ORIGINAL ARTICLE

Exploring diatom diversity through cultures - a case study from the Bow River, Canada

Oliver Skibbe,[1*] Nelida Abarca,[1] Francine Forrest,[2] Petra Werner[3]

[1]Botanic Garden and Botanical Museum Berlin-Dahlem, Freie Universität Berlin, Berlin, Germany; [2]Red Deer River Watershed Alliance, Red Deer, Alberta, Canada; [3]Diatoms as Bioindicators, Berlin, Germany

ABSTRACT

Diatom cultures can help answer taxonomic, biogeographic and ecological questions on a local and global scale. Unialgal cultures are derived from a single cell and provide abundant material for morphological and molecular analyses. The link between the historic morphological species concept and molecular data is becoming increasingly important with the use of eDNA metabarcoding. Additionally, cultures provide insights into the life cycle of diatoms and thereby complement taxonomy and species ecology. In this study, we present an approach to extract benthic diatoms from an environmental sample to generate unialgal cultures. We explored diatom diversity in preserved assemblages and by culturing as many different taxa as possible from benthic freshwater samples taken on the same day from the Bow River in Calgary, Canada. With both methods we found a total of 221 different benthic diatom taxa, of which 182 were identified in the preserved diatom assemblages. Interestingly, an additional 39 taxa only appeared in the cultures. In total 129 strains were cultivated, representing 71 different taxa. This study includes pictures of living cells demonstrating the additional merits of unialgal cultures, as they provide information on plastid details, auxospores and endosymbionts. Both, the identification of the diatom assemblages and the generation and identification of strains provide the foundation for additional water quality assessment tools, taxonomic insights, and molecular references libraries.

INTRODUCTION

Similar to many rivers around the world, the Bow River (Alberta, Canada) experiences natural and anthropogenic pressures that influence water quality and can affect its uses. The Bow River Basin is the most regulated and populated basin in Alberta and provides water for aquatic life, municipal drinking water, hydroelectric generation, irrigation, industrial, agricultural, wastewater assimilation and recreational purposes (City of Calgary, 2021). The physical, chemical and biological characteristics of the Bow River are influenced by natural variation in climate and vegetation (Culp *et al*., 1992) along with many urban, agricultural and industrial point and non-point sources (City of Calgary, 2021). Watershed planning initiatives are ongoing to manage and maintain the health of the Bow River (City of Calgary, 2021).

It is difficult to assess changes over time in major rivers with multiple stressors and often multiple tools are required. Chemistry, macrophytes and periphyton biomass have been monitored intermittently since the 1980s on the Bow River (Sosiak, 2002; Saffron and Anderson, 2009; Kirkwood *et al*., 2007; Chung, 2013; Dixon, 2014). Many studies have focussed on assessing the impacts of the City of Calgary's wastewater treatment plant discharges on the Bow River using trophic indicators (nutrients, dissolved oxygen, periphytic biomass; Sosiak, 2002; Chung, 2013). However, limited studies have identified diatoms to species level and used them as bio-indicators (Saffron and Anderson, 2009). This study inventoried periphytic diatoms to species level in the Bow River reach upstream of Calgary's downtown city centre.

For decades diatoms have been used successfully as bio-indicators (Stevenson *et al*., 2010; Charles *et al*., 2021), as they are particularly sensitive to pH, salt and nutrient levels. A diatom cell cycle takes only a few days, yet biofilm communities can be stable for weeks to months

Corresponding author: o.skibbe@bo.berlin

Key words: benthic diatoms; Bow River; diatom plastids; live observations; single-cell isolation; unialgal cultures.

Citation: Skibbe O, Abarca N, Forrest F, Werner P. Exploring diatom diversity through cultures - a case study from the Bow River, Canada. J. Limnol. 2022;81:2095.

Edited by: Francesca Bona, *Department of Life Sciences and Systems Biology, University of Turin, Italy.*

Received: 26 September 2022.
Accepted: 18 November 2022.

Publisher's note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

©Copyright: the Author(s), 2022
Licensee PAGEPress, Italy
J. Limnol., 2022; 81:2095
DOI: 10.4081/jlimnol.2022.2095

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

Non-commercial use only

pagepress

OPEN ACCESS

(Rimet *et al*., 2009). Therefore, diatom assemblages reflect the water quality of this integrated time period. Correspondingly, in Europe diatoms are one of four standard bio-indicators for monitoring water quality (Council of the European Communities, 2000; Kelly *et al*., 2009).

A correct identification of taxa is the foundation of every diatom-index for water quality assessments and also a requirement for studies on taxon-specific ecological preferences, geographic diatom distribution and invasive species (Smol and Stoermer, 2010; Bennett *et al*., 2014; Kahlert *et al*., 2016; Werner *et al*., 2016). Taxon identification requires a precise description of species. For the delimitation of morphological similar taxa and the description of new species, diatom cultures are an indispensable source of taxonomic information. Unialgal cultures are derived from a single cell and provide abundant material for refined taxonomic analyses. They display the morphological variability of genetically identical cells (Abarca *et al*., 2014, 2020; Jahn *et al*., 2020; Mora *et al*., 2021). For museums and collections, permanent slides made from cultures are reliable records (vouchers) of a taxon, as they have much less ambiguity compared to natural samples that may contain valves of several morphologically similar but genetically different taxa. When cultures go through auxosporulation, they can also show the cardinal points in the diatom life cycle. They allow for the examination of living cells, their physiology and their cellular features including the number, shape and arrangement of plastids. These observations complement the findings derived from valve morphology and may provide valuable insights into the phylogenetic position of a species (Cox and Williams, 2000; Cox and Williams, 2006; Cox, 2009). Thus, pictures and observations of living diatoms during their life cycle should ideally be included in the morphological, molecular and eco-physiological description of a taxon.

The best way to compile the diatom diversity of a region is to complement the morphological methods with molecular methods (Mora *et al*., 2019), e.g., by using environmental and sedimentary DNA metabarcoding (Zimmermann *et al*., 2015; Mora *et al*., 2019). Such molecular studies on current and past natural diatom communities require reliable and extensive reference libraries. Diatom reference libraries link a unique DNA-barcode to a morphologically described diatom taxon (Zimmermann *et al*., 2014). Currently DNA-based environmental studies are still restricted by the incompleteness of the reference libraries (Borrego-Ramos *et al*., 2021; Capo *et al*., 2021). The best source of sufficient DNA and valve material for reference libraries are cultures (Mann and Chepurnow, 2004; Skibbe *et al*., 2018).

Diatom cultures have many additional merits (Mann and Chepurnow, 2004). Over the past decades they have often been used for physiological and autecological studies (Durbin, 1974; Claquin *et al*., 2006; D'Alelio *et al*., 2010; Trobajo *et al*., 2011; Prelle *et al*., 2019; Bilcke *et al*., 2021) and for studies on phylogenetic and bio-geographic relationships among taxa (Abarca *et al*., 2014; Kermarrec *et al*., 2014). However, environmental studies based on diatom cultures are still scarce (Novis *et al*., 2012; Mora *et al*., 2019; Ribeiro *et al*., 2020; Ashworth *et al*., 2022). This especially holds true for studies involving benthic species, as the majority of protocols for generating pure cultures (Bold, 1942; Pringsheim, 1954; Lewin, 1959; Hoshaw and Rosowski, 1973; Daste *et al*., 1983; Perumal *et al*., 2015) are best suited for the isolation of planktic species.

The purpose of this study was to provide an approach for culturing benthic diatoms and to explore diatom diversity of a site. Thus, we assessed how many diatom taxa could be taken into short term (several months) culture from natural diatom assemblages, using four benthic samples from the Bow River. We i) identified the diatom assemblages using light microscopy (LM); ii) cultured as many taxa as possible from the same assemblages using three different culture media; and iii) captured images of both living cells and cleaned valves. Culturing success was measured with (A) the percentage of number of strain taxa from the number of taxa identified with LM and (B) the number of rare species, i.e., taxa that were captured in the cultures and not found on the assemblage slides. Using live pictures, this study further highlights several merits of generating unialgal diatom cultures.

METHODS

Bow River and sampling sites

The Bow River originates in the Canadian Rocky Mountains of southern Alberta and flows over 625 km east through subalpine forests, aspen parkland, and mixed grasslands to converge with the Oldman River and form the South Saskatchewan River (Fig. 1).

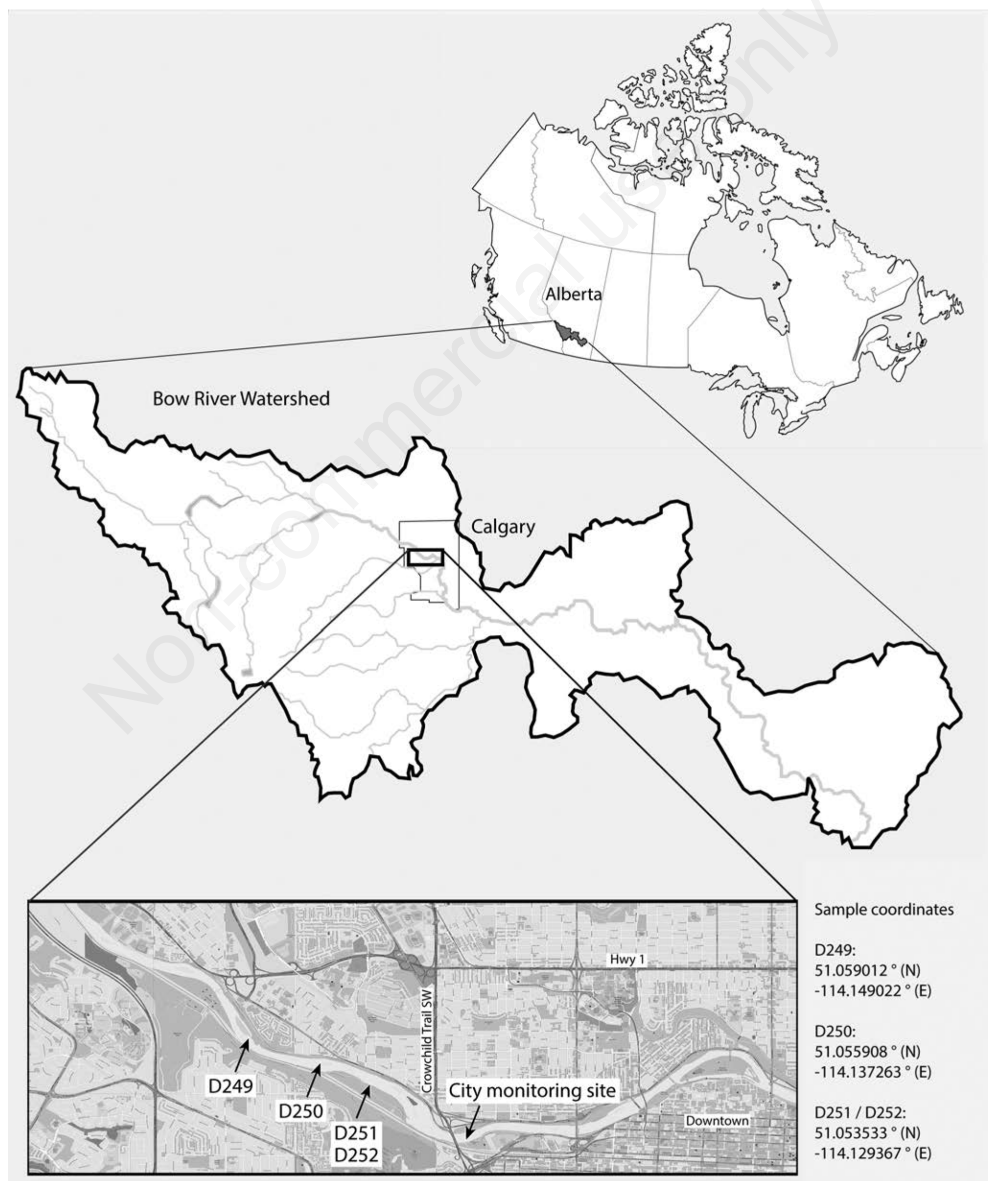
Periphyton samples were collected from four locations within a Bow River reach just upstream of the City of Calgary's downtown centre on June 17, 2018 (Fig. 1). The daily flow on the day of sampling was reported as 148 $m^3 s^{-1}$ (Water Survey of Canada, 2019), which is typical of mid May to mid June conditions when flows increase with mountain snowmelt and peak on average at 200 $m^3 s^{-1}$. Flows recede thereafter to return to average base flow levels of approximately 60 $m^3 s^{-1}$ by late fall (Water Survey of Canada, 2019).

Water quality conditions were measured on June 12, 2018 by the City of Calgary staff at a long-term monitoring site approximately 2.5 km downstream of the first periphyton sampling location and about 1 km downstream of the last sampling location (Fig. 1, Tab. 1; City of Cal-

Tab. 1. Summary of environmental, monthly spring conditions (April, May, June) in the Bow River at the City monitoring site (Fig. 1) for the 2003-2018 period and measurements of June 12, 2018 (City of Calgary, *unpublished*), i.e., five days prior to diatom sampling.

	Units	n	Minimum	Maximum	Median	June 12, 2018
Temperature*	°C	53	1.0	16.8	9.6	12.0
pH*		55	8.2	8.6	8.4	8.4
Specific conductivity*	µS/cm	54	205.5	378.6	316.5	273.0
Total alkalinity as $CaCO_3$	mg/L	53	104.7	156.4	128.6	112.0
Dissolved oxygen*	mg/L	54	8.6	15.1	10.9	10.7
Total phosphorus	µg/L	55	3	117	9	9
Total dissolved phosphorus	µg/L	54	1	23	2	6
Total nitrogen	mg/L	53	0.060	0.837	0.231	0.092
Silica	mg/L	21	2.71	6.51	3.28	NA

**Denotes parameter measured in the field, other parameters were measured at the City Laboratory: n, number of cases; NA, not available.*



Non-commercial use only

Fig. 1. Bow River watershed and location of the four periphyton sampling sites (samples D249-252) and the long-term City of Calgary water quality monitoring site (Tab. 1) in Calgary, Alberta, Canada.

gary, *unpublished*). June 12 conditions were similar to historical spring conditions (April, May and June of 2003-2018) in this reach of the Bow River. Conditions are generally low in nutrients with median TP and TN concentrations of 9 µg L^{-1} (n=55) and 231 µg L^{-1} (n=53), respectively (Tab. 1; City of Calgary, *unpublished*.). The Bow River is a freshwater and alkaline river with spring conductivity ranging from 206-379 µS cm^{-1} (n=54) and a pH range of 8.2-8.6 (n=55) (Tab. 1).

Sampling

The samples were collected in 20-30 cm water depth from the Bow River (Fig. 1) in the afternoon of June 17, 2018. About 20-30 mL of periphyton was collected from sand and some submerged plants for sample D249, from stones for samples D250 and D251 and from various macrophytes for sample D252. The living samples were sealed in 50 ml plastic vials, transported to Berlin (Germany) by airplane and then each sample was divided into two subsamples three days after sampling. One subsample was used for culturing. The other subsample was preserved with ethanol for subsequent analysis of the diatom assemblage.

Diatom isolation and culturing

Culture media

To maximize the number of cultured taxa, three different culture media were used. Two of those media were obtained ready-made: AlgaGro (AG) from Carolina Biological Supply Company (USA) and Walne's Medium (Wal) from VitaPlankton UG, Germany. According to the manufacturer the AG-medium is mineral-based (pers. comm.) and was designed for a large variety of algal groups, while the Wal-medium includes B vitamins (Walne, 1970; Andersen, 2005) and is commonly used for the enrichment of microalgae in marine aquaculture. Stock solutions of both media were diluted following the manufacturer's recommendations using Volvic mineral water.

Additionally, the AG medium was modified to create a third, humic medium (AG+HC). Humic substances have been reported to stimulate growth of certain marine and freshwater species (Prakash *et al*., 1973; Chen and Wang, 2008; Martin *et al*., 2014). There is no standard method to extract humic substances from soil extracts. To ensure reproducibility, we used synthetic humic substances. The basis of all humic substances are phenolic molecules like hydrochinone (Gerke, 2018). For the AG+HC medium, we added an aged and brownish solution of hydrochinone sulfonic acid, in which the monomers had polymerized creating an artificial humic system, to the AG medium (1 mg L^{-1} final concentration). From previous tests it was evident that some taxa grew particularly well in this modified medium (O. Skibbe, *personal observation*).

Enrichment cultures

Unialgal cultures are usually established by transplanting single living cells from their natural environment and community into an artificial environment. Both the act of mechanical removal and the exposure to different physical and chemical environmental factors may negatively affect the vitality of the cells. Additionally, many microalgae have strong interdependencies with other microorganisms and are difficult to culture in sterile culture media (Ashworth and Morris, 2016). Thus, we used an intermediate step in our approach (Fig. 2). We started with a multitude of enrichment cultures in three aforementioned media: small subsamples (e.g., grains of sand, small plant fragments with epiphytic diatoms, or 1-2 mL of detritus) were transferred from the collected environmental samples to 5 cm (diameter) Petri dishes filled with sterile-filtered liquid culture media. Thereafter, the remaining sample was extensively screened for larger and uncommon diatom taxa using a stereo microscope (Olympus SZH) and glass capillaries.

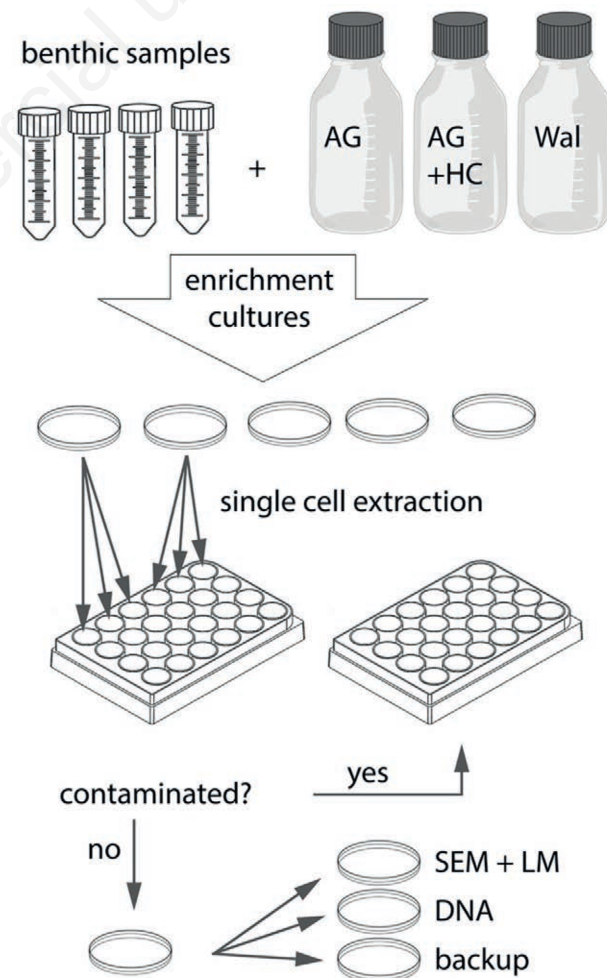


Fig. 2. Diagram of culturing benthic diatoms (for details see text).

Non-commercial use only

These selected cells were either transferred into existing enrichment cultures or gathered in another vial with culture medium. In this manner, a multitude of starter cultures were prepared that grew into enrichment cultures (Fig. 2). Due to heterogeneous distribution of diatoms in the original sample and random early colonization effects, the species assemblages often develop differently in enrichment cultures from the same sample. Thus, to maximize the number of cultured taxa our approach included a high number of replicates of enrichment cultures for each medium (about three to ten for each sample).

As the presence or absence of grazers may greatly influence the species abundance and composition in the enrichment cultures, invertebrates (crustaceans, worms, snails) and large protozoa (ciliates, heliozoa) were removed as much as possible. The presence of small heterotrophic nanoflagellates and amoeba on the other hand often stimulates diatom growth in cultures, as bacterivorous protozoa prohibit overgrowth of bacteria and also release nutrients (Sherr and Sherr, 2002). Thus, these organisms were left in the enrichment cultures.

Over the span of several weeks or months, the enrichment cultures were kept in an incubator (Memmert IPP) with refitted LED light (5000 K LED light, set to 300 Lux, 12:12h daylight) at 20°C. During this time, diatoms propagated and also adapted to the culture conditions, thereby increasing the chance of successful consecutive cell isolations.

Isolation of cells and removal of contaminants

For several weeks after incubation, the diatom taxa that grew well were isolated from the enrichment cultures (Fig. 2), in an attempt to maximize the number of cultured taxa. For this purpose, single cells were selected using an inverted microscope (Olympus CKX53). Each cell was transferred to one well of a 24-well cell culture plate (Sarstedt, Germany) using hand-drawn glass capillaries. Each well contained 1 mL of culture medium. If densities of diatoms and protists in the enrichment cultures were very high, an additional step of separation and washing was necessary, in which case a small number of target diatoms were transferred from the enrichment culture into a clean petri dish. Then, we carefully selected single cells and ensured that no protists were attached before transferring to micro wells.

Cell reproduction and absence of contaminants were monitored at 400x magnification every two weeks. Eukaryotic contaminants were removed where possible from the isolates to facilitate future molecular analysis, as the presence of phylogenetically related protist taxa may interfere with diatom-specific PCA primers used for DNA sequencing. Typical contaminants found in our cultures were heterotrophic flagellated or amoeboid protists and green algae. Particular attention was paid to heterotrophic chrysophytes, whose DNA is amplified by diatom-specific primers (Pérez-Burillo *et al*., 2022). Whenever contaminations were detected, single diatom cells were once again isolated. Multiple re-isolations of the same strain sometimes led to strain-duplicates.

Short term culturing

After diatom densities had increased and cells covered the bottom of the wells, isolates were transferred from 24-well plates to 5 cm Petri dishes and kept in the incubators to generate more material. Typically, a few weeks later, densities were sufficient to split the cultures into three subcultures, again in 5 cm Petri dishes: One subculture was grown for morphological analysis (LM, SEM), one for future DNA-analysis and the third was stored frozen as a back-up (Fig. 2). One to three weeks after splitting, subcultures were usually ready for harvest. Prior to harvesting the diatoms, images of living cells of each culture were taken to document protoplast details, such as plastids. In this paper we use the term ‘plastids’ and not ‘chloroplasts’ for the photosynthetic organelles of diatoms, because they are secondary plastids derived from red algae and not directly related to the chloroplasts of green algae and plants (McFadden and van Dooren, 2004). LM micrographs were taken with a Zeiss AxioCam HRc camera on a Axio Imager M2 microscope using a 100/1.4 Plan-Apochromat objective and differential interference contrast. Images were selected and arranged to emphasize and compare features of interest using Adobe Photoshop and Illustrator.

Preparation of permanent diatom slides and SEM stubs

For the diatom assemblage samples and the preserved culture subsamples, cell contents were oxidized using 35% hydrogen peroxide and rinsed using centrifugation (10 min at 2000 rpm). Cleaned valves were mounted on glass slides for LM using Naphrax. For SEM the valves were dried on 10x10 mm silicon wafer chips and mounted on stubs. Unsputtered valves were examined at 1kV accelerating voltage using a Hitachi SU 800 SEM.

Assessment of preserved diatom assemblages

For the identification of the diatom assemblages, a minimum of 400 benthic valves per sample were counted using a 100x objective (1.4 aperture) with the microscope Zeiss Axio-Scope A1. Diatoms were counted, if the valve was intact to at least 50%. Subsequently, the slides were scanned for a minimum of 30 additional rare taxa. All taxa were photographed using an AxioCam ICm1 camera and differential interference contrast. Images were arranged and cropped using Adobe Photoshop and Illustrator.

Morphological identification

Identification of taxa was based on light microscopy using Krammer and Lange-Bertalot (1988-2004), Lange-

Non-commercial use only

Bertalot and Moser (1994), Lange-Bertalot and Metzeltin (1996), Krammer (1997a, 1997b, 2000, 2002, 2003), Reichardt (1999), Lange-Bertalot (2001), Potapova and Ponader (2004), Levkov (2009), Potapova (2009), Hofmann *et al*. (2011), Lange-Bertalot *et al*. (2011), English and Potapova (2012), Reichardt (2012), Novais *et al*. (2015), Wetzel *et al*. (2015), Levkov *et al*. (2016), Jahn *et al*. (2017), Lange-Bertalot *et al*. (2017), Hatcher (2018), Bahls *et al*. (2018), Reichardt (2018), Bahls (2019) and Wynne (2019).

Whenever possible, identification literature from Canada and the USA was consulted, but often European literature had to be used. Taxa were designated as 'sp.', if no similarity to a described taxon was found. Taxa were designated as 'species affinis' (aff.), if they looked similar to a described species (and are probably closely related), but differed from this species in a way that suggests that the named taxon is probably another (unknown) species. Taxa were designated as 'confer' (cf.), if they shared most but not all of their characteristics with an established taxon from identification literature (and are probably identical). The term *sensu lato* (s.l.) after the name of a taxon, such as *Achnanthidium minutissimum* s.l., signifies that this taxon probably comprises a complex of species or many different taxa which have not yet been taxonomically evaluated in detail. LM pictures were presented of identified taxa categorised as ambiguous (sp., aff., cf., s.l.) to enable comparison of counting results among diatomists (Dreßler *et al*., 2015).

We used two methods to evaluate our results. For documenting the diversity, we used species count with the highest taxonomic resolution feasible (based on LM evaluation). For comparing the species counts between the preserved assemblage and the cultures we based our analysis on species resolution to the sensu lato level in order to achieve a robust assessment of culturing success.

RESULTS

Preserved diatom assemblages

A total of 182 different diatom taxa were identified on the slides from the four samples including ten planktic taxa (Tab. 2).

Non-commercial use only

Tab. 2. List and relative abundances of taxa from preserved assemblages from four samples of the Bow River.

Taxon / substrate	D2 49 (Sand)	D2 50 (Stone)	D2 51 (Stone)	D2 52 (MP)	Comment
Achnanthidium jackii Rabenhorst	3.9	5.4	6.7	3.7	
Achnanthidium kranzii (Lange-Bertalot) Round & Bukhtiyarova	0.2				
Achnanthidium minutissimum (Kützing) Czarnecki **s.l.**					
Achnanthidium minutissimum (<2.5um wide)	4.8	9.3	7.1	14	
A. minutissimium (Kützing) Czarnecki var. *minutissimum*	15.3	22.8	31.6	26.1	
Achnanthidium neomicrocephalum Lange-Bertalot & Staab				0.2	
Achnanthidium pyrenaicum (Hustedt) Kobayasi	0.9				
Achnanthidium cf. *rivulare*	0.4	1.2	0.6	1.8	
Achnanthidium rosenstockii (Lange-Bertalot) Lange-Bertalot	1.4				
Achnanthidium straubianum (Lange-Bertalot) Lange-Bertalot	3.0	0.7	x		
Adlafia bryophila (J.B.Petersen) Lange-Bertalot	x*				
Adlafia detenta (Hustedt) Heudre, C.E.Wetzel & Ector	x				
Amphora copulata (Kützing) Schoeman & R.E.M.Archibald	x	x	x	x	
Amphora eximia J.R.Carter				x	
Amphora indistincta Levkov	0.9	0.2		0.2	
Amphora cf. *neglecta* f. *densestriata*				0.5	
Amphora ovalis Kützing			x		
Amphora pediculus (Kützing) Grunow	2.5	0.2			
Asterionella formosa Hassall (P)	0.7	0.5	1.7	0.2	
Brachysira neoexilis Lange-Bertalot	3.0	3.0	1.7	0.5	
Caloneis sp. 3 (*Caloneis* aff. *bacillum*)				0.5	similar to *Caloneis* sp. 1
Caloneis alpestris (Grunow) Cleve	x				[1]
Caloneis cf. *alpestris*				0.5	
Cavinula cocconeiformis (W.Gregory ex Greville) D.G.Mann & A.J.Stickle	0.2				
Cocconeis neodiminuta Krammer		x			

To be continued on next page

Tab. 2. Continued from previous page.

Taxon / substrate	D2 49 (Sand)	D2 50 (Stone)	D2 51 (Stone)	D2 52 (MP)	Comment
Cocconeis pediculus Ehrenberg	x	x	x	x	
Cocconeis placentula Ehrenberg s.l.	0.5	x		x	
Cyclotella comensis Grunow (P)	0.7	0.9	0.9	0.5	
Cyclotella distinguenda Hustedt (P)		x	0.2		
Cyclotella ocellata Pantocsek (P)	0.5				
Cymbella hantzschiana Krammer			x		
Cymbella vulgata Krammer	0.5	x	3.9	0.5	sensu Krammer, 2002
Cymbopleura amphicephala (Nägeli ex Kützing) Krammer	x				
Cymbopleura cf. *incerta* var. *grunowii*			x		
Delicatophycus cf. *montana*		x	0.6		
Delicatophycus sparsistriatus M.J.Wynne	0.5	0.2	x	x	
Denticula tenuis Kützing	0.2	0.2	x	0.2	
Diatoma moniliformis (Kützing) D.M.Williams	7.7	16.3	17.6	12.4	sensu Potapova, 2009
Diatoma problematica Lange-Bertalot	0.5	0.2	0.6	0.2	[1]
Diatoma tenuis C.Agardh (P)	3.2	3.0	8.2	5.0	
Didymosphenia geminata (Lyngbye) Mart.Schmidt	x	0.2	x	0.5	
Diploneis oculata (Brébisson) Cleve	0.2				[1]: Plate 66: 28-30.
Diploneis cf. *puella*				x	
Diploneis separanda Lange-Bertalot				x	
Encyonema cespitosum Kützing	0.2	1.2	0.4	0.7	
Encyonema evergladianum Krammer		0.5			
Encyonema minutum (Hilse) D.G.Mann		x	0.4		
Encyonema prostratum (Berkeley) Kützing		x			
Encyonema silesiacum (Bleisch) D.G.Mann s.l.:					
Encyonema silesiacum var. *altense* Krammer	0.5		x		sensu Krammer 1997a, 1997b
Encyonema silesiacum var. *latum* Krammer		x			sensu Krammer 1997a, 1997b
Encyonema silesiacum (Bleisch) D.G.Mann var. *silesiacum*	0.2			0.2	sensu Krammer 1997a, 1997b
Encyonema silesiacum cf. var. *silesiacum*				0.5	sensu Krammer 1997a, 1997b
Encyonema ventricosum (C.Agardh) Grunow morphotype 1	0.5	1.9	2.4	3.2	sensu Krammer 1997a, 1997b
Encyonema ventricosum var. *angustum* Krammer	0.5	0.9		2.5	sensu Krammer 1997a, 1997b
Encyonopsis cesatiformis Krammer	0.2	x	x	0.5	
Encyonopsis microcephala (Grunow) Krammer	x			0.5	
Encyonopsis minuta Krammer & E.Reichardt	0.7	1.6	0.6	1.1	
Encyonopsis subminuta Krammer & E.Reichardt	1.1	7.5	3.4	5.7	
Encyonopsis tavirana Krammer	2.7				
Eucocconeis flexella (Kützing) F.Meister	0.2	0.7	x	x	
Eucocconeis laevis (Østrup) Lange-Bertalot **s.l.**					
Eucocconeis quadratarea (Østrup) Lange-Bertalot	1.1	x	0.2	x	
Fallacia lucinensis (Hustedt) D.G.Mann		x			
Fallacia subhamulata (Grunow) D.G.Mann		0.5	x	0.5	
Fragilaria cf. *austriaca*			0.2		
Fragilaria capucina var. *capucina* Desmazières	1.8	0.7	x	0.7	
Fragilaria capucina cf. var. *capucina*			0.2		
Fragilaria crotonensis Kitton (P)		0.2			
Fragilaria cf. *crotonensis* (P)				0.2	
Fragilaria cf. *drouotiana*		x			
Fragilaria gracilis Østrup				1.4	
Fragilaria mesolepta Rabenhorst		0.2			

To be continued on next page

Non-commercial use only

Tab. 2. Continued from previous page.

Taxon / substrate	D2 49 (Sand)	D2 50 (Stone)	D2 51 (Stone)	D2 52 (MP)	Comment
Fragilaria perdelicatissima Lange-Bertalot & Van de Vijver	0.9			0.5	
Fragilaria cf. *perdelicatissima*			0.2		
Fragilaria cf. *perminuta*			0.2	0.7	
Fragilaria sandellii Lange-Bertalot & Van de Vijver	0.2				
Fragilaria saxoplanctonica Lange-Bertalot & S.Ulrich		0.7	1.1	0.2	
Fragilaria tenera Lange-Bertalot var. *tenera*	0.2			0.2	
Fragilaria cf. *tenera* var. *tenera*	0.2*				
Fragilaria vaucheriae (Kützing) J.B.Petersen		x			
Geissleria decussis (Østrup) Lange-Bertalot & Metzeltin	x				
Gomphonella olivacea (Hornem.) Rabh.	x	x	x	0.5	syn.: *Gomphonema olivaceum*
Gomphonema sp. 1 (*Gomphonema* aff. *minusculum*)		5.8	0.2	0.5	
Gomphonema sp. 2			0.4		
Gomphonema sp. 3 (*Gomphonema* aff. *minusculum*)		0.7			
Gomphonema cf. *clavatulum*	0.2*				
Gomphonema lateripunctatum E.Reichardt & Lange-Bertalot	0.7	x	0.4	x	
Gomphonema cf. *micropus*			x		
Gomphonema cf. *minusculum*	0.5		0.2	x	
Gomphonema minutum (C.Agardh) C.Agardh	0.5		x		
Gomphonema olivaceoides Hustedt		x	0.2	x	
Gomphonema cf. *pseudobohemicum*				0.2	
Gyrosigma acuminatum (Kützing) Rabenhorst				x*	
Halamphora thumensis (Ant.Mayer) Levkov	0.5	x			
Hannaea arcus (Ehrenberg) R.M.Patrick				0.2	
Hantzschia amphioxys (Ehrenberg) Grunow sensu stricto				x*	
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski		x*		x	
Hippodonta cf. *hungarica*		x			
Karayevia clevei (Grunow) Bukhtiyarova var. *clevei*	0.5	x			
Karayevia clevei (Hustedt) Bukhtiyarova **var. *rostrata***	0.2				
Krasskella cf. *kriegerana*	0.2				
Luticola cohnii (Hilse) D.G.Mann				x	
Mastogloia lacustris (Grunow) Grunow				x	
Melosira varians C.Agardh		0.2			
Navicula sp. (*Navicula* aff. *wendlingii*)		x			
Navicula antonii Lange-Bertalot	0.2			0.2	
Navicula capitatoradiata H.Germain ex Gasse	x		x	x	
***Navicula caterva-reichardtiana* s.l.**		x		x	
Navicula cryptotenella Lange-Bertalot	0.2	0.5	0.4	0.7	
Navicula* cf. *exilis		x			
Navicula cf. *gottlandica*			x	0.2	
Navicula gregaria Donkin				x	
Navicula cf. *lacuum*	x				
Navicula lanceolata Ehrenberg	x				
Navicula libonensis Schoeman	0.2				
Navicula cf. *libonensis*	x				
Navicula moskalii Metzeltin, Witkowski & Lange-Bertalot		x		x	
Navicula pseudolanceolata Lange-Bertalot	x		0.2		
Navicula radiosa Kützing	x	x	x	x	

To be continued on next page

Non-commercial use only

Tab. 2. Continued from previous page.

Taxon / substrate	D2 49 (Sand)	D2 50 (Stone)	D2 51 (Stone)	D2 52 (MP)	Comment
Navicula tripunctata (O.F.Müller) Bory				x	
Navicula veneta Kützing		0.5		x	
Navicula viridula (Kützing) Ehrenberg	0.5				
Neidiomorpha cf. *binodis*	x			x	
Nitzschia sp. 1 (*Nitzschia* aff. *pura -puriformis -sublinearis*)	0.7	0.2	0.4	0.5	
Nitzschia sp. 2	0.2				
Nitzschia cf. *acidoclinata*	0.2	0.5			
Nitzschia cf. *adamata*	x	0.2			
Nitzschia amphibia Grunow	x			0.5	
Nitzschia angustata (W.Smith) Grunow	0.2	0.2	x	0.2	
Nitzschia archibaldii Lange-Bertalot	2.3				
Nitzschia capitellata Hustedt	0.2	x		x	
Nitzschia denticula Grunow	0.7	x	x	x	
Nitzschia dissipata (Kützing) Rabenhorst ssp. *dissipata*	1.6	0.5	0.2	0.2	
Nitzschia dissipata (Hantzsch) Grunow var. *media*	1.6	0.2	x		
Nitzschia draveillensis Coste & Ricard (P)	0.2				
Nitzschia fonticola (Grunow) Grunow	0.5	0.5	0.2	0.2	
Nitzschia frustulum (Kützing) Grunow var. *frustulum*	0.2				
Nitzschia cf. *graciliformis* (P)		0.2			
Nitzschia lacuum Lange-Bertalot	0.2	0.7	0.2	x	
Nitzschia microcephala Grunow			0.2		
Nitzschia oligotraphenta (Lange-Bertalot) Lange-Bertalot			x		
Nitzschia palea (Kützing) W.Smith **s.l.**	0.5	x		0.2*	
Nitzschia cf. *paleacea*	2.5			0.5	
Nitzschia pura Hustedt	0.5	0.2	0.2	0.2	[1]
Nitzschia pusilla Grunow				0.2	
Nitzschia recta Hantzsch ex Rabenhorst	0.5	0.2	x	0.2	
Nitzschia solgensis A.Cleve				0.2	
Nitzschia supralitorea Lange-Bertalot	0.2				
Nitzschia tabellaria (Grunow) Grunow		x			
Nitzschia tenuis W.Smith			x		
Nitzschia valdestriata Aleem & Hustedt	x				
Odontidium mesodon (Kützing) Kützing		x			syn.: *Diatoma mesodon*
Parlibellus protracta (Grunow) Witkowski, Lange-Bertalot & Metzeltin	x				
Pennales (= aff. *Sellaphora joubaudii* [1] = aff. *S. seminulum* [2])		x			
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot s.l.	0.2			x	
Planothidium rostratum (Østrup) Lange-Bertalot **s.l.**	0.2				
Platessa conspicua (Ant.Mayer) Lange-Bertalot	0.2*				
Platessa ziegleri (Lange-Bertalot) Lange-Bertalot		x			
Psammothidium daonense (Lange-Bertalot) Lange-Bertalot	x				
Psammothidium cf. *levanderi*	0.2				
Pseudostaurosira sp. (aff. *P. parasitica* var. *parasitica*)	x				
Pseudostaurosira brevistriata (Grunow) D.M.Williams & Round	3.4	0.2	0.9	0.5	
Pseudostaurosira parasitica var. *parasitica* (W.Smith) E.Morales	0.2	x	x		
Reimeria sinuata (W.Gregory) Kociolek & Stoermer	x	x	x	x	
Sellaphora nigri (De Notaris) C.E.Wetzel & Ector **s.l.**	x	x			syn: *Eolimna minima* [1]
Sellaphora pupula (Kützing) Mereschkovsky **s.l.**	1.6	x	x	0.5	
Sellaphora stroemii (Hustedt) H.Kobayasi	x	x	x	x	

To be continued on next page

Non-commercial use only

Each sample included 80 to 111 taxa, of which 31 to 43 taxa were only found during the scan for rare taxa. *Achnanthidium minutissimum* (Kützing) Czarnecki var. *minutissimum* (Fig. 10) dominated the assemblages with 15-32% relative abundance, while *Diatoma moniliformis* (Kützing) D.M.Williams sensu Potapova (2009) (8–18%) and slender cells of *A. minutissimum* s.l. (<2.5 µm wide; 5-14%; Figs. 12-14) were co-dominant. Abundant taxa included planktic *Diatoma tenuis* C. Agardh (3-8%) and benthic *Encyonopsis subminuta* Krammer & E. Reichardt (1-7%), *Achnanthidium jackii* Rabenhorst (4-7%, Figs. 5-7) and *Staurosira venter* (Ehrenberg) Cleve & J.D.Möller (0.4–6%, Figs. 44-46). *Gomphonema* sp. 1 (Figs. 88-93) occurred with 6% in one sample (D250) and was identified in two other samples with 0.2% and 0.5%, respectively. The remaining taxa contributed a maximum of 4% to a sample. Planktic diatoms contributed relative abundances of 5-11% per sample to the assemblages (Tab. 2). Cleaned valve images are presented for all taxa that could only be identified and categorised as ambiguous (sp., aff., cf., s.l.) with the identification literature cited together with otherwise discussed taxa and ambiguously identified or similar taxa from cultures (Figs. 3-154). We were very strict with the identification, i.e., as soon as one feature of a taxon did not match the description in the identification literature to 100%, we labelled the species with a 'cf.'. Thus, we ensured that our counting results can be compared among diatomists (Dreßler *et al*., 2015).

Diatom cultures

During the course of six months more than 300 single diatom cells were isolated from the four benthic diatom samples of the Bow River. From these a total of 129 strains were successfully established representing 106 clones (Tab. 3). Twenty-three strains were duplicates. The 106 different clones represent 71 different taxa when basing the identification predominantly on light microscopy. A total of 70 cultures grew in the Wal medium, 28 in AG+HC medium, 27 in AG medium. Four strains first grew in AG+HC medium and were transferred to Wal medium after four weeks because of weak growth. These strains were all duplicates of *Encyonema temperei* Krammer (Tab. 3). Twelve of the 71 cultured taxa grew in two different media (Tab. 3). Overall, the Wal medium was suitable for many diatom taxa, but also stimulated growth of bacteria

Non-commercial use only

Tab. 2. Continued from previous page.

Taxon / substrate	D2 49 (Sand)	D2 50 (Stone)	D2 51 (Stone)	D2 52 (MP)	Comment
***Sellaphora* cf. *stroemii* 2**	x				
Stauroneis separanda Lange-Bertalot & Werum	x				
Stauroneis smithii Grunow var. *smithii*	x	x			
Staurosira construens Ehrenberg	0.2	0.2	x	x	
Staurosira venter (Ehrenberg) Cleve & J.D.Möller	6.2	0.5	0.4	1.1	
Staurosirella leptost. (Ehrenberg) D.M.Williams & Round var. *leptostauron*	1.1	x	0.2	x	
Staurosirella neopinnata (Ehrenberg) E.A.Morales, C.E.Wetzel *et al.* **s.l.**	3.9	1.2	1.3	1.1	
***Surirella* sp.** (syn: *Cymatopleura* sp.)'			x		twisted form
Surirella apiculata W.Smith (syn: *Cymatopleura solea* var. *apiculata*)		x			
Surirella lacrimula J.D.English	x		x		English and Potapova, 2012
Surirella librile (Ehrenberg) Ehrenberg (syn: *Cymatopleura solea* var. *solea*)	x	x	x		
Surirella cf. *visurgis*		x			
Tabellaria flocculosa (Roth) Kützing				0.2	
Ulnaria sp.		0.2	0.2	0.2	
Ulnaria acus (Kützing) Aboal s.l.	0.5	1.2	0.6	1.4	
Ulnaria biceps (Kützing) Compère		4.0	1.5	2.7	
Ulnaria ulna (Nitzsch) Compère			0.2		
#Taxa: (max. taxonomic resolution): 182	**111**	**95**	**80**	**94**	
#Taxa: (sensu lato resolution): 178	**110**	**94**	**79**	**92**	
#only found during scan (x)	**31**	**43**	**34**	**34**	
Sum of counted valves	**439**	**429**	**465**	**437**	

*MP, maccrophytes; Bold, Taxa were successfully taken in culture (Tab. 3); x, only found during scan, *only broken valves found; (P), planktic taxon; [1], sensu Hofmann et al., 2011; [2], sensu Wetzel et al., 2015.*

Tab. 3. List of taxa taken into culture from the four samples of the Bow River.

Taxon	D2 49	D2 50	D2 51	D2 52	Culture medium	Comments
Achnanthidium minutissimum (Kützing) Czarnecki **s.l.**						
Achnanthidium lineare W.Smith	■				AG+HC	
A. minutissimum (Kützing) Czarnecki var. *minutissimum*			■		AG+HC	
Amphora sp.	■			■	AG+HC, Wal	
Amphora affinis Kützing s.l.	■			■	AG+HC, Wal	
Amphora ovalis Kützing	■				Wal	
Caloneis sp. 1		■			Wal	
Caloneis sp. 2	■				Wal	
Caloneis amphisbaena (Bory) Cleve	■				Wal	
Caloneis cf. *leptosoma*				■	Wal	
Cocconeis pediculus Ehrenberg	■				AG	
Cymbella proxima Reimer	■				Wal	
Cymbopleura sp.	■				AG+HC	
Cymbopleura cf. *florentina*	■				AG	Krammer, 2003
Cymbopleura lata (Grunow ex Cleve) Krammer	■				Wal	Auxospores
Cymbopleura naviculiformis (Auerswald ex Heiberg) Krammer	■				Wal	
Diatoma moniliformis (Kützing) D.M.Williams	■				AG	
Diploneis sp.	■		■		AG+HC, Wal	
Diploneis cf. *separanda*				■	AG, AG+HC	
Encyonema cespitosum Kützing				■	Wal	
Encyonema cf. *silesiacum* s.l.				■	Wal	
Encyonema temperei Krammer				■	AG+HC, Wal	
Encyonopsis subminuta Krammer & E.Reichardt				■	Wal	
Entomoneis ornata (Bailey) Reimer	■				Wal	
Eucocconeis flexella (Kützing) F.Meister	■	■			AG, Wal	
Eucocconeis laevis (Østrup) Lange-Bertalot **s.l.**			■		AG+HC	
Eunotia arcus Ehrenberg				■	Wal	
Fallacia pygmaea (Kützing) Stickle & D.G.Mann		■			AG	
Geissleria decussis (Østrup) Lange-Bertalot & Metzeltin	■				AG+HC	
Gomphonema capitatum Ehrenberg		■			AG	
Halamphora thumensis (A.Mayer) Levkov	■				Wal	
Iconella cf. *hibernica (Campylodiscus* cf. *hibernicus)*	■				Wal	
Iconella cf. *splendida*	■				Wal	
Karayevia clevei (Hustedt) Bukhtiyarova **var. *rostrata***	■				AG+HC	
Navicula capitatoradiata H.Germain ex Gasse	■			■	AG+HC, Wal	
***Navicula caterva-reichardtiana* s.l.**		■			AG	
Navicula* cf. *exilis				■	Wal	
Navicula gregaria Donkin	■				Wal	
Navicula libonensis Schoeman	■				Wal	
Navicula radiosa Kützing				■	AG+HC	
Navicula* cf. *tripunctata (O.F.Müller) Bory				■	Wal	
Navicula veneta Kützing	■				AG, Wal	
Navicula viridulacalcis Lange-Bertalot	■				AG+HC	
Neidiomorpha binodiformis (Krammer) Cantonati, Lange-Bertalot & N.Angeli	■				Wal	
Neidium affine (Ehrenberg) Pfitzer	■				AG	
Neidium ampliatum Ehrenberg s.l.	■				AG+HC, Wal	
Nitzschia amphibia Grunow	■				AG	
Nitzschia denticula Grunow			■		AG	
Nitzschia lacuum Lange-Bertalot		■			AG+HC	

To be continued on next page

Non-commercial use only

and bacterivorous protists, i.e., in consequence led to contaminated strains that needed further purification. The most common contaminants were heterotrophic protozoa (bodonids, cercomonads, choanoflagellates) and mixotrophic or colourless chrysophytes, especially '*Spumella*-like' flagellates as defined by Grossmann *et al*. (2016).

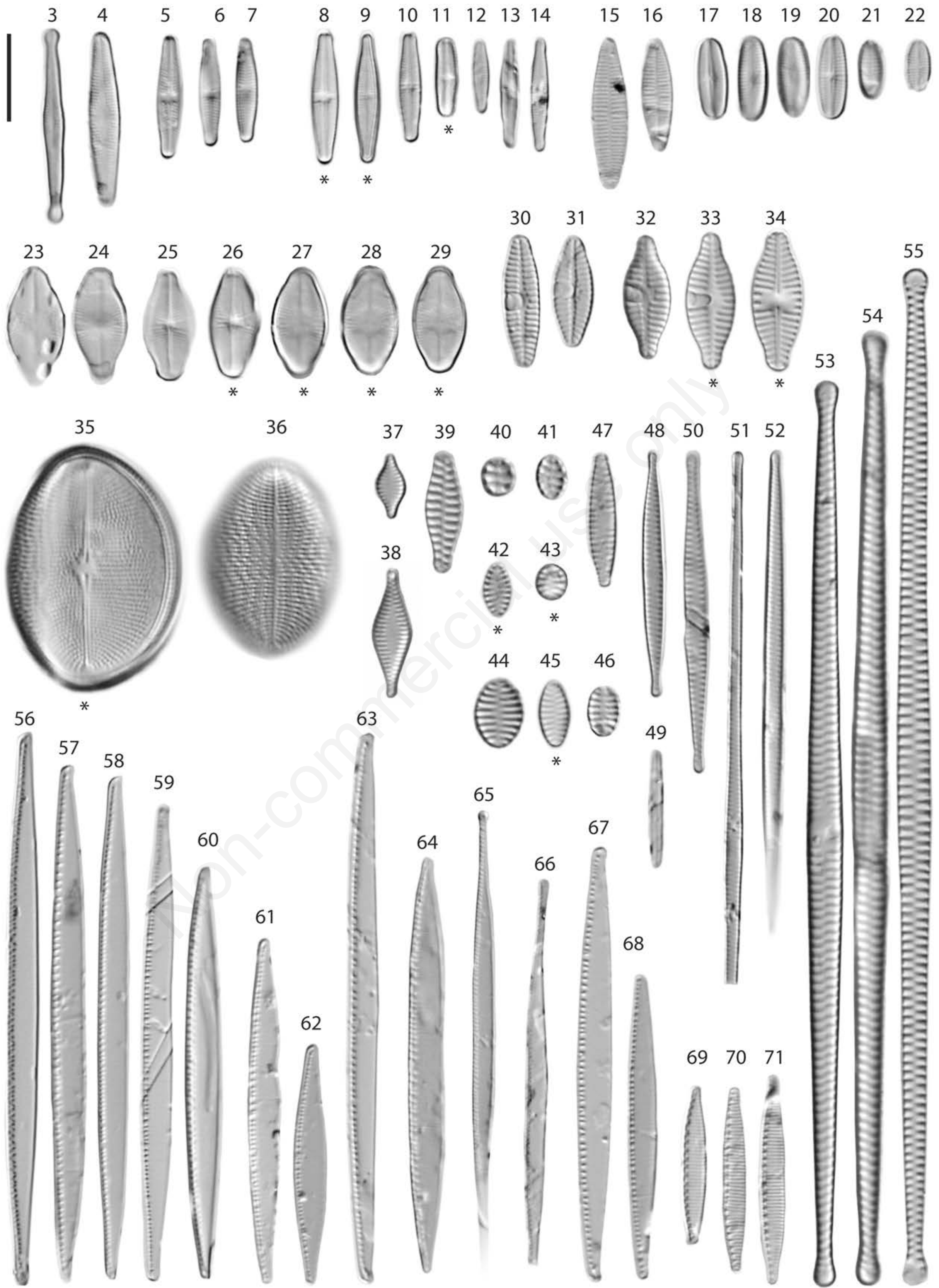
Already in the enrichment cultures, several different diatom life-forms were discernible. Aside from taxa that are generally considered epiphytic but nevertheless grew on non-organic surfaces like the bottom of the Petri dish (e.g., *Gomphonema capitatum* Ehrenberg, *Cocconeis pediculus* Ehrenberg), there were taxa that preferred an epipelic lifestyle and were mostly found in detritus conglomerates (*Nitzschia*, *Pinnularia*, *Neidium*) and also typically epipsammic taxa covering the surface of sand grains (*Karayevia, Planothidium rostratum* (Østrup) Lange-Bertalot, Fig. 162). A peculiarity of the sand sample (D249) was the presence of large twisted diatoms such as *Iconella* cf. *hibernica* (formerly *Campylodiscus* cf. *hibernicus*, Fig. 163), *Entomoneis ornata* (Figs. 158-161) and *Surirella* sp. (Figs. 152, 155-157; Tab. 3). Additionally, overall species diversity was exceptionally high in the sand sample as 142 of 221 observed taxa occurred here, among them were 43 of 71 cultured taxa (Tabs. 2 and 3).

Figs. 3-71. Cleaned valve pictures of taxa categorised as ambiguous (sp., aff., cf., s.l.) or otherwise mentioned taxa from the preserved assemblages and from the cultures. Valves from cultures are marked with an asterisk (*): (3) *Achnanthidium neomicrocephalum*; (4) *A. kranzii*; (5-7) *A. jackii*; (8-14) *A. minutissimum* s.l.; (8-10) *A. minutissimum* var. *minutissimum*; (8-9) Culture D251_006 (11) *A. lineare*, Culture D249_025; (12-14) A. *minutissimum* <2.5µm wide; (15-16) *A. pyrenaicum*; (17-21) *A.* cf. *rivulare;* (22) *A. straubianum;* (23-29) *Eucocconeis laevis* s.l.; (23-25) *E. quadratarea;* (26-29) Culture D251_007; (30-31) *Planothidium frequentissimum* s.l.; (32-34) *P. rostratum* s.l.; (33-34) Culture D249_045; (35-36) *Cocconeis pediculus*; (35) Culture D249_039; (37) *Pseudostaurosira* sp.; (38) *P. parasitica* var. *parasitica*; (39-41) *Staurosirella neopinnata* s.l.; (42-43) *Staurosira venter* s.l.; (42) Culture D249_049; (43) Culture D252_007; (44-46) *Staurosira venter*; (45) Culture D249_048; (47) *Fragilaria capucina* cf. var. *capucina*; (48) *F.* cf. *austriaca*; (49) *Krasskella* cf. *kriegerana*; (50) *Fragilaria* cf. *crotonensis*; (51) *F.* cf. *tenera* var. *tenera;* (52) *F. tenera* var. *tenera*; (53-55) *Ulnaria* sp.; (56-60) *Nitzschia* sp. 1 (*N.* aff. *pura-puriformis-sublinearis*); (61-62) *Nitzschia pura* sensu Hofmann *et al*., 2011*;* (63) *Nitzschia* sp. 2; (64) *N.* cf. *adamata*; (65) *N.* cf. *graciliformis*; (66) *N.* cf. *paleacea*; (67-68) *N. palea* s.l.; (69-71) *N.* cf. *acidoclinata.* Scale bar: 10 µm.

Tab. 3. Continued from previous page.

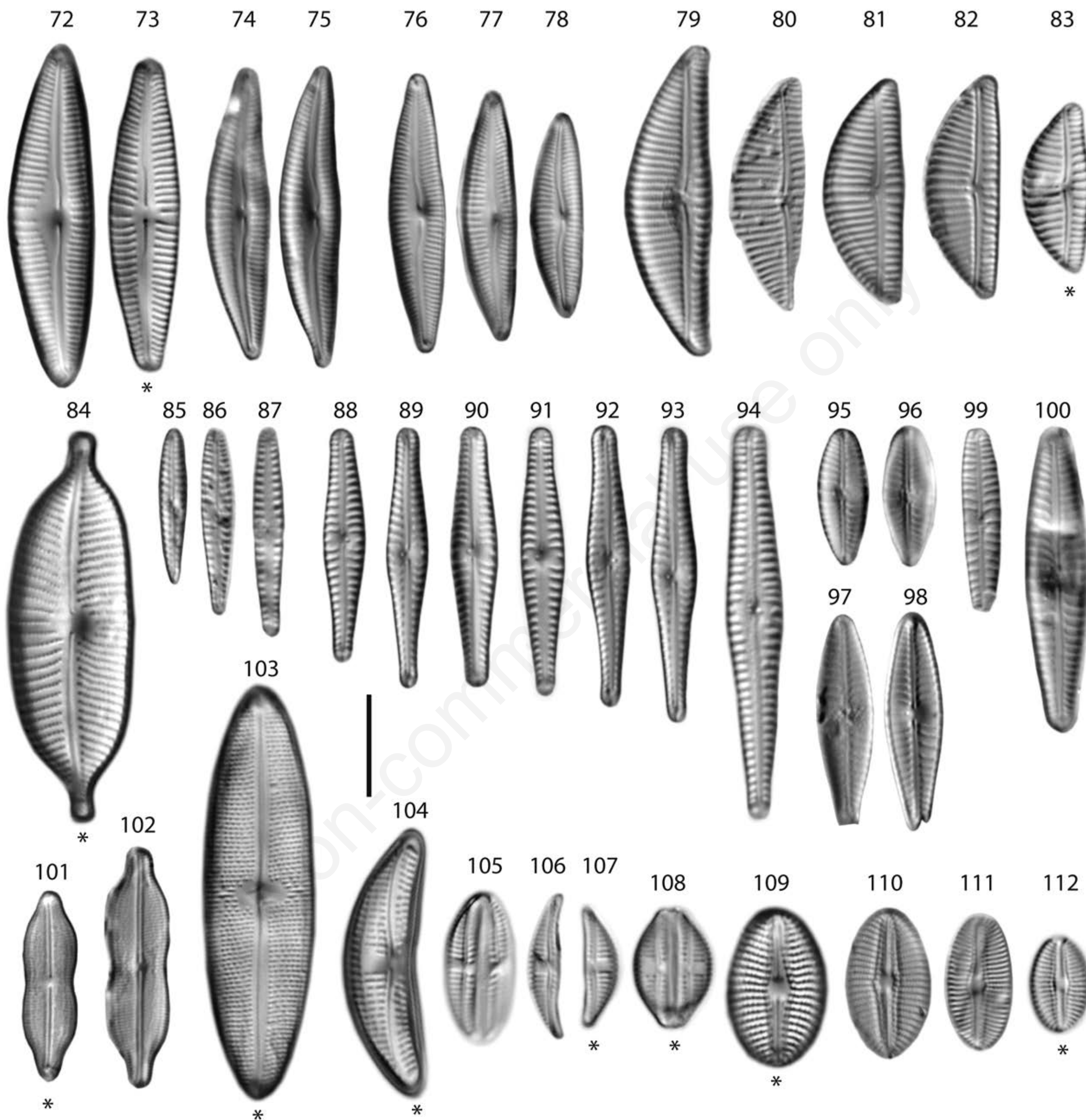
Taxon	D2 49	D2 50	D2 51	D2 52	Culture medium	Comments
Nitzschia palea (Kützing) W.Smith **s.l.**		■			AG	
Pinnularia sp.				■	Wal	
Pinnularia cf. *viridiformis*	■				AG+HC	
Placoneis placentula (Ehrenberg) Mereschkowsky	■				AG+HC	
Planothidium rostratum (Østrup) Lange-Bertalot **s.l.**	■				Wal	
Pseudostaurosira parasitica E.Morales var. *subconstricta*		■			AG	
Rhopalodia gibba (Ehrenberg) O.Müller var. *gibba*		■			AG	
Sellaphora mutatoides Lange-Bertalot & Metzeltin	■				AG	Auxospores
Sellaphora nigri (De Notaris) Wetzel & Ector **s.l.**		■			Wal	*Eolimna minima*
Sellaphora pupula (Kützing) Mereschkovsky **s.l.**	■				AG+HC, Wal	
Sellaphora cf. *saugerresii* (syn: S. seminulum [1])	■				Wal	Auxospores
Sellaphora* cf. *stroemii 1		■			AG+HC	
Sellaphora cf. *vitabunda*	■				Wal	
Stauroneis cf. *subgracilis*	■				Wal	
Staurosira venter (Ehrenberg) Cleve & J.D.Möller	■				AG	
Staurosira venter s.l.	■			■	AG, Wal	
Staurosirella neopinnata (Ehrenberg) E.A.Morales *et al.* **s.l.**		■			Wal	
***Surirella* sp.**	■				Wal	
Surirella angusta Kützing		■			AG	
Surirella lacrimula J.D.English		■			AG	
Surirella undulata (Ehrenberg) Ehrenberg			■		AG	*syn: C. elliptica*
Surirella visurgis Hustedt	■				Wal	
Tryblionella brunoi (Lange-Bertalot) Cantonati & Lange-Bertalot	■				AG+HC	
#Taxa: 71	**43**	**14**	**5**	**15**		

Bold, taxa also found in the preserved assemblages (Tab. 2); [1], sensu Hofmann et al., 2011.

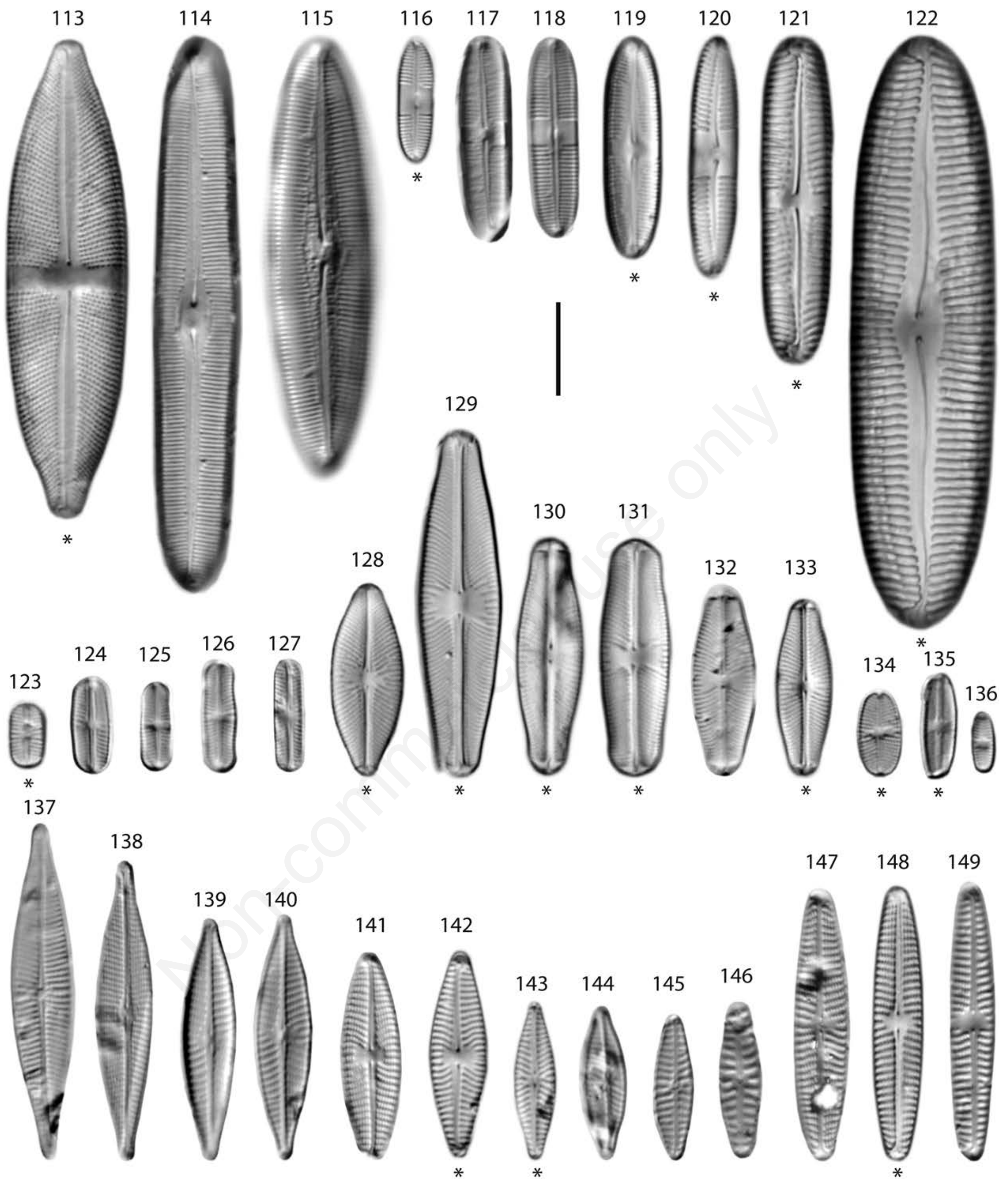


Non-commercial use only

Unfortunately, diatoms had suffered during transport. Some species were abundant (*Didymosphenia geminata* (Lyngbye) Mart.Schmidt) or present (*Surirella apiculata* W.Smith (formerly *Cymatopleura solea* var. *apiculata*) and *Surirella librile* (Ehrenberg) Ehrenberg (formerly *Cymatopleura solea* var. *solea*)), but not viable.



Figs. 72-112. Cleaned valve pictures of taxa categorised as ambiguous (sp., aff., cf., s.l.) or otherwise mentioned taxa from the preserved assemblages and from the cultures. Valves from cultures are marked with an asterisk (*): (72) *Cymbopleura incerta* var. *grunowii;* (73) *C.* cf. *florentina*, Culture D249_056; (74-75) *Delicatophycus sparsistriatus*; (76-78) *D.* cf. *montana*; (79-82) *Encyonema silesiacum* s.l.; (79) *Encyonema silesiacum* var. *latum*; (80) *E. silesiacum* var. *silesiacum*; (81) *E. silesiacum* cf. var. *silesiacum*; (82) *E. silesiacum* var. *altense*; (83) *E.* cf. *silesiacum* s.l.; Culture D252_012; (84) *Cymbopleura* sp., Culture D249_057; (85-87) *Gomphonema* cf. *minusculum*; (88-93) *Gomphonema* sp. 1 (*G.* aff. *minusculum*); (94) *Gomphonema* sp. 3 (*G.* aff. *minusculum*); (95-96) *Gomphonema* sp. 2; (97-98) *G.* cf. *pseudobohemicum*; (99) *G.* cf. *clavatulum*; (100) *G.* cf. *micropus*; (101) *Neidiomorpha binodiformis,* Culture D249_058; (102) *N.* cf. *binodis*; (103) *Neidium ampliatum* s.l.; Culture D249_061; (104) *Amphora affinis* s.l.; Culture D249_005; (105-106) *A.* cf. *neglecta* f. *densestriata*; (107-108) *Amphora* sp.; (107) Culture D252_015; (108) Culture 249_028; (109) *Diploneis* sp., Culture D249_003; (110) *Diploneis* cf. *puella*; (111) *D. separanda;* (112) *D.* cf. *separanda,* Culture D251_005. Scale bar: 10 µm.

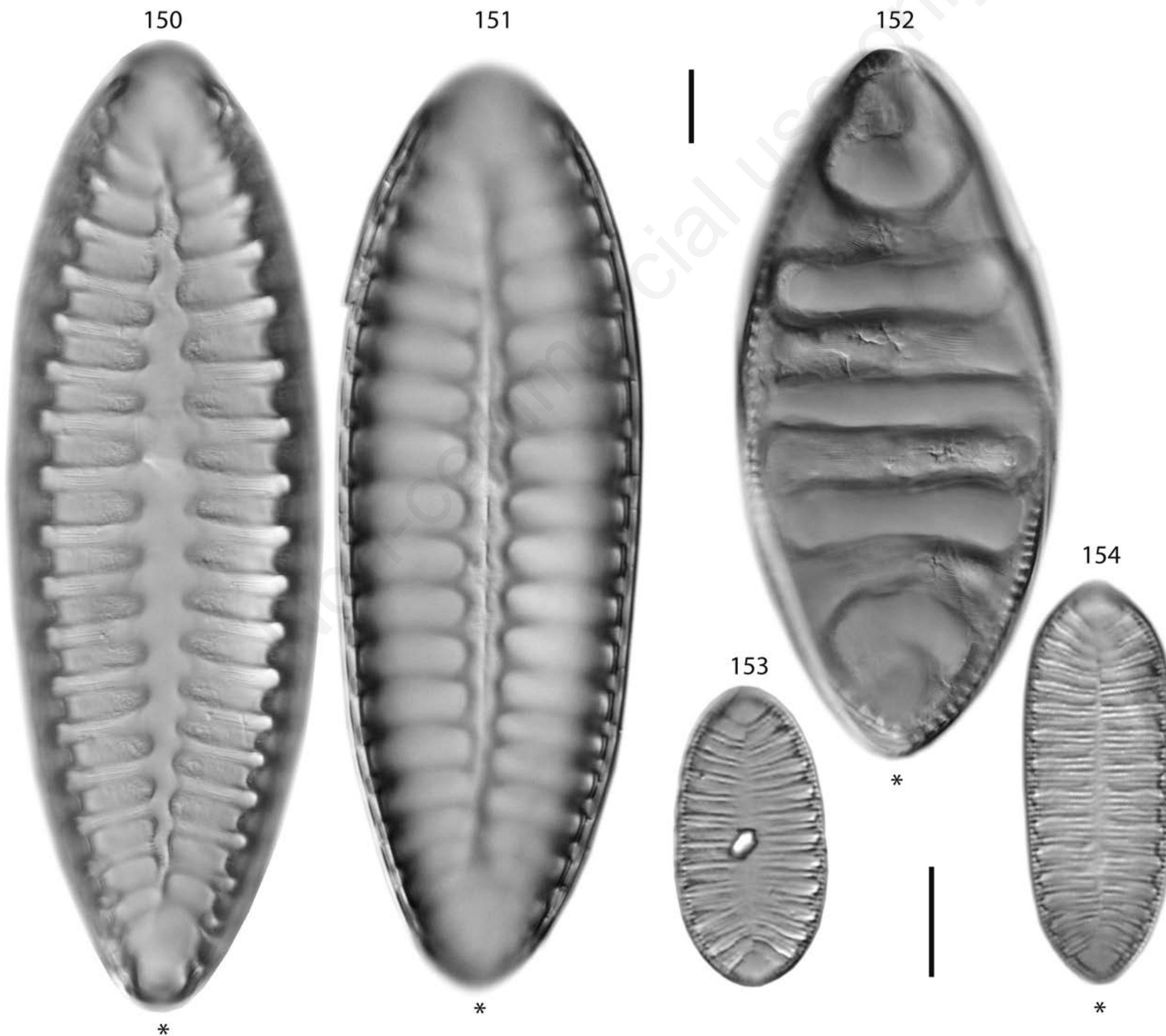


Non-commercial use only

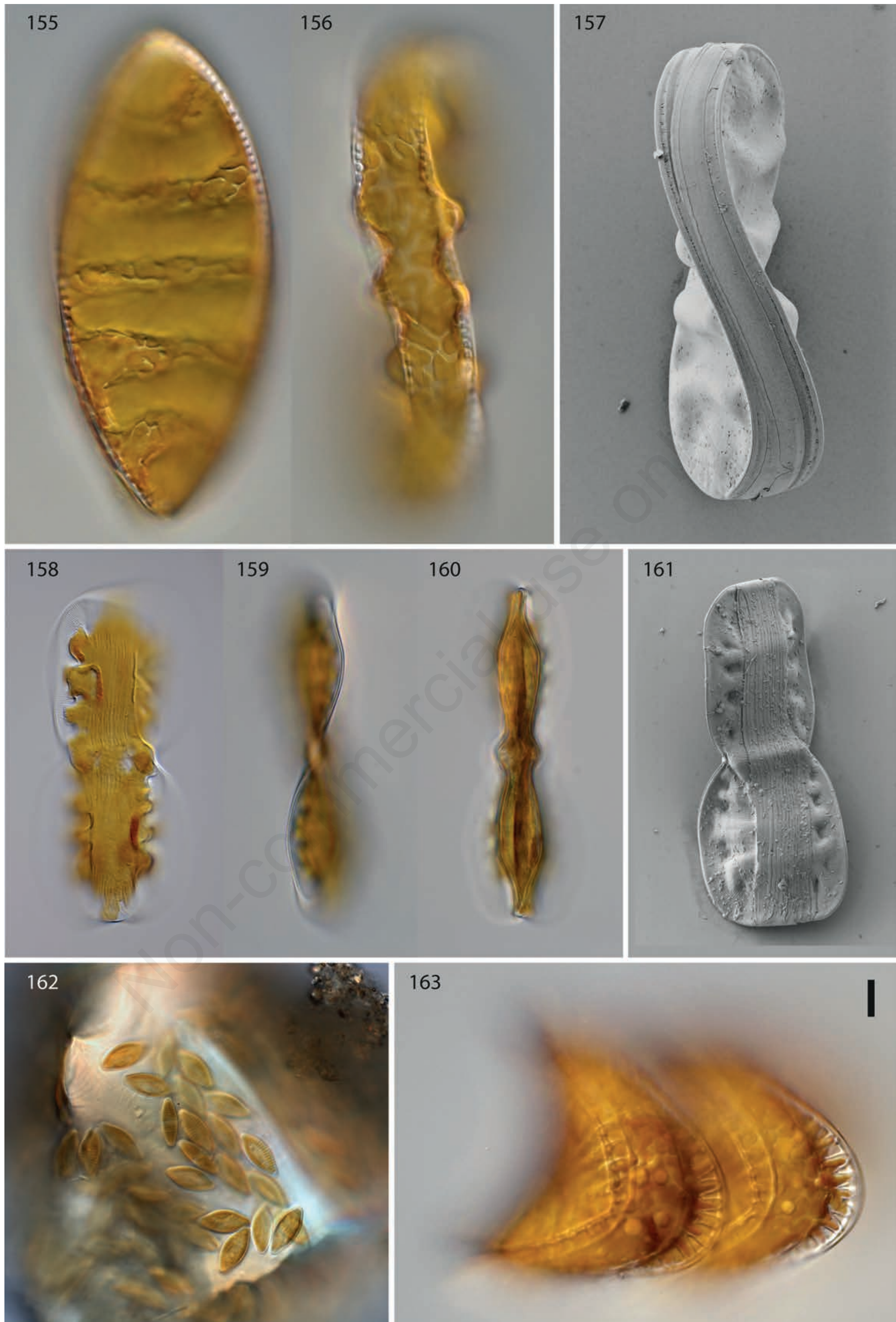
Figs. 113-149. Cleaned valve pictures of taxa categorized as ambiguous (sp., aff., cf., s.l.) or otherwise mentioned taxa from the preserved assemblages and from the cultures. Valves from cultures are marked with an asterisk (*): (113) *Stauroneis* cf. *subgracilis,* Culture D249_060; (114) *Caloneis* cf. *alpestris*; (115) *C. alpestris*; (116) *Caloneis* sp. 2, Culture D249_064; (117-118) *Caloneis* sp. 3, two valves of the same frustule; (119) *Caloneis* sp. 1, Culture D250_013; (120) *Caloneis* cf. *leptosoma,* Culture D252_010; (121) *Pinnularia* sp., Culture D252_004; (122) *Pinnularia* cf. *viridiformis,* Culture D249_021; (123) *Sellaphora* cf. *stroemii* 1, Culture D250_017; (124-125) *S. stroemii*; (126) *S.* cf. *stroemii* 2; (127) Pennales (aff. *S. joubaudii* sensu Hofmann *et al*., 2011); (128-129) *S. mutatoides,* Culture D249_052; (130-133) *S. pupula* s.l.; (130) Culture D249_044; (131) Culture D249_014; (133) Culture D249_033; (134) *S.* cf. *vitabunda,* Culture D249_080; (135-136) *S. nigri* s.l.; (135) Culture D250_012; (137-138) *N.* cf. *gottlandica*; (139-140) *Navicula* sp. (*N.* aff. *wendlingii*) two valves of the same frustule**;** (141-142) *N.* cf. *exilis*; (142) Culture D252_001; (143-144) *N. caterva-reichardtiana* s.l.; (143) Culture D250_007; (145) *N.* cf. *lacuum*; (146) *Hippodonta* cf. *hungarica*; (147) *N.* cf. *libonensis*; (148-149) *N. libonensis*; (148) Culture D249_026. Scale bar: 10 µm.

Others re-appeared a few weeks after incubation with the culture media. The earliest successful cultures were *Diploneis* cf. *separanda* (Fig. 112), *Eucocconeis flexella* (Kützing) F.Meister, *Navicula capitatoradiata* H.Germain ex Gasse, *Navicula* cf. *exilis* (Fig. 142), *Navicula tripunctata* (O.F.Müller) Bory, *Pinnularia* sp. (Fig. 121), *Sellaphora pupula* (Kützing) Mereschkovsky s.l. (Figs. 130-133) and *Sellaphora* cf. *saugerresii* (Figs. 209-213). Apparently, these taxa were generally fast growing and/or the used media were particularly suitable for them. Even 10-14 weeks after sampling some formerly overlooked taxa were still discovered in the enrichment cultures and successfully cultured, e.g. *Caloneis amphisbaena* (Bory) Cleve, *Iconella* cf. *hibernica* (Fig. 163), *Encyonema temperei* and *Sellaphora* cf. *stroemii* 1 (Fig. 123) (Tab. 3). *Encyonema prostratum* (Berkeley) Kützing was also found late and then isolated several times. Unfortunately, these strains died after a month.

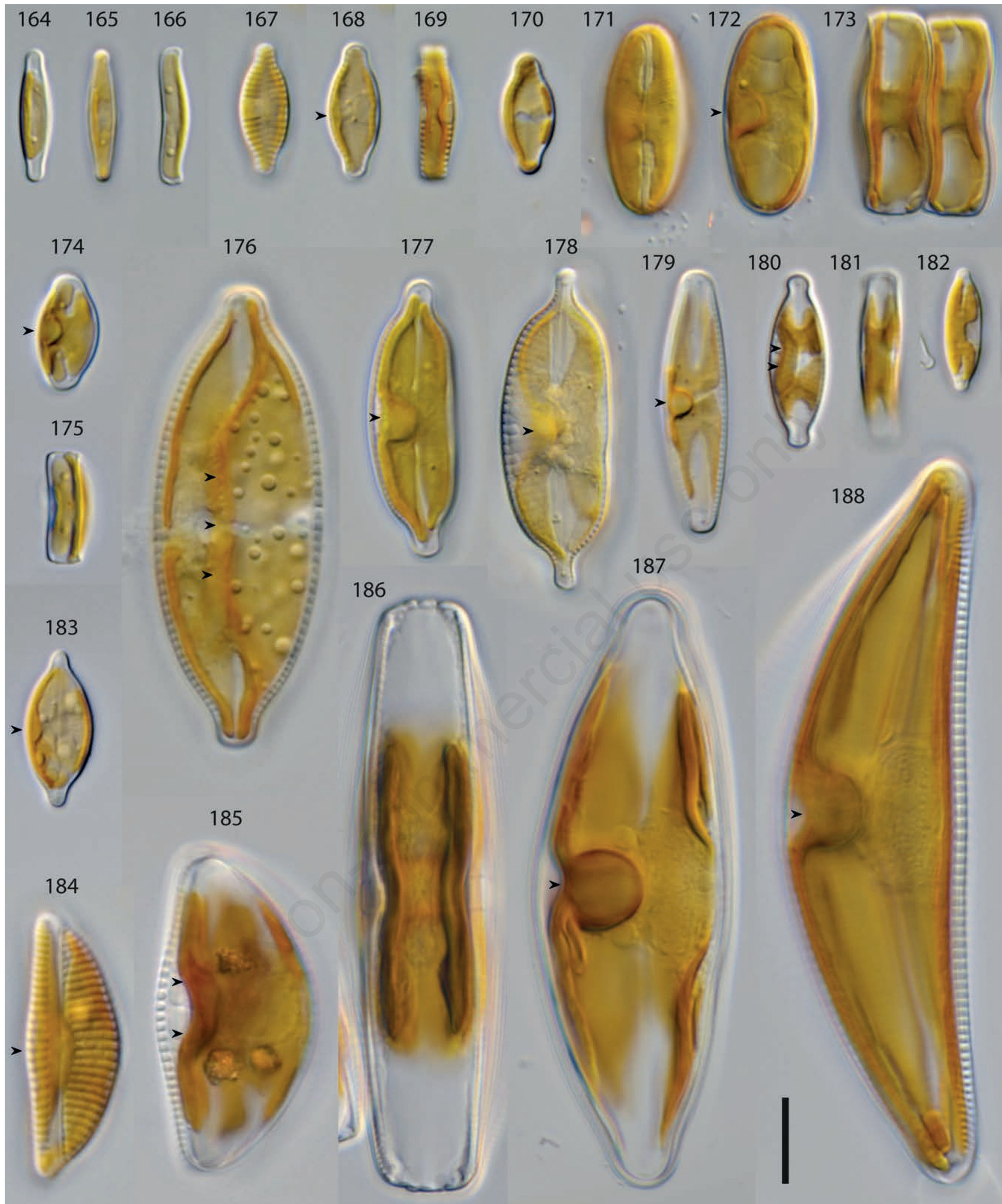
Live pictures were taken to document the various plastid shapes (Figs. 164-236). For example, *Pinnularia* contained plastids in a naviculoid arrangement with one plate-like plastid on each side (Figs. 195-197), while the related genus *Caloneis* often has a bridge between the two plastids to form one H-shaped plastid (in valve view) (Fig. 189). In this regard, the *Caloneis* plastid resembles the plastid of the genus *Sellaphora* and *Fallacia* (Mann and Stickle, 2009). During the cell cycle, the observed plastid shapes of *Sellaphora* varied from H-shapes to N-shapes and other forms (Figs. 201-229).



Figs. 150-154. Cleaned valve pictures of taxa categorised as ambiguous (sp., aff., cf., s.l.) or otherwise mentioned taxa from the preserved assemblages and from the cultures. Valves from cultures are marked with an asterisk (*): (150-151) *Iconella* cf. *splendida,* Culture D249_002, two focal planes of the same valve; (152) *Surirella* sp. Culture D249_007; (153) *Surirella* cf. *visurgis*; (154) *S. visurgis*, Culture D249_062. Scale bars: 10µm, (150-152) reduced scale, upper scale bar.



Figs. 155-163. Light micrographs of living cultured taxa and SEM pictures of twisted valve shapes and living epipsammon: (155-157) *Surirella* sp.*;* (155-156) Culture D249_007; (157) Culture D249_006; (158-161) *Entomoneis ornata;* (158-160) Culture D249_009; (161) D249_008; (162) *Karayevia* and *Planothidium* growing on sand grain (enrichment culture); (163) *Iconella* cf. *hibernica* (Syn. *Campylodiscus* cf. *hibernicus*)*,* Culture D249_073. Scale bar: 10 µm.



Figs. 164-188. Light micrographs of living cultured taxa featuring the plastids in Monoraphida and Cymbellales: (164-166) *Achnanthidium minutissimum* s.l., Culture D251_006; (167-170) *Planothidium rostratum* s.l.; (167-169) Culture D249_045; (170) Culture D249_063; (171-173) *Eucocconeis flexella*, Culture D249_050; (174-175) *Eucocconeis laevis* s.l., Culture D251_007; (176) *Placoneis placentula,* Culture D249_017; (177) *Cymbopleura naviculiformis,* Culture D249_029; (178) *Cymbopleura* sp., Culture D249_057; (179) *C. florentina,* Culture D249_056; (180-181) *Geissleria decussis,* Culture D249_024; (182) *Encyonopsis subminuta,* Culture D252_011; (183) *Karayevia clevei* var. *rostrata,* Culture D249_023; (184) *Encyonema cespitosum,* Culture D252_009; (185) *E. temperei,* Culture D252_017; (186-187) *Cymbopleura lata,* Culture D249_011b; (188) *Cymbella proxima,* Culture D249_012. Arrowhead(s) pointing to pyrenoid inside the isthmus. Scale bar: 10 µm.

Non-Commercial use only

Figs. 189-236. Light micrographs of living cultured taxa featuring the plastids in Naviculales: (189-191) *Caloneis* sp. 1, Culture D250_013; (192-194) *C.* cf. *leptosoma,* Culture D252_010; (195-197) *Pinnularia* sp.*,* Culture D252_004; (198-200) *Caloneis* sp. 2, Culture D249_064; (201-204) *Sellaphora* cf. *vitabunda,* Culture D249_080*;* (205-208) *S.* cf. *stroemii* 1*,* Culture D250_017; (209-213) *S.* cf. *saugerresii,* Culture D249_046; (209-210) prior to auxosporulation; (211) early stage of auxosporulation; (212) late stage of auxosporulation; (213) result of auxosporulation; (214-221) *S. pupula* s.l.; (214-217) Culture D249_033; (218) Culture D249_044; (219-221) Culture D249_014; (222-225) *S. mutatoides,* Culture D249_052; (226-229) *S. nigri* s.l.; Culture D250_012; (230-231) *Neidium ampliatum* s.l., Culture D249_069; (232-234) *Neidiomorpha binodiformis,* Culture D249_058c (arrows point to putative endosymbionts); (235-236) *Neidium affine,* Culture D249_051. Arrowhead points to pyrenoid. Scale bar: 10 µm.

In large *Sellaphora* species, more plastid details were visible, such as the triangular pyrenoid in *S. pupula* (Figs. 216, 220). The cymbelloid genera *Cymbopleura* (Figs. 177-179, 186-187)*, Geissleria* (Figs. 180-181) and *Placoneis* (Fig 176) displayed their typical double-layered plastid (Figs. 181, 186) consisting of two H- or K-shaped units that are connected by a bridge (isthmus). This bridge is often located near the centre of one girdle side. It contains a more or less conspicuous lenticular pyrenoid (Figs. 177-179, 187-188).

Similar to the Cymbellales, a lobed double-layered plastid was also found in many monoraphid taxa like *Eucocconeis flexella* (Figs. 171-173) and *Planothidium rostratum* s.l. (Figs. 167-170). Here the fully extended plastids covered both valve faces completely, but the lobed plastid-outlines were often difficult to distinguish. In contrast, *Achnanthidium minutissimum* s.l. featured a single-layered plate-like plastid that was typically located beneath the araphid valve (Figs. 164-166). As another example of plastid variability, the differing plastid shapes of *Neidium* and the newly established *Neidiomorpha* are presented (Figs. 230-236).

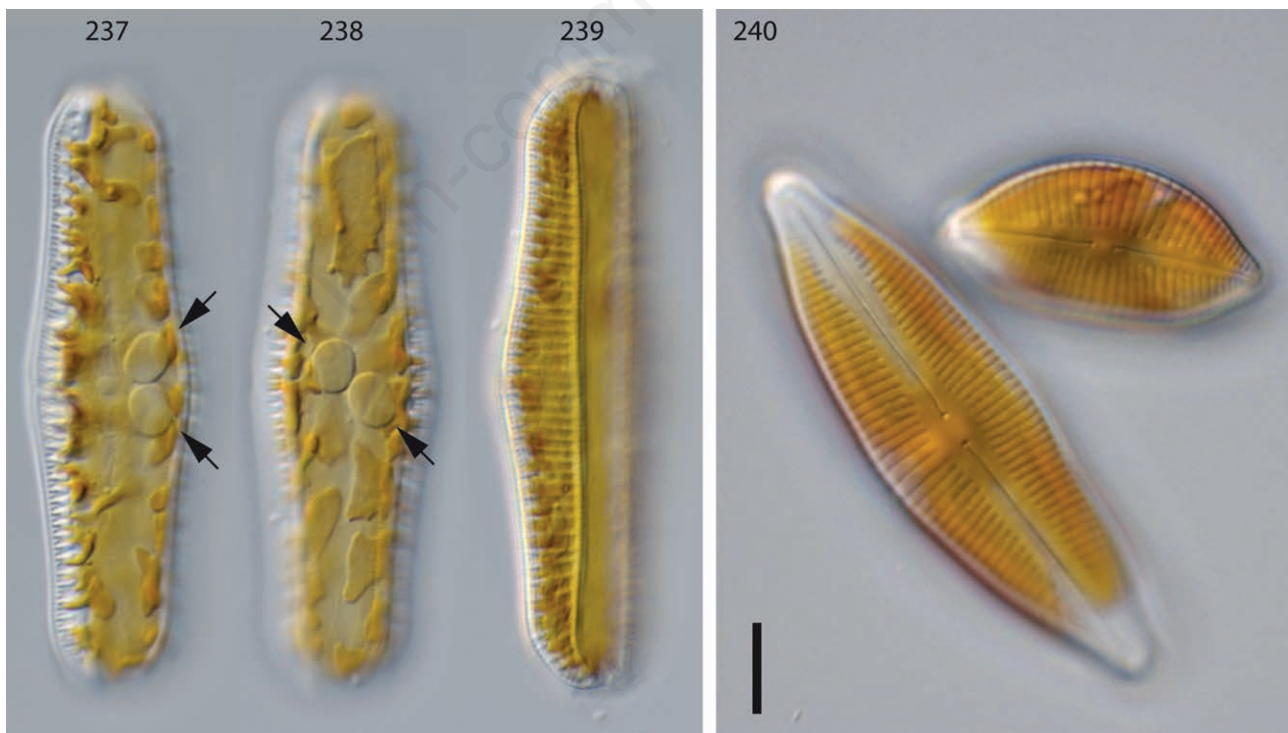
Auxospores were only detected in one culture of *Sellaphora* cf. *saugerresii* (Fig. 209-213). However, the sudden appearance of large cells in the cultures of small-celled *Sellaphora mutatoides* Lange-Bertalot & Metzeltin (Figs. 128-129) and *Cymbopleura lata* (Grunow ex Cleve) Krammer (Fig. 240) indicated that auxosporulation occurred here as well. Likewise, the cultures of *Cocconeis pediculus* contained distinctly larger cells compared to those found in the original diatom assemblages (Figs. 35-36), suggesting that auxosporulation happened in culture, likely in the enrichment cultures.

It was also noteworthy that in *Rhopalodia gibba* (Ehrenberg) O.Müller var. *gibba* cultures (Figs. 237-238) large, pale blue-green spheroid bodies (endosymbionts) were present and also in *Neidiomorpha binodiformis* (Krammer) Cantonati, Lange-Bertalot & N.Angeli much smaller putative endosymbionts (Fig. 234) were recognizable.

Comparison of cultures and preserved assemblages

Overall 31 (17%) of the 178 benthic diatom taxa identified in the four preserved assemblages were successfully cultured (based on taxonomic sensu lato level; Tabs. 2 and 3). The congruence between assemblage and cultures differed from sample to sample: 12 of 110 taxa (11%) in D249, six of 94 taxa (6%) in D250, three of 79 taxa (4%) in D251 and five of 92 taxa (5%) in D252).

Non-commercial use only



Figs. 237-240. Light micrographs of living cultured taxa: (237-239) *Rhopalodia gibba* var. *gibba* with endosymbionts (arrows), Culture D250_002; (240) two cells of *Cymbopleura lata* showing size difference before and after auxosporulation, Culture D249_011b. Scale bar: 10 µm.

Cultured taxa included four of the six abundant (>4%) benthic species, i.e., *Achnanthidium minutissimum* s.l., *Diatoma moniliformis*, *Encyonopsis subminuta* and *Staurosira venter*. The highest number of taxa occurred in the genus *Nitzschia*. Of the 28 *Nitzschia* taxa found in the assemblage, only four were taken into culture. *Caloneis* sp. 3 from the assemblage (Fig. 117-118) resembled the culture *Caloneis* sp.1 (Fig. 119). Some species *sensu lato* from the preserved assemblages were also cultured, but may represent different taxa. For example, *Eucocconeis laevis* s.l. was identified in the preserved assemblages as *Eucocconeis quadratarea* (Figs. 23-25), while the valve morphology in the clonal culture had higher variability (Figs. 26-29). *E. quadratarea* differs from *E. laevis* sensu stricto by the central area on the raphe-less valve that is unilaterally expanded to the margin (Krammer and Lange-Bertalot, 1991). The clonal culture displayed both shapes of central areas, the ones typical for *E. quadratarea* and central areas not reaching the margin which is typical for *E. laevis* sensu stricto, and also transitional central area shapes (Figs. 27-29). Similarly, *Encyonema silesiacum* s.l. was represented by different varieties in the preserved assemblages (Figs. 79-82) and present only as *Encyonema* cf. *silesiacum s*.l. in the cultures (Fig. 83). Additionally, 39 strains of rare taxa were cultured that were not found in the preserved assemblages: 31 taxa in D249, eight taxa in D250, two taxa in D251 and ten taxa in D252 (based on taxonomic sensu lato level; Tabs. 2 and 3).

DISCUSSION

Challenges of culturing benthic diatoms

Using a technically simple approach (Fig. 2) with three different culture media, we established diatom cultures of 71 different taxa from just four samples of the Bow River (Tab. 3). This approach was based on the knowledge that many diatom cells would not survive a direct transfer from the water sample to fresh culture medium. Thus, enrichment cultures were used to promote diatom individuals that were able to grow well under the given culture conditions. Additionally, the approach included the use of different culture media that had proven to be suitable for many different benthic diatom taxa. The manual skills required can be learned and improve quickly with experience. The challenge is that many benthic taxa adhere to the substrate and are easily destroyed during removal. For novice cultivators we suggest starting with large, unattached taxa such as *Pinnularia*-species and try small, well-attached taxa with gained experience. However, even for skilled experts some taxa (e.g., small monoraphids) are extremely challenging and require a lot of time and perseverance. Repeated isolations increased the success, as some isolates were contaminated with flagellates or small rhizopods while others failed due to damaged cells. All these factors can quickly lead to a high number of isolates that are no longer manageable. Thus, many species found in the preserved samples were not successfully cultured.

The use of different media helped the culturing success. For example, some taxa exclusively grew in AG+HC medium, such as *A. minutissimum* s.l. and *Eucocconeis laevis* (Østrup) Lange-Bertalot (Tab. 3). Possibly they were adapted to or even dependant on the presence of humic substances from decomposing detritus. The different substrates (sand, stones and macrophytes) provided very diverse microhabitats, particularly the sand substrate as found in other studies (Round and Bukhtiyarova, 1996; Stevenson *et al*., 1996), which also had the highest number of taxa in this study (Tabs. 2 and 3). The habitat diversity explains the co-existence of many different taxa (Stevenson *et al*., 1996; Taxböck *et al.*, 2020). Thus, by providing the different culture media, optimal growth conditions for a high number of taxa were given. The multitude of successfully grown taxa (Tab. 3) indicates that our approach using several culture media was highly suitable for culturing benthic diatoms from this oligo-mesotrophic river. An increased diversity of culture media and also some variation of culture conditions (e.g., temperature, agitation, light intensity or day/night cycle) could potentially stimulate the growth of even more stenoecious and less competitive species and thus improve culture success.

Culturing success

Strains were established for 17% of the benthic diatom taxa that were identified in the four sample assemblages (Tab. 2). Some taxa were identified in the preserved samples that did not survive the transport (e.g., the common *Didymosphenia geminata*), while some taxa were probably already dead during sampling, but their cell walls were still present in the assemblage. Several taxa (e.g., *Encyonema prostratum*) were common in the enrichment cultures but failed to grow when isolated, despite several replicates, i.e., the offered culture conditions were not suitable. The abundant (>4% in at least one sample) *Achnanthidium jackii* (Figs. 5-7) and *Gomphonema* sp. 1 (Figs. 88-93) were also not found alive or successfully brought into culture (Tab. 2). Additionally, the discrepancy may have been due to oversight or unintentional selection bias of the researcher during diatom extraction.

Interestingly, 39 of the 71 different cultured taxa were not found in the preserved diatom assemblages despite an intensive search for rare taxa. Notably they included large taxa, such as *Caloneis amphisbaena*, *Iconella* cf. *hibernica* (Fig. 163), *Cymbella proxima* Reimer, *Cymbopleura lata*, *Encyonema temperei*, *Iconella* cf. *splendida* (Figs.

150-151), *Placoneis placentula* (Ehrenberg) Mereschkowsky (Fig. 176), *Pinnularia* sp. (Fig. 121), *Pinnularia* cf. *viridiformis* (Fig. 122), *Rhopalodia gibba* var. *gibba* (Figs. 237-239) and *Surirella undulata* (Ehrenberg) Ehrenberg (Tab. 3). This bias reflects the presented approach of actively searching the original samples for rare and large taxa and adding them to the enrichment cultures. Overall, both methods of diversity identification (assemblage analysis and cultures) complemented each other well. In a similar study on streams in Mexico (Mora *et al.*, 2019) diatom diversity was identified using three methods (cultures, LM valve counts and additionally eDNA analysis) and it was also demonstrated that each method discovered taxa that were not found with the other methods. Thus, we expect that future eDNA-analyses of Bow River samples will detect the presence of more taxa than those here presented.

Taxonomic resolution and assignment

The proportion of cultured taxa (Tab. 3) in relation to the number of taxa found in the preserved assemblages (Tab. 2) may change when taxa are differently aligned due to changes in taxonomy. LM pictures presented of identified taxa categorised as ambiguous (sp., aff., cf., s.l.) facilitate a re-adjustment with future taxonomic findings. Correspondingly, a change in the delimitation between taxa within a species complex, such as *Encyonema silesiacum* s.l. (Figs. 79-83) and *Sellaphora pupula* s.l. (Figs. 130-133), will influence the numerical results. For example, one culture strain (*Caloneis* sp.1, Fig. 119) resembles a very rare similar taxon in the preserved assemblage (*Caloneis* sp. 3, Figs. 117-118). Due to its low abundance in the sample, it cannot be verified if both represent the same taxon. An integrative morphological (SEM) and molecular analysis of the culture may help to decide whether *Caloneis* sp.1 is a new species.

We did not discriminate among taxa of *Achnanthidium minutissimum* s.l. (Figs. 8-14), because the taxa of this complex can currently not be well distinguished from each other morphologically (e.g., Potapova and Hamilton, 2007). In the preserved assemblages we found at least two morphodemes, one similar to *Achnanthidium minutissimum* var. *minutissimum* (Fig. 10) and one that resembled the *Achnanthes minutissima* var. "Sippe mit besonders schmalen Schalen" (taxon with particularly slender valves, i.e. 1.8-2.7 µm; p. 60 and p. 312 in Krammer and Lange-Bertalot, 2004) (Figs. 12-14). Similarly, the two unialgal cultures of *Achnanthidium minutissimum* s.l. may also represent two different taxa. One was also similar to *A. minutissimum* var. *minutissimum* (Fig. 8-9) and the other was similar to *Achnanthidium lineare* W.Smith (synonym *Achnanthidium minutissimum* var. *inconspicua* sensu Novais *et al.*, 2015) (Fig. 11). However, none of the cultured *Achnanthidium* strains resembled the abundant slender cells (<2.5 µm width) of *Achnanthidium minutissimum* s.l. from the assemblages. It remains unclear, if this slender taxon is an independent taxon or if the variability of the cultured taxa had not captured the slender forms. A molecular analysis of these cultures may help to identify the taxa of *A. minutissimum* s.l., as demonstrated, for example, for the *A. minutissimum*-complex from the Arctic by Pinseel *et al*. (2017).

The delimitation of *Eucocconeis laevis* and related taxa is problematic (Enache and Potapova, 2012). Although valves from the preserved assemblage could be assigned to *E. quadratarea* (synonym *E. laevis* var. *quadratarea*, Figs. 23-25), the variability of the culture strain (Figs. 26-29) clearly demonstrates that the unilateral extension of the central area to the margin is not a distinct feature, supporting that the taxonomic concepts of *Eucocconeis laevis* s.l. are vague and require further investigation (Enache and Potapova, 2012). Thus, the analysis of *E. laevis* s.l. cultures can help to further identify the morphological variability of this species.

The taxa identified here within both the diatom assemblages and the cultures can serve as reference for diatom assemblages of the Bow River and also contribute to a taxonomically curated Canadian molecular reference library. Presence or absence of these taxa may be used as an indication tool to assess and monitor the water quality of the river in the future. A prerequisite for such a tool is a sufficient taxonomic resolution (Werner *et al*., 2016), ideally in combination with an established extensive reference library. The distinction between morphological varieties and forms within populations of high phenotypic plasticity remains necessary, because the morphodemes may contain ecological information (Cox, 2014). These morphologically similar taxa may even co-occur within the same water bodies, due to slightly different ecological requirements or the occupation of different micro-habitats. In an evolutionary context, species groups can be indicators for radiative speciation based on ecological diversification, as has been substantiated for *Aneumastus stroesei* s.l. (Stelbrink *et al*., 2018). Cultures will help to discriminate such complexes (Mann *et al*., 2004; Vanormelingen *et al*., 2013; Çiftçi *et al*., 2022), thereby improving their use as bio-indicators.

An additional application would be the detection of potentially invasive or endangered taxa that may have been overlooked at this time. For example, the invasion of *Cymbella janischii* (A.W.F.Schmidt) De Toni from the United States to Japan was recently detected based on the combination of morphological and molecular investigations (Kato-Unoki *et al*., 2022). Similarly, biogeographic questions may be answered by comparing the DNA-sequences of the culture strains of this study with eDNA or culture strains from different regions. Paleolimnological studies using sedimentary eDNA also rely on a compre-

Non-commercial use only

hensive reference library but are currently limited by sparsely populated databases (Capo *et al*., 2021).

Additional merits of diatom cultures

A high diversity of diatom taxa was extracted, especially from the sand sample (Tabs. 2 and 3). Many of the successfully cultured species are epipsammic taxa (attached to sand grains) like *A. minutissimum*, *Karayevia clevei* (Grunow) Bukhtiyarova, *Planothidium rostratum* and *Staurosirella neopinnata* (Ehrenberg) E.A.Morales, C.E.Wetzel, Haworth & Ector (Fig. 162, Tab. 3). Among them are many "notoriously difficult taxa with many infraspecific forms" (Round and Bukhtiyarova, 1996). Morphological and molecular data from cultures may help to untangle such taxonomically difficult to distinguish taxa. Among the multitude of small-celled sand dwellers, a couple of large-celled taxa with twisted shapes (*Surirella* sp., *Iconella* cf. *hibernica, Entomoneis ornata* (Bailey) Reimer, Figs. 155-161, 163) stood out and were successfully grown. The functional value of the twisted shapes is unknown. Possibly, the valve geometry prevents cells from drifting or helps them "drill" into finely grained sediments. For diatoms living in low light environments twisted shapes may also be advantageous for gathering light from all sides. Moreover, we observed that living cells of *Surirella* sp. and *Iconella* cf. *hibernica* were able to quickly change their spatial orientation according to the incidence of light by using the twisted curvature of their raphe. Under low light conditions cells rested horizontally on the bottom of the petri dish, while under bright illumination they pivoted to an upright position, possibly to reduce over-exposure.

Live images documented the various plastid shapes in cultured diatoms (Figs. 164-236). All *Pinnularia*-strains featured two typical lateral plate-like plastids (Figs. 195-197). In contrast, the *Caloneis* strains displayed both lateral plate-like plastids similar to *Pinnularia* (*Caloneis* cf. *leptosoma*, Figs. 192-194) and also H-shaped plastids (*Caloneis* sp. 1, Figs. 189-191). This inconsistency in plastid shapes supports the impression that *Caloneis* is not a monophyletic group (Cox, 1988; Mann, 2001; Bruder *et al*., 2008). However, plastid shapes are not a constant and permanent trait within a population as they change throughout the mitotic cell cycle (Mann, 1989). It may even happen that all observed cells in a synchronously dividing culture are temporarily missing a feature that would be clearly visible during interphase. But it is more common that the cells of actively dividing cultures display multiple diverging plastid shapes at the same time. This shape diversity was also evident in many *Sellaphora* cultures (Figs. 201-229). The main characteristic separating *Sellaphora* from *Navicula* is the shape of its plastid during interphase (Mann, 1989). Many small *Sellaphora* taxa may have been falsely assigned to the genus *Navicula*, as they were described based on valve features only. Recently, the taxonomy was revised for some of these *Sellaphora* taxa (Wetzel *et al*., 2015), but live observations were still missing. Our observations now confirm that the single plastid of *Sellaphora nigri* (De Notaris) Wetzel & Ector (formerly *Navicula nigri* De Notaris; Figs. 226-229) and *Sellaphora saugerresi* (formerly *Navicla saugerresii;* Figs. 209-213) is indeed typical for the genus *Sellaphora.* The plastid was H-shaped and consisted of two large girdle-appressed plates connected by an isthmus (Figs. 209, 227; Mann and Stickle, 2009). Thus, the observed plastid shapes support the transfer of these two species from *Navicula* to *Sellaphora* (Wetzel *et al*., 2015).

Sometimes pyrenoids were observed inside the plastids (Figs. 168, 172, 174, 177, 179, 183, 187, 188, 216, 220). Pyrenoids are crystal-like proteinaceous structures of the plastid that are apparently involved in the fixation of inorganic carbon and supposedly play an important role in carbon concentrating mechanisms (Jungnick *et al*., 2014; Kroth, 2015; Kroth and Matsuda, 2022). A single offset pyrenoid was clearly visible in the plastids of *Sellaphora pupula* s.l. (Figs. 216, 220), but not in the otherwise similar species *S. mutatoides* (Figs. 222-224) or other *Sellaphora* taxa isolated from the Bow River (Figs. 201-213, 226-229). In *Planothidium* taxa featuring a horseshoe area (e.g., *P. lanceolatum* (Brébisson ex Kützing) Lange-Bertalot) or cavum (e.g., *P. rostratum*) there appears to be a striking spatial relationship between these structures and the location of the pyrenoid (Figs. 167-170). We hypothesize that horseshoe and cavum might act as supporting structures (maybe diffusion barriers) for the carbon concentrating mechanism (CCM) in the pyrenoid, as a CCM is necessary to provide a locally increased steady-state CO_2 concentration for Rubisco (Roberts *et al*., 2007). Many details of diatom pyrenoid function are still unknown (Kroth and Matsuda, 2022). In unstained cells, pyrenoids can be difficult to observe and there was not enough funding available for extensive studies. Hence, more culture strains are needed to provide abundant material for pyrenoid observations and further investigations.

In valve view, the lobed plastids of Cymbellales often appear H- to K-shaped with a pronounced lateral pyrenoid (Figs. 177-187). On closer inspection, the plastid of the Cymbellales actually consists of two super-imposed layers with more or less identical outline (four-lobed) that are adjacent to both valve faces. These two layers are connected by an isthmus that contains the pyrenoid (Figs. 180-181, 186-187). The pyrenoid can be as big as the adjacent nucleus (Figs. 185, 187,188). In the genera *Cymbella*, *Cymbopleura* and *Encyonema* the isthmus is located in a lateral position near the girdle. In *Cymbella* and *Cymbopleura* cells the isthmus is located on the dorsal side (Figs. 177, 179, 187, 188), while in *Encyonema* cells it is on the ventral side (Fig. 185). Although the arrangement of organelles in *Cymbella*, *Cymbopleura* and

Non-commercial use only

Encyonema appears inconsistent at first, there is actually a correlation between the ontogenetic valve orientation and the location of plastid and nucleus in the cell. The side where the plastid's isthmus is located corresponds to the ontogenetically secondary side of the valves (Geitler, 1981; Mann, 1984), i.e., in *Cymbella* first the ventral valve side develops during mitosis, then the dorsal side, while *Encyonema* cells first grow the dorsal side.

The cells of *Geissleria decussis* (Østrup) Lange-Bertalot & Metzeltin also featured a laterally positioned isthmus (Fig. 180). This position was contrary to the notion that *Geissleria* and *Placoneis* share a common plastid morphology where the isthmus and pyrenoid are located in the centre of the cell unlike the aforementioned genera (Cox, 1987; Nakov *et al*., 2014), but it was in accordance with other recent publications that depict cells from both genera with a lateral isthmus (Kulikovskiy *et al*., 2014; Kezlya *et al*., 2021). Apparently, the plastid shapes of small *Geissleria* and some small *Placoneis* do not differ significantly from the plastids of the other Cymbellales, while other *Placoneis* taxa like *Placoneis placentula* (Ehrenberg) Mereschkowsky feature a central isthmus (Fig. 176, O. Skibbe, pers. obs.). Thus, the molecular investigation of *Placoneis* and other Cymbellales may provide some taxonomic surprises.

Within the monoraphids both single layered, plate like plastids as well as complex double layered, lobed plastids occurred (Figs. 164-175). A simple plate-like plastid, located under the araphid valve, was found in *Achnanthidium minutissimum* s.l. In some cells, the plastid appeared condensed (Fig. 164), in others it covered the entire valve area and was bent along one girdle side (Figs. 165-166). This difference may be due to the plastid changing shape during cell cycle or be caused by varying light levels. The comparatively low light levels during incubation may have stimulated the plastids to fully unfold, while the plastids possibly contracted due to the high light intensity during microscopic observation. The location of the plastid may be an adaption to a more or less surface-attached lifestyle, with the rapheless valve facing the light and the raphe valve mostly oriented to the substrate. The same holds true for *Cocconeis pediculus* cells which contained a similar plate-like, often C-shaped plastid.

In contrast to the above mentioned monoraphid taxa with one-layered plastids, the cells of *Eucocconeis laevis*, *Eucocconeis flexella* and *Planothidium rostratum* s.l. featured a markedly two-layered plastid arrangement similar to the Cymbellales, which was especially visible in girdle view (Figs. 169, 173, 175). The plastid apparently consists of two sections that are connected by a lateral isthmus: a more or less plate-like section adjacent to the rapheless valve and a more or less H-shaped section adjacent to the raphe valve. The difference in plastid shape and arrangement supports the notion that monoraphids are not a monophyletic group and warrants further phylogenetic and molecular analyses.

Live images also showed a discrepancy in plastid shape and arrangement between the related genera *Neidium* (Figs. 230-231, 235-236) and *Neidiomorpha* (Figs. 232-234). The new genus *Neidiomorpha* was mainly separated from *Neidium* on the basis of differing valve morphology (Cantonati *et al*., 2010). The differences in plastid shapes confirmed in this study were also proposed to be relevant for the separation of the new genus (Cantonati *et al*., 2010). Our observations attest that protoplast features provide useful arguments to clarify phylogenetic relationships within and among genera that were historically created based on valve morphology only (Cox and Williams, 2000; Cox and Williams, 2006; Cox, 2009).

For *Sellaphora* cf. *saugerresii* auxospores were documented in culture (Fig. 211-212), while for *Cocconeis pediculus, Cymbopleura lata* and *Sellaphora mutatoides* auxosporulation was only inferred by the appearance of large cells in the culture (compare Fig. 36 with Fig. 35, see Fig. 240 and compare Fig. 128 with Fig. 129, respectively). Thus, the cardinal points in the life cycle of these taxa have been recorded, as valves from cultures encompass the complete size range of these strains and demonstrate changing morphological valve features. The result of auxosporulation is particularly remarkable for *S. mutatoides*, as the large cells of this species were less strongly tapered compared to the small cells (Figs. 128-129) and thus were more similar to those of *Sellaphora pupula* s.l. (Fig. 130-133). Further research is needed to better differentiate both taxa from each other based on molecular and morphological criteria (this study; Mann *et al*., 2008; Mann, 2010).

Our cultures of *Rhopalodia gibba* var. *gibba* contained endosymbionts (Figs. 237-238). The existence of these blue-green "spheroid bodies" in the genera *Epithemia* and *Rhopalodia* was already noticed in the 19th century (Pfitzer, 1871), but their cyanobacterial nature and their role as nitrogen fixing endosymbionts was only recognized much later (Drum and Pankratz, 1965; Geitler, 1977; Floener and Bothe, 1980; Prechtl *et al*., 2004). For rhopalodiacean diatoms these endosymbionts are considered to be obligate (Nakayama and Inagaki, 2017), but their number varies. In laboratory experiments the average number of endosymbionts in *R. gibba* cells increased from 2.2 to 3.8 when transferred to a nitrogen-limited environment (DeYoe *et al*., 1992). In contrast, no noticeable endosymbionts were found in many *Rhopalodia* and *Epithemia* strains isolated from eutrophic lakes and rivers in Germany (O. Skibbe, pers. obs.). It is conceivable that the diatoms lost their endosymbionts as the result of decades of eutrophication. Thus, a critical (molecular, taxonomic, cytological) comparison of the existing populations from different world regions (e.g., Canada and Germany) seems necessary. The indicator values should be reassessed when basing water

quality assessments only on valve morphology. Similarly, our detection of putative endosymbionts in *Neidiomorpha binodiformis* (Figs. 232-234) warrants further investigation. It may be worthwhile to survey the occurrence and abundance of endosymbionts in diatom communities as a measure of nitrogen availability in aquatic environments.

CONCLUSIONS

We identified a total of 221 different taxa from the Bow River based on preserved assemblages and cultures (Tabs. 2 and 3). Of these taxa, 151 were exclusively identified in the preserved assemblages and 39 taxa solely appeared in the cultures, demonstrating that both methods complement each other well. Some taxa identified in the preserved samples were not found alive. We suspect that they did not survive the transport or were already dead during sampling. Despite their rare occurrence, some large or conspicuous taxa were successfully brought into culture due to the selective process of manual cell extraction from the natural assemblages and enrichment cultures. In addition, culture media and culture environment may have promoted the growth of some rather rare species. Overall, the compiled data of Canadian diatom diversity may serve as a basis for future taxonomic, biogeographic and ecological studies.

A technically simple approach to culture benthic diatoms is presented in detail. We established cultures for 71 different taxa from just four samples that were taken the same day. Some of the extracted species were cultured for the first time ever, and many had not been comprehensively described from live observations. Our cultures provided many insights into protoplast structures and their dynamic transformation during the diatom life cycle. We hope that the merits of unialgal cultures will inspire more research on living diatoms. Additionally, data derived from clone cultures are of particular importance to address taxonomic questions and resolve species complexes, e.g., in the genera *Achnanthidium*, *Encyonema, Eucocconeis* and *Sellaphora*. The cultures generated copious material for valve morphology and future molecular analyses, for which DNA has already been extracted. Thus, the cultured taxa will contribute to a Canadian molecular reference library, which can serve in the future as another management tool for monitoring, thereby helping river watershed managers to better assess ecosystem health.

ACKNOWLEDGEMENTS

We would like to thank Dave Park, Andrew Paul, Jim Wagner and Fred Wrona from the Government of Alberta for their initial project support and Eric Camm for sharing City of Calgary data. Many thanks are also due to Juliane Bettig and Jana Bansemer for assistance in the lab. Kristina van Riswyck kindly took pictures of oxidized valves from cultures. We are grateful to Regine Jahn, Juliane Bettig, Wolf-Henning Kusber and Jonas Zimmermann for endorsement and providing useful feedback on this manuscript.

This work received funding from the Verein der Freunde des Botanischen Gartens und Botanischen Museums Berlin Dahlem e.V. and the Federal Ministry of Education and Research (German Barcode of Life 2 Diatoms, GBOL2, grant number 01LI1501E).

We acknowledge support by the OpenAccess Publication Fund of Freie Universität Berlin.

REFERENCES

Abarca N, Jahn R, Zimmermann J, Enke N, 2014. Does the cosmopolitan diatom *Gomphonema parvulum* (Kützing) Kützing have a biogeography? PLoS One 9:e86885.

Abarca N, Zimmermann J, Kusber WH, Mora D, Van AT, Skibbe O, Jahn R, 2020. Defining the core group of the genus *Gomphonema* Ehrenberg with molecular and morphological methods. Bot. Lett. 167:114-159.

Andersen RA, 2005. Algal culturing techniques. Academic Press: 578 pp.

Ashworth MP, and Morris JJ, 2016. Axenic microalgal cultures overlook the complexity of the phycosphere marketplace. Perspectives in Phycology 107-111.

Ashworth M, Majewska R, Frankovich TA, Sullivan M, Bosak S, Filek K, Van de Vijver B, Arendt M, Schwenter J, Nel R, Robinson NJ, Gary MP, Theriot EC, Stacy NI, Lam DW, Perrault JR, Manire CA, Manning SR, 2022. Culturing putatively obligate epizoic diatoms: Insights for the evolution and ecology of diatoms and their host. Sci. Rep.12:15116.

Bahls LL, 2019. Diatoms from Western North America. 2. *Cymbellafalsa*, *Cymbopleura* and *Delicatophycus* (Bacillariophyta) - Taxonomy, Ecology, Biogeography. The Montana Diatom Collection, Helena: 114 pp.

Bahls L, Boynton B, Johnston B, 2018. Atlas of diatoms (Bacillariophyta) from diverse habitats in remote regions of western Canada. PhytoKeys 105:1-186.

Bennett JR, Sisson DR, Smol JP, Cumming BF, Possingham HP, Buckley YM, 2014. Optimizing taxonomic resolution and sampling effort to design cost-effective ecological models for environmental assessment. J. Appl. Phycol. 51:1722-1732.

Bilcke G, Osuna-Cruz CM, Santana Silva M, Poulsen N, D'hondt S, Bulankova P, Vyverman W, De Veylder L, Vandepoele K, 2021. Diurnal transcript profiling of the diatom *Seminavis robusta* reveals adaptations to a benthic lifestyle. Plant J. 107:315-336.

Bold HC, 1942. The cultivation of algae. Bot. Rev. 8:69−138.

Borrego-Ramos M, Bécares E, García P, Nistal A., Blanco S, 2021. Epiphytic diatom-based biomonitoring in Mediterranean ponds: Traditional microscopy versus metabarcoding approaches. Water 13:1351.

Bruder K, Sato S, Medlin L, 2008. Morphological and molecular investigations of naviculoid diatoms IV. *Pinnularia* vs. *Caloneis*. Diatom 24:8-24.

Cantonati M, Lange-Bertalot H, Angeli N, 2010. *Neidiomorpha*

Non-commercial use only

gen. nov. (Bacillariophyta): A new freshwater diatom genus separated from *Neidium* Pfitzer. Bot. Stud. 51:195-202.

Capo E, Giguet-Covex C, Rouillard A, Nota K, Heintzman PD, Vuillemin A, et al., 2021. Lake sedimentary DNA research on past terrestrial and aquatic biodiversity: Overview and recommendations. Quaternary 4:6.

Charles DF, Kelly MG, Stevenson RJ, Poikane S, Theroux S, Zgrundo A, Cantonati M, 2021. Benthic algae assessments in the EU and the US: Striving for consistency in the face of great ecological diversity. Ecol. Indic. 121:107082.

Chen M, Wang WX, 2008. Accelerated uptake by phytoplankton of iron bound to humic acids. Aquat. Biol. 3:155−166.

Chung C, 2013. Diel oxygen cycles in the Bow River: Relationships to Calgary's urban nutrient footprint and periphyton and macrophyte biomass. M.Sc. Thesis. University of Calgary.

Çiftçi O, Alverson AJ, van Bodegom P, Roberts WR, Mertens A, Van de Vijver B, Trobajo R, Mann DG, Pirovano W, van Eijk I, Gravendeel B, 2022. Phylotranscriptomics reveals the reticulate evolutionary history of a widespread diatom species complex. J. Phycol. 58:643-656.

City of Calgary, 2021. Bow River Basin Council. Accessed 17 October 2021. The City of Calgary, Water Resources, Calgary. Available from: https://www.brbc.ab.ca/ecr/

Claquin P, Leynaert A, Sferratore A, Garnier J, Ragueneau O, 2006. Physiological ecology of diatoms along the river-sea continuum, p. 121-138. In: Ittekkot V, Unger D, Humborg C, An NT (eds.), The silicon cycle: human perturbations and impacts on aquatic systems. Island Press.

Council of the European Communities, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities 327:1-72.

Cox EJ, 1987. *Placoneis* Mereschkowsky: the re-evaluation of a diatom genus originally characterized by its chloroplast type. Diatom Res. 2:145–157.

Cox EJ, 1988. Variation within the genus *Pinnularia* Ehrenb.: further evidence for the use of live material in diatom systematics?, p. 437-447. In: Round FE (ed.), Proceedings of the Ninth International Diatom Symposium, Bristol, Biopress.

Cox EJ, 2009. What's in a name? – Diatom classification should reflect systematic relationships. Acta Bot. Croat. 68:443-454.

Cox EJ, Williams D, 2000. Systematics of naviculoid diatoms: The interrelationships of some taxa with a stauros. Eur. J. Phycol. 35:273-282.

Cox EJ, Williams D, 2006. Systematics of naviculoid diatoms (Bacillariophyta): A preliminary analysis of protoplast and frustule characters for family and order level classification. System. Biodiver. 4:385-399.

Cox EJ, 2014. Diatom identification in the face of changing species concepts and evidence of phenotypic plasticity. J. Micropalaeontol. 33:111–120.

Culp JM, Hamilton HR, Sosiak AJ, Davies RW, 1992. Longitudinal zonation of the biota and water quality of the Bow River system in Alberta, Canada, p. 31−49. In: Becker CD, Neitzel DA (eds.), Water quality in North American River. Battelle Press, Columbus.

D'Alelio D, d'Alcala MR, Dubroca L, Zingone A, Montresor M, 2010. The time for sex: a biennial life cycle in a marine planktonic diatom. Limnol. Oceanogr. *55*:106-114.

Daste P, Neuville D, Victor-Baptiste B, 1983. A simple procedure for obtaining clonal isolation of diatoms. Br. Phycol. J. 18:1−3.

DeYoe HR, Lowe RL, Marks JC, 1992. Effects of nitrogen and phosphorus on the endosymbiont load of *Rhopalodia gibba* and *Epithemia turgida* (Bacillariophyceae). J. Phycol. 28:773-777.

Dixon J, 2014. Calgary Watershed Report, A summary of surface water quality in the Bow and Elbow Watersheds, 2010. The City of Calgary, Calgary.

Dreßler M, Verweij G, Kistenich S, Kahlert M, Werner P, 2015. Applied use of taxonomy: lessons learned from the first German intercalibration exercise for benthic diatoms. Acta Bot. Croat. 74:211-232.

Durbin EG, 1974. Studies on the autecology of the marine diatom *Thalassiosira nordenskioldii* Cleve. I. The influence of daylength, light intensity, and temperature on growth. J. Phycol. 10:220-225.

Drum RW, Pankratz S, 1965. Fine structure of an unusual cytoplasmic inclusion in the diatom genus *Rhopalodia*. Protoplasma 60:141-149.

English JD, Potapova MG, 2012. Ontogenetic and interspecific valve shape variation in the Pinnatae group of the genus *Surirella* and the description of *S. lacrimula* sp. nov. Diatom Res. 27:9-27.

Enache MD, Potapova M, 2012. A new species of *Eucocconeis* (Bacillariophyceae) from North America. Diatom Res. 27:75-80.

Floener L, Bothe H, 1980. Nitrogen fixation in *Rhopalodia gibba*, a diatom containing blue-greenish inclusions symbiotically, p. 541-552. In: Schwemmler W, Schenk HEA (eds.), Endocytobiology: Endosymbiosis and cell biology, a synthesis of recent research. Walter de Gruyter, Berlin, New York.

Geitler L, 1977. [Zur Entwicklungsgeschichte der Epithemiaceen *Epithemia*, *Rhopalodia* und *Denticula* (Diatomophyceae) und ihrer vermutlich symbiotischen Sphäroidkörper].[Article in German]. Plant Syst. Evol. 128:259-275.

Geitler L, 1981. [Die Lage des Chromatophors in Beziehung zur Systematik von *Cymbella*-Arten (Bacillariophyceae)].[Article in German]. Plant Syst. Evol. 138:153-156.

Gerke J, 2018. Concepts and misconceptions of humic substances as the stable part of soil organic matter: A review. Agronomy 8:76.

Grossmann L, Bock C, Schweikert M, Boenigk J, 2016. Small but manifold-hidden diversity in "*Spumella*-like flagellates". J. Eukar. Microbiol. 63:419.

Hatcher K, 2018. *Surirella undulata*. In: Diatoms of North America. Accessed 16 October 2020. Available from: https://diatoms.org/species/surirella-undulata

Hofmann G, Werum M, Lange-Bertalot H, 2011. [Diatomeen im Süßwasser-Benthos von Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis. Über 700 der häufigsten Arten und ihre Ökologie].[Book in German]. A.R.G. Gantner Verlag K.G. Rugell: 908 pp.

Hoshaw RW, Rosowski JR, 1973. Methods for microscopic algae, p. 53–67. In: Stein JR (ed.), Handbook of phycolog-

Non-commercial use only

ical methods: Culture methods and growth measurements. Cambridge University Press, Cambridge.
Jahn R, Kusber WH, Cocquyt C, 2017. Differentiating *Iconella* from *Surirella* (Bacillariophyceae): Typifying four Ehrenberg names and a preliminary checklist of the African taxa. PhytoKeys 82:73-112.
Jahn R, Abarca N, Kusber WH, Skibbe O, Zimmermann J, Mora D, 2020. Integrative taxonomic description of two new species of the *Cocconeis placentula* group (Bacillariophyceae) from Korea based on unialgal strains. Algae 35:303-324.
Jungnick N, Ma Y, Mukherjee B, Cronan J, Speed D, Laborde S, Longstreth D, Moroney J, 2014. The carbon concentrating mechanism in *Chlamydomonas reinhardtii*: Finding the missing pieces. Photosyn. Res. 121:159-173.
Kahlert M, Ács É, Almeida SF, Blanco S, Dreßler M, Ector L, Karjalainen SM, Liess A, Mertens A, van der Wal J, Vilbaste S, Werner P, 2016. Quality assurance of diatom counts in Europe: towards harmonized datasets. Hydrobiologia 772:1-14.
Kato-Unoki Y, Kurihara A, Kuge T, Shimasaki Y, Suzawa Y, Mayama S, 2022. Genetic evidence for the invasion of *Cymbella janischii* (A. Schmidt) De Toni, 1891 in Japan. BioInvasions Record 11:409-415.
Kelly M, Bennett C, Coste M, Delgado C, Delmas F, Denys L, Ector L, Fauville C, Ferréol M, Golub M, 2009. A comparison of national approaches to setting ecological status boundaries in phytobenthos assessment for the European Water Framework Directive: results of an intercalibration exercise. Hydrobiologia 621:169-182.
Kermarrec L, Franc A, Rimet F, Chaumeil P, Frigerio J. M, Humbert JF, Bouchez A, 2014. A next-generation sequencing approach to river biomonitoring using benthic diatoms. Freshwater Sci. 33:349-363.
Kezlya E, Glushchenko A, Maltsev Y, Gusev E, Genkal S, Kociolek JP, Kulikovskiy M, 2021. Three new species of *Placoneis* Mereschkowsky (Bacillariophyceae: Cymbellales) with comments on cryptic diversity in the *P. elginensis*-Group. Water 13:z3276.
Kirkwood A, Shea R, Jackson L, McCauley E, 2007. *Didymosphenia geminata* in two Alberta headwater rivers: an emerging invasive species that challenges conventional views on algal bloom development. Can. J. Fish. Aquat. Sci. 64:1703-1709.
Krammer K, 1997a. [Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa, Teil 1. Allgemeines und *Encyonema* part)].[Book in German]. Bibliotheca Diatomologica 36. Cramer, Berlin and Stuttgart: 382 pp.
Krammer K, 1997b. [Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa, Teil 2. *Encyonema* part., *Encyonopsis* und *Cymbellopsis*)].[Book in German]. Bibliotheca Diatomologica 37. Cramer, Berlin and Stuttgart: 469 pp.
Krammer K, 2000. The genus *Pinnularia*. In: Lange-Bertalot H (ed.), Diatoms of Europe, Vol. 1. A.R.G. Gantner Verlag, Ruggell.
Krammer K, 2002. *Cymbella*. In: Lange-Bertalot H (ed.), Diatoms of Europe, Vol. 3. A.R.G. Gantner Verlag, Ruggell.
Krammer K, 2003. *Cymbopleura*, *Delicata*, *Navicymbula*, *Gophocymbelloides*, *Afrocymbella*. In: Lange-Bertalot H (ed.), Diatoms of Europe, Vol. 4. A.R.G. Gantner Verlag, Ruggell.
Krammer K, Lange-Bertalot H, 1988. [Süßwasserflora von Mitteleuropa, Bacillariophyceae. 2/2: Bacillariaceae, Epithemiaceae, Surirellaceae)].[Book in German]. Spektrum Akademischer Verlag, Heidelberg and Berlin: 596 pp.
Krammer K, Lange-Bertalot H, 1991. [Süßwasserflora von Mitteleuropa, Bacillariophyceae. 2/3: Centrales, Fragilariaceae, Eunotiaceae)].[Book in German]. Spektrum Akademischer Verlag, Heidelberg and Berlin: 576 pp.
Krammer K Lange-Bertalot H, 1997. [Süßwasserflora von Mitteleuropa, Bacillariophyceae. 2/1: Naviculaceae)].[Book in German]. Spektrum Akademischer Verlag, Heidelberg and Berlin: 876 pp.
Krammer K, Lange-Bertalot H, 2004. [Süßwasserflora von Mitteleuropa, Bacillariophyceae. 2/4: Achnanthaceae (ergänzter Nachdruck)].[Book in German]. Spektrum Akademischer Verlag, Heidelberg and Berlin: 437 pp.
Kroth PG, 2015. The biodiversity of carbon assimilation. J. Plant Physiol. 172:76-81.
Kroth PG, Matsuda Y, 2022. Carbohydrate metabolism, p. 465-492. In: Falciatore A, Mock T (eds.), The molecular life of diatoms. Springer, Cham.
Kulikovskiy M, Gusev E, Andreeva S, Annenkova N, 2014. Phylogenetic position of the diatom genus *Geissleria* Lange-Bertalot and Metzeltin and description of two new species from Siberian mountain lakes. Phytotaxa 177:249-260.
Lange-Bertalot H, 2001. *Navicula* sensu stricto. 10 Genera Separated from Navicula sensu lato, *Frustulia*. In: Lange-Bertalot H (ed.), Diatoms of Europe, Vol 2. A.R.G. Gantner Verlag, Ruggell.
Lange-Bertalot H, Metzeltin D, 1996. [Oligotrophie-Indikatoren. 800 Taxa repräsentativ für drei diverse Seen-Typen].[Book in German]. Iconographia Diatomologica Vol. 2. Koeltz Scientific Books, Königstein: 390 pp.
Lange-Bertalot H, Moser G, 1994. [*Brachysira*. Monographie der Gattung].[Article in German].Bibliotheca Diatomologica 29:1-212.
Lange-Bertalot H, Bak M, Witkowski A, Tagliaventi N, 2011. *Eunotia* and some related genera. In: Lange-Bertalot H (ed.), Diatoms of Europe Vol. 6. A.R.G. Gantner Verlag, Ruggell.
Lange-Bertalot H, Hofmann G, Werum M, Cantonati M, 2017. Freshwater benthic diatoms of Central Europe: Over 800 common species used in ecological assessment. Koeltz Botanical Books, Schmitten-Oberreifenberg: 942 pp.
Levkov Z, 2009. *Amphora* sensu lato. In: Lange-Bertalot H (ed.), Diatoms of Europe Vol. 5. A.R.G. Gantner Verlag, Ruggell.
Levkov Z, Lange-Bertalot H, Mitić-Kopanja D, Reichardt E, 2016. The Diatom Genus *Gomphonema* from the Republic of Macedonia. In: Lange-Bertalot H (ed.), Diatoms of Europe Vol. 8. Koeltz Botanical Books, Oberreifenberg.
Lewin RA, 1959. The isolation of algae. Rev. Algol. 4:181−197.
Mann DG, 1984. An ontogenetic approach to diatom systematics. p. 113-144 In: Cassie, V (ed.), Proceedings of the Seventh International Diatom Symposium. O. Koeltz, Koenigstein.
Mann DG, 1989. The diatom genus *Sellaphora*: Separation from *Navicula*. Br. Phycol. J. 24:1-20.
Mann DG, 2001. A discussion of *Caloneis* and related genera. Diatom 17:29-36.
Mann DG, 2010. Discovering diatom species: is a long history

Non-commercial use only

of disagreements about species-level taxonomy now at an end? Plant Ecol. Evol. 143:251–264.

Mann DG, Chepurnow VA, 2004. What have the Romans ever done for us? The past and future contribution of culture studies to diatom systematics. Nova Hedwigia 79:237−291.

Mann DG, McDonald SM, Bayer M, Droop SJ, Chepurnov VA, Loke RE, Ciobanu A, du Buf JM, 2004. The *Sellaphora pupula* species complex (Bacillariophyceae): morphometric analysis, ultrastructure and mating data provide evidence for five new species. Phycologia 43:459-482.

Mann DG, Stickle A, 2009. Cytological characteristics of the Sellaphoraceae. Acta Bot. Croat. 68:239-250.

Mann DG, Thomas SJ, Evans KM, 2008. Revision of the diatom genus *Sellaphora*: a first account of the larger species in the British Isles. Fottea 8:15–78.

Martin M, Gebühr C, Mártire D, Wiltshire K, 2014. Characterization of a humic acid extracted from marine sediment and its influence on the growth of marine diatoms. J. Mar. Biol. Assoc. UK 94:895-906.

McFadden GI, van Dooren GG, 2004. Evolution: Red algal genome affirms a common origin of all plastids. Curr. Biol. 14:R514-R516.

Mora D, Abarca N, Proft S, Grau J, Enke N, Carmona J, Skibbe O, Jahn R., Zimmerman J, 2019. Morphology and metabarcoding: a test with stream diatoms from Mexico highlights complementarity of identification methods. Freshwater Sci. 38:448-464.

Mora D, Stancheva R, Jahn R, 2021. *Cocconeis czarneckii* sp. nov. (Bacillariophyta): a new diatom species from Lake Okoboji (Iowa, USA), based on the strain UTEX FD23. Phycologia 61:60-74.

Nakayama T, Inagaki Y, 2017. Genomic divergence within non-photosynthetic cyanobacterial endosymbionts in rhopalodiacean diatoms. Sci. Rep. 7:13075.

Nakov T, Ruck E, Galachyants Y, Spaulding S, Theriot E, 2014. Molecular phylogeny of the Cymbellales (Bacillariophyceae, Heterokontophyta) with a comparison of models for accommodating rate variation across sites. Phycologia 53:359-373.

Novais MH, Juettner I, Van de Vijver B, Morais MM, Hoffmann L, Ector L, 2015. Morphological variability within the *Achnanthidium minutissimum* species complex (Bacillariophyta): comparison between the type material of *Achnanthes minutissima* and related taxa, and new freshwater *Achnanthidium* species from Portugal. Phytotaxa 224:101-139.

Novis P, Braidwood J, Kilroy C, 2012. Small diatoms (Bacillariophyta) in cultures from the Styx River, New Zealand, including descriptions of three new species. Phytotaxa 64:11-45.

Perumal P, Prasath BB, Santhanam P, Ananth S, Devi AS, Kumar SD, 2015. Isolation and culture of microalgae. In: Perumal S, Thirunavukkarasu AR, Pachiappan P (eds.), Advances in Marine and Brackishwater Aquaculture. Springer, New Delhi.

Pfitzer E, 1871. [Untersuchungen über Bau und Entwicklung der Bacillariaceen (Diatomaceen)].[Book in German]. Adolph Marcus, Bonn.

Pinseel E, Vanormelingen P, Hamilton PB, Vyverman W, Van de Vijver B, Kopalova K, 2017. Molecular and morphological characterization of the *Achnanthidium minutissimum* complex (Bacillariophyta) in Petuniabukta (Spitsbergen, High Arctic) including the description of *A. digitatum* sp. nov. Eur. J. Phycol. 52:264-280.

Pérez-Burillo J, Valoti G, Witkowski A, Prado P, Mann DG, Trobajo R, 2022. Assessment of marine benthic diatom communities: insights from a combined morphological-metabarcoding approach in Mediterranean shallow coastal waters. Mar. Pollut. Bull. 174:113-183.

Potapova M, 2009. *Diatoma moniliformis*. In: Diatoms of North America. Accessed 16 October 2020. Available from: https://diatoms.org/species/diatoma_moniliformis

Potapova MG, Ponader KC, 2004. Two common North American diatoms, *Achnanthidium rivulare* sp. nov. and *A. deflexum* (Reimer) Kingston: morphology, ecology and comparison with related species. Diatom Res. 19:33-57.

Potapova M, Hamilton PB, 2007. Morphological and ecological variation within the *Achnanthidium minutissimum* (Bacillariophyceae) species complex. J. Phycol. 43:561-575.

Prakash A, Rashid MA, Jensen A, Subbarao DV, 1973. Influence of humic substances on growth of marine phytoplankton-diatoms. Limnol. Oceanogr. 18:516−524.

Prechtl J, Kneip C, Lockhart P, Wenderoth K, Maier UG, 2004. Intracellular spheroid bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin. Mol. Biol. Evol. 21:1477−81.

Prelle L, Graiff A, Gründling-Pfaff S, Sommer V, Kuriyama K, Karsten U, 2019. Photosynthesis and respiration of Baltic Sea benthic diatoms to changing environmental conditions and growth responses of selected species as affected by an adjacent peatland (Hütelmoor). Front. Microbiol. 10:1500.

Pringsheim EG, 1954. [Algenreinkulturen ihre Herstellung und Erhaltung].[Book in German]. Gustav Fischer Verlag, Jena: 108 pp.

Reichardt E, 1999. [Zur Revision der Gattung *Gomphonema*].[Book in German]. Iconographia Diatomologica 8. Ganter Verlag, Rugell: 203 pp.

Reichardt E, 2012. [Neue Diatomeen (Bacillariophyceae) aus dem Gebiet der Stadt Treuchtlingen].[Article in German]. Berichte der Bayerischen Botanischen Gesellschaft 82:19-32.

Reichardt E, 2018. [Die Diatomeen im Gebiet der Stadt Treuchtlingen].[Book in German]. Vol. 1 and 2. Selbstverlag der Bayerischen Botanischen Gesellschaft, München: 1184 pp.

Ribeiro CG, dos Santos AL, Gourvil P, Le Gall F, Marie D, Tragin M, Probert I, Vaulot D, 2020. Culturable diversity of Arctic phytoplankton during pack ice melting. Elementa: Science of the Anthropocene 8:6.

Rimet F, Ector L, Cauchie HM, Hoffmann L, 2009. Changes in diatom-dominated biofilms during simulated improvements in water quality: implications for diatom-based monitoring in rivers. Eur. J. Phycol. 44:567−577.

Roberts K, Granum E, Leegood RC, Raven JA, 2007. Carbon acquisition by diatoms. Photosyn. Res. 93:79-88.

Round F, Bukhtiyarova L, 1996. Epipsammic diatoms - Communities of British rivers. Diatom Res. 11:363-372.

Saffron K, Anderson AM, 2009. Review of benthic invertebrates and epilithic algae at long-term monitoring sites in the Bow River. Alberta Environment, Edmonton: 126 pp.

Sherr EB, Sherr BF, 2002. Significance of predation by protists

Non-commercial use only

in aquatic microbial food webs. Antonie Van Leeuwenhoek 81:293-308.

Skibbe O, Zimmermann J, Kusber WH, Abarca N, Buczkó K, Jahn R, 2018. *Gomphoneis tegelensis* sp. nov. (Bacillariophyceae): a morphological and molecular investigation based on selected single cells. Diatom Res. 33:251-262.

Smol JP, Stoermer EF, 2010. The diatoms: applications for the environmental and earth sciences. Cambridge University Press.

Sosiak A, 2002. Longterm response of periphyton and macrophytes to reduced municipal nutrient loading to the Bow River (Alberta, Canada). Can. J. Fish. Aquat. Sci. 59:987−1001.

Stelbrink B, Jovanovska E, Levkov Z, Ognjanova-Rumenova N, Wilke T, Albrecht C, 2018. Diatoms do radiate: Evidence for a freshwater species flock. J. Evol. Biol. 31:1969-1975.

Stevenson RJ, Bothwell ML, Lowe RL, Thorp JH, 1996. Algal ecology: Freshwater benthic ecosystems. Academic Press, San Diego.

Stevenson RJ, Pan YD, van Dam H, 2010. Assessing environmental conditions in rivers and streams with diatoms, p. 57–85. In: Smol JP, Stoermer EF (eds.), The diatoms. Applications for the environmental and earth sciences. Cambridge University Press, Cambridge.

Taxböck L, Karger DN, Kessler M, Spitale D, Cantonati M, 2020. Diatom species richness in Swiss springs increases with habitat complexity and elevation. Water 12:449.

Trobajo R, Rovira L, Mann DG, Cox EJ, 2011. Effects of salinity on growth and on valve morphology of five estuarine diatoms. Phycol. Res. 59:83-90.

Vanormelingen P, Evans K, Chepurnov V, Vyverman W, Mann D, 2013. Molecular species discovery in the diatom *Sellaphora* and its congruence with mating trials. Fottea 13:133-148.

Walne PR, 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*. H.M. Stationery Office: 62 pp.

Water Survey of Canada, 2019. Bow River at Calgary station 05BH004. Accessed 17 October 2021. Available from: https://wateroffice.ec.gc.ca/mainmenu/historical_data_index_e.html

Werner P, Adler S, Dreßler M, 2016. Effects of counting variances on water quality assessments: implications from four benthic diatom samples, each counted by 40 diatomists. J. Appl. Phycol. 28:2287-2297.

Wetzel CE, Ector L, Van de Vijver B, Compère P, Mann DG, 2015. Morphology, typification and critical analysis of some ecologically important small naviculoid species (Bacillariophyta). Fottea 15:203–234.

Wynne MJ, 2019. *Delicatophycus* gen. nov.: a validation of "*Delicata* Krammer" inval. Gomphonemataceae, Bacillariophyta. Notulae Algarum 97:1-3.

Zimmermann J, Abarca N, Enke N, Skibbe O, Kusber WH, Jahn R, 2014. Taxonomic reference libraries for environmental barcoding: a best practice example from diatom research. PloS one 9:e108793.

Zimmermann J, Glöckner G, Jahn R, Enke N, Gemeinholzer B, 2015. Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. Mol. Ecol. Resour. 15:526-542.

Non-commercial use only