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From microbial mats in Puck Bay to the Szczecin Diatom Culture Collection. A three decade long research on marine benthic diatoms inspired by Professor Helmut Pankow

Abstract

During the second half of the 1980s and first half of the 1990s the first author has been visiting Professor Helmut Pankow's algological Research Group (Arbeitskreis Algologie) at the University of Rostock. This activity has been carried out within a frame of scientific cooperation between the Universities of Gdańsk and Rostock. Research visits to the University of Rostock have been dedicated to the taxonomy and ecology of the unicellular organisms inhabiting microbial mats in the littoral zone of Puck Bay in Poland. Through these efforts, the importance of diatoms within the microbial mats has been revealed, which has focused the scientific career of the senior author on marine diatoms from the littoral zone worldwide. While this research has previously been based purely on cleaned diatom frustules and their morphology, it has now reached the point where cytological features (chloroplasts), cell cycle (sexual reproduction) and molecular markers (nuclear and chloroplasts encoded genes) are applied to characterize these organisms. This has been made possible through the establishment of a culture collection at the University of Szczecin (due to changes in employment). About 700 strains of diatoms from marine littoral are now grown and are available through the Szczecin Diatom Culture Collection (SZCZ) for cytological and molecular studies on the taxonomy, biogeography and phylogeny of diatoms.

Keywords: Professor Helmut Pankow, Rostock University, Algology, microbial mats, diatoms, marine littoral, culture collection, molecular markers

1 Introduction

During the period of 1985-1996, within a framework of scientific cooperation between partner Universities in Gdańsk and Rostock, the first author conducted numerous research visits to Rostock. The major aim of these visits was to analyze the species composition of unicellular organisms inhabiting microbial mats in the littoral and supralittoral of Puck Bay, Poland. Visits to Rostock University and the laboratory of Professor Helmut Pankow helped in the identification of the Cyanobacteria and other photosynthetic bacteria (*Beggiattoa* spp. and purple sulfur bacteria), green algae (Chlorophyta) and diatoms (Bacillariophyta) found in these mats. It was an introduction to the techniques of sampling, processing those samples and of microscopic identification, resulting in the publication of species composition lists of the microbial mat biota in the Puck Bay coastal shallows (WITKOWSKI 1990, 1994b). In the early 1990s, the scientific focus changed from investigations of general floristic species composition to that of diatoms specifically. This early period of diatomological studies has resulted in a general description of the species compositions of diatom assemblages of the Puck Bay coastal shallows, in addition to the description of a few dozen new species (WITKOWSKI 1991, 1992, WITKOWSKI & LANGE-BERTALOT 1993, WITKOWSKI et al. 1996, WITKOWSKI et al. 1998) and even new genera (WITKOWSKI et al. 1997, 2000). This research of the microbial mat biota of Puck Bay was pioneering in the Baltic Sea basin. During the first decade of the 21st century, research on species composition and eco-physiology of the so-called “wind flats” of the western Baltic Sea was performed (e.g. WOELFEL et al. 2007, HEYL et al. 2010). Studies on microbial mats developing in quartz sandy sediments of the temperate climatic zone is rather limited, as their fossilization potential is believed to be rather low, though not negligible (e.g. WITKOWSKI 1990). Major research on this topic has been principally performed in the North Sea tidal flats (e.g. NOFFKE et al. 1996, 1997a).

Regardless of locality, diatoms seem to play an important role in the stabilization of the sediment (PATTERSON 1989, NOFFKE et al. 1997b, UNDERWOOD & PATERSON 2003). Unique environmental conditions, shallow water, changes in water inundation and frequent exposure to strong irradiation, high organic matter content and high content of organic matter degradation products (e.g. hydrogen sulfide) result in specialized diatom assemblages, such as the assemblage characterized by high abundances of very small-celled diatoms (WITKOWSKI 1990, 1991a, PNIEWSKI 2010). As the research on marine littoral benthic diatom assemblages has expanded, many of the diatom taxa described from the Puck Bay microbial mat seem to exhibit a world wide geographic distribution. This group includes *Amicula speculum* (Witkowski) Witkowski, *Cocconeis hauniensis* Witkowski, *Fragilaria gedanensis* Witkowski, *Fragilaria geocollegarum* Witkowski & Lange-Bertalot, *Fragilaria guenter-grassii* Witkowski & Lange-Bertalot, *Opephora krumbeinii* Witkowski, Witak & Stachura and *Navicula wiktoria* Witkowski & Lange-Bertalot (e.g. WITKOWSKI et al. 2000, MORALES 2002, RIBEIRO 2010, SEDDON et al. 2011). Interestingly, in the microbial mat of Puck Bay there occur also a group of taxa which have been established elsewhere, but apparently have worldwide distribution. Here included are: *Opephora mutabilis* (Grun.) Sabbe & Wyverman, *Catenula adhaerens* (Mereschkowsky) Mereschkowsky, *Planothidium delicatulum* (Kützing) Round & Bukhtiyarova, *Navicula vimineoides* Giffen, (GIFFEN 1975, SUNDBÄCK & MEDLIN 1986, SABBE 1997, WITKOWSKI et al. 2000). From these assemblages and reports we see further examples of marine benthic diatoms exhibiting a cosmopolitan distribution.

The idea of cosmopolitanism among marine littoral diatoms has always been a strongly-debated issue. Among the most extreme opinions, FINLAY et al. (2002) proposed there should be no dispersal limit to diatoms due to their microscopic size and fast reproduction rates: the “everything occurs everywhere” hypothesis. Purely morphological approaches have been shown to be inconclusive on their own in the light of expanding use of molecular methods (DNA sequence analysis) — for biogeography and phylogeny estimation of both freshwater and marine diatoms (e.g. MEDLIN & KACZMARSKA 2004, SORHANNUS 2004, THERIOT et al. 2010, RIMET et al. 2011, ASHWORTH et al. 2013, TROBAJO et al. 2013). As DNA data in these studies have forced us to reconsider the evolution of diatoms, it became obvious that morphological approaches must be combined with molecular methods. Further progress in studying the biogeography of marine benthic diatoms by means of these molecular methods has been made possible with the establishment of the culture collection of diatoms at the Palaeoceanology Unit, Faculty of Geosciences at the University of Szczecin in 2010. This was founded by the Polish Ministry of Science and Higher Education and further developed with assistance from the National Science Centre in 2012. In this paper we introduce and describe the Szczecin Diatom Culture Collection (SZCZ), highlighting the process of isolation and purification of clonal cultures, which began in late 2011. Clonal cultures are harvested in order to extract the genomic DNA, which is then amplified by PCR and sent out for DNA sequencing. Routinely three genes are amplified and sequenced: nuclear-encoded ribosomal RNA (SSU) and chloroplast-encoded *rbc*L and *psb*C, which are three of the most commonly-sequenced genes across diatoms (THERIOT et al. 2010). In parallel, cleaned material from the extracted cultures is examined and documented by light (LM) and electron microscopy (EM).

2 Methods

2.1 Sampling and cultures

2.1.1 Procedure for growing monoclonal diatom cultures at the University of Szczecin.

The culturing process starts with samples taken from marine littoral zones. Whenever possible, parameters such as salinity, temperature, pH, oxygen content and oxygen saturation are measured at the sampling site by means of a portable instrument (HI 9828 of Hanna Instruments). Samples are collected into small plastic tubes (25 ml) from sediment, small pieces of seaweeds or from dead mineral detritus of organic origin (such as mollusk shells). If the substrate is either rocky or composed of large cobbles, scrapings from those rocks or boulders are also taken into the tubes. Frequently material is also sampled from the subtidal zone with a 20 µm plankton net. Special care is taken that the tubes are not filled with a mixture of sample and the sea water entirely to leave some air inside. Our experience shows that samples left without an access to the air are usually dying during the transport to the laboratory facilities. To prolong the life of the sampled material during expeditions, the Palaeoceanology Unit team will typically add a few ml of culture medium (f/2 medium) to the sampled material. Samples collected in multi-day expeditions are also stored in natural light, although care is taken in regions with very

strong sun (e.g. in South Africa), as the elevated temperatures from excess solar irradiation can stress and kill the collected diatoms.

Before starting field sample processing in laboratory in Szczecin, lab surfaces and hands are disinfected to avoid contamination by other diatoms or bacteria and fungus which can contaminate the strains. In the first step, a small amount of the field sample is transferred into plastic petri dish and enriched with f/2 culture medium (GUILLARD 1975) with the salinity of the medium based on the field measurements or on data from the literature. Freshly enriched samples are regularly (daily and later on weekly) checked under the inverted microscope to observe the ratio of live versus dead diatom cells. After as many as a dozen days, single cells of living diatoms are isolated by means of micropipettes under the inverted microscope by means of capillary tube technique (ANDERSON & KAWACHI 2005). A drop of sample with an isolated diatom cell is transferred onto a microscope slide. A drop of culture medium might be added at this stage to a drop of sample placed on a microscopic slide in order to dilute any suspended particles that were isolated with the diatom cell. Additional (separate) drops of culture medium are placed on microscopic slide near the isolated diatom cell. The isolated diatom cell is then transferred into one of the fresh drops, leaving unwanted particles in the previous drop of culture medium. This operation is repeated several times until only the desired, isolated diatom cell remains in a drop of culture medium. Glass pipettes are washed or replaced entirely each time the cell is transferred in order to avoid contamination. After these cleaning steps, the selected diatom cell is placed either in plastic petri dish or DNA well plate filled with culture medium. The Petri dish is labeled with the date of inoculation, type of culture medium and name of strain and put into a growth chamber. During the period of ca. one to two weeks, the inoculated petri dish (DNA well plate) is checked for diatom growth and any potential contamination. Successfully-growing diatom cells are kept in the growth chamber, while in petri dishes with contamination (bacteria, fungus or other microeukaryotes) the cleaning process is repeated. Diatom strains in the growth chamber are grown in enriched seawater (f/2)-medium at 18°C under a 12 h light–12 h dark cycle, illuminated by 50 µmol m–2 s–1 of white light.

Once the culture in a petri dish has been demonstrated to be uncontaminated and monoclonal, the strain is ready for further analyses. Pictures of diatom plastids are taken; portions of the cultured material is taken for genomic DNA analysis, and part is taken for microscopic slides as a voucher for the Palaeoceanology Unit diatom collection (SZCZ), and the rest is stored in illuminated refrigerator. Once the slides are prepared and the DNA is extracted Szczecin diatom culture collection number is given to it with the prefix of SZCZ, the name of the person registering it and 5 digits. The record of SZCZ_P_00001, means strain number 1 in the Szczecin diatom culture collection registered by Przemysław Dąbek (Tab. 1). Registered strains are re-inoculated at regular intervals to keep the strains alive.

2.2 Microscopy

Live cells of the cultured clones are photographed in counting chambers using a Nikon TS300 inverted microscope (Nikon Corporation, Tokyo, Japan) equipped with a 100× PlanApochromatic oil immersion lens (n.a.=1.40) and differential interference contrast (DIC) optics. For light microscopy (LM), scanning (SEM) and transmission (TEM) electron microscopy, suspensions of the diatom cultures are boiled with 12 ml of hydrogen peroxide for a few hours. An alternative method for

processing the cultured cells is based on the use of a Rorax® bio-power-gel (Erdal Rex, Germany), a strong domestic drain cleaner, modified from SATO et al. (2008). The cleaned diatom suspension is mounted with Naphrax® (Brunel Microscopes Ltd, Wiltshire, U.K.) for LM observations, which are conducted with a Zeiss Axio Imager 2M (Carl Zeiss, Jena, Germany) using a 100× oil immersion PlanApo objective (n.a.=1.46). For scanning electron microscopy (SEM) examination, few drops of cleaned material are put onto Whatman Nuclepore polycarbonate membranes (Fisher Scientific, Schwerte, Germany) and allowed to dry. Dry membranes are mounted onto aluminum stubs and coated with gold-palladium. SEM observations were made at the Warsaw University of Technology, Faculty of Materials Science and Engineering using a Hitachi SEM/STEM S-5500 or SU8000 and at the J.W. Goethe University, Frankfurt am Main, Germany by means of a Hitachi SEM/STEM S-4500.

2.2.1 DNA extraction and PCR

Depending on the cell density, several milliliters of cell suspension in the exponential phase growth are centrifuged for 15 min at 8,000rpm to harvest cells for genomic DNA extraction. For this, the Genomic DNA NucleoSpin Plant II Kit ® (Macherey-Nagel, Germany) is applied in accordance to the manufacturer`s instruction. The small subunit (SSU) of nuclear ribosomal RNA, and two chloroplast genes (rbcL and psbC) are amplified using the primers and following the protocols of THERIOT et al. (2010). PCR products are visualized in 1% agarose (Maximus, Poland) gel and then are purified using Exonuclease I & Polar-BAP (EURx, Gdańsk, Poland) protocol (Witkowski et al. 2014). The PCR products are sent to oligo.pl DNA Sequencing Laboratory IBB PAS, Warsaw, Poland for Sanger sequencing with the use of BigDye Terminator v. 3.1 chemistry and ABI3730 xl sequencer.

2.2.2 Phylogenetic analysis

Maximum likelihood (ML) trees of the diatoms are constructed with three markers (SSU, rbcL and psbC). Phylogenetic trees are constructed with our own sequences and those accessed from the GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The datasets are partitioned by gene and codon position (in case of chloroplast markers). The analysis typically consists of 20 times of ML research and 1000 bootstrap replicates were conducted using rapid Bootstrap analysis by RAxML v8.1. The best-scoring ML tree drawn with bootstrap values are chosen as the final tree.

3 Results and discussion

3.1 Taxonomy

At present, almost 700 diatom strains are currently housed in the Szczecin Diatom Culture Collection (SZCZ). The largest number of strains originate from the coasts of southern Africa (Madagascar, Mozambique, Republic of South Africa, Namibia and Angola; Figs 1-3) and east and south-east Asia (Korea, China and

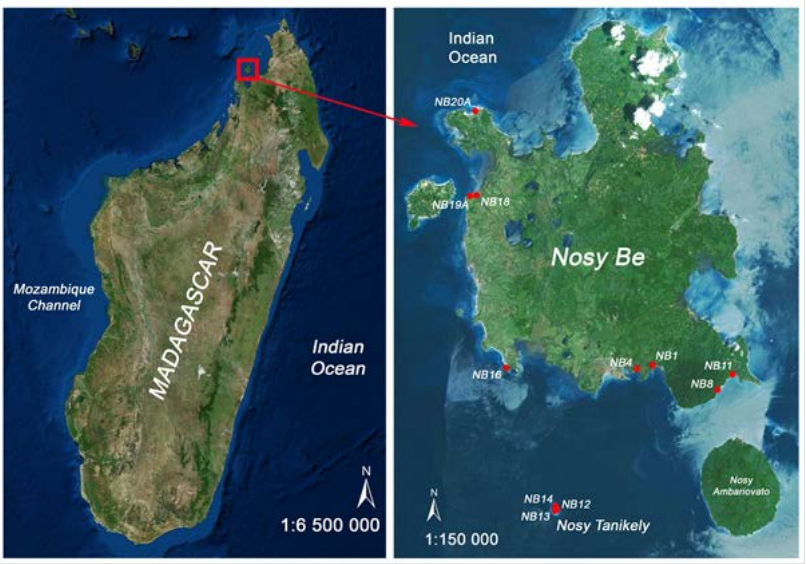

Fig. 1 Location of sampling sites in Nosy Be NW Madagascar

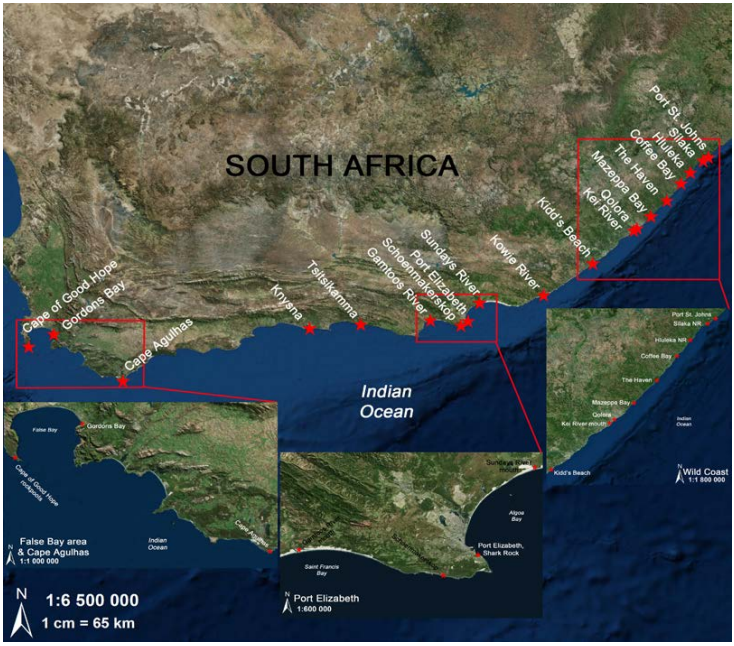

Fig. 2 Location of sampling sites in South Africa, south and east coasts.

Vietnam). Less numerous are the strains which originate from the Gulf of Gdańsk and Puck Bay microbial mats (Baltic Sea), the Algarve coast in Portugal, Tagus Estuary (Lisbon), Adriatic Sea (Croatia), Turkish coasts (Mediterranean, Aegean, Marmara and Black Seas Fig. 4), Canary Islands, Florida, Gulf of Mexico (Corpus Christi, Texas), Martinique, New Zealand and French Polynesia. In addition, a few diatom strains are being grown from high latitudes in the north (Nuuk Fiord, Greenland) and in the south (King George Island, Antarctica).

In the study areas, diatom sampling is accompanied by environmental parameters. Most of the samples are taken from the fully oceanic salinities; however, the Gulf of Gdańsk culture medium is prepared with 7 psu sea water, whereas the Zhuhai region (South China Sea, Pearl River Estuary) is prepared with 10 psu seawater, the Marmara Sea and Black Sea with 15 psu seawater and Tagus Estuary with 20 psu sea water.

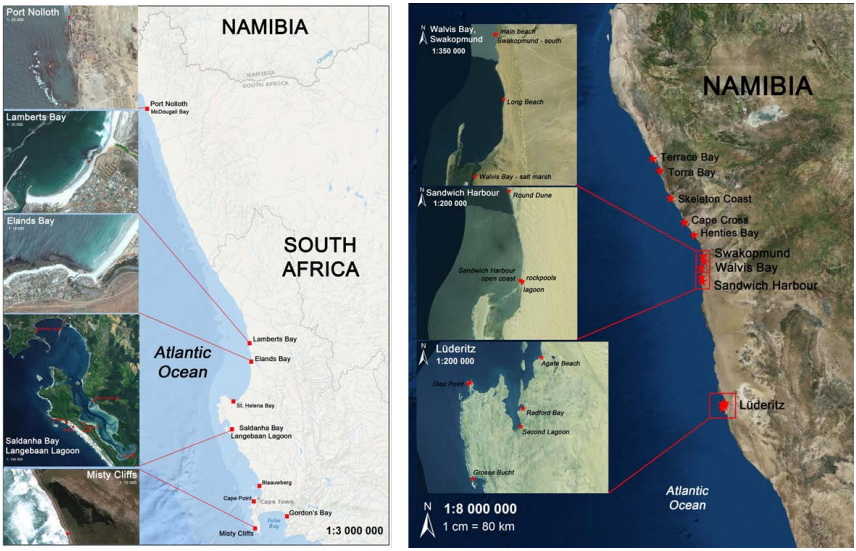

Fig. 3 Location of sampling sites along the South Africa West- and Namibia coast

Special efforts have been taken to collect and isolate the diatom taxa living within a biofilm grown on loggerhead turtles nesting at the Turkish coast of the Aegean Sea and from Holothurians sampled in Nosy Tanikely, Madagascar. At present, 37 diatom strains of potentially diatoms epizoic on loggerhead turtles and a few on Holothuria are successfully grown in the Szczecin Diatom Culture Collection.

The original field material from which the diatoms are sampled for culturing are cleaned of organic matter and the material is prepared for further study by means of light and electron microscopy. The aim of this approach is to find, identify and document natural population of diatoms isolated in culture. Some diatoms can significantly change their morphology in the culture (e.g., *Odontella aurita* Fig. 5) or

representatives of the family Cymatosiraceae). The culture conditions provide them unlimited opportunities to grow and reproduce by supplying an optimal concentrations of nutrients and light, in contrast to the natural conditions, which may limit their growth due to the nutrients depletion, variability of other physico-chemical conditions of the water or zooplankton activity.

Most of the diatom clones grown in our culture collection represent taxa identified to the generic level. Their identification as a matter of routine procedure based on observation in the inverted microscope with the 40x objective lens. Such observations are usually carried out during the isolation and purification of the respective clones. In parallel, chloroplasts are photo-documented in monoclonal cultures, which can assist in the identification of taxa. Our collection contains diatoms from a diversity of taxonomic families; Table 1 shows the first page of our Index Table, but the complete table will be accessible soon online at:

http://www.marinebenthicdiatoms.univ.szczecin.pl.

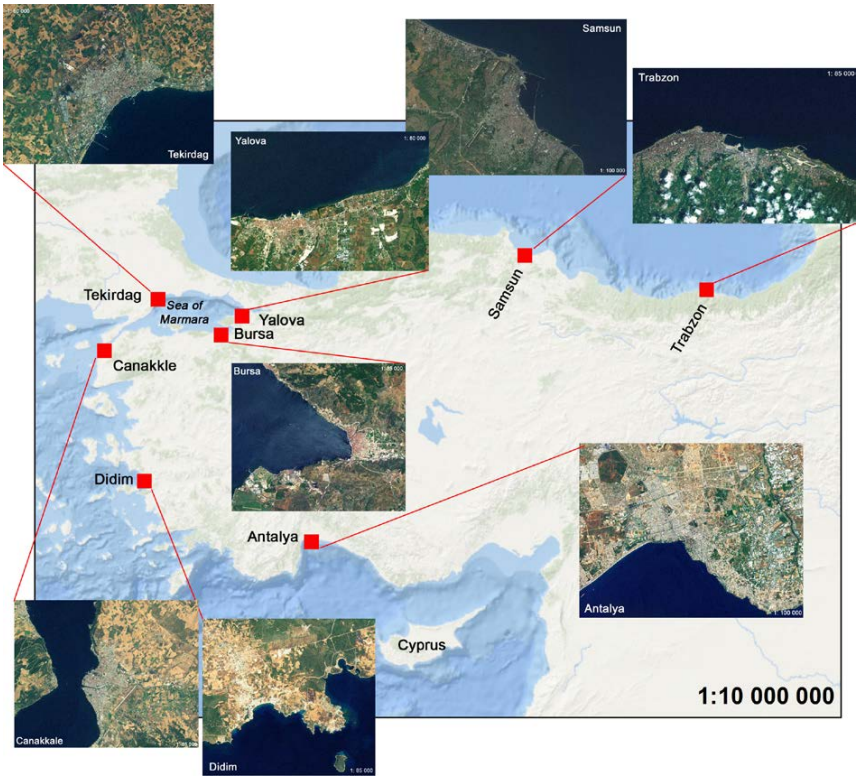

Fig. 4 Location of the sampling sites along the coasts of Turkey

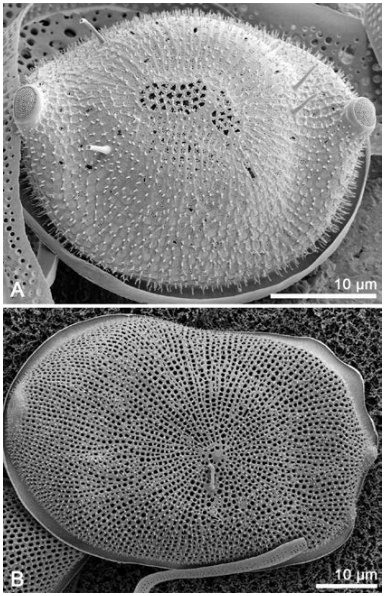

Fig. 5 SEM images of *Odontella* sp. (strain SZCZ_P_00160 from Szczecin Culture Collection) from the Langebaan Lagoon west coast (South Africa).

Out of ca. 700 clones 40 represent radial centrics with *Melosira, Paralia, Cyclotella, Actinoptychus, Actinocyclus* and a few *Thalassiosira* spp. The number of multipolar centrics amounts to 20, whereas of bipolar centrics including Biddulphiaceae and Cymatosiraceae amount to 60. Some taxa of Cymatosiraceae recently described as new to science are grown in culture as well. Araphid forms, represented mostly by Fragilariaceae and Plagiogrammaceae are ca. 100 clones. Three distinct clades represent monoraphid diatoms. The first one represented by 18 clones belongs in *Achnanthes* sp., the second with 16 clones in Cocconeidaceae (*Cocconeis, Planothidium* and some most likely new genera) and the third one with 8 clones involving *Schizostauron* spp. is grouping together with Stauroneidaceae. It seems that the highest number of clones (more than 400) represents naviculoid diatoms (e.g. *Navicula, Seminavis, Pinnularia, Amphora, Halamphora, Caloneis, Parlibellus, Biremis, Lunella, Amicula*) and Bacillariaceae (*Nitzschia, Psammodictyon, Tryblionella, Bacillaria*), Rhopalodiaceae (*Auricula, Rhopalodia, Protokeelia*), Entomoneidaceae (*Entomoneis*) and Surirellaceae (*Surirella, Petrodictyon*).

Following identification, clonal cultures are re-inoculated, indexed in the culture collection and harvested. From part of harvested clonal culture DNA is extracted and amplified, whereas the remaining part is cleaned from cell content and the voucher slides for LM and suspended material for EM investigations are prepared. We find DNA extractions and sequencing important for identifying diatoms based solely on DNA sequences. This is one of the most important feature tasks of our culture collection; our database of vouchered sequenced genomic DNA, is a very important reference for metagenomic work conducted with environmental DNA extractions and descriptive phylogenetic work, in addition to the biogeography issues we discuss in the following section.

3.2 Biogeography

With such a broad sampling, several diatom taxa in the SZCZ Diatom Culture Collection can be considered to be cosmopolitan in geographic distribution, such as *Odontella* cf. *aurita, Odontella* cf. *rostrata, Melosira nummuloides, M. moniliformis, Plagiogrammopsis vanheurckii, Achnanthes* cf. *brevipes, Nitzschia aurariae, Nanofrustulum* spp., *Dimeregramma minor, Entomoneis* spp., *Planothidium*

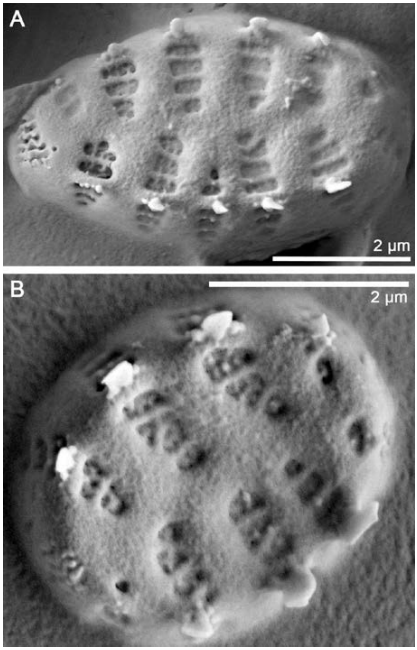

Fig. 6 SEM images of *Opephora* cf. *mutabilis* from the Puck Bay littoral zone (strain SZCZ_Ch_00153) from Szczecin Culture Collection.

delicatulum, Amphora cf. *helenensis*, *Halamphora* spp. and *Opephora* cf. *mutabilis* (Fig. 6). Particularly interesting is *Nanofrustulum* spp., which was thus far isolated from all oceans except the Antarctic waters. Our *Nanofrustulum* sp. strains originate from Greenland (Nuuk fjord), Baltic Sea (Puck Bay), Adriatic Sea (Croatia), Mediterranean Sea (Turkey), Atlantic coasts (Portugal, Angola, Namibia and South Africa), Indian Ocean (Madagascar, Mozambique) and Pacific Ocean (Korea, South China Sea and Vietnam). As numerous are *Odontella* cf. *aurita* clones, which originate from Portugal, Canary Islands, Namibia, South Africa, Madagascar, Vietnam and China. Similarly widespread in terms of geography are clones of *Achnanthes* cf. *brevipes*. For some clones of these widespread taxa, the rbcL gene has been sequenced and downloaded from GenBank, in some cases with puzzling results (Fig. 7). For example, strains identified as *Nanofrustulum* have been resolved in two different clades across the phylogenetic tree of diatoms. Even more as our unpublished results show they can group with other *Nanofrustulum* taxa, or with the unrelated genera e.g. with *Dimeregramma* (LI & WITKOWSKI unpublished observations). We are not able yet to determine the reason of such "wandering" of *Nanofrustulum* taxa across the diatom phylogenetic tree, though with morphological analyses of *Nanofrustulum* in progress, we expect some possible solutions to this discrepancy during further study.

A large group of taxa in the SZCZ Diatom Culture Collection appear to be widespread, but bound to a certain climatic zone. Such taxa, like *Biddulphia tridens, Schizostauron* spp., *Cocconeis* spp., *Astartiella* sp., *Delphineis* sp., *Seminavis* sp., *Cymatosira lorenziana*, have been isolated from the littoral zone of the subtropical and tropical regions from several different ocean basins. They have been isolated from samples collected from the tropical waters across the western Pacific (China and Vietnam), Indian Ocean (Madagascar, Mozambique), and the western Atlantic (Florida and Gulf of Mexico). Results of our morphological and molecular studies show that while some of these taxa do appear cosmopolitan across climate zones (e.g., *B. tridens* and *C. lorenziana*) we find numerous strains which are potentially new species e.g., *Astartiella* sp., *Cocconeis* sp. and even new genera (gen. and sp. indet. monoraphid). Additionally, there are numerous strains grown in the SZCZ Diatom Culture Collection known only from one sampling site and never appear again in either culture or in cleaned natural samples. Examples of these strains include *Diploneis* sp. (Canary Island – SZCZ_P_00035), *Rhopalodia* cf. *pacifica*

Krammer (Corpus Christi, Gulf of Mexico – SZCZ_Ch_00163) or *Minutocellus africana* Dąbek & Witkowski (Lamberts Bay, South Africa, in Dąbek et al. 2014, SZCZ_P_00074).

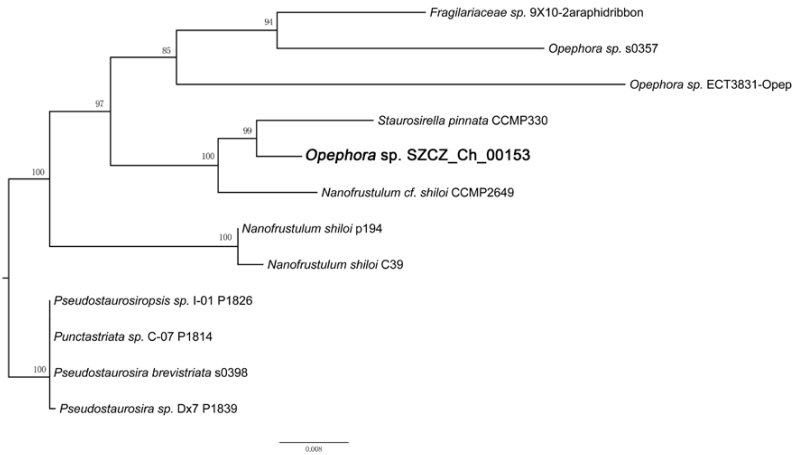

Fig. 7 Close up of the phylogenetic tree of diatoms to show changing position of *Nanofrustulum* spp.

3.3 Physiological experiments

Establishing the culture collection has enabled us also to design and perform ecophysiological experiments on certain diatom species like *Odontella aurita* or *Melosia nummuloides* using different environmental factors (i.e., temperature, salinity or concentration of basic nutrients – N, P, Si). The aim of this study is to define the tolerance ranges under these varying conditions of the selected taxa and to determine the limits of the maximum range of occurrence for those diatoms in the oceans. It turned out, e.g., that *Odontella aurita* strain, isolated from the Langebaan Lagoon (South Africa), is mostly limited by phosphorus (DĄBEK, 2014 – unpublished observations). A deficit of this nutrient in the water negatively affects the growth rate and cell number in the culture. In addition, depletion of silica usually limits the auxospore production. The taxa referred to are considered widespread marine diatoms occurring all over the world in the different habitats, e.g., attached to the sand grains, rocks, macroalgae or floating in the water. Therefore, they are particularly interesting to perform such experiments to find out what conditions are optimal for their growth.

Table 1 First page of the Szczecin Culture Collection index.

Strain Number	Taxon	Origin of the sample / date	LM Slide (number)	DNA extraction (date)	LM	Live	SEM	Remarks
					Availability of images			
SZCZ_P_00001	*Seminavis* sp.	Schoenmakerskop, Port Elizabeth, Eastern Cape Province, South Africa / 22.4.2013	✓	21.6.2013	✓	✓		P8-205/1/f4
SZCZ_P_00002	*Seminavis* sp. 1	Sandwich Harbour - open coast, Namibia / 10.4.2013	✓	21.6.2013		☐		P31-138/2/G1
SZCZ_P_00003	*Seminavis* sp.	Schoenmakerskop, Port Elizabeth, Eastern Cape Province, South Africa / 22.4.2013	✓	21.6.2013		✓		P34-205/1/F6
SZCZ_P_00004	*Cymatosira lorenziana*	Indian Ocean, Bazaruto sand beach / 24.5.2013	✓	15.7.2013		✓		P89-Ba/5/G2
SZCZ_P_00005	*Nitzschia/ Entomoneis*	Qolora 27, sediment 2m deep, Eastern Cape Province, South Africa / 1-21.6.2012	✓	✓	☐	✓		P184
SZCZ_P_00006	*Amphora* sp.	Dias Point - brown diatom sand from rockpool, Namibia	✓	15.7.2013		✓	✓	P95-102/8/B2
SZCZ_P_00007	*Navicula* ss	Long Beach, rocks, Namibia - 11.4.2013	✓	15.7.2013		✓		P100 - 145/6/G11
SZCZ_P_00008	*Auricula* sp.	Indian Ocean, Tofo Sand, 22.5.2013	✓	21.6.2013		✓	✓	P32 - TS/2/D11
SZCZ_P_00009	*Amphora* sp.	Dias Point - brown diatom sand from rockpool, Namibia	✓	15.7.2013		✓	✓	P96-102/8/G2
SZCZ_P_00010	*Nanofrustulum* sp.	Sandwich Harbour - open coast, Namibia / 10.4.2013	✓	21.6.2013		✓		P33-138/1/C12

4 Conclusion

It must be stated clearly that all diatom strains grown in the SZCZ Diatom Culture Collection at the University of Szczecin are freely accessible for to the scientific community in the spirit of cooperation and collaboration. A list of the available strains kept in culture will be soon accessible online. At present, intensive cooperation is realized between the SZCZ Diatom Culture Collection and University of Texas in Austin, Research Station in Karadag in Crimea, Nelson Mandela Metropolitan University in Port Elizabeth, South Africa, Institute of Agriculture and Food Research and Technology (IRTA), Sant Carles de la Ràpita, Spain, and Perpignan University, France. The first results of joint research have already been published and included description of a new *Biremis* species from Pacific Ocean coast of Panama. Using molecular and auxosporulation data we have been able showing that *Biremis* as a genus is closely related to Neidiaceae not to Scoliotropidaceae (WITKOWSKI et al. 2014). The current task is revision of Plagiogrammaceae in terms of morphological and molecular data. In this paper a new taxa will be described and new knowledge on phylogeny of the Plagiogrammaceae will be published (Li, Ashworth, Witkowski unpublished observations). Also experiments on auxosporulation of monoraphid taxa of *Schizostauron* are realized in collaboration (Davidovich N. et al. unpublished observations). These global collaborations are the realization of the early inspiration of the first author by Professor Pankow in studying marine littoral benthic diatoms. In the last 30 years since those important initial studies on the diatoms of microbial mats in Puck Bay, we are now building a set of morphological, physiological and molecular tools to ask bigger questions about the biogeography and phylogeny of marine diatoms. Our preliminary data both agree and disagree with the Finlay et al. (2002) hypothesis: everything is not everywhere, although we also find some examples of widely-distributed benthic diatoms which support this hypothesis. Molecular methods are not a panacea on the problems with geographic distribution of diatoms as microbial eukaryotes, but are just another way to study this problem. Morphological data in combination with information on cell biology, life cycle and reproduction processes are also very important for solving all these puzzles.

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