



Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXV

Biodiversity of microphytobenthos combining morphological and molecular approaches

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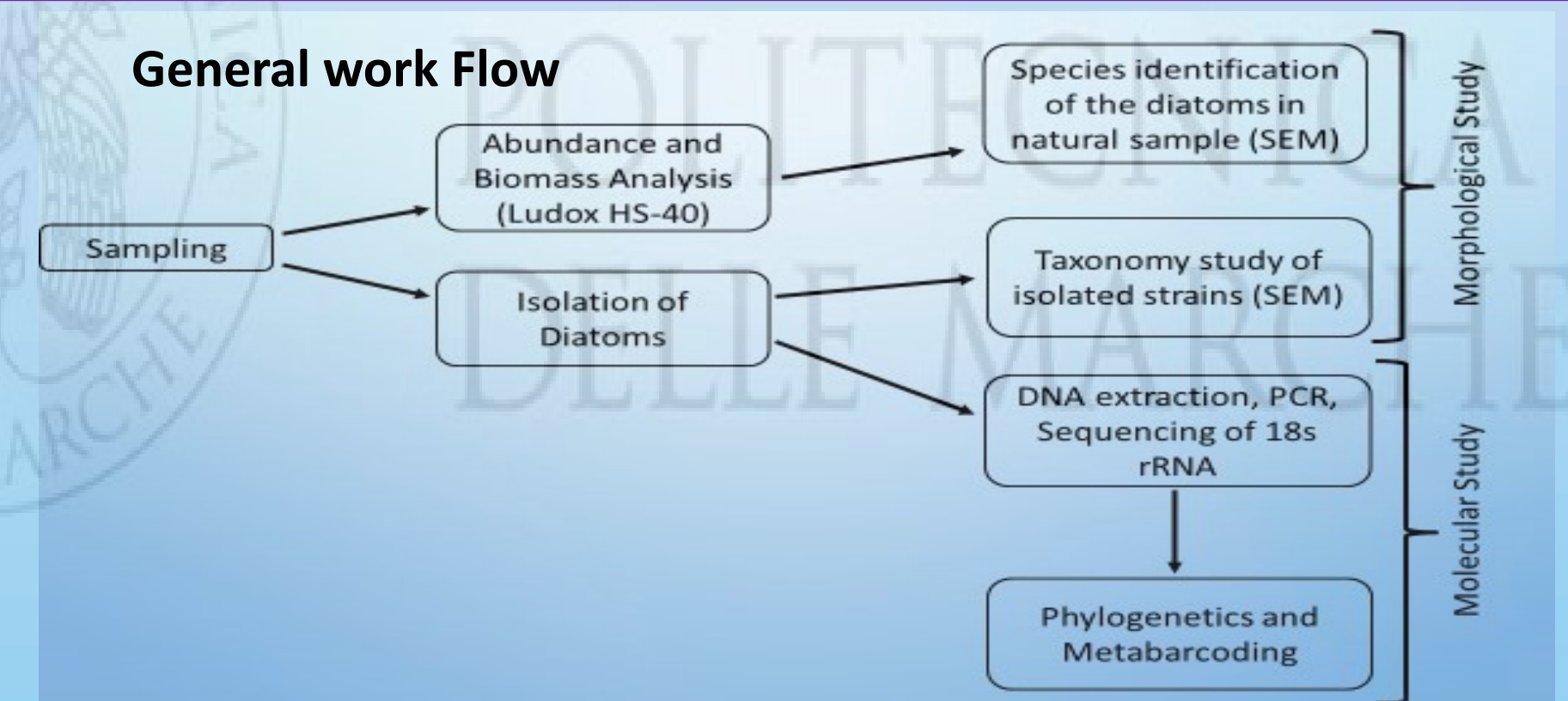
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Introduction: Diatoms are photosynthetic unicellular eukaryotes which are ubiquitous and dominant in the of microphytobenthos (MPB) community. MPB have major contribution in nutrient cycling, carbon transfer and benthic-pelagic coupling (Marques da Silva et al., 2017). They form a biofilm on the sediment which is important to stabilize the sediment and to exchange nutrients between sediment and water column. In the past 30 years, functions of the MPB are studied vastly as compared to their community structure (i.e., species taxonomy, distribution, and diversity) (Park et al., 2014). This is because of the difficulties in the extraction/isolation of cells from sediment grains and limited comprehensive taxonomic monographs which makes the species identification problematic. There are few diatomist who has worked on the taxonomy of diatom communities grown on hard substrata or as epiphytes and epizoic (Totti et al., 2007, 2009, Romagnoli et al., 2014). Biodiversity analysis of benthic diatom communities with polyphasic approach can advance the knowledge of their taxonomy as well as it will improve the quality of HTS analyses and its application in ecological and biomonitoring programs.

Aims and objectives:

1. To study the seasonal variability of epipelagic diatom communities at two Adriatic sites (Senigallia and Portonovo) and its relationships with environmental factors
2. To characterize selected isolated strains from Adriatic and Tyrrhenian Seas through morphological and molecular analysis
3. To study biodiversity of the entire microphytobenthic community through metabarcoding analysis of sediment samples collected in the Gulf of the Naples and in the Adriatic Sea
4. To add taxonomically validated sequences to reference databases
5. To describe new species



Methods

Environmental parameters

- Physical (Temperature, Salinity, Oxygen concentration, pH) parameters measured in the water column using CTD
- Water nutrient analysis (surface and bottom) analysed through QUAATRO Technicon autoanalyzer
- Granulometry analysis: very fine sands (63-125 μm) at both stations

Abundance and biomass analysis

1. **Diatom/Sediment separation:** Gradient centrifugation Ludox HS-40 (Mélédér 2007)
2. **Microscopic analysis (Identification and Counting):** Utermöhl apparatus

Methods

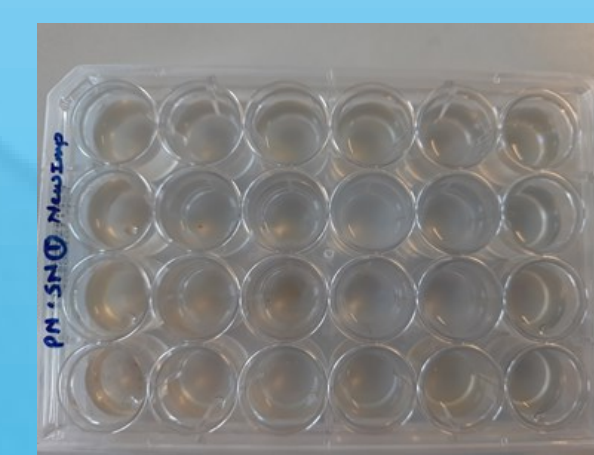
Isolation of diatoms (live strains)

Strain isolation for ultrastructural analysis (SEM/TEM) and molecular analysis (PCR and Sanger sequencing of 18S rDNA).

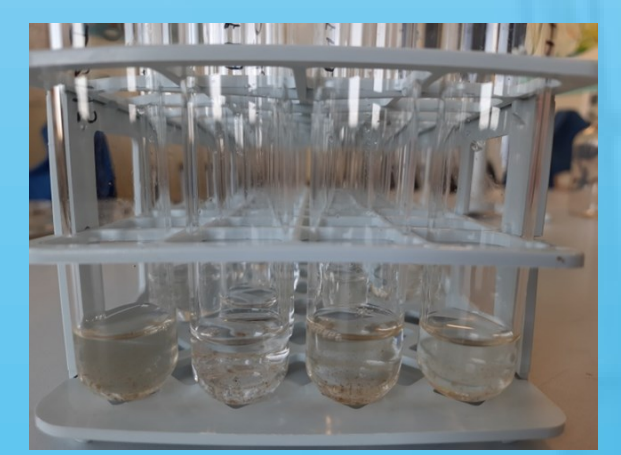
1. **Coverslip method**
2. **Single cell picking with microcapillary pipette**



Coverslip Method



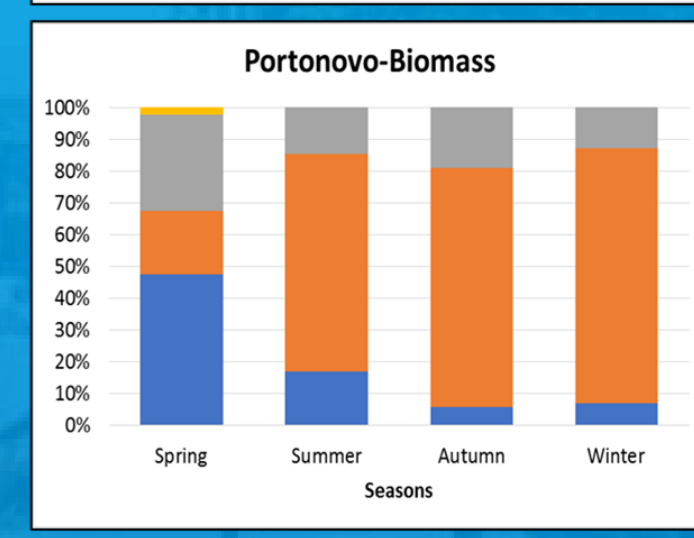
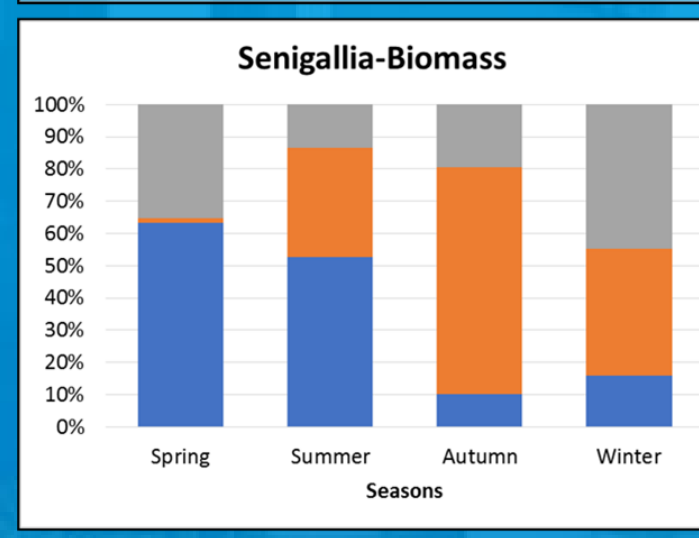
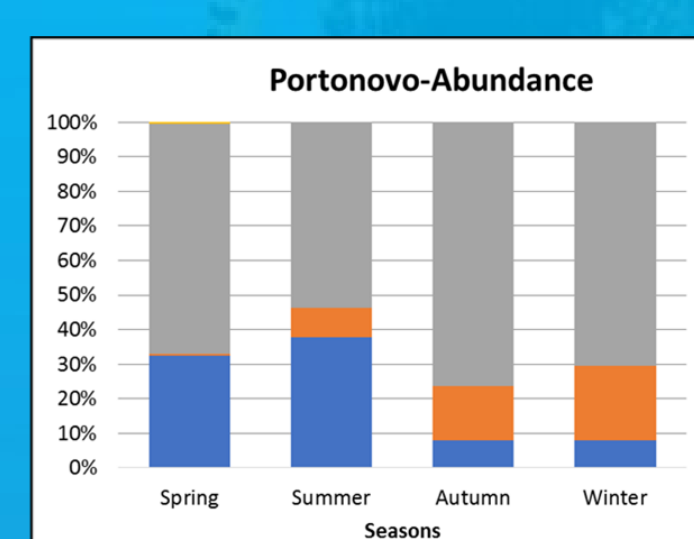
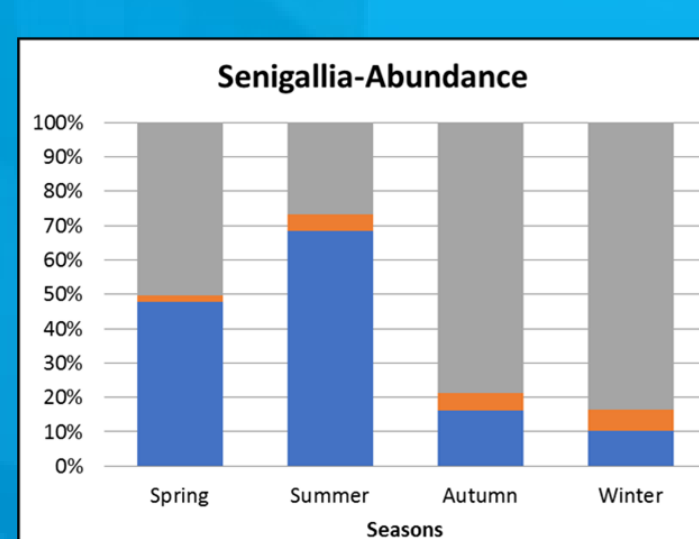
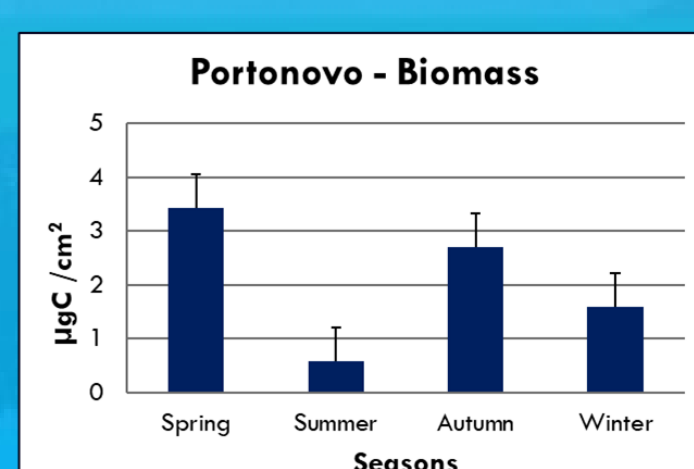
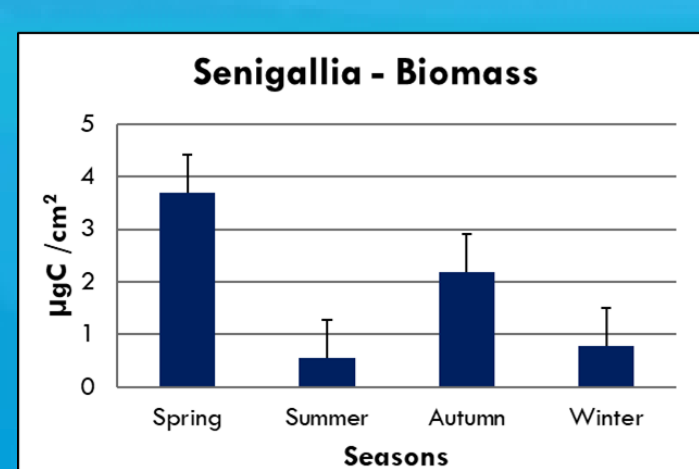
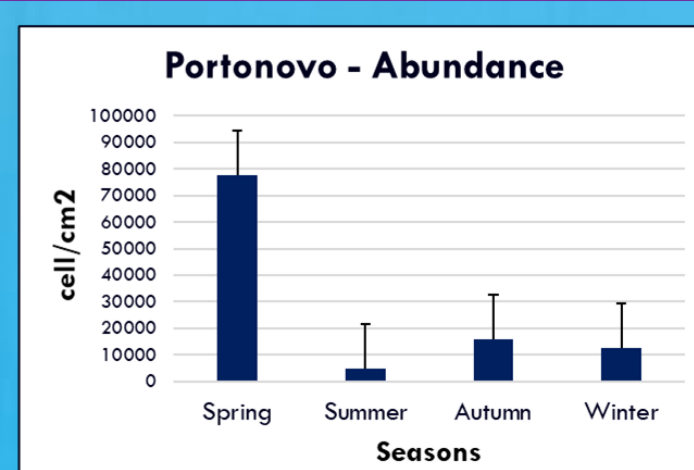
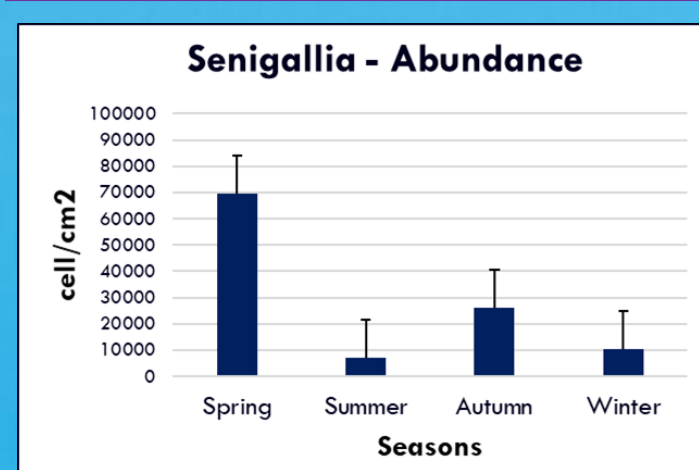
Microwell plate with single cell of diatoms in each



Strains of diatoms maintained in tubes

Results

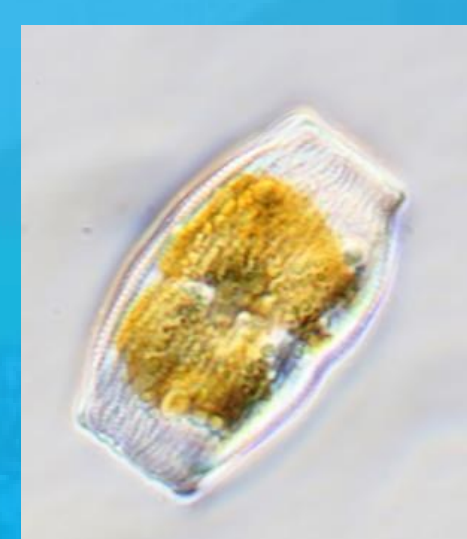
Benthic diatom abundance and biomass in different seasons



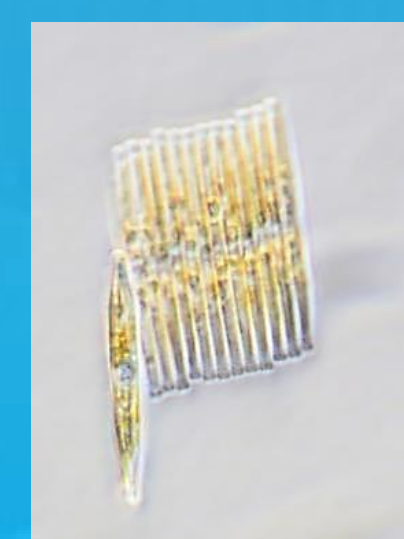
■ Adnate ■ Plocon ■ Motile ■ Erect



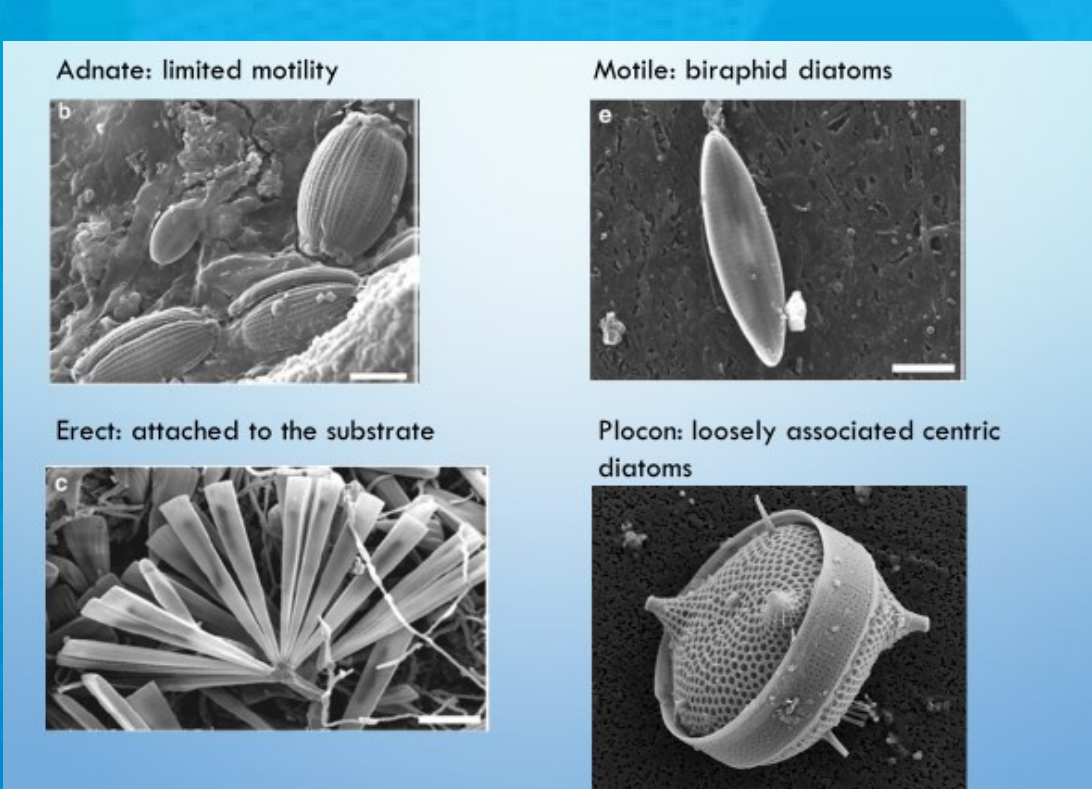
Nitzschia cf. sigma



Amphora sp.



Bacillaria paxillifera



Abundance and biomass of diatom growth forms

Results

1. **59 strains isolated:** 19 from Portonovo, 18 from Senigallia, 22 from Naples
2. **Light microscopy** done for all the strains
3. **Electron Microscopy:** Preparation and SEM/TEM observation in progress

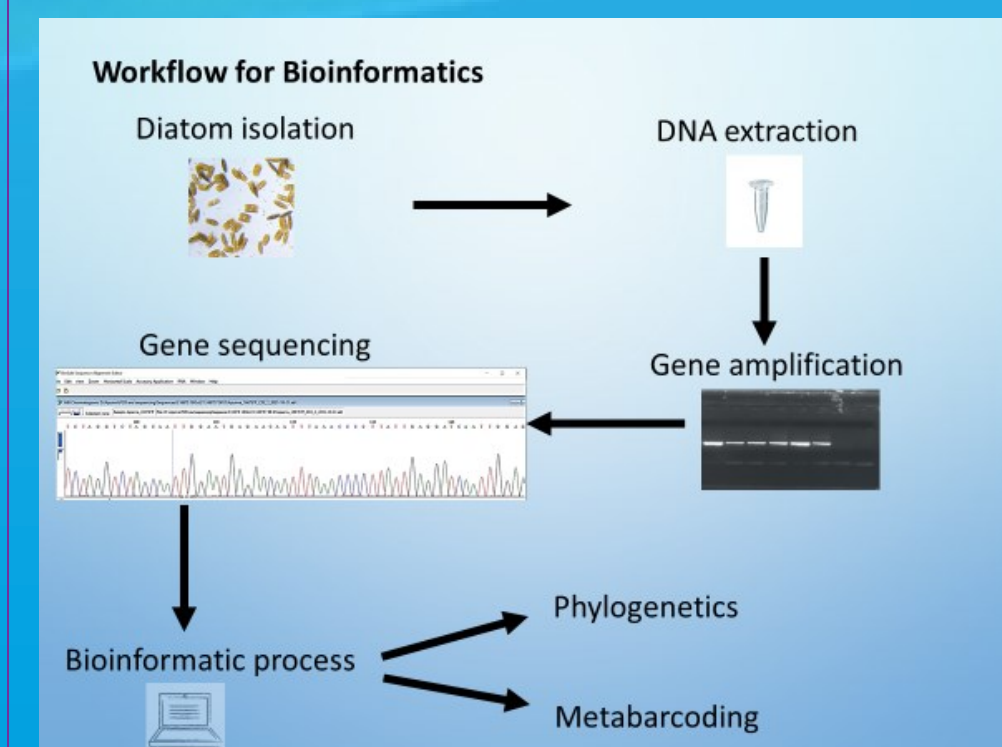
Molecular study: 18S rRNA gene amplification done for all the strains and 33 sequences submitted to in house database of SZN

1. Phylogenetic studies

- In total 542 sequences were manually aligned using Se-AI program
- To understand the phylogenetic position of the isolated sequences Maximum likelihood tree was prepared using MEGA-X

2. Metabarcoding studies (On-going)

- This study will produce taxonomically validated annotated sequences which will provide structure of MPB community

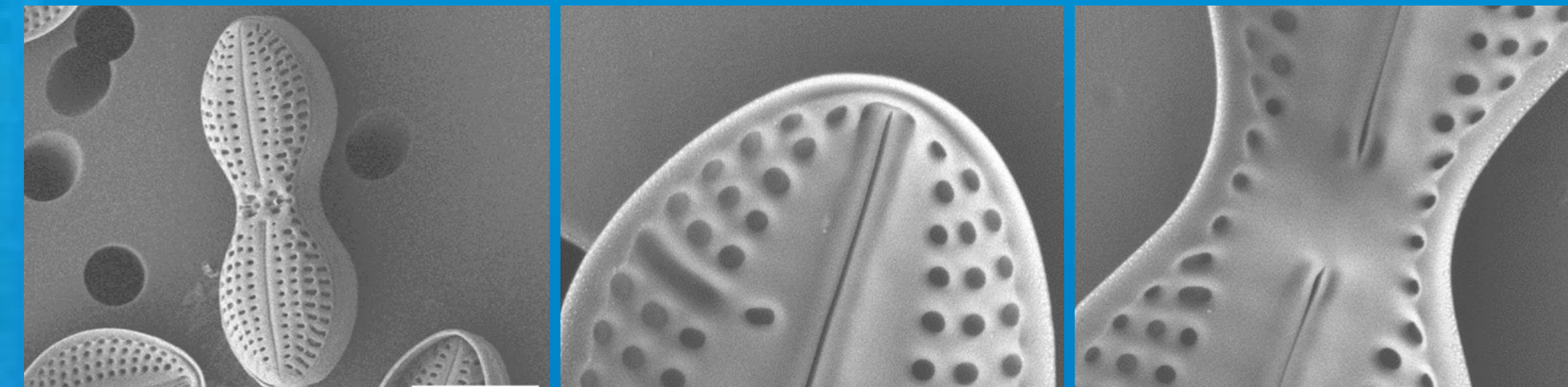


Example of the species Identification based on morphological and molecular data

- BLAST of the 18S rRNA gene on the Genebank gave **96.21%** similarity to *Diploneis* sp.
- Morphological study with different characters suggested the species *Diploneis weissflogiopsis* (Pennesi et.al. 2017)



Light microscopic photograph



Scanning electron microscopic photograph

References

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