Key findings and outcomes of the COZADIMO project:

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Summary

Over the 18 months of the project we have made a systematic review and examination of the diatom flora of the Congo and Zambezi drainage basins with the aim of documenting and describing taxa new to science as well as taxa with problematic identifications and those which are common and can be potentially used as indicator organisms.

We also documented the diatom genera of tropical Africa and the included these taxa in an illustrated guide for their identification (over 90 genera). This guide will be published in 2015 as a volume of the book series Abc Taxa (see Appendix 2). The guide is aimed at local scientists in order to introduce the most recent nomenclatural and taxonomic concepts on which to eventually base a water quality monitoring system.

Project outcomes

1. Description of new taxa

A number of diatoms from the study region were described or are being described as new to science:

Cavinula lilandae

Diploneis fenestrata

Actinellopsis murpheyi (new genus)

Stenopterobia cataractum

Navicula nielsfogedii

Eunotia leonardii

Eunotia fuseyi

Without description and delimitation, especially of common taxa the eventual application of diatom indices is not possible.

Supporting scientific papers:

Cocquyt, C. De Haan, M. And Taylor, J.C. (2013) *Cavinula lilandae*(Bacillariophyta), a new diatom species from the Congo Basin, Central Africa. *Diatom Research* 28 (1-2):157-164.

Fofana C.A.K., Sow, E.H., Taylor J.C., Ector, L. & van de Vijver B. (2014) *Placoneis cocquytiae* a new raphid diatom (Bacillariopyceae) from the Senegal River (Senegal, West Africa). Phytotaxa 161(2): 139-147.

Cocquyt C., Taylor J.C. & Wetzel C.E. (2014) *Stenopterobia cataractarum sp. nov*. (Bacillariophyta), a new benthic diatom from a waterfall in Zambia, Africa. Phytotaxa 158: 76-84.

Taylor J.C., Karthick B., Kociolek J.P., Wetzel C.E. & Cocquyt C. (2014) *Actinellopsis murphyi gen. et spec. nov*.: A new small celled freshwater diatom (Bacillariophyta, Eunotiales) from Zambia. Phytotaxa 178(2): 128-137.

Taylor J.C., Karthick B., Cocquyt C. & Lang P. (2014) *Diploneis fenestrata* spec. nov. (Bacillariophyta), a new aerophilic diatom species from Zambia, Africa. Phytotaxa 167: 79-88.

Cocquyt C. & Taylor J.C. New and interesting *Surirella* taxa (Surirellaceae, Bacillariophyta) from the Congo basin (DR Congo) – working title. To be submitted to European Journal of Taxonomy.

2. Resolution of taxonomic issues and identification of common taxa

We resolved issues pertaining to the identification of the common tropical diatom species *Eunotia zygodon* and resolved its common conflation with *Euntia monodon* var. *tropica*. As previously stated we cannot apply diatom indices without being certain of the identity of the organisms occurring in those samples.

Supporting scientific papers:

Large celled *Eunotia* (Bacillariophyta) from the Democratic Republic of the Congo, tropical central Africa – working title. To be submitted to Plant Ecology and Evolution.

Navicula nielsfogedii J.C. Taylor & C. Cocquyt, a new diatom (Bacillariophyta) species from tropical and sub-tropical Africa – working title. To be submitted to Fottea.

3. Recommendations for diatom-index application in central Africa

Extract from Appendix 1 – Submitted for publication on invitation from the Royal Society for Overseas Science

For the foreseeable future the application of **species** level diatom indices will not be possible in central Africa, the reasons for this are manifold but include in particular the following:

- Diatom species, and even some genera, from this region are relatively unknown and many un-described.

- Published literature from the region on species identification and environmental tolerances is scarce but necessary to construct indices.

- Lack of expertise in central Africa to identify diatoms.

In South Africa when index testing began, although exhaustive knowledge of the local taxa and their requirements was not known, these indices were applied in river monitoring programs to reflect water quality. Although this is far from an ideal situation, the inclusion of these techniques allowed for sample and data collection which in turn could be used to fill taxonomic gaps and gain information on species distribution and environmental tolerances. Coupled with the production of guides for identification and methodology the technique has now gained impetus and has become a routinely used part of the suite of biomonitoring tools nationally.

We would propose a similar solution for central Africa. As discussed, species identifications cannot be made with any great certainty, however genus identifications may be simpler to achieve. Recently diatom taxonomy and nomenclature have undergone a number of changes, in particular very large diatom genera have been split into more natural groups. These groups also very often have very specific environmental tolerance. In essence there are now more genera so that we can expect greater resolution in the use of genus-based indices. If accurately identified taxa could be used for the calculation of a genus level index then this in turn could be used to indicate, with some degree of accuracy, reigning environmental conditions. A region specific guide is a vital resource. Such guides are by no means exhaustive, neither do they pretend to be, but instead provide a first introduction into the world of diatoms for many students and other interested workers. Once a reliable guide (coupled with relevant methodology) to the genera of tropical Africa is available, scientists in central Africa can receive adequate training in diatom identification and begin with the application and testing of indices based on diatom genus level.

4. Tools for future diatom index application in central Africa (see Appendix 2)

The application of diatom-based monitoring is not possible without accurate and up to date literature. However, diatom literature (as is true for much other scientific literature) is prohibitively expensive. For this reason during the COZADIMO project we focused on the production of a genus level diatom identification guide. The guide provides information on the structure and ecology of the genera as well as illustrates these genera from living material and cleaned African material. We have also produced digital stylized drawings so that key features of the genera can be easily highlighted. This work will be published as a volume of the series 'AbcTaxa' which is provided free of charge to African scientists. The scheduled date for completion of this volume is June 2015 with publication before the end of 2015. Examples of the layout are given in Appendix 2.

5. Establishment of collaborative efforts for taxonomic resolution

This collaboration established in this project had unforeseen benefits. It allowed J. Taylor to collaborate with more specialists in the field of diatom studies than just the project leader. Collaboration from within the Botanic Garden allowed for the checking of diatom material which is important not only for Africa but internationally. The Botanic Garden Meise is a rich repository for type material going back to the early 1800s. This type material is easily accessible and allowed for systematic studies of two important diatom taxa. Visits were also made to other European institution such as the University of Szczecin, Poland to seek taxonomic advice from leading scientists in the field.

Supporting scientific papers:

Taylor J.C., Cocquyt C., Karthick B. & Van de Vijver B. (2014). Analysis of the type of *Achnanthes exigua* Grunow (Bacillariophyta) with the description of a new Antarctic species. Fottea 14(1): 43-51.

Dalu T., Taylor J.C., Richoux N.B., Froneman P.W. (accepted) A re-examination of the type material of *Entomoneis paludosa* (W Smith) Reimer and its morphology and distribution in African waters. Fottea. TBA.

6. Other outcomes:

Taylor J.C. & Cocquyt C. (2014) A guide to the diatom genera of tropical Africa – building capacity for diatom identification for local researchers. Poster presented at the 2nd Annual Meeting on Plant Ecology and Evolution. Louvain-la-Neuve, Belgium, 14 November 2014: 19.

Cocquyt C. & Taylor J.C. (2014) Diatom diversity of some acid rivers and streams in the vicinity of Yangambi (Oriental Province, DR Congo). Oral presentation at the 1st International Conference on Biodiversity in the Congo Basin, Kisangani, 6-10 June 2014: 75-76.

Taylor J.C. (2014). Diatom research in southern and central Africa: Historical perspectives and current activities. Invited oral presentation at the Royal Academy for Overseas Sciences. Brussels, Belgium, 20 May 2014.

Taylor J.C., Cocquyt C., Lang P. & van Rensburg L. (2012) The Use of Diatoms for Monitoring River Water Quality in the Zambezi and Congo Sister Basins. Poster presented at the GAPSYM6, 6th Symposium of the Ghent Africa Platform. Ghent, Belgium, 7 December 2012.

Taylor J.C Cocquyt C., & van Rensburg L. (2012) Diatoms from the Congo and Zambezi Sister Basins – A First Overview. AMPEE1. Poster presented at the 1st Annual Meeting on Plant Ecology and Evolution. National Botanic Garden of Belgium, Meise, Belgium, 30 November 2012.

Lang P., Taylor J.C & Bertolli L. (2012) River Diatom Biodiversity and Biointegrity Assessment in Zambia: A Conservation Perspective. Poster presented at the ECCB Glasgow 2012. 3rd European Congress of Conservation Biology. SECC, Glasgow, UK, 28 August - 1 September 2012.

Taylor J.C. , Cocquyt C., Lang P. & van Rensburg L. (2012) The Use of Diatoms for Monitoring River Water Quality in the Zambezi and Congo Sister Basins. Poster presented at the 22nd International Diatom Symposium, Ghent, Belgium, 26 -31 August 2012.

7. Project duration:

Start date – April 2012 (1 month)

Continuation – August to end December 2012 (6 months)

Continuation – August to end December 2013 (5 months)

Final period – May to November 2013 (7 months)

Total – 18 months

APPENDIX 1 – Manuscript of invited lecture to the Royal Academy for Overseas Science (Belgium)

Diatom research in southern and central Africa: Historical perspectives and current activities

by

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KEYWORDS. – Diatoms, southern Africa, central Africa, taxonomy, biomonitoring.

SUMMARY. - Diatom research in southern and central Africa has a rich history spanning more than a century; however, much of this research was on marine environments and the African Great Lakes. In this paper we focus on the less studied riverine habitats. Research on riverine diatoms can be loosely grouped into taxonomic and ecological studies, although we will show in this paper that these two disciplines, of necessity, overlap. We briefly summarize relevant diatom-based indices for inferring water quality in rivers and streams and systematically report on recent efforts to use diatoms for ecological monitoring in southern and central Africa. We deal in particular with the challenges faced when studying the central African diatom flora and introduce the problem of diatom species concept drift. We also relate these concepts to water quality monitoring. We discuss solutions and current efforts to resolve these issues. Importantly our work also focuses on equipping scientists based in these regions with the tools for further studies.

TREFWOORDEN. – Diatomeeën, zuidelijk Afrika, centraal Afrika, taxonomie, biomonitoring.

SAMENVATTING. – Diatomeeënonderzoek in zuidelijke en centraal Afrika kent een rijk historisch verleden van meer dan een eeuw. Een groot deel van dit onderzoek was echter toegespitst op marine milieus en op de Grote Afrikaanse Slenkmeren. In het huidige manuscript richten we ons op de minder bestudeerde habitatten, namelijk de rivieren. Onderzoek op lotische diatomeeën kan ruwweg onderverdeeld worden in taxonomisch en ecologische studies niettegenstaande we in dit manuscript zullen aantonen dat beide disciplines, uit noodzaak, overlappen. We geven een kort overzicht van op diatomeeën gebaseerde relevante indices om de waterkwaliteit van rivieren en beken te bepalen, alsook geven we een systematisch overzicht van recent geleverde inspanningen om diatomeeën te gebruiken in ecologische monitoring in zuidelijke en centraal Afrika. Speciale aandacht gaat uit naar de uitdagingen die ondernomen moeten worden wanneer de centraal Afrikaanse diatomeeënflora wordt bestudeerd, en we introduceren het probleem van de "drift van het concept van diatomeeënsoort". We betrekken we deze concepten eveneens bij de monitoring van de waterkwaliteit. Verder bespreken we mogelijke oplossingen en actueel ondernomen inspanningen om deze problemen te verhelpen. Belangrijk is dat ons werk zich ook concentreert op het uitrusten van wetenschappers die verblijven in deze gebieden met middelen voor verdere studies.

MOTS CLEF. – Diatomées, Afrique méridionale, Afrique centrale, taxonomie, bio-monitoring.

RÉSUMÉ. – La recherche sur les diatomées en Afrique méridionale et centrale connaît une histoire riche et longue de plus d'un siècle. Cependant, la majeure partie de cette recherche concernait les milieux marins et les Grands Lacs Africains. Dans ce manuscrit nous mettons l'accent sur les habitats des rivières moins étudiés. La recherche sur les diatomées lotiques peut être divisée de manière générale entre l'étude taxonomique et l'étude écologique, mais nous montrerons dans cet article que ces deux disciplines, par nécessité, coïncident. Nous résumons brièvement les indices basés sur les diatomées pour déterminer la qualité de l'eau des rivières et ruisseaux et nous rapportons méthodiquement les efforts récents dans l'utilisation les diatomées pour le monitoring écologique en Afrique méridionale et centrale. Nous traitons plus particulièrement des défis rencontrés lors de l'étude de la flore des diatomées en Afrique centrale, et nous introduisons le problème de la dérivation du concept d'espèce. Nous mettons également ces concepts en relation avec le monitoring de la qualité de l'eau, et nous débattons des solutions et des efforts actuels pour résoudre ces problèmes. Plus important encore, notre travail se concentre aussi sur l'accompagnement des scientifiques basés dans ces régions pour leur fournir les outils nécessaires à des études ultérieures.

1. Introduction

Diatoms occur in all types of aquatic ecosystems, also extending into damp sub-aerial habitats. A golden-brown mucilaginous film on the surface of a substrate indicates the presence of benthic diatoms. Planktonic diatoms occur free-living in the water column of rivers, streams, ponds and dams. The diatoms (Bacillariophyta) comprise a ubiquitous, highly successful and distinctive group of unicellular algae, whose most obvious distinguishing characteristic is the possession of a siliceous cell wall (frustule). The frustule, unique to the diatoms, is composed chiefly of hydrated amorphous silica, but which may also contain other trace elements, and comprises two almost equal halves known as the valves. Each valve is composed of two parts: the valve face and valve mantle which is connected almost at right angles to the valve face. Closely-united to the valve mantle are the girdle bands or copulae (Round et al., 1997). As autotrophs, diatoms contribute significantly to the carbon productivity of ecosystems, forming together with other algae the base of aquatic food chains (Cox, 1996) and are responsible for the production of significant amounts of oxygen.

2. Historical notes

2.1. THE STUDY OF DIATOMS IN SOUTHERN AFRICA

The freshwater diatom flora of southern Africa received much attention in the past. The investigations were initiated in the middle 19th century by people such as Ehrenberg (e.g. Ehrenberg, 1845) and Cleve (e.g. Cleve, 1881). Their work was continued into the 20th Century by notable specialists, including Fritsch (e.g. Fritsch, 1918) and co-worker Rich (e.g. Rich, 1932). During the 1950's and 1960's the acclaimed diatom specialist, Dr. Bela J. Cholnoky, produced over 40 papers dealing with many of the diatom species found in southern Africa (e.g. Cholnoky, 1960). Later, Giffen published much valuable work in the 1960's and 1970's, dealing with marine and estuarine diatoms along with several accounts of freshwater species to be found in the Eastern Cape region (e.g. Giffen, 1966). The work of Schoeman and Archibald in the late 1970's and early 1980's has made an invaluable contribution to the knowledge of both the taxonomy and ecology of the diatoms. The most noted work of these two authors being 'The diatom Flora of Southern Africa', the first volume of which was published in 1976 (Schoeman & Archibald, 1976-1980). Further important contributions by these two authors include a detailed investigation of the Genus *Amphora* in a series of papers entitled 'Observations on *Amphora* species (Bacillariophyceae) in the British Natural History Museum' (e.g. Schoeman & Archibald, 1986).

2.1. THE STUDY OF DIATOMS IN CENTRAL AFRICA

Studies on the diatom flora of rivers and streams in central Africa have been rather restricted compared to the East African Great Lakes (Lakes Malawi, Tanganyika and Victoria). A short overview of the algal studies on the African Great Lakes and smaller lakes is given by Cocquyt (2006). Among the earliest diatom reports on lotic ecosystems in central Africa not related to the great lakes are West (1907) and Müller (1903, 1904, 1905, 1911) followed in the mid 20 century by Hustedt (1949) and at the end of last century by Mpawenayo (1996). Diatom reports from rivers and small water-bodies in DR Congo, formerly Belgian Congo and Zaire, are limited: up to the present around 260 diatom taxa were reported by Kufferath (1948, 1956a, b), Cholnoky (1964) Compère (1989, 1995) and Golama (1996), although some unpublished theses at universities in DR Congo were conducted. In Zambia and Congo-Brazzaville on the other hand, no publications dealing with diatoms have been produced. But since 2013 some papers dealing with the description of new diatom species from the Congo and Zambezi basins were published, e.g., Cocquyt et al. 2013, 2014, Taylor et al.

2014a, b. Attempts to use diatoms as a tool for water quality of rivers in central Africa started only recently on some small rivers in the vicinity of Gombe Stream National Park in Tanzania (Bellinger et al. 2006).

2.2. The use of diatoms as indicator organisms in South Africa

The potential of diatoms as indicators of water quality was realised in South Africa many years ago. Cholnoky (1968) describes the application of the Thomasson (1925) community analysis, which he adapted to determine water quality using benthic diatom community composition. Use of the Thomasson community analysis allows for comparisons to be made between sites in the same river, or it may be used to track changes at a single site. One aspect of water chemistry is chosen for study, e.g. the amount of nitrogenous effluent. First the sum of all the species of the genus *Nitzschia* within a particular diatom community is calculated as an abundance value. The genus *Nitzschia* is known generally to be nitrogen heterotrophic (able to utilise organically bound nitrogen), and therefore the relative abundance of this genus in a sample gives a reflection of the amount of nitrogenous pollution at the study site. Similarly, abundance values of the acidobiontic diatom genus *Eunotia* can be used to track a pH gradient in a river system. Cholnoky (1968) obtained good results using this index, but the user of the Thomasson analysis method needs to have an in-depth knowledge of the autecology of individual diatom genera and species to draw accurate environmental conclusions based on diatom community composition. Cholnoky's application of the Thomasson analysis method was a forerunner of modern autecological indices, which have since become more accurate due to the development of correspondence analysis, with the advantage of being able to assign exact tolerance limits for chemical variables to not only genera, but also species.

Archibald (1972) attempted to relate diversity in some diatom communities to water quality. The diversity index approach proved to be unsuccessful, with Archibald concluding that diversity of species within a particular diatom community provides an unreliable reflection of water quality. Although Archibald's attempt to use diatoms as bio-indicators failed, the diversity approach was a parallel development in water quality monitoring with European countries in using microalgae to monitor water quality.

Schoeman (1976) used diatom indicator groups in the assessment of water quality. Schoeman simplified the community analysis method of Cholnoky (discussed above) by dividing diatom associations into four groups, each with their own particular ecological requirements. Only the groups or associations were then reflected in the table of results, instead of the lengthy tables used by Cholnoky. Schoeman concluded that these diatom associations or groupings could be successfully employed to assess the quality of running waters especially in regard to the trophic status. Round

(1993) also came to the conclusion that Schoeman (1976) found a good fit between groups of diatoms and chemical levels in the Jukskei-Crocodile River system, and went on to comment that the species used were similar to those in Europe.

In 1979 Lange-Bertalot developed a monitoring system based on groups of diatoms with similar tolerances towards pollution. Lange-Bertalot's "saprobian" classification system proved, after certain modifications, to be highly successful. Schoeman (1979) tested Lange-Bertalot's (1979) method in the upper Hennops River, South Africa and found a good correlation between the species composition of the diatom communities studied and the water quality. Unfortunately, this parallel development with Europe in the study of the application of diatoms as bio-indicator organisms terminated then in South Africa with Schoeman's (1979) work.

Diatoms, as indicators of water quality, were only again investigated in depth in South Africa by Bate et al. (2002). The investigation attempted to relate a descriptive index, based on a dataset for the environmental tolerances of diatom species found in the Netherlands, to water quality in South Africa. The environmental variables generated by the van Dam et al. (1994) index include: pH, conductivity, oxygen requirements, trophic status, saprobian 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) came to the conclusion that benthic diatoms could be useful and that they give a time-integrated indication of specific water quality components. However, Bate and co-workers went on to state that the particular data set tested in their study (that of van Dam et al., 1994), could not be transposed directly for use under South African conditions.

2.3. Development of European diatom-based indices

The various European diatom indices can be divided into different classes. The majority of the indices used are based on the weighted average equation of Zelinka & Marvan (1961) and have the basic form:

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$$index = \frac{\sum_{j=1}^{n} a_j s_j v_j}{\sum_{j=1}^{n} a_j v_j}$$

where a_j = abundance (proportion) of species j in a sample, v_j = indicator value and s_j = pollution sensitivity of species j. The performance of the indices depends on the values given to the constants s (indicator value) and v (pollution sensitivity of species) for each taxon and the values of the index ranges from 1 to an upper limit equal to the highest value of s. Diatom indices differ in the number of species used and in the values of s and v which have been attributed after compiling the data from literature and from canonical correspondence analysis (Prygiel & Coste, 1993).

In 1979 Descy proposed the first true diatom index using the equation of Zelinka & Marvan (1961) on the basis of an investigation carried out on the Belgian section of the Sambre and Meuse Rivers (Prygiel et al., 1999).

Using Descy's method (Descy, 1979) Coste (in CEMAGREF, 1982) proposed an index known as the Specific Pollution sensitivity Index (SPI). The SPI index is based on 189 surveys carried out during a national monitoring programme in the period 1977 to 1980 at sites in the Rhône-Méditerranée-Corse basin. The index has been updated since 1982 in order to incorporate changes in taxonomy and new knowledge of diatom ecology.

Following the SPI, a Generic Diatom Index (GDI) was proposed (Coste & Ayphassorho, 1991) containing 174 taxa, including new genera, proposed by Round et al. (1990).

Leclerq & Maquet (1987) applied the method of Descy (1979) to the Belgian Ardennes watercourses (Samson catchment area). The authors proposed new s and v values for 210 species, following an exhaustive compilation of the autecological data in scientific literature. The index was updated (Leclercq, 1995), and now includes 403 species.

In 1991 Descy & Coste developed a diatom index for use in general water quality monitoring across Europe. The Commission for Economical Community index (or CEC) is calculated from a two-entry table, which contains 208 taxa. Horizontally, there are 8 groups of taxa ranked according to decreasing tolerance for pollution by biodegradable organic matter; vertically from left to right, there are 4 subgroups of the more stenoecous species representing the upstream-downstream succession along a theoretical running water ecosystem.

The Artois-Picardie Diatom Index (APDI; Prygiel et al., 1996) was the result of the need expressed by French water management for a technique for wide application in monitoring networks. The APDI was designed to combine ease of use and reliability with standardised techniques. An attempt was made to reduce the number of units to be counted, the level of identification and a reduction in number of taxa to those of the most significance for index calculation (i.e. those taxa with a high indicator value). The requirements for ease of use and reliability were met by combining the most recent version of the GDI index and the SPI index, yielding an index based on the identification of 45 genera and 91 species.

The wide use of GDI and SPI in France lead to the creation of the Biological Diatom Index (BDI; Lenoir & Coste, 1996) to meet the need for an index capable of being applied to monitoring networks throughout the whole of France. The BDI was designed on the basis of 1332 biological and physico-chemical surveys and includes 1028 diatom species and varieties. To maximise the usability of the BDI, morphologically similar species that are difficult for the non-specialist to identify with light microscopy were combined. This reduced the number of taxa. Rare species (less than 5% of the inventory) were eliminated from the list, which resulted in 209 taxa being kept (Prygiel & Coste, 1999).

Dell'Uomo (1996) proposed an index known as the Eutrophication/Pollution Index (EPI). The EPI was designed on the basis of investigations concerning 8 measurement stations in the river Chienti, a watercourse in the Central Apennines, Italy. The EPI is a specific sensitivity index, which integrates the saprobic (pollution tolerance), the trophic (trophic levels) and halobic (specific salinity requirements) aspects attributed to 93 diatom species.

Slàdeček (1986) applied the method of Descy (1979) in the context of the saprobic system. Saprobity refers to the differing levels of tolerance or sensitivity towards organic pollution (domestic and industrial). The values within the formula of Zelinka & Marvan (1961) of s (pollution sensitivity) and v (indicator value) are attributed to 323 species according to their affinity for organic material expressed in the measurement of BOD5 (Biochemical Oxygen Demand; Slàdeček, 1973, 1986).

Schiefele and Kohmann in Hofmann (1996) proposed a Trophic Diatom Index (TDI) on the basis of a three year study of 31 sampling sites in 5 German federal states. Indicator values relating to dissolved inorganic phosphate (DIP), total phosphate (TP), nitrate and ammonia were calculated for 105 diatom species. The formula of the trophic diatom index conforms to the saprobic index of Zelinka & Marvan (1961), and is intended to be its trophic counterpart. As a measure of the indicator quality, species-specific tolerances are weighted (1 to 7) and included into the calculation. Analogous to the saprobic system, the TDI divides quality status into seven levels covering oligotrophic to hypereutrophic conditions. This TDI index is only calibrated for mesotrophic to hypereutrophic conditions (Prygiel et al., 1999).

A similar Trophic Diatom Index (TDI) was proposed by Kelly & Whitton (1995), this based on investigations at 70 sites representing 14 hydrographical basins located in England and Scotland. The TDI index is not a general quality index, but should be considered an auxiliary tool for decision-making on phosphorus treatment in wastewater plants. The index should not be used on its own but should be complimented by the percentage of organic pollution-tolerant taxa. Easy identification and high indicator values were the criteria for the selection of 86 taxa. A sensitivity value between 1 and 5 was given to each taxon, depending on the concentration at which taxa were most abundant. The final value is comprised between 1 (very low nutrient concentrations) and 5 (very high nutrient concentrations). This technique is original in that, while working with species and genera in a way, which is analogous to APDI (Prygiel et al., 1996), it also takes into account the cell size of the species. A number of changes have been implemented since the 1995 Kelly & Whitton paper, namely scale extension from 1-5 to 1-100, removal of predominantly planktonic taxa from the calculation of the index and slight changes to pollution sensitivity and indicator values for some taxa (Prygiel et al., 1999).

Diatom analyses are included in the European Water Framework Directive of 2000 and with implementation date of 22 December 2003.

3. Recent application of diatom indices in southern Africa

3.1. Why use diatoms as indicator organisms?

No single group of organisms is best suited for detecting the diversity of environmental perturbations associated with human activities (Kelly, 2002). If the maintenance of ecosystem integrity is the aim of environmental management of a river system, the need to monitor the status of different taxonomic groups is vital. Diatoms provide interpretable indications of specific changes in water quality, whereas invertebrate and fish assemblages may better reflect the impact of changes in the physical habitat in addition to certain chemical changes (McCormick & Cairns, 1994).

Round (1991) lists several reasons why animal (fish and aquatic macroinvertebrates) components of an ecosystem may not provide a satisfactory index system. Animals have complex reproductive cycles which are often linked to the seasons; animals are largely motile and this may cause difficulty during sampling; animals may have many different life stages and may undergo metamorphosis; animals have specific habitats and niches; they are actively grazed; and closely linked to flow conditions, rendering in their uneven distribution from headwaters to estuaries. In addition, watercourses that are too deep or dangerous to wade across may prove difficult if not impossible to evaluate using a macro-invertebrate index along the length of the river.

Diatoms have several advantages over the animal (fish and aquatic macroinvertebrates) component of streams and rivers. Diatoms are an abundant, diverse and important component of algal assemblages in freshwater bodies. Diatoms comprise a large portion of total algal biomass over a broad spectrum of trophic levels (Kreis et al., 1985). While diatoms collectively show a broad range of tolerance along a gradient of aquatic productivity, individual species have specific habitat and water chemistry requirements (Patrick & Reimer, 1966; Werner, 1977; Round et al., 1990). In addition, diatom communities live in open waters of lakes (plankton), or primarily in association with plants (epiphyton), rocks (epilithon), sand (epipsammon) or mud (epipelon) in littoral, nearshore habitats.

Eutrophication of surface waters has a severe influence on general water quality. Numerous problems are posed in the chemical monitoring of eutrophication. Criteria for assessing trophic status from total phosphorus concentrations are based on annual average values (OECD, 1982) and, in turn, criteria for assessing trophic status from total nitrogen are based on averages for the summer months (DWAF, 1995). The ratio between these two elements needs to be determined before an accurate assessment of trophic status can be made.

It is generally accepted that invertebrate-based indices do not provide a reliable indication of eutrophication. For this reason it is better to take direct measurements of the photosynthetic community (Kelly, 1998). Diatoms are the preferred organisms used in bio-monitoring of eutrophication as they are sensitive to changes in nutrient concentrations (Pan et al., 1996), supply rates and ratios (e.g., Si:P; Tilman, 1977; Tilman et al., 1982). Because diatoms are primarily photo-autotrophic organisms, they are directly affected by changes in nutrient and light availability (Tilman et al., 1982). Each taxon has a specific optimum and tolerance limit for nutrients, which can usually be quantified to a high degree of certainty (e.g. P: Hall & Smoll, 1992; Reavie et al., 1995; Fritz et al., 1993; Bennion, 1994, 1995; Bennion et al., 1996; N: Christie & Smol 1993).

Diatom assemblages are typically species rich. This diversity of diatoms in different population densities, composition and overall abundance, contains considerable ecological information. Moreover, the large number of taxa provides redundancies of information and important internal checks in datasets, which increase confidence of environmental inferences (Dixit et al., 1992).

In addition to the above factors the response of diatoms to perturbation and recovery is rapid (Zeeb et al., 1994). Diatoms have one of the shortest generation times of all biological indicator groups (Rott, 1991). They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration success. Rapid immigration rates and the lack of physical dispersal barriers ensure that there is little lag-time between perturbation and response (Vinebrooke, 1996).

Round (1993) lists numerous reasons why diatoms are useful tools for bio-monitoring, amongst which the following bear special relevance to the southern and central African situation: diatom-based methods are cost effective; data is comparable (national and international); techniques are rapid and accurate; identifications and counts can be done by trained non-specialists, if they are provided with illustrated guides. Diatom-based indices could be particularly valuable in assessing rivers because a one-time assay of species composition of diatom assemblages in the system could provide better characterisations of physical and chemical conditions than conventional physico-chemical techniques (Stevenson & Pan, 1999). In addition, by sampling stream biota, a reflection of the biological integrity of the stream may be gained. The structure of the community may not directly reflect the measured concentrations of water quality variables. This may be due to a number of reasons: either the chemical constituent was not sampled for or, if sampled it was below the levels of detection in the particular laboratory performing the analysis, or, either synergistic or antagonistic reactions took place between several chemical constituents within the stream or river. For this reason, measuring the integrity of the biotic community sampled, rather than just the relationship between biota and chemical concentrations, provides an indication of general stream health, as stream biota are directly exposed to all the elements within the particular water body which they inhabit. The community structure of a selected group of organisms provides an integrated reflection of all the chemical variables that influence that particular group of biota.

Taxonomic difficulties may also be avoided by using a simplified diatom index such as the Generic Diatom Index (GDI) of Coste & Ayphassorho (1991). GDI allows for the determination of water quality at a particular site, based on the identification of diatoms to the genus level. GDI index has been found comparable to indices such as the Specific Pollution sensitivity Index (SPI; CEMAGREF, 1982), which is based on a large number of taxa (Kelly et al., 1995; Kwandrans et al., 1998). The genus-level approach has also proved to be successful in Taiwanese waters using a specific index based on only six genera and the ratio of occurrence between these six genera (Wu & Kow, 2002). Strong correlations were found between the Taiwanese generic index and other diatom-based indices of water quality (Wu & Kow, 2002). Currently this may be the only sensible approach to adopt for central African rivers (see discussion below).

3.2. The application of European diatom-based indices in south Africa

Diatom indices developed in Europe were identified as possible useful tools for monitoring water quality and were tested in South Africa. Taylor (2004) and Taylor et al. (2007a; 2007b) examined the used of numerical diatom indices for indicating water quality in some of the most important river systems in South Africa. In general, these studies conclude that European, diatom-based numerical water quality indices could be used with success in South Africa. However, Taylor et al (2007b) highlighted the following potential problems:

i) The list of taxa included in the indices needs to be adapted to the studied region. Most European diatom indices may be used in many regions and also in South Africa as they are based on the ecology of widely distributed or cosmopolitan taxa. However, special attention should be paid to taxa occurring in pristine water (e.g. *Achnanthidium standeri (*Cholnoky) J.C. Taylor, Morales & Ector) as well as endemic taxa which are absent in the indices reference lists (e.g. *Gomphonema venusta* Passy, Kociolek & R. L. Lowe). When these taxa are abundant, water quality may be misinterpreted.

ii) Diatom taxonomy is undergoing rapid changes, especially at the genus level. Local floras, guides and methods to be used must be consistent. Some European indices (as discussed above) have been proposed in the seventies or in the eighties and have never been revised. Thus, several common and abundant taxa, some of which being newly or recently described, may not be taken into account and lead to erroneous results. There are also several different approaches to taxonomy when calculating index scores. For example, *Achnanthidium pyrenaicum* (Hustedt) H. Kobayasi is part of the BDI (Biological Diatom Index, Lenoir & Coste 1996) taxa list, even if lumped with *Achnanthidium minutissimum* (Kützing) Czarnecki, but is not considered in other European indices such as TDI (Trophic Diatom Index, Kelly & Whitton 1995) for example. Such an exclusion will possibly change index scores as these two taxa have a different ecology, *A. pyrenaicum* is characteristic of pristine calcareous rivers while *A. minutissimum* is considered as a cosmopolitan pioneer taxon. In the case of BDI, many taxa have been lumped because of the difficulty to separate them in routine surveillance, even if their ecology is different.

iii) It has been highlighted in other studies that classification systems based on species tolerances should be carefully considered as built, to a greater or lesser extent, from local data. For example, Rott et al. (2003) noted that when using BDI, resulting index scores classified Austrian rivers as relatively good, even though large nutrient loads should have lead then to be classified as eutrophic, poor quality rivers. It should be noted that BDI was developed from data collected from the French national monitoring network which was aimed almost solely at monitoring impacts on water quality.

The index approach was deemed useful in South Africa to provide information on water quality impacts on rivers and streams. The studies also demonstrated that many widely distributed diatom species have similar environmental tolerances to those recorded for these species in Europe and elsewhere.

4. Diatom taxonomy and diatom species concept drift

When diatoms were first systematically studied and documented, a brief description was accompanied by an often minute drawing (e.g. Kutzing, 1844). These drawings were also made from freshly collected material or material dried to thin sheets of mica. Surprisingly despite this some species concepts remain remarkably stable (see discussion in Taylor et al., 2014c). However, others are far less stable, especially when the original author of the taxon did not provide a clear species concept (see discussion below). As diatom studies advanced, diatoms were documented, again mostly with drawings, but from cleaned material mounted in media with a high refractive index allowing the morrphology of the cells to be better observed, and such drawings are open to interpretation. When photomicroscopy became more accessible and commonly used species concepts could be firmly established. Added to this the rules for botanical nomenclature changed in 1958 necessitating the description of a species from a single type slide/sample allowing less room for error in interpretation of the original authors concept by later workers. Drawings of cells are always open to interpretation and shifts come about as one author identifies what he assumes to be a previously described taxon and then in turn often illustrate this taxon themselves (their concept). This new illustration may sometime be easier available to the scientific community than the original. If the concepts of the species differ the newer illustration can take precedence simply because of the availability of literature. This has been in particular true for Africa as literature, even now with the availability of electronic resources, still remains difficult for central African scientists to obtain locally. With drawings as illustrations these subtle shifts can occur several times distancing the species far from its original concept and perhaps even from the generic classification. As mentioned before, as photographic floras were published, species concepts became more stable but these stable concepts may still have been based on misinterpretation. Several attempts to remedy this problem have been made: Schoeman and Archibald (1976-1980) set about describing in detail the diatom flora of South Africa by checking the type slide of each species they discussed, collecting together the original drawings, light microscope and electron microscope images. Unfortunately for a number of reasons this work was discontinued.

Recently however, there has been a resurgence within the diatom community of the notion that we cannot accurately describe and discuss the ecology of taxa without referring to the type material (e.g. Taylor et al., 2014c, Morales et al., 2013, Wetzel et al., 2013). However such research is time consuming and the investment of time may not be necessary to successfully apply indices. For this reason the use of species based indices is not currently recommended for central Africa. We propose for the moment that genus based indices be used in this region.

5. Conclusions and recommendations for the use of diatom indices in central Africa

For the foreseeable future the application of species level diatom indices will not be possible in central Africa, the reasons for this are manifold but include in particular the following:
- diatom species, and even some genera, from this region are relatively unknown and many un-described;
- published literature from the region on species identification and environmental tolerances is scarce but necessary to construct indices;
- lack of expertise in central Africa to identify diatoms.

In South Africa when index testing began, although exhaustive knowledge of the local taxa and their requirements was not known, these indices were applied in river monitoring programs to reflect water quality (Taylor et al., 2007a). Although this is far from an ideal situation, the inclusion of these techniques allowed for sample and data collection which in turn could be used to fill taxonomic gaps (e.g. Taylor et al., 2010) and gain information on species distribution and environmental tolerances. Coupled with the production of guides for identification and methodology (e.g. Taylor et al., 2007b; 2007c) the technique has now gained impetus and has become a routinely used part of the suite of biomonitoring tools nationally.

We would propose a similar solution for central Africa. As discussed, species identifications cannot be made with any great certainty, however genus identifications may be simpler to achieve. Recently diatom taxonomy and nomenclature have undergone a number of changes, in particular very large diatom genera have been split into more natural groups. These splits also very often have very specific environmental tolerance (e.g. *Humidophila* (Lange-Bertalot & Werum) R. L. Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová). In essence there are now more genera so that we can expect greater resolution in the use of genus-based indices. If accurately identified taxa could be used for the calculation of a genus level index and this in turn could be used to indicate, with some degree of accuracy, reigning environmental conditions. A region specific guide is a vital resource. Such guides are by no means exhaustive, neither do they pretend to be, but instead provide a first introduction into the world of diatoms for many students and other interested workers. Once a reliable guide (coupled with relevant methodology) to the genera of tropical Africa is available, scientists in central Africa can receive adequate training in diatom identification and begin with the application and testing of indices based on diatom genus level.

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APPENDIX 2 – Sample pages from proposed volume of 'AbcTaxa' – scheduled for publication mid 2015

Bacillariophyceae I Cymbellales I Anomoeoneidaceae I *Anomoeoneis*

Anomoeoneis Pfitzer 1871

Type species: *Anomoeoneis sphaerophora* Pfitzer

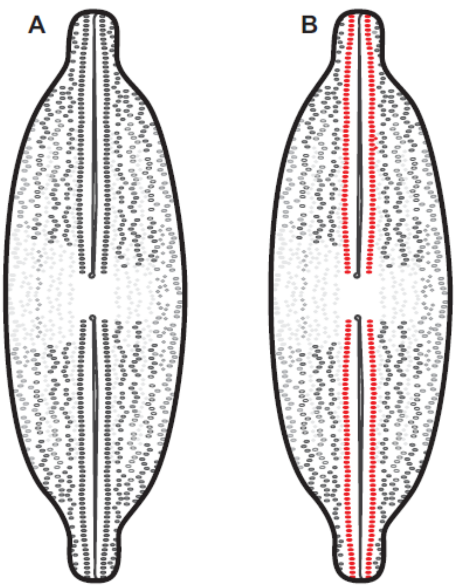
SYNONYM:
Brachysira Kützing 1836 pro parte

Characteristics - This genus is most noticeably distinguished in light microscopy by the scattered areolae on the valve face forming uneven **transapical lines** (Fig. X; A). A number of '**ghost areolae**' (pictured as light grey dots Fig. X) are found on the valve face and are especially visible in the **central area (**Fig. X;H), these areolae do not perforate the **valve face**.

Plastid structure - The single plastid is large and occupies most of the cell (Fig. X; A, B), it has two lobes, one appressed to each valve face forming a H-shape when seen from the girdle (Fig. X; C). One large pyrenoid is found adjacent to the cell margin. The plastid arrangement is similar to that of *Cymbella* and *Gomphonema*, hence its placement in the order Cymbellales.

Identification of species - Species and varieties in this genus are distinguished based on cell size and shape as well as the shape of the apices.

Ecology - Cells solitary, motile. Commonly found in waters of higher electrolyte content.



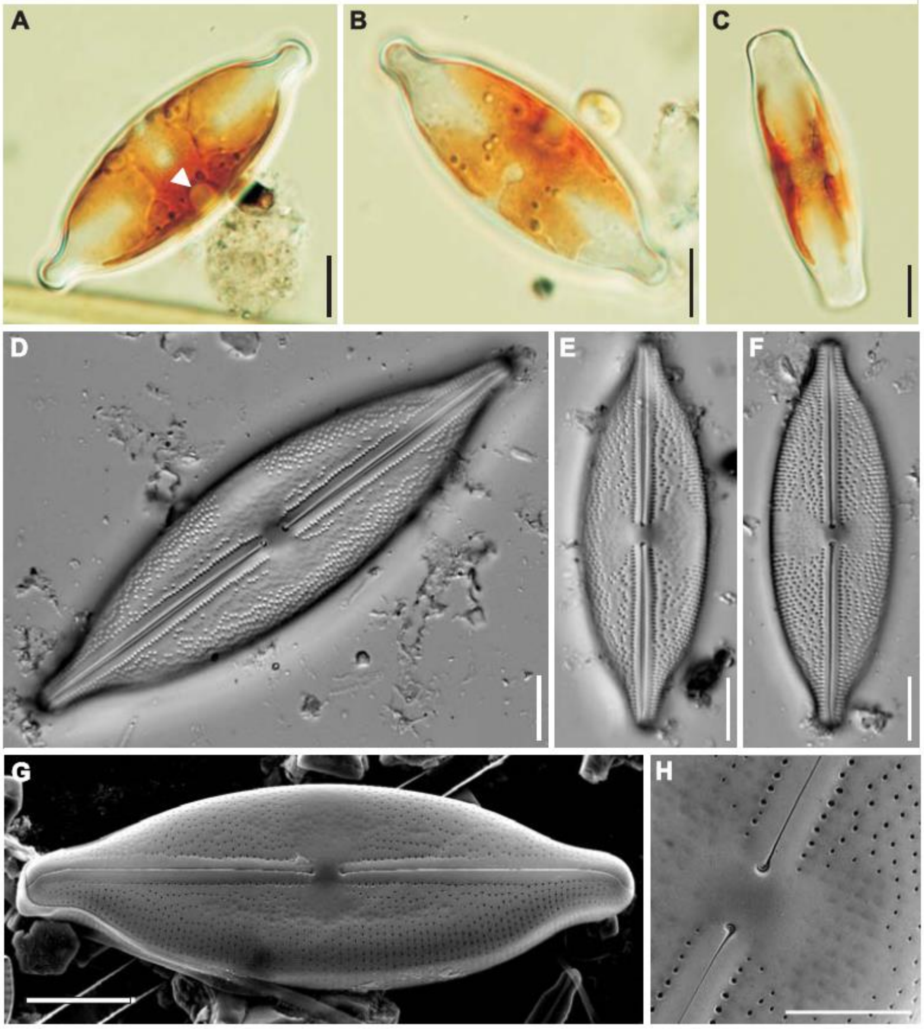


Fig X. *Anomoeoneis sphaerophora.* **A**. Living cell, note pyrenoid (arrow) next to the center right hand margin. **B**. Living cell, note shape of plastid. **C**. Living cell, girdle view, note bridge between the two plates of the plastid. **D-F**. LM of cleaned valves. **G**. SEM of complete cleaned valve. **H**. SEM detail of central raphe endings. Scale bars = 10 µm (A-G), 5 µm (H).

Mastogloia **(Thwaites) W. Smith 1856**

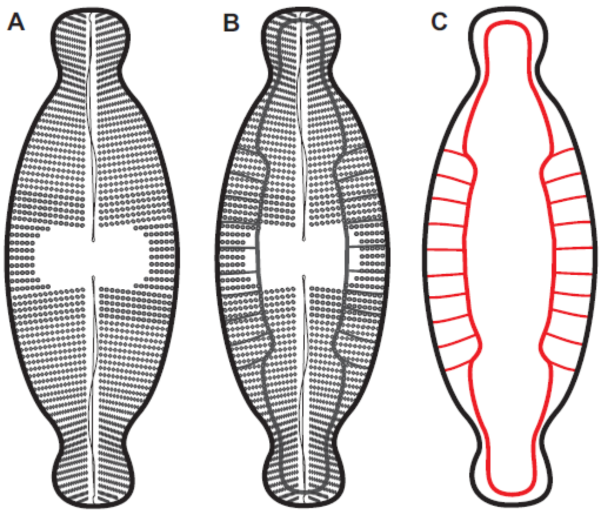
Type species: *Mastogloia dansei* (Thwaites) Thwaites ex W. Smith

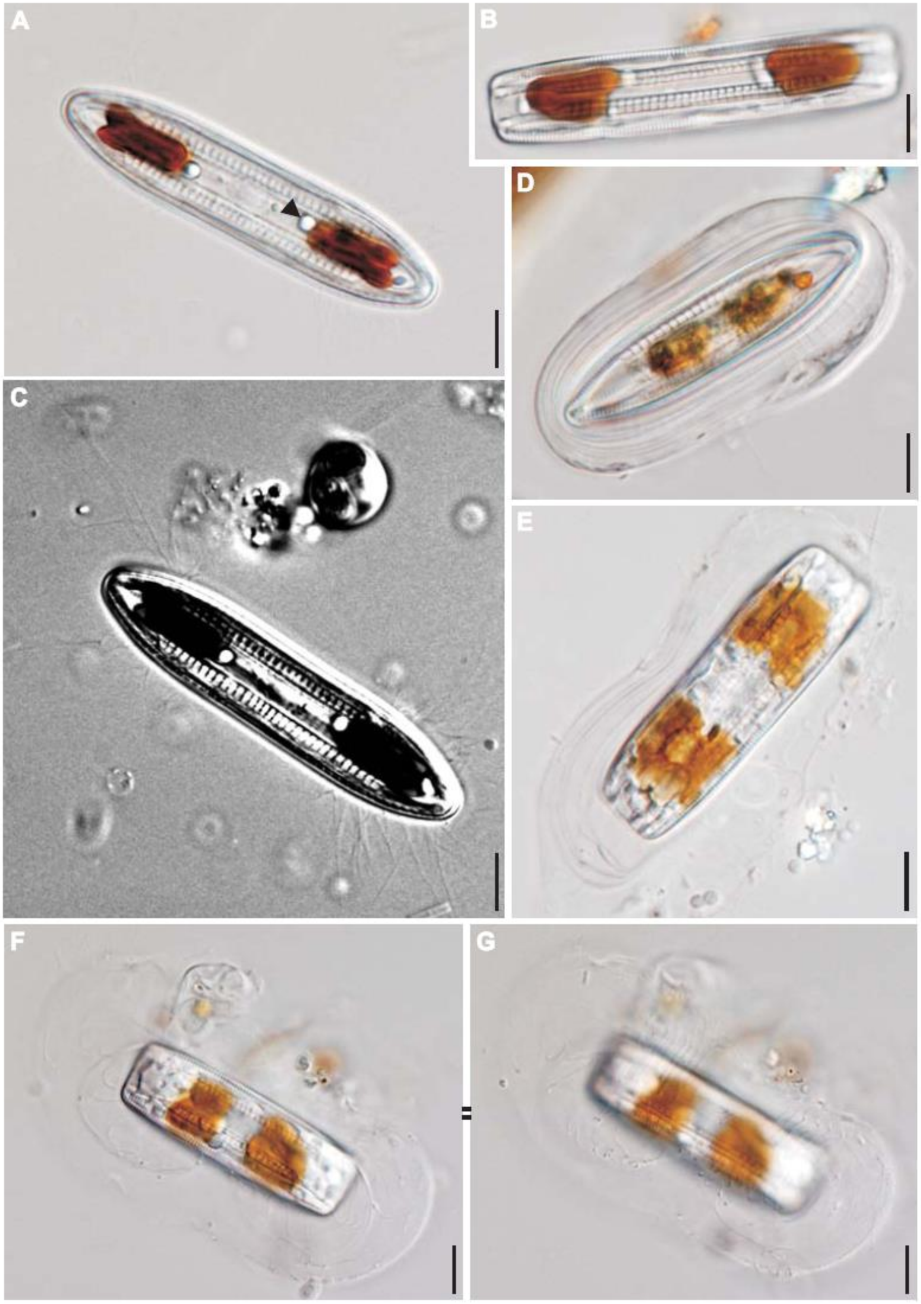
Characteristics - This genus is most noticeably distinguished in light microscopy by the **partecta** or chambers (Fig X; B) associated with the first girdle band or **valvocopula** (Fig. X; C). When seen from the girdle in SEM the large perforations extending into the **partecta** are clearly visible. The raphe also usually appears highly sinuous and complex. Areolae are large and clearly visible in LM.
In living cells mucilage threads are exuded from the **parteca** (Fig X; C), this mucilage often encapsulates the entire (as illustrated in Fig. X; C-G) and may play some role in allowing these cells to survive dessication and other unfavorable circumstances such as shifts in osmotic pressure.

Plastid structure - There are two small double lobed plastids found at each end of the cell (Fig. X; A, B) with a pyrenoid between the two lobes of each plastid (Fig. X; E).

Identification of species - Species in this genus are distinguished based on cell size and shape as well as the shape of the apices. Striae density and orientation are also of importance as well as the size of the areolae.

Ecology - Cells solitary, motile or encased in mucilage. The majority of species are brackish or marine but some are also found in fresh waters of higher electrolyte content and calcium rich waters .




A
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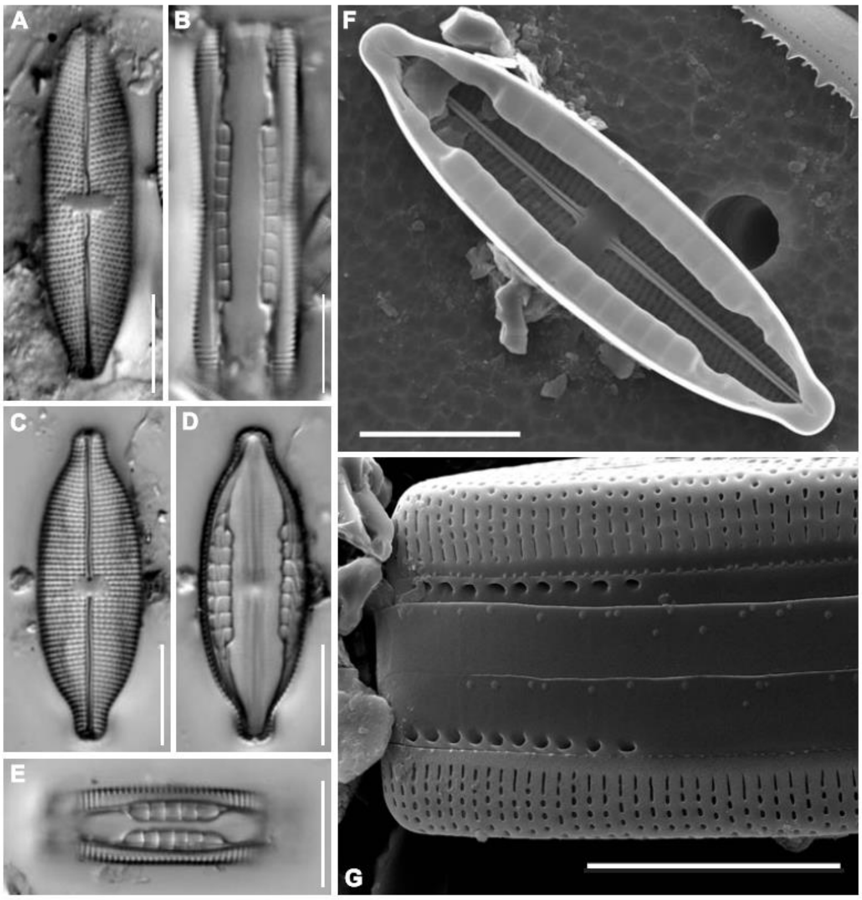

Fig X. *Mastogloia* spp. **A-E**. LM of cleaned valves. **A, C.** Valve view. **B, E.** Girdle view **D**. Detail of the valvocopula. **F**. SEM internal view of valvocopula showing the partecta. G. SEM of girdle note external openings of the partecta through which the mucilage is exuded. Scale bars = 10 µm (A-F), 3 µm (G).

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Fig X. *Mastogloia*. **A**. Living cell, note the lipid bodies associated with each plastid (arrow). **B**. Living cell, girdle view. **C**. Living cell (high contrast), mucilage threads protruding from the partecta. **D-G**. Living cells encapsulated in mucilage, note threads protruding from partecta. Scale bars = 10 µm (A-G).