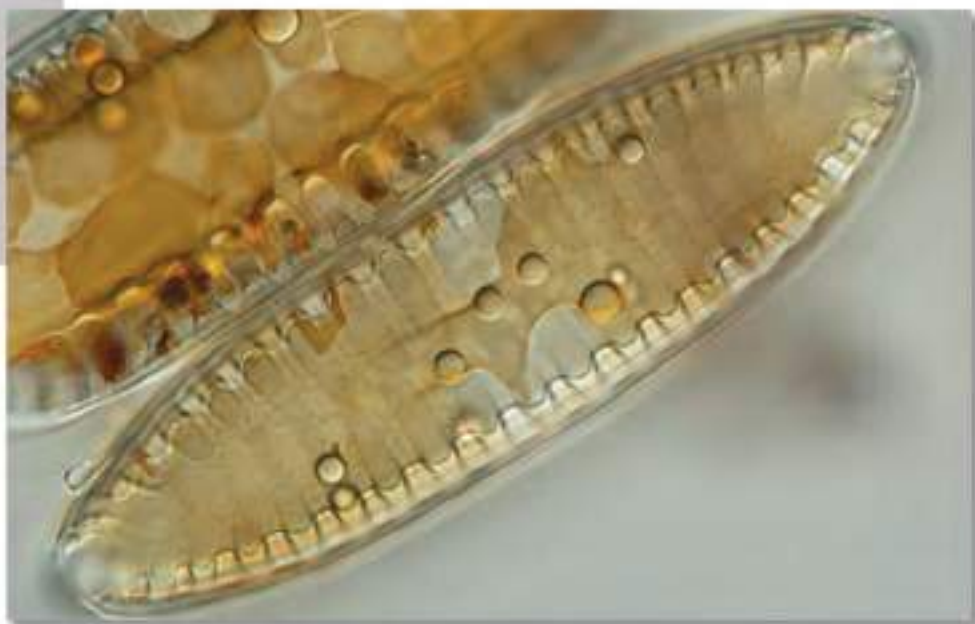


# Diatoms from the Congo and Zambezi Basins - Methodologies and identification of the genera

J.C. Taylor  
C. Cocquyt



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# Abc Taxa

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Dedicated to Capacity Building  
in Taxonomy and  
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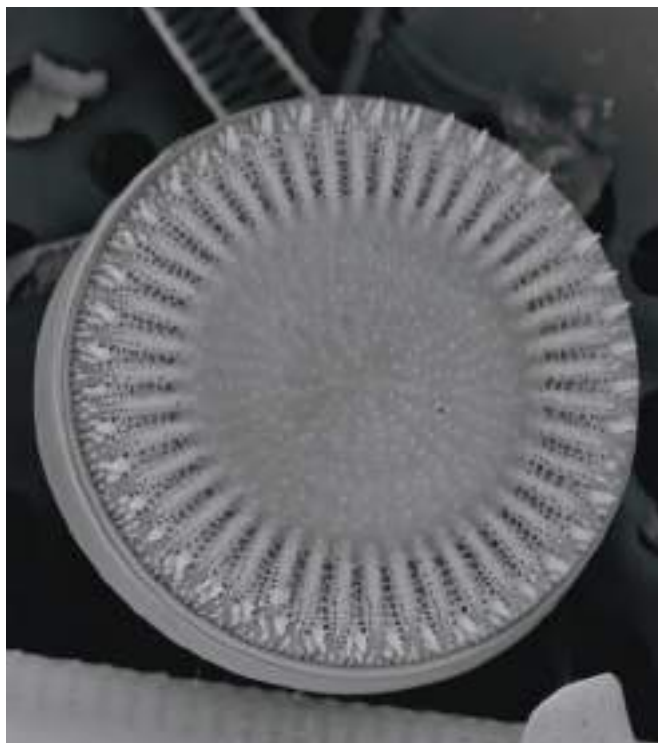
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# Diatoms from the Congo and Zambezi Basins - Methodologies and identification of the genera



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**Front cover:** *Surirella* Turpin

**Half-title page:** *Cyclotella meneghiniana* Kützinger

## Preface

The Congo river basin is, after the Amazon, the second largest in the world and the Zambezi river is the fourth longest river in Africa. Both rivers and their catchments are of prime importance to millions of people. These human populations continue to increase. For example, the population in the Congo basin (c 777,000 square km<sup>2</sup>) experiences an annual increase of c 1.7 million people. This creates rising demands (food, fuel shelter) at a great cost to the forest and to the river itself. As a result, wildlife and fish stocks rapidly decrease, mainly because of the largely uncontrolled trade in bush meat and because of overfishing. Moreover, waters in the catchments are experiencing rapid eutrophication, because of the countless domestic fires that are daily lit to cook food. Ashes are then washed into the water ways by torrential tropical rains. The fires themselves demand massive logging, which causes erosion and further degradation of water quality in these basins.

Measures need to be taken to stop the degradation of tropical river catchments in general, and those of the Congo and Zambezi rivers in particular, and many are already in place. Some of these measures deal with birth control, others impose sustainable hunting and fishing activities. Monitoring of biodiversity and water quality is urgently needed. Most of such interventions demands education, formation and training of local people.

One of the major drawbacks faced by programmes studying the biodiversity of tropical river catchments is the lack of taxonomic knowledge (also called the taxonomic impediment). Yet, estimations of fish stocks, identification of bush meat sold at local markets, the use of aquatic organisms to determine water quality, and many other monitoring activities that could provide scientifically underpinned recommendations to management, largely depend on good taxonomy.

Abc Taxa offers a welcomed forum to disseminate knowledge on a taxon that reveals itself as indicative to water quality: namely diatoms. Over the past decades, water quality monitoring research has produced a long set of (mostly locally applicable) Biotic Indices (BIs) using different biological groups. Such BIs have the advantage that they monitor the health of aquatic communities which are the result of time averaged effects of potential pollution events, that could easily be missed by point measurements of water chemistry, especially in flowing rivers. The first wave of BI largely dealt with fish, macrophytes and macro-invertebrates. Since about 20 years, however, there is an increased use of diatoms in water quality monitoring, mostly (of course) in the northern hemisphere where the taxonomy of these algae is much better known. The present book will remedy the taxonomic impediment of diatoms in the Congo and Zambezi catchments and will allow the start of new monitoring programs and the refinement of running ones.

The editors of Abc Taxa are to be congratulated for the production of this high - level monograph and so are the authors of this highly appreciated volume. May this series see light to many forthcoming issues that will relieve the taxonomic impediment in the Global South.

Koen Martens

Head of Research RBINS

Editor in Chief European Journal of Taxonomy

## **Abstract**

Diatom research has historically been well established in Central Africa but more commonly directed towards the phytoplankton of large bodies of standing water. Recently there has been considerable international research interest on using diatoms as indicators of water quality. Usually attached diatoms originating from rivers and streams are used for this purpose. Diatom taxonomy has undergone considerable changes during the last 3 decades with many new diatom genera being established, these diatom genera are ecologically relevant in terms of establishing water quality conditions. This volume sets out to introduce researchers to the latest concepts in collection and preparation methodology as well as diatom taxonomy and nomenclature. This is achieved by illustrating and discussing methodological concepts, providing a fully illustrated glossary and illustrating, by a variety of means, the most common diatom genera occurring in the Congo and Zambezi catchment region.

**Key words** - *Bacillariophyta*, morphology, taxonomy, tropical Africa, water quality

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## 1. Introduction

### 1.1. Diatom research in Central Africa

The African Great Lakes, Tanganyika, Malawi and Victoria and surrounding regions attracted the interest of several phycologists at the end of the 19<sup>th</sup> century. The first publication appeared in 1880 written by Dickie who reported on attached algae on the aquatic phanerogams (seed-bearing plants) of Lake Malawi (also called Lake Nyasa or Lake Nyassa): he found a total of 38 taxa of which 31 are diatoms. The research on the diatoms of Lake Malawi/Nyasa and lotic ecosystems in the surroundings was continued by Müller (1897, 1903, 1904, 1905, 1910) resulting in the description of 126 new diatom taxa (species, varieties and forms). Müller also examined material from Lake Victoria from which he described another ten new diatom taxa, and one new variety from Mount Kilimanjaro. West (1907) reported on the algae of the three Great Lakes including a total of 58 diatom taxa, 26 from Lake Malawi, 19 from Lake Victoria and 37 from Lake Tanganyika. The publication included the description of nine new taxa, all from Lake Tanganyika. Several diatoms from this lake, new to science were depicted by Hustedt in Schmidt's atlas of diatoms (Schmidt 1914, 1922, 1925), followed later (Hustedt in Huber-Pestalozzi 1942) by an elaborated description. In addition to the studies of Müller the diatoms from Lake Victoria were studied by Ostenfeld (1908, 1909) who reported 15 and 9 taxa respectively, Schröder (1911) described a new *Rhizosolenia* species (now transferred to the genus *Urosolenia*), Virieux (1913) reported 25 taxa and one new variety, Woloszyńska (1914) reported 34 species of which two were new species, one a new variety and one a new forma, and Bachmann (1933) mentioned 18 taxa including one new variety. A first overview of all taxa reported from the Great Lakes was published by Van Meel in 1954, followed by an updated checklist in 1993 (Cocquyt *et al.* 1993). Later the lacustrine and riverine algal diversity in the African Great Lakes area was discussed in Cocquyt (2006). Besides the Great Lakes, diatom investigation in the region was also carried out in rivers, ponds, etc., e.g., Bachmann (1938), Caljon (1987, 1988) and Mpawenayo (1996).

Diatom research in D.R. Congo, formerly the Republic of Zaire (1971-1997) and Belgian Congo (1908-1960) started with Zanon (1938) who studied the diatoms from the region of Lake Kivu. He reported 263 taxa belonging to 33 genera. Seventeen taxa were new to science of which ten were *Pinnularia* species; the others belonging to *Cocconeis*, *Cymbella*, *Eunotia*, *Neidium* and *Synedra*. In the mid-20<sup>th</sup> century Hustedt (1949) published a treatise on the diatoms of the Albert National Park in Belgian Congo, nowadays the Virunga National Park in D.R. Congo. Among the 55 new taxa, 25 belong to *Nitzschia*, the others are spread over 12 other genera. Some research was also done on the Congo River. In 1948 Kufferath reported 25 taxa in the plankton of the Congo River near Makanza, previously called Nouvelle-Anvers, midway between Kisangani and Kinshasa, including one new *Nitzschia* species (Kufferath 1956a). In 1956 he mentioned eight taxa near the isle of Mateba (Kufferath 1956a) and 44 taxa of which 10 were new

from Banana Beach (Kufferath 1956b), both localities are close to the mouth of the Congo River in the Atlantic Ocean (Kufferath 1956a) and include marine species. Not only the Congo River but its tributaries including the Lindi River, the Tshopo River and small rivers and ponds in Kisangani were studied by Golama (1996) who found 278 diatom taxa. A new *Gomphonema* was described from the Tshopo River (Compère 1995). Some years earlier a new *Stauroneis* was described from a fish pond in Kinshasa (Compère 1989). Cholnoky (1970) described three new species among the 93 taxa he observed in the Bangweulu swamps. In addition to the papers mentioned, there also exist a limited number of unpublished thesis studies conducted at universities in D.R. Congo.

Diatom research in Zambia started only recently, with exception of studies carried out in the Bangweulu swamps by Cholnoky (1970). Lake Kariba, located on the border of Zambia and Zimbabwe was also the subject of algal investigations. Thomasson (1965) reported ten diatom taxa, *Aulacoseira granulata* being the dominant species. Diatom communities and their seasonal succession were studied by Hancock (1979) as well as the epiphytic diatom community on underwater leaves of *Salvinia molesta* D.S. Mitchell (water fern) (Hancock 1985). Cronberg (1997) mentioned the presence of 155 algal species in Lake Kariba based on a study of 152 plankton samples collected between 1986 and 1990. Among these only thirteen taxa belong to the diatoms, seven are *Aulacoseira* species. A later study in this lake (Muzavazi *et al.* 2008) also reported twelve diatom species, of which only four could be assigned a species name.

## **1.2. Diatom research related to water quality in Central Africa**

Attempts to use diatoms as a tool for water quality of rivers in Central Africa started only recently. Some small rivers and streams south of Gombe Stream National Park, Tanzania (Bellinger *et al.* 2006) have been the subject of such an investigation. A number of indicator species tolerant to eutrophication were found and the Trophic Diatom Index (TDI) (Kelly & Whitton 1995) values showed significantly higher impact in deforested than in forested streams.

Utete *et al.* (2013) studied the impact of aquaculture on the water quality in Lake Kariba based on physical and chemical variables, but not using diatoms. In East Africa some research was done over the last few decades on diatoms in relation to water quality, mainly through PhD theses in Kenya and Ethiopia (e.g., Lung'ayia 2002, Beyene 2010, Beyene *et al.* 2009, 2014).

This is in contrast to South Africa which has a long history of diatom research related to ecological research and water quality (Taylor & Cocquyt in press). Cholnoky can be considered as the founder of diatom studies in South Africa. His intensive and extensive studies on the taxonomy and ecology of the diatoms was also the start of the South African Diatom collection, nowadays owned by the South African Institute for Aquatic Biodiversity and housed at the North-

West University in Potchefstroom, South Africa. Cholnoky had little faith in only the chemical analysis of water quality and stated that “*the chemical and physical characteristics of a water body could be determined more reliably and easily through a study of the diatom associations found living in it*” (Cholnoky 1968). The application by Cholnoky of the Thomasson (1925) community analysis on benthic diatom community composition was a forerunner of modern autecological indices. The method allows comparisons between sites in the same river, or the tracking of changes at a single site, but only one aspect of the water chemistry is chosen. When we consider for example the amount of nitrogenous effluent, the sum of all specimens belonging to the genus *Nitzschia* is calculated as an abundance value relative to the total of the cells enumerated from a particular diatom community.

Why the genus *Nitzschia*? *Nitzschia* is a genus known generally to be nitrogen heterotrophic and be able to utilise organically bound nitrogen. Therefore the relative abundance of this genus in a sample gives a reflection of the amount of nitrogenous pollution at the site studied. A higher percentage indicates a higher degree of impact in terms of nitrogenous effluent. Another example is that a pH gradient can be tracked in a river system by using the abundance values of the diatom genus *Eunotia*. This genus is known to prefer acid environments, to be acidobiontic. Cholnoky (1968) obtained good results using this index. However, the user of the Thomasson analysis method needs to have an in-depth knowledge of the autecology of individual diatom genera and species to be able to draw accurate environmental conclusions based on diatom community composition. Several years later, Archibald (1972) and Schoeman (1976) attempted to develop better approaches using diatoms in water quality monitoring. Their development was parallel to the development in water quality monitoring in Europe. The first proved to be unsuccessful. Schoeman (1976) simplified the community analysis method used by Cholnoky: he divided the diatom associations into four groups, each with their own particular ecological requirements. The table of results is thus shortened compared to the long species tables used by Cholnoky.

Schoeman (1976, 1979) came to the conclusion that these diatom groupings (or associations) could be successfully employed to assess the quality of running waters especially in regard to their trophic status. Unfortunately the investigation of diatoms as indicator species in South African freshwater ecosystems was then interrupted to be restarted at the beginning of the 21<sup>st</sup> century. Bate *et al.* (2002) attempted to relate a descriptive index, based on a dataset for the environmental tolerances of diatom species found in the Netherlands (van Dam *et al.* 1994), to water quality in South Africa. The “van Dam *et al.* index” includes pH, conductivity, oxygen requirements, trophic status, saprobic status and habitat requirements of a selected number of diatom species found in waters of the Netherlands (van Dam *et al.* 1994). Bate *et al.* (2002) concluded that benthic diatoms could be useful for water quality investigation in South Africa and that they give a time-integrated indication of specific water quality components, but that the particular data set, generated in the Netherlands, could not be transposed directly for use under South African conditions.

Taylor (2004) and Taylor *et al.* (2007a, b) continued this investigation by testing several numerical diatom indices developed in Europe for indicating water quality in some of the most important river systems in South Africa. They concluded that in general these European indices could be used with success in South Africa but that there are, however, some potential problems (Taylor *et al.* 2007b). In particular, the list of taxa included in the indices needs to be adapted to the studied region. Although most European diatom indices may be used in many regions as they are based on the ecology of widely distributed or cosmopolitan taxa, special attention should be paid to taxa occurring in pristine waters and to endemic taxa, absent in the indices reference lists. When these taxa are abundant the inferred water quality may be misinterpreted.

Another problem that arises is the rapid changes in diatom taxonomy, especially at the genus level. Some European indices were erected in the seventies or in the eighties of the last century and have never been revised. The positive result in the study of Taylor *et al.* (2007b) is they demonstrated that many widely distributed diatom species found in South Africa have similar environmental tolerances to those recorded for these species in Europe and elsewhere.

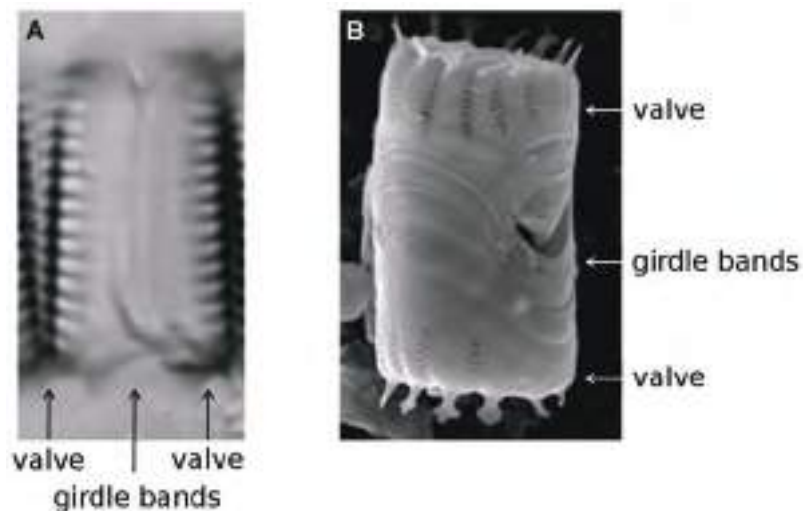
### **1.3. Aim of the present diatom book**

The aim of the present work on diatoms is twofold. On the one hand we want to encourage and facilitate the study of diatoms as a useful tool for water quality monitoring. On the other hand we want to give an overview of the most common diatom genera which can be observed in the Congo and Zambezi basins. Accurate identifications at this level form the basis for further taxonomic studies. Nomenclatural and taxonomic changes and the description of numerous new diatom species and genera during the past decades make the study of diatoms in tropical Africa complex. In the present work the recently accepted diatom taxonomy at genus level is illustrated for the most common genera of tropical Africa using schematic computer generated drawings. On these drawings the typical characteristics, or characteristics important for identification of species, are highlighted in red, often on a duplicate of the drawing. Moreover, light microscopic micrographs are presented from cleaned material, all from the Congo and Zambezi basins, to show the typical valve ornamentations on which identification is based. Where possible light microscopic micrographs from living material are given to show the plastid(s) structure typical for the genera; these micrographs however are mainly from Southern African material. Only if both authors of the present work were completely certain of the species identity the species name is added to the figure captions. For most genera the ultrastructures of the diatom valves are illustrated with photographs taken with a scanning electron microscope; the material used originates from the Congo and Zambezi basins. A scale bar is added to all micrographs to indicate the size of the valves and the ultrastructures. Light and scanning electron microscope investigations were performed at the North-West University, Potchefstroom, South Africa and at the Botanic Garden Meise, Belgium.

## 2. Definition of a diatom

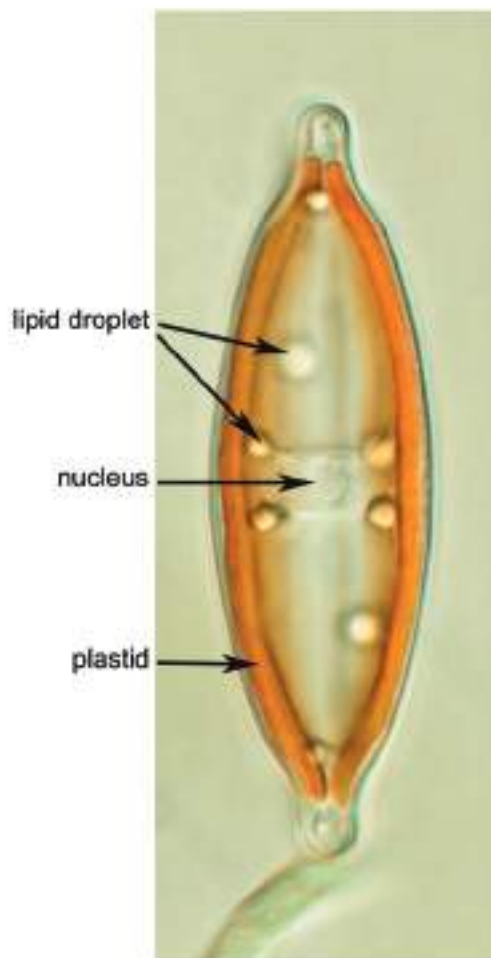
Diatoms or Bacillariophyta are a major group (phylum) of microscopic eukaryotic algae, unicellular but often forming colonies. The cell wall, called a frustule, is highly differentiated and heavily impregnated with silica (hydrated silicon dioxide) and is composed of two valves connected by girdle bands (Fig. 1). The valves and girdle bands fit together very tightly preventing flux of material across the cell wall, which can only take place through openings (pores and slits) in the frustule. A thin layer of organic material (membrane) is also present on the outside of the cell wall. All diatoms probably secrete polysaccharides; some may diffuse in the surrounding environment while others may remain around the cell as stalks, pads, threads or even capsules. This thin organic layer obscures the details of the silica cell wall ornamentations which are used for identification. For this reason diatom cells must be cleaned (oxidation to remove the organic material) before making permanent light microscopic slides and before making preparations for scanning electron microscopic investigation.

The origin of the diatoms may go back to the early Jurassic period (201.3 Ma) or even before, although well-documented fossil records only extend to the middle Cretaceous (127-89 Ma). The diatoms found in the Upper Cretaceous sediments are all of marine origin; most genera are now extinct. Evidence for the presence of freshwater species is found from the late Eocene (38 Ma) and Miocene (23 Ma) onwards.



**Fig. 1.** Diatom frustules, composed of two valves connected by girdle bands. A. Light microcopy (LM). B. Scanning electron microscopy (SEM).

Algae traditionally formed part of botanical studies as they were considered in the past as plants: they are photosynthetic organisms, making their own organic material using sunlight (autotrophs). In the system of the five kingdoms (Whittaker 1969), they are part of the Protista (the other kingdoms are the Plantae, Animalia, Fungi and Monera or Bacteria); later the Monera were divided into the Eubacteria and the Achaebacteria (Woese & Fox 1977). A more recent system (Woese *et al.* 1990) divides all living organisms in three domains: Bacteria, Archaea and Eukarya or Eukaryota. The diatoms are part of the last domain as they possess a true nucleus encapsulated in a nuclear membrane.



**Fig. 2.** Living diatom cell showing the plastid, lipid droplets and the nucleus.

### 3. Living diatoms

Diatoms are a major component of the primary producers in aquatic ecosystems. They can live free in the water column or be attached to a substratum. When they are free living, floating in the water column, they are called planktonic. When the entire life cycle takes place in the water column they are euplanktonic; when they become suspended in the plankton after being detached from the substratum they are known as tychoplanktonic. Tychoplankton occur mostly near-shore in the littoral zone of lakes. In rivers, suspended benthic organisms form part of what is known as the potamoplankton. Species that live on the bottom of a waterbody are called benthos. Benthic species can be attached to the bottom and are then sessile, or they can be motile (or both). The bottom can consist of a hard substratum such as stones, pebbles, boulders, rocks, or of loose sediments such as sand, mud, silt, clay. The term periphyton is used for species attached on submerged substrata; depending on the substratum we can have epiphytic species when they are attached to aquatic plants, epipellic when they are living on sediments and mud, epixylic when they are attached on wood, epilithic when they are living on stones, rocks, boulders, etc., and epipsammic when they are attached to sand grains.

### 4. Morphology of the diatom cell

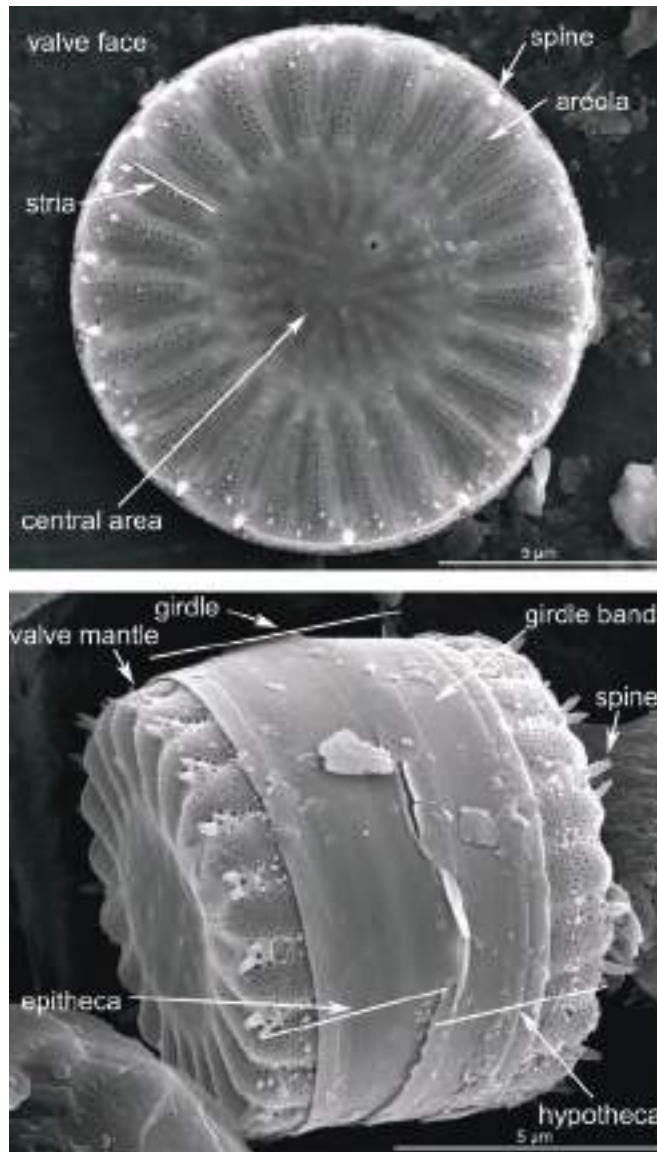
Diatoms are unicellular algae with a siliceous cell wall, the frustule. Besides the photosynthetic pigments chlorophyll a and c and fucoxanthin they possess  $\beta$ -carotene, diatoxanthin, diadinoxanthin, violaxanthin, antheraxanthin, and zeaxanthin and storage products (oil/lipid droplets) (Fig. 2, previous page). Two main groups, based on cell symmetry can be distinguished: the radially symmetric or centric diatoms (Fig. 3) and the bilaterally symmetric or pennate diatoms (Fig. 4). In freshwater, the first are commonly found suspended in the water column while the second are more typical of benthic habitats or are temporarily re-suspended in the water column, although several *Nitzschia* (pennate diatom genus) are a typical component of the phytoplankton in tropical African lakes.

Other aquatic organisms can also possess siliceous structures which can be confused with diatoms. The first are structures called spicules, formed by sponges. They are composed of a solid silica body having a central empty canal almost as long as the entire length of the spicule. Several kinds of spicules exist, e.g., gemmasclere, microsclere and megasclere (Fig. 5).

The second type of siliceous structures are phytoliths, silica bodies formed in or between plant cells. Phytoliths are also solid silica structures with a very large diversity of shapes, often genus specific (Fig. 6). The solid silica structure can have ornamentations, such as cones which can be surrounded by smaller, satellite cones.

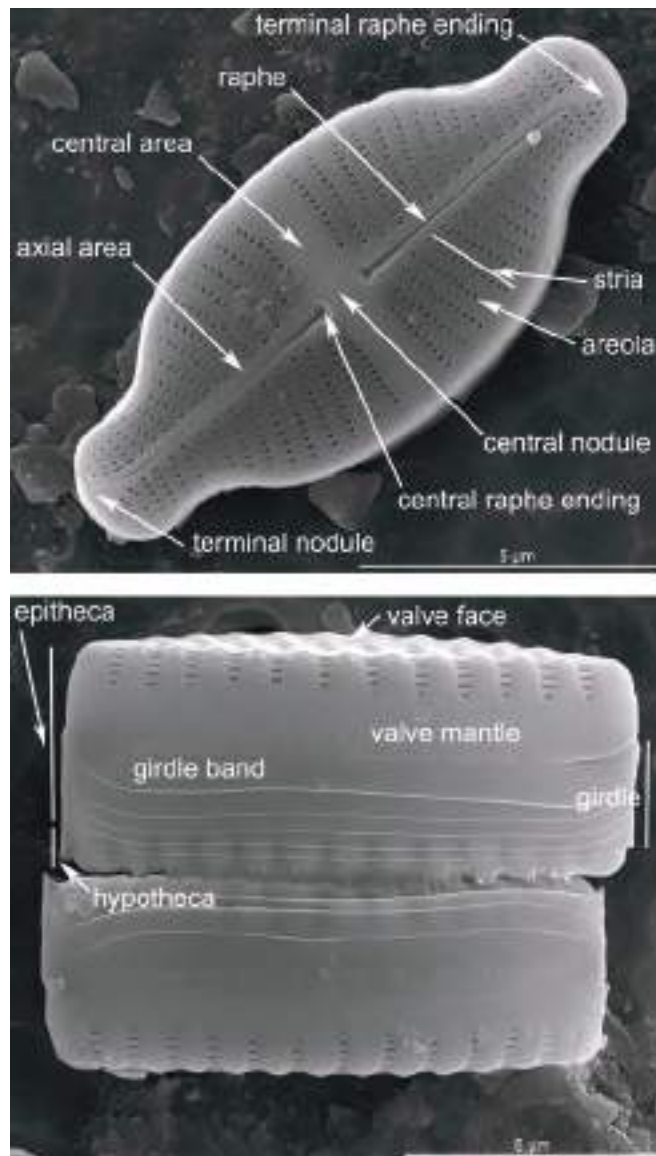
A third silica structure we can mention are cysts, formed by other algae such as Chrysophyta and Dinophyta. Cysts are characterized by an apical pore and the





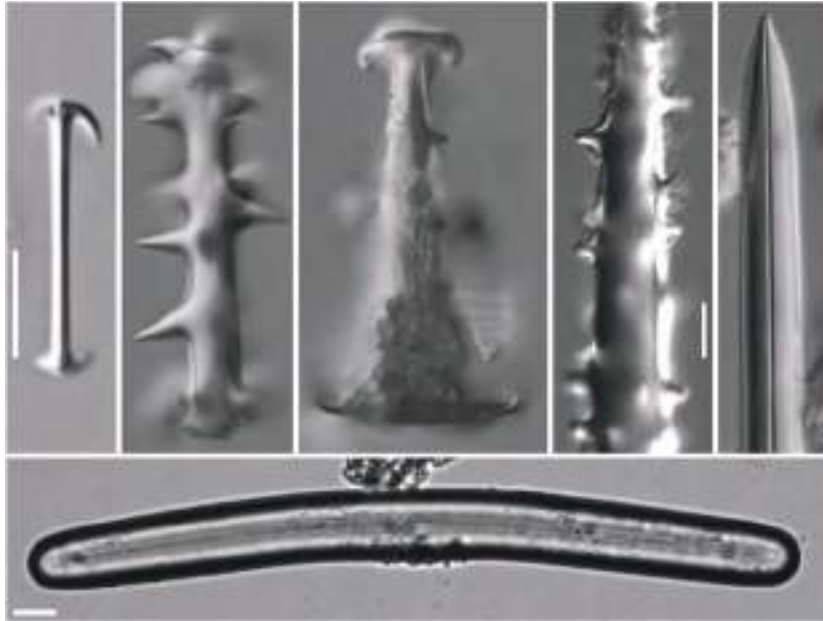
**Fig. 3.** Centric diatom showing the different structures. SEM. Top: valve view. Below: girdle view of an entire frustule composed of two valves and several girdle bands.



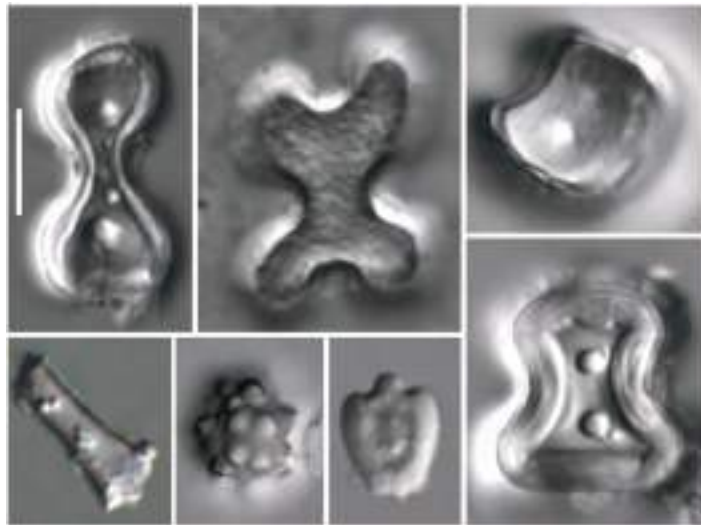


**Fig. 4.** Pennate diatom showing the different structures. SEM. Top: valve view. Below: girdle view of two entire frustules composed of two valves and several girdle bands.

wall can be smooth or ornamented (spines, verrucae, ridges) (Fig. 7). Another structure that can be confused with a diatom frustule is the lorica, a shell-like protection, of *Trachelomonas*, a genus within the Euglenophyta (Fig. 7). The wall



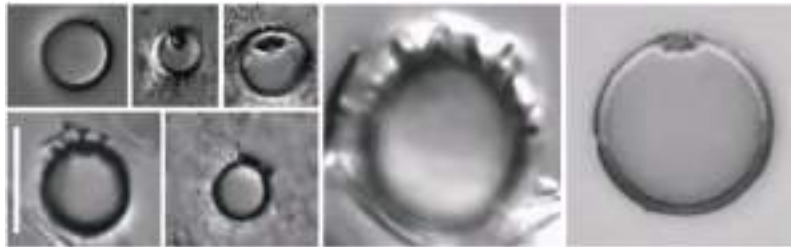
**Fig. 5.** Sponge spicules, different types. LM. Scale bars = 10  $\mu\text{m}$ .



**Fig. 6.** Phytoliths, different types. LM. Scale bar = 10  $\mu\text{m}$ .

of the lorica, in which silica can be incorporated, can be smooth or ornamented with small spines and is, like the mentioned cysts, characterized by an apical gap from which the flagellum protrudes.

Note that all the pictures presented of these silica bodies (sponges, phytoliths, cysts and lorica) are from material sampled in the Congo and Zambezi basins.

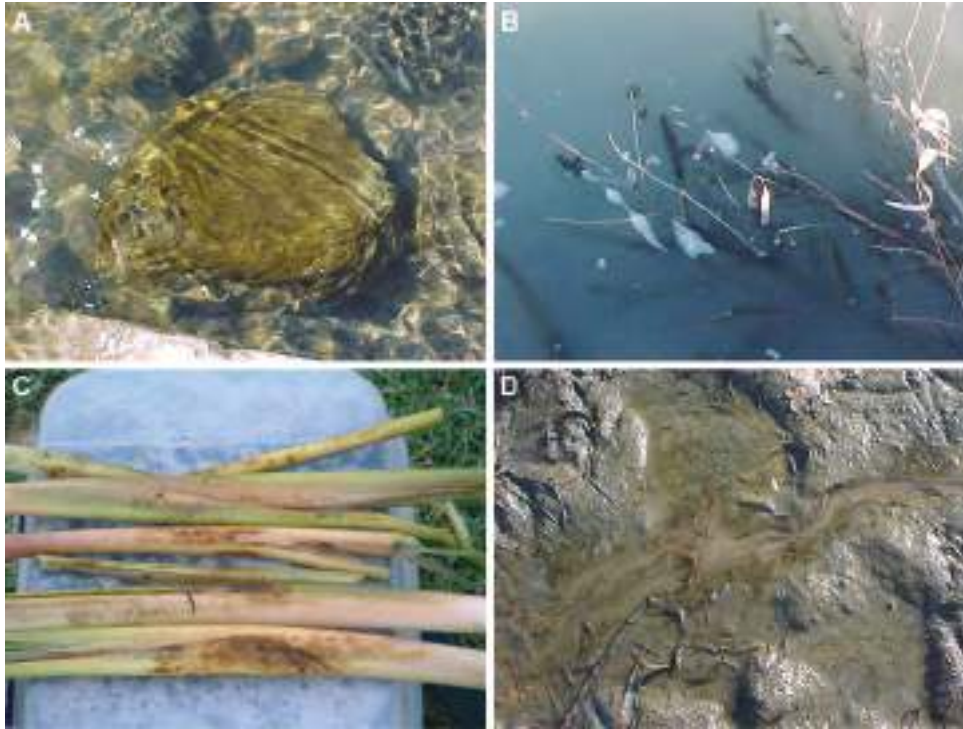


**Fig. 7.** Different types of Chrysophyte cysts on the left, a Dinophyte cyst in the centre, and the lorica of a *Trachelomonas* (Euglenophyte) on the right. LM. Scale bar = 10  $\mu$ m.

## 5. How to recognize diatoms in natural environments

Periphytic (attached) diatom communities may be seen as a thin golden-brown film covering the substrata (Fig. 8). This film can be very thin but can also become thicker and much more obvious during certain times of the year when environmental conditions such as light, temperature and nutrient availability favour diatom growth. The film formed by the diatoms feels slimy or mucilaginous. Attached diatoms grow on all kinds of substrata including solid substrata such as pebbles, boulders and rocks and submerged stems and leaves of aquatic and/or submerged macrophytes (Fig. 8). Man-made objects such as paper, plastic bags and glass may be colonized by diatoms. Diatoms may even be found in soils, with several taxa adapted to survive desiccation. Diatoms form a component of the phytoplankton community where they live in suspension, or attached to other algae. An essential aspect when using diatoms to infer ecological conditions is to sample well colonized substrata. It takes several weeks for an uncolonized substratum to be fully colonized by diatoms. During that time a process of succession in diatom species and abundances can be observed.

The colonization of a substratum by diatoms takes place in various phases and by different diatoms during succession (Bijkerk 2014). Pioneer diatoms, often belonging to small *Achnantheidium* sp. (e.g., *A. minutissimum*), affix themselves to the substratum by small mucilaginous stalks. During succession they are overgrown by other species belonging to among others *Gomphonema*, *Encyonema*, *Rhopalodia* spp. These are attached also to the substratum by mucilaginous stalks but longer ones, or they are attached to the other diatoms or their stalks. The diatom film found



**Fig. 8.** Periphytic diatom communities on various substrata. A. Thick diatom layer (biofilm) attached to boulders. B. Thick diatom layer on submerged parts of tree branches. C. Thin diatom layer attached to submerged stems of *Phragmites australis* (Cavanilles) Trinius ex Steudel. D. Diatom layer on mud.

on a substratum can thus be compared to a forest with several layers such as the canopy, the understory, the shrub and the ground layer.

Diatoms can be attached in various ways to the substratum: mucilage stalks, mucilage tubes, mucilage pads (Fig. 9). Diatoms often form colonies, in particular this adaption allow planktonic species to remain suspended in the water column. The colonies can have the form of a chain, be stellate or zigzag (Fig. 10).

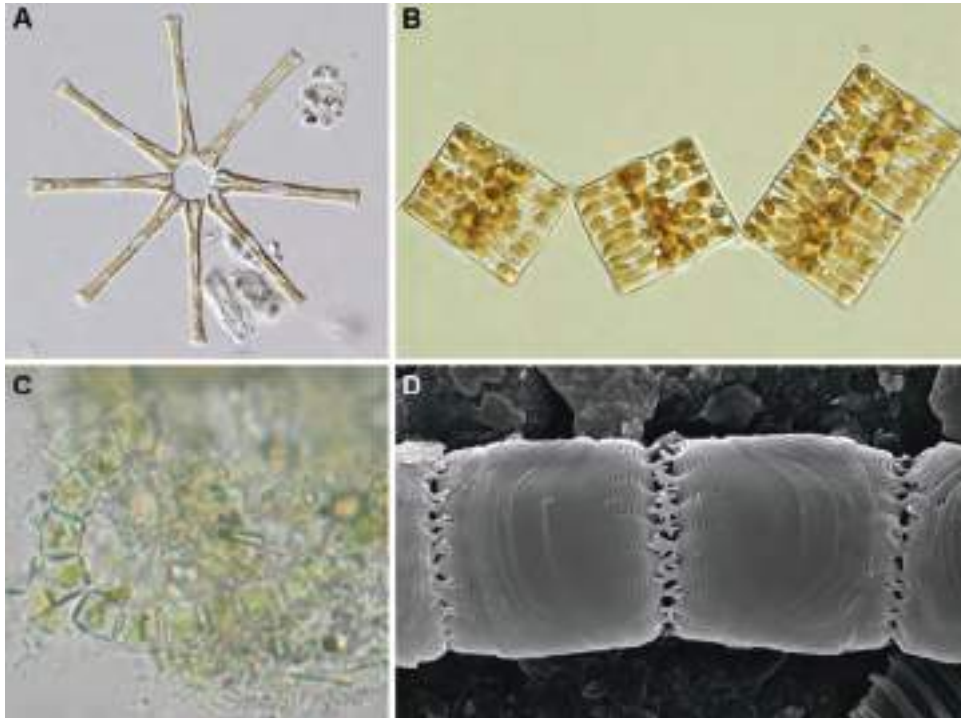
## 6. The role of diatoms in aquatic food webs

Diatoms are key organisms in aquatic ecosystems, together with representatives of the other micro-algae. They are autotrophic, making their own organic material from inorganic nutrients and sunlight through photosynthesis. Phosphorus (as dissolved orthophosphate) and nitrogen (as nitrate, nitrite or in the form of ammonium ions) are



**Fig. 9.** Living diatom cells. LM. A. *Cymbella* sp. attached to a substratum with a mucilage stalk. B. *Encyonema caespitosum* inhabiting a mucilage tube. C. Cells of *Gomphonema* sp. with dichotomously branching mucilage stalks. D. Cells of *Achnanthes minutissimum* attached to a *Lyngbya* sp. (filamentous bluegreen alga) by means of short mucilage stalks. E. Chain forming cells of *Melosira varians* attached to each other by mucilage pads. F. *Afrocybella barkeri* attached to a substratum with short mucilage stalks.





**Fig. 10.** Various types of colonies formed by diatom cells. A-C: LM, D: SEM.  
 A. *Asterionella formosa* cells forming a stellate colony. B. *Tabellaria flocculosa* cells forming a zigzag colony; cells are attached to each other at the corners by mucilage pads. C. *Staurosirella* sp. cells forming a chain; cells are attached to each other, valve face to valve face, by connecting spines. D. *Staurosira* sp., detail of connecting spines.

among the most important nutrients for diatom growth, iron and other trace elements are also necessary. Being part of primary production they lie at the base of the food web and are consumed by heterotrophic organisms which are secondary producers. The consumers of diatoms range from microscopic ciliates to grazing molluscs and plankton filtering fishes. Changes in the environment will first become apparent at the base of the food web i.e., changes within the algae community including diatoms. For this reason diatoms are often used as bio-indicators in the study of water quality. The advantage of using diatoms in water quality analyses is that, besides the ease of sampling (section 7) they can be preserved for relatively long periods.

Identification of diatoms is based on the morphologic characteristics of the frustule/valve, a rigid structure (cell wall) composed of silica, and for this reason samples can be fixed in situ and stored with little concern that the cell wall will deform or rapidly

degrade. Immediate analysis is not required in contrast to phytoplankton analyses. However it is still worth examining the living communities and noting not only the other micro-algae and their interactions with the diatoms, but also the amount of dead diatoms (empty frustules). In lakes especially more than half of the observed diatoms can be empty frustules and sometimes only a minor portion are in fact living diatom cells.

## **7. Field collection methodology**

### **7.1. Epilithon**

The preferred substratum used for the diatom-based monitoring for water quality assessment in riverine environments comprises cobbles and small boulders or rocks. Most of the diatom indices developed and tested throughout the world can be applied using the diatom communities found on this substratum.

The main advantage with using the epilithon is that cobbles and small boulders are generally widely available throughout the entire length of a river or stream, from the source up to the mouth. Moreover the epilithic diatom community has been studied intensively around the world, their ecology is better known and the performance of the major diatom-based indices on this substratum is well understood at least in the case of Europe, North America and South Africa. In the absence of this substratum alternative man made substrates can be used such as bricks, pieces of concrete, bridge supports, pillars, channel walls, etc., and in the absence of these artificial substrata can be introduced. However, if this is the case sampling should only be done after these artificial substrates have been submerged for at least four weeks, allowing diatoms to colonize them. A disadvantage of this method is that one is not sure if the diatom community has already attained its climax structure after the four weeks and there is also the rather high probability that the substratum is removed by third party or animals before sampling can take place. Sampling from this substratum is achieved as follows. At least five cobbles or pebbles, of a size that can safely be picked up, are removed from the river or stream. The upper surfaces, those exposed to the flow of the river, are then firmly brushed with a small plastic brush such as a tooth or nail brush (or a knife) into a collection tray. All the substrata are pooled together and form a single sample. This sample is then well mixed and placed in a suitable labelled bottle. Other hard substrata which cannot be removed from the river may be sampled *in situ*. This is best achieved by scraping the substrata with a knife, spoon or with a tooth brush. The tooth brush tends to trap the biofilm rather well between its bristles and the brush can then be repeatedly rinsed into the sampling tray or other receptacle until sufficient material has been collected. Preservation in the field is recommended except when the cells are to be examined in their living state. Preservation is achieved using ethanol to a final concentration of 20% by volume. In the absence of ethanol formalin may be used but if unbuffered it is not suitable for long term preservation. After each sampling the apparatus used (tooth brush, knife, collection tray) must be thoroughly cleaned with distilled water before collecting a new sample in order to

avoid contamination. If distilled water cannot be carried into the field, the apparatus should be washed after the sampling event in the stream and then again in the stream at the new sampling site before collecting the sample.

## 7.2. Epiphyton

Although cobbles and small boulders are generally widely available, this is not the case for many rivers in D.R. Congo among which a prime example is the Congo River. Even if such a substratum is available the depth and velocity of flow of this river make obtaining them dangerous. An alternative is then to sample the diatom community growing on permanently submerged, usually rooted, parts of plants, belonging either to submerged macrophytes such as *Potamogeton* spp., *Ceratophyllum* spp., or emergent macrophytes, such as *Phragmites* spp., *Vossia cuspidata* (Roxburgh) Griffith, *Papyrus* spp. It is also possible to sample from *Eichhornia crassipes* (Martius) Solms and *Pistia stratiotes* Linnaeus but it is better to avoid these as they are floating plants and are easily transported and thus not representative of the sampling locality. In the European Integrated Water Policy *Phragmites* is often used as substratum. *Phragmites* was subjected to intensive studies, among others on the colonization by diatoms (e.g., STOWA 2014). In these studies it was found that maximal diatom growth on *Phragmites* stems was attained after five weeks and species diversity stayed more or less constant after seven weeks (Van Dam in STOWA 2014). Species quantity and composition is also dependent on the place the plant is growing in relation to the river bank. Therefore it is important that different parts of different plants are collected: at least 5 pieces of about 5 cm each on which a layer of diatoms/biofilm is clearly visible. These pieces are put together in a plastic zip bag with as little water as possible from the sampling locality. Close the bag and rub the bag with the plant pieces firmly between your hands so that the attached diatoms come loose from the substratum. A brown-greenish liquid will become visible, containing the diatoms. If the plant material is too dry, add a small amount of distilled water. Put the brown-greenish liquid in a bottle and fix immediately in the field with ethanol or formalin, see comments in section 7.1.

## 7.3. Epipsammon

Diatoms growing on sand grains, epipsammon, form colonies which are different from the epilithic and epiphytic communities. Sand as substratum is subject to abrasion as a result of movement of the grains where only strongly attached taxa can survive. Some typical taxa are *Cavinula lilandae* Cocquyt, M. de Haan & J.C. Taylor recently described from a stream in D.R. Congo and *Cymbellonitzschia minima* Hustedt, an endemic species of the East African Great Lakes. This habitat is not recommended for studies for biomonitoring, however it may form an important part of biodiversity studies. Sampling may be achieved in several ways. If the visible brownish biofilm is thick enough it can be gently scraped from the surface with a spoon or sucked up using a large pipette or syringe. Alternatively the very top layer of sand can be collected and rinsed to free the diatoms from the grains. Motile diatoms may be sampled by collecting the top layer of sand and returning this wet and unpreserved to



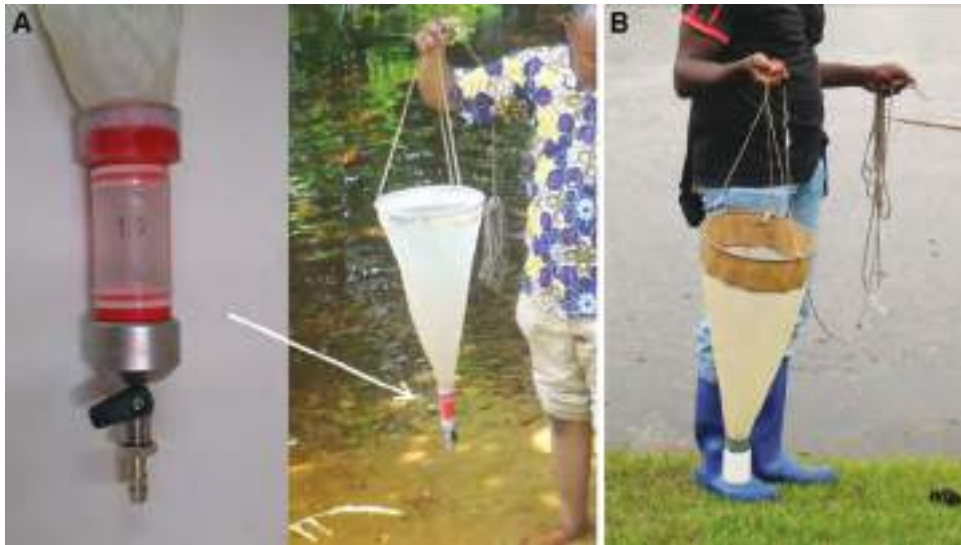
the laboratory. The sand is then placed in a Petri dish and either cover glasses or lens tissue is placed over the moist surface of the sand. The diatoms will migrate towards the light and in so doing stick to either the tissue or the glass. The tissue or glass can then be removed and rinsed in order to collect the diatom cells for examination.

#### **7.4. Phytoplankton**

Diatoms are one of the algal groups composing the phytoplankton. They can thus also be studied as part of a phytoplankton investigation. Sampling can be done in a quantitative way or, when only relative composition is needed, in a semi-quantitative way.

The quantitative method is mostly used to study the entire phytoplankton community, not only the diatoms. Depending on the trophic state of the water body the sample can be collected in a bottle which can range in volume from 1 l for oligotrophic waters to 50 ml for eutrophic waters. The preservation is done *in situ* with an alkaline iodine solution (Lugol's). When it is not possible to analyze the samples shortly after sampling (within a few days) formalin should be added to the sample in the field just after the fixation with Lugol's. Samples must be kept in the dark and preferably but not obligatory in a refrigerator or a cool box at around 4°C. Prior to analysis the sample is concentrated by settling for 24 to 48 hours for 1 l. To preserve the phytoplankton sample after analysis, buffered formalin must be added as in an alkaline environment dissolution of the diatom frustules is slowly taking place.

The semi-quantitative method is based on the sampling with a phytoplankton net. Best is to use a net with mesh size of 10 µm. Experience in tropical Africa has demonstrated that the diatom communities are composed of small cells, often smaller than 20 µm and even 10 µm. However, in eutrophic systems the meshes are quickly blocked preventing the water to be filtered; nets with larger mesh size can be used but the mesh size must never exceed 27 µm. One must be aware that the results of analyses based on such samples may not be representative of true algal communities as small organisms are not be retained in such a net sample. The phytoplankton net should be held in the stream of moving waters for a couple of minutes, avoiding suspension of benthic material. The concentrated sample at the bottom of the net should then be put in a storage bottle and fixed with ethanol or buffered formalin. In shallow standing waters the phytoplankton net should be dragged back and forth just below the surface. Again care should be taken that benthic material is not disturbed and included in the sample. For standing waters deeper than 10 m, such as lakes and dams, where vertical stratification can occur in the water column, a vertical haul with the phytoplankton net must be taken. The depth of the haul depends on the depth of the photic zone (up to where light penetration is sufficient for algal growth). In oligotrophic lakes such as Lake Tanganyika, the vertical haul may be up to 40 m depth; in other small oligotrophic deep crater lakes a haul of up to 25 m is recommended.



**Fig. 11.** Phytoplankton net. A. Commercial net (Hydrobios) with removable net bucket with a mesh screen which aids in concentration of the sample and a small tap at the bottom to decant the sample. B. Self-made net with screw thread adapted to a particular plastic bottle, in the depicted case a polyethylene bottle of 100 ml.

In order to aid the passage of water through the net it may be moved gently back and forth but care should be taken that none of the water is lost from the net.

The phytoplankton net is composed of a supporting ring made of stainless steel to which a conical net bag is attached. Usually the ring has a diameter of 25 cm and the bag is around 50 cm long. The sample is concentrated in a removable container or bucket on the end of the net with an opening covered by a mesh screen (Fig. 11 A), the sample is transferred to a bottle by opening a small tap. As the commercial nets (e.g., Hydrobios) are rather expensive, it is also possible to use homemade nets. Special material for phytoplankton nets with mesh size of 10  $\mu\text{m}$  is available from specialized (web)shops. Make sure to use a stainless steel or aluminium ring to avoid corrosion. Instead of a concentration removable net bucket, an adaption piece to screw the bottles used for storing samples can be made (Fig. 11 B).

## 7.5. Terrestrial or soil diatoms

Soil diatoms, also called aerophilic diatoms, are a special group with many adaptations for the microclimate they live in which can be relatively arid compared to the permanently wet condition of aquatic environments.

Collecting can be done from moist sub-aerial, aerial and arid aerial habitats. It is recommended that six sub-samples of about 5  $\text{cm}^2$  should be taken within a radius

of 10 m to cover the local variability. To sample arid or dry soils remove carefully any detritus and other material covering the surface of the soil using a knife or a spoon. Collect the soil with a knife or spoon to a depth of about 1 cm. The six subsamples thus collected should be placed in a paper envelope and not in a plastic bottle, this is to lessen the chances for the growth of bacteria and fungi due to the accumulation of moisture in a bottle. Dry or semi-dry rock faces and seep zones can also be sampled in a similar fashion. The biofilm is scraped from the surface of the rock using a spoon or knife. It is often not possible to cover a 10 m radius and thus several subsamples within the area covered by the biofilm should be collected. Depending on the amount of moisture present the sample can be stored in either paper envelopes or plastic bottles

## 7.6. Environmental parameters

During sampling, especially for water quality investigation, it is essential to note several characteristics of the water body as well as to measure some environmental parameters.

- Hydrological characteristics: stream velocity  
lake, river or channel depth  
river or channel width
- Physical variables: water temperature  
turbidity  
sampling depth  
coordinates (collected using a GPS)
- Chemical variables: pH  
electrical conductivity (EC) also called specific electricity  
dissolved oxygen (DO)
- Nutrients: samples for measuring nitrogen:  
nitrates ( $\text{NO}_3\text{-N}$ )  
nitrites ( $\text{NO}_2\text{-N}$ )  
ammonium ( $\text{NH}_4\text{-N}$ )  
phosphorus:  
orthophosphate ( $\text{PO}_4\text{-N}$ )  
total phosphate (TP)  
soluble reactive phosphorous (SRP)  
silica, iron, ... is recommended if the equipment for  
measuring these parameters is available.
- Others: sampling site shaded or not

## **7.7. Annotations**

It is essential to take notes during a sample collection trip. Besides the date and time of sampling all information mentioned in the previous section must be noted in the field during or just after the sampling. It is very difficult to remember everything on return back to the laboratory as most of the time collection trips have several sampling occasions and sampling sites.

How to take notes in the field? Do you have to use a field notebook or is it better to use prepared field record forms? Both have their advantages and disadvantages. Field record forms have the advantage that all information needed to be noted in the field is clearly indicated on a A4 sheet and have only to be filled in. It also allows for a standard data set to be collected at each site. The disadvantage is that separate sheets easily get lost. Forms must be clearly and logically composed. An example is given in Fig. 12. This example of a field record form can be adapted to the specific needs and type of sampling that will be conducted.

A notebook has the advantage that all the collected information from different sampling trips remains together. Notebooks are easy to store and must be kept with the relevant sample or slide collection if these are stored in a herbarium, museum, etc. A disadvantage of using a notebook is that one must be careful not to forget to include necessary information on the sample/sampling site, which may be the case for novice collectors; but once sampling becomes a routine, taking notes will also become routine.

## **7.8. Sample numbering and labeling**

The correct numbering and labeling of the samples is also essential during a sampling trip.

Individual labels for each sample should be provided. It is recommended that sample information be written on self-adhesive labels with a pencil to avoid smudging if exposed to water. Once the label has been stuck onto the sampling bottle/vial, transparent tape can then be placed over the label and extend onto the bottle/vial to prevent damage such as abrasion and fading of the label during transport and storage (Fig. 13).

When a routine sampling is planned, labels can be made in advance in the office before starting the field work.

Field annotations in a field notebook or on a field record form together with the corresponding label on the sampling bottle are crucial for successful sampling and sample archiving. A sample without a label and without related field information is scientifically useless.

River: \_\_\_\_\_ Site: \_\_\_\_\_  
Date: \_\_\_\_\_ Sample number: \_\_\_\_\_  
Sample collected by: \_\_\_\_\_

---

### Physical records

Width: \_\_\_\_\_ Depth: \_\_\_\_\_

### Substrate (record estimated percentage)

bedrock	<input type="checkbox"/>	boulders/cobbles	<input type="checkbox"/>
pebbles/gravel	<input type="checkbox"/>	peat	<input type="checkbox"/>
sand	<input type="checkbox"/>	silt/clay	<input type="checkbox"/>

### Estimated percentage of boulders and cobbles covered by:

Filamentous algae ☐ Other macrophytes ☐

### Shading (record estimated percentage)

Left bank	None <input type="checkbox"/>	Broken <input type="checkbox"/>	Dense <input type="checkbox"/>
Right bank	None <input type="checkbox"/>	Broken <input type="checkbox"/>	Dense <input type="checkbox"/>

### Habitat

Pool ☐ Run ☐ Riffle ☐ Slack ☐

### Water clarity

Clear ☐ Cloudy ☐ Turbid ☐

### Bed stability

Firm ☐ Stable ☐ Unstable ☐ Soft ☐

### Chemical records

pH:	type of meter used:
Conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ):	type of meter used:
Water temperature ( $^{\circ}\text{C}$ ):	type of meter used:

### Photograph

Facing upstream: \_\_\_\_\_ Facing downstream: \_\_\_\_\_

Remark: It is important to include an immovable structure in a photograph as a reference for future comparison e.g. a bridge

**Fig. 12.** Example of a field record form to accompany a diatom sampling trip in a river or stream.



**Fig. 13.** Field collection methodology. A-B. Collecting samples with a phytoplankton net. C-D. Measuring of physical and chemical variables E-F. Taking notes in a field notebook and labeling of a sampling bottle.

## **8. Laboratory methodology**

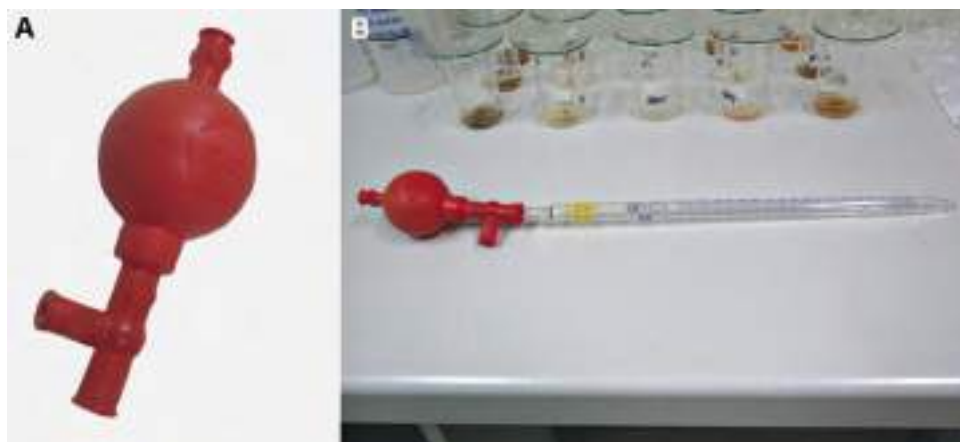
### **8.1. Cleaning samples for diatom investigation**

Diatom cells are covered on the outside of their silica cell wall by a thin layer of organic material (membrane). This thin layer obscures morphologic features, such as the perforations and the raphe, needed for determination of the diatom species. The sample will also contain detritus, protists, bacteria and soft-bodied algae which are the usual components of a biofilm; this material will also obscure the structures of the diatom cell when viewed at high magnification. Therefore the organic material, not only in and around the diatom cell itself but also other organic material present in the sample, must be removed to obtain cleaned material for making permanent diatom slides. After such cleaning process all that remains are the resistant silica cell walls of the diatoms and occasionally other siliceous structures such as sponge spicules or phytoliths.

#### **Material needed for the cleaning or oxidation of samples**

- Beakers (heat-resistant glass) with a total volume of 100 or 250 ml depending on the sample volume and concentration.
- Watch glasses (heat-resistant).
- Hot plate for heating the material (to be used inside a fume cabinet).
- Bottles/vials (preferably glass).
- Pipettes.
- Safety pipette filler (Propipette) (Fig. 14).
- Reagents: peroxide ( $\text{H}_2\text{O}_2$ ) 30 %,  
potassium permanganate ( $\text{KMnO}_4$ ) for organic rich samples  
hydrogen chloride ( $\text{HCl}$ ) for samples from environments rich  
in salts, especially calcareous waters.
- Waste bottles for disposal of hazardous compounds.
- Permanent marker.
- Centrifuge for 10 ml centrifuge tubes and centrifuge tubes (optional).





**Fig. 14.** A. Safety pipette filler. B. Safety pipette filler mounted on a pipette.

## Protocol

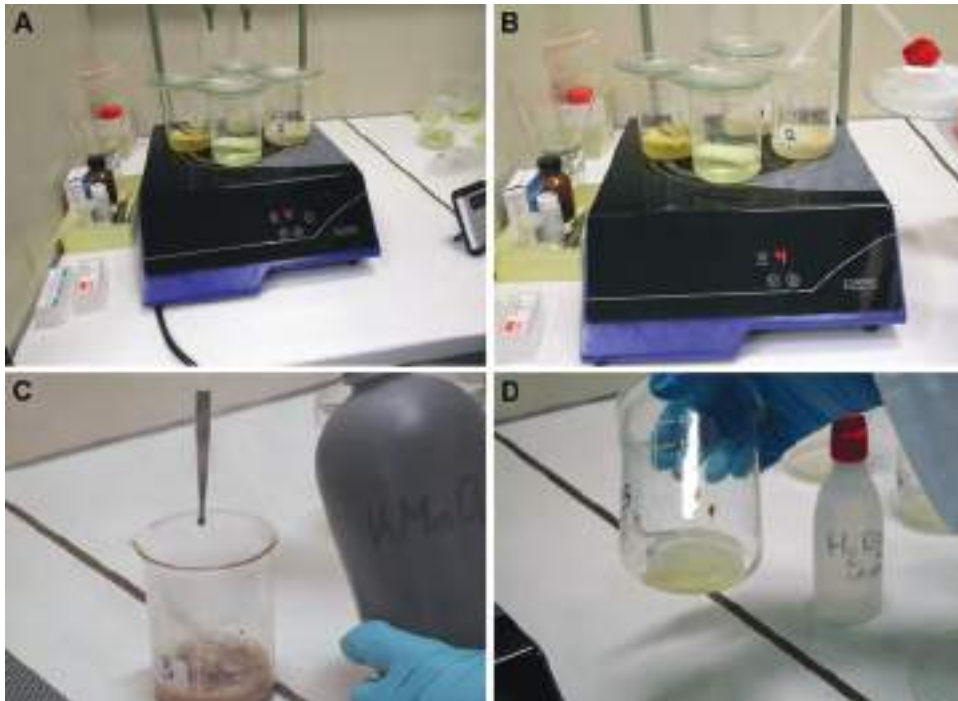
As with sampling it is important that the final scatter of diatom cells on the slide is representative of the original sample. For this reason it is important to mix or shake the sample well at each stage in the process mentioned below.

- Allow the sample to stand for 24 h in the laboratory to allow the diatoms to settle at the bottom of the bottle. This is best done soon after the field sampling.
- After the period of 24 h to allow for settling, decant the supernatant liquid from the sample bottle (the supernatant liquid must be very clear) taking care not re-suspend any of the settled material which will cause the loss of diatom material. A better method, but more time consuming, is to remove off the supernatant liquid using a pipette provided with a safety pipette filler (follow the instructions in the product operating manual).
- Shake the remaining thick suspension well in the sample bottle to homogenize the material containing the concentrated diatoms and pour a part of the suspension (about 5 to 10 ml of the concentrated sample depending on the concentration of the material and the present organic material) into a heat-resistant beaker.
- Cover the heat-resistant beaker with a watch glass (heat-resistant) to prevent cross-contamination between the samples.
- Mark the heat-resistant beaker in several places with the sample number using a permanent marker.



- Add 10 to 20 ml  $\text{H}_2\text{O}_2$  (30 %) to the concentrated sample in the heat-resistant beaker.
- Put the heat-resistant beaker with the material on the hot plate inside a fume cabinet at about 90-100°C for 2 to 3 h; the samples should be regularly observed to prevent boiling-over or drying out. The heat-resistant beaker should always be covered with a heat-resistant watch glass to prevent contamination between the beakers. Contamination can happen not only due to splashing of material if boiling becomes too vigorous but also due to diatoms present in the steam of the boiling material. (Fig. 15)
- When the material is very rich in organic material, add some drops of potassium permanganate ( $\text{KMnO}_4$ ) to complete the oxidation process. The number of  $\text{KMnO}_4$  drops needed depends on concentration of organic material in the sample: drops must be added until the suspension clears leaving a straw coloured supernatant and a precipitate ranging in colour from brown to grey to white depending of the geology of the sample site.
- Leave the cleaned solution in the heat-resistant beaker to cool down.
- Rinse the sample with distilled water. This can be done by centrifugation or by allowing the material to settle out for 24 h. For centrifugation the cleaned material must be transferred to 10 ml centrifuge tubes. Before pouring the cleaned solution from the beakers, the beakers must be vigorously swirled to re-suspend the diatoms: heavier particles such as sand grains particles will fall to the bottom of the beaker. Centrifugation is done for 10 min at a rotation speed of 3000 rpm (rotations per minute). After centrifugation the supernatant is decanted or pipetted off using a pipette provided with a safety pipette filler. This washing or rinsing is repeated 4 times; the pH of the final sample should be more or less circumneutral. When the preparation of permanent diatoms slides is not urgent, or when a centrifuge is not available, the material can be rinsed by leaving the material in the beaker or in a centrifuge tube to settle during for 24 h before removing the supernatant by decantation or by pipetting off. Again washing should be repeated 4 times.

The above protocol is only one of many that exist. Additional methodologies can be found in the literature, e.g. STOWA 2014, Taylor *et al.* 2007c.



**Fig. 15.** Removing organic material in a diatom sample: part of the material is placed in a heat-resistant beaker, covered with a heat-resistant watch glass to prevent contamination between the beakers, and placed on a hot plate inside a fume cabinet at about 90-100°C for 2 to 3 hours. For material rich in organic material some drops of  $\text{KMnO}_4$  are added to complete the oxidation process.

## 8.2. Permanent diatom slide preparation

Material needed for making permanent diatom slides

- Pasteur pipettes or automatic micro-pipette when quantitative slides are needed (Fig. 16).
- Diatom specific mounting media/mountant with high refraction index ( $\text{RI} \geq 1.7$ ), for example Naphrax ( $\text{RI} 1.73$ ) or Pleurax ( $\text{RI} 1.73$ ).
- Hot plate
- Microscope slides
- Cover slips (example: 20 x 20 mm, 22 x 22 mm, 22 x 40 mm)

## Slide preparation

There are a number of slide preparation methods. The method chosen is less important than the final result. Diatom slides used for ecological and taxonomical studies are most often of the type known as 'strewn mounts'. These mounts represent as closely as possible the structure of the diatom community as collected in the original sample. In order to count or photograph cells on the slide it is important that they do not lie over each other and thus obscure each other (high concentration of material). It is also important that the sample is not highly dilute, in this case enumeration of the sample becomes very difficult and time consuming. A good rule of thumb is that a diatom strewn mount should have 5 – 50 cells visible in the field of the microscope at 1000 x magnification. This of course may not always be possible, especially if the sample has a high sediment content but it can be accepted as a general best practice guideline.

Take an aliquot of the cleaned sample and place in a test tube or other suitable vessel. Add one drop of ammonium chloride 10% solution to the tube and mix with the diatom material.

Put a drop of cleaned sample on a cover slip (about 0.5 - 2 ml depending on the size of the cover slip). If necessary concentrate the cleaned sample or dilute a part of the cleaned sample to a slightly cloudy solution. This is the most important step, if too little material is used the concentration of diatoms on the slide will be too low and making enumeration difficult, if too much material is used the diatom cells will lie over each other and it becomes impossible to identify individual cells. There is also no hard and fast rule for the dilution step, it will depend on the ratio of diatom cells to fine sediment, the initial concentration of diatoms in the field sample etc. This step requires practice and with experience it will be possible to successfully estimate the required concentration.

The volume of liquid placed on the cover slip is also of importance. The surface of the cover slip should only just be covered with liquid, if too much of the liquid (sample) is placed on the cover slip it will lead to unusual drying patterns – most often with the majority of the cells being deposited at the centre of the cover slip.

Let dry the sample on the cover slip. This can be done by two ways:

- air-dry, takes approximately 24 h, best to cover to prevent dust settling on the sample;
- hot plate dry, takes about 1-2 h, very slight warming around 40-50 °C, to avoid the material drying in rings, or leave under a 60 W incandescent lamp - there is less chance with overheating with this method.

At this stage the cover slip can be placed (still with the diatom facing up) on a microscope slide and viewed at low magnification under a light microscope. This is in order to determine if the appropriate concentration of diatom cells is present. If so then the slide can be made into a permanent mount as described below.

Mount in a high resolution mountant. The following mounting media are generally used: Naphrax (RI 1.73) or Pleurax (RI 1.73).

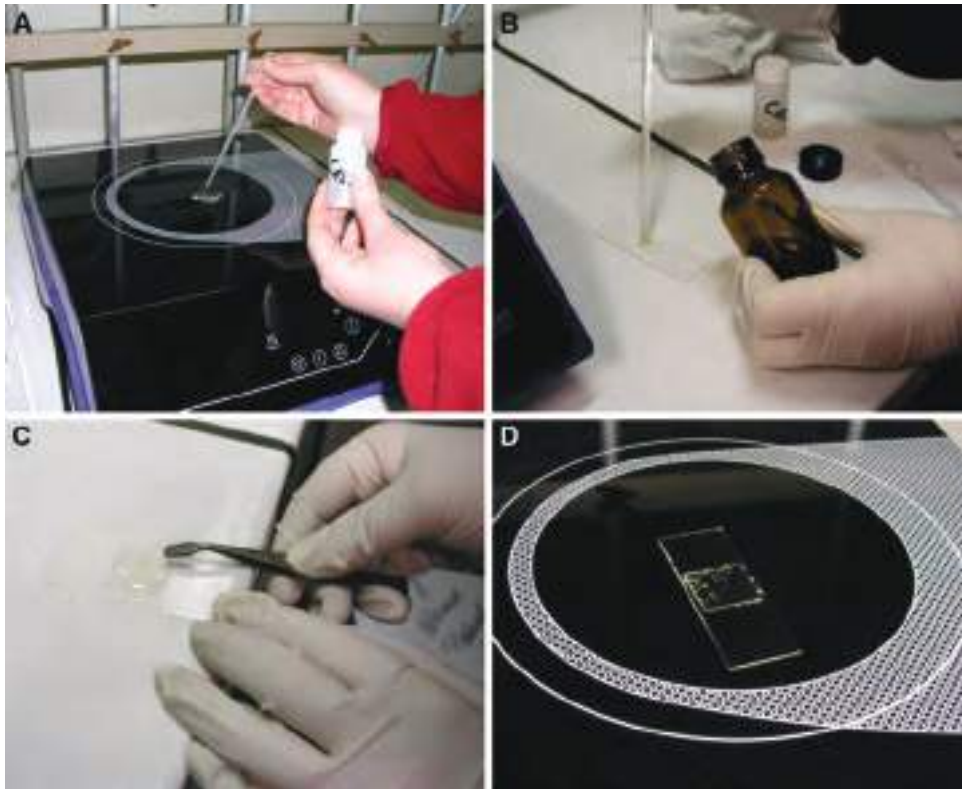
Perform the following procedures out doors or in a fume cabinet. Do not inhale gasses or fumes.

**Naphrax:** (Fig. 16)

- Put a drop of the mounting media on the microscope slide.
- Put the cover slip with the dried samples at a 90-degree angle on the microscope slide alongside the drop of Naphrax.
- Carefully lower the cover slip.
- Put the microscope slide with the cover slip on the hot plate at ~100 °C.
- Allow to 'boil' for 2 min (to remove the toluene – the solvent).
- Remove the microscope slide from the hot plate and let it cool down.

**Pleurax:**

- Place the cover slip, diatoms upward, onto the hot plate.
- Put a drop or two (depending on size of the cover slip) of the mounting media on the cover slip.
- Heat the sample until smoke starts to rise from the mounting media, allow this to continue for about 30 to 45 sec. The Pleurax burns easily so be careful not to overheat it at this stage.
- Carefully invert a clean microscope slide onto the cover slip, do not force the slide onto the Pleurax as this will cause it to be squeezed out of the edges. The cover slip should just be 'caught' by the slide.
- Turn the slide over and heat until the mounting media gently bubbles (~ 90 °C).
- Heat the slide until all air bubbles have been driven out.
- Remove the microscope slide from the hot plate and let it cool down.
- Once completely cool try to chip a small portion of the mountant at the edge of the cover slip. If the mountant is brittle the slide is cured. If viscous return to the hotplate and heat for another minute and test again.



**Fig. 16.** Making a permanent diatom slide from cleaned diatom material. A. A drop of cleaned sample is placed on a cover slip and dried on a hot plate. B. A drop of the mounting media is placed on the microscope slide. C. The cover slip with the dried samples is held at a 90-degree angle on the microscope slide alongside the drop of mounting media and is carefully lowered. D. The microscope slide with the cover slip is placed on the hot plate at ~100 °C to boil.

### 8.3. Preparation for Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is used in diatom taxonomy to study the ultrastructure of the diatom frustules (valves and girdle bands). The valve and girdle ornamentations and perforations are often not fully discernible in light microscopy and can only be studied in detail using SEM. During SEM investigations a three dimensional impression of the structure of the diatom silica cell wall is gained but on the other hand it is not possible to look through the valve as can be easily done with LM. Diatoms with their silica cell wall do not require extra cleaning steps for SEM examinations, other than those explained in section 8.1. in contrast to other, soft-bodied algae which may require critical point drying. However, for water quality monitoring purposes these in-depth investigations using SEM are usually not essential. It should however be taken into account that a large number

of new species have been encountered during water quality studies and in this case it becomes important to document such taxa with SEM if such facilities are available.

Aluminium stubs are used for mounting material for SEM studies as aluminium is a conductive material. Cleaned diatom material can be transferred to these aluminium stubs in several ways:

- a drop of cleaned material can be placed directly on an aluminium stub and air dried;
- a drop of cleaned material can be put on a small cover slip (diameter of 12 mm) and air dried: once the material is totally dry the cover slip is fixed on the aluminium stub with carbon tape or a special conductive glue;
- a drop of cleaned material can be filtered through a Millipore® filter ( $\leq 2.5 \mu\text{m}$ ); after the filter is totally air dried it is fixed on the aluminium stub with a carbon tape or a special conductive glue.

After mounting the diatoms the stubs are sputter coated with gold or gold palladium. Material is then ready for examination with the scanning electron microscope. Depending on the microscope an acceleration voltage of 10-15 kV is usually adequate for diatom examination, although the new generation of field emission scanning electron microscopes work at low voltages as low as 1 kV.

Stubs with the material must be completely dehydrated before entering in the SEM. Therefore they must be kept under very low humidity conditions. Stubs should always be placed in a dessicator containing silica gel for 24 hours to make sure they are completely dehydrated before SEM examination.

#### **8.4. Storage of samples and permanent diatoms slides**

It is always interesting to keep a portion of the original, untreated samples. If a sample is lost during preparation, if there is still a portion of the original material available this can then be used to replace the lost sample. In addition, other researchers may wish to study the material if further investigations are needed using LM or SEM, or especially in case new techniques are developed in the future. The same applies to the cleaned material. Therefore it is essential that the bottle/vials in which the material is preserved are well labelled.

Original uncleaned material is already fixed just after the sampling (formalin of ethanol); the cleaned material on the other hand is not. Therefore ethanol should be added to the bottle/vial to reach a final concentration of 20 % by volume to prevent the growth of micro-organisms such as bacteria, green algae and fungi. Alternatively some thymol crystals (2-isopropyl-5-methylphenol) can be added

to the bottles/vials or the material can simply be allowed to dry out (the bottle should still be kept sealed after drying).

Permanent diatom slides should be well labelled (country, site, locality, river, coordinates, date of collection and name of collector) and stored, preferably in a herbarium or diatom collection to facilitate cross-referencing. Besides their importance for taxonomists diatom slides are, from the point of view of water quality, very important as they provide a permanent historical record of water quality conditions at a site. They should be stored in order to ensure that they can be accessed for future analyses (e.g., hind-casting water quality). Moreover it is recommended that at least two slides are prepared from each sample. One of these should be lodged in the appropriate national herbarium or in another herbarium or institution where its future is assured. A database with all information on the material and on the preparation should be deposited together with the slides.

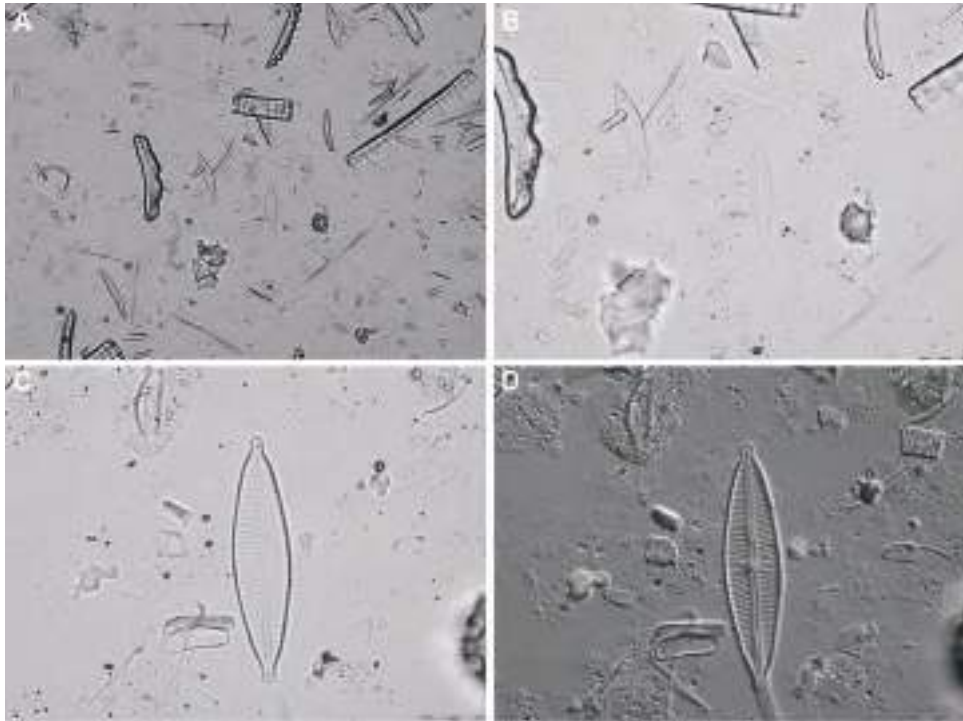
## **9. Diatom analysis**

### **9.1. Light microscopic investigation**

Diatom taxa in manuals, guides and books are usually depicted as a series of neatly aligned pictures. In the past these pictures were handmade drawings by the authors, e.g., Ehrenberg, Hustedt, Chohnoky. Around the second half of the 20<sup>th</sup> century the first photographic illustrations appeared. For tropical Africa we can mention here among others Hustedt (1942, 1949) later followed by Gasse (1986) and Cocquyt (1998). The diatom cells are illustrated in valve view showing the morphological characteristics used for determination. Seldom the girdle view is shown, except if it has a typical shape such as in several *Gomphonema* and *Surirella* species. Valve fragments or broken pieces are not or are rarely depicted. The diatom cells observed during investigation are seldom lying in a nice valve view, but are orientated at different angles, obliquely or in girdle view, and may be damaged or in fragments.

Different types of microscopic illumination exist, bright field, dark field, phase contrast, differential interference contrast (DIC), giving slightly different images than those found in the identification guides which use a selection of the best pictures the authors possess. Diatom counts can be easily done with bright field for routine investigation. However for taxonomic purpose more details are often needed which can't be (easily) observed using bright field. Phase contrast and/or differential interference contrast are then recommended (Fig. 17).





**Fig. 17.** View of a permanent slide of cleaned diatom cells mounted in Naphrax at different microscopic magnifications and with different illumination. A. Low magnification (200x). B. Medium magnification (400x). C. High magnification (1000x) using bright field. D. High magnification (1000x) using differential interference contrast (DIC).

## 10. Glossary

The glossary provides an overview of the most important terms used in diatom taxonomy. All terms indicated in bold in the discussion of the genera, in the present book, are included as well as some outdated terms that are commonly used in the literature, especially in older publications. To make the glossary assessable for French speaking researchers, the English terms are translated into French followed by a short description in French and illustrated using the pictures as in the English glossary (section 10.2 ). English and French terms are all put in alphabetical order.



## 10.1. Glossary

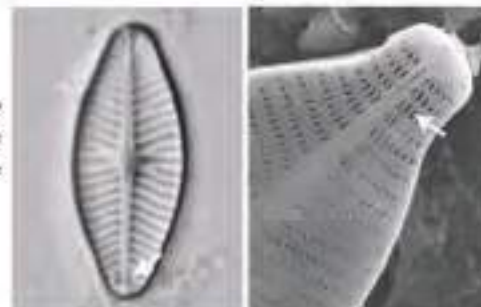
**Adnate:** cell attached by the raphe bearing valve face to the substratum.

**Aerophilic:** occurring in well oxygenated habitats such as on mosses, wet stones, moist earth.

**Alveolus - alveoli:** stria composed of a transversely linear chamber in the valve wall having many small openings to the exterior valve face and one large opening to the interior valve face.



**Annulae:** structure composed of one to four transapical striae interrupting the typical striae near the apices. Structure present only in the genus *Geissleria*.



**Apex - apices:** the extremity of the valve in pennate diatoms, also called pole/poles.

**Apical axis:** the longitudinal axis of the valve face in pennate diatoms passing through the poles.

**Apical nodule:** highly silicified part of the diatom valve near the apex, where the raphe furrow ends; also known as the polar nodule.

**Apical pole:** extremity in pennate diatoms

**Apical pore field:** area with small pores or perforations through the cell wall near one or both valve extremities in pennate diatoms. It is the place where mucopolysaccharides (mucilage) forming stalks and pads are secreted.



**Araphid:** pennate diatom valve without raphe slit.

**Areola - areolae:** round or nearly round perforation in the silica cell wall, also called punctum. The areolae are usually aligned and form striae.



**Axial area:** hyaline area within the pennate diatoms located on the valve face along the longitudinal axis, between the raphe slit, if present, and the striae.

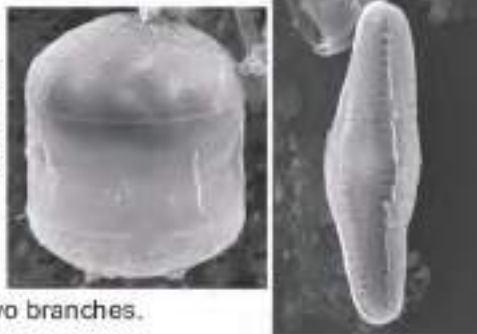
**Axial costa:** narrow siliceous ridge along the axial area, parallel to and bordering the raphe.



**Axial plate:** siliceous plate present on the internal part of the valve covering the internal openings of the areolae. The plate is present in some *Gomphonoid* species where its margin is visible as a longitudinal line in light microscopy.



**Auxospore:** special cell formed during sexual reproduction after the fusion of the gametes. This cell is larger than the daughter cells and the maximum size of the diatom is thus re-established.



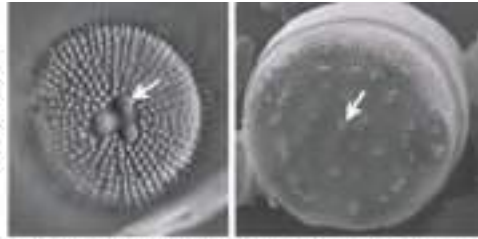
**Bifurcate:** a structure that is divided in two branches.

**Biraphid:** pennate diatom with a raphe on both valves.

**Biseriate:** composed of two parts; a biseriate stria is composed of a double row of areolae.



**Carinoportula:** central process, restricted to the genus *Orthoseira*; the internal openings are simple, the external openings are composed of well-defined collars.



**Central area:** hyaline area (without areolae) in the middle of the valve face at the position of the central nodule within the pennates. In centric diatoms areolae are present in the central area

**Central fissure:** central raphe slit ending near the central nodule, may be enlarged or curved.

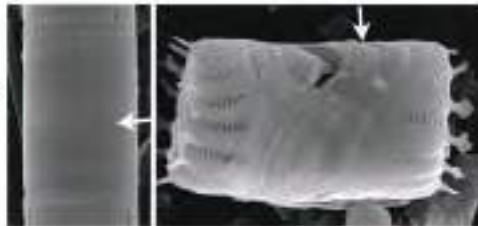


**Central nodule:** more heavily silicified part of the valve wall in between the central raphe fissures.



**Chrysolaminarin:** reserve material, a polysaccharide, present in diatoms and Chrysophytes.

**Cingulum - cingula:** series of siliceous bands associated with one valve only.



**Clavate:** club-shaped.

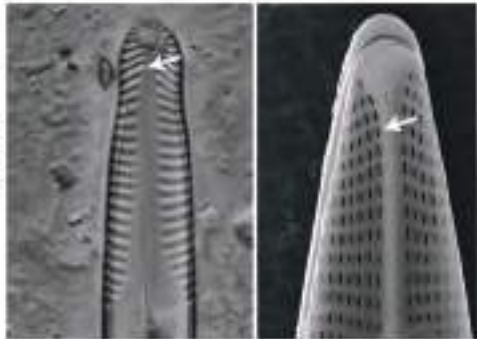
**Collum:** narrow and hyaline area on the valve mantle within the genus *Aulacoseira*. A small furrow, the sulcus or "Ringleiste" divides the collum from the part of the valve mantle bearing the areolae.



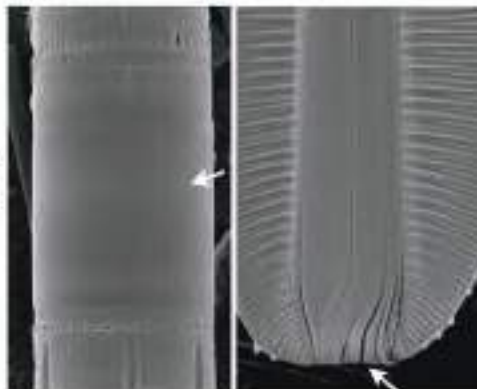
**Conopeum - conopea:** delicate siliceous flap lying along the apical axis and covering a part of the external valve face; can be slightly to distinctly elevated and partly or totally covering the striae and may extend up to the valve margin.



**Convergent:** striae are convergent when they are turned away from the central nodule and oriented to the terminal nodule.

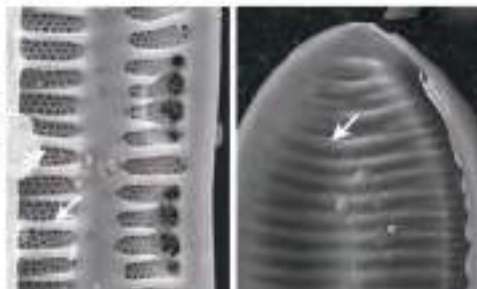


**Copula - copulae:** siliceous band in between both valves; also called intercalary band or girdle band.



**Cosmopolite:** occurring everywhere on earth in corresponding habitats.

**Costa - costae:** rib-like thickenings and non-ornamented part of the valve face parallel to the striae.





**Craticula - craticulae:** auxiliary structure on the internal valve face composed of a stemum and strong solid transverse bars, formed under conditions of higher osmotic pressure.



**Cribrum - cribra:** type of pore occlusion (perforated siliceous plate).

**Cruciform:** shape of a cross.

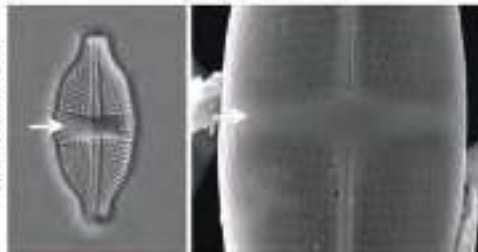
**Dorsal side:** in diatoms asymmetrical to the apical axis, the side of the valve that is the most convex.



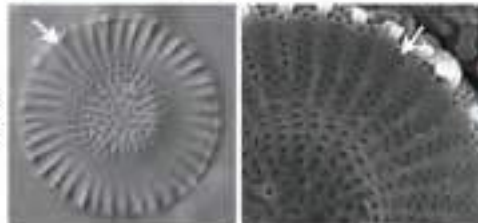
**Dorsiventral:** valve with distinguishable dorsal and ventral sides.

**Epitheca:** the larger and older of the two valves composing the diatom frustule.

**Fascia - fasciae:** thick hyaline siliceous area, extending from the central area to the valve margin, in some pennate diatoms, and formed by secondary deposition of silica in depressions of the valve face.



**Fascicle:** series or bundle of rows of areolae oriented radially in some centric diatoms.



**Fibula - fibulae:** internal siliceous support of the canal that contains the raphe; also called keel puncta or carinal dot in older literature.

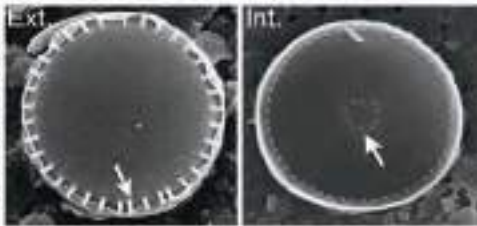


**Foot pole:** the narrower pole, extremity in heteropolar pennate diatoms.

**Foramen - foramina:** type of pore occlusion.

**Frustule:** diatom cell wall composed of silica, and having two valves and associated girdle bands.

**Fultoportula - fultoportulae:** or strutted process; tubular process in some centric diatoms, associated with the secretion of  $\beta$ -chitin. The tubular central process is accompanied by two or more satellite pores in internal valve view; a tube or a simple pore in the valve wall in external valve view.



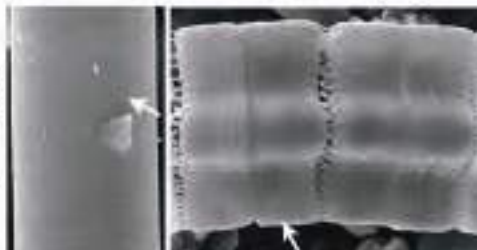
**Fusiform:** the shape of a spindle with the broadest part mid-valve and tapering to both ends.



**Ghost striae:** faint striae, composed of areolae that do not perforate the valve wall.

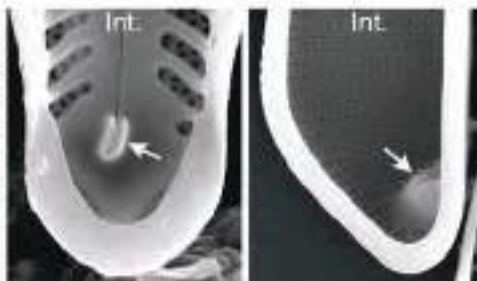
**Girdle:** series of siliceous bands associated with the valve; also called cingulum.

**Girdle band:** one of the bands associated with the valve.

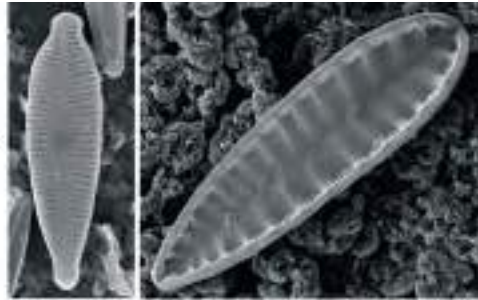


**Head pole:** the largest pole, extremity in heteropolar pennate diatoms.

**Helictoglossa - helictoglossae:** structure with the shape of a pair of lips found at the end of the terminal raphe fissures near the internal ends of the valve in many pennate diatoms bearing a raphe; in the past also called an infundibulum.



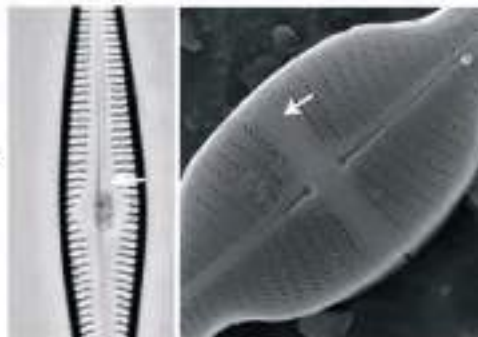
**Heteropolar:** asymmetric valve; having a different shape of poles or apices.



**Heterovalvar:** frustule composed of two different valves; difference can be in the presence or absence of a raphe or in the valve ornamentation.

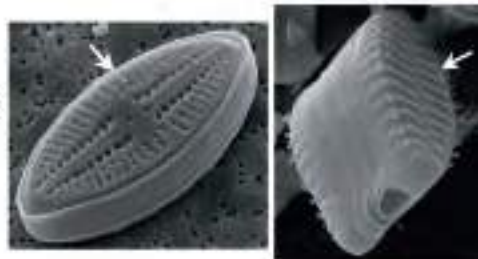


**Hyaline area:** area on the valve without perforations or ornamentations.



**Hymen:** type of pore occlusion.

**Hypotheca:** the smaller and younger valve of the diatom frustule.

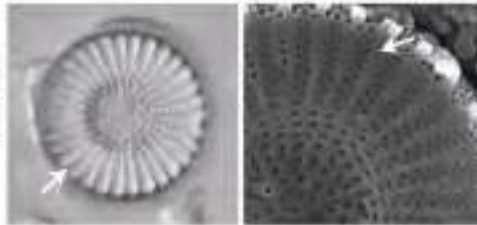


**Infundibulum:** structure with the shape of a pair of lips found at the end of the terminal raphe fissures near the internal ends of the valve in many pennate diatoms bearing a raphe; former name for a helictoglossa.

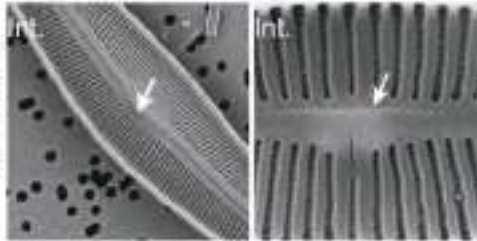


**Intercalary band:** siliceous band in between both valves; also called copula or girdle band.

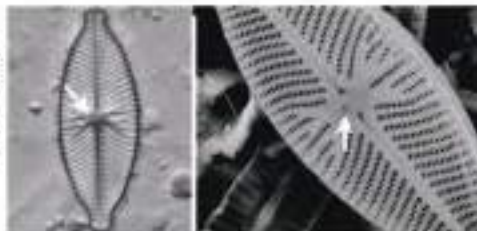
**Interfascicle:** rib-like thickened and unornamented part of the valve face in centric diatoms running parallel to the striae; also called a costa.



**Intermissio:** internal slit which connects the central raphe fissures in some cymbelloid taxa; instead of distinct internal central raphe endings, a fissure is present.



**Isolated punctum:** round opening in the valve wall in the central area clearly separated from the areolae of the striae. Present in genera such as *Geissleria*, *Placconeis*.



**Isopolar:** valve symmetric; both apices having the same shape and size.

**Keel:** elevated ridge bearing the raphe, formed by a folding of the valve wall. Present in genera such as *Nitzschia*, *Surirella*, *Cymatopleura*, *Campylodiscus*.

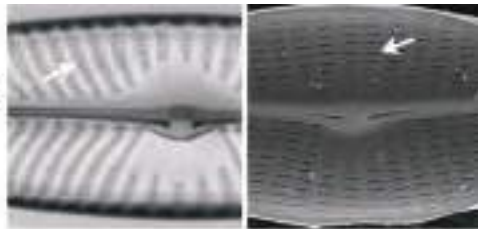


**Keel puncta:** internal siliceous support of the canal that contains the raphe; old name for fibula.

**Ligula - ligulae:** siliceous projection of a girdle band which fills the gap in the next band.

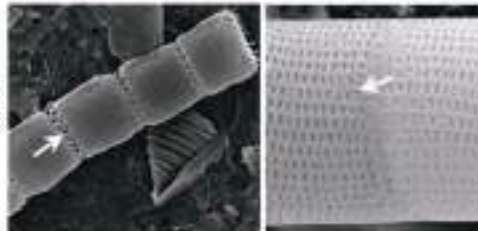


**Lineola - lineolae:** areola elongated in apical direction.



**Lineolate stria:** stria composed of areolae elongated in apical direction.

**Linking spine:** spine, silica extension of the valve, joining frustules together to form a chain.



**Longitudinal canal:** Chamber with the shape of a tube in the internal valve, oriented along the apical axis. Present in the genus *Neidium*.



**Longitudinal rib:** longitudinal silica structure present on the valve face at each side of the raphe and crossing the striae.



**Lunate:** shape of a crescent moon.

**Mantle:** vertical part of the valve, surrounding the valve face at usually 90°.

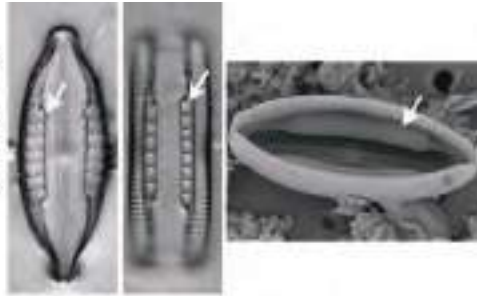


**Monoraphid:** pennate diatom with a raphe on one valve.

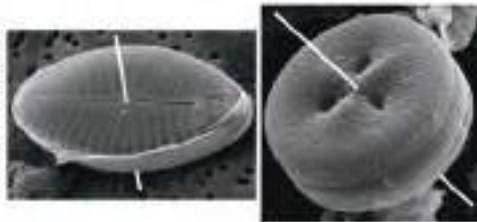
**Ocellus - ocelli:** eye-like structure composed of small pores surrounded by a shallow rim of silica. Present on the junction of the valve face and valve mantle in the genus *Pleurosira*. Secretes mucopolysaccharide pads that join the cells together.



**Partectum - partecta:** bulbous chamber on the inside of the valvocopula, only present in *Mastogloia*. These chambers are usually arranged in a row along each side of the valvocopula, forming together the partectal ring.



**Pervalvar axis:** the axis of the valve which is perpendicular to the center of the valve face; in pennate diatoms it is the point where the apical and the transapical axes meet; in the centric diatoms it is the point where the striae come together.



**Polar nodule:** more silicified part of the diatom valve near the apex, where the raphe furrow ends; also known as the apical nodule.



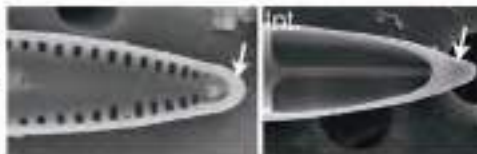
**Porca - porcae:** transapical undulation of the valve face in the genus *Surirella*; also called a corrugation ridge.



**Primary side:** the side of the valve formed from the initial branches of the raphe sternum during valve formation in raphid diatoms.

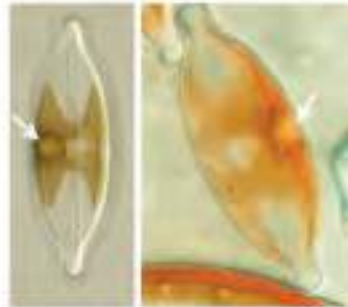


**Pseudoseptum - pseudosepta:** silica plate in the internal cell extending from the wall of the valve.

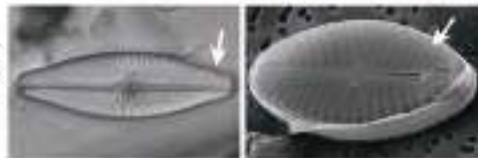


**Punctum:** round or ovate perforation in the silica cell wall, also called an areola.

**Pyrenoid:** structure present in the chloroplast of algae which is responsible for the CO<sub>2</sub> fixation and not for the production of starch as proposed in the past; it is often surrounded with starch granules.



**Radiate:** striae are radiate when they are orientated away from the central nodule.



**Raphe:** slit or fissure through the valve face in the mono- and biraphids, often located along the apical axis.



**Raphe canal:** cylindrical structure bearing the raphe and more or less closed on the internal side of the valve.

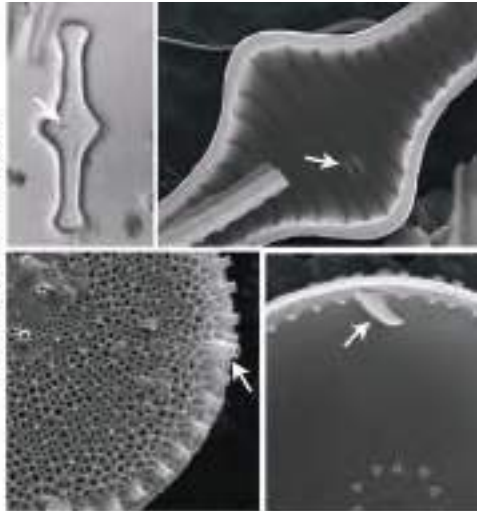


**Raphe keel:** well pronounced elevated ridge, formed by folding of the valve wall, on the junction of the valve face and valve mantle. Present in genera such as *Nitzschia*, *Surirella*, *Cymatopleura* and *Campylodiscus*.





**Rimoportula** or labiate process: tubular process in some centric and pennate diatoms, associated with the secretion of mucopolysaccharides (mucilage) and other carbon compounds. On the internal valve face the opening of the process has the shape of a pair of lips; on the external valve face the opening is a tube extending out from the valve, or a simple pore in the valve wall.



**Ringleiste:** small silica ledge dividing the collum from the part of the valve mantle bearing the areolae. Only present in the genus *Aulacoseira*.



**Secondary side:** the side of the valve formed after the primary side by fusion of the silica branches extending from the centre and the extremities of the raphe sternum during valve formation in the raphid diatoms. Junction where fusion takes place is known as the Voight discordance.



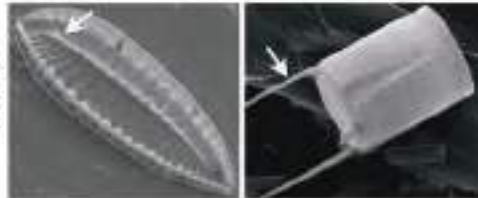
**Septum - septa:** silica plate in the internal cell extending from the wall of a girdle band.



**Seta - setae:** simple or robust silica extension of the valve, longer than a spine. Present in the genus *Chaetoceros*. The setae join the cells together allowing them to form chains.



**Spine:** sharp pointed silica extension of the valve, solid or hollow, very long or tiny, arising at different places on the external valve face in different taxa.



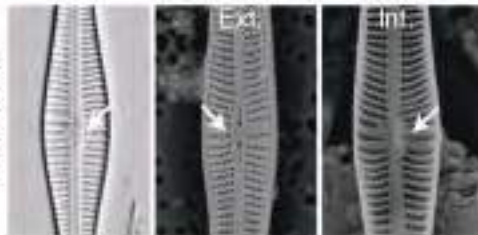
**Stauros:** hyaline thickening in the central area; in the cell ontogeny formed differently from a fascia; present only in the genus *Stauroneis*.



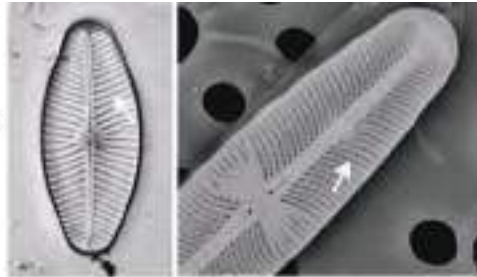
**Sternum - sterna:** thick siliceous structure of the valve face along the apical axis in pennate diatoms; it is the ontogenic center of the pennates. The sternum often contains the raphe, and may be positioned centrally as in *Navicula*, or marginally as in *Eunotia*.



**Stigma - stigmata:** opening in the valve wall in the central area distinct in structure from an areola; externally a round or elongate opening, internally it has the shape of a slit or a more complex structure.

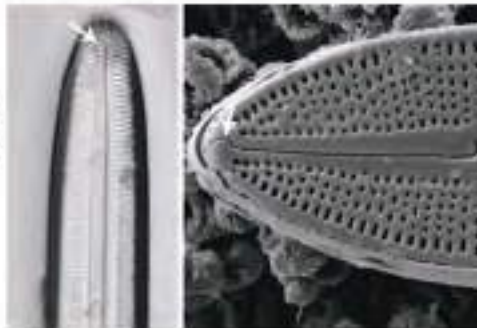


**Stria - striae:** a row of pores, areolae on the valve.



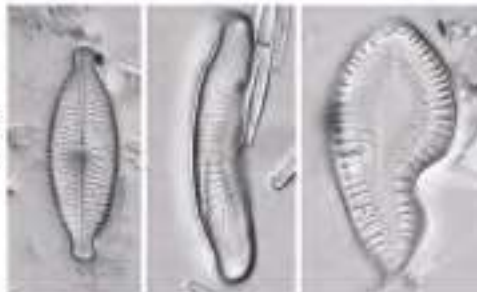
**Stria density:** number of striae present on the valve, expressed as number in 10  $\mu\text{m}$ . In centric diatoms, it is the number of striae in 10  $\mu\text{m}$  measured at the circumference.

**Terminal fissure:** terminal raphe slit ending near the pole/terminal nodule, may be expanded or curved.



**Terminal nodule:** more heavily silicified part of the valve wall near a pole and a terminal raphe slit; the polar or apical nodule.

**Teratologic form:** deformations and abnormalities in the valve ornamentation.



**Theca:** part of the frustule composed of a valve and its corresponding girdle bands.

**Transapical axis:** the short axis of a pennate diatom valve, crossing the middle of the valve face; axis perpendicular to the apical axis.





**Ubiquitous:** occurring everywhere on earth.

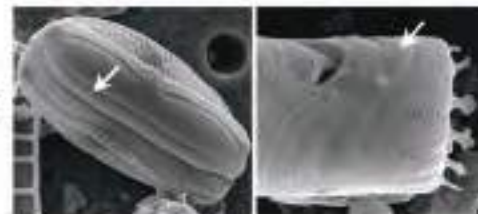
**Uniseriate:** stria composed of a single row of areolae.



**Valve:** part of a frustule, composed of a flat part, the valve face, and an extension, usually at 90°, the valve mantle.

**Valve view:** view of the frustule turned so that the valve face is visible.

**Valvocopula:** the girdle band in contact with the valve; the first girdle band.

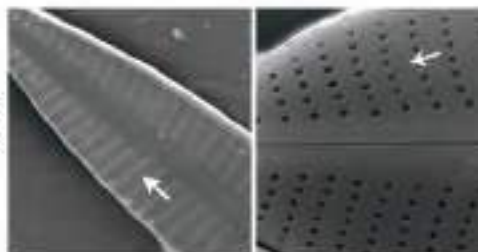


**Velum:** type of pore occlusion.

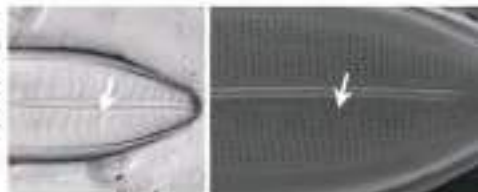
**Ventral side:** in diatoms asymmetrical to the apical axis, the side of the valve that is straight to slightly convex or concave.



**Virga - virgae:** solid siliceous rib between regularly aligned areolae, also called interstria/interstriae.



**Voigt fault or Voigt discordance:** discontinuity in the striae on the secondary side of the valve at the point where the two branches are joined to each other during valve formation.



**Vola - volae:** type of pore occlusion.

## 10.2. Translation of English terms into French

<b>alveolus:</b> alvéole	<b>fusiform:</b> fusiforme
<b>annulae:</b> annulae	<b>ghost striae:</b> stries fantômes
<b>apex:</b> apex	<b>girdle:</b> ceinture
<b>apical axis:</b> axe apical	<b>girdle band:</b> bande de ceinture
<b>apical nodule:</b> nodule apical	<b>head pole:</b> pôle apical
<b>apical pole:</b> pôle apical	<b>helictoglossa:</b> hélictoglosse
<b>apical pore field:</b> champs de pores apicaux.	<b>heteropolar:</b> hétéropolaire
<b>araphid:</b> araphide	<b>heterovalvar:</b> hétérovalvaire
<b>areola:</b> aréole	<b>hyaline area:</b> aire hyaline
<b>axial area:</b> aire axiale	<b>hymen:</b> hymen
<b>axial costa:</b> côte axiale	<b>hypotheca:</b> hypothèque
<b>axial plate:</b> plaque axiale	<b>infundibulum:</b> infundibulum
<b>auxospore:</b> auxospore	<b>intercalary band:</b> bande intercalaire
<b>bifurcate:</b> bifurqué	<b>interfascicle:</b> côte
<b>biraphid:</b> biraphide	<b>intermissio:</b> intermission
<b>biseriate:</b> bisérié	<b>isolated punctum:</b> point isolé
<b>carinoportula:</b> carinoportule	<b>isopolar:</b> isopolaire
<b>central area:</b> aire centrale	<b>keel:</b> carène
<b>central fissure:</b> fissure centrale	<b>keel puncta:</b> fibule
<b>central nodule:</b> nodule central	<b>ligula:</b> ligule
<b>chrysolaminarin:</b> chrysolaminarine	<b>lineola:</b> linéole
<b>cingulum:</b> cingulum	<b>lineolate stria:</b> strie linéolée
<b>clavate:</b> allongé	<b>linking spine:</b> épine de jonction
<b>collum:</b> collet	<b>longitudinal canal:</b> canal longitudinal
<b>conopeum:</b> conopeum	<b>longitudinal rib:</b> côte longitudinale
<b>convergent:</b> convergente	<b>lunate:</b> luniforme
<b>copula:</b> bande intercalaire	<b>mantle:</b> manteau
<b>cosmopolite:</b> cosmopolite	<b>monoraphid:</b> monoraphide
<b>costa:</b> côte	<b>ocellus:</b> ocellus
<b>craticula:</b> craticule	<b>partectum:</b> locule
<b>cribrum:</b> cribrum	<b>pervalvar axis:</b> axe pervalvaire
<b>cruciform:</b> cruciforme	<b>polar nodule:</b> nodule polaire
<b>dorsal side:</b> côté dorsal	<b>porca:</b> porca
<b>epithec:</b> épithèque	<b>primary side:</b> côté primaire
<b>fascia:</b> fascia	<b>pseudoseptum:</b> pseudoseptum
<b>fascicle:</b> fascicule	<b>punctum:</b> point
<b>fibula:</b> fibule	<b>pyrenoid:</b> pyrénioïde
<b>foot pole:</b> apex/pôle podal	<b>radiate:</b> radiaire
<b>foramen:</b> foramen	<b>raphe:</b> raphé
<b>frustule:</b> frustule	<b>raphe canal:</b> canal raphéen
<b>fultoportula:</b> fultoportule	<b>raphe keel:</b> carène du raphé
	<b>rimoportula:</b> rimoportule
	<b>Ringleiste:</b> Ringleiste

**secondary side:** côté secondaire  
**septum:** septum  
**seta:** seta  
**spine:** épine  
**stauros:** stauros  
**sternum:** sternum  
**stigma:** stigma  
**stria:** strie  
**stria density:** densité des stries  
**terminal fissure:** fissure terminale  
**terminal nodule:** nodule terminal  
**teratologic form:** forme tératologique

**theca:** thèque  
**transapical axis:** axe transversal  
**ubiquitous:** ubiquiste  
**uniseriate:** unisériel  
**valve:** valve  
**valvocopula:** valvocopula  
**velum:** vélum  
**ventral side:** côté ventral  
**Voigt fault / Voigt discordance:**  
défaut de Voigt  
**vola:** vola

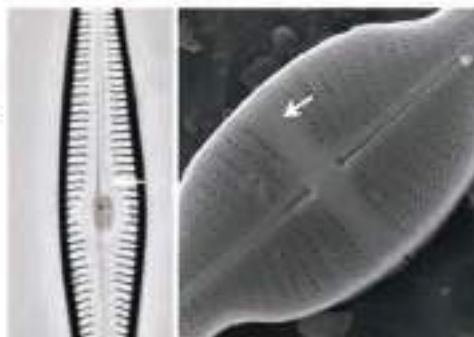
### 10.3. Glossaire

**Adné:** attaché au substrat par la surface de la valve à raphé.

**Aire axiale:** espace hyalin sur la surface valvaire le long de l'axe longitudinal, entre le raphé, si présent, et les stries chez les diatomées pennées.

**Aire centrale:** espace hyalin au centre de la valve à hauteur du nodule central si présent, dépourvue d'aréoles.

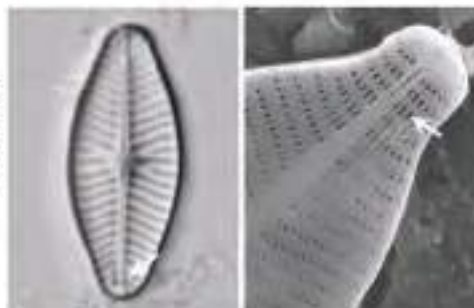
**Aire hyaline:** zone de la valve sans perforation ni ornementation.



**Alvéole:** strie composée d'une chambre transversalement linéaire dans la paroi de la valve avec de petites ouvertures multiples à la face extérieure et de grandes ouvertures à la face intérieure de la valve.



**Annulae:** structure composée d'une à quatre stries transapicales (perpendiculaires) interrompant les stries typiques vers les apices. Structure restreinte au genre *Geissleria*.



**Apex - apices:** chez les diatomées pennées extrémité de la valve, aussi appelée pôle.

**Apex apical:** chez les diatomées pennées hétéropolaires extrémité de la valve la plus large, aussi appelée pôle apical.

**Apex podal:** chez les diatomées pennées hétéropolaires extrémité de la valve la plus fine, aussi appelée pôle podal ou pôle basal.

**Araphide:** diatomée pennée sans fissure raphéenne sur les deux valves.

**Aréole:** ou point, perforation ronde ou presque ronde de la paroi en silice. Les aréoles sont généralement alignées formant une strie.



**Axe apical:** axe longitudinal de la face valvaire des diatomées pennées reliant les apices.

**Axe pervalvaire:** axe de la valve qui est perpendiculaire vers le centre de la surface de la valve; dans les diatomées pennées c'est le point de rencontre entre les axes apical et transversal; dans les diatomées centriques c'est le point de rencontre des stries.

**Axe transversal:** axe de la valve le plus court, passant le centre de la surface de la valve; axe perpendiculaire à l'axe apical.

**Auxospore:** cellule spéciale formée dans la reproduction sexuelle après la fusion des gamètes ; la cellule formée est plus grande que les cellules filles et la taille maximale de la diatomée est rétablie.

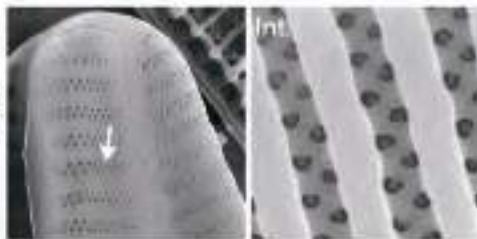


**Bande de ceinture ou bande intercalaire:** une des bandes siliceuses associées à la valve.

**Bifurqué:** structure qui est divisée en deux parties.

**Biraphide:** diatomée pennée qui porte un raphé sur chaque valve.

**Bisérié:** composé de deux parties; les stries bisériées portent deux lignes d'aréoles.



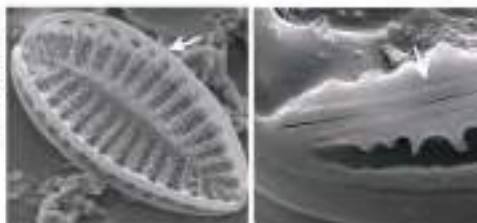
**Canal longitudinal:** chambre en forme de tube dans la valve interne orientée le long de l'axe apical. Présent dans le genre *Neidium*.



**Canal raphéen:** structure cylindrique plus ou moins fermée à l'intérieur de la valve, portant le raphé.



**Carène:** côte élevée qui contient le raphé, formée par un pli de la paroi de la valve. Présent dans des genres comme *Nitzschia*, *Surirella*, *Cymatopleura*, *Campylodiscus*.



**Carène du raphé:** structure ressemblant à une crête très distincte au bord de la valve où passe le raphé, formé par un pli de la paroi de la valve. Présent dans des genres comme *Nitzschia*, *Surirella*, *Cymatopleura*, *Campylodiscus*.

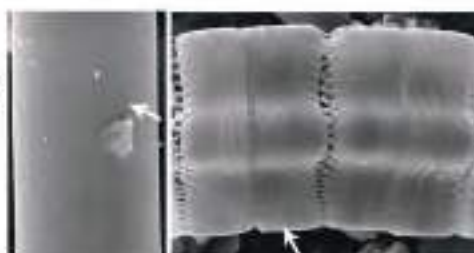




**Carinoportule:** processus central restreint au genre *Orthoseira*; les ouvertures internes sont simples, les ouvertures externes sont composées des cols bien définis.



**Ceinture:** série de bandes associées à la valve; aussi appelée cingulum.

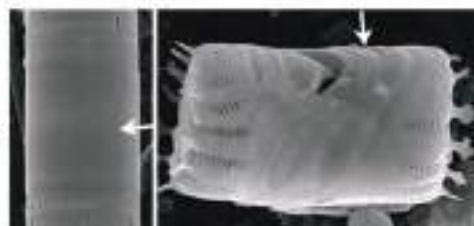


**Champs de pores apicaux:** zone de pores ou perforations très fines à travers la paroi près d'une ou des deux extrémités de la valve des diatomées pennées. C'est la zone où des mucopolysaccharides qui forment des tiges sont.



**Chrysolaminarine:** un polysaccharide de réserve chez les diatomées et les Chrysophytes.

**Cingulum:** série de bandes siliceuses associées à une valve.



**Clavé:** en forme d'une massue.

**Collet:** aire étroite et hyaline du manteau de la valve chez les *Aulacoseira*.

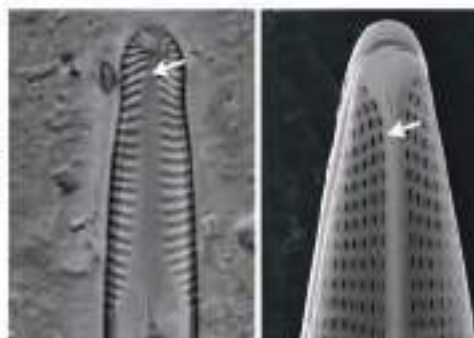




**Conopeum:** fine couverture siliceuse sur la surface externe de la valve le long de l'axe apical; peut être légèrement à distinctement élevé et couvrir partiellement ou totalement les stries et être étendu jusqu'au bord de la valve.

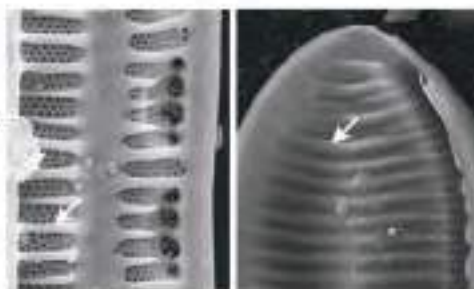


**Convergente:** les stries sont convergentes quand elles sont détournées du nodule central et orientées vers le nodule terminal.



**Cosmopolite:** présent partout au monde dans les mêmes habitats.

**Côte:** partie de la valve plus épaisse et non ornementée parallèle aux stries.



**Côte axiale:** bord siliceux étroit le long de l'axe axiale, parallèle au et encadrant le raphé.



**Côte longitudinale:** structure siliceuse longitudinale sur la surface de la valve à de chaque côté du raphé en croissant les stries.



**Côté dorsal:** dans les diatomées asymétriques en vue de l'axe apical, le côté de la valve le plus convexe.



**Côté primaire:** côté de la valve formé par les branches initiales du sternum raphéen dans l'ontogénie des diatomées à raphé.



**Côté secondaire:** côté formé après le côté primaire par la fusion des branches de silice allongeant du centre et des extrémités du sternum raphéen dans l'ontogénie des diatomées à raphé.



**Côté ventral:** dans les diatomées asymétriques en vue de l'axe apical, le côté de la valve qui est droit, faiblement convexe ou concave.



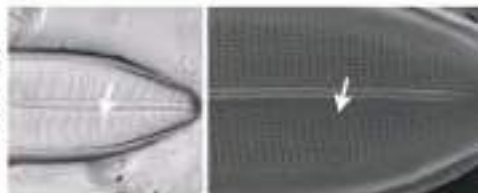
**Craticule:** structure en surplus de la surface interne d'une valve composée d'un sternum et des barres transversales solides, formée sous des conditions de haute pression osmotique.



**Cribum:** type de couverture d'un pore.

**Cruciforme:** en forme de croix

**Défait de Voigt:** discontinuité dans les stries dans le côté secondaire à l'endroit où les deux branches se fusionnent lors de l'ontogénie des diatomées à raphé.

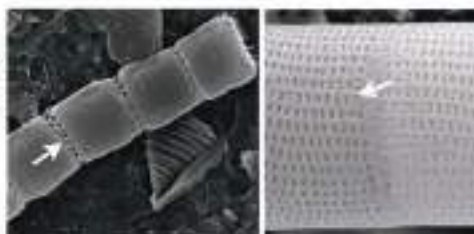


**Densité des stries:** nombre de stries sur la valve, exprimé en nombre par 10  $\mu\text{m}$ . Pour les diatomées centriques le nombre de stries sur 10  $\mu\text{m}$  de la circonférence.

**Épine:** prolongement aigu siliceux de la valve, massif ou creux, très long ou minuscule, qui apparaît dans les différents taxons à différents endroits sur la surface externe de la valve.

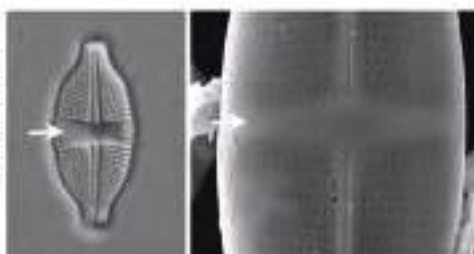


**Épine de jonction:** épine, prolongement aigu siliceux de la valve, qui réunit une des frustules en une chaîne.

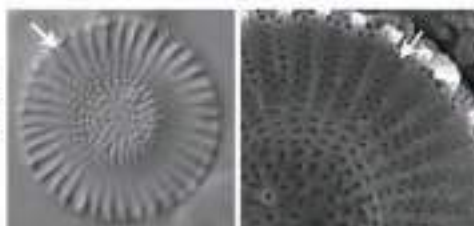


**Épithèque:** valve la plus large et la plus vieille des deux valves d'une frustule de diatomée.

**Fascia:** aire hyaline épaisse siliceuse, étendue de l'aire centrale vers les bords de la valve chez quelques diatomées pennées, formée par dépôt secondaire de silice dans des dépressions dans la surface de la valve.



**Fascicule:** série ou groupe d'aréoles orientées radialement chez certaines diatomées centriques.



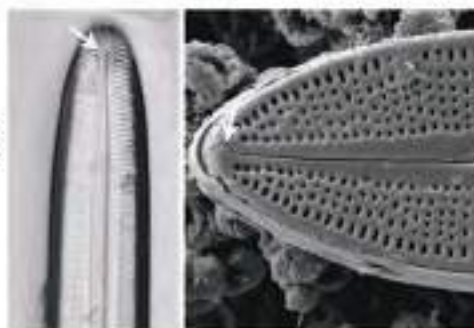
**Fibule:** support siliceux interne du canal qui contient le raphé.



**Fissure centrale:** extrémité de la fente raphéenne près du nodule central; peut être élargie ou courbée.



**Fissure terminale:** extrémité de la fente raphéenne près du nodule terminal; peut être élargie ou courbée.



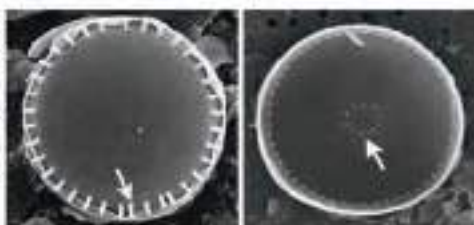
**Foramen:** type de couverture d'un pore.

**Forme tératologique:** déformations et anomalies dans les ornements de la valve.



**Frustule:** cellule d'une diatomée composée de silice, et de deux valves et les bandes connectives associées.

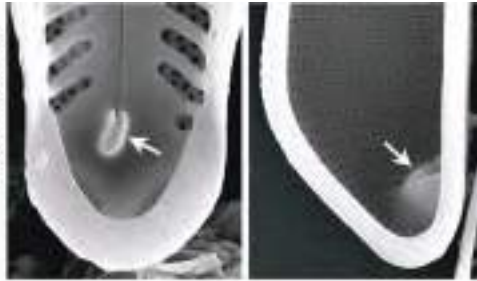
**Fultoportule:** processus tubulaire chez certaines diatomées centriques, associé à la sécrétion de  $\beta$ -chitine. En vue intérieure de la valve le processus central est entouré de deux ou plusieurs pores satellites; en vue extérieure un tube ou un pore simple dans la paroi de la valve.



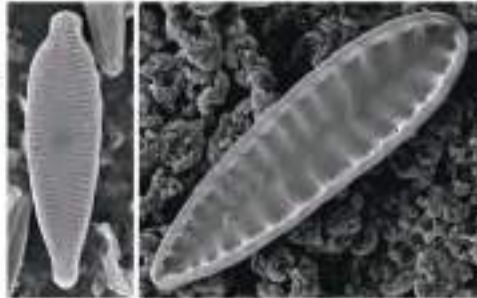
**Fusiform:** comme un fuseau, avec la partie plus large au centre et devenant plus étroite vers les extrémités.



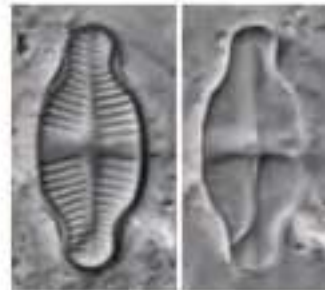
**Hélictoglosse:** structure en forme de lèvres à l'extrémité de la fissure terminale du raphé vers les extrémités internes de la valve dans beaucoup de diatomées pennées à raphé; autrefois désigné comme infundibulum.



**Hétéropolaire:** valve asymétrique; présentant une différence dans la forme des pôles ou de l'axe apical.



**Hétérovalvaire:** frustule composée de deux valves différentes; la différence peut être dans la présence ou l'absence d'un raphé ou dans l'ornementation de la surface des valves.



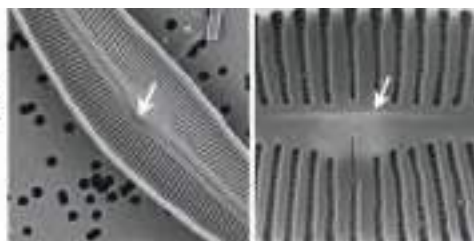
**Hymen:** type de couverture d'un pore.

**Hypothèque:** Valve la plus petite et la plus jeune d'une frustule.



**Infundibulum:** structure en forme de lèvres à l'extrémité de la fissure terminale du raphé vers les extrémités internes de la valve dans beaucoup de diatomées pennées à raphé : ancien nom pour hélictoglosse.

**Intermission:** fissure interne qui lie les fissures centrales chez quelques taxons cymbelloïdes.



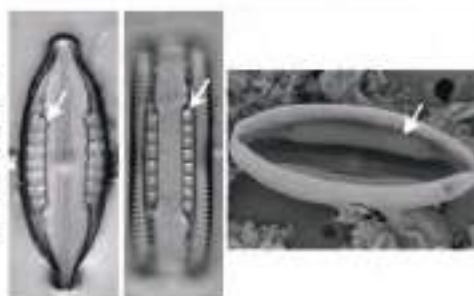
**Isopolaire:** valve qui a une forme identique de chaque côté de l'axe transapical; les deux extrémités ont la même forme et taille.



**Ligule:** expansion siliceuse d'une bande connective qui remplit le sillon, causée par une faille dans la bande, de la bande connective suivante.



**Linéole:** aréole allongée en direction apicale.



**Locule ou partectum:** chambre globulaire à l'intérieur de la valvocopula, présente uniquement dans le genre Mastogloia. Les chambres sont rangées sur une ligne le long de chaque côté de la valvocopula en formant un anneau partectal.

**Luniforme:** en forme de lune, de croissant.

**Manteau:** hauteur d'une valve, partie dressée de valve qui entoure la face de la valve.



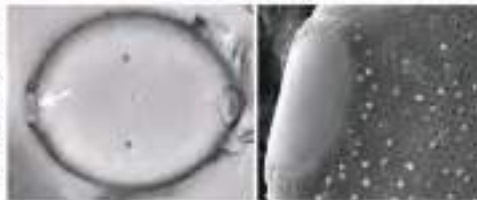
**Monoraphide:** diatomée pennée qui porte un raphé sur une des deux valves.

**Nodule apical, polaire ou terminal:** partie de la valve plus épaisse, située près d'un pôle où la fente raphéenne se termine.

**Nodule central:** partie de la valve plus épaisse entre les fissures centrales du raphé.



**Ocellus:** structure en forme d'œil, composée de petits pores entourés d'une côte siliceuse peu profonde. Présente à la transition de la surface de la valve et le manteau dans le genre *Pleurosira*. Responsable de la sécrétion de polysaccharides qui unissent les cellules.



**Plaque axiale:** plaque siliceuse qui se trouve dans la partie interne d'une valve et qui couvre les ouvertures internes des aréoles. La plaque existe chez quelques représentants du genre *Gomphoneis* où le bord est visible au microscope optique comme une ligne longitudinale.



**Point:** ou aréole, perforation ronde ou ovale de la paroi en silice.



**Point isolé:** perforation ronde de la paroi en silice à hauteur de l'aire centrale, nettement séparé des aréoles des stries.





**Pôle:** chez les diatomées pennées, extrémité de la valve, aussi appelée apex.

**Pôle apical:** chez les diatomées pennées hétéropolaires, extrémité de la valve la plus large.

**Pôle basal ou pôle podal:** chez les diatomées pennées hétéropolaires, extrémité de la valve la plus fine, aussi appelée apex podal.

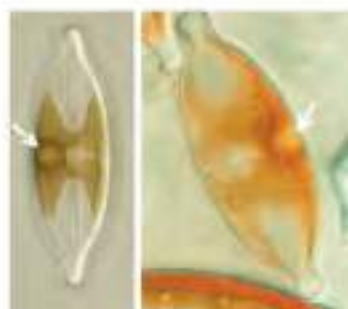
**Porca:** ondulation transapicale de la surface de la valve dans le genre *Sunrella*.



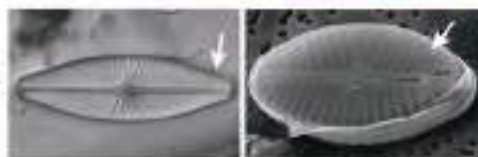
**Pseudoseptum:** plaque de silice avancée à l'intérieur de la cellule étendue de la valve.



**Pyrénoïde:** structure chez les algues dans le chloroplaste qui est responsable de la fixation de CO<sub>2</sub>, et pas de la production de l'amidon comme supposé autrefois; elle est souvent enveloppée des grains ou d'une gaine d'amidon.



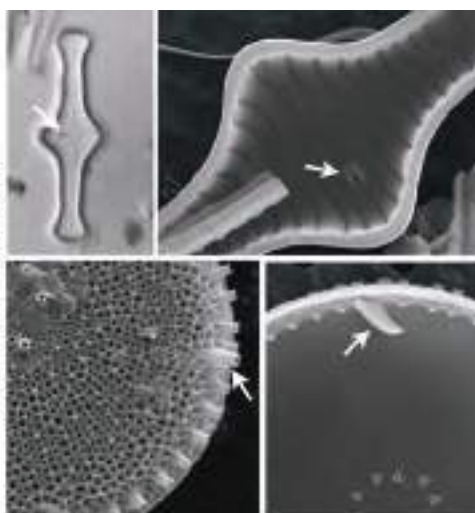
**Radiaire:** les stries sont radiaires quand elles sont rayonnantes à partir du nodule central.



**Raphé:** fente dans la surface de la valve chez les mono- et biraphides, souvent localisée le long de l'axe apical.



**Rimoportule ou processus labié:** processus tubulaire de quelques diatomées centriques et pennées, associé à la sécrétion de polysaccharides et d'autres substances contenant du carbone. En vue intérieure de la valve le processus se voit comme une ouverture en forme de lèvres; en vue extérieure un tube



**Ringleiste:** petit rebord qui sépare le collet de la partie du manteau à aréoles chez le genre *Aulacoseira*.



**Septum:** plaque de silice avancée à l'intérieur de la cellule étendue d'une bande connective.



**Seta:** prolongement simple ou robuste de la valve, plus allongé qu'une épine. Présent dans le genre *Chaetoceros*. Les setae connectent les cellules pour former des chaînes.



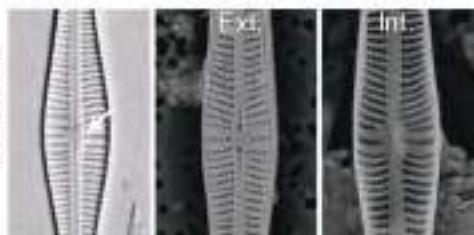
**Stauros:** partie hyaline épaisse présente dans l'aire centrale, formée différemment d'un fascia dans l'ontogénie de la cellule. Uniquement présent dans le genre *Stauroneis*.



**Sternum:** structure épaisse siliceuse allongée le long de l'axe apical chez les diatomées pennées; c'est le centre ontogénique des pennées. Le sternum contient souvent le raphé, et peut se trouver au centre, comme chez *Navicula*, ou marginal comme chez *Eunotia*.



**Stigma:** perforation de la paroi en silice à hauteur de l'aire centrale, différente d'une aréole; ouverture ronde à l'extérieur et une fente à l'intérieur, ou structure très complexe.



**Strie:** rangée de pores, d'aréoles sur la valve.



**Stries fantômes:** stries floues, composées d'aréoles ne perforant pas la paroi de la valve.

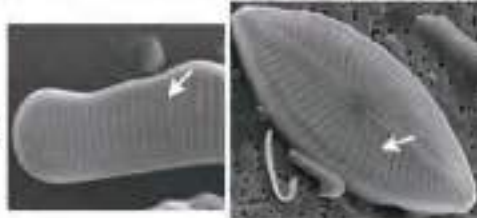


**Strie lineolée:** strie composée de linéoles (aréoles allongées en direction apicale)

**Thèque:** partie d'une frustule composée de la valve et des bandes intercalaires associées à cette valve.

**Ubiquiste:** présent partout dans le monde.

**Unisérie:** les stries unisériées portent une seule ligne d'aréoles



**Valve:** partie d'une frustule, composée d'une partie aplatie, la surface de la valve, et d'une partie dressée, le manteau.

**Valvocopula:** bande connective en contact avec la valve; c'est la première bande de la ceinture.



**Vélum:** type de couverture d'un pore.

**Vue valvaire:** vue de la frustule quand la face de la valve est visible.

**Vola:** type de couverture d'un pore.

## 11. Classification of the diatoms

In the present work ninety-one diatom genera are illustrated, covering most of the genera which can be observed in tropical Africa. The classification used is after Round *et al.* (1990) with some modifications when it concerns genera described after 1990. This classification is more complex than the before used “centrics” and “pennates”. We will cite here only the genera discussed in the taxonomic part of the present book.

The divisions given are: class (-phyceae), subclass (-phycidae), order (-ales), family (-aceae) and genus. Note that the names are written in italics from genus level on. Taxa lower than genus most often used in diatom taxonomy are: species, variety and forma.

### Division Bacillariophyta

Class: Coscinodiscophyceae Round & R.M. Crawford

Subclass: Thalassiosirophycidae Round & R.M. Crawford

Order: Thassiosirales Glezer & Makarova

Family: Thalassiosiraceae Lebour

Genus: *Thalassiosira* Cleve

Family: Stephanodiscaceae Glezer & Makarova

Genus: *Cycostephanos* Round

*Cyclotella* Kützinger ex Brébisson

*Discostella* Houk & Klee

*Pantocsekiella* Kiss & Ács

*Stephanodiscus* Ehrenberg

Subclass: Coscinodiscohycidae Round & R.M. Crawford

Order: Melosirales R.M. Crawford

Family: Melosiraceae Kützinger

Genus: *Melosira* C. Agardh

Order: Aulacoseirales R. M. Crawford

Family: Aulacoseiraceae R.M. Crawford

Genus: *Aulacoseira* Thwaites

Order: Orthoseirales R. M. Crawford

Family: Orthoseiraceae R.M. Crawford

Genus: *Orthoseira* Thwaites

Subclass: Biddulphiophycidae Round & R.M. Crawford

Order: Triceratiales Round & R.M. Crawford

Family: Triceratiaceae (Schütt) Lemmermann

Genus: *Pleurosira* (Meneghini) Trevisan  
 Subclass: Rhizosoleniophycidae Round & R.M. Crawford  
 Order: Rhizosoleniales Silva  
 Family: Rhizosoleniaceae De Toni  
 Genus: *Urosolenia* Round & R.M. Crawford

Class: Fragilariophyceae Round  
 Subclass: Fragilariophycidae Round  
 Order: Fragilariales Silva  
 Family: Fragilariaceae Greville  
 Genus: *Asterionella* Hassall  
*Ctenophora* Grunow ex D.M. Williams & Round  
*Diatoma* Bory  
*Fragilaria* Lyngbye  
*Fragilariforma* D. M. Williams & Round  
*Meridion* C. Agardh  
*Pseudostaurosira* D.M. Williams & Round  
*Staurosira* Ehrenberg  
*Staurosirella* D.M. Williams & Round  
*Tabularia* Kützing ex D.M. Williams & Round  
*Ulnaria* (Kützing) Compère

Order: Tabellariales Round  
 Family: Tabellariaceae Kützing  
 Genus: *Tabellaria* (Ehrenberg) Kützing

Class: Bacillariophyceae Haeckel  
 Subclass: Eunotiophycidae D.G. Mann  
 Order: Eunotiales Silva  
 Family: Eunotiaceae Kützing  
 Genus: *Actinella* F.W. Lewis  
*Actinellopsis* J.C. Taylor, B. Karthick & Kociolek  
*Desmogonium* Ehrenberg  
*Eunotia* Ehrenberg



Subclass: Bacillariophycidae D.G. Mann

Order: Mastogloiales D.G. Mann

Family: Mastogloiaceae Mereschkowsky

Genus: *Mastogloia* Thwaites ex W. Smith

Order: Cymbellales D.G. Mann

Family: Rhoicospheniaceae Chen & Zhu

Genus: *Rhoicosphenia* Grunow

Family: Anomoeoneidaceae D.G. Mann

Genus: *Anomoeoneis* Pfitzer

Family: Cymbellaceae Greville

Genus: *Afrocybella* Krammer

*Cymbella* C. Agardh

*Cymbopleura* (Krammer) Krammer

*Encyonema* Kützing

*Encyonopsis* Krammer

*Placoneis* Mereschkowsky

Family: Gomphonemataceae Kützing

Genus: *Gomphonema* Ehrenberg

*Gomphosphenia* Lange-Bertalot

Order: Achnanthales Silva

Family: Achnanthaceae Kützing

Genus: *Achnanthes* Bory

*Lemnicola* Round and Basson

*Psammothidium* Bukhtiyarova & Round

Family: Cocconeidaceae Kützing

Genus: *Anorthoneis* Grunow

*Cocconeis* Ehrenberg

Family: Achnanthidiaceae D.G. Mann

Genus: *Achnanthidium* Kützing

*Planothidium* Round & Bukhtiyarova

Order: Naviculales Bessey

Family: Cavinulaceae D.G. Mann

Genus: *Cavinula* D.G. Mann & Stickle

Family: Diadesmidaceae D.G. Mann

Genus: *Diadesmis* Kützing

*Humidophila* R.L. Lowe, Kociolek, J.R.  
Johansen, Van de Vijver, Lange-

Bertalot & Kopalová  
*Luticola* D.G. Mann  
 Family: Amphipleuraceae Grunow  
 Genus: *Amphipleura* Kützing  
*Frustulia* Rabenhorst  
 Family: Brachysiraceae D.G. Mann  
 Genus: *Brachysira* Kützing  
 Family: Neidiaceae Mereschowsky  
 Genus: *Neidium* Pfitzer  
 Family: Sellaphoraceae Mereschowsky  
 Genus: *Fallacia* Stickle  
*Pseudofallacia* Y. Liu, Kociolek & Q.X.  
 Wang  
*Sellaphora* Mereschowsky  
 Family: Pinnulariaceae D. G. Mann  
 Genus: *Caloneis* Cleve  
*Pinnularia* Ehrenberg  
 Family: Diploneidaceae D.G. Mann  
 Genus: *Diploneis* (Ehrenberg) Cleve  
 Family: Naviculaceae Kützing  
 Genus: *Adlafia* Gerd Moser, Lange-Bertalot  
 & Metzeltin  
*Capartogramma* Kufferath  
*Eolimna* Lange-Bertalot & W. Schiller  
*Fistulifera* Lange-Bertalot  
*Geissleria* Lange-Bertalot & Metzeltin  
*Hippodonta* Lange-Bertalot, Metzeltin &  
 Witkowski  
*Kobayasiella* Lange-Bertalot  
*Mayamaea* Lange-Bertalot  
*Navicula* Bory  
*Nupela* Vyverman & Compère  
*Seminavis* D.G. Mann  
 Family: Pleurosigmataceae Mereschowsky  
 Genus: *Gyrosigma* Hassall  
*Pleurosigma* W. Smith

- Family: Stauroneidaceae D.G. Mann
  - Genus: *Craticula* Grunow
  - Stauroneis* Ehrenberg
- Family: incertae sedis
  - Genus: *Envekadea* Van de Vijver, Gligora, Hinz, Kralj & Cocquyt
- Order: Thalassiophysales D.G. Mann
  - Family: Catenulaceae Mereschowsky
    - Genus: *Amphora* Ehrenberg ex Kützing
    - Halamphora* (Cleve) Levkov
- Order: Bacillariales Hendey
  - Family: Bacillariaceae Ehrenberg
    - Genus: *Bacillaria* J. F. Gmelin
    - Denticula* Kützing
    - Gomphonitzschia* Grunow
    - Hantzschia* Grunow
    - Nitzschia* Hassall
    - Simonsenia* Lange-Bertalot
    - Tryblionella* W. Smith
- Order: Rhopalodiales D. G. Mann
  - Family: Rhopalodiaceae (Karsten) Topachevs'kyj & Oksiyuk
    - Genus: *Epithemia* Kützing
    - Rhopalodia* O. Müller
- Order: Surirellales D. G. Mann
  - Family: Entomoneidaceae D.G. Mann
    - Genus: *Crucicostulifera* J.C. Taylor & Lange-Bertalot
  - Family: Surirellaceae Kützing
    - Genus: *Campylodiscus* Ehrenberg ex Kützing
    - Cymatopleura* W. Smith
    - Stenopterobia* (Brébisson) Van Heurck
    - Surirella* Turpin

## 12. Diatom genera

## ***Thalassiosira* Cleve 1873**

Type species: *Thalassiosira nordenskiöldii* Cleve

SYNONYM:

*Coscinodiscus* Ehrenberg 1839 pro parte

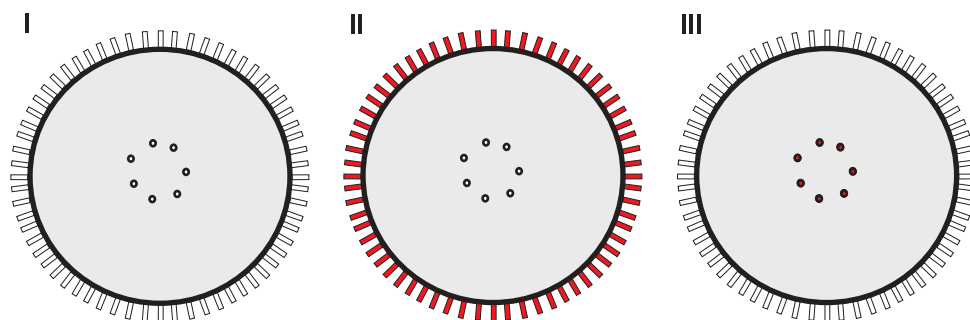
**Characteristics** – Cells **centric**, striae radiate, not arranged in **fascicles**, areolae may be difficult to discern under LM. A row of prominent **ful topoportulae** (strutted processes) present at junction of valve face and mantle (II, Fig. 18: F) which at first glance may resemble spines. Valve face **ful topoportulae** (strutted processes) present and usually arranged in a ring in the centre of the valve face (III, Fig. 18: D, G, H). One **rimoportula** present (Fig. 18: I).

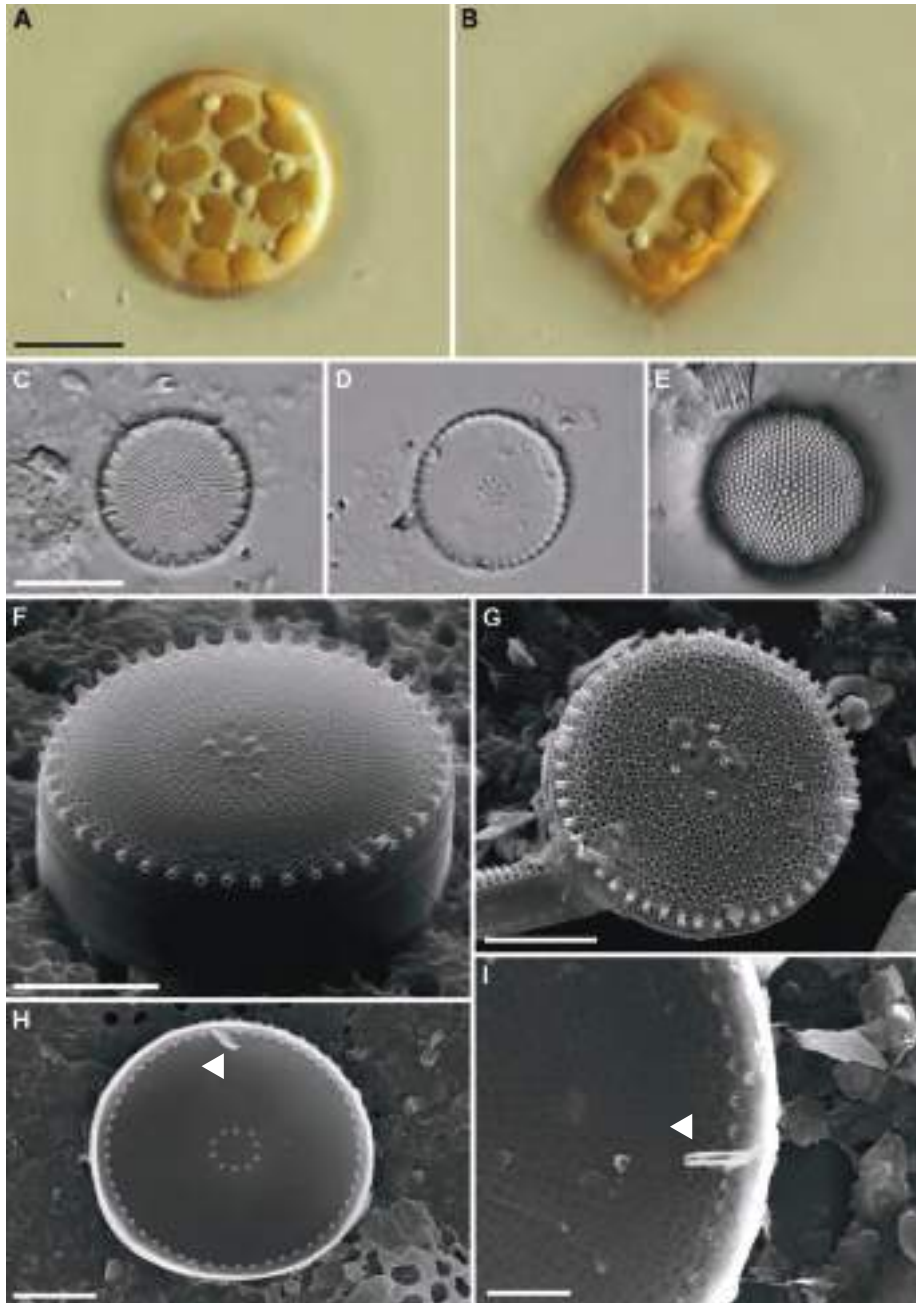
**Plastid structure** – Cells with small discoid plastids (Fig. 18: A-B) and a number of scattered lipid bodies (Fig. 18: A-B).

**Identification of species** – Cell diameter, size and number of the areolae, placement and structure of the marginal and valve face **ful topoportulae**.

Note: Many important cell characteristics can only be observed using SEM.

**Ecology** – Cells planktonic may become entrained in the benthos. Found in waters with medium to high conductivity and higher trophic levels.





**Fig. 18.** *Thalassiosira* spp. **A-E.** LM. **A-B.** Living cells of *T. weissflogii*. **A.** Valve view. **B.** Girdle view. **F-I.** SEM, *T. weissflogii*. **F-G.** External view of valve, note central valve face fultoportulae and marginal ring of marginal fultoportula. **H-I.**

Internal view of valve, note internal opening of valve face and marginal fultoportula and one rimoportula (arrow).

Scale bars = 10 µm (A-E), 5 µm (F-H), 1 µm (I).

## ***Cyclostephanos* Round 1988**

Type species: *Cyclostephanos novaezeelandiae* (Cleve) Round

SYNONYM:

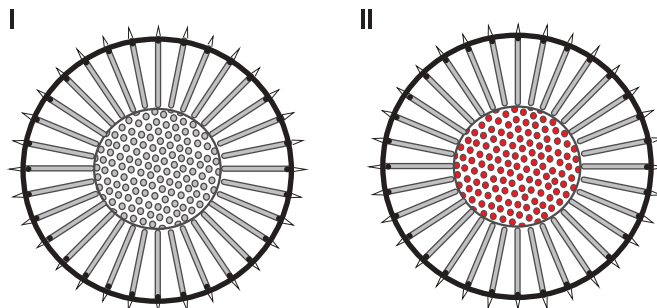
*Stephanodiscus* Ehrenberg 1845 pro parte

**Characteristics** – Cells **centric** with radiate striae. Striae near the valve margins arranged in bundles (**fascicles**) separated by **interfascicular costae** which extend from the valve margin approximately half way across the valve face where they fuse together. Central area composed of irregularly spaced areolae (II). Spines present at junction of valve face and valve margin at the end of each **costa**. Valve face **fultoportulae** (strutted processes) present on valve face and below the spines on the valve margin. Several **rimoportulae** present near the spines.

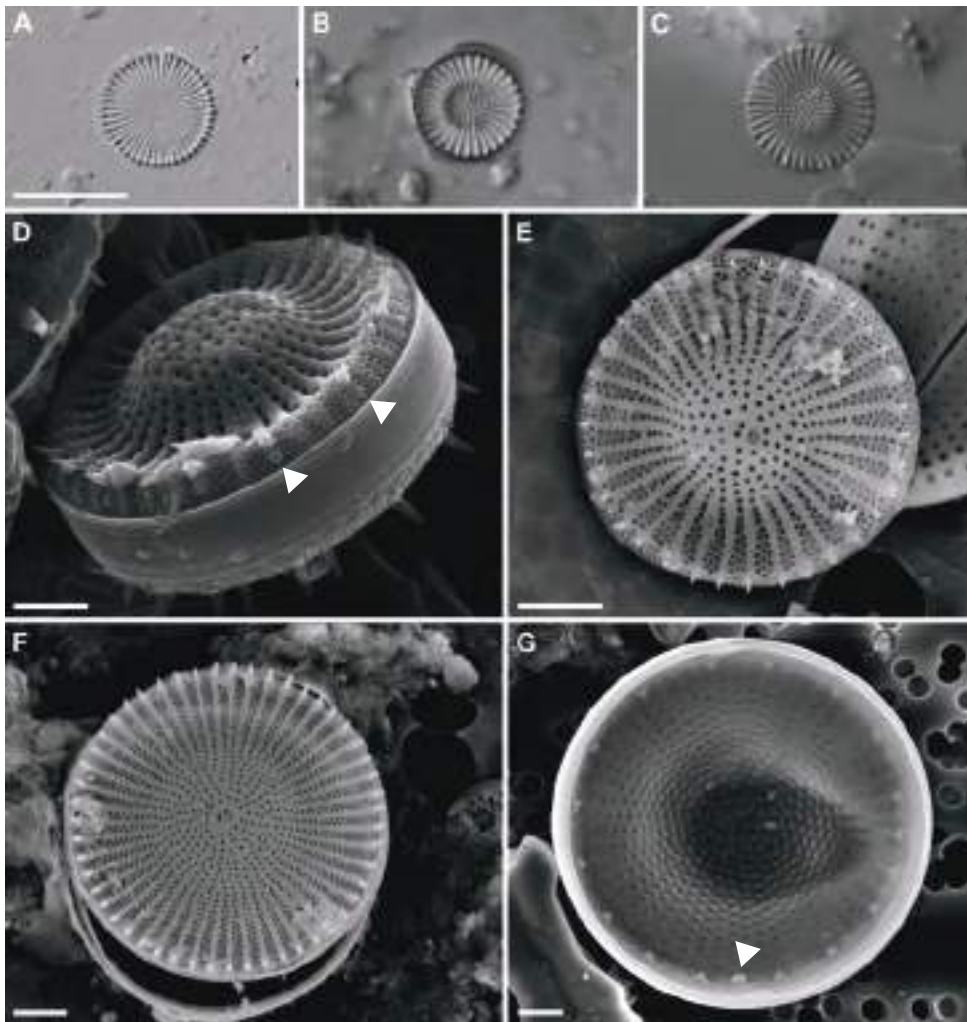
**Plastid structure** – Cells with small discoid plastids.

**Identification of species** – Cell diameter, number of striae, **fascicles** and **costae** as well as structure of costae. Note: Many important cell characteristics, such as the branching of the striae on the valve mantle, can only be observed using SEM.

**Ecology** – Cells solitary not forming chains, planktonic may become entrained in the benthos. Found in waters with medium conductivity and ranging from oligotrophic to eutrophic conditions.







**Fig. 19.** *Cyclostephanos* spp. **A-C.** LM. **A-C.** Valve views. **D-G.** SEM. **D.** Oblique view showing a ring of spines at the junction of the valve face and mantle, and some fulcrapertures on the mantle (arrows). **E-F.** External view of valve. **G.** Internal view of valve showing the internal openings of the valve and marginal fulcrapertures and the rimopertula (arrow).  
Scale bars = 10  $\mu\text{m}$  (A-C), 2  $\mu\text{m}$  (D-E), 1  $\mu\text{m}$  (F-G).

## ***Cyclotella* Kützing ex Brébisson 1838**

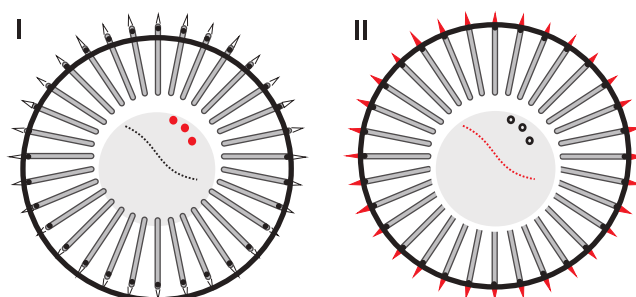
*Cyclotella tecta* Håkansson & R. Ross

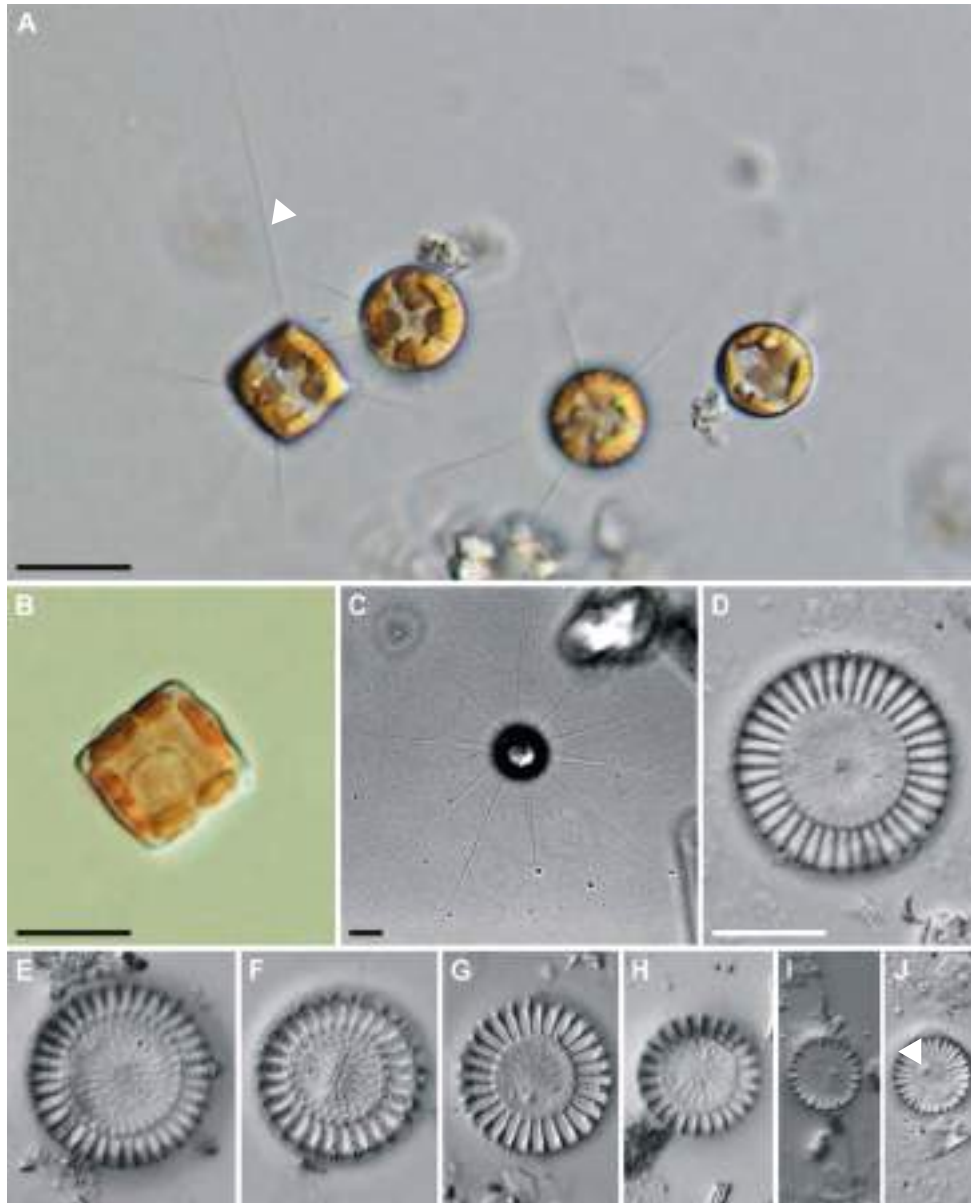
**Characteristics** – Cells **centric** with radiate striae. Striae separated by robust **interfascicular costae** which extend from approximately half way across the valve face to the valve margin, leaving an open central area which may undulate slightly (II). Spines can be present at junction of valve face and valve mantle at the end of each **costa** (II). Valve face **fultoportulae** (strutted processes) are often present on valve face towards the center (I, Fig. 21: G, H) and below the spines on the valve margin. One **rimoportula** present on the valve mantle.

**Plastid structure** – Cells with small discoid plastids, scattered lipid bodies (Fig. 20: A).

**Identification of species** – Cell diameter, number of striae and costae as well as structure of the costae. Presence or absence of valve face **fultoportulae**, number and distribution of marginal **fultoportulae**. Presence or absence of an undulation in the central area. Presence or absence of spines. Note: Many important cell characteristics can only be observed using SEM.

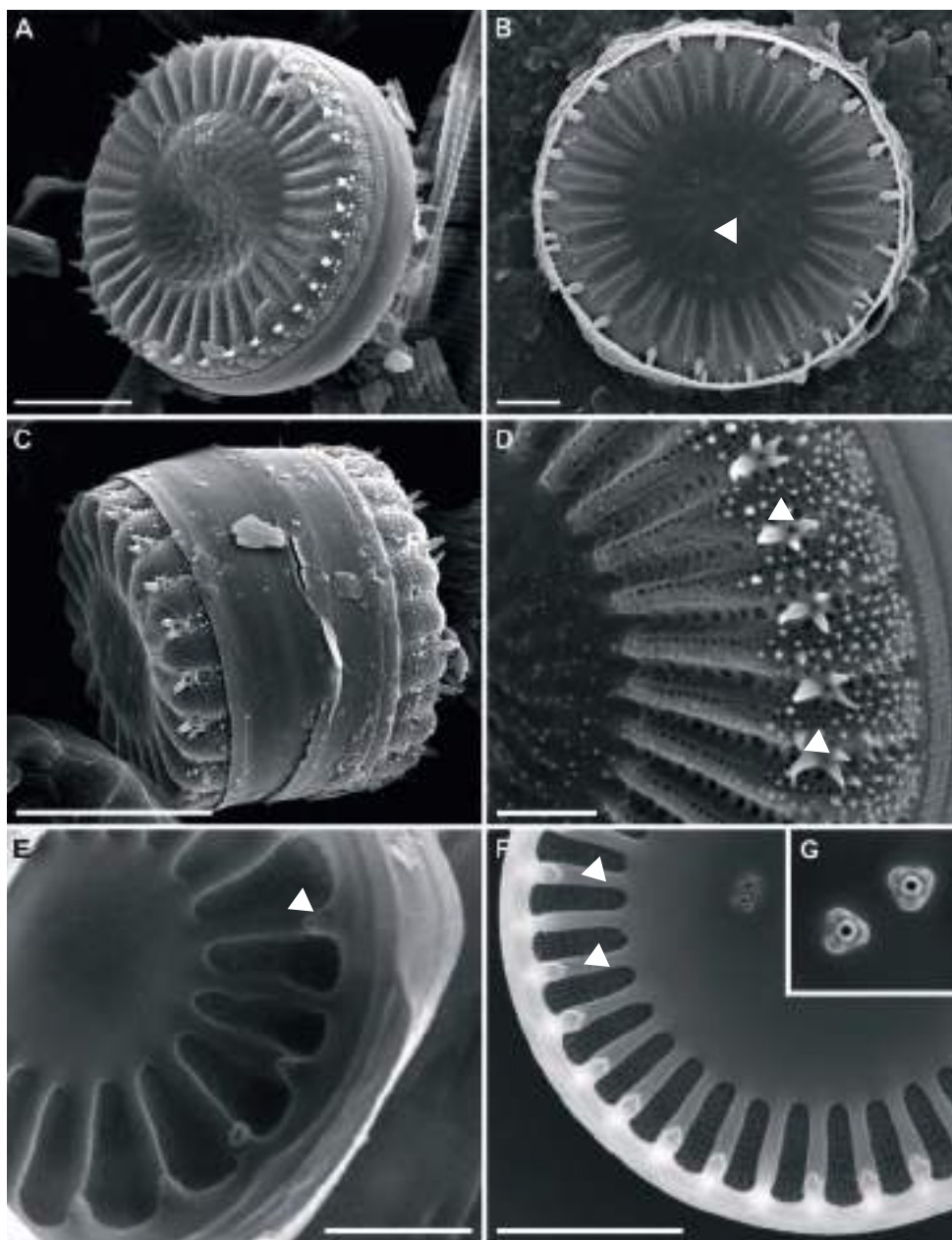
**Ecology** – Cells, solitary or in pairs but not forming chains, planktonic may become entrained in the benthos. Cells exude chitin threads (Fig. 20: A) from **fultoportulae** allowing them to remain suspended for a longer time in the water column. Found in waters with medium to high conductivity and higher trophic levels.





**Fig. 20.** *Cyclotella* spp. **A-J.** LM. **A.** Living cells, valve and girdle views of *Cyclotella meneghiniana* Kützinger, note chitin threads (arrow). **B.** Living cell, girdle view. **C.** Cleaned cell, showing the chitin threads. **D-H.** *C. meneghiniana*, valve views of cleaned cells. **I-J.** *C. atomus* Hustedt, valve views, note rimoportula (arrow).

Scale bars = 10 µm.



**Fig. 21.** *Cyclotella* spp. A-F. SEM. **A-D.** *C. meneghiniana*. **A.** Oblique view, note central undulation. **B.** Valve view, note external opening of rimoportula (arrow). **C.** Girdle view. **D.** Detail of the mantle, note the marginal fuloportulae (arrows). **E-G.** Internal view of valve, note internal opening of valve face and marginal fuloportulae (arrows). **G.** Detail of valve face fuloportulae with 3 satellite pores. Scale bars = 5  $\mu$ m (A-F).

## ***Discostella*** Houk & Klee 2004

Type species: *Discostella stelligera* (Cleve & Grunow) Houk & Klee

SYNONYM:

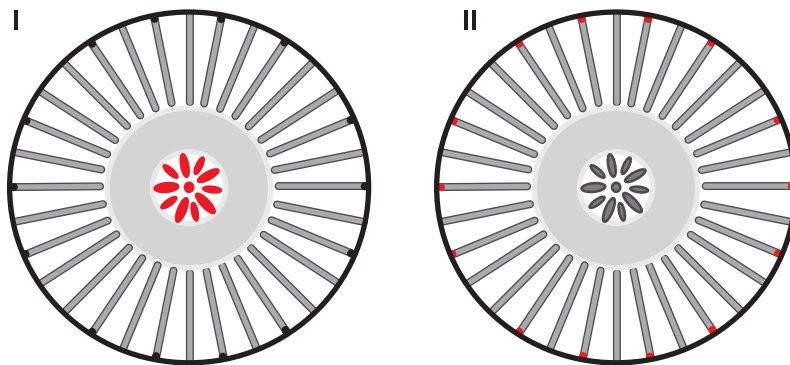
*Cyclotella* Kützinger ex Brébisson 1838 pro parte

**Characteristics** – Cells **centric**, short striae, separated by robust **costae**, extending from the margin approximately half way across the valve face. Central area thickened silica with large perforations in a more or less stellate pattern (I). Cells lack marginal spines. Marginal **fuloportulae**, only visible with SEM, present at the margin on every second or third costa (II, Fig. 22: J, L, M).

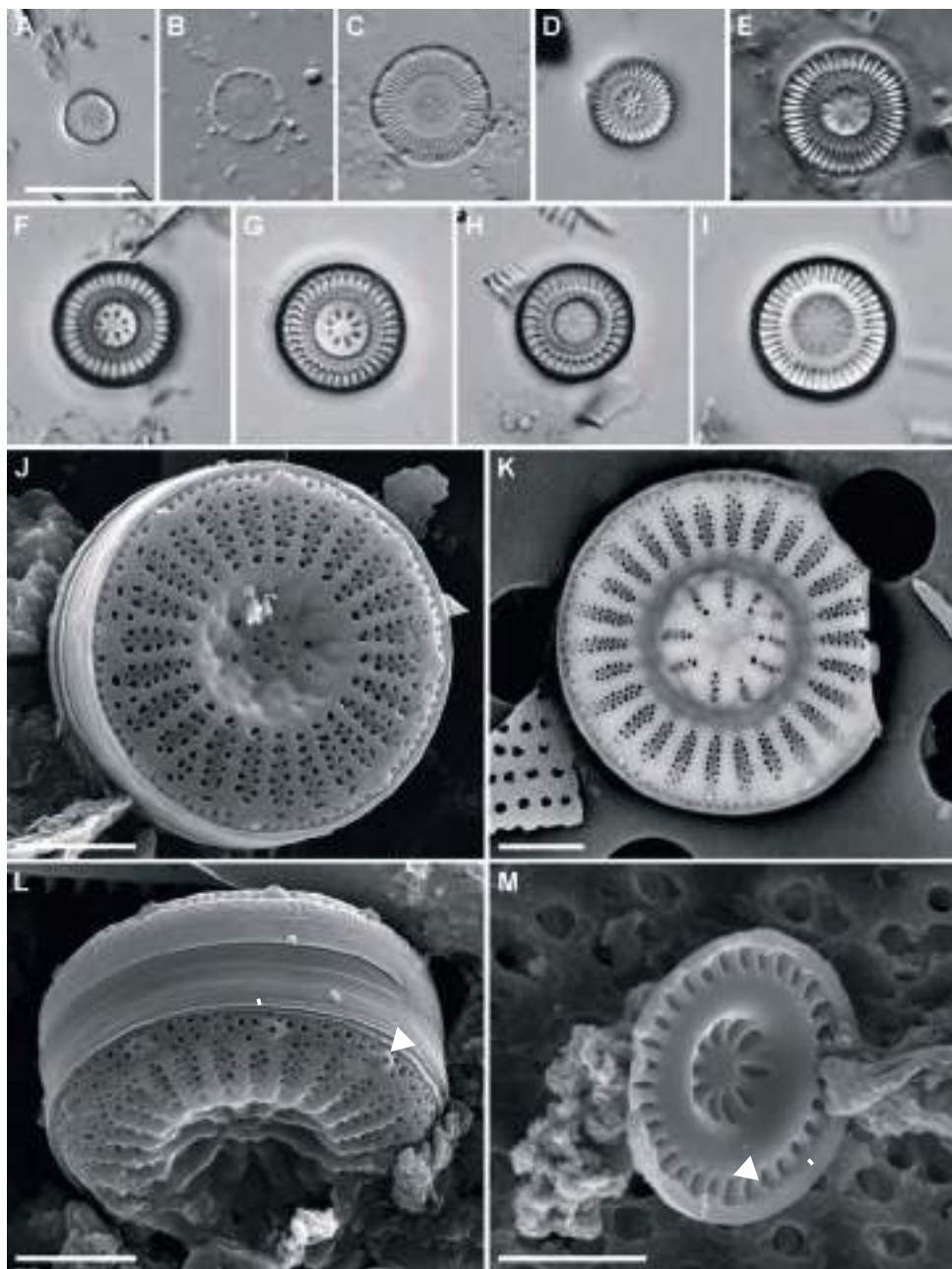
**Plastid structure** – Cells with small granular plastids.

**Identification of species** – Species can be identified by cell size and density and structure of the striae. The shape, structure and configuration of the ornamentation of the central area are important.

**Ecology** – Cells planktonic may become entrained in the benthos. Found in oligotrophic to mesotrophic waters with moderate conductivity.







**Fig. 22.** *Discostella* spp. **A-I.** LM, cleaned material. **A-C.** Valve views of *Discostella woltereckii* (Hustedt) Houk & Klee. **D-I.** Valve views of *D. stelligera*. **J-M.** SEM. **J-K.** External view of valve of *D. stelligera*. **L.** Oblique external view of valve of *D. stelligera*, note external openings of marginal fultoportulae (arrow). **M.** Oblique internal view of valve of *D. stelligera*, note internal openings of marginal fultoportulae (arrow)

Scale bars = 10 µm (A-I), 2 µm (J-L), 5 µm (M).



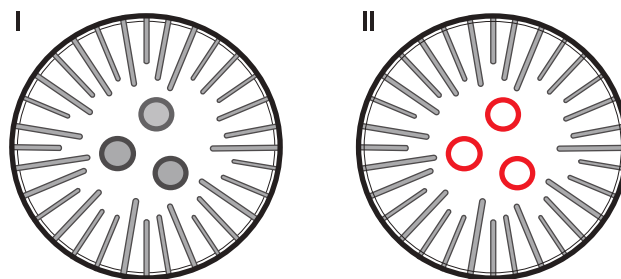
***Pantocsekiella*** K.T. Kiss & Ács 2016*Pantocsekiella ocellata* (Pantocsek) K.T. Kiss & Ács**SYNONYM:***Cyclotella* Kützing ex Brébisson 1838 pro parte

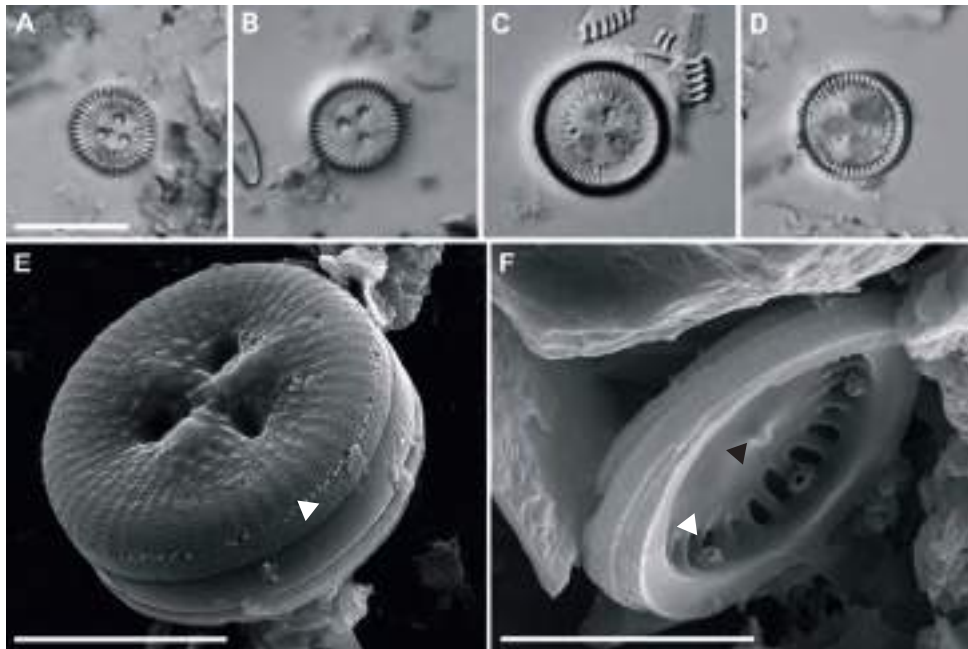
**Characteristics** – Cells **centric** with radiate striae. Striae separated by robust **interfascicular costae** which can differ in length and extend from approximately half way across the valve face to the valve margin, leaving an open central area which has large circular depressions (lacunae) (II, Fig. 23: E, F). Weakly developed spines can be present at junction of valve face and valve mantle at the end of each **costa**. Valve face **fuloportulae** (strutted processes) are often present on valve face towards the center (Fig. 23: E, F) and below the spines on the valve margin. One **rimoportula** present on the valve mantle.

**Plastid structure** – Cells with small discoid plastids, scattered lipid bodies (see *Cyclotella*).

**Identification of species** – Cell diameter, number of striae and costae as well as structure of the costae. Number and distribution of circular depressions. Note: Many important cell characteristics can only be observed using SEM.

**Ecology** – Cells, solitary or in pairs but not forming chains, planktonic may become entrained in the benthos. Found in waters with medium conductivity and higher trophic levels.





**Fig. 23.** *Pantocsekiella* spp. **A-D.** LM. **A-C.** *Pantocsekiella ocellata*, valve views of cleaned cells. **D.** *Pantocsekiella* sp., valve view. **E-F.** SEM. *P. ocellata*. **E.** External oblique view of valve, note external openings of fultoportulae (arrow). **F.** Internal view of valve, note internal opening of valve face (black arrow) and marginal fultoportulae (white arrow).  
Scale bars = 10 µm (A-D), 5 µm (E), 3 µm (F).

***Stephanodiscus* Ehrenberg 1845**

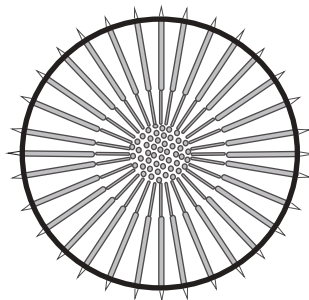
Type species: *Stephanodiscus niagarae* Ehrenberg

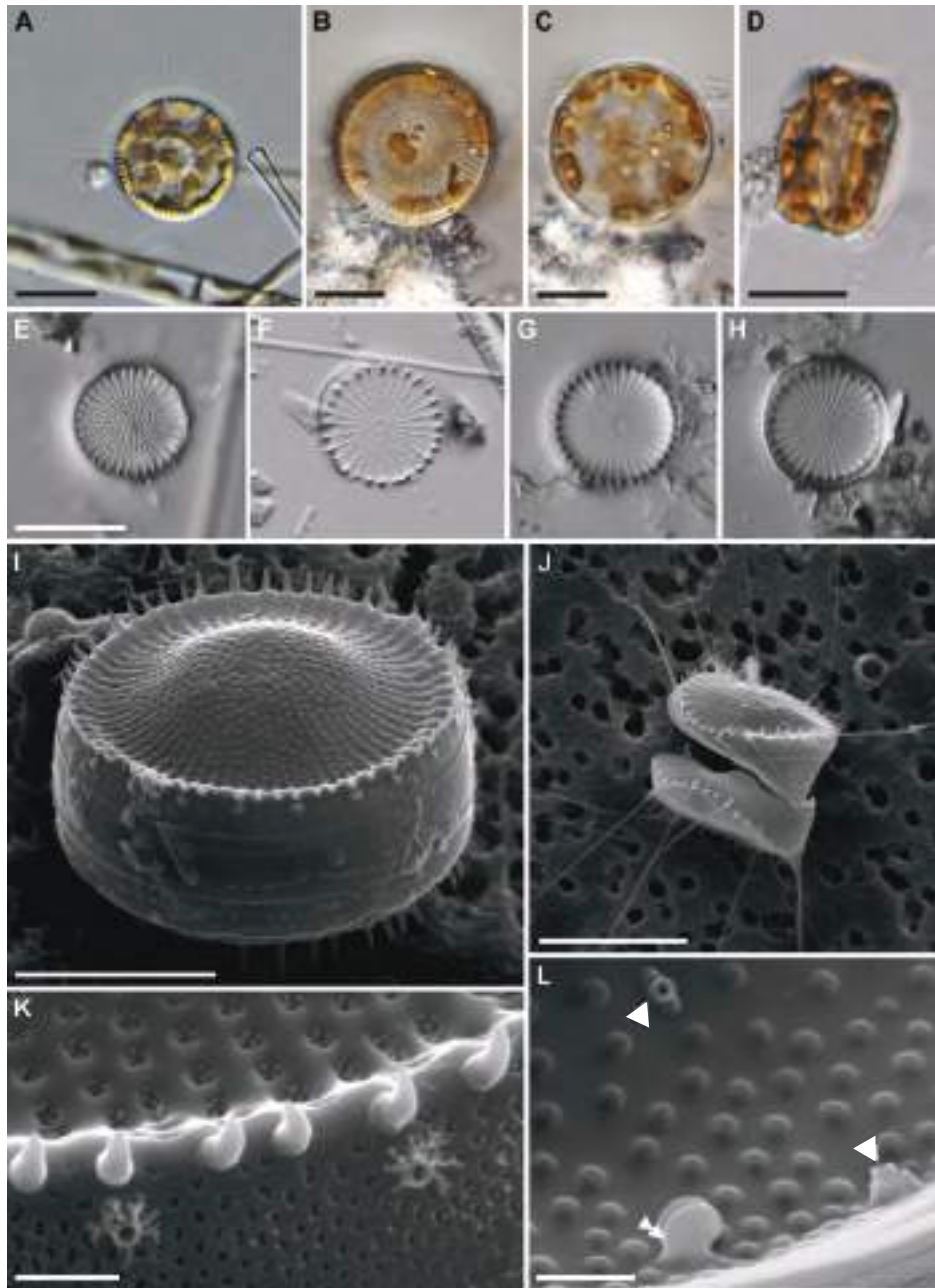
**Characteristics** – Cells **centric** with radiate striae. Valve face flat or concentrically undulate. Striae composed of 2-3 rows of areolae and are combined in **fascicles** (rows of 2-3 areolae) by **interfascicular costae** which extend from the margin of the valve face to the centre of valve face where they fuse together. Spines present at junction of valve face and mantle at the end of each **costa**. Valve face **fultoportulae** (strutted processes) present on valve face and below the spines on the valve mantle. One **rimoportula** present on the valve mantle.

**Plastid structure** – Cells with small discoid plastids (Fig. 24: A-C).

**Identification of species** – Cell diameter, number of striae and costae as well as structure of costae. Note: Many important cell characteristics can only be observed using SEM.

**Ecology** – Cells planktonic may become entrained in the benthos. Chitin threads (Fig. 24: A) exuded from marginal fultoportulae increase surface area, slowing sinking through the water column. Found in waters with medium conductivity and higher trophic levels.





**Fig. 24.** *Stephanodiscus* spp. **A-H.** LM. **A-D.** Living cells. **E-H.** Cleaned cells. **I-L.** SEM. **I.** Oblique view. **J.** External view of valve, note chitin threads. **K.** External view of valve margin, note marginal spines and marginal fultoportulae. **L.** Internal view of valve, note valve and marginal fultoportulae (arrows) and rimoportula (double arrow).

Scale bars = 10  $\mu\text{m}$  (A-J), 1  $\mu\text{m}$  (K-L).

***Melosira* C. Agardh 1824**

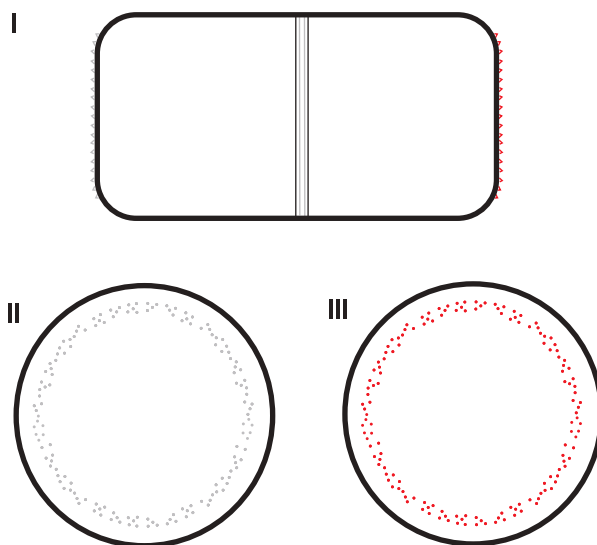
Type species: *Melosira nummuloides* C. Agardh

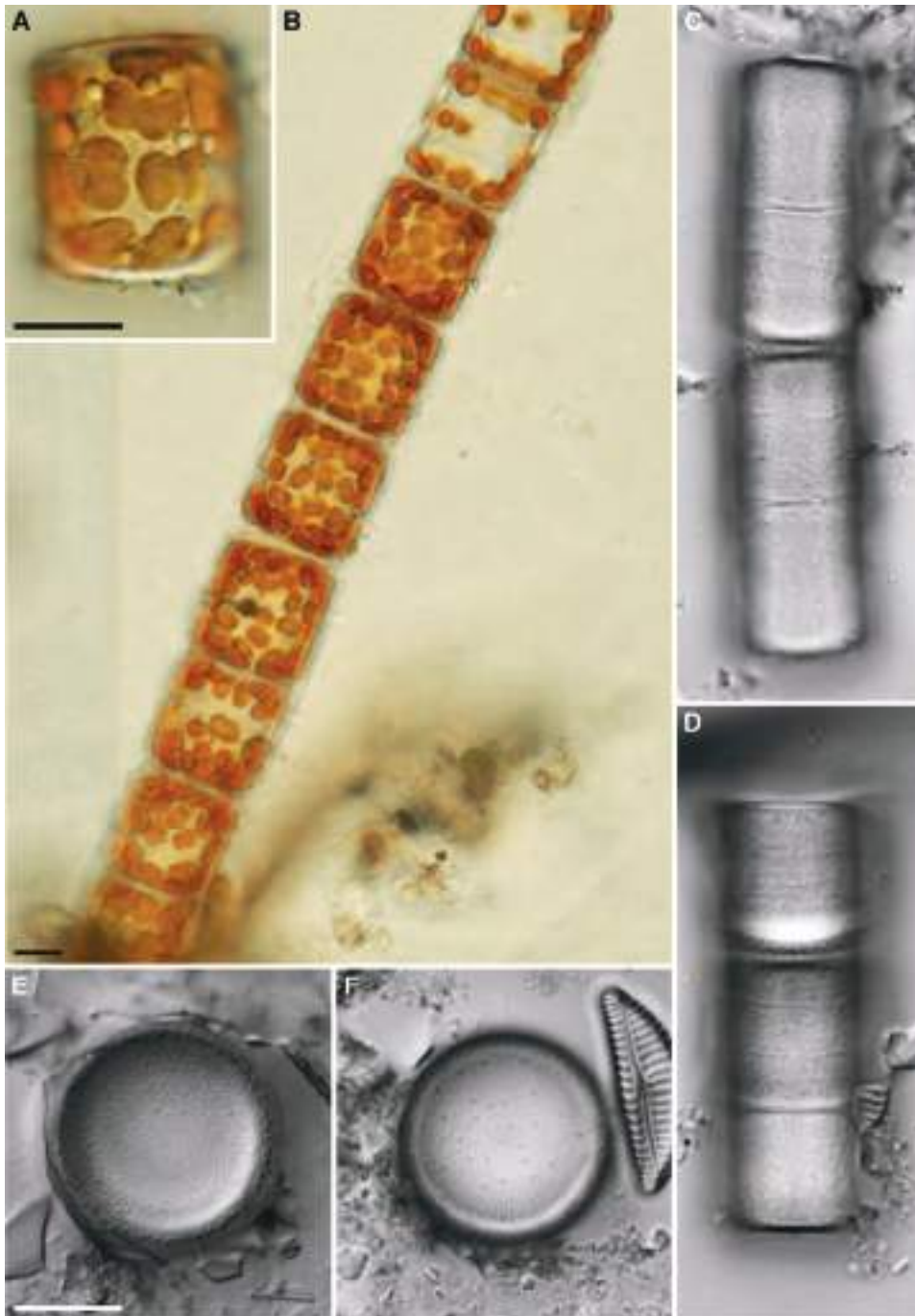
**Characteristics** – Cells **centric**, valve mantle is rather deep, cells often observed in girdle view (I). Areolae very small, scattered over the valve face only visible with SEM. **Rimoportulae** small (Fig. 26: C-D, F), scattered over valve face, usually not possible to resolve using LM. Valve face bears a large number of scattered silica granules which can be seen both under LM and SEM (Fig. 25: E-F; Fig. 26:A-B). When seen in valve view under LM a ring of very small spines can be observed around the periphery of the cell close to the valve margin (III; Fig. 25: E-F; Fig. 26: A-B).

**Plastid structure** – Cells with small plate-like plastids that may be lobed or circular, found around the periphery of the cell (Fig. 25: A-B).

**Identification of species** – Up till now only one species known from tropical African freshwaters: *Melosira varians* C. Agardh.

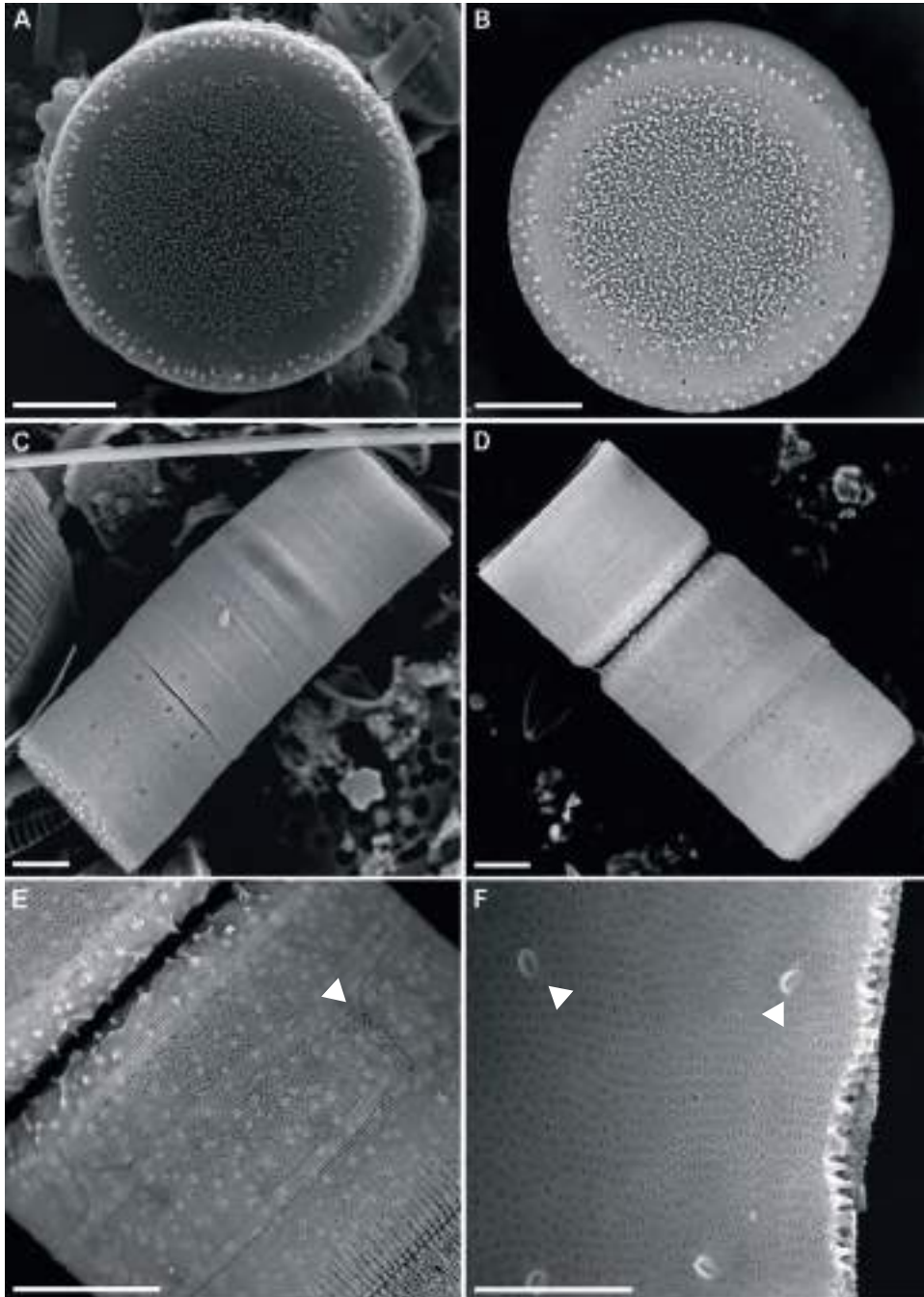
**Ecology** – Cells joined face-to-face by mucilage pads forming long chain like-colonies with the terminal cell attached to the substrate by the same means. Found in the benthos, and may be re-suspended in the plankton, of eutrophic waters with moderate to high conductivities.





**Fig. 25.** *Melosira varians*. **A-F.** LM. **A-B.** Living cells, firdle view. **C-F.** Cleaned cells. **C-D.** girdle view. **E-F.** Valve view.  
Scale bars = 10 µm.





**Fig. 26.** *Melosira varians*. **A-F.** SEM. **A-B.** External view of valves. **C-D.** Girdle views. **E.** Detail of girdle bands, note ligula (arrow). **F.** Internal view of valve, note rimoportulae (arrows).

Scale bars = 10  $\mu\text{m}$  (A-D), 5  $\mu\text{m}$  (E), 2  $\mu\text{m}$  (F).

## ***Aulacoseira* Thwaites 1848**

Type species: *Aulacoseira crenulata* (Ehrenberg) Thwaites

SYNONYM:

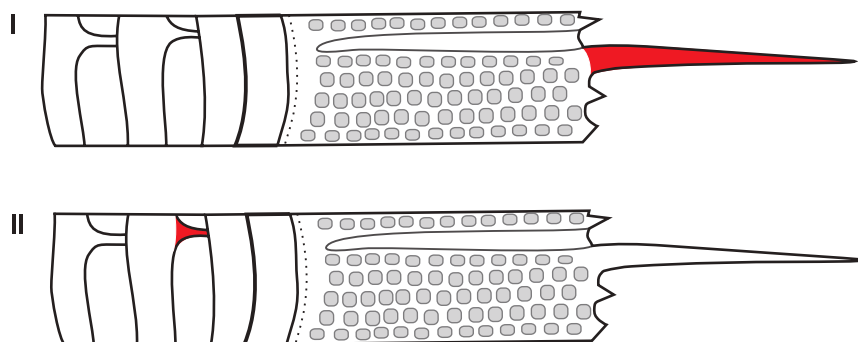
*Melosira* C. Agardh 1824 pro parte

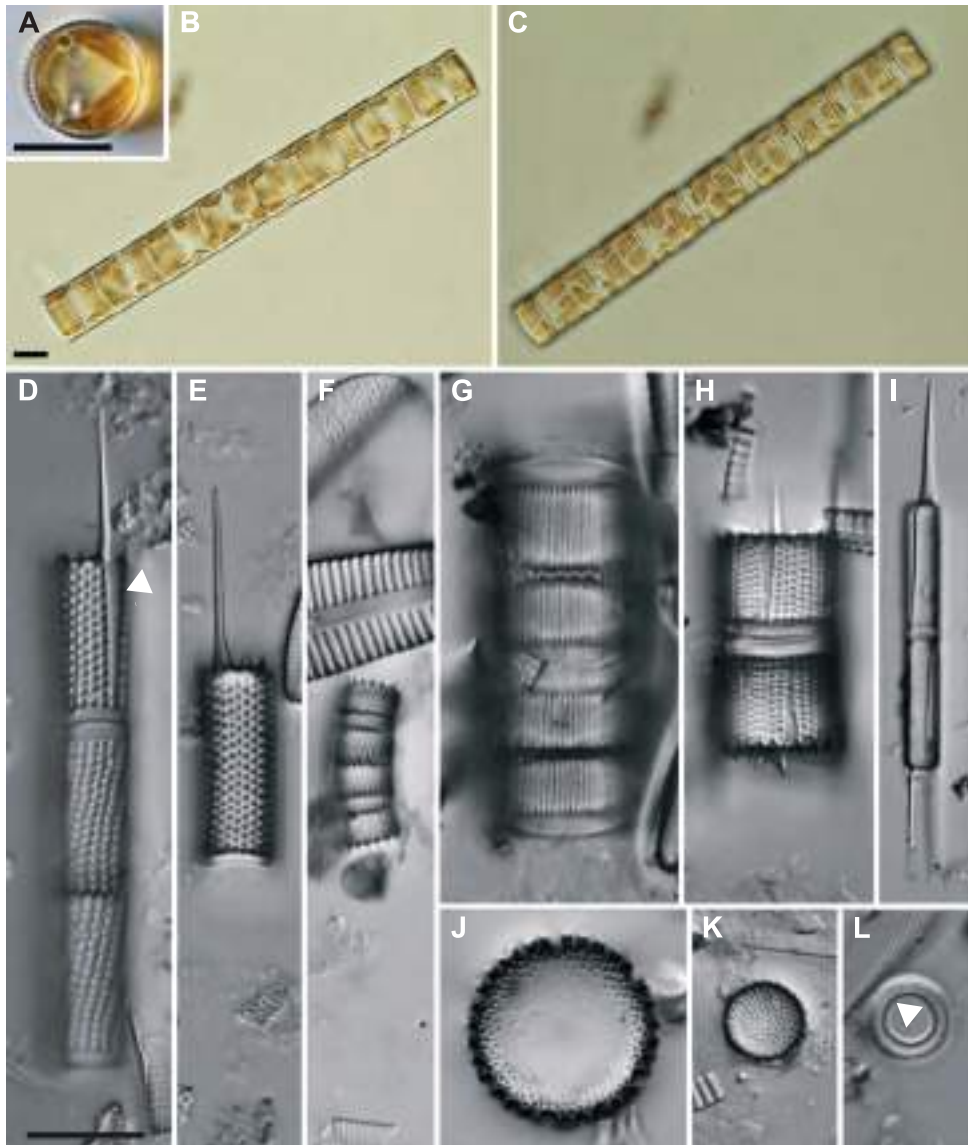
**Characteristics** – A **centric** diatom genus with the **valve mantle** most often deeper than the diameter of the **valve face**, for this reason cells are mostly seen in girdle view. Areolae usually large and easily discernable in LM but may be rather small in some cases (e.g. *Aulacoseira herzogii* (Lemmermann) Simonsen; Fig. 27: I). Spines are present including long linking spines (I). Girdle composed of both open and closed bands, a **ligula** or tongue-like structure is present (II, Fig. 28: E).

**Plastid structure** – Many small disc-like plastids (Fig. 27: B-C).

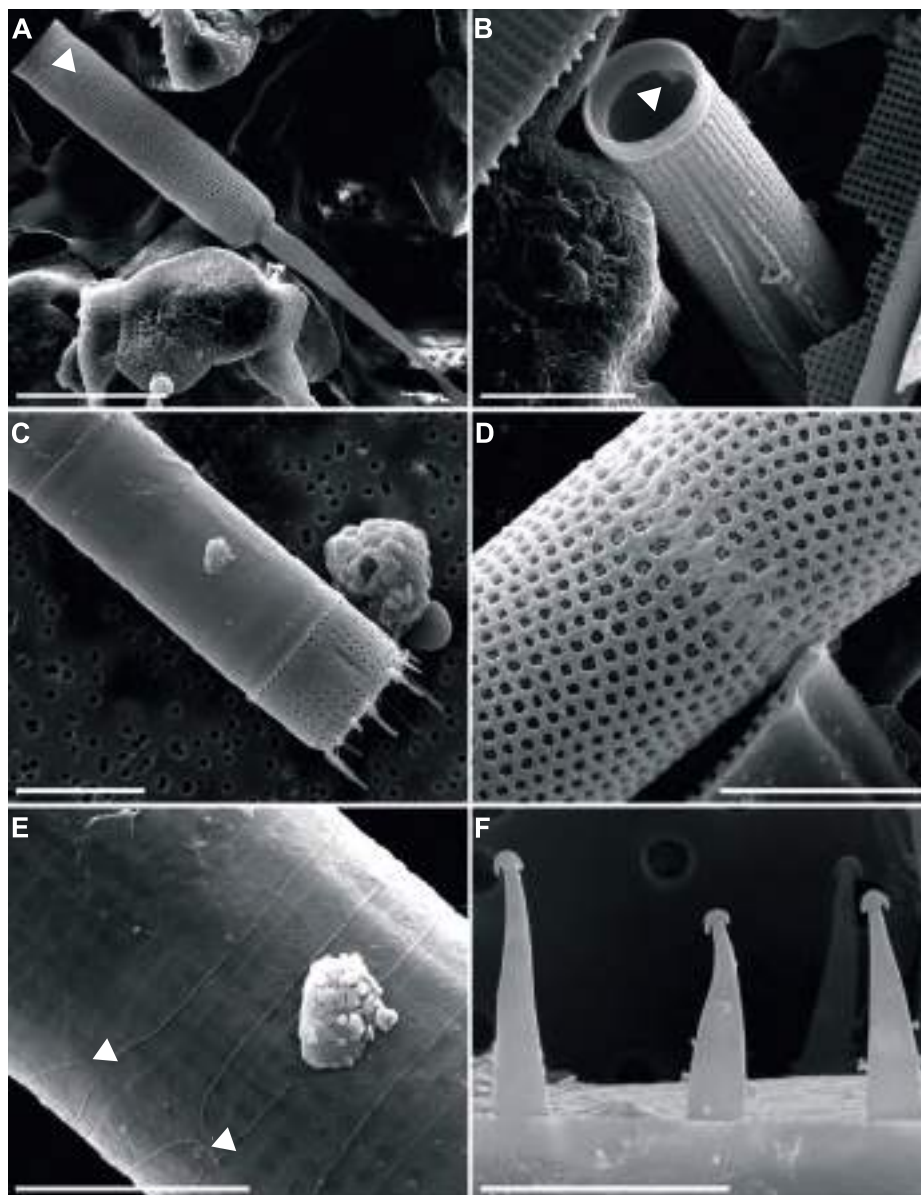
**Identification of species** – Depth of the valve mantle is very important together with the orientation and dimensions of the striae and type and length of spines present (e.g. *Aulacoseira granulata* (Ehrenberg) Simonsen has long and short linking spines while *Aulacoseira ambigua* (Grunow) Simonsen only has short linking spines and *Aulacoseira herzogii* only has long spines).

**Ecology** – Cells colonial forming chains, planktonic in a wide range of water qualities. The increased surface area of these chain-like colonies helps to prevent sinking through the water column.





**Fig. 27.** *Aulacoseira* spp. **A-L.** LM. **A.** Living cell of *Aulacoseira ambigua*, valve view, note position of plastids - appressed to valve mantle. **B-C.** Living cells of *A. ambigua* forming a filamentous colony or chain, different foci of the same filament. **D-E.** Girdle view of *A. granulata*, note long linking spines and associated groove in the mantle (arrow). **F.** Girdle view of *A. subarctica* (O. Müller) E.Y. Haworth. **G.** Girdle view of *Aulacoseira* sp. **H.** Girdle view of *A. muzzanensis* (F. Meister) Krammer, note relatively shorter linking spines as compared with *A. granulata*. **I.** Girdle view of *A. herzogii*. **J-L.** Valve views of various *Aulacoseira* species showing distribution of areolae on valve face and position of the ringleiste (arrow). Scale bars = 10 µm (A-H).



**Fig. 28.** *Aulacoseira* spp. **A-F.** SEM. **A.** Girdle view of *Aulacoseira herzogii*, note external opening of the rimoportula (arrow). **B.** Oblique view of *A. herzogii* showing groove in the valve mantle occupied by the linking spine, note internal opening of the rimoportula (arrow). **C.** Girdle view of *Aulacoseira* sp. showing valve mantle and associated copulae. **D.** Girdle view of *A. ambigua* showing the structure of the areolae and the short linking spines. **E.** Detail of the structure of the copulae of *Aulacoseira* sp., note the ligulae (arrows). **F.** Detail of the complex structure of the linking spines of *Aulacoseira* sp.  
Scale bars = 10  $\mu$ m (A), 5  $\mu$ m (B-D), 3  $\mu$ m (E), 2  $\mu$ m (F).

***Orthoseira* Thwaites 1848**

Type species: *Orthoseira americana* (Kützting) S.A. Spaulding & Kociolek

SYNONYM:

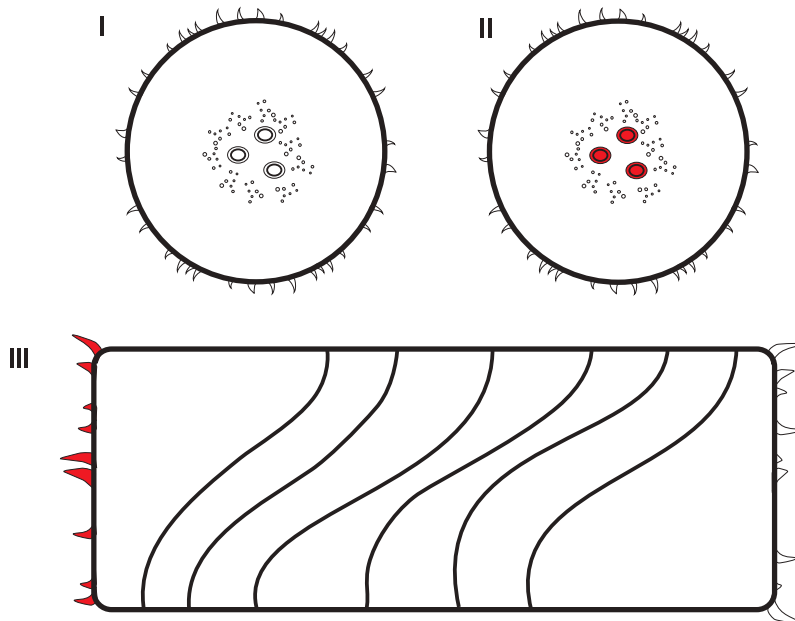
*Melosira* C. Agardh 1824 pro parte

**Characteristics** – Cells **centric**, valve mantle is rather deep, cells often observed in girdle view. Girdle composed of multiple bands (**copulae**) (Fig. 30: C, H; Fig. 31: D). Valve face bears unique structures in the centre (II; **carinoportulae**). A ring of spines is found around the periphery of the cell close to the junction of the valve face and mantle (III; Fig. 30: A; Fig. 31: B-C) but may be difficult to observe using LM (Fig. 30 B-F).

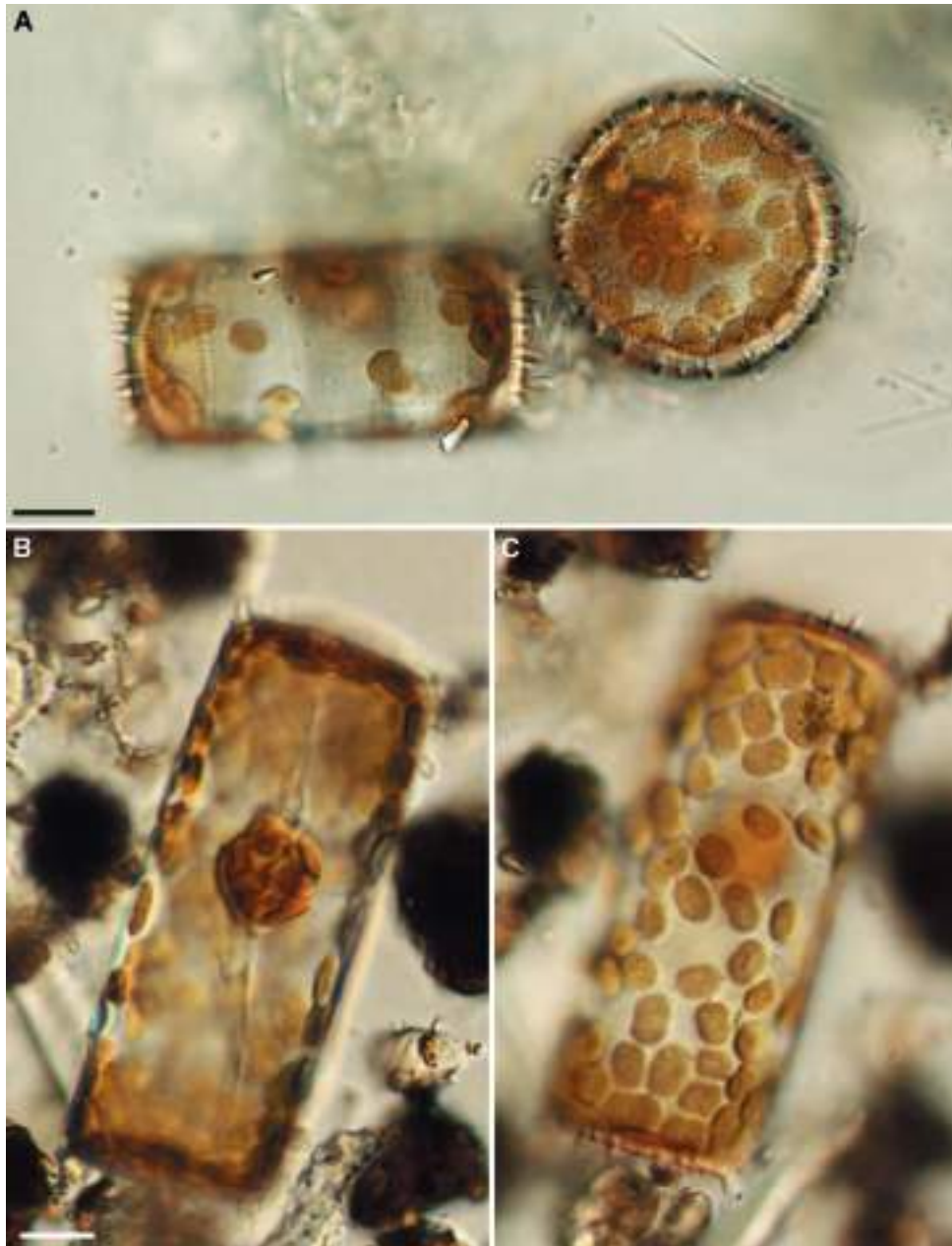
**Plastid structure** – Cells with many small discoid plastids (Fig. 29), found in the peripheral cytoplasm (Fig. 29: A, C) as well as clustered in the cytoplasm surrounding the nucleus (Fig. 29: B).

**Identification of species** – Based on SEM.

**Ecology** – Cells linked by spines to form short chains. Generally found in sub-aerial habitats, sometimes washed into rivers, streams and lakes.

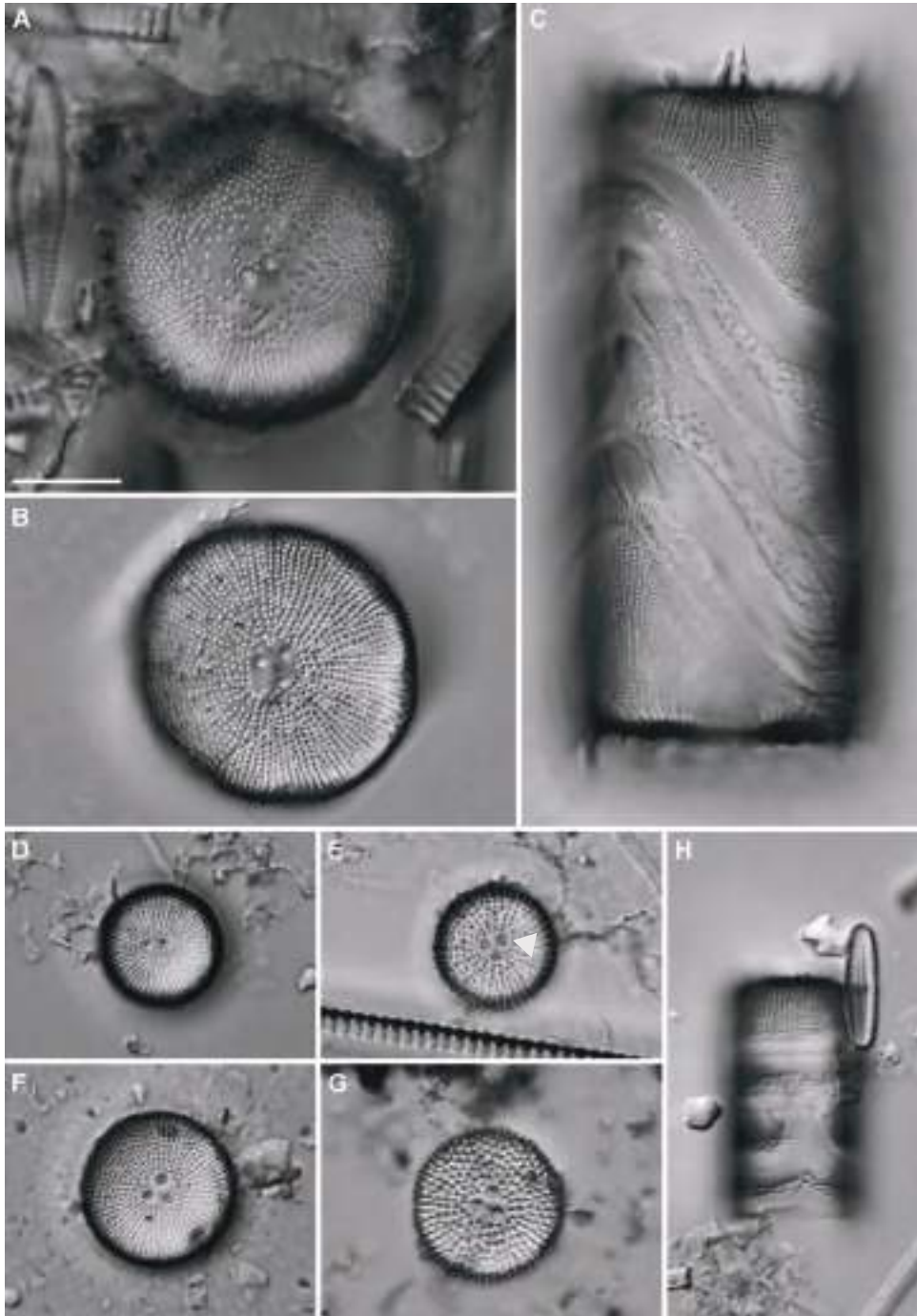




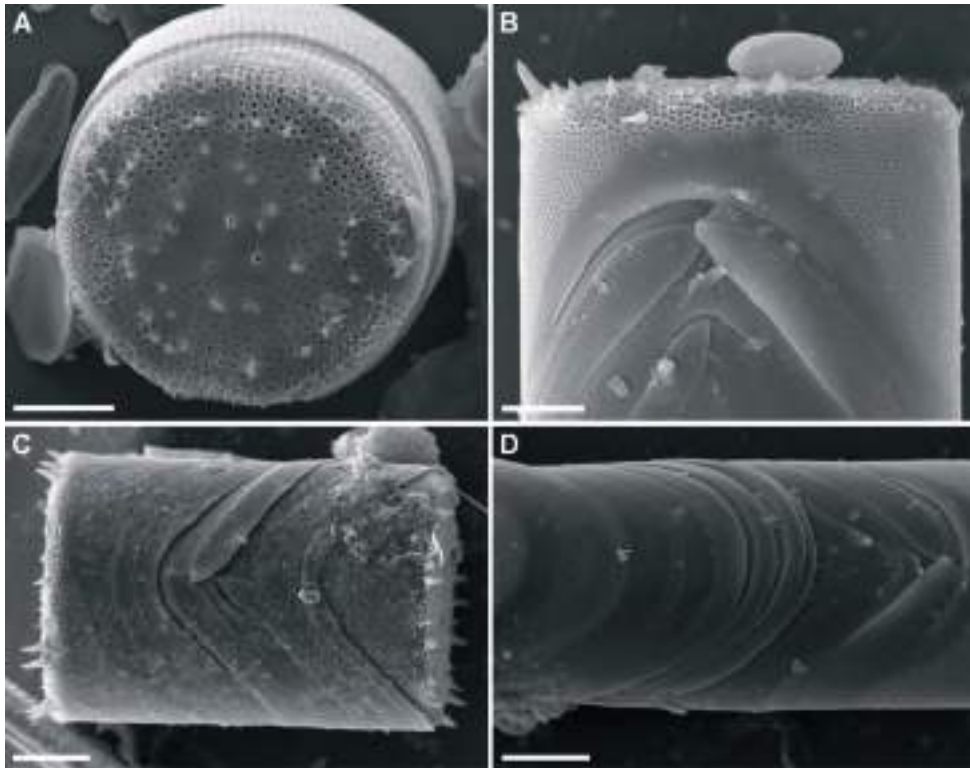


**Fig. 29.** *Orthoseira* sp. **A-C.** LM, Living cells. **A.** Valve view and girdle view.  
**B-C.** Girdle view of the same cell at different foci.  
Scale bars = 10  $\mu$ m.





**Fig. 30.** *Orthoseira* spp. **A-H.** LM, cleaned material. **A-B, D-G.** Valve views, note the carinoportulae (arrow - **E**). **C, H.** Girdle views.  
Scale bar = 10 µm.



**Fig. 31.** *Orthoseira* spp. **A-D.** SEM. **A.** External view of valve. **B-C.** Girdle views, note the spines at the junction of the valve face and mantle. **D.** Detail of the girdle bands.

Scale bars = 10  $\mu\text{m}$  (A, C-D), 5  $\mu\text{m}$  (B).