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Taxonomy and molecular phylogeny of monoraphid marine benthic diatoms with an account on their oil production potential

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ABSTRACT

In a forwarded PhD thesis, the research on monoraphid diatom taxa originating from marine littoral worldwide is presented. The taxonomic position of diatoms selected for this study was uncertain or inappropriately designated. Species belonging in *Schizostauron* and *Astartiella*, are often misidentified due to their confusing taxonomic history or lightly silicified frustule impeding proper identification. *Cocconeis* on the other hand, is a polyphyletic genus, to which almost all cocconeid taxa are assigned, with no regard to ultrastructure or molecular phylogeny. *Cocconeis* is among genera, that are the most frequently recorded in ecological surveys, especially in the fresh water habitats. A strain of *Schizostauron trachyderma* was selected for plastidic and mitochondrial genome sequencing analyses because its morphology was very well documented and three-gene based phylogeny was already available from previous studies. Moreover, this species was also selected for lipid capacity analysis, because of availability of multiple monoclonal cultures and undemanding behaviour in culture.

In the first part of the thesis the taxonomic and phylogenetic position of *Schizostauron* and *Astartiella* is discussed with morphological characters analysis and DNA sequence data. Two new combinations are proposed and three *Schizostauron* species new to science are described, along with six *Astartiella* species new to science. Description of new taxa was based on 28 cultures and wild specimens from samples that cultures were isolated from. The data comprising of species description included plastid shape and number, valve morphology and ultrastructure description based on LM, SEM and TEM microphotographs and focus ion beam (FIB)

nanocuts. Phylogenetic position of these taxa is reconstructed based on three molecular markers SSU, *rbc*L, *psb*C.

The second part consists of summation of Achnanthales assemblages, that occurred on Raivavae, an island on South Pacific. Cladistic analysis of *Cocconeis vaiamanuensis* and allied taxa (*Achnanthidium*, *Karayevia*, *Lemnicola*, *Pauliella*, *Planothidium*, *Platessa*, *Psammothidium*, *Rossithidium*) with formal analysis of ultrastructure of *Cocconeis* was provided. Moreover, the phylogeny of *Cocconeis* spp. was reconstructed, based on sequences available online and one sequence of *Cocconeis* cf. *sigillata* isolated from Chinese littoral zone in Laoshan Shangquan in the Yellow Sea coastal area. The phylogenetic reconstruction based on *rbc*L gene database of available *Cocconeis* taxa indicate that the genus in not monophyletic.

In the third part the complete mitochondrial and plastid genome of *S. trachyderma* was assembled and genes were annotated. Based on the limited data that is available in online repositories, the multigene phylogeny was reconstructed for both organellar genomes. The data was compared with the genomes of closely related diatom species that were available in online repository. This analysis is both, supplementary to the phylogenetic analysis with similar results indicating that *Schizostauron* is related to members of the Stauroneidaceae family, and additional to expand the reference molecular database for future research of marine monoraphid benthic diatoms. The two genomes are the first monoraphid diatom genomes published online.

The fourth part concerns analysis of the lipid production capacity that includes five strains of *Schizostauron* spp. (four of *S. trachyderma* and one of *Schizostauron kajotkei*). One of the strains originated from initial cell division, isolated from sexually reproducing *S. trachyderma* mixture. The sexual reproduction process was induced in *S. trachyderma* monoclonal strains in order to observe its mating type. Such data is the additional indication to *Schizostauron* genus identity. Moreover, mating experiment was necessary in order to isolate initial cells for further research. Without the biggest cells of this species, the lipid capacity analysis would not be possible. The growth rate of the strains was calculated and the cells dimensions were taken. After 14-day experiment, the cells of each strain were stained with Nile Red fluorescent stain. The value of fluorescence was measured with both flow cytometry and confocal microscopy in order to find the relationship of cells size and fluorescence intensity/lipid accumulation capacity. Both, flow cytometry measurements and lipid droplets metrics results indicate the positive relation of *S. trachyderma* cells size to the lipids droplet radius.

The research that is presented in forwarded PhD thesis deals with current problems in diatomology and is characterized by a multidisciplinary approach to taxonomy and phylogeny in general. Their results, in all their aspects, are innovative and are the first of their kind for monoraphid marine benthic diatoms.. Furthermore, documenting the life cycle of *S. trachyderma* helps to better understand the evolutionary history of a monoraphid diatom lineage that is distinct from the monoraphid familied Achnanthaceae, Cocconeidaceae and Achnanthidiaceae, for which research of this type is still pending. Finally, the documentation of sexual reproduction enabled for the development of a cell that was nearly twice the size of the mother cell, which was used in the lipid capacity experiment. The unique technique that was applied to lipid droplets measurements will hopefully aid in improving the process of optimizing biomass growth of diatoms.

Key words: *Astartiella*, *Cocconeis*, *Schizostauron*, phylogeny, genome, diatom sexual reproduction, life cycle, lipid

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