Chrysophyta

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Abstract

The *chrysophytes* (more than 1,200 described species) are unicellular or colonial algae characterized by *heterokont* flagella and chloroplasts with *chlorophyll a* and *c,* and by their endogenous silicified *stomatocysts*. They occur mainly as phytoplankton in temperate freshwaters, and their distribution is ecologically determined, mainly by temperature and pH.

Cells are naked or in many cases surrounded by an envelope, e.g., of species-specific silica scales manufactured from the chloroplast ER and *Golgi* vesicles and transported to the cell membrane and extruded. Photoreceptor systems include a swelling on the short flagellum and a corresponding stigma in one of the chloroplasts. Photosynthesis results in *chrysolaminaran*. But in many species, e.g., in colorless species, organic compounds can be taken up from the water or by phagocytosis. Life history includes mitotic divisions and *encystment*. In many species, *sexuality* – cell fusion followed by *encystment* of the zygote – has been observed. Classification was traditionally based on morphological criteria, including ultrastructure, but in recent years molecular methods have resulted in profound changes in our concepts of relationships and evolution.

Keywords

Occurrence • Ecology • Cell construction • Life history • Cultivation • Classification • Phylogeny

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Introduction

General Characteristics

The Phylum Chrysophyta is a group of golden-brown microscopic algae and related colorless forms, most of them flagellates (Fig. 1). About 1,200 species in about 112 genera (Kristiansen and Preisig 2001) have been estimated, but many more species will certainly be described. The classification of the phylum with the classes Chrysophyceae and Synurophyceae is shown in Table 1, but the latter class, erected in 1987, should now again be included in the Chrysophyceae due to several recently published molecular investigations (Takishita et al. 2009; Del Campo and Massana 2011; Yang et al. 2012). On the other hand, several taxa previously associated with
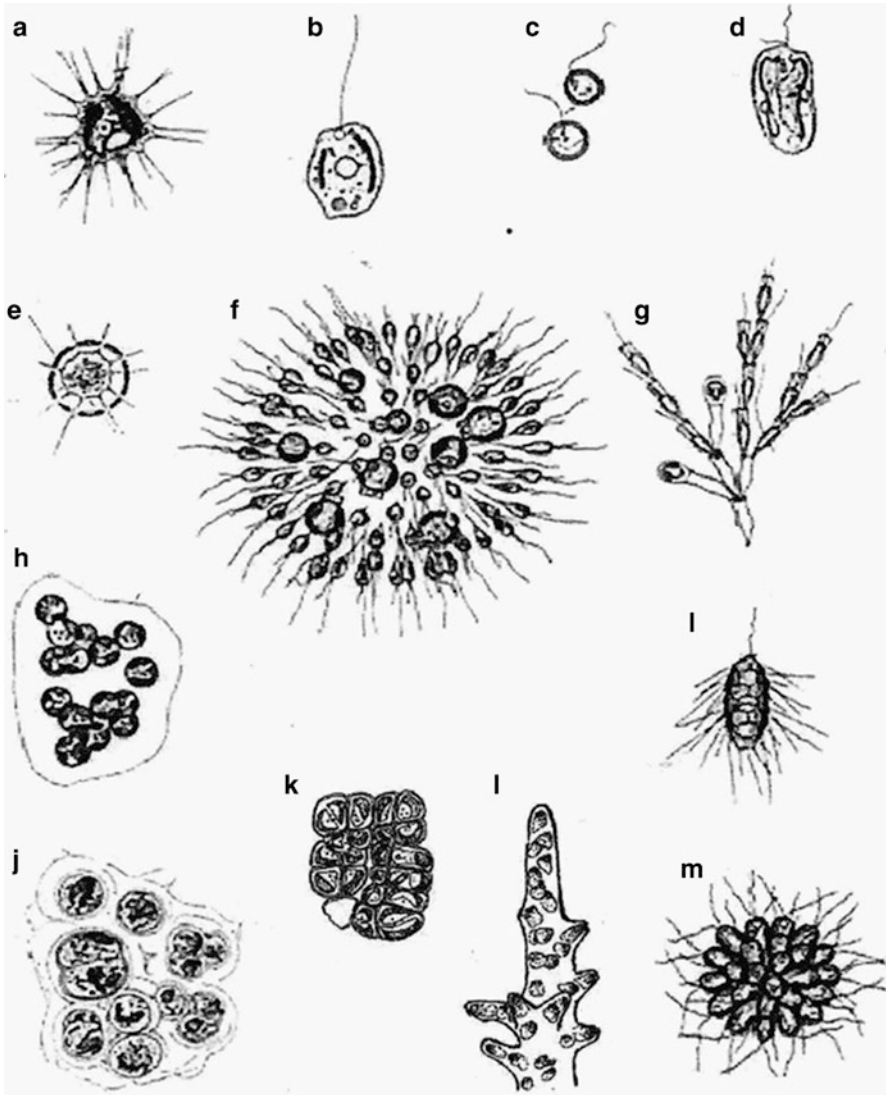
Fig. 1 Morphological diversity within the Chrysophyta. *A. Chrysamoeba. B. Chromulina, C. Chrysococcus, D. Ochromonas, E. Chrysothecopsis*, *F. Uroglena, G. Dinobryon, H. Chrysocapsa, I. Mallomonas, J. Gloeochrysis, K. Phaeoplaca, L.*, *Hydrurus, M. Synura* (After Kristiansen 2005, with alterations)

chrysophytes have been shown to belong to other evolutionary lineages and separated as independent classes: Phaeothamniophyceae, Dictyochophyceae, Pelagophyceae, and Bicosoecophyceae. Accordingly, they are not included here.

A survey of all the genera has been given in the "Encyclopedia of Chrysophyte Genera" (Kristiansen and Preisig 2001).

Table 1 Classification of the Chrysophytes

Traditional classification based on morphological data (after Preisig in Kristiansen and Preisig 2001, altered)	Updated classification based on molecular data
Class CHRYSOPHYCEAE Pascher 1914	**Class CHRYSOPHYCEAE** Pascher 1914
Order **CHROMULINALES** Pascher 1910	Order **OCHROMONADALES** Pascher 1910
Family **Chromulinaceae** Engl. 1897	Order **CHROMULINALES** Pascher 1910
Family **Dinobryaceae** Ehrenb. 1834	
Family **Paraphysomonadaceae** Preisig and Hibberd 1983	Order **PARAPHYSOMONADIDA** Scoble et Cavalier-Smith 2014
Family **Chrysolepidomonadaceae** M. C. Peters & R. A. Andersen 1993	
Family **Chrysamoebaceae** Poche 1913	
Family **Chrysocapsaceae** Pascher 1912	Order **CHRYSOSACCALES** Bourrelly 1957
Family **Chrysosphaeraceae** Pascher 1914	
Family **Chrysothallaceae** Hub.-Pest. 1941	
Order **HIBBERDIALES** R. A. Andersen 1989	Order **HIBBERDIALES** R. A. Andersen 1989
Family **Hibberdiaceae** R. A. Andersen 1989	
Family **Stylococcaceae** Lemmerm. 1899	
Order **HYDRURALES** Pascher 1931	Order **HYDRURALES** Pascher 1931
Family **Hydruraceae** Rostaf. 1881	
Class SYNUROPHYCEAE R. A. Andersen 1987	
Order **SYNURALES** R. A. Andersen 1987	Order **SYNURALES** R. A. Andersen 1987
Family **Mallomonadaceae** Diesing 1866	
Family **Synuraceae** Lemmerm. 1899	Order **SEGREGATALES** Boenigk et Grossmann 2016
	Order **APOIKIIDA** Boenigk et Grossmann 2016

Occurrence

The great majority of described species are found in plankton of fresh water. Some others are epibiotic or neustonic (i.e., attached to the water surface). A few species are benthic, e.g., found attached to the bottom in streaming mountain rivers. Relatively few known species occur as marine plankton. For example, species of the colorless genus *Paraphysomonas* may play an important role during the formation of sea ice (Ikävalko 2001). However, a recent culture-independent analysis of chrysophyte diversity revealed the existence of several unknown, marine clades (del Campo and Massana 2011) raising the question of the major predominance of chrysophytes in freshwater habitats.

Some chrysophyte species are very common and cosmopolitan, others are rare with peculiar disjunct distributions; however, our knowledge is still very fragmentary but rapidly increasing. Due to the rising number of investigations undertaken almost all over the world, the knowledge of the global distribution of the chrysophytes has increased considerably, especially for the silica-scaled forms, because of their reliable EM identification and documentation based on the silica scales. Thus a number of distribution types have been established (Kristiansen 2001). Of the ∼250 species of silica-scaled chrysophytes, about 50 species are widely distributed or cosmopolitan. They have dispersed to suitable localities almost all over the world (Kristiansen 2000). The other species have more or less restricted distributions determined by climatic, historic, ecological, and dispersal factors. The following distribution types have been recognized: Northern temperate-subarctic-arctic species, species with bipolar distribution, and tropical species. A large group of species are endemic, having only been found within a restricted area. In fact, almost all new species start as endemic for the type locality, but most of them sooner or later will also be found in other localities and thus loose endemic status. In 2004, of the 172 described *Mallomonas* species, 69 were considered endemic (Kristiansen and Lind 2005).

The distribution of a species is due to dispersal, mainly of stomatocysts, by birds and by air. Thus the distribution pattern at a given time depends on several factors: dispersal capacity of the species, available vectors, suitable available habitats, and, perhaps most important, sufficient time (Kristiansen 2008). This is in contrast to the ubiquity hypothesis advocated mainly by Finlay and Clarke (1999) that all species are everywhere, only the environment determines the occurrence. This problem is still under discussion, and a sort of compromise has been offered by Řezáčová and Neustupa (2007). However, the ubiquity hypothesis has been contradicted, e.g., by studies in North America where quite similar neighboring water bodies had different floras of silica-scaled chrysophytes (Siver and Lott 2012b).

In addition, the rapidly increasing amount of molecular investigations has revealed the existence of cryptic lineages within the presumably cosmopolitan species, showing restricted distribution patterns. For example, the cosmopolitan *Synura petersenii* s.l. (Fig. 2) has been shown to consist of at least 10 well-defined species, some of them occurring in geographically separated areas (Boo et al. 2010; Kynčlová et al. 2010; Škaloud et al. 2012, 2014). Probably the most striking example is the distribution pattern of *S. hibernica* restricted to an extremely small biogeographic area of western Ireland (Škaloud et al. 2014).

Literature

Important identification works: The most comprehensive identification work today on freshwater chrysophytes is in the "Süsswasserflora von Mitteleuropa" by Starmach (1985) and Kristiansen and Preisig (2007), the latter based on electron microscopy of silica scales. In addition, there are regional floras from, e.g., British Isles and North America, where the chrysophytes have been treated by Kristiansen and Preisig (2011), Siver (2003), and Nicholls and Wujek (2003), respectively.

Fig. 2 Silica scale of *Synura petersenii.* × 20,000
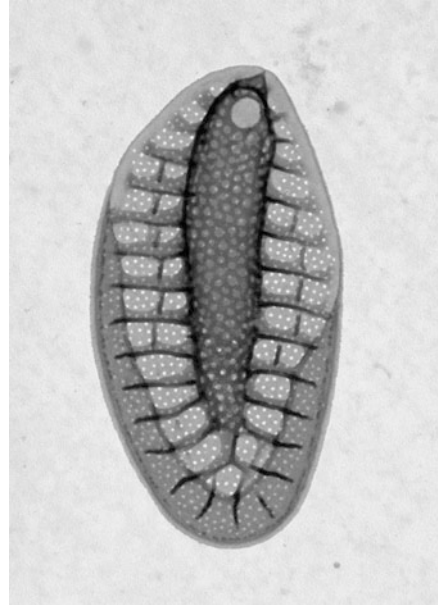
A survey of all chrysophyte genera has been compiled by Kristiansen and Preisig (2001). A general account of chrysophytes and their biology has been given by Kristiansen (2005). Further useful references are Pienaar (1980), Kristiansen and Takahashi (1982), and Round (1986).

The scale-bearing species, as seen in the electron microscope, were first surveyed by Takahashi (1978) and, more recently, the Synurales by Kristiansen and Preisig (2007). This was supplemented by the work on *Paraphysomonas* and related genera by Preisig and Hibberd (1982, 1983) and by Scoble and Cavalier-Smith (2014). A recent review on the taxonomy of silica-scaled chrysophytes has been published by Škaloud et al. (2013).

History of Knowledge

Knowledge of the chrysophytes was initiated by the Danish naturalist O. F. Müller who, in his famous work *Animalcula Infusoria* (1786), depicted and named the colorless *Volvox vegetans,* which is now known as *Anthophysa vegetans.* A systematic survey of microorganisms, among them many chrysophytes, culminated in Ehrenberg's (1838) magnificent work, in which species of *Synura, Dinobryon,* and *Uroglena* were depicted and described.

The first precise descriptions of chrysophytes are found in the authoritative work of Stein (1878); many of his illustrations are still used in modern textbooks.

Many species were subsequently described and placed with other flagellates in the animal kingdom. Pascher (1913, 1914, and in a long series of papers) established the botanical position of these algae. He defined the class Chrysophyceae, showing also how chrysophytes resemble the diatoms, the brown algae, and others. He demonstrated parallel evolution in the major algal groups: like other algal taxa, the Chrysophyceae evolved from flagellates to multicellular organization levels, which retained swarmers of the ancestral flagellated types. Based on these principles, Bourrelly (1957) published his *Recherches sur les Chrysophycées,* including all available light microscopy information on these protists. Later, Bourrelly (1965) considered flagellar number as the main taxonomic criterion.

Knowledge of the chrysophytes has advanced considerably since then by the introduction of electron microscopic techniques that reveal cell structure, flagellar systems, and cell envelopes. Understanding of their taxonomy has greatly progressed, so that life cycles and sexuality can be recognized, and studies on the ecology and distribution of the individual species can be carried out. Chlorophylls and the accessory pigments have been identified and their functions elucidated by improved biochemical methods (Kristiansen 2005). However, our knowledge is still fragmentary and based on investigations of rather few species. Introduction of molecular methods has greatly enhanced our understanding of taxonomic relationships, as will be discussed in the final chapter.

Practical Importance

The practical use of chrysophytes is restricted to the laboratory: *Ochromonas* species have served as experimental organisms for many investigations of general biological importance, viz., the freshwater species *Ochromonas danica* for secretion of organic compounds such as vitamins into the environment (Aaronson et al. 1971). *Poteriochromonas malhamensis* has been used for determining the toxicity of lead compounds (tetraethyl lead) as antiknock additives to gasoline (Röderer 1980).

Because of their narrow ecological spectra, silica-scaled chrysophytes can serve as indicators for changes in trophic conditions, in particular of pH in lakes (Smol et al. 1984; Siver and Hamer 1990). Silica structures, such as stomatocysts and scales (Figs. 3 and 4), are used in sediment studies in geology and limnology, often together with pollen analysis, to study the history of lakes (e.g., Nygaard 1956; Munch 1980; Smol 1980; Adam and Mahood 1981; Carney and Sandgren 1983; Cronberg 1986; Siver and Smol 1993; Siver and Marsicano 1996). Changes in pH (acidification) and anthropogenic influence can readily be followed.

Some chrysophytes, e.g., the genera *Synura* and *Uroglena*, may become a nuisance when they occur in great quantities, because they excrete fishy-smelling ketones and aldehydes (Collins and Kalnins 1972). They may foul drinking water reservoirs (Watson et al. 2001; Watson and Satchwill 2003).

Fig. 3 Stomatocyst of
Mallomonas teilingii within
the scaly envelope
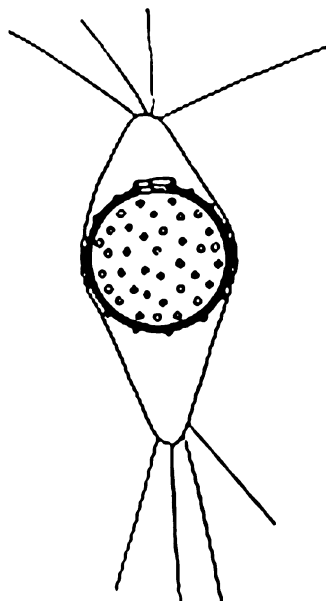
Fig. 4 Silica scale of
Mallomonas acaroides

Habitats and Ecology

Chrysophytes occur mainly as phytoplankton, and standard phytoplankton methods are used in their collection. Although planktonic species are obtained in plankton nets of suitable mesh, e.g., 20 μm, a great many nanoplankton species pass through. These must be obtained directly from water samples brought to the laboratory. Most chrysophytes are very fragile; thus, transport to the laboratory should take place in a thermos or on ice and living material should be examined as soon as possible. Immediate preservation of field samples for light microscopy and counting is made by Lugol's solution modified with the addition of acetic acid; glutaraldehyde is used for electron microscopy.

Material from water samples should be concentrated (by filtration or centrifugation) for examination in the laboratory. To determine species diversity and abundance, an inverted microscope is indispensable. Lugol-fixed material is inspected in sedimentation chambers of defined volume viewed from below in an inverted microscope for quantification; this is also a way to detect many very small forms.

To detect and identify many of the scale-covered species, electron microscopic examination is required. Material is dried on formvar-coated grids and often shadow casting with a heavy metal (e.g., gold-palladium or chromium) is necessary to enhance contrast and to show three-dimensional structures in TEM. SEM is increasingly used for identification (e.g., Siver 1991).

Most chrysophytes occur as plankton in lakes and ponds. Only few, such as *Hydrurus*, are found attached to stones in running waters (Parker et al. 1973). Some few species occur as neuston attached to the surface layer; *Chromophyton* may cover small forest ponds with a golden layer, in the quantity of two million cells per cm^2 (Molisch 1901; Frølund 1977).

Typical freshwater chrysophyte habitats are humic, neutral, or slightly acidic lakes and ponds with a moderate supply of nutrients. Here the chrysophytes may constitute the main phytoplankton biomass. In more acidic, low nutrient, or alkaline waters, few species occur but sometimes at high cell numbers. Ponds surrounded by agricultural land, unless polluted by cattle, are often very rich in chrysophytes. Species of scaled chrysophytes can be arranged along a trophic gradient in relation to their trophic demands, their trophic scores (Siver and Marsicano 1996).

Many species have well-defined occurrence ranges regarding pH; they can thus be arranged as acidobiontic, acidophilic, indifferent, alkaliphilic, and acidobiontic species (compare Kristiansen 1975 and 2005). The ecological tolerances of species differ greatly even between species of which many are distinguishable only by electron microscopy (Fig. 5). *Synura sphagnicola* (Fig. 6), for example, occurs only in acidic water, while other *Synura* species occur only in alkaline water or are more broadly adapted, e.g., the nearly ubiquitous *S. petersenii*. However, as already mentioned, this species has been shown to include a number of cryptic species with presumably different ecological preferences.
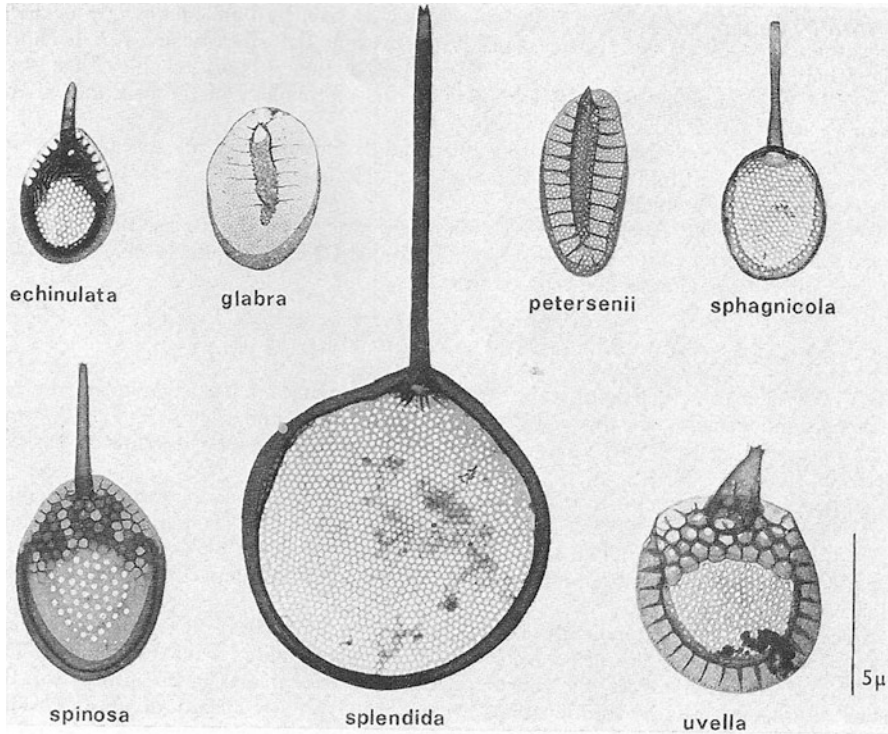
Fig. 5 Scales of species of *Synura*, originally defined on structural characters, but now additional molecular information is necessary

Most species have their main occurrence in spring, often just after ice break. Many species are restricted to cold or cool water, thus in temperate regions occurring in spring and autumn, others prefer warmer water in summer.

There are only few true marine species described (Scoble and Cavalier-Smith 2014). Until recently, the sea was considered to be crowded with chrysophytes, but as several "splinter groups", e.g., Dictyochophyceae (Ostroff et al. 1980; Moestrup and Thomsen 1990), Phaeothamniophyceae (McLachlan et al. 1971), and Pelagophyceae (Lewin et al. 1977) have been shown to have other affinities (Moestrup 1995), the number of marine species has been reduced considerably. Among the most abundant former marine chrysophytes are the silicoflagellates (Dictyochophyceae). However, as already mentioned, the marine diversity of true chrysophytes is probably much greater than previously realized (del Campo and Massana 2011).

Species of *Paraphysomonas* (Preisig and Hibberd 1982) are found both in fresh and sea water, and they may occur in quantities during sea ice formation (Ikävalko 2001). As colorless phagotrophic organisms attached to "marine snow," they may play an important role in the marine food web (Lim et al. 1999).

Fig. 6 *Synura sphagnicola* a
motile, colonial scale-bearing
chrysophyte. × 400 (From
Korshikov 1927)
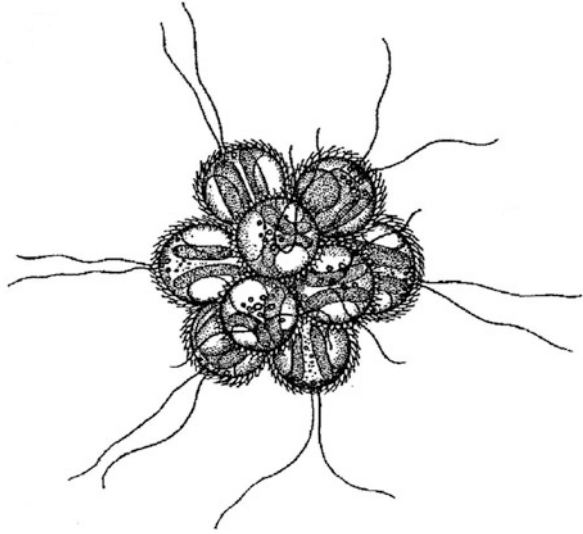
Fig. 7 *Dinobryon
cylindricum* var. *alpinum,* a
colonial, loricate chrysophyte.
× 600
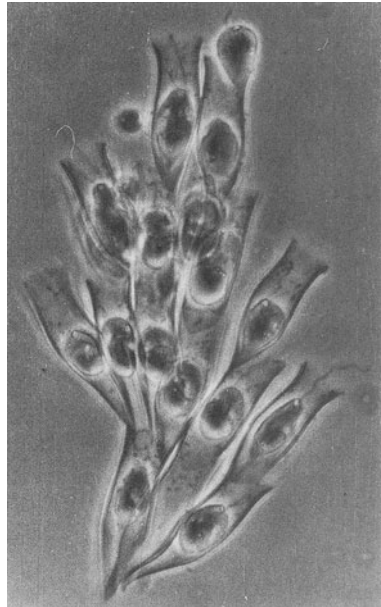
All chrysophytes form endogenous cysts (statospores, stomatocysts) during their
life history. In *Dinobryon cylindricum* (Fig. 7), encystment occurs either in the
exponential phase of population growth (intrinsic, mainly sexual resting cysts) or
in the stationary phase (extrinsic, induced by nutrient depletion). Two clones must be

present in order to produce sexual cysts in *Dinobryon cylindricum,* whereas asexual cysts are produced by individuals, pioneers in a new habitat. These *Dinobryon* produce asexual cysts at a low rate, which gradually slows down during the end of the growth period. They produce sexual cysts rapidly during rapid growth. These two strategies result in almost the same number of cysts. The cysts sink into the sediment; the germination rate during the next spring is unknown (Sandgren 1983a, b). *Dinobryon* stomatocysts in surface waters of an arctic lake germinated during the same summer, whereas those in the sediment only germinated the next spring, when turnover exposed them to light (Sheath et al. 1975).

Chrysophytes excrete a great variety of organic compounds (Aaronson et al. 1971), corresponding to 20% of the carbon they fix by photosynthesis. These compounds include carbohydrates, enzymes, and vitamins and are utilized by bacteria and heterotrophic protists. Small chrysophytes, together with cryptomonads and prymnesiophytes, make up an important part of the nanoplankton of many lakes where they are the main food for zooplankton.

Because *Dinobryon* has an effective phosphate-uptake mechanism, it is especially abundant in waters with low phosphate concentrations (Lehmann 1976). However, most species (excluding Synurales) are mixotrophic, partly covering carbon and phosphorus demand by ingestion of bacteria (Sanders and Porter 1988).

Silica is required for scale-bearing species. *Synura* and *Paraphysomonas* require silica in the water at a concentration of at least 1 μM in order to grow well; they are able to deplete a medium almost completely of silica. Very low silica content results in unstable colony structure and failure to form cysts and scales. The silica require-ment is further demonstrated by the inhibitory effect of germanium dioxide on growth (Klaveness and Guillard 1975; Lee 1978).

Most of the chrysophytes have chlorophyll *a*- and *c*-containing chloroplasts and can photosynthetically utilize inorganic carbon from CO_2 in the synthesis of organic compounds. An exogenous supply of organic carbon compounds, e.g., vitamins of the B group, mainly B_{12}, is also necessary. This will normally be present in the water, either excreted by bacteria, released by the decomposition of algal cells, or brought by sewage. Organic compounds are also obtained by phagocytosis of particulate food by many species. Colorless forms are exclusively dependent on phagocytosis and/or uptake of dissolved organic compounds (Pringsheim 1952).

Characterization and Recognition

Cell Structure

The Chrysophyta, a group of protists containing single-celled individuals as well as quite complex colonial forms, can briefly be defined by the following biochemical and structural criteria: chloroplasts with chlorophylls *a* and *c* (Andersen and Mulkey 1983) but lacking *b,* fucoxanthin as the most important accessory pigment, β-1, 3-glucan (chrysolaminaran) as storage product, swarmers with heterokont flagella (i.e., one long hairy and one shorter smooth, the latter in many cases only to be

Fig. 8 A longitudinal thin section of a *Dinobryon* cell showing the position of most organelles. The chloroplasts (**a**), one of them with a stigma (**b**) in juxtaposition to the flagellar swelling (**c**). Several mitochondrial sections (**d**) with tubular cristae. A vacuole (**e**) is above the nucleus (**f**) and two lipid droplets (**g**). The golgi body (**h**) can be seen. The chrysolaminaran storage vacuole (**i**) occupies a major portion of the posterior part of the cell. × 8,700
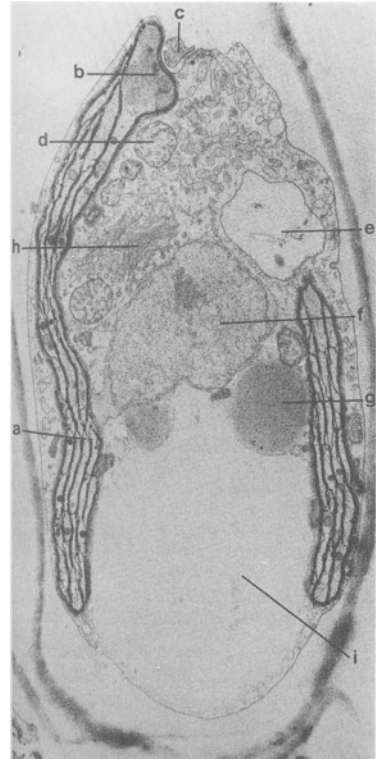

detected by EM). Endogenous silicified cysts (stomatocysts) are present throughout the class.

The basic morphological type in the Chrysophyceae is the motile cell or swarmer (flagellate), from which other structural types or organization levels presumably evolved (Pascher 1914). The swarmer cell is naked or surrounded by an envelope; it occurs either solitary or in colonies. It is provided with one or two visible flagella; contractile vacuoles, most often located anteriorly, are present, and in most cases a chloroplast with a stigma (eyespot) is also present (Kristiansen 1986, 2005).

Many species have a tendency to form lobed or branched cytoplasmic extensions. In some species, the cell is amoeboid during the greater part of its life history (rhizopodial organization level), and either motile or sessile. The palmelloid level of organization is characterized by immotile cells located within mucilage as the dominant stage of the life cycle. Many motile species have such a stage during their life cycle as well. The coccoid level of organization, in which the cell is immotile and surrounded by a distinct wall, is displayed by a few genera.

Chrysophyte cells exhibit a number of structural characteristics by which they can be distinguished from other protists (Figs. 8 and 11), including distinctive flagellar basal bodies and subsurface microtubules, golgi appressed to the nucleus,

Fig. 9 Detail of the lorica of *Dinobryon pediforme,* showing the fibrillar construction. ×40,000
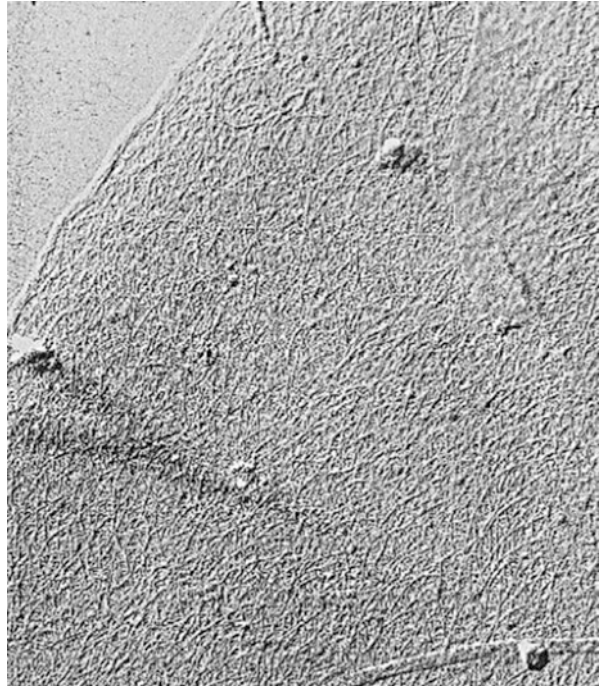

chloroplast endoplasmic reticulum, and a flagellar swelling opposite the distal face of the plastid with the stigma (Bold and Wynne 1978).

Most chrysophytes occur as naked cells. The cell membrane is in direct contact with the water; in *Ochromonas,* it is covered with a fuzzy layer and with surface blebs and vesicles. These may serve to trap bacteria and other particles that are subsequently engulfed as food (Kahan et al. 1978).

In many chrysophytes, the cells are surrounded by a wall or lorica of several different shapes. For example, it is vase- or beaker-shaped in *Dinobryon,* flask-shaped in *Lagynion,* or globular in *Chrysococcus.* The lorica consists of imbricate scales in *Epipyxis;* in *Bitrichia,* it has a peculiar double construction. In *Lagynion,* the lorica is fixed to a substratum. A ring-shaped part of the lorica fastens *Chrysopyxis* around an algal filament (Kristiansen 1972). The lorica is an interwoven system of fine fibrils consisting of cellulose; or in some cases it consists of chitin (Herth et al. 1977). In *Dinobryon,* the cellulosic fibrils are secreted during rotation of the protoplast and thus show a more or less helical arrangement (Franke and Herth 1973; Herth 1979; Fig. 9). In *Chrysococcus,* the dark and opaque lorica is impregnated with manganese and iron compounds. In *Ochromonas,* simple lorica forerunners have been observed (Schnepf et al. 1968).

Cells of several genera, mainly in the order Synurales, are covered by an armor of silica scales, spines, and bristles. By means of X-ray microanalysis, they have been proved to be composed of silica, which is consistent with the inhibition of scale formation by germanium dioxide (Klaveness and Guillard 1975; Lee 1978). An
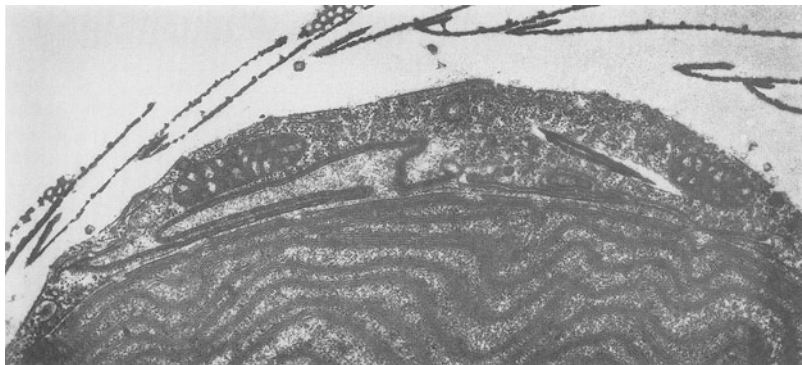
Fig. 10 *Synura petersenii,* formation of silica scales from the chloroplast. *Above*, part of the scaly armour is seen. × 17,500

additional organic component has been demonstrated in *Synura* scales (McGrory and Leadbeater 1981).

Silica scales and associated structures are produced internally; two different but related mechanisms are involved. In the Synurales, scale deposition vesicles are produced from the chloroplast endoplasmic reticulum (CER) on the outer side of the chloroplast. In *Synura* (Schnepf and Deichgräber 1969), the adjacent part of the CER bulges into such vesicles (of golgi body origin), functioning as molds for the scales (Fig. 10). "Hairy" golgi body vesicles that transport material fuse with the scale-producing vesicle. The mature scale is extruded from the cell and brought into correct position in relation to the other scales and the cell surface. *Mallomonas* bristles are formed in a similar way. They are initiated as flat sheets and then rolled into hollow tubes, which are then hinged to the scales (Wujek and Kristiansen 1978; Mignot and Brugerolle 1982). Beech et al. (1990) have shown the mechanism in *Mallomonas splendens*, how the bristles are extruded and brought in correct position and then with their foot glued to the scale.

In the Paraphysomonadida, scale production takes place somewhat differently. One vesicle produces scales while another vesicle from the endoplasmic reticulum functions as a mold (Preisig and Hibberd 1983).

Scale structure is species specific and very complicated, and it was understood only after electron microscopy came into common use. A scale generally consists of a perforated basal plate provided with ribs, spines, and other ornamentation (Fig. 5). In *Mallomonas,* some scales bear long, often complicated, bristles (Asmund and Kristiansen 1986; Kristiansen 2002). Scanning EM shows the three-dimensional structure of the scales (e.g., Siver 1991). Scales are deposited on the cell surface in an imbricate, often screwlike pattern. Several scale types are produced in the same cell and deposited on the surface in a definite sequence, as apical, body, and caudal scales (e.g., Belcher 1969b). Organic surface scales of a complicated flowerpot-like shape that cover both cell and flagella have been reported in *Sphaleromantis* (Manton and Harris 1966). A species of *Chromulina* is covered with simple oval scales (Pienaar 1977).

The flagellar system shows a complicated structure and an interesting evolution. The primitive heterokont condition is the presence of two dissimilar flagella: one flimmer (mastigonemate, hairy) flagellum and one shorter, smooth flagellum, both inserted apically in the cell. In more advanced forms, the short flagellum may be somewhat or almost completely reduced and/or transformed into a photoreceptor (Hibberd 1976).

Basal bodies are located anteriorly in the cell, in most cases at an angle to each other. Only in *Mallomonas* and *Synura* are they parallel. These basal bodies are interconnected by a system of fibers and connected by a fibrous band to the stigma region of the chloroplast (Kristiansen and Walne 1976). Systems of microtubules spread as microtubular roots below the cell membrane, and a rhizoplast proceeds into the cell and connects with the nuclear envelope (Figs. 11 and 12). In the transitional region above the basal body, the transitional helix (Hibberd 1979) is a general feature.

The longer, hairy flagellum is most often forwardly directed, beating with uniplanar sine waves starting from the base (Jarosch 1970). It has two rows of mastigonemes (flagellar hairs) causing the pulling effect of its movement. In *Ochromonas,* the mastigonemes are single in one row, in tufts in the other. Each mastigoneme consists of a base and a stiff shaft and bears two terminal and several lateral filaments (Bouck 1971). These mastigonemes are produced in the perinuclear space between the nucleus and chloroplast (Leedale et al. 1970). They are transported via golgi vesicles to the base of the flagellum. These vesicles fuse with the plasmalemma, thus the mastigonemes become extracellular and are transferred to the plasmalemma of the flagellum (Hill and Outka 1974). The short flagellum bears fine lateral filaments. The short flagellum, generally directed laterally, beats in helical waves. It may bear a swelling or be completely transformed into a photoreceptor. In some genera it is reduced so that it is only visible by electron microscopy (Belcher 1969a; Belcher and Swale 1967), accordingly these have originally been considered uniflagellate.

Small and simple flagellar scales occur in *Synura* and *Mallomonas* (Hibberd 1973; Bradley 1966). In *Sphaleromantis*, they are similar to the rather complicated body scales, making the flagella appear coarse and stiff (Manton and Harris 1966).

Photoreceptor systems are present in almost all motile chrysophytes; they consist of a swelling on the short flagellum with the photoreceptor and a stigma (often called the "eyespot") functioning as a screen. The stigma, located anteriorly in a chloroplast lobe in juxtaposition to the photoreceptor (Fig. 12), consists of a number of red (carotene) lipid droplets densely arranged just within the chloroplast membranes. A stigma is present in most motile chrysophytes; it does not occur in *Chrysamoeba,* some species of *Chrysococcus* (Belcher and Swale 1972a), in Synurales, and in most colorless forms (Hibberd 1976).

The swelling is placed proximally on the smooth flagellum and often has a complicated internal structure. In *Sphaleromantis* (Manton and Harris 1966), *Chromulina* (Belcher and Swale 1967), and *Chrysococcus* (Belcher 1969a), this flagellum is very short, so that it almost exclusively consists of a photoreceptor and is placed in a pocket in direct juxtaposition to the stigma. In *Mallomonas*, it is reduced
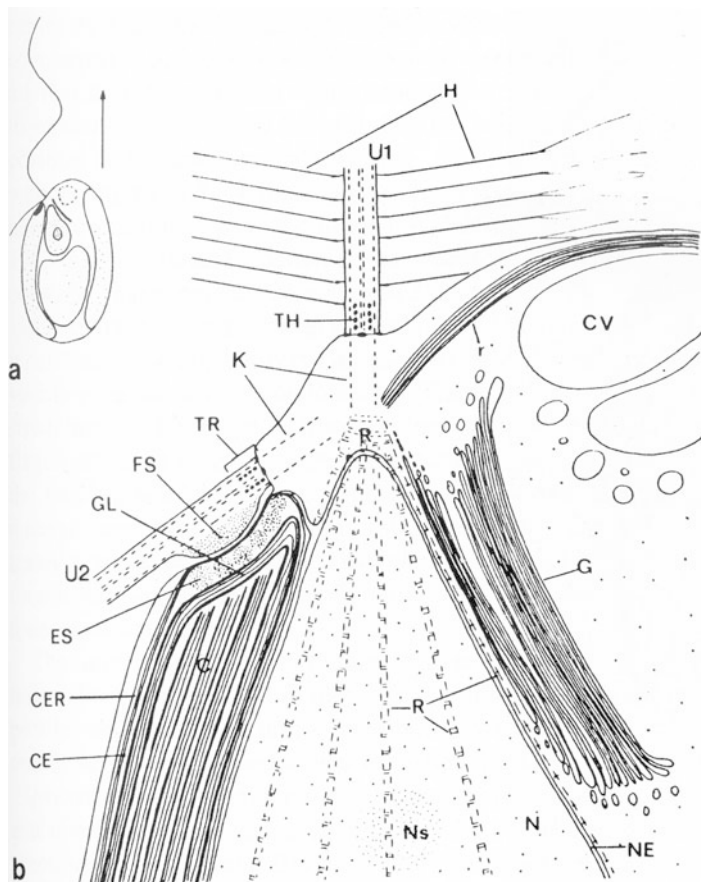
Fig. 11 Basic organization of a chrysophycean cell. (**a**) Diagram showing the flagella and other important organelles as seen with the light microscope (chloroplasts, eyespot, nucleus, golgi body, chrysolaminaran vacuole). (**b**) Diagram of anterior part of cell as seen in thin section with the transmission electron microscope: *C* chloroplast, *CE* chloroplast envelope, *CER* chloroplast endoplasmic reticulum, *CV* contractile vacuole, *ES* eyespot, *G* golgi body, *GL* girdle lamella, *H* flagellar hairs, *K* flagellar basal bodies, *N* nucleus, *Ns* nucleolus, *NE* nuclear envelope, *R* rhizoplast, *r* microtubular flagellar root, *TH* transitional helix, *TR* transitional region, *U1* anteriorly directed flimmer flagellum, *U2* laterally directed smooth flagellum (With permission from: D. J. Hibberd 1976, *Bot. Journ. Linn. Soc.* 72: 55–80, Copyright 1976, The Linnean Society of London)

to a peduncle, hardly protruding beyond the scale cover, and bearing the photoreceptor (Bourrelly 1957). Since no stigma is present in this genus, the shading effect may be due to the chloroplast itself.

In colorless forms, where the chloroplast has been lost or reduced to a leucoplast, there is most often also a reduction of the photoreceptor system. In the genus *Paraphysomonas,* a colorless counterpart to *Spiniferomonas*, there are all transitions from stigma-bearing species with complete photoreceptor system to species without

Fig. 12 Flagellar swelling
with photoreceptor in
juxtaposition to stigma-
bearing part of the chloroplast
(*Dinobryon*), × 64,000 (EM:
J. Kristiansen, from
Kristiansen and Walne (1977),
with permission from British
Phycological Society)
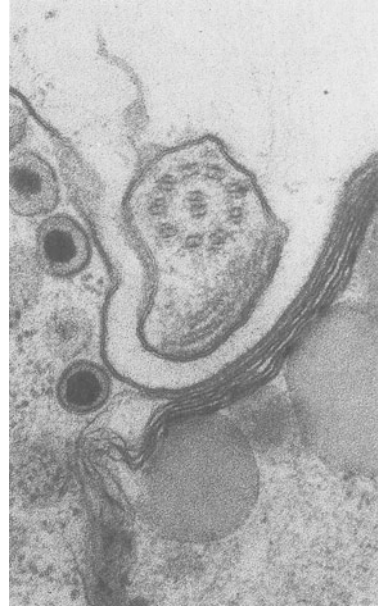
stigma but still with the leucoplast in juxtaposition to the flagellar swelling, and finally to species without stigma and swelling, and with no spatial relationship between leucoplast and flagellum (Preisig and Hibberd 1982, 1983). A similar reduction series is present in *Spumella,* a colorless counterpart to *Ochromonas* (Mignot 1977).

The nucleus surrounded by a double nuclear membrane is normally located in the center of the cell. In most cases, the outer nuclear membrane is continuous with the chloroplast ER, and the nucleus is thus intimately associated with the chloroplast (Fig. 13).

Close to the nucleus is the golgi body (Fig. 14). In most cases it consists of a single but very conspicuous set of vesicles often visible even in the light microscope. There are several golgi structures in *Hydrurus*. A close association exists between the nucleus and the forming face of the golgi: vesicles cut off from the outer nuclear membrane fuse to form golgi cisternae. Vesicles released from the edges of these cisternae are associated with the formation of scales, transport of flagellar hairs, and exocytosis of various substances.

The mitochondria have tubular cristae. The number of mitochondria per cell is difficult to discern. Many mitochondria profiles may be seen in thin sections, but they usually represent one or very few long and coiled mitochondria.

Microtubules occur mainly as peripheral systems below the cell membranes, emanating as microtubular bundles from the basal bodies as flagellar roots. They serve as a cytoskeleton to maintain cell shape. *Ochromonas* cells treated with colchicine, which prevents the assembly of microtubules, lose their specific shape

Fig. 13 Transverse section of a *Dinobryon* cell, showing the nucleus and the chloroplast wihin the chloroplast ER. On the inner face of the chloroplasts the periplastidial reticulum is seen. × 16,100. EM

Fig. 14 The relation between the golgi body (*above*), nucleus (*below*), and chloroplast (*right*) in *Dinobryon.* × 30,200. EM

and become spherical (Bouck and Brown 1973). Massively developed microtubular systems occur in the tetrahedral swarmers of *Hydrurus* and *Chrysonebula* (Hoffman et al. 1986; Hibberd 1977a). A bundle of microtubules is situated in the stalk of *Poteriochromonas* (Péterfi 1969).

Most species possess one or two plastids (Fig. 12). The plastids are often lobed and located in close connection with the nucleus. They are surrounded by four membranes (Gibbs 1962), the outermost of which, called the chloroplast endoplasmic reticulum, is continuous with the outer nuclear membrane. The compartment between the next membrane and the inner chloroplast membranes contains the periplastidial reticulum, which functions in the transport of proteins into the plastid (Gibbs 1979).

The chloroplast contains photosynthetic lamellae, each consisting of three thylakoids. A girdle lamella is present, except in *Mallomonas* and *Synura.* The chloroplast DNA is ring-shaped and located just within the girdle lamella. Pyrenoids are either immersed or semi-immersed in the plastid; they are sometimes traversed by thylakoids. Only in *Hydrurus* are they stalked. Colorless chrysophytes have leucoplasts, e.g*., Spumella, Heterochromulina,* and *Paraphysomonas.* In *Anthophysa* and some species of *Paraphysomonas,* the small leucoplast even possesses a stigma (Belcher and Swale 1972b; Preisig and Hibberd 1983).

The chloroplasts of the chrysophytes contain chlorophyll *a* as the main photosynthetic pigment. In addition, chlorophylls of the *c*-group occur, normally both *c*1 and c2, but in *Mallomonas* and *Synura* only *c*1. The golden-brown color of the plastid is due to the occurrence of accessory pigments, mainly xanthophylls; the most important is fucoxanthin, comprising up to 75% of the total pigment in *Ochromonas danica.* Diatoxanthin has been demonstrated in *Sphaleromantis* and *Ochromonas,* diadinoxanthin in *Sphaleromantis* (Aaronson and Baker 1959). β-carotene is present in all chrysophytes. Carotene is concentrated in the part of the plastid differentiated as the stigma.

The product of photosynthesis is chrysolaminaran (chrysose or leucosin). It is a β-1,3-glucan and is deposited as a peculiarly refringent storage product in a posterior vacuole. Lipids are deposited in small vesicles in the cytoplasm. The chrysophytes are known to produce a great variety of fatty acids.

It is doubtful if any entirely photoautotrophic chrysophytes exist. In darkness, *Ochromonas* can grow osmotrophically on dissolved organic compounds, in which case the plastids will eventually be reduced (Pringsheim 1952). Many photosynthetic naked chrysophytes are capable of phagocytosis. Cell membrane flow transports trapped particles to the apex where phagocytosis occurs. Chrysophytes take up any particles, even inorganic ones. Bacteria, small algae, and quite large diatoms that completely distort the cell may be ingested. The phagocytic vacuole is then transported to a special digestion vacuole at the posterior end of the cell (Cole and Wynne 1974). Rhizopodial species are especially adapted for this feeding method. The food uptake mechanism has been studied in detail first in *Ochromonas* (Doflein 1922), showing how bacteria were trapped in a cytoplasmic basket and then engulfed. In *Epipyxis* (Wetherbee and Andersen 1992; Andersen and Wetherbee 1992), food particles, e.g., bacteria, are captured by the flagella and brought into the cytoplasmic feeding basket supported by one of the flagellar roots and from there transported into a digestion vacuole.

Life History

The swarmer is the predominant stage in the life cycle at the monadoid level of organization. However, swarmers also occur as a regular phase in the life cycles of most species at other levels. Swarmers fall into two main types: *Ochromonas*-like swarmers have two flagella, while in *Chromulina* swarmers only one is visible. A special swarmer of tetrahedral shape occurs in *Hydrurus* (Joyon 1963) and *Chrysonebula.* In some coccoid and filamentous forms, the cell divides into several immotile offspring cells (called autospores) liberated by rupture of the parent cell wall.

Sexuality was believed to be rare among the chrysophytes; although it mostly escapes attention and requires much patience to demonstrate, it is likely to be more prevalent. Sex is most often observed in small loricate monads (i.e., surrounded by a special envelope) such as *Kephyrion, Stenocalyx, Chrysolykos,* and the solitary *Dinobryon* species. Undifferentiated cells act as gametes, fuse apically, and produce a globular zygote. The empty loricae of the gametes, which remain attached to the zygote, make it easily recognizable (Fott 1959).

In colonial species of *Dinobryon,* sexuality has also proved to be of great importance. Cyst formation involves autogamic processes (fusion of nuclei formed by a prior mitosis) or gametic fusion of cells liberated from male colonies with loricate cells in female colonies to form zygotic cysts (Sandgren 1981).

In *Synura* and *Mallomonas*, normal scale-bearing cells act as gametes, with posterior fusion (Wawrik 1972). *Synura* is heterothallic; sexuality is induced at high cell density. Single cells liberated from male colonies act as gametes and copulate with cells in female colonies; subsequently the zygotes encyst and remain in the colony (Sandgren and Flanagin 1986).

The endogenous cyst, the stomatocyst (often also called the "statospore"), the characteristic resting stage of the Chrysophyceae, has a very special morphology: a globular, silicified wall with an opening called a porus, closed by a pectic plug. In many species the porus is surrounded by a collar. The stomatocyst wall may be smooth or bear ornamentation, including protuberances such as spines in various arrays depending on species (Fig. 3). In *Hydrurus* and a few other genera, a distinctive stomatocyst occurs that is ellipsoidal with an equatorial ring.

The stomatocysts are usually classified following an artificial taxonomy based upon size and shape, the outer wall ornamentation, as well as pore and collar morphology (Kamenik 2010). Guidelines for the description and nomenclature of stomatocysts have been worked out by Cronberg and Sandgren (1986), and the stomatocyst atlas by Duff et al. (1995) contained 240 taxa. But already in 2001 an enlarged edition was necessary (Wilkinson et al. 2001).

Stomatocyst formation has been studied in detail in *Ochromonas*, *Mallomonas*, and *Dinobryon* by electron microscopy (Hibberd 1977b; Andersen 1982; Sandgren 1980a, b). Two basic types of cyst formation are known, but they have in common the internal formation of the silica wall in the silicalemma, a silica-depositing vesicle

derived from the Golgi body. At maturity, the porus is closed by a plug of fibrillar pectic material.

In *Ochromonas tuberculata* and *Mallomonas caudata*, the uninucleate cell transforms directly into a cyst. The internal silica wall is formed by deposition on a basal lamella, and the porus is formed by resorption of part of the already deposited wall. The cyst also contains one nucleus. The external cytoplasm disintegrates after having deposited the external wall structures. In *Ochromonas sphaerocystis*, the external cytoplasm does not disintegrate but is retracted through the porus. In *Dinobryon,* the process is more complicated. The cell moves to the lorica mouth and secretes a surrounding encystment chamber. After formation of the silica-depositing vesicle, the cyst wall is produced with the porus preformed. After the wall ornamentation has been deposited, the remaining external cytoplasm is retracted through the porus and the plug is formed.

Cyst germination has been examined in only a few species. The plug dissolves and a motile naked cell escapes. In *Ochromonas,* a single normal swarmer separates; in *Leukochrysis* and *Kybotion,* small amoeboid cells emerge. In *Mallomonas,* the germination products are small scaleless monads. In *Dinobryon,* a special germination chamber is formed from the porus of the stomatocyst. The cell divides twice to form four cells that wander into this germination chamber, from where they are eventually released as naked, free-swimming monads (Sheath et al. 1975).

Cell division is longitudinal, and in motile cells it starts from the anterior end of the cell. In scale-bearing forms, the scaly armor appears to be reestablished as division proceeds. Mitosis, studied in detail only in *Ochromonas* and a few others, is of a special type: the rhizoplasts from the two basal bodies act as poles for the organization of the spindle microtubules (Slankis and Gibbs 1972).

Maintenance and Cultivation

General algal culture methods, including those for chrysophytes, are described in Andersen (2005) and references given there. Table 2 presents culture media used with success for chrysophytes.

For ultrastructural and many taxonomical investigations, pure cultures are not always necessary. Enough material may be obtained in other ways: by collecting naturally occurring high concentrations (blooms) or by concentration of motile cells (e.g., *Synura*) using their phototactic behavior.

Crude cultures to enrich for rare chrysophytes may consist only of the natural sample placed in a cool north-facing window, and successively several chrysophyte species will appear, e.g., attached to the water surface. In many cases, enrichment cultures with nutrients added are more adequate. For freshwater nanoplanktonic species of *Spiniferomonas,* Preisig and Hibberd (1982, 1983) added modified Chu 10 medium to their natural water samples, after larger organisms had been filtered off.

Table 2 Examples of culture media

Chromulina placentula	Chu 10 modif.	Belcher and Swale 1967
Chrysococcus cordifieris	Pringsheim biphasic	Belcher and Swale 1972a
Chrysococcus rufescens	Pringsheim biphasic	Belcher 1969a
Dinobryon divergens	Dy-III-medium	Lehmann 1976
Mallomonas papillosa	Pringsheim biphasic	Belcher 1969b
Ochromonas danica	Aaronson and Baker	Aaronson and Baker 1959
Ochromonas minuta	Pringsheim org. Medium	Hill and Outka 1974
Ochromonas sphaerocystis	Chu 10 modif.	Andersen 1982
Ochromonas tuberculata	Bold's Basal + leaf extract	Hibberd 1977b
Paraphysomonas spp.	Lake water + Chu 10 modif.	Preisig and Hibberd 1982
	Sea water +1iver extract	Caron et al. 1999
Phaeaster pascheri	Pringsheim biphasic	Belcher 1969c
Poteriochromonas spp.	Pringsheim org. Medium	Schnepf et al. 1968
Synura petersenii	WC modif.	Klaveness and Guillard 1975
	Waris modif. Enriched	Schnepf and Deichgräber 1969
Standard chrysophyte medium	Dy-V-medium	Andersen et a1. 2005

Naked marine chrysophytes can be cultured by the addition of modified Erdschreiber medium to original water samples.

Many chrysophytes, because they are extremely fragile and delicate, do not tolerate the procedures necessary to get them into unialgal or axenic culture. For unialgal cultures, vitamins, and other organic growth factors must be included in the media. In chemically defined media necessary for most physiological investigations, these organic compounds must be added as specific vitamins, amino acids, etc. Media based on soil or liver extract, although they contribute a wide and undefined spectrum of vitamins and other organic and inorganic nutrients, unpredictably support growth of some organisms and not of others.

Erdschreiber solution, rich in phosphate and nitrate with added soil extract, is one of the media frequently used. Pringsheim's biphasic soil water medium is one of the most successful for growing freshwater chrysophytes, e.g., *Mallomonas papillosa, Chrysococcus cordiformis,* and *Phaeaster pascheri.* The soil in the bottom of the culture vessel slowly releases small amounts of nutrients.

Bold's Basal Medium, an inorganic synthetic medium, has been adapted for chrysophytes such as *Synura petersenii* and *Ochromonas tuberculata*; organics such as vitamin mixtures, leaf-, soil or peat-extract are added.

Chu 10, an inorganic medium containing silica, with addition of organic compounds is useful for silica-scale-bearing algae.

A standard medium for all chrysophytes is Dy V (Andersen 2005), based on Lehman's original Dy III medium for *Dinobryon* (Lehman 1976).

Highly enriched media, such as Pringsheim's organic medium (Pringsheim 1952) containing glucose, liver extract, yeast extract, and tryptone have been used for the cultivation of mixotrophic forms such as *Ochromonas minuta* and

Poteriochromonas malhamensis. In nonaxenic cultures, *Ochromomas* feeds on the bacteria that grow in the medium.

Colorless chrysophytes (e.g., *Anthophysa vegetans* and *Spumella elongata*) can grow in soil-water medium if the necessary extra organic nutrition such as starch or a barley seed are added (Belcher 1976). The phagotrophic *Paraphysomonas* species feed on bacteria naturally growing in nonaxenic culture (Lee 1978) or in sea or lake water enriched with liver extract or Chu 10 (Caron et al. 1999; Preisig and Hibberd 1982).

Chrysophyte cultures are maintained by numerous Culture Collections. Among these may be mentioned the following: UTEX, Austin, Texas, USA; NIVA, Oslo, Norway; CCAP, Oban, Scotland, UK; CAUP, Prague, Czech Republic; NCMA, Bigelow, Maine, USA; EPSAG, Göttingen, Germany.

Evolutionary History

Fossil Records

The siliceous structures of Chrysophyceae are very resistant and therefore common in many geological deposits, providing a better understanding of the evolutionary origin and stratigrafic distribution of these algae. Chrysophycean cysts (stomatocysts) are more heavily silicified than the other siliceous structures (scales and bristles), and so they are more likely to be present in the older sediments. On the other hand, natural classification is almost impossible as the stomatocyst descriptions are rarely accompanied by descriptions of their vegetative cells (Duff et al. 1995).

Cysts from freshwater deposits are grouped into an artificial family Chrysostomataceae, whereas those from marine sediments are grouped into the equally artificial Archaeomonadaceae. Since different genera could produce similar or even identical stomatocysts (Findenig et al. 2010), their fossil records are primarily important for the timing of the evolutionary origin of chrysophytes. Siliceous scales and bristles are generally preserved for a shorter geologic period (Siver et al. 2009; Siver and Wolfe 2005). However, in contrast to the stomatocysts, they could also be used to trace the evolutionary history and diversification of particular chrysophyte genera, or even species.

Although the oldest known chrysophyte-like structures have been reported from the Cambrian sediments (Allison and Hilgert 1986), their affinity to the Chrysophyceae is doubtful as they do not resemble any siliceous structures of modern taxa. Therefore, they may belong to any unrelated or even extinct lineage. The oldest certain fossils of chrysophytes are represented by siliceous stomatocysts of Archaemonadaceae, recovered from Tertiary or Upper Cretaceous marine deposits (Riaux-Gobin and Stumm 2006). At present, the oldest stomatocysts are from Southern Ocean sediments of Lower Cretaceous (Aptian-Albian, $\sim$ 112 Ma),

which may indicate the initiation of silicification within chrysophyte algae (Harwood and Gersonde 1990). In addition, since the stomatocysts are commonly found in fossil marine sediments, chrysophytes are presumed to have a marine origin (Tappan 1980).

The oldest records of fossilized chrysophyte scales and bristles have been reported from the Paleogene age. Recently, the oldest known microfossils of scales have been recovered from a Paleocene kimberlite deposit ($\sim$ 60 Ma) by Siver et al. (2013a). The scales could be assigned to the genus *Synura*, though two of four taxa discovered represent presumably extinct species. Scales and bristles of other genera of silica-scaled chrysophytes (*Mallomonas*, *Spiniferomonas*) are known from younger, Middle Eocene freshwater deposits ($\sim$ 47 Ma; Siver and Wolfe 2005, Siver et al. 2009; Siver and Lott 2012a). Other chrysophyte fossils are very rare. Identifiable remnants of *Dinobryon*, *Lagynion*, and *Cyrtophora* have been found in coprolites from Wyoming, dating from the Upper Eocene (Tappan 1980).

In general, the fossil record of chrysophytes is still very incomplete and poorly understood; there is much work to be done before it can be utilized to infer the timing of their evolutionary origin and to trace the diversification of particular lineages. Therefore, the origin and divergence times of extant genera are primarily estimated based on molecular clock calculations. According to the reconstruction of stramenopile diversification times, chrysophytes most likely originated in the Permian ($\sim$ 279 Ma; Brown and Sorhannus 2010). This estimation is in accordance with the study of Jo et al. (2013), who estimated the origin of the chrysophytes as $\sim$ 250 Ma. Interestingly, the diversification of *Mallomonas* species was dated to -133-119 Ma (Jo et al. 2013; Siver et al. 2013b, 2015), implying that this genus evolved much earlier than the paleontological record indicates.

Classification

The first systematics of chrysophyte algae was introduced by Pascher (1913), who stressed the organization levels as foundations for taxonomy, with the flagellar number being of major importance. Uniflagellate organisms were placed in the Chromulinales, whereas those organisms possessing two flagella of reportedly equal length were classified in the Isochrysidales. Organisms having two unequal flagella were grouped in the Ochromonadales. A year later, Pascher (1914) established the class Chrysophyceae, encompassing those protists with golden brown pigmentation. However, he took a different approach to their classification, placing emphasis upon vegetative life forms (flagellate, capsoid, or amoeboid) rather than the number and shape of flagella. In his newly proposed system, all flagellates were classified in the order Chrysomonadales.

Pascher's classification was widely accepted in the years that followed. Bourrelly (1957, 1965) divided the Chrysophyceae into three subclasses: the

Acontochrysophycidae (no flagella), the Heterochrysophycidae (one flagellum or two unequal flagella), and the Isochrysophycidae (two equal flagella). Within the Heterochrysophycidae, he recognized two orders: the Chromulinales (one flagellum) and the Ochromonadales (two flagella). All chrysophycean genera forming siliceous scales and spines were united in the family Synuraceae, within the Ochromonadales. Later on, Silva (1980) has pointed out that the name Mallomonadaceae has priority over Synuraceae.

The subsequent ultrastructural studies have shown that the number of flagella is a quantitative character based on reduction of the short flagellum, having no taxonomic value (Kristiansen 1986). Preisig and Hibberd (1983) used the ultrastructural features to split the silica-scaled chrysophytes into the families Mallomonadaceae (the parallel insertion of flagellar basal bodies, presence of girdle lamella and flagellar scales, and lack of stigma) and Paraphysomonadaceae. In his review, Kristiansen (1986) followed this classification scheme, raising the families to an order status, the Mallomonadales and Ochromonadales. Increasing evidence of morphological and chemical (unique chlorophyll composition) differentiation of Mallomonadales culminated in their establishing as an independent class, the Synurophyceae (Andersen 1987). (Table 1).

However, several recently published phylogenies of Stramenopiles or chrysophyte algae (e.g., Ben Ali et al. 2002; Takishita et al. 2009; Del Campo and Massana 2011; Yang et al. 2012; Škaloud et al. 2013; Scoble and Cavalier-Smith 2014) show the close affinity of Synurophyceae with Chrysophyceae, with the former class often nested within the paraphyletic Chrysophyceae. Therefore, the two classes should be combined again, with the synurophyte algae being members of the order within Chrysophyceae, the Synurales.

Phylogeny

Phylogenetic relationships among the chrysophyte taxa were first inferred by Andersen et al. (1999), who investigated SSU rDNA sequences. Both the NJ (neighbourjoining) and MP (maximum parsimony) analyses resolved the seven distinct clades. Later, Andersen (2007) improved the dataset considerably by including several new chrysophyte taxa and conducted Bayesian phylogenetic analyses of the nuclear SSU rDNA and rbcL genes. The newly published sequences of the genus *Chrysosphaerella* were inferred as distantly related to *Paraphysomonas*, indicating the artificial concept of the Paraphysomonadaceae.

A detailed SSU rDNA phylogeny of chrysophyte algae, based on the data set including nearly all available sequences from cultured species and environmental DNA, was published by Klaveness et al. (2011). More recently, Scoble and Cavalier-Smith (2014) published a detailed phylogenetic reconstruction of Chrysophyta based on 239 SSU rDNA sequences, showing the existence of diverse chrysophyte-related environmental clades EC1 and EC2. In their investigations of heterotrophic *Spumella*-like flagellates, Findenig et al. (2010) and Grossmann et al. (2016)

demonstrated a significant cryptic diversity of these organisms forming a number of distinct lineages across the Chrysophyta. Accordingly, 7 new genera (*Acrispumella*, *Apoikiospumella*, *Chromulinospumella*, *Cornospumella*, *Pedospumella*, *Poteriospumella*, and *Segregatospumella*) and 2 new orders (Apoikiida and Segregatales) have been described.

According to the phylogenetic reconstruction based on recently available SSU rDNA and *rbc*L sequences of morphologically well-characterized taxa, nine orders can be presently recognized within the Chrysophyta (Fig. 15):

Ochromonadales Pascher 1910
This order represents the most diverse lineage, comprising a number of flagellate genera, including the solitary flagellates (e.g., *Ochromonas*, *Spumella*), colonial forms (e.g., *Uroglena*, *Chrysonephele*), or loricate monads (e.g., *Dinobryon*, *Poterioochromonas*). The morphologically similar loricate genera *Dinobryon* and *Epipyxis* occupy separate, phylogenetically distant positions within the order. Similarly, the phylogenetic reconstruction indicates several independent losses of plastid during the evolution of the lineage.

Chromulinales Pascher 1910
The order comprises the solitary (e.g., *Chromulina*, *Oikomonas*) and colonial (*Chrysosphaerella*, *Cyclonexis*) flagellates, as well as the amoeboid organisms (*Chrysamoeba*). The colonial genus *Chrysosphaerella* produces siliceous spines and scales.

Apoikiida Boenigk et Grossmann 2016
The order includes two heterotrophic, bacterivorous biflagellated genera: a colonial genus *Apoikia* forming swimming colonies of cells held together by mucilage and a solitary genus *Apoikiospumella*.

Chrysosaccales Bourrelly 1954
This order includes the morphologically diverse assemblage of taxa, including the *Ochromonas*- or *Chromulina*-like flagellates, coccoid chrysophytes (e.g., *Chrysosphaera*), cells embedded in mucilage (*Chrysosaccus*) or amoeboid loricate organisms (*Lagynion*).

Hydrurales Pascher 1931
The order presently comprises three morphologically distinct genera – colonial, freshwater *Hydrurus* forming macroscopic thalli usually growing in cold water, pseudoparenchymatous marine chrysophyte *Phaeoplaca*, and the *Ochromonas*-like flagellate isolated from Antarctic sea ice.

Hibberdiales R. A. Andersen 1989
The order groups the colonial organisms having the palmelloid level of organization. Cells either secrete a buoyant mucilaginous material to which the cells adhere (*Kremastochrysis*), are closed in a spherical capsoid colonies (e.g., *Hibberdia*,
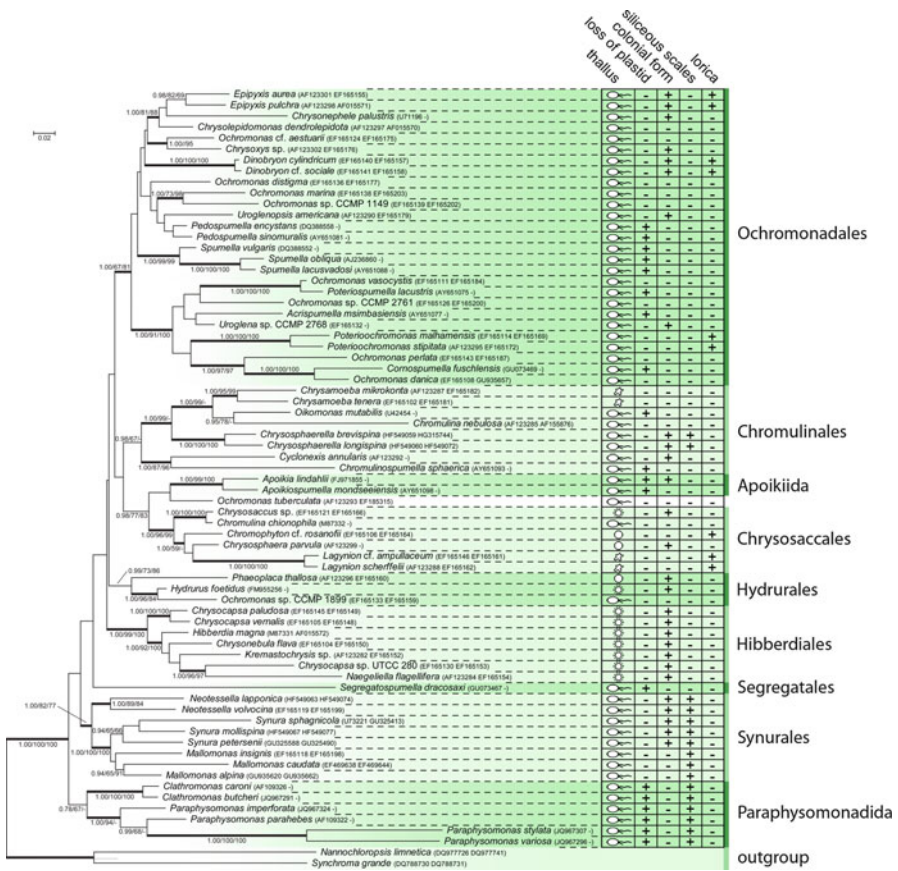
Fig. 15 Phylogeny of Chrysophyta, obtained by a Bayesian analysis of the combined and partitioned SSU rDNA + *rbc*L dataset using a GTR+G+I model for all partitions. Values at the nodes indicate statistical support estimated by three methods – MrBayes posterior-node probability (*left*), maximum-likelihood bootstrap (*middle*), and maximum parsimony bootstrap (*right*). Thick branches represent nodes receiving the highest PP support (1.00). GenBank accession numbers for the concatenated sequences (SSU rDNA and rbcL, respectively) accompany each species name. Scale bar shows the estimated number of substitutions per site. Basic characteristics of depicted chrysophyte taxa are provided in a graphical form. Thallus: the vegetative form is graphically indicated, including the monadoid, amoeboid, coccoid, and palmelloid organization. Other symbols indicate the presence/absence of plastid loss, colonial form, siliceous scales, and loricate structures

Chrysonebula), or they are grouped in a center of mucilaginous matrix extending a number of gelatinous tubes (*Naegeliella*).

Segregatales Boenigk et Grossmann 2016
The order currently comprises a single organism *Segregatospumella dracosaxi*, a bacteriovorous heterotrophic flagellate living in fresh water.

Synurales R. A. Andersen 1987

The order comprise three genera of autotrophic scale-bearing flagellates, namely, the solitary *Mallomonas* and the colonial *Synura* and *Neotessella*. The two former genera comprise the most common chrysophycean members of the freshwater planktonic communities worldwide.

Paraphysomonadida Scoble et Cavalier-Smith 2014

The order includes solitary, heterotrophic, flagellated genera *Paraphysomonas* and *Clathromonas*. Cells are covered with siliceous spine, basket, or plate scales. In addition, the order probably comprises a diverse, morphologically uncharacterized, environmental clade EC1.

Even though DNA sequence data are still lacking for several morphologically distinct genera (e.g., *Eusphaerella*, *Spiniferomonas*), the reconstructed phylogeny enables some inferences about evolutionary trends in the chrysophytes. First, the Synurales has the position as a nested group within the Chrysophyceae. Therefore, its recognition as a separate class, the Synurophyceae, is obviously not correct (see the "Classification" section) and should not be followed.

Second, the basal position of the Paraphysomonadida, a group with a significant portion of marine organisms, corroborates the hypothesis that the chrysophytes are of a marine origin (Tappan 1980).

Third, the silica-scaled chrysophytes do not form a monophyletic group, indicating either at least two independent origins of the ability to produce the siliceous structures (at the base of the Chrysophyta and within the Chromulinales) or multiple independent losses of this ability during the chrysophyte evolution.

Fourth, the phylogenetic reconstruction also indicates at least 10 independent reductions and losses of plastids, after which many distinct genera evolved.

Fifth, the morphologically simplest chrysophyte genera, *Ochromonas* and *Chromulina*, are both polyphyletic. The genus *Ochromonas* forms at least nine independent lineages, within the orders Ochromonadales, Hydrurales, and in a sister position to the order Apoikiida. The *Ochromonas* swarmer type might therefore be considered as the most primitive chrysophycean form from which the other morphological types evolved.

In general, the evolutionary history of the chrysophytes seems to be very complex, with several independent origins of morphologically similar taxa. Sequencing of additional taxa, together with extending the fossil data, will undoubtedly yield deeper insight into the evolution of this remarkable group of protist organisms.

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