

Süßwasserflora von Mitteleuropa
Freshwater Flora of Central Europe

B. Büdel · G. Gärtner · L. Krienitz
M. Schagerl (Hrsg. / Eds.)

Øyvind Moestrup · António J. Calado

Dinophyceae

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*Dedicated to the memory of
Hans Rudolf Preisig (1949–2011)*

Preface

The present work began in 2004 with an e-mail from professor Hans R. Preisig, Zürich, Switzerland, in which he suggested to the first author to write a new version of the dinoflagellate volume for the Süßwasserflora von Mitteleuropa series, the previous version being nearly sold out. After some hesitation it was decided to take up the challenge, pending that some manpower could be obtained to assist collecting literature and to help solving some of the many taxonomic problems. In February 2005 a 2-year grant was obtained from the Villum-Kann Rasmussen Fund, which allowed Karin Lindberg to be attached to the project. The new edition of the flora was considered a challenge as the previous edition had received some criticism for the merging of many species. The number of species in the previous edition was 169, in 30 genera. The present version includes some 350 species in 65 genera, in other words a doubling in number of both species and genera.

It was decided to consult all original descriptions of freshwater dinoflagellates to evaluate whether they should be considered independent species. This was a major task, as the first dinoflagellate was described by O. F. Müller in 1773. Many species were described in the 1800s and early 1900s, sometimes in a very sketchy way, which made them difficult to identify and some of the literature difficult to obtain. In 2010 António José Calado became part of the project, the illustrations being one of his many responsibilities.

In early 2011 the work was speeded up during a 3-month stay for both of us at the Freie Universität, Berlin, by invitation of professor Klaus Hausmann. From then on, one chapter after the other was completed.

Literature was sometimes a problem but we have been able to obtain and scan all original descriptions into the computer, thanks to help from many colleagues, who not only assisted by obtaining difficult literature but also by translating selected parts from what to both of us were (are) rather exotic languages.

It was obviously not the intention to solve all the taxonomic problems before completing the manuscript, but during the preparation several problems were tackled, especially—by chance—concerning the thin-covered species, the woloszynskioids, and later also some of the thecate genera. A very great deal more needs to be done, however, and we hope that the present volume may serve as an inspiration for others to take up some of the taxonomic questions remaining.

It is an often repeated idea that freshwater dinoflagellates are distributed globally, and while it is true that many morphospecies are very widely distributed, data on their genetic setup have been accumulating only very slowly. Thus, evaluation of the identity of a certain morphospecies remains somewhat uncertain: do cryptic species exist in different parts of the world? Are some morphologically similar species identified from different geographical areas in fact different species? This also touches upon the species concept. It is, however, becoming apparent that clones of the same morphospecies are not always genetically identical when sampled in different parts of the world, and this raises questions on how much variation should be allowed before a species should be divided into two species, subspecies or cryptic species.

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Classification of the dinoflagellates is still somewhat unsatisfactory. This applies not only to the thin-covered or naked species, which are notoriously difficult, but also to many groups of thecate species. Assignment to genus is presently a major problem, as many of the old genera have been found to be polyphyletic and in need of better definition and circumscription, considering all available morphological, biochemical and genetic information.

In 2000, Daugbjerg *et al.* published an article on the naked species, in which the genera *Gymnodinium* and *Gyrodinium* were re-defined using a combination of ultrastructural and molecular data. Two additional genera were erected, and during the following years the genera *Amphidinium* and *Katodinium* were similarly re-circumscribed. However, many species formerly included on the old genera could not be assigned to the newly defined genera, pending lack of information. This problem applies also to many thecate species, not the least to those presently included in the genus *Peridiniopsis*. In the present book we have used the new generic names and circumscriptions as much as possible. The large assemblage of insufficiently studied naked species we have included in *Gymnodinium*, until more information can be obtained. This has resulted in numerous new combinations, many of which will be merged in the future or transferred to new genera. While we have included in the book all described species which we feel can be recognized, a number of species has been assembled in a chapter comprising taxa which we think have been described in such an incomplete way that identification is not possible. In some cases the original names were found to be illegitimate and in need of replacement, or the descriptions did not conform with the new generic concepts, and we have transferred the species to other genera. Two new genera are described: *Matvienkoella* and *Speroidium*, as are three new families, Amphidiniaceae, Gyrodiniaceae and Sphaerodiniaceae, and two new orders: Amphidinales and Tovelliales. There is little doubt that the number of genera will continue to grow in the future when more information becomes available, perhaps also the number of families and orders, but the total number of dinoflagellate species in freshwater is difficult to assess. Some of the described taxa will fall into synonymy while new ones will be described. More than 10 years have passed since this work began, and Hans Preisig sadly did not live to see the completed book. We dedicate the book to his memory. His premature death deprived Switzerland of one of its most capable freshwater phycologists, and we lost a dear friend.

Øjvind Moestrup and António J. Calado

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We wish to thank Hans Preisig, Zürich, for the invitation to write this book.

In Denmark, we thank the Villum-Kann Rasmussen Foundation for a 2-year scholarship to Karin Lindberg, who helped solving some of the many taxonomic problems associated with thin-covered freshwater dinoflagellates. We thank Karin for her enthusiasm, persistence and hard work throughout this agreeable period.

In Germany, it is a pleasure to thank Klaus Hausmann, Berlin, for inviting us to spend three months in his lab at the Freie Universität, working full time on the manuscript, and for assisting us (helped by several librarians at the “Botanischer Garten und Botanisches Museum Berlin, Dahlem”) in obtaining copies of the old literature, some of which was very difficult to find.

In Portugal, we thank Sandra Carla Craveiro, Aveiro, for helping throughout, notably during our stay in Berlin by making bibliographic material accumulated by the second author available to us over the internet, often within minutes of our request. She is also thanked for reading the entire manuscript, pointing out mistakes and inconsistencies, and helped with several figures.

In Japan, we thank Kazuya Takahashi, Yamagata, for providing drawings of *Leiocephalum pseudosanguineum* and *Asulcocephalum miricentonis*.

We finally wish to thank Karin Rengefors, Lund, Anke Kremp, Helsinki, and Gert Hansen, Copenhagen, for accepting our invitations to contribute chapters on ecology and on culturing of freshwater dinoflagellates.

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Part I

Introduction



Some highlights of the 200-year history of exploration of freshwater dinoflagellates, from O.F. Müller to Pierre Borelly

In the following, an account is given on some of the work on freshwater dinoflagellates during the first 200 years of exploration. Readers interested in earlier work on protists may consult the interesting and detailed account of protist research provided by Saville Kent (1880), the British Museum, covering the period from Leeuwenhoek's first observations (*ca.* 1675) to F. von Stein. It includes a small amount of information on dinoflagellates. For a general account of dinoflagellate exploration, see Taylor (1987), who concentrated on the marine species.

The Earliest Times

The history of freshwater dinoflagellate research may be said to begin in 1773, when Otto Friedrich Müller (1730–1784), a private teacher in the home of a wealthy Danish family at Frederiksdal, near Copenhagen, Denmark, published an account of the microscopic organisms from a number of lakes north of Copenhagen, including the species *Bursaria hirundinella* and *Vorticella cincta* (Müller 1773). Illustrations only appeared after his death (Müller 1786), and the former species was subsequently redescribed in 1793 as a member of the new genus *Ceratium* by the German Franz von Paula Schrank (1747–1835), the oldest generic name of a dinoflagellate still in use. Müller's second species represented the first description of a peridinioid.

Half a century passed before Christian Gottfried Ehrenberg (1795–1876) in Berlin published on dinoflagellates and other microscopic organisms, the work culminating in the first major book devoted to the study of protists, or “Infusionstierchen” (small infusoria animals) as Ehrenberg called them, *Die Infusionstierchen als vollkommene Organismen* (Ehrenberg 1838) (the term infusoria was coined by the German Martin Frobenius Ledermüller in Nürnberg as early as 1763, the term protist by Ernst Haeckel in 1866). This rare milestone book is now available on the internet. Ehrenberg gave the first formal name to the dinoflagellate group in 1830, as a “section” of family Epitepicha, and later as family Peridinea, which included also a number of volvocalean green algae (Ehrenberg 1830, p. 38; Ehrenberg 1831). The name was based on *Peridinium*, a generic name he gave in the *Infusionstierchen ...* to a number of dinoflagellate species, naked or armored. Most have subsequently been transferred to other genera. In German, Ehrenberg created the name “Kranztierchen”, Kranz meaning “ring”, as Ehrenberg believed the cingulum to carry a ring of cilia. In 1836, Ehrenberg observed a large eyespot

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in some species of dinoflagellates, and for these he created the generic name *Glenodinium*, “gleno” meaning eye.

Ehrenberg was the Hon. Secretary of the Prussian Academy, professor of zoology and natural history, and a recognized authority, to the extent that subsequent researchers confirmed his erroneous report of cilia in the cingulum. Indeed, Édouard Claparède (1830–1871) and Johannes Lachmann (1832–1860), authors of three major volumes on protists and other small organisms, published between 1858 and 1861, created the name *Cilioflagellata* for the group, in reference to the row of cilia in the cingulum and the flagellum in the sulcus. Even Friedrich von Stein (1818–1885) in Prague repeated Ehrenberg’s now 45-year-old mistake in his four-volume series on *Infusionsthier*, which contains some of the most detailed and beautiful drawings of freshwater and marine dinoflagellates ever published. Among Stein’s many new species and genera were *Gymnodinium* and *Hemidinium*, the former separated from *Peridinium* by lacking a cell cover of cellulosic plates, and the latter by having only half a cingulum. It was due to the sharp eyes of a young German researcher, Georg Albrecht Klebs (1857–1918) that the mistake of cingular cilia finally came out in the open, and from then on all could see a flagellum in the cingulum rather than a ring of cilia. Klebs’ (1883) observation resulted in instant demise of the term cilioflagellates, and after that dinoflagellates were no longer considered intermediate between flagellates and ciliates. Klebs considered the biflagellate condition to be typical of the group.

The End of the 1800s

The period around the turn of the century saw works by many dinoflagellate experts, most of them publishing in German. August Jacob Schilling (1865–?) was a German student of Klebs’ at a time when Klebs was employed by the Botanical Institute at the University of Basel, Switzerland, where he served as Rector in 1892–93. Schilling’s doctoral dissertation (Schilling 1891a) dealt with freshwater dinoflagellates, which by now had grown to 6 genera: *Hemidinium*, *Gymnodinium*, *Amphidinium*, *Glenodinium*, *Peridinium* and *Ceratium*. *Hemidinium* was thought to be restricted to fresh water, while *Amphidinium* had been described back in 1859 by Claparède & Lachmann for some marine species. Schilling apparently collected near Basel but he gave practically no information about localities. He increased the number of described species from 16 to 25, but his drawings are small and lacking in detail, and this has resulted in uncertainty on the identity of some of the species, in fact most of the 9 new species he described: 5 species of *Gymnodinium*, 3 species of *Glenodinium* and 1 species of *Peridinium*. The same year (1891) a Swiss, Eugène Penard (1855–1954), published the results of 3 months’ work on dinoflagellates from Lac Léman (Lake Geneva). He collected 11 species in the lake, 4 of which were described as new. To one he gave the rather confusing name *Glenodinium gymnodinium*, most likely the same species described later by J. Wołoszyńska as *Peridinium polonicum* (Wołoszyńska 1916), and now known as *Naiadinium polonicum*. Penard’s drawings are of a higher quality than Schilling’s and his descriptions more detailed, thus allowing recognition of, among others, his new species *Gymnodinium helveticum*, currently known as the only commonly found species of *Gyrodinium* in fresh water, *G. helveticum*.

While these studies were going on for freshwater dinoflagellates, major progress was being made on marine species by Otto Bütschli (1848–1920) and Franz Schütt (1859–1921), both in Germany, the former creating the first nomenclatural system for amphiesmal plates that caught on (based on an earlier system of Stein, and which Schütt compared with a floral diagram) (Bütschli 1885). A description of the various labelling systems is provided on page 23–25. Schütt created the now generally used terms amphiesma and pusule (Schütt 1895).

The Golden Period

During the first part of the 1900s, three further German-speaking men and one German writing woman dominated research on freshwater dinoflagellates: Ernst Lemmermann in Bremen, followed by Erich Lindemann in Berlin, Adolf Pascher at the German University in Prague, the university where Stein had worked only a short time before and where he at one time served as Rector, and Jadwiga Wołoszyńska in Lviv (German Lemberg, Polish Lvów), and shortly afterwards in Cracow. They all wrote in German, thus most of the classical literature on freshwater dinoflagellates is in German. In fact, at this time there were leading dinoflagellate experts working in each of the major towns Prague, Berlin, Cracow, Bremen and Heidelberg. It was a golden period for freshwater dinoflagellate research, but one is left with the impression that the experts rarely or never cooperated, nor had much contact with one another.

Lemmermann (1867–1915) was born in Bremen where he was employed for his entire career, first as schoolteacher, but working part-time at the herbarium. In 1909 he became full-time botanical assistant at the Museum für Natur-, Völker- und Handelskunde and he remained as such until his premature death from poor health. Lemmermann had close contact with Swedish phycologists and taught himself to speak and read Swedish during a three-month visit to Northern Sweden in 1907. He had previously received fixed plankton samples from various parts of Sweden, especially from O. Borge, Stockholm, and O. Nordstedt, Lund, and this resulted in a 209-page publication on plankton from Swedish waters in 1904. He described here *Peridiniopsis borgei*, the type species of *Peridiniopsis*, from a lake in Uppland, collected 1900. Lemmermann had a very broad general knowledge of freshwater algae, which enabled him to write or edit, in 1907–1910, a 712-page book on the freshwater algae of “Mark Brandenburg and adjacent areas”, in fact covering most of northern Germany. The dinoflagellate chapter, 120 pages long, includes a general account of dinoflagellate structure, occurrence, cell division, etc., followed by 12 pages of literature (283 references). The book contains descriptions of 11 genera and 79 species, the first milestone after Stein (1883). The number of described species had thus increased by a factor of 3 during the 20 years following Schilling and Pénard in 1891, and the number of genera had nearly doubled. New taxa described by Lemmermann in the book include the enigmatic and rarely observed *Lophodinium* (from South America!), still not obtained in culture, but refound several times.

Klebs' paper from 1912, 29 years after he clarified the cilioflagellate confusion, is another milestone in dinoflagellate research. Based mainly on material collected during his stay in Java, he described in this work, among other species, no less than

five of the presently known 10 genera of coccoïd freshwater dinoflagellates: *Cystodinium*, *Tetradinium*, *Hypnodinium*, *Stylodinium* and *Phytodinium*. *Cystodinium* had actually been seen and illustrated by Stein (1883) who believed it to be a resting stage of another dinoflagellate, but Klebs clarified this problem.

When Lemmermann died in 1915, several others took over. Adolf Pascher (see below) started his new project on the Süßwasserflora from Germany, Austria and Switzerland, and he asked Schilling, now in Darmstadt, Germany, to cover the dinoflagellates (1913). This is somewhat surprising, considering that Schilling had contributed little to dinoflagellate research since his dissertation 22 years before. The quality of Schilling's illustrations now improved, however, and the number of genera grew to 13, including those described by Klebs the previous year. The number of species totaled 52, plus 9 uncertain.

Erich Lindemann (1888–1945) served as high school teacher, first in present-day Poland, then after the First World War in Tempelhof, Berlin, retiring in 1924. He later worked at the Landesanstalt für Wasser, Boden- und Lufthygiene in Berlin-Dahlem, and produced, while writing scientific articles and working as editor of a new journal (*Schriften für Süßwasser- und Meereskunde*, from 1923), within a period of a few years no less than two large treatises of dinoflagellates: first (1925) in the Eyferth-Schoenichen series on “Natural history of microscopical freshwater organisms”, mentioned by the editor W. Schoenichen as not intended to compete with the “large, more specialized volumes” on freshwater organisms, but to introduce the organisms to the “friends of the world of microscopic freshwater organisms”. Nevertheless, Lindemann's chapter on dinoflagellates was one of the most detailed treatments of freshwater dinoflagellates at the time, 15 years after Lemmermann's work. The number of genera in Lindemann's first treatise had now risen to 17, including his own *Kolkwitzella*, but the number of species included was only 58, many lesser known species having been left out. Three years later Lindemann produced a detailed general account of dinoflagellates (this time both marine and freshwater) in one of the “specialized volumes”, Engler's *Die natürlichen Pflanzenfamilien* (1928). It started with ten pages of references in very small script and proceeded to genus-level (not species level) accounts of dinoflagellates. Lindemann described several new species during his short career, but many of these have not been accepted as separate species. In the 1928 account he must have felt the need to create higher level taxa, however, naming no less than 23 new dinoflagellate families, mostly comprising marine species. Lindemann was very interested in the culturing of dinoflagellates, and was given space (but apparently not funding!) at the Plant Physiology Institute of the University of Berlin. His culturing and life cycle experiments were done using a north window as light source (Lindemann 1929). Two years later, after publishing a very detailed account of dinoflagellates collected from present-day Indonesia and given to him for taxonomic treatment (Lindemann 1931), he suddenly stopped publishing.

Lindemann's contemporary Adolf Pascher (1881–1945) worked in Prague. Having been born in Bohemia, he moved to Prague as a student, remaining there for the rest of his life. He first published on freshwater algae in 1903, and over the years developed into the world's leading freshwater phycologist, his career only terminating after the Red Army entered Prague in May 1945, when he and his wife are reported having committed suicide. A main interest of Pascher's was taxonomy and phylogeny, and many of his reports of new species and genera touch upon phylogeny.

His publications are characterized by detailed observations, usually documented by high-quality illustrations, and they remain an important source of knowledge and inspiration. Pascher's influence on phycology during the first half of the 1900s was very great, not the least after he initiated the *Süßwasserflora* series, of which the present volume is part, and became editor of *Archiv für Protistenkunde*, for many years the leading journal on protists. According to Geitler "Pascher laid down a new foundation for protistological and cytological research" (Geitler 1946). New genera of dinoflagellates described by Pascher are *Cystodinedria*, *Dinamoebidium* and *Dinothrix*, in addition to the very unusual *Desmomastix* and *Desmocapsa*, which are still to be refound.

While Lindemann and Pascher worked in Germany and at the German university in Prague, respectively, Jadwiga Wołoszyńska (1882–1951) studied freshwater dinoflagellates in Poland, located at first in Lviv (then known as Lemberg and capital of the Habsburg Kingdom of Galicia and Lodomeria) and subsequently in Cracow, and years later surviving the Nazi occupation of Poland (1939–1945) by going underground. In the period 1915–1930 Wołoszyńska described a large number of dinoflagellate species, mostly from (present-day) southern Poland. She used a special staining technique that allowed her to study the amphiesmal vesicles, which in dinoflagellates with very thin cover can otherwise be difficult or impossible to see in the light microscope. She produced some of the most detailed drawings of freshwater dinoflagellate amphiesmal structure ever published, rivalling with the detailed information on size, shape and arrangement of amphiesmal vesicles of thin-covered or naked dinoflagellates that is currently obtained with the scanning electron microscope. Wołoszyńska described several new genera of dinoflagellates: *Sphaerodinium*, *Glenodiniopsis*, *Staszicella* and *Amphidiniopsis*.

In Switzerland, Robert Hippolyte Chodat (1865–1934), professor of botany in Geneva and working on higher plants, but also a major contributor to our understanding of morphological variation in green algae on the basis of pure cultures, also found the time to describe, in 1924 from Grand-Saint-Bernard, the unusual dinoflagellate *Bernardinium*, now known to be one of the few genera of the Tovelliales. It was recently shown to be exceptional also by occurring both with and without chloroplasts, the chloroplasts in the former case sometimes apparently non-functional (Fawcett & Parrow 2014).

The 1930–1950s: Times of Compilation, Lefèvre and Schiller

France appeared in earnest on the freshwater dinoflagellate world map with the 210-page monograph by Marcel Lefèvre (1897–1975) at the Natural History Museum in Paris, covering the genus *Peridinium* (and *Peridiniopsis*), and appearing in 1932. It contains descriptions of all freshwater species of the genus known at the time, in addition to forms and varieties, the only monograph so far devoted to this important group of freshwater dinoflagellates. The book contains no less than 915 drawings, in addition to some of the first light photomicrographs of freshwater dinoflagellates. The latter were taken by Lefèvre himself, and the book constitutes a work which will probably never be repeated nor surpassed. Lefèvre described

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34 species in the book and at the end provided a list of the taxa he considered to be insufficiently known (including *P. limbatum*!). Taxa thought to belong to related genera (including *Peridinium berlinense*, now *Tyrannodinium edax*), were also listed, as were species or varieties considered to be based on teratological (abnormal) specimens (altogether 11, all described by Lindemann!).

Descriptions of all known freshwater and marine dinoflagellates known at the time were compiled by Josef Schiller (1877–1960) in Vienna, in a two-volume work, *Dinoflagellatae* (Peridineae), the complete volumes appearing in 1933 and 1937 as part of *Rabenhorst's Kryptogamen-Flora*. Schiller's volumes are a compilation rather than a critical work but even so, these books remain, now 80 years on, an indispensable tool that dinoflagellate taxonomists consult regularly. In later publications Schiller went on to describe many new species of dinoflagellates himself, but his artistic talent was not well developed nor were his descriptions sufficiently detailed. Thus many of his taxa are difficult or impossible to identify.

In Hungary, Géza Entz jr. (1875–1943) published extensively on dinoflagellates from Hungary during the period 1905–1930. His 50-page article of *Peridiniopsis borgei* is probably the most detailed account of a single dinoflagellate published (Entz 1926).

The 1950–1970s

One of the most important floras on freshwater dinoflagellates was published by the Swiss medical doctor Gottfried Edouard Huber-Pestalozzi (1877–1966) in Zürich in 1950, as part of the series he edited, *Das Phytoplankton des Süßwassers*. 210 pages are devoted to dinoflagellates, all descriptions being as complete as possible and containing information about ecology and geographical distribution. As mentioned by Fott in his obituary of Huber-Pestalozzi (Fott 1967), all students of biology and ecology of fresh water have used these volumes. Indeed, it is difficult to envisage how he found the time to attend to his medical patients, as his hydrobiological work was done at the same time as his work as medical doctor. Switzerland had previously seen the publication of an early, general account of freshwater microorganisms, which included a few dinoflagellates (Perty 1852), and the above-mentioned accounts of the dinoflagellates from Lac Léman (Penard 1891), from near Basel (Schilling 1891a) and from the alpine area around Grand-Saint-Bernard (Chodat 1924).

Additional, very careful work on freshwater dinoflagellates from Switzerland was published by Hans Rudolf Christen (1924–2011), Winterthur, a famous chemist who also wrote a much-used textbook on chemistry. Christen's few but carefully prepared publications contain descriptions of several new species of dinoflagellates, thus his monograph of *Katodinium* (Christen 1961) contains 9 new species or combinations, out of a total of 23 species discussed.

Another freshwater phycologist who described and illustrated several new dinoflagellate species is Heinrich Leonhards Skuja (1892–1972). Skuja began his career in his native Latvia but fled to Sweden in 1944 when The Red Army occupied Latvia, spending the rest of his life and career at Uppsala University. Skuja covered many parts of freshwater phycology, and among his major works are the books on freshwater algae from central Sweden in 1948 and 1956 and from Lapland (Northern

Sweden) 1964, large works which will remain of lasting value both internationally and for Swedish freshwater biology. His descriptions are very detailed, and his hundreds of drawings are of a rare artistic quality, and have served as inspiration to many later phycologists.

At the National History Museum in Paris Pierre Bourrelly (1910–1995) continued in the tradition of Lefèvre and developed into one of the most knowledgeable freshwater phycologists. His 3 volumes on *Algues d'Eaux Douces* are standard books on freshwater algae (Bourrelly 1966 et seq.), which made Paris a reputed center for research on freshwater algae, and forced many a student to improve his or her proficiency in the French language. Bourrelly's 65-page treatment of the dinoflagellates appeared in 1970 (second edition 1985) and included original drawings of the genera. Bourrelly also provided keys to identification of orders, families and genera. Bourrelly marks the transition to the period in which electron microscopes began to play important roles for taxonomy and description of species. Bourrelly described *Thompsodinium*, based on Rufus Thompson's description of *Peridinium intermedium* from Kansas.

A new genus, *Bourrellyella* was named after Bourrelly by Baumeister (1957b) for a species of *Dinastridium*. Bourrelly preferred to retain this poorly known species within *Dinastridium* but it would not come as a surprise if future research reinstates *Bourrellyella* as a separate genus.

The World Outside Europe

From the account above, the strong emphasis given to European freshwater dinoflagellates is striking but not surprising. Tropical dinoflagellates were, however, treated by European specialists from time to time when preserved samples were brought back and given to the specialists. In fact tropical dinoflagellates were until relatively recently studied mainly from preserved samples.

In Asia, Ceylon (Sri Lanka) was initially one of the best-studied areas, as material was examined by Apstein (1907) and Lemmermann (1907b). Lemmermann (1905b) when given material from Singapore and Java, collected by W. Volz, reported one species of *Ceratium* and four species of *Peridinium*. One of the latter was considered to be undescribed and was named after the collector, *Peridinium volzii*, from Singapore. Additional material from Java was given to Wołoszyńska, collected by her supervisor Marian Raciborski, and this resulted in one of Wołoszyńska's first publications (1912). She found 8 species of dinoflagellates, three of which she considered new: *Peridinium raciborskii*, *P. gutwinski*, and *P. treubii*, the latter subsequently considered by Lefèvre to be a form of *Peridinium cunningtonii*. She was given additional material many years later, from both Java and Sumatra (Wołoszyńska 1930) and described new forms and varieties, in addition to *Peridinium parvulum* and *P. steinmannii*, both of which we consider to be part of the *Parvodinium inconspicuum* complex. The Danish high-school teacher and limnologist Gunnar Nygaard (1903–2002) also received material from Java, and described *Peridinium keyense* (Nygaard 1926). Lindemann (1931), however, gave the most detailed account of freshwater dinoflagellates from the Sunda Islands Sumatra, Java and Bali, and while many of his new species have not been accepted, *Glenodiniopsis pretiosa*, *Peridinium baliense*, and *Peridinium playfairii* are included in

the present book, although all three are in need of more studies. We have found no studies on freshwater dinoflagellates from China, Korea or Japan from this period, in which the emphasis seems to have been on marine species.

In North America, apart from the early description of what is now known as *Ceratium carolinianum* from South Carolina, Georgia and Florida (Bailey 1851), studies on freshwater dinoflagellates began in the late 1920s when Prescott (1928) reported 8 species from Iowa. The number doubled to 16 when Eddy (1930), in a large article, provided a general account of all freshwater species known at the time, including *Peridinium wisconsinense*. Further important work was done by Rufus Henney Thompson (1908–1980), first from Maryland (Thompson 1947), and for the remainder of his life from Lawrence, Kansas. Thompson's most important contributions on freshwater dinoflagellates are from 1949 and 1951. The 1949 article contains what are undoubtedly the most beautiful drawings of coccoid (Phytophycocystis) dinoflagellates published. Rufus Thompson's works are generally illustrated with drawings of a rare artistic quality, and it is perhaps no coincidence that in his 1951 article he named a new genus *Woloszynskia*, after his Polish predecessor J. Wołoszyńska, who was endowed with a similar artistic talent. In the 1951 article Thompson described 5 new species, including *Glenodinium ambiguum* (now *Kansodinium ambiguum*), *Peridinium intermedium* (now *Thompsodinium intermedium*) and the unusual *Sphaerodinium fimbriatum*, which most likely does not belong in *Sphaerodinium*. The studies from North America were recently compiled and reviewed by Susan Carty (Carty 2003, 2014).

Latin American studies on freshwater dinoflagellates began in the early 1900s, when Daday from Budapest (1905) was given preserved plankton material collected in Paraguay 1902–1903. He found four species of dinoflagellates, two assigned to *Glenodinium* and two to *Peridinium*. Cells of one of the former had very unusual longitudinal ridges, and Daday described them in considerable detail under the name *Glenodinium polylophum*. The structure was so unusual, however, that Lemmermann shortly afterwards transferred it to the new genus *Lophodinium* (Lemmermann 1910).

Dinoflagellates from fresh waters of Panama were reported on by Nygaard (Ostenfeld & Nygaard 1925), including the description of the widespread species *Peridinium gatunense*, and from the Caribbean, e. g. by Bourrelly & Manguin (1952), who provided a general account of the freshwater algae from Guadeloupe, including 9 taxa of dinoflagellates.

African freshwater dinoflagellates have received little attention, and in most countries the first studies still remain to be done. Wołoszyńska (1914), in material from Lake Victoria reported 4 species of *Peridinium* and 2 of *Ceratium*. Researchers at the museum in Paris provided scattered information from the former French colonies Ivory Coast (Couté & Iltis 1984, 12 species, incl. *Peridinium crenulatum* sp. nov.), Mali and Madagascar.

Australia was put firmly on the freshwater dinoflagellate map by George Israel Playfair (1871–1922) who in 1912 published on plankton algae in the Sydney water supply, including two dinoflagellate species and several forms. In 1920 a more detailed article appeared on the Peridineae of New South Wales, containing descriptions of 18 species and several forms, most of which were illustrated. Several were described as new, including *Peridinium striolatum* and *Glenodinium australicum*. It will be interesting to compare Playfair's many forms and species with material

from elsewhere, using molecular methods. Following Playfair, little happened regarding freshwater dinoflagellates in Australia for about half a century until in the 1980s Peter Tyler in Tasmania started publishing on freshwater phytoplankton. In 1987–1988 the new genus *Thecadinium* was described by Croome, Hallegraeff & Tyler and the same years saw the description of two new species of *Prorocentrum* from fresh water (Croome & Tyler 1987). For a recent article on Australian freshwater dinoflagellates, see Ling *et al.* (1989).



The dinoflagellate cell

In the following is given a short description of the dinoflagellate cell, with emphasis on features of the freshwater species.

General Cell Morphology, Morphological Types

Freshwater dinoflagellates fall into three morphological groups of which the flagellated, motile type is the most common. Selected examples are illustrated in **Fig. 1a–k**. The order Dinamoebidiales (order 2), with a single, little-known benthic or epiphytic species in fresh water, contains amoeboid cells (**Fig. 1a**), while species of order 10, Phytodiniales and order 11, Gloeodinales, display coccoid, non-motile vegetative stages attached to other algae, mosses or vascular plants (**Fig. 1b, c**). The remaining species are motile bi-flagellated cells. In two orders the flagella are apical: order 1, Desmomastigales, whose single species has been seen once only, 102 years ago (**Fig. 1d**), and order 12, Procentrales, a mainly marine order (**Fig. 1e**). In the remaining 7 orders, cells have the typical dinoflagellate structure (**Fig. 1f–k**): the cell is divided into two parts, the epicone and the hypocone, by a more or less transverse furrow, the cingulum, in which the transverse, undulating flagellum is located. The proximal ends of the two flagella attach to the cell on what is termed the ventral side of the cell. While the longitudinal flagellum is located in the longitudinal furrow, the sulcus, it usually extends beyond the sulcus and can be visible behind the swimming cell. The cingulum is rarely circular, more commonly it descends in a shallow helix, the proximal and the distal ends displaced one or more cingulum widths. Sometimes the helix is steep, with the cingulum ends far apart (**Fig. 1i**). In many species the sulcus also extends for a short distance onto the epicone, but very rarely reaches the apical end of the cell. The position of the cingulum on the cell varies between genera, thus in order 3, Amphidinales, the cingulum is located near the apical end of the cell (**Fig. 1f**), the two flagella inserting at the base of a short finger-like epicone. In the other orders the position and orientation of the cingulum varies considerably as illustrated in **Fig. 1g–j**, and this was initially used as a generic character. In most species the distal end of the cingulum joins up with the sulcus on the ventral side of the cell, but in a few species the cingulum is incomplete, terminating on the dorsal side of the cell, as in *Hemidinium* (**Fig. 1k**) or it extends to the ventral side but does not reach the sulcus (e. g. *Borghiella tenuissima*, q.v.).

14 The dinoflagellate cell

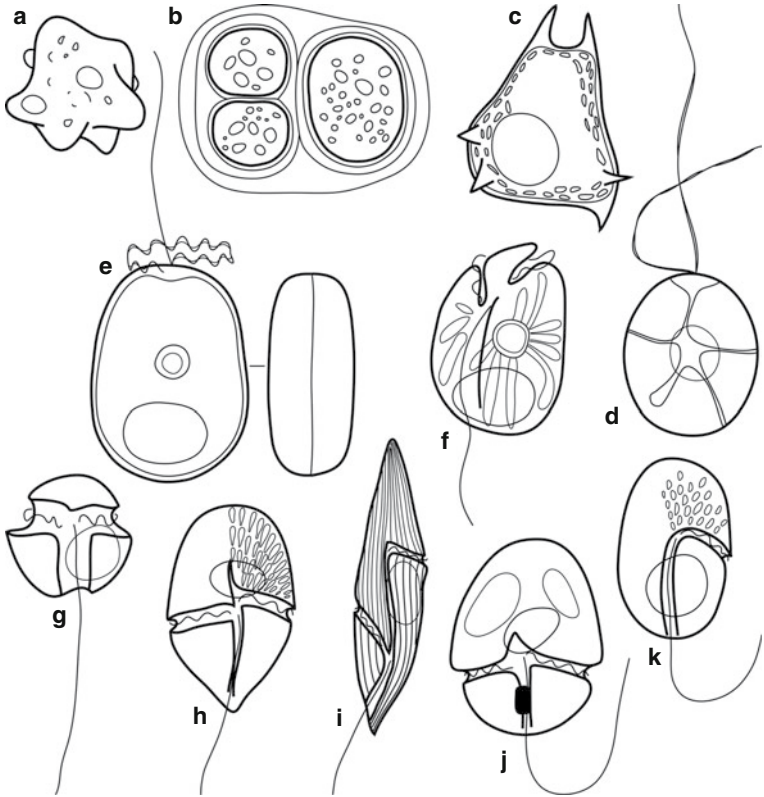


Fig. 1 Morphological types of freshwater dinoflagellates. **a** *Dinamoebidium*, **b** *Gloeodinium*, **c** *Tetradinium*, **d** *Desmomastix*, **e** *Prorocentrum* frontal (valve) and lateral views, **f** *Amphidinium*, **g** *Prosoaulax*, **h** *Gymnodinium*, **i** *Gyrodinium* (marine species), **j** *Opisthoaulax*, **k** *Hemidinium*

The Nucleus

The nucleus of dinoflagellates has a number of special features, and Dodge (1965) created the term mesokaryon for this particular type of eukaryotic nucleus, which he considered to be intermediate between eukaryotic nuclei and nucleoids of prokaryotes. The term was later replaced with dinokaryon (Fensome *et al.* 1993), and Fensome *et al.* considered it to be characteristic of the nucleus in members of their first subdivision Dinokaryota, in contrast to the nucleus in the parasitic species of the second subdivision Syndinea. The chromosomes of the Dinokaryota are visible

in the interphase cell (Fig. 2), while this is not the case in Syndinea. Chromosomes contain very large amounts of DNA, some of the highest known in eukaryotic cells. Dinoflagellate nuclei were for a long time considered to lack basic proteins, histones, but recently, Roy & Morse (2012) demonstrated that a full suite of histone and histone-modifying genes are transcribed in the marine species *Lingulodinium polyedrum*. However, the level of histone proteins accumulated was below current level of detection. The significance of the low levels of histones in dinoflagellates is not clear.

While the nucleus is separated from the cell protoplasm by a two-membrane nuclear envelope as in other eukaryotes, the envelope displays some variation. In some species of the Gymnodiniales, a series of nuclear chambers are present, lined on both the cytoplasmic and the nucleoplasmic side by the nuclear membranes. Normal-looking pores interconnect each chamber to the nucleoplasm, but no such pores have been found between the nuclear chambers and the cytoplasm. Nuclear chambers occur in many species of the Gymnodiniales, but they are not a general feature of the order, lacking for example in *Nusuttodinium* and, among marine species, in the family Warnowiaceae.

Chloroplasts

Chloroplast diversity in dinoflagellates is probably unparalleled in any other group of protists, and new variations are still reported regularly. Morphologically, chloroplasts occur as individual plate-like bodies, some of which may contain one or more pyrenoids, or they may form a network in the outer part of the cell, or throughout the cell. More rarely a single stellate chloroplast is present, or several chloroplasts join into a single stellate cluster, each chloroplast extending from a central pyrenoid (a compound pyrenoid). Chloroplasts are typically separated from the cytoplasm by three chloroplast membranes, and the chloroplast thylakoids are arranged in triplets (Fig. 2), occasionally more irregularly. The ultrastructure of the pyrenoids is very diverse, as observed in the electron microscope, and is often a species characteristic. Single or paired thylakoids enter into the pyrenoid lumen in many species. In light-grown cells, large amounts of starch may accumulate in the cytoplasm, often around the pyrenoids.

The color of dinoflagellate chloroplasts, when studied in the light microscope, varies between species from yellow-brown or yellowish to brown or green, rarely reddish or blue. The origin of the chloroplasts is generally accepted to be other eukaryotic cells, the chloroplasts usually the only remains of the eukaryotic cells engulfed in the past and retained. In the freshwater species treated in the present book, the chloroplasts in most species are thought to originate from three different groups of algae. The *peridinin* type is the most common and occurs in most orders, the chloroplast believed to be the remains of a red algal chloroplast, although all morphological resemblance to red algal chloroplasts has been lost. In the *fucoxanthin* type chloroplasts are of diatom origin. This type of chloroplasts characterizes the peridinialean family Kryptoperidiniaceae, represented in freshwater by 10 species of the genera *Unruhdinium* and *Durinskia* included in the present account. In fact a whole diatom cell is here located within the cytoplasm of the dinoflagellate host,

The pigment composition of the different types of chloroplasts reflects the origin of the chloroplasts. In the *peridinin* type major changes must have occurred in the original red algal chloroplast: the red algal phycobilins disappeared and the red algal chlorophyll a was joined by chlorophyll c. The brownish color of the chloroplasts is caused by the carotenoid peridinin, a carotenoid not known to occur in red algae. Species containing the *fucoxanthin* type contain a relatively little-modified diatom chloroplast, including the yellow or brown carotenoid fucoxanthin. Cells with the *cryptomonad* type of chloroplast contain a little-modified cryptomonad chloroplast.



Fig. 2 Diagrammatic representation of the dinoflagellate cell. Only structures exclusive to dinoflagellates are shown, not features that are general in eukaryotic cells. **a** longitudinal view as seen from the cell's left. A surface view (closer to the observer) is shown on the epicone (top right) and on the transverse flagellum (tf); the remaining part represents the cell cut open longitudinally at about sulcus level. The cell represented is heterotrophic and feeds using a peduncle (pe) supported by a single row of microtubules (MSP, microtubular strand of the peduncle) that is accompanied by electron opaque vesicles (eov) presumably filled with digestive enzymes. The ribbon-like appearance of the tf results from the greater length of the helicoidal axoneme compared to the fibrous strand (fs) that lies closer to the cell surface. Flagellar hairs are shown only along the first turn of the tf. A flagellar canal (fc) and its limiting striated fibrous collar (sc) is shown for the longitudinal flagellum (lf). A pusule (pu) made of a convoluted tubule wrapped by a vesicle (shown in light grey) connects with the fc. Microtubular root 1, the longitudinal microtubular root (LMR) marks the position of the sulcus and is shown here in front of a type E eyespot (e). Amphiesmal vesicles in nearly frontal view (avf) and underlying peripheral microtubules (pm) are shown on the upper-right side of the diagram. Amphiesmal vesicles are shown in profile (avp) where the surface was cut open. The typical large nucleus (N), with a nucleolus (nu) and condensed chromosomes (chr) is shown in the hypocone. Mitochondrial profiles (mit) show the inner membrane with the typical tubular cristae constricted at the base. **b** profile of amphiesma with a trichocyst ready to discharge (tr). The tr is perpendicular to the cell surface and its enveloping membrane is continuous with the plasmalemma at the trichocyst pore (trp). A transverse section of the tr is shown on the bottom-left. The amphiesmal vesicles shown contain polysaccharide material forming plates (plt) and are underlain by peripheral microtubules (pm) represented in cross-section. A chloroplast lobe (C) lies in the peripheral cytoplasm. **c** flagellar canal (fc) and pusule, here in the form of several pusular vesicles (puv) closely associated with an enveloping vesicle (epv). The flagellum inserts at the bottom of the fc, where the centriolar-type basal body (BB) appearance changes into the axoneme (ax). A fibrous collar (sc) surrounds the exit area of the fc. The plasmalemma (pl) is continuous with the fc membrane and with the flagellar membrane. Flat vesicles (pv) are common next to the peripheral microtubules (pm). su, suture between amphiesmal vesicles (avp). **d** detail of peridinin-type of chloroplast showing three membranes in the envelope (ce) and internal lamellae with thylakoids (thl) in groups of three

Flagella and Flagellar Structure

The ultrastructure of the flagellar apparatus is often used in phylogenetic and taxonomic studies, but being invisible in the light microscope, the details will not be discussed here. The two flagella move differently, the transverse flagellum moving in regular helical waves of variable speed within the cingulum, the longitudinal flagellum beating less regularly and usually extending behind the cell. During cell division, the transverse flagellum is reduced to its basal body, and it subsequently grows out into a new, longitudinal flagellum, while the two new flagella being formed both become transverse flagella (flagellar transformation). The flagellar apparatus is connected to the eyespot by a major microtubular flagellar root situated in the sulcus floor.

Apical Furrow Apparatus

Naked or thin-covered species typically possess an apical furrow system. Such a system has been known since the days of Wołoszyńska in the early half of the 1900s, but it can be difficult to see in the light microscope (Wołoszyńska observed cells stained in a special way) and it was rarely reported by other researchers. The apical furrow apparatus is sometimes visible in high-resolution light microscopes but details are most clearly visible in the scanning electron microscope. Care must be taken to prevent cells from collapsing during preparation for scanning electron microscopy (critical point drying is very useful) as the finer details will otherwise be impossible to see. Considerable variation is presently being found in the arrangement and structure of the apical furrow apparatus in different species of naked or thin-covered dinoflagellates, and this variation is often phylogenetically informative, different taxonomic groups (families or orders) differing in the path and structure of the apical furrow apparatus. The function of the apical furrow is not known.

Special Pigments, Reserve Material

The main reserve material of dinoflagellates is starch which may accumulate in large amounts in the cytoplasm, especially when cells grow in strong light. Lipid globules are also commonly present, occasionally sufficiently large to be mistaken for eyespots, especially when tinged with carotenoid pigments. A rare pigment is the carotenoid astaxanthin, which occurs in some species of the genus *Tovellia*, the best known being *Tovellia sanguinea*, formerly found in large numbers in Lake Tovel in Northern Italy and staining the lake water red.

The Eyespot (Stigma)

An eyespot is a characteristic feature of many freshwater dinoflagellates. Its position in the cell is very constant on the ventral side of the cell, in the proximal part of the sulcus or slightly further back towards the antapex. This constant position is probably related to it being attached to a broad microtubular flagellar root underneath the cell membrane of the eyespot region, the root connecting to the flagellar bases and establishing what is probably a functional relationship between the eyespot and the flagellar bases. The eyespot is typically red or orange, and orange droplets of similar color located elsewhere in the cell are most likely carotenoid-rich oil droplets. Some diversity of eyespot structure in dinoflagellates was noted already by Dodge (1969) in several marine species, and subsequent studies have shown more diversity in eyespot structure within dinoflagellates than within any other group of protists. Five morphological types were diagrammed by Moestrup & Daugbjerg (2007), and labelled Types A–E. A Type F was subsequently reported by Craveiro *et al.* (2010). All of these types are known to occur in freshwater species (Fig. 3). The more complex ocelloid type of light receptor known from the marine Warnowiaceae has not been found in fresh water. It was discovered, somewhat unexpectedly, that the occurrence of the different eyespot types is phylogenetically significant. In other words, certain taxonomic groups of dinoflagellates are characterized by a certain type of eyespot. The least unusual type is Type A, which resembles the eyespot of many other algal groups in being formed by one or several layers of carotenoid-rich globules inside a chloroplast, near the surface facing the sulcus. A similar type is known in green algae, chrysophytes and many other heterokonts, and in cryptomonads. In the dinoflagellates it is common in the Peridinales and Thoracosphaerales. Type B is similar but the eyespot is overlain on the sulcus side by a layer of vesicles with crystalloid contents. It characterizes many species of the suessialean family Borghiellaceae. Type C is unusual by not being part of a chloroplast. In the light microscope it appears like a typical eyespot but in the electron microscope it is seen to be formed by a group of carotenoid-rich oil globules, which often appear partially fused in layers. The globules are not surrounded by membranes, but are located in the typical eyespot position. It is characteristic of the family Tovelliaceae, which in the present account is separated into the order Tovelliales. Type D occurs only in the peridinialean family Kryptoperidiniaceae. It is thought to represent a transformed chloroplast in which most thylakoids have disappeared, to be replaced with carotenoid-rich droplets. This modified chloroplast/eyespot is believed to be of red algal origin while the functional chloroplasts in the same species originate from diatoms. Type E is an exceptional type, which in the electron microscope looks somewhat like a loose stack of Golgi-body cisternae, but each cisterna is filled with crystalloid-like material. In the light microscope it looks like a typical, sometimes lightly colored, eyespot. It is restricted to species of the family Suessiaceae and has no equivalent in any other algal group, although somewhat similar structures have been described from ciliates. The latest type found, Type F, was detected in the suessialean family Sphaerodiniaceae. In the light microscope it is horseshoe shaped and, like Type C, it is not part of a chloroplast. A single layer of crystal-containing units overlies more or less fused globules that are not bounded by any membranes. The actual photoreceptor has not been identified in any of these types of eyespot.

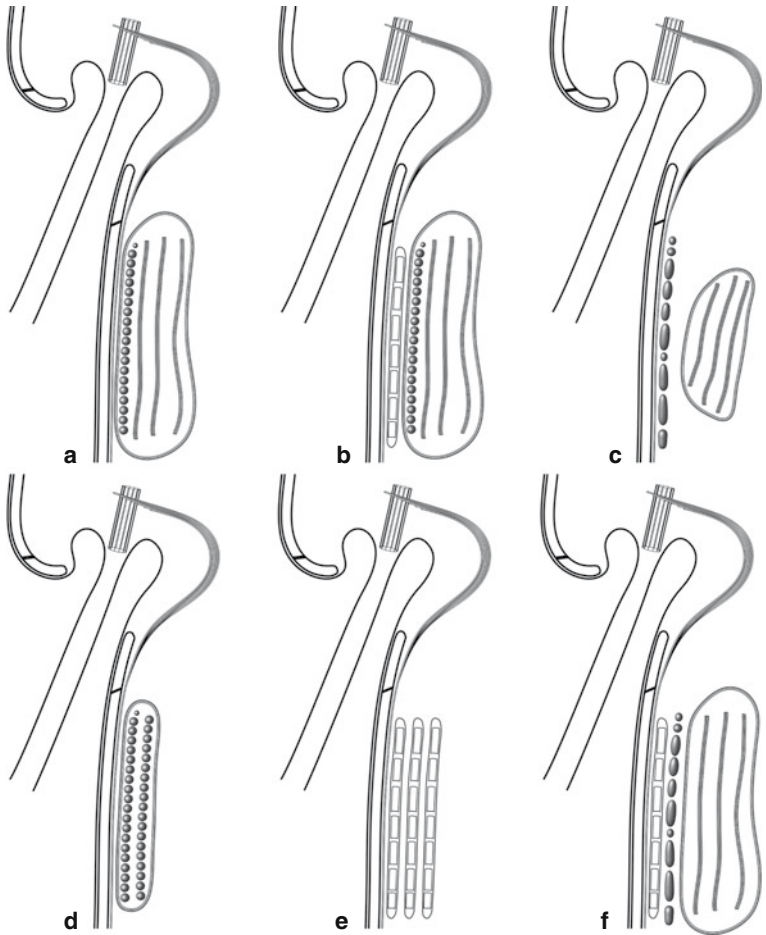


Fig. 3 Eyespot types in freshwater dinoflagellates. Diagrams of sulcal area viewed from the left of the cell, with the multi-stranded longitudinal microtubular root (r1) extending downward from the left side of the longitudinal basal body to lie underneath amphiesmal vesicles lining the sulcus. Amphiesmal vesicles vary extensively in size and contents. **a** type A, found in many members of orders Peridiniales and Thoracosphaerales; **b** type B, found in the family Borghiellaceae, but also in *Peridiniopsis borgei*; **c** type C, typical of the Tovelliaceae (chloroplasts may be absent); **d** type D, found only in the Kryptoperidiniaceae; **e** type E, common in the order Suessiales; **f** type F, so far found only in *Sphaerodinium*

The Pusule

The organelle known as the pusule is confined to dinoflagellates. In the light microscope it may be seen as a more or less spherical vacuole-like structure, which can be very conspicuous. It was named by Schütt (1895). In the electron microscope the pusule system is often found to be highly complex. The most typical part of the pusule system consists of small vesicles, tubules or canals lined by three membranes, a pusular membrane towards the lumen of the canal and two additional membranes (of a wrapping vesicle) on the cytoplasmic side, the two inner membranes usually closely appressed. The complexity is perhaps best illustrated by *Naiadinium polonicum*. Two different pusular systems were found, one associated with the canal of the transverse flagellum, the other with that of the longitudinal flagellum. The simplest type was associated with the transverse flagellum canal, and comprised a number of three-membraned vesicles which emptied directly into the flagellar canal. The other system, associated with the longitudinal flagellum canal, was very complex, however, and made of three different components. The innermost component comprised *ca.* 70 narrow pusular tubules, each measuring up to 9 μm in length, which emptied into the second component, a more or less ovoid collecting chamber. This chamber then connected to the flagellar canal through the third component, a pusular canal estimated to measure *ca.* 13 μm in length (Craveiro *et al.* 2015).

In other species the system is simpler but in most species the pusular system has not been examined in detail. In all members of the Tovelliaceae examined, the inner membrane is covered on the lumen side by a dense layer of short, elongate bodies. Obviously such extensive and complex systems must serve important functions in the cell, but what these functions are is still disputed. Parts of the pusular system have been reported to pulsate or contract but there is little recent evidence to support this claim. Schütt (1895) observed slow changes in pusular size of marine species studied under the light microscope and Entz (1926) reported variations in the volume of the large sac pusule of the freshwater *Peridiniopsis borgei*. Both increase and decrease in volume were seen, but no regular pulsation. Schütt speculated, however, that slow pulsation might take place. The vesicular components associated with the pusular system indicate that it may serve a function in excretion, perhaps associated with osmoregulation. In freshwater dinoflagellates the pusule system may have taken over the osmoregulatory function of contractile vacuoles. Uptake of molecules has also been suggested. In marine species, where the pusule may be very conspicuous, the function is even more obscure.

Note on contractile vacuoles: the contractile vacuole is well known in thin-walled or naked freshwater protists, but absent in dinoflagellates. Skuja claimed contractile vacuoles to be present in *Kolkwitiella acuta* (as *Diplopsalis acuta*) (Skuja 1948, p. 372) and in *Opisthoaulax woloszynskae* (as *Massartia woloszynskae* var. *notata*; Skuja 1956, p. 362). In *Kolkwitiella acuta* Skuja described in detail that a contractile vacuole in the hypocone contracted regularly every 1–2 min. In *Opisthoaulax* he reported that a large contractile vacuole was formed by fusion of several smaller ones, the large vacuole contracting every 20–30 min. We have independently examined both *Kolkwitiella acuta* and species of *Opisthoaulax* without seeing contraction of vacuoles in the cytoplasm. Skuja's observations therefore remain an enigma.