 2023 at the latest. No prolonged funding is needed for WP1.

WP2 - Bottenfauna/Benthic macroinvertebrates

The WP has addressed whether increased resolution of diverse and taxonomically complex groups of benthic invertebrates, using barcoding/metabarcoding, help to improve monitoring and assessment of human-induced impact.

1a. Performed activities

Metabarcoding of benthic macroinvertebrates from archived samples from national monitoring for the years 2006 and 2017 from 12 lakes were analysed using BF2+BR2 and 16S markers (WP2.1), using only CO1 for 25 lakes and 25 streams in 2017 (preliminary analyses) and from the full set of c. 100 archived lake (littoral, profundal) from 2020 and fresh (2021) samples.

1b. Preliminary results & conclusions

- Metabarcoding of 12 lakes with varying water chemistry identified 78 species of Chironomidae: 69 identified to species and 9 to genus: 6 not previously recorded in Sweden, and 1 not even in Europe. As only c. 20 species are identified in routine monitoring, metabarcoding thus resulted in a marked increase in taxonomic resolution.
- Analyses of among-year differences (2006 and 2017) showed no systematic differences, suggesting that the method worked equally well for older and more recent samples.
- Analyses with 16S-markers, done to test if this additional method would provide a better separation of closely related species, showed that the use of COI was sufficient to identify most species.
- Preliminary metabarcoding results from the archived samples (n=25 lakes and 25 streams from 2017) showed that metabarcoding resulted, as expected, in greater number of species. Furthermore, results for classifying the ecological status of lakes are very encouraging as all five metrics showed significant relationships between morphologically- and metabarcoding-derived indices and EQRs.

Main conclusions

- COI is sufficient to identify most species.
- Results comparing morphology and metabarcoding are very encouraging: (i) metabarcoding resulted in a higher number of species identified, (ii) calculation of national assessment metrics showed significant correlations using morphology and metabarcoding and (iii) we found high coherence in classifications of ecological status. Combined these results suggest that it should be possible to replace morphology-based assessment of ecological status with metabarcoding.

2. Project plan

COVID-19 resulted in considerable delays in field sampling and metabarcoding and difficulties in employing technicians per the proposal.

- Field sampling was carried out in autumn of 2022 and analyses of archived samples has been delayed by a large backlog of samples to process.
- We anticipate that DNA-barcoding results will be available by August/September 2022, at which time we plan to employ a researcher to help with data treatment and analyses.

3. Project deliveries

- Presentations at HaV annual meetings

4. Future time plan

Due to COVID-19 restrictions the project experienced a number of delays in field sampling and in analyses (see above). Given that we are behind in analysing the metabarcoding, we hope to have an extension of the project until 30th of June 2023.