

Developments in the taxonomy of silica-scaled chrysophytes – from morphological and ultrastructural to molecular approaches

Pavel Škaloud, Jørgen Kristiansen and Magda Škaloudová

P. Škaloud (skaloud@natur.cuni.cz) and M. Škaloudová, Dept of Botany, Faculty of Science, Charles Univ. in Prague, Benátská 2, CZ-128 01 Praha 2, Czech Republic. – J. Kristiansen, Dept of Biology, Faculty of Science, Univ. of Copenhagen, Øster Farimagsgade 2D, DK-1353, Copenhagen K, Denmark.

Taxonomy in silica-scaled chrysophytes has gone through three morphological phases. From primary studies of the cell morphology in the 18th century, the focus was in the 20th century replaced by studies of the silica structures of the cell envelope. Now, in the latest decades the importance of DNA sequencing has been recognized, not only to support the taxonomic framework but also to obtain new understanding of taxonomic relations among particular taxa. In the first part of this review, we provide a historical overview of the developments in the taxonomy of scale-bearing chrysophytes. In the second part, we present a phylogenetic reconstruction of chrysophyte algae, updated by newly obtained SSU rDNA and *rbcL* sequences of several isolated *Synura*, *Mallomonas* and *Chryso-sphaerella* species. We detected significant incongruence between the phylogenies obtained from the different datasets, with the SSU rDNA phylogram being the most congruent with the morphological data. Significant saturation of the first *rbcL* codon position could indicate the presence of positive selection in the *rbcL* dataset. Within the Synurales, the relationships revealed by the phylogenetic analyses highlight the artificial infragenetic classification of *Mallomonas* and *Synura*, and the occurrence of cryptic diversity within a number of traditionally defined species. Finally, three new combinations are proposed based on the phylogenetic analyses: *Tessellaria lapponica*, *Synura asmundiae* and *S. bjoerkii*.

Silica-scaled chrysophytes belong to the class Synurophyceae and the family Paraphysomonadaceae in the class Chrysophyceae. Historically, their taxonomy has passed through several stages, and will here be discussed from morphological, light-microscopical, electron-microscopical, and molecular viewpoints. After the beginning in the 19th century with purely coarse-morphological concepts of cell construction, the scaly envelopes of these genera were discovered, and light microscope (LM) studies of the silica structures were initiated and came into focus for the taxonomy and identification. Later, electron microscopy (EM) opened up for new possibilities, and the ultrastructure of scales became the standard tool for taxonomy. Several genera were exclusively described and studied by EM.

The increasing need for understanding the taxonomic value of minor structural differences and the growing importance of tracing evolutionary relationships was met by the introduction of molecular techniques, which during the last decade have become indispensable in taxonomic research and have not only confirmed and refined established knowledge, but to a high degree also shown unexpected relationships.

The genera discussed here will mainly be *Synura* and *Mallomonas* in the Synurophyceae and *Chryso-sphaerella*, *Spiniferomonas* and *Paraphysomonas* in the Chrysophyceae (Fig. 1).

Morphological delineation of silica-scaled chrysophytes

Morphology of vegetative cells

Cell morphology was the only tool available for the early chrysophyte researchers. After some preliminary attempts by Müller (1786), Ehrenberg (1838) described and pictured *Synura uvella* as globular colonies of elongate cells (Fig. 2). Stein (1878) provided more detailed drawings – useful even to-day – of *Synura uvella* with its chloroplasts, and even pictured the ‘hairy’ structure of the periplast, without trying to explain it. In the beginning of the twentieth century, Lemmermann (1904) and Pascher (1910) used the reticulate or warty structure of the periplast for describing two additional species (or varieties), *S. reticulata* Lemmermann and *S. verrucosa* Pascher, possibly as members of the same series of variation, in their treatment for the ‘Süßwasserflora’ (Pascher 1913). It was not clear what status they really should be given, due to the rather vague distinguishing characters. Conrad (1920) was convinced that there were not three species, but all transitions, which even could be found in the same sample. Awerinzew (1912) had unsuccessfully tried to document the nature of the hexangular pattern of the periplast by photomicrography.

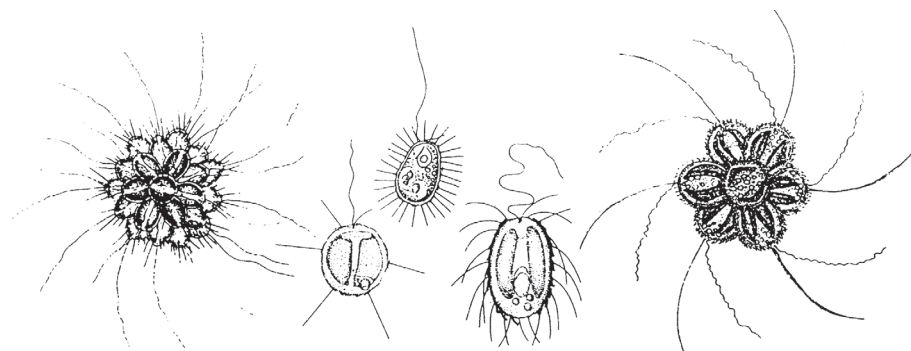


Figure 1. The morphology of the most important scale-bearing chrysophyte genera (from left to right): *Chrysosphaerella*, *Spiniferomonas*, *Paraphysomonas*, *Mallomonas* and *Synura* (Kristiansen 2008).

Only one *Synura* species showed sufficiently deviating cell structure to be useful as a taxonomic marker, viz. *Synura sphagnicola*, originally described in the independent genus *Skadowskiella* (Korshikov 1927) because of its axial instead of parietal chloroplasts. For purely morphological reasons the genus *Chlorodesmos* was erected, with filiform colonies (Phillips 1884). A similar case is the genus *Mallomonas*, described by Perty (1852) as ‘hairy monads’ (Fig. 2), including two species; but already by Seligo (1893) the true nature of the hairs was discovered – see later. However, in the following years several species were described solely on cell shape and number and arrangement of the bristles, as seen from the monograph by Conrad (1920). As late as 1961 Skvortzow described 10 new species that cannot be identified based on such vague characters.

The genus *Chrysosphaerella* has a rather confused history. It was originally described as *Actinoglena klebsiana* by Zacharias (1897), as a colonial chrysomonad with long radiating needles but no flagella. However, already in 1899 Lemmermann found flagella, and also a periplast like in *Synura*, and the organism was transferred to *Synura*. The needles, not normally found in *Synura*, were considered as possibly only seasonal in occurrence. At the same time Lauterborn (1899) found a similar organism with long

needles and a layer of short needles surrounding the colony, *Chrysosphaerella longispina*. However, he was not sure of its identity with Zacharias’s species.

As late as 1954 a new *Chrysosphaerella* species was described based on light-microscopy (LM) without study of spines and scales (Schiller 1954). *Physomonas* (*Paraphysomonas* de Saedeleer) was described by Stokes (1885) as colourless stalked monads with radiating bristles attached to the cell surface. The first *Spiniferomonas* species were described but misinterpreted as e.g. *Mallomonas globosa* (Schiller 1954), as the scales could not be investigated.

Light-microscopy of silica structures

As seen from the above, early taxonomy in these genera was hampered by the lack of suitable reliable characters. An opening for a solution of this problem was given by Petersen (1918) in what he thought was *Synura uvella*. In dry preparations and by staining he found that the envelope surrounding the cell was composed of silica scales of a characteristic morphology (Fig. 3). Korshikov (1929) was inspired by Petersen and by investigating *Synura* with this method he could define both *S. uvella* (Fig. 4) and five additional species. And further: Petersen’s species could not be identical with that of Stein with a spiny cell surface, but had to be

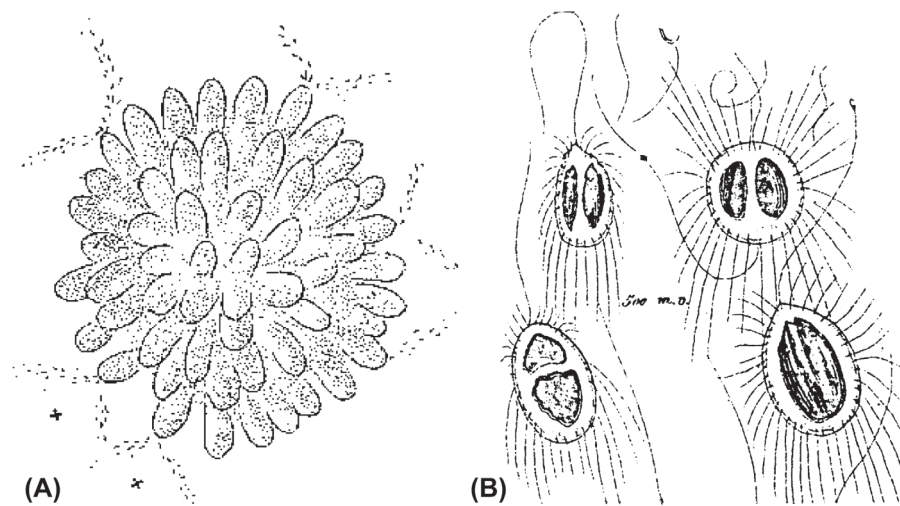


Figure 2. Earliest drawings of silica-scaled chrysophytes. Colonial *Synura uvella* (Ehrenberg 1838) and unicellular *Mallomonas acaroides* (Perty 1852).

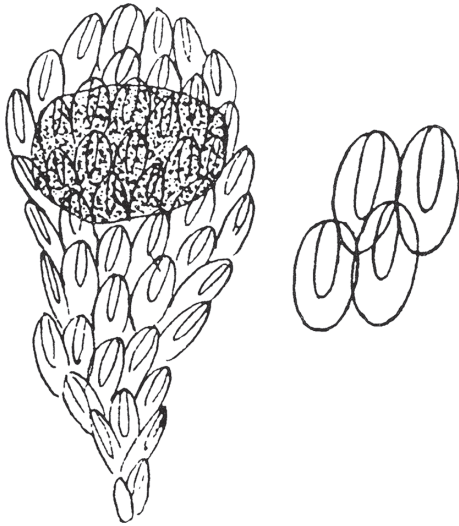


Figure 3. *Synura petersenii* – first drawing of a scale cover and scales, LM (Petersen 1918).

described as a new species, *Synura petersenii*. Likewise, Bioret (1931) also inspired by Petersen, examined *Synura* material in dry preparations, and he found scales similar to three of the types described by Korshikov, but refrained from taxonomic conclusions. Korshikov's species concept was adopted in subsequent identification keys, such as Huber-Pestalozzi (1941).

The structure of the *Mallomonas* envelope started to be solved already by Seligo's (1893) accurate description of scales and bristles in *Lepidotodon dubium*, which was the forerunner of what is now known as *Mallomonas caudata*. However, this organism got a confused fate. Ivanov (1899) described the species, but unfortunately had the scales mixed up with scales from another species: *M. acaroides* (of which he by the way also described the scale structure; Fig. 5). Further, Lemmermann at the same time described *M. fastigiata* (Lemmermann 1899), with Zacharias as author (!), and transferred Seligo's species to *Mallomonas*. So, in 1910 Lemmermann in his 'Kryptogamenflora der Mark Brandenburg' could enumerate the three species *M. dubia*, *M. fastigiata* and *M. caudata*, which later by Krieger (1930) were united to *M. caudata*.

Scale and bristle structure soon became an integrated part in the descriptions of *Mallomonas* species, such as it is seen from the identification works by Krieger (1930) and Conrad (1933), published almost simultaneously. In the latter scale morphology was used – as far as possible – to divide the genus into several sections.

The chrysophyte volume of 'Phytoplankton des Süßwassers' (Huber-Pestalozzi 1941) with 56 accepted species was mainly based on Conrad's work. Starmach (1985), in his volume of 'Süßwasserflora', included 126 species of *Mallomonas*, but still only part of them had known scale structure, and cell shape was still a main criterion. Accordingly, many of the species included could in fact not be identified.

The structure of scales and spines of *Chrysophaerella* was studied by Korshikov (1942) and used in distinguishing between *C. longispina* and the new species *C. brevispina*. But as late as in 1954 a third species *C. setifera* was published without such information (Schiller 1954) and thus cannot be identified. *Paraphysomonas* scales of the only known species (*P. vestita*) were described by Korshikov (1929) as siliceous, with his method used for *Synura*, as circular plates bearing a central spine.

As have been understood, LM observations of the silica structures were soon found unsatisfactory, even if they to some extent were used in species identification. The use of phase contrast and other advanced techniques were a help, but the nature of light itself set a limit for resolution in the LM.

Ultrastructure of silica structures

Structures less than 0.2 μm may not be resolved by LM, but here the electron beam with its immensely shorter wave length and a thousand times better resolving power of the transmission electron microscope (TEM) made the observation of much finer details possible, and shadow casting and later scanning electron microscopy (SEM) showed the three-dimensional structure of the scales and bristles.

Almost simultaneously several *Synura* investigations by EM were published, by Manton (1955), Petersen and Hansen (1956) and Fott and Ludvík (1957). This gave rise to numerous studies with descriptions of new taxa. By studies of the scales, the filiform colonies described under the name of *Chlorodesmos* (Phillips 1884) could be shown to be a morphological expression of *Synura spinosa* (Calado and

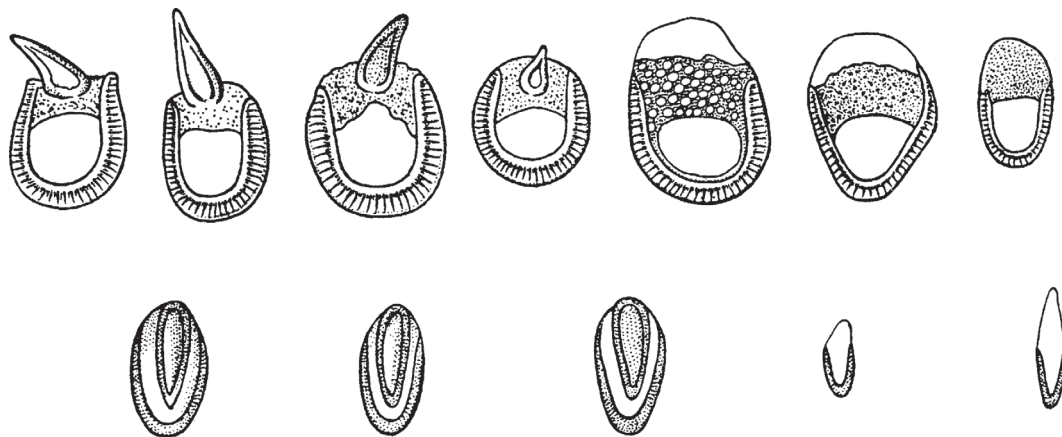


Figure 4. *Synura uvella* and *S. petersenii* – scale variation in the same cell, LM (Korshikov 1929).

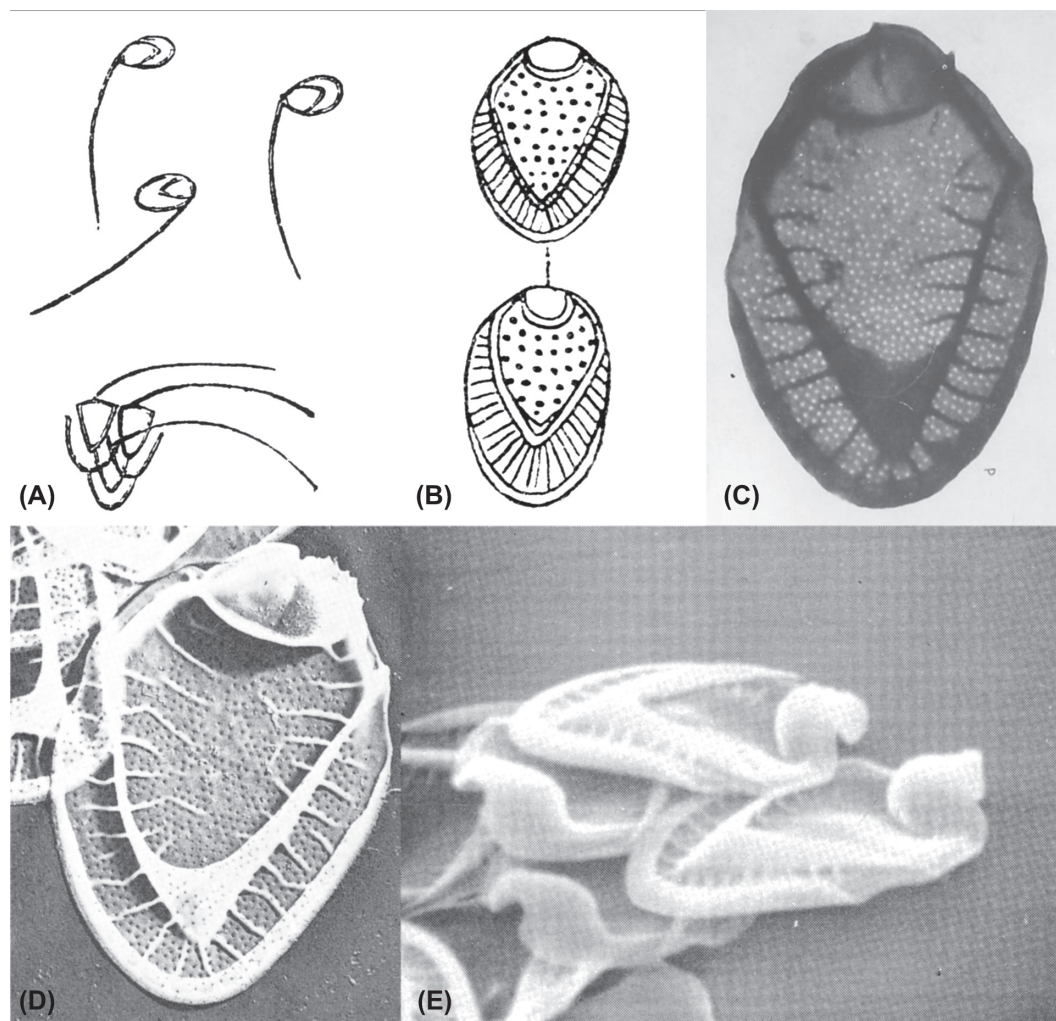


Figure 5. Scales of *Mallomonas acaroides*. (A) Early drawings of scale structure as viewed in light microscope (LM) – Ivanov (1899), (B) Detailed structure investigated by Bourrelly (1957) using LM, (C) First scales viewed in transmission electron microscopy (TEM) – Asmund and Kristiansen (1986), (D) Scale studied by shadow-cast TEM – Kristiansen (1975b), (E) First scanning electron microscopy (SEM) micrographs of scale structure – Kristiansen (1971).

Rino 1994). Similarly, Ehrenberg's *Syncrypta volvox* has been shown to be identical with *Synura sphagnicola* (Kristiansen 1988). Now, more than 20 taxa are listed in the latest edition of the 'Süßwasserflora von Mitteleuropa' (Kristiansen and Preisig 2007; Fig. 6). In the most recent description of new *Synura* species (Škaloud et al. 2012), the ultrastructural study of the scales is supplemented and supported by molecular data.

Similarly, the EM study of *Mallomonas* was initiated by Asmund (1955) on *Mallomonas caudata* and during the following years several other species were studied by her, and also by Fott (1955). The first scanning micrographs of *Mallomonas* scales (Fig. 5) were published by Kristiansen (1971). Later on, SEM was exclusively used by Siver (1991) in his *Mallomonas* monograph. The wealth of studies on this genus was summarized by Asmund and Kristiansen (1986) and Kristiansen (2002). Based on the ultrastructure of scales and bristles, altogether 163 taxa were recognized and the genus could be divided in sections and series. In this work, the recognition of two types of bristles, viz. craspedodont and notacanthic, was also important.

The genus *Mallomonopsis* had been described as having two visible flagella, but ultrastructure of scales and bristles showed its correct position in *Mallomonas*, which by the way also has two flagella, one of which is not visible in LM (Kristiansen 2002).

One case which has not yet been solved is the genus *Conradiella* described by Pascher (1925); instead of separate scales it has rings surrounding the cell. However, it has not been possible to obtain material for EM investigation, and the genus may be based on misinterpretation of *Mallomonas* cells (Kristiansen 1988, Kristiansen and Preisig 2007).

The first EM study of the colourless *Paraphysomonas* was made by Houwink (1952); later came the impressive monograph on the ultrastructure of both of scales and cells (Preisig and Hibberd 1982, 1983). Three types of scales, viz. plate, spine, and crown scales could be distinguished. Variations in these and their various combinations defined about 50 species, most of which were new. This study resulted in establishing the new family Paraphysomonadaceae as separate from Mallomonadaceae, see below.

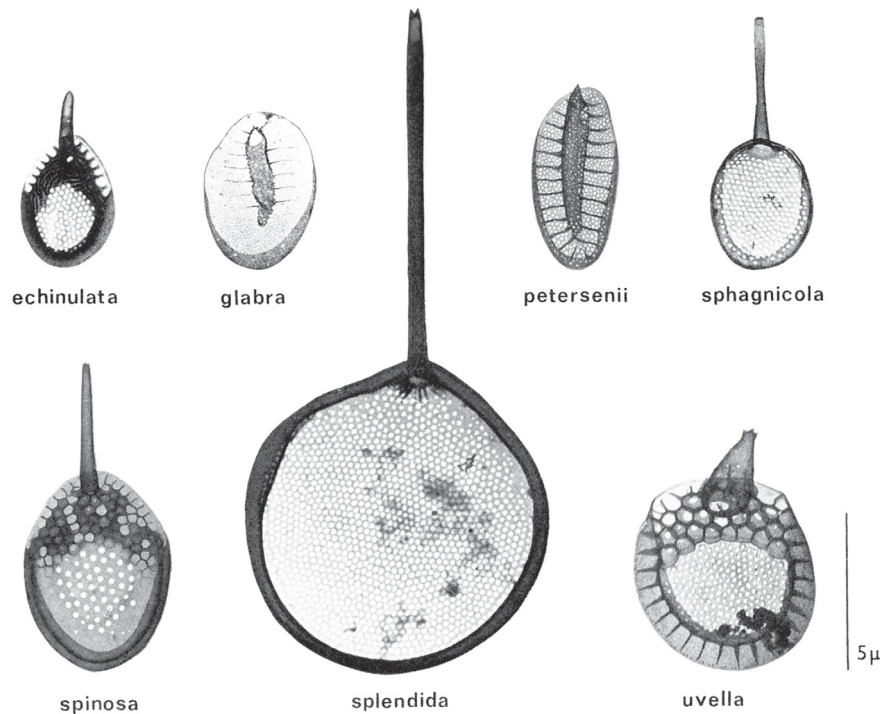


Figure 6. Scale types in *Synura*, TEM (Kristiansen 1975a).

The related, pigmented, genus *Spiniferomonas* comprising very small monads was established by Takahashi (1973) with seven species, but many more have been described since. A few had previously been described as species of *Mallomonas*, but their specific identity is unknown. As the key character separating these two genera is the presence or absence of a chloroplast, there have been some discussions concerning their delimitation. At present about 26 species of *Spiniferomonas* are known.

The genus *Chrysophaerella* was known from LM studies, but only EM clarified the relation between the colonial type species *C. longispina* and other later described species, some of which were solitary. The latter were also considered as species of *Spiniferomonas* (Nicholls 1984), but Kristiansen and Tong (1989) showed the taxonomic importance of the spine base: species with simple spine base should be classified as *Spiniferomonas*, whereas those with any spine base elaboration (hole, septum, double disc) should be placed in *Chrysophaerella*.

Classification of silica-scaled chrysophytes

In the initial stage of chrysophycean classification, the three earliest described genera of silica-scaled chrysophytes (*Synura*, *Mallomonas*, *Chrysophaerella*) were incorporated into the family Chrysomonadina (Stein 1878, Klebs 1892) comprising a loose collection of various brown-coloured flagellates. In his treatment of this family in 'Süßwasserflora', Pascher (1913) introduced the first systematics of chrysophyte algae, based on the number and length of flagella. Uniflagellate organisms (including *Mallomonas* and *Chrysophaerella*) were placed in the Chromulinales, whereas organisms possessing two flagella of equal length (including *Synura* and

Chlorodesmos) were classified in the Isochrysidales. Organisms having two unequal flagella were grouped in the Ochromonadales. One year later, Pascher (1914) established the class Chrysophyceae, encompassing 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 following years. However, the morphological heterogeneity of flagellates led many authors to divide Chrysomonadales into a number of families. For example, Fritsch (1935) divided the chrysophycean flagellates possessing siliceous scales into the families Mallomonadaceae and Synuraceae. Smith (1938) modified the Pascher's original classification and defined families on the basis of the number and length of flagella. The genera *Mallomonas* and *Synura* were classified in the Chromulinae and Isochrysidineae, respectively. Later, Bourrelly (1957, 1968) also recognized the significance of Pascher's (1912) observations on flagellar morphology and divided the Chrysophyceae into three subclasses: the Acontochrysophyceae (no flagella), the Heterochrysophyceae (one flagellum or two unequal flagella) and the Isochrysophyceae (two equal flagella). Within the Heterochrysophyceae, he recognized two orders, the Chromulinales (one flagellum) and the Ochromonadales (two flagella). All chrysophycean genera forming siliceous scales and spines (currently accepted *Mallomonas*, *Synura*, *Conradiella*, *Chrysophaerella* and *Paraphysomonas*) were united in the family Synuraceae (Ochromonadales). Starmach (1968) followed Bourrelly's classification scheme, and included also the genus *Chrysodidymus* in Synuraceae.

A few years later, a new genus of silica-scaled chrysophytes, *Spiniferomonas*, was erected by Takahashi (1973) who classified it in Synuraceae. Later on, Silva (1980) pointed out that the name Mallomonadaceae has priority over Synuraceae. Consequently, Preisig and Hibberd (1982) referred all chrysophycean genera having siliceous scales to Mallomonadaceae. However, one year later they showed that the fine cell structure of *Chrysosphaerella*, *Paraphysomonas*, *Spiniferomonas* and the newly described genus *Polylepidomonas* is much more similar to that of *Ochromonas* and *Chromulina* than to *Mallomonas* and *Synura* (Preisig and Hibberd 1983). Therefore, they established the new family Paraphysomonadaceae to comprise those scale-bearing flagellates sharing a generally similar internal structure (perpendicular orientation of flagella, lack of flagellar scales and girdle lamella of chloroplast, presence of stigma). In his review, Kristiansen (1986a) followed this classification scheme, and split silica-scaled chrysonomads into two orders, Mallomonadales (incl. Mallomonadaceae) and Ochromonadales (incl. Paraphysomonadaceae).

Increasing evidence of morphological differentiation between Mallomonadaceae and other chrysophycean taxa culminated in the description of a new class of Stramenopiles, the Synurophyceae (Andersen 1987). Andersen (1987, 1989) delineated the Synurophyceae from the Chrysophyceae on the basis of the absence of photosynthetic pigment c_2 , the parallel insertion of flagellar basal bodies, the lack of an eyespot, posteriorly located contractile vacuoles, and the bilaterally symmetrical silica scales. The genera of Synurophyceae were placed in the order Synurales consisting of two families, the colonial Synuraceae (*Synura*, *Chrysodidymus*) and the single-celled Mallomonadaceae (*Mallomonas*, *Conradiella*). At the same time, Tyler et al. (1989) rediscovered the rare Australian endemic colonial chrysonomad *Tessellaria*, and classified it within Synurophyceae. The putative mucilaginous envelope has been shown to consist of a layer of overlapping scales, strongly resembling the morphology of *Synura lapponica*. However, Pipes et al. (1991) pointed out the uncertainty about the classification of *Tessellaria*, as it possesses both synurophycean and chrysophycean ultrastructural features.

The current classification of silica-scaled chrysophytes recognizes two classes, Chrysophyceae and Synurophyceae (Preisig 1995, Kristiansen and Preisig 2007). Chrysophycean genera (*Chrysosphaerella*, *Paraphysomonas*, *Spiniferomonas*, *Polylepidomonas*) belong in Paraphysomonadaceae, one of the families of Chromulinales. The single synurophycean order Synurales comprises two families, the colonial Synuraceae (including the scale bearing *Chrysodidymus*, *Synura* and *Tessellaria*) and the single-celled Mallomonadaceae (comprising the genera *Mallomonas* and *Conradiella*).

As described above, it is obvious that no proper identifications could be done without EM. The problem then arose of harmonizing EM identifications with older LM descriptions, as names based on these still occurred in surveys and floras. Often these problems could not be solved. In the *Mallomonas* monographs (Asmund and Kristiansen 1986, Kristiansen 2002) only species with known scale ultrastructure were accepted. The same procedure was followed in the newest edition of the 'Süßwasserflora' (Kristiansen and Preisig 2007).

Molecular phylogenetic investigations

Phylogenetic relationships of silica-scaled chrysophytes

During the last century, a solid foundation was built to identify and describe new species and varieties of the silica-scaled chrysophytes. Many investigations were made all around the world, including the biogeographic studies characterizing the various distribution types (Kristiansen 2001). However, new problems arose, including the taxonomical evaluation of minute differences in the morphology of siliceous structures. Moreover, it became essential to clarify the classification of silica-scaled chrysophytes by estimating the degree of relationship among chrysophyte taxa. Newly emerging techniques of molecular biology opened the way to resolve these problems.

The first DNA sequences of silica-scaled chrysophytes were published in the early 1990s, as parts of studies investigating the general phylogeny of Stramenopiles organisms (Ariztia et al. 1991, Bhattacharya et al. 1992). The earliest phylogenetic study focusing on scale bearing chrysophytes was published by Lavau et al. (1997), who performed a MP (maximum parsimony) analyses based on nuclear SSU rDNA data and scale characteristics of 17 species of *Mallomonas*, *Synura*, and *Tessellaria*. The study supported the Synurophyceae as a monophyletic assemblage, with *Tessellaria volvocina* inferred to have a basal position within the class. However, independent parsimony analyses of molecular data and scale characteristics did not recover the genera *Mallomonas* and *Synura* as monophyletic. In contrast, analysis on the combined dataset resolved these genera monophyletic, but with a very weak bootstrap support. Two years later, Caron et al. (1999) investigated the phylogenetic position of the scale-bearing, heterotrophic genus *Paraphysomonas*. The molecular phylogenetic analyses corroborated the independent origin of siliceous scales in Synurophyceae and Paraphysomonadaceae, as proposed by Preisig and Hibberd (1983). Almost concurrently, Andersen et al. (1999) conducted NJ (neighbor-joining) and MP analyses of a broad taxon sample of Chrysophyceae, using SSU rDNA sequences. The analyses resolved Synurophyceae as one of seven Chrysophycean clades, which cast doubt on its distinctness as a class level taxon. In addition, there was no bootstrap support for separating the Chrysophyceae from the Synurophyceae. In congruence with the DNA-based parsimony analysis of Lavau et al. (1997), the genera *Mallomonas* and *Synura* were not inferred reciprocally monophyletic.

More recently, Andersen (2007) published a Bayesian phylogenetic analysis of the Chrysophyceae and the Synurophyceae using nuclear SSU rDNA and *rbcL* sequences from more than 90 taxa. Despite the considerably broadened taxon sampling, the separation of the two classes still remained unresolved. The *rbcL* phylogram did not support a monophyletic Synurophyceae, since *Tessellaria* was placed in a sister position to all other Chrysophycean taxa. The SSU rDNA phylogeny provided strong statistical support for a monophyletic Synurophyceae, but the Chrysophyceae was not resolved. The combined analysis provided weak support for separating those classes. Further, the combined analysis suggested the reciprocal monophyly of the Synurophycean genera *Synura* and *Mallomonas*, though the *rbcL* gene alone

resolved them as polyphyletic. Later, Andersen (2007) published the first sequences of *Chryso-sphaerella*, another genus of scale bearing chrysophytes. According to the SSU rDNA analysis, *Chryso-sphaerella* is neither related to Synurophyceae nor to the genus *Paraphysomonas*, indicating the artificial concept of the Paraphysomonadaceae. Shortly after, Kim et al. (2007) published a ML analyses of several strains of *Mallomonas caudata* and allied species, based on SSU rDNA and *rbcL* sequences. Contrary to the analyses of Andersen (2007), the monophyly of genera *Synura* and *Mallomonas* was recovered in the *rbcL* analysis, though without bootstrap support.

The most recent phylogenies of chrysophyte algae were published in 2011. First, Jo et al. (2011) examined the phylogenetic relationships among 18 species of *Mallomonas*, analyzing sequence data from the nuclear SSU, LSU rDNA and *rbcL* gene. Bayesian and ML analyses revealed that *Mallomonas* consists of two strongly supported clades, differentiated by the presence of a V rib on the shield of the silica scales. Since no *Synura* species were included in the analysis, the relationship and monophyly of these two genera were not tested. Del Campo and Massana (2011) presented a SSU rDNA phylogeny of chrysophyte algae, including a number of environmental sequences. The analysis revealed the presence of significant hidden diversity within the genus *Paraphysomonas* and placed Synurophyceae nested within several Chrysophyceae clades. Finally, Klaveness et al. (2011) published the so far most detailed SSU rDNA phylogeny of chrysophyte algae, based on a data set including nearly all available sequences from cultured species and environmental DNA. The Synurophyceae did not obtain statistical support in ML and Bayesian analyses; although a relative large number of *Mallomonas* and *Synura* species were included in the analysis, only the latter genus obtained moderate statistical support.

In general, all above-mentioned studies have shown that the classes Chrysophyceae and Synurophyceae are more closely related to each other than to other groups of Stramenopiles algae. Moreover, Synurophyceae is often resolved as nested within a paraphyletic Chrysophyceae. Although recognizing Synurophyceae as a separate class seems rather incorrect, the final decision to invalidate this taxon should be based on a multi-gene phylogeny of Stramenopiles. Recently, such a multigene phylogenetic analysis was published by Yang et al. (2012), recovering Synurophyceae as a distinct taxon, but closely related to (a 10-gene dataset), or even nested within (a five-gene dataset), the Chrysophyceae.

Molecular evidence for cryptic diversity

Along with the molecular phylogenetic studies investigating the relationships among particular chrysophyte taxa, several studies have focused on the cryptic diversity present within nominal species of silica-scaled chrysophytes. Most of these studies investigated the cryptic diversity within a common freshwater species, *Synura petersenii*. The first molecular analysis was performed by Wee et al. (2001), who investigated the genetic variability of 15 isolates of *S. petersenii*, using the ITS rDNA region. A MP analysis revealed the existence of two well-supported clades. Whereas the first one exhibited

a cosmopolitan distribution, the second was restricted to North America. Later, Kynčlová et al. (2010) broadened the dataset by adding another 21 ITS rDNA sequences of newly isolated strains and a new ML analysis revealed the existence of six different clades within *S. petersenii*. In addition, traditional morphological analyses and geometric morphometrics of silica scales revealed significant phenotypic differences between all inferred clades. The results provided robust evidence for the presence of cryptic species within *S. petersenii*.

Almost concurrently, Boo et al. (2010) published a multigene phylogeny of almost 100 *S. petersenii* isolates, confirming the high degree of cryptic, species-level diversity within this species. Their results indicated the existence of both cosmopolitan and regionally endemic cryptic species. The taxonomic assessment of the observed cryptic diversity was published by Škaloud et al. (2012), who redefined the species concept within the *S. petersenii* morphotype, and recognized six cryptic lineages as separate species *S. americana*, *S. conopea*, *S. glabra*, *S. macropora*, *S. petersenii* and *S. truttae*. In addition, the morphological distinction of all the newly defined species allowed to trace their distribution based on previously published reports, indicating the significant underestimation of their distribution in previous studies based on molecular investigations only.

Apart from studies focusing on the cryptic diversity within *S. petersenii*, Kim et al. (2007) investigated the genetic diversity of six isolates of *Mallomonas caudata*. Despite using slowly evolving molecular markers such as SSU rDNA and *rbcL*, the sequences were not exactly identical, suggesting some degree of population differentiation or cryptic speciation.

As described above, evolution and genetic relationships of species are still poorly known in silica-scaled chrysophytes. Some genera still remain molecularly uncharacterized (e.g. *Spiniferomonas*), and published sequences from the others represent only a small portion of all described species. Up to date, molecular data have been obtained for 25 *Mallomonas* and 11 *Synura* taxa, representing about 16% of all currently accepted species and infraspecific taxa. The genus *Chryso-sphaerella* is so far characterized by two sequences obtained from uncultured, isolated colonies, without any detailed species determination (Andersen 2007). In addition, the monophyly of the most common autotrophic genera *Synura* and *Mallomonas* is still questioned.

Therefore, we aimed to present the updated phylogeny of chrysophyte algae, including newly obtained sequences from several isolated *Synura*, *Mallomonas* and *Chryso-sphaerella* species. We hope that adding new taxa, including morphologically distinct and rare species, will considerably improve our knowledge about the evolution of silica-scaled chrysophytes and the relationships of *Mallomonas* and *Synura* species.

Methods

Newly isolated taxa

In total, we successfully isolated and cultured 12 taxa of silica-scaled chrysophytes, the majority being cultivated for the first time since their description (Table 1). We focused mainly on

Table 1. Origin and GenBank accession numbers of analyzed strains.

Taxon	Strain	Locality	Geographic coordinates	SSU rDNA	<i>rbcl</i>
<i>Chrysophaerella brevispina</i>	S 74.D5	Dráčovské pools, Czech Republic	49°23'46.7"N, 14°71'30.7"E	HF549059	–
<i>C. longispina</i>	S 61A.B4	Tvillinghullerne, Bornholm, Denmark	55°13'34.5"N, 14°94'00.7"E	HF549060	HF549072
<i>Mallomonas kalinae</i>	CAUP B601	a small peat bog, Ostrov, Czech Republic	50°80'983"N, 14°045'53"E	HF549061	HF549073
<i>M. papillosa</i>	CCMP 476	a salt march, England, UK	–	HF549062	–
<i>Synura lapponica</i>	S 59.C4	Helgassjön, Sweden	56°95'61.1"N, 14°71'63.0"E	HF549063	HF549074
<i>S. macracantha</i>	S 90.B5	Tehriselkä, Finland	61°75'65.2"N, 26°48'58.1"E	HF549064	HF549075
<i>S. mammillosa</i>	S IE.105A	Caha Lakes, Beara, Ireland	51°72'17.0"N, 9°66'02.9"W	HF549065	HF549076
<i>S. mammillosa</i>	S 89.C3	unnamed lake near Koivula, Finland	62°25'02.9"N, 26°58'00.0"E	HF549066	–
<i>S. mollispina</i>	S 71.C10	Podhradská pool, Czech Republic	50°46'11.2"N, 14°91'17.6"E	HF549067	HF549077
<i>S. multidentata</i>	S 90.C11	Heinäjärvi, Finland	62°88'12.5"N, 25°49'74.9"E	HF549068	HF549078
<i>S. petersenii</i> f. <i>asmundiae</i>	S 90.D11	Rutajärvi, Finland	61°91'40.3"N, 26°08'15.9"E	HF549069	HF549079
<i>S. petersenii</i> f. <i>bjoerkii</i>	SC 57.A6	a small canal, Store Mosse, Sweden	57°30'64.0"N, 14°01'25.7"E	HF549070	HF549080
<i>S. spinosa</i>	S 74.D2	Veselské sand quarries, Czech Republic	49°15'36.7"N, 14°70'98.6"E	–	HF549081
<i>S. splendida</i>	S 90.E4	Rutajärvi, Finland	61°91'40.3"N, 26°08'15.9"E	HF549071	HF549082

the genus *Synura*, which represents the most undersampled genus from the perspective of the morphological diversity of silica scales. The new isolates belong to all three sections of the genus (sensu Kristiansen and Preisig 2007): Lapponica, *Synura* and Petersenianae (Fig. 7). *Synura lapponica*, the sole species of the section Lapponica, is characterized by oval scales with upturned edge and a central, rounded knob. Six isolates belong to the section *Synura*, defined by oval body scales bearing a distal spine: *S. mollispina*, *S. multidentata*, *S. spinosa*, *S. splendida* and two isolates of *S. mammillosa*. Finally, we isolated three morphologically distinct representatives of the section Petersenianae, which is characterized by rather lanceolate body scales possessing a median keel: *S. macracantha*, *S. petersenii* f. *asmundiae* and *S. petersenii* f. *bjoerkii*. Two species of the genus *Chrysophaerella* were successfully brought into the culture: *C. brevispina* and *C. longispina*. In addition, we obtained sequence data from two cultured *Mallomonas* species, *M. kalinae* and *M. papillosa*, obtained from the Culture Collection of Algae of Charles Univ. in Prague (CAUP) and the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA). The cultures were isolated and cultivated as described in Kynčlová et al. (2010).

Molecular data and phylogenetic analyses

For DNA isolation, the cells were centrifuged in PCR tubes (6000 rpm for 3 min), and 50 ml of the InstaGene matrix was added to the pellet. The solution was vortex mixed for 10 s, incubated at 56°C for 30 min and heated to 99°C for 8 min. After vortex mixing a second time, the tubes were centrifuged at 12000 rpm for 2 min, and the supernatant was directly used as a PCR template. Two molecular markers were amplified by PCR: nuclear SSU rDNA and chloroplast *rbcl*. The amplification of SSU rDNA was performed as described in Škaloud et al. (2012), using the primers 18S-F and 18S-R (Katana et al. 2001). The amplification of the

rbcl marker was performed according to Jo et al. (2011), using the primers *rbcl*_2F (Daugbjerg and Andersen 1997) and *rbcl*_R3 (Jo et al. 2011). The PCR products were purified and sequenced by Macrogen in Seoul, Korea. The sequence data were submitted to The Barcode of Life Database (BOLD, <www.boldsystems.org>) and to GenBank under accession numbers HF549059–HF549082 (Table 1).

Two different alignments were constructed for the phylogenetic analyses, based on the SSU rDNA and *rbcl* sequences. The sequences were selected according to the publications of Andersen (2007) and Klaveness et al. (2011) to encompass all chrysophycean lineages. After including newly determined sequences, the final matrices contained 111 SSU rDNA and 91 *rbcl* sequences, respectively. The outgroup taxa (*Synchroma* and *Nannochloropsis*) were selected based on the results of the recent multigene phylogenetic analysis of Stramenopiles published by Yang et al. (2012). The SSU rDNA sequences were aligned using MAFFT, ver. 6, applying the Q-INS-i strategy (Kato et al. 2002). Then, poorly aligned positions were eliminated using the program Gblocks, ver. 0.91b (Talavera and Castresana 2007). The final alignment was 1686 bp long. The *rbcl* sequences were manually aligned using MEGA 5 (Tamura et al. 2011), resulting in an 1025 bp long alignment. Suitable substitution models for the entire SSU rDNA dataset and individual *rbcl* codon positions were selected using MEGA 5. The GTR + G + I model was estimated as the most appropriate for all partitions. The strength of the phylogenetic signal versus noise in SSU rDNA and *rbcl* codon partitions was assessed by plotting the uncorrected p-distance against the corrected GTR + G + I distance using PAUP, ver. 4.0b10 (Swofford 2002). To remove saturated nucleotide sites of the 1st and 3rd *rbcl* codon partitions, we applied a modified site-stripping approach (Waddell et al. 1999). Site-specific rates were calculated with the 'Substitution Rates' standard analysis implemented in HyPhy

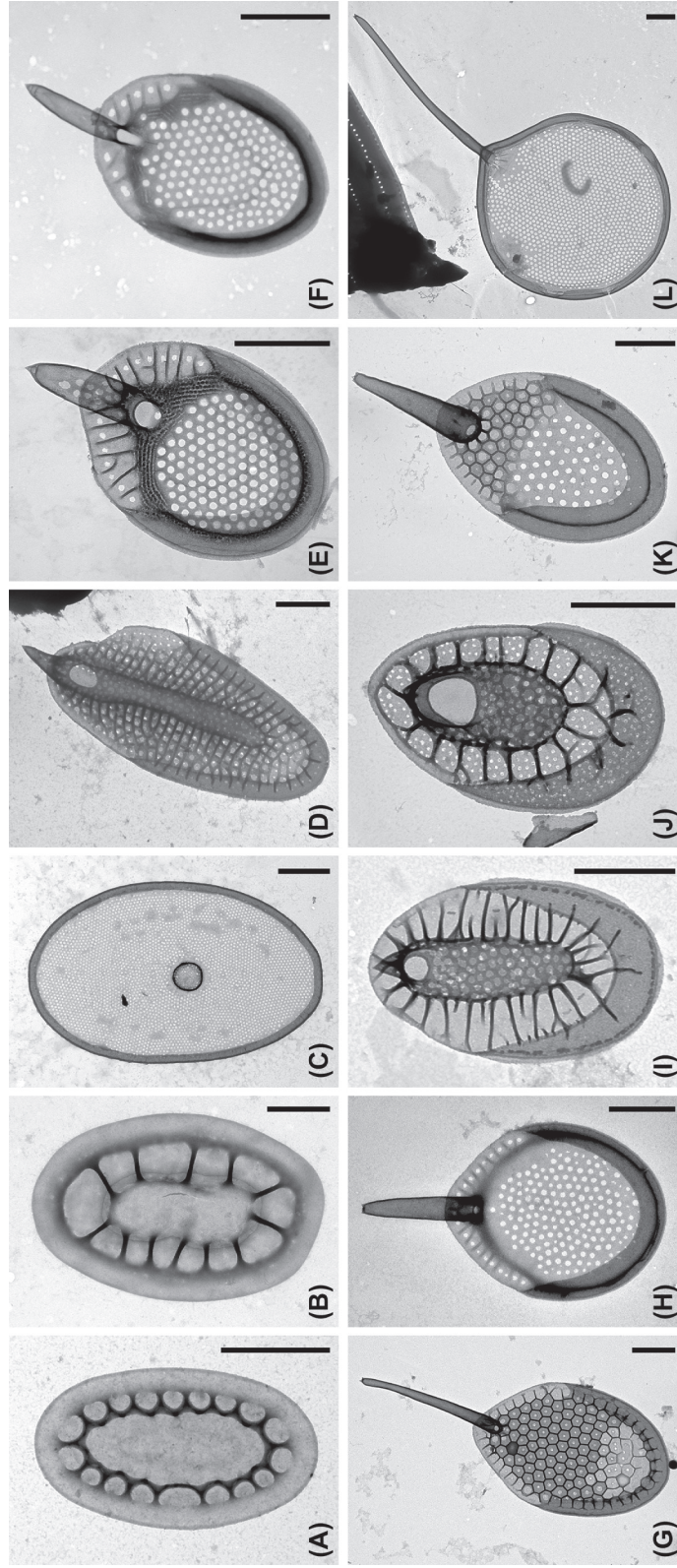


Figure 7. Scale morphology of newly isolated strains. (A) *Chrysophaerella brevispina* S 74.D5, (B) *C. longispina* S 61A.B4, (C) *Synura lapponica* S 59.C4, (D) *S. macracantha* S 90.B5, (E) *S. mammillosa* S IE.105A, (F) *S. mammillosa* S 89.C3, (G) *S. mollispina* S 71.C10, (H) *S. multidentata* S 90.C11, (I) *S. petersenii* f. *asmundiae* S 90.D11, (J) *S. petersenii* f. *bjoerkii* SC 57.A6, (K) *S. spinosa* S 74.D2, (L) *S. splendida* S 90.E4. Scale bars represent 1 μm .

(Pond et al. 2005), under a global GTR + G + I model using the inferred ML phylogeny as a guide tree. 8% (1st codon position of *rbcL*) and 27% (3rd codon position of *rbcL*) of fast-evolving sites were removed using SiteStripper (Verbruggen 2012), according to the rates file generated in HyPhy. The final, stripped *rbcL* alignment was 784 bp long.

The phylogenetic trees were inferred with Bayesian inference (BI) by using MrBayes ver. 3.1 (Ronquist and Huelsenbeck 2003). Analysis of the *rbcL* dataset was carried out on a partitioned dataset to differentiate among codon positions. All parameters were unlinked among partitions. Two parallel Markov chain Monte Carlo (MCMC) runs were carried out for three million generations each with one cold and three heated chains. Trees and parameters were sampled for every 100 generations. Convergence of the two cold chains was checked and 'burnin' was determined by use of the 'sump' command. Bootstrap analyses were performed by ML and weighted parsimony (wMP) criteria by using GARLI, ver. 2.0 (Zwickl 2006) and PAUP, respectively. ML analyses consisted of 100 replicates, using default settings and the automatic termination set at 100 000 generations, under the unpartitioned SSU rDNA and partitioned *rbcL* datasets. The wMP bootstrapping (1000 replicates) was performed using heuristic searches with 1000 random sequence addition replicates, TBR swapping, and random addition of sequences (the number was limited to 10 000 for each replicate). The weight of the characters was assigned using the rescaled consistency index on a scale of 0–1000. New weights were based on the mean of the fit values for each character over all of the trees in memory.

Results

The SSU rDNA analysis revealed the phylogenetic position of silica-scaled chrysophytes as three different lineages within Chrysophyceae (Fig. 8). The genus *Paraphysomonas* formed a distinct lineage, which was inferred to be basal to other chrysophycean taxa. However, the basal position was not statistically supported. Two newly isolated *Chryso-sphaerella* species formed a well-supported clade, together with the sequence of an uncultured *Chryso-sphaerella* summer isolate. This clade was closely related to the amoeboid genus *Chrysamoeba*, and the flagellates *Oikomonas mutabilis* and *Chromulina nebulosa*. The phylogenetic analysis revealed a monophyletic, supported Synurales, consisting of the scale-bearing genera *Mallomonas*, *Synura* and *Tessellaria*. Newly isolated *Synura lapponica* formed a strongly supported clade together with *Tessellaria volvocina*, in a basal position within Synurales. All other *Synura* taxa constituted a monophyletic, though unsupported clade. Most of the newly isolated *Synura* taxa were inferred to be related to morphologically similar species. All three representatives of the section Petersenianae formed a well-supported clade with *S. petersenii* and allied species. Two *S. mammillosa* isolates were closely related to the Australian isolate of *S. mammillosa* (MUCC 298). However, all three *S. mammillosa* isolates differed in their SSU rDNA sequences, and formed a paraphyletic assemblage with *S. echinulata*. *Synura multidentata* was inferred in a position

basal to this clade. This species is morphologically similar to *S. mammillosa* and *S. echinulata* by the absence of a meshwork covering the scale. *S. mollispina* clustered together with morphologically similar species *S. spinosa* and *S. curtispina*, and a long-branching clade of *S. sphagnicola* and *Synura* sp. The morphologically distinct *S. splendida* formed a separate clade, unrelated to any other species.

Similarly to the genus *Synura*, all *Mallomonas* taxa comprised in a single, but unsupported, monophyletic clade. Interestingly, the newly obtained SSU rDNA sequence of *Mallomonas kalinae* (CAUP B601) was identical to those previously obtained from *M. papillosa* (CCMP A3807) and *M. cf. rasilis* (MUCC 292). The strain of *M. papillosa* was isolated by Andersen in 1984, who later re-identified it as *M. rasilis*, and changed the strain number to CCMP 479. However, we checked the morphology of the strain CCMP 479 and found that it actually represents *M. kalinae* (Fig. 9). Similarly, the strain of *M. cf. rasilis* MUCC 292 in fact represents *M. kalinae*, according to the scale micrographs published by Lavau et al. (1997). In fact, Lavau et al. (1997) themselves stated that the strain represents an intermediate between *M. rasilis* and *M. paxillata*. To determine the real phylogenetic position of *M. papillosa*, we ordered, morphologically checked, and sequenced the strain CCMP 476. The strain took a distinct position within the *Mallomonas* lineage, having no close relationship to any other sequenced *Mallomonas* species (Fig. 8).

As compared to the SSU rDNA analysis, the *rbcL* phylogenetic tree in general exhibited lower support values of internal branches, resulting in less resolution of taxonomic relationships (Fig. 10). Synurales were resolved as paraphyletic, with the *Poterioochromonas* clade nested within. In addition, the genera *Mallomonas* and *Synura* were recovered as paraphyletic, as well, divided into five and three clades, respectively. In general, the SSU rDNA and *rbcL* phylogenies were incongruent, with the SSU rDNA topology being more congruent with the morphological data. To check for possible saturation of the *rbcL* dataset, the strength of the phylogenetic signal vs noise was assessed for the SSU rDNA and different *rbcL* codon partitions. The saturation plots of the 1st and 3rd *rbcL* codon partitions were found to strongly level off with increasing genetic distance, indicating their significant saturation (Fig. 11). To eliminate the deleterious effects of substitution saturation on the reconstructed topology, we removed saturated nucleotide sites by the site-stripping method, and inferred the *rbcL* phylogram based on the updated, stripped alignment. Though the resulting phylogenetic analysis revealed Synurales as monophyletic, the genera *Mallomonas* and *Synura* were still recovered as paraphyletic, divided into four and three clades, respectively (Supplementary material Appendix 1 Fig. A1). Neither complete deletion of the 3rd *rbcL* codon partition, nor an analysis based on the translated amino acid sequence data improved the topology of Chrysophyceae to be more congruent with the SSU rDNA topology and hence the morphological data (trees not shown).

Although the topologies from independent analyses of SSU rDNA (Fig. 8) and the stripped *rbcL* dataset (Supplementary material Appendix 1 Fig. A1) were obviously

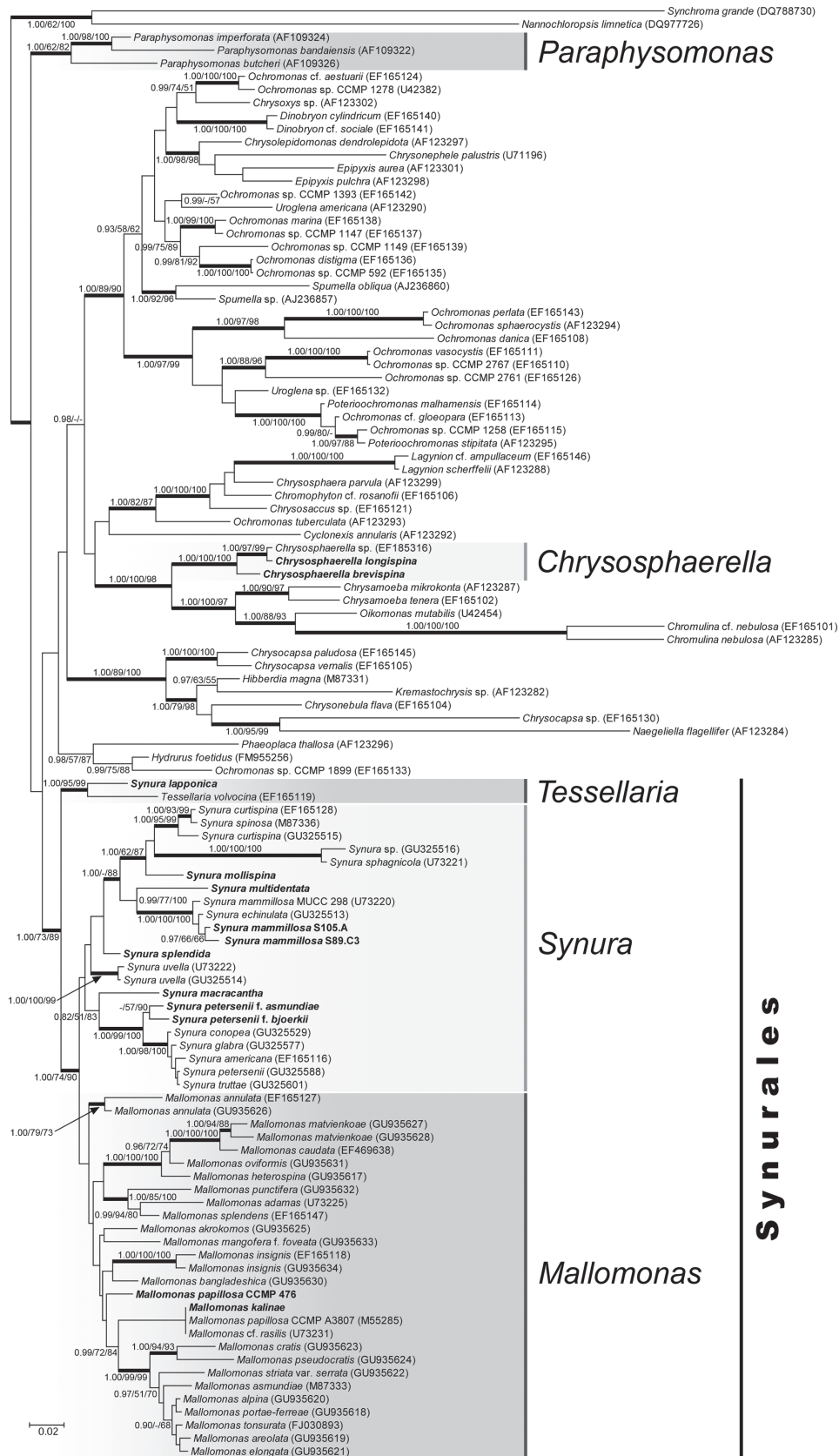


Figure 8. Bayesian analysis of Chrysophyceae based on the SSU rDNA dataset using a GTR + G + I nucleotide substitution model. Values at the nodes indicate statistical support estimated by three methods – MrBayes posterior-node probability (left), maximum-likelihood bootstrap (middle), and maximum parsimony bootstrap (right). Thick branches represent nodes receiving the highest PP support (1.00). Lineages composed of silica-scaled chrysophytes are highlighted. Newly obtained sequences are given in bold. The scale bar shows the estimated number of substitutions per site.

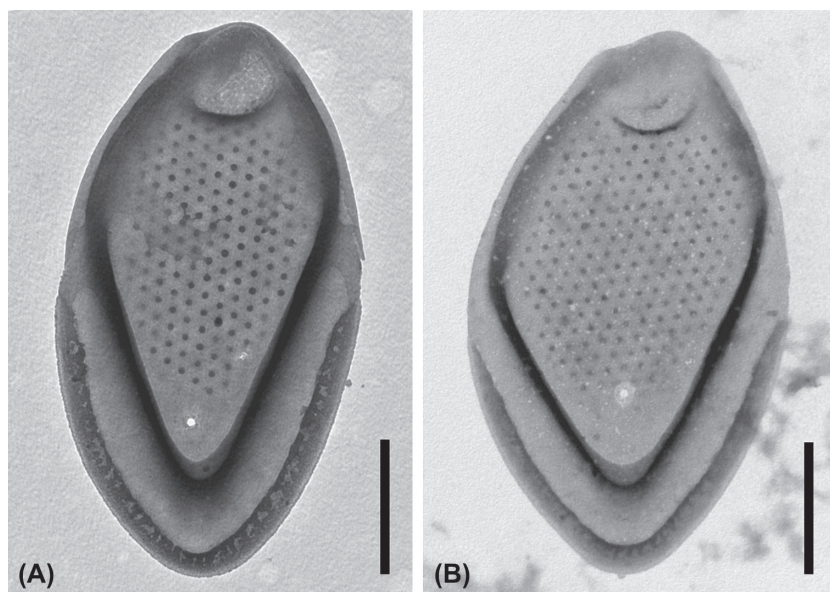


Figure 9. Scale morphology of investigated *Mallomonas kalinae* strains. (A) CAUP B601, (B) CCMP 479. Scale bars represent 1 μm .

different from one another, almost none of the cases of incongruence involved branches from the *rbcL* trees that possessed the highest posterior probability support (1.00). The single exception was the strong relationship of *Synura mollispina* and *S. spinosa*, which was revealed by the *rbcL* analysis only. Therefore, we combined both alignments to infer the chrysophyte phylogeny based on the larger data set. Results of the concatenated SSU rDNA + stripped *rbcL* analysis (Supplementary material Appendix 1 Fig. A2) revealed substantial topological congruence with the SSU rDNA analysis (Fig. 8), indicating that the *rbcL* data are, at least in part, being overwhelmed. In addition, the concatenated phylogeny led to increasing statistical support for the monophyly of both Synurales and the genus *Synura*, the latter even receiving the highest Bayesian posterior probability value.

To conclude, our results indicate that the current infrageneric classification of *Mallomonas* and *Synura*, based on morphological similarities, in some cases does not correlate with the relationships revealed by molecular phylogenetic analyses (Fig. 12). In *Mallomonas*, the sections Planae, Papillosae and Striatae were found to be polyphyletic or paraphyletic. For example, *M. bangladeshica* was recovered as distantly related to the other members of the section Planae: *M. matvienkoeae*, *M. oviformis*, and *M. caudata*. Similarly, two analyzed species of the section Papillosae, *M. papillosa* and *M. kalinae*, did not form a monophyletic group despite of their morphological similarity. In *Synura*, species of the series Spinosae and Splendidae (sensu Wee 1997) did not form monophyletic groups. *S. splendida* and *S. sphagnicola*, united to a single series based on their rather simple scale ultrastructure, were found to be obviously unrelated to each other. Similarly, species traditionally classified into the series Spinosae formed two unrelated lineages, which even could be morphologically characterized by the presence or absence of the meshwork covering the scale.

Discussion

Phylogenetic inferences

The classical morphological studies on cell construction, and later on silica scales, have resulted in a detailed knowledge of structure and ultrastructure but have not given a satisfactory understanding of taxonomic relationships and phylogeny of chrysophyte algae. Thus they have now been supplemented and replaced by molecular studies which have led to a tremendous progress, even if many problems still remain unresolved. One of the most notable difficulties in resolving the chrysophyte tree of life is the incongruence between phylogenies obtained from different sequences, in particular nuclear SSU rDNA and chloroplast *rbcL* genes. This incongruence was observed and briefly noted by Andersen (2007) and Kim et al. (2007), but without giving any explanation of this phenomenon.

According to our analyses, the SSU rDNA phylogenies are more congruent with the morphological data, suggesting that they may better correlate with the real species tree than the *rbcL* topologies. The saturation plots revealed a significant over-saturation of some *rbcL* nucleotide positions; however, the topological incongruence persisted though the site-stripping method was used to remove such positions from the dataset. The cause of this incongruence remains unknown, but we have an idea that may provide an explanation of this phenomenon: the positive selection in the *rbcL* gene. The positive selection in the *rbcL* gene, leading to the fixation of adaptive substitutions in contrasting environments, has recently been reported for cyanobacteria (Miller 2003) and higher plants (Kapralov and Filatov 2006, 2007). Positive selection may obscure the ancestral signal in phylogenetic reconstructions, and could thus significantly affect the resulting topology. Recently, adaptive evolution of the *rbcL* gene induced by the physiological adaptations to declining atmospheric CO_2 has been proposed to occur

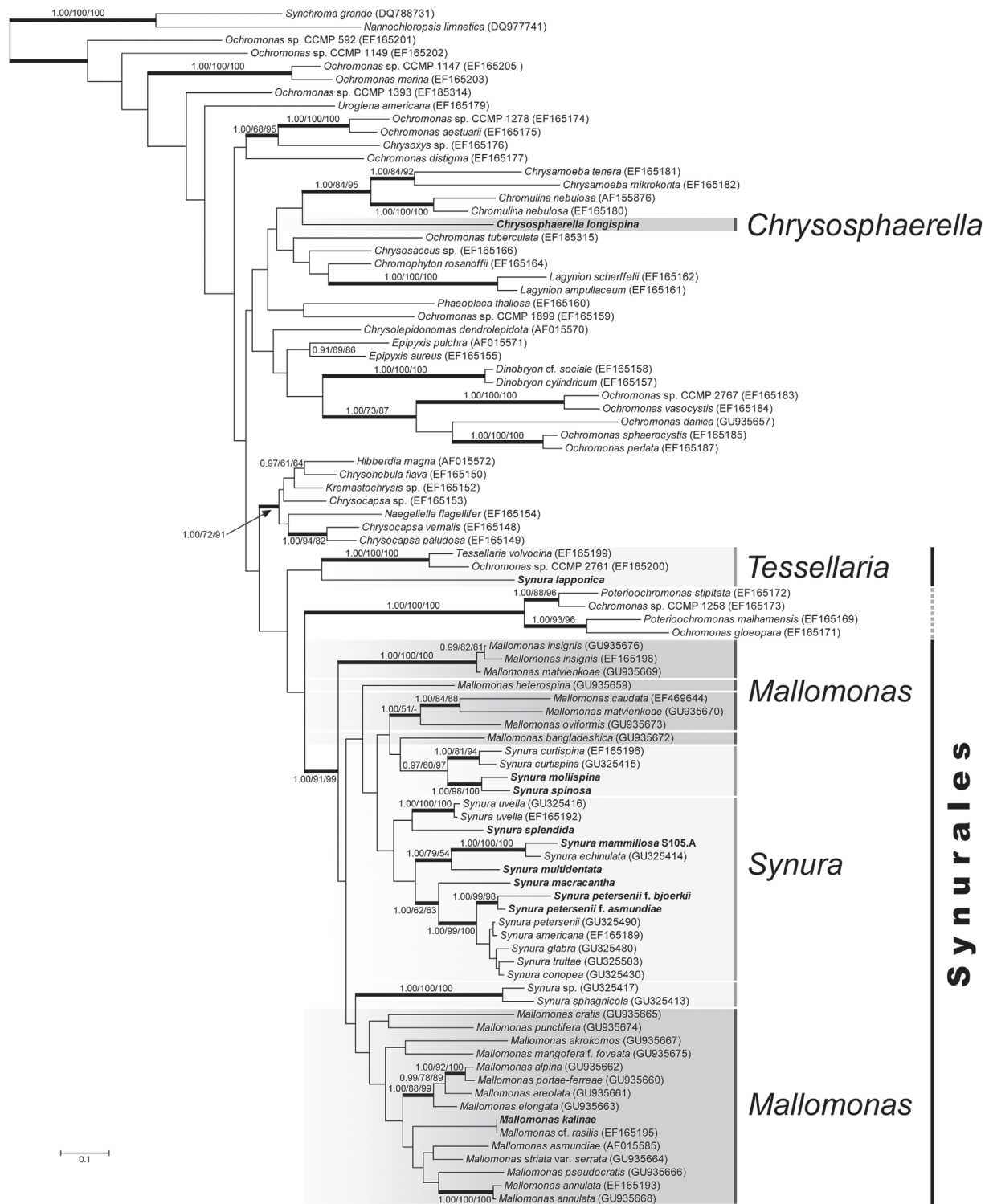


Figure 10. Bayesian analysis of Chrysophyceae, based on the partitioned *rbcL* dataset using a GTR + G + I model for all codon partitions. Values at the nodes indicate statistical support estimated by three methods – MrBayes posterior-node probability (left), maximum-likelihood bootstrap (middle), and maximum parsimony bootstrap (right). Thick branches represent nodes receiving the highest PP support (1.00). Lineages composed of silica-scaled chrysophytes are highlighted. Newly obtained sequences are given in bold. The scale bar shows the estimated number of substitutions per site.

during the diversification of Chromista lineages (Young et al. 2012). Detailed statistical evaluation of positive selection in our *rbcL* dataset is beyond the scope of this review. However, the significant, non-expected saturation of the 1st codon

position we observed in our data (Fig. 11) could be explained just by the existence of widespread positive selection on *rbcL* in chrysophyte algae (Källersjö et al. 1998, Rydin and Källersjö 2002). Obviously, conducting a wider,

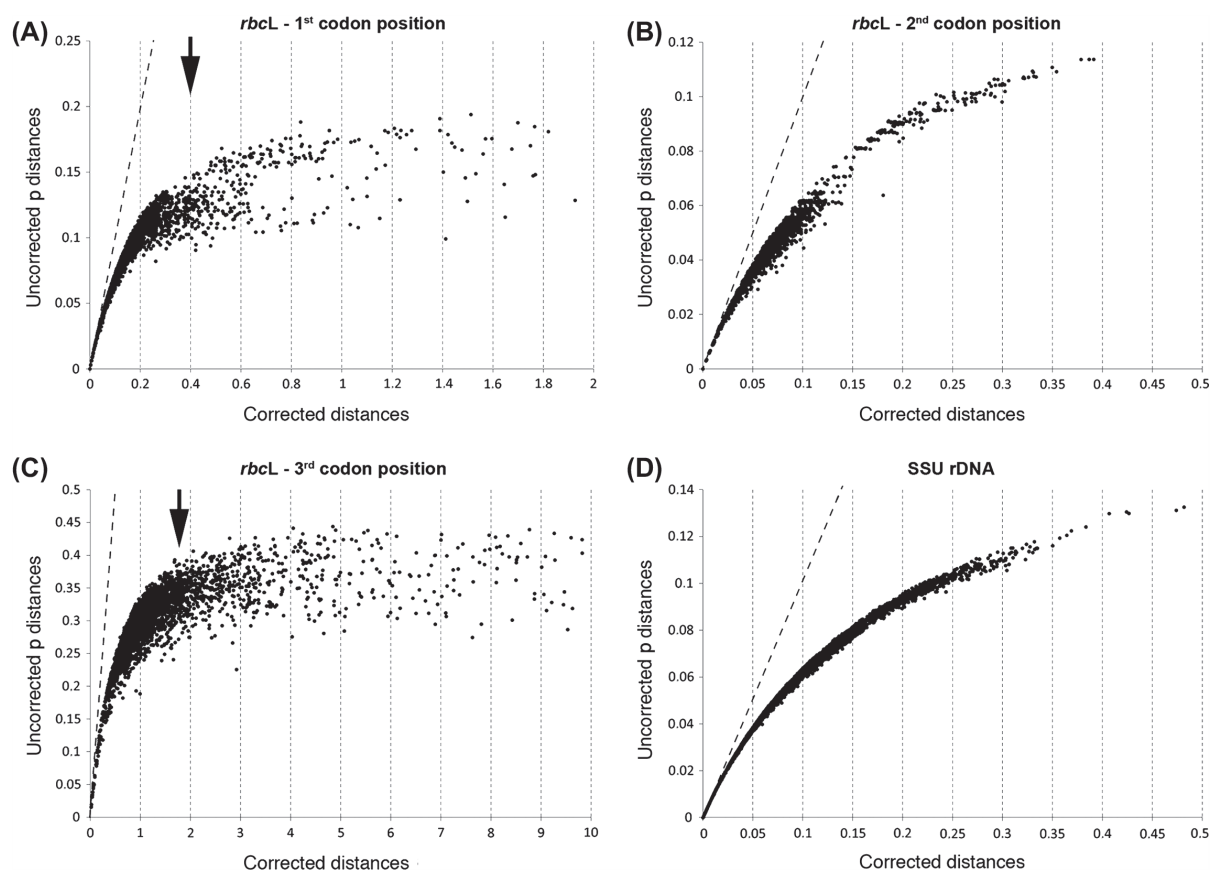


Figure 11. Analysis of substitutional saturation. The graphs visualize the saturation of the first (A), second (B) and third (C) codon position of the *rbcL* gene, and the SSU rDNA dataset (D), by plotting ML-corrected distances against uncorrected p-distances (black dots). Strong curving of saturation plots indicates the significant saturation of molecular datasets. The lowest corrected distances used for removal of fast-evolving sites are indicated by arrows.

phylogeny-based study of positive selection in the *rbcL* gene of Chrysophyceae would be of high value. Until then, we propose to carefully assess phylogenetic reconstructions based solely on chloroplast data.

Despite the topological incongruence of both single gene phylogenies, both SSU rDNA and concatenated SSU rDNA + stripped *rbcL* analyses point to some interesting conclusions of relevance to the classification of silica-scaled chrysophytes. First, we confirmed that silica-scaled chrysophytes are much more unrelated than expected. According to the most recent classification of Chrysophyte algae (Preisig 1995, Kristiansen and Preisig 2001), scale-bearing chrysophytes are found in two higher taxa, namely Synurales and Paraphysomonadaceae, the latter comprising the genera *Chryso-sphaerella*, *Paraphysomonas*, *Polylepidomonas* and *Spiniferomonas*. However, we demonstrated that the scale-bearing genus *Chryso-sphaerella* is unrelated to *Paraphysomonas*, indicating the obvious polyphyly of the family (Fig. 8, Supplementary material Appendix 1 Fig. A2). The phylogenetic position of *Chryso-sphaerella* has already been shown by Andersen (2007), on the basis of uncultured, undetermined isolates. Our sequence data, obtained from the cultured species *C. brevispina* and *C. longispina*, corroborated this finding, resolving *Chryso-sphaerella* as closely related to the naked genera *Chrysoamoeba*, *Chromulina* and *Oikomonas*.

Second, the artificial infrageneric classification of *Mallomonas*, recently reported by Jo et al. (2011), was also observed in *Synura* (Fig. 12). In the most recent taxonomic treatment of synurophyte algae (Kristiansen and Preisig 2007), the genus *Synura* is divided into three sections according to the morphological similarities of their silica scales: Lapponica, Petersenianae and *Synura*. Wee (1997) also recognized three sections, namely Lapponica, Petersenianae and Uvellae, the latter further divided into the series Splendidae and Spinosae. However, the phylogenetic analyses presented here do not correlate with any of these two infrageneric classifications. We revealed that *S. lapponica*, single member of the section Lapponica, in fact belongs to the genus *Tessellaria*. Within *Synura*, the section Petersenianae/Peterseniae was recovered as monophyletic, however, members of the section *Synura/Uvellae* were revealed as phylogenetically far distant, forming a paraphyletic assemblage (Fig. 12). To ensure that the subgeneric classification corresponds to monophyletic units, we propose a revised classification that recognizes five instead of three sections: Peterseniae (*S. australiensis*, *S. longisquama*, *S. macracantha*, *S. petersenii* and allied species), Spinosae (*S. curtispina*, *S. mollispina*, *S. nygaardii*, *S. sphagnicola*, *S. spinosa*), Echinulatae (*S. biseriata*, *S. echinulata*, *S. leptorrhabda*, *S. mammillosa*, *S. multidentata*), Splendidae (*S. splendida*).

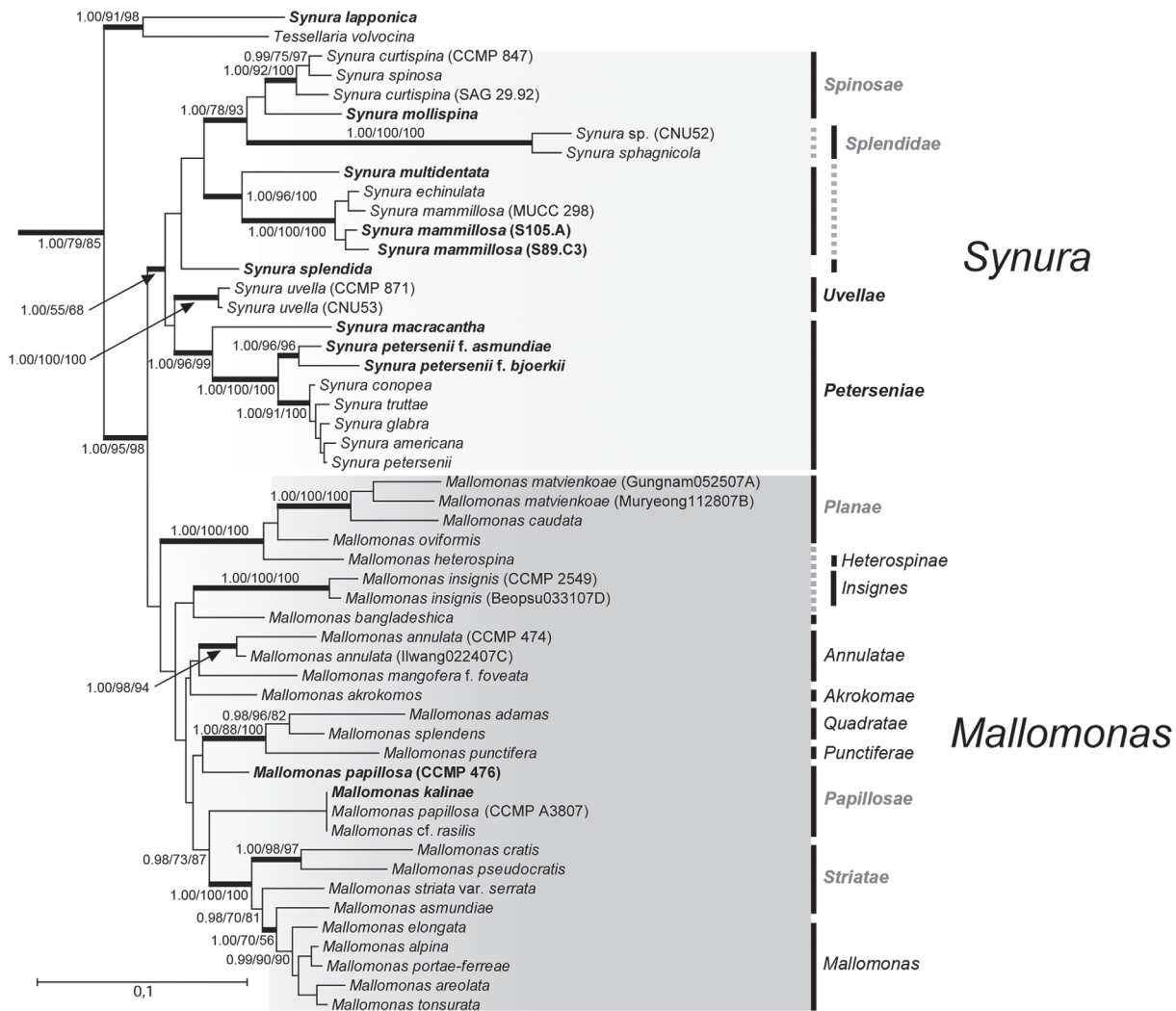


Figure 12. Bayesian analysis of Synurales, based on the combined and partitioned SSU rDNA + *rbcL* dataset using a GTR + G + I model for all partitions. Values at the nodes indicate statistical support estimated by three methods – MrBayes posterior-node probability (left), maximum-likelihood bootstrap (middle), and maximum parsimony bootstrap (right). Thick branches represent nodes receiving the highest PP support (1.00). Infrageneric classification of the genera *Mallomonas* (sensu Kristiansen and Preisig 2007) and *Synura* (sensu Wee 1997) is indicated next to the tree. Newly obtained sequences are given in bold. The scale bar shows the estimated number of substitutions per site.

and Uvellae (*S. uvella*). The phylogenetic position of the molecularly uncharacterized *S. punctulosa* is still unknown, but its morphological distinctness suggests it could be classified in a separate, sixth section.

Finally, in concordance with several recently published phylogenies of Stramenopiles (Ben Ali et al. 2002, Takishita et al. 2009, Del Campo and Massana 2011, Yang et al. 2012) our phylogenetic reconstructions show the close affinity of Chrysophyceae and Synurophyceae, with the latter class nested within the paraphyletic Chrysophyceae. Therefore, we tend not to follow the recognition of Synurophyceae as a separate Stramenopiles class, but rather to classify the synurophyte algae as members of a single order within Chrysophyceae, Synurales.

Species concept of silica-scaled chrysophytes

The well-established species concept of silica-scaled chrysophytes is almost exclusively based on morphology of silica

structures studied by a transmission or scanning electron microscopy. It is even considered as one of the best among the protists, as the silica structures give us extra morphological criteria that extend beyond standard phenotypic taxonomical tools used in microorganisms. Notwithstanding, the correct species identification could be hindered by the presence of infraspecific variation and continuous morphological gradients in scale structure (Kristiansen 1986b). The incorrect interpretation of morphological differences as infraspecific variation only is well documented in the *Synura petersenii* species complex (Boo et al. 2010, Kynčlová et al. 2010), resulting in the recent recognition of a number of cryptic species (Škaloud et al. 2012). Similarly, our phylogenetic analyses clearly indicate that two *S. petersenii* formae (*S. petersenii* f. *asmundiae*, *S. petersenii* f. *bjoerkii*) are genetically so distinct that they should be considered as separate species (Fig. 12).

Despite the efficient species concept, the existence of morphologically similar species could frequently lead in

their incorrect delimitation. For example, prior its formal description *Mallomonas kalinae* was in several floristic studies often assigned to the morphologically similar species *M. rasilis* (Řezáčová 2006). Moreover, its SSU rDNA and *rbcL* sequences were submitted in GenBank under the wrong species names *M. cf. rasilis* (U73231, EF165195) and *M. papillosa* (M55285). In addition, cryptic speciation leading to the underestimation of real species diversity occurs within a number of traditionally defined, nominal species of silica-scaled chrysophytes. Our phylogenetic reconstructions indicate the presence of the cryptic diversity at least within the nominal species *S. curtispina*, *S. mammillosa*, *S. sphagnicola* and *S. uvella* (Fig. 12).

Taxonomical consequences

The presented phylogenetic data indicate that *Synura lapponica* should be formally transferred to the genus *Tessellaria*. The close affinity of these two species has already been proposed by various authors (Lavau et al. 1997, Goldstein et al. 2005, Němcová and Pichrtová 2009), as they share several unique morphological features. Our phylogenetic reconstructions clearly corroborate the relationship between *Tessellaria volvocina* and *S. lapponica*, so that we propose the new combination *Tessellaria lapponica*. Further, the distinct phylogenetic position of both analyzed *S. petersenii* forms (*S. petersenii* f. *asmundiae*, *S. petersenii* f. *bjoerkii*) warrants their recognition as separate species, *S. asmundiae* and *S. bjoerkii*. These new nomenclatural combinations are also corroborated by apparent morphological differences. Both *S. asmundiae* and *S. bjoerkii* have distinctly smaller scales than *S. petersenii* s. str. The dorsal ridge is transformed into a very broad, stout and acute spine. In some apical *S. asmundiae* scales there are also apically rounded spines. *S. asmundiae* scales have densely arranged anastomosing struts (Fig. 7I), while those of *S. bjoerkii* are short and more simple (Fig. 7J).

Tessellaria lapponica (Skuja) Škaloud, Kristiansen & Škaloudová comb. nov.

Basionym: *Synura lapponica* Skuja (1956, pp. 275–276).

Type: Sweden, Lappland, swampy ponds and lakes around Abisko, Pl. 47, Fig. 10–14, Pl. 48, Fig. 1–2p (iconotype).

Synura asmundiae (Cronberg & Kristiansen) Škaloud, Kristiansen & Škaloudová comb. nov.

Basionym: *Synura petersenii* f. *asmundiae* Cronberg & Kristiansen (1980, p. 610).

Type: Sweden, Småland, Lake Fiolen, Fig. 14A (iconotype).

Synura bjoerkii (Cronberg & Kristiansen) Škaloud, Kristiansen & Škaloudová comb. nov.

Basionym: *Synura petersenii* f. *bjoerkii* Cronberg & Kristiansen (1980, p. 612).

Type: Sweden, Småland, Lake Frejen, Fig. 14B–E (iconotype).

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References

- Andersen, R. A. 1987. Synurophyceae classis nov., a new class of algae. – *Am. J. Bot.* 74: 337–353.
- Andersen, R. A. 1989. The Synurophyceae and their relation to other golden algae. – *Nova Hedwigia Beih.* 95: 1–26.
- Andersen, R. A. 2007. Molecular systematics of the Chrysophyceae and Synurophyceae. – In: Brodie, J. and Lewis, J. (eds), *Unravelling the algae*. CRC Press, pp. 285–313.
- Andersen, R. A. et al. 1999. Phylogenetic analysis of the SSU rRNA from members of the Chrysophyceae. – *Protist* 150: 245–263.
- Ariztia, E. V. et al. 1991. A new phylogeny for chromophyte algae using 16S-like rRNA sequences from *Mallomonas papillosa* (Synurophyceae) and *Tribonema aequale* (Xanthophyceae). – *J. Phycol.* 27: 428–436.
- Asmund, B. 1955. Electron microscope observations on *Mallomonas caudata* and some remarks on its occurrence in four Danish ponds. – *Bot. Tidsskr.* 52: 163–168.
- Asmund, B. and Kristiansen, J. 1986. The genus *Mallomonas* (Chrysophyceae). A taxonomic survey based on the ultrastructure of silica scales and bristles. – *Opera Bot.* 85: 1–128.
- Awerinzew, S. 1912. Beiträge zur Kenntnis der Protozoen, II. Über die Struktur der Hülle bei *Synura uvella*. – *Arch. Protistenk.* 25: 6–7.
- Ben Ali, A. et al. 2002. Evolutionary relationships among heterokont algae (the autotrophic Stramenopiles) based on combined analyses of small and large subunit ribosomal RNA. – *Protist* 153: 123–132.
- Bhattacharya, D. et al. 1992. Algae containing chlorophylls a + c are paraphyletic: molecular evolutionary analysis of the Chromophyta. – *Evolution* 46: 1801–1817.
- Biorêt, G. 1931. Les écailles de *Synura uvella* Stein. – *Travaux Crypt. déd. à Louis Mangin*. Mus. Nat. d'Hist. Nat. Paris: 219–226.
- Boo, S. M. et al. 2010. Complex phylogeographic patterns in the freshwater alga *Synura* provide new insights into ubiquity vs endemism in microbial eukaryotes. – *Mol. Ecol.* 19: 4328–4338.
- Bourrelly, P. 1957. Recherches sur les Chrysophycées. – *Rev. Algol. Mém. Hors-Série* 1: 1–409.
- Bourrelly, P. 1968. Les Algues d' eau douce II: Les Algues jaunes et brunes. – Éditions N. Boubée et Cie, Paris.
- Calado, A. J. and Rino, J. A. 1994. *Chlorodesmos hispidus*, a morphological expression of *Synura spinosa* (Synurophyceae). – *Nord. J. Bot.* 14: 235–239.
- Caron, D. A. et al. 1999. Molecular genetic analysis of the heterotrophic chrysophyte genus *Paraphysomonas* with design of rRNA-targeted oligonucleotide probes for two species. – *J. Phycol.* 35: 824–837.
- Conrad, W. 1920. Contributions à l'étude des Chrysomonadines. – *Bull. Cl. Sci. Acad. R. Belg.*, 5me sér., 6: 167–189.
- Conrad, W. 1933. Revision du genre *Mallomonas* Perty (1852) incl. *Pseudo-Mallomonas* Chodat (1920). – *Mém. Mus. R. Hist. Nat. Belg.* 56: 1–82.
- Cronberg, G. and Kristiansen, J. 1980. Synuraceae and other Chrysophyceae from central Småland, Sweden. – *Bot. Not.* 133: 595–618.
- Daugbjerg, N. and Andersen, R. A. 1997. Phylogenetic analysis of the *rbcL* sequences from haptophytes and heterokont algae suggest their chloroplasts are unrelated. – *Mol. Biol. Evol.* 14: 1242–1251.

- Del Campo, J. and Massana, R. 2011. Emerging diversity within Chrysophytes, Choanoflagellates and Bicosoecids based on molecular surveys. – *Protist* 162: 435–448.
- Ehrenberg, C. G. 1838. Die Infusionsthierchen als vollkommene Organismen I–II. – Leipzig.
- Fott, B. 1955. Scales of *Mallomonas* observed in the electron microscope. – *Preslia* 27: 280–282.
- Fott, B. and Ludvík, J. 1957. Die submikroskopische Struktur der Kieselschuppen bei *Synura* und ihre Bedeutung für die Taxonomie der Gattung. – *Preslia* 29: 5–16.
- Fritsch, F. E. 1935. The structure and reproduction of the algae. I. – Cambridge.
- Goldstein, M. et al. 2005. Morphology and reproduction of *Synura lapponica* (Synurophyceae). – *Phycologia* 44: 566–571.
- Houwink, A. L. 1952. Die Pellikularschuppen und die Geißel der Physomonas vestita Stokes. – *Z. Wiss. Mikrosk.* 60: 402–404.
- Huber-Pestalozzi, G. 1941. Das Phytoplankton des Süßwassers. 2,1. Chrysophyceen, farblose Flagellaten, Heterokonten. – In: Thienemann, A. (ed.), Die Binnengewässer XVI, 2, 1. Schweizerbartsche Verlagsbuchhandlung, Stuttgart.
- Ivanov, L. 1899. Beitrag zur Kenntniss der Morphologie und Systematik der Chrysomonaden. – *Bull. Acad. Imp. Sci. St Petersburg* 11: 247–262.
- Jo, B. Y. et al. 2011. Studies on ultrastructure and three-gene phylogeny of the genus *Mallomonas* (Synurophyceae). – *J. Phycol.* 47: 415–425.
- Källersjö, M. et al. 1998. Simultaneous parsimony jackknife analysis of 2538 rbcL DNA sequences reveals support for major clades of green plants, land plants, seed plants and flowering plants. – *Plant. Syst. Evol.* 213: 259–287.
- Kapralov, M. V. and Filatov, D. A. 2006. Molecular adaptation during adaptive radiation in the Hawaiian endemic genus *Schizococcus*. – *PLoS One* 1: e8.
- Kapralov, M. V. and Filatov, D. A. 2007. Widespread positive selection in the photosynthetic Rubisco enzyme. – *BMC Evol. Biol.* 7: 73.
- Katana, A. et al. 2001. Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. – *J. Phycol.* 37: 443–451.
- Katoh, K. et al. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. – *Nucleic Acid Res.* 30: 3059–3066.
- Kim, H. S. et al. 2007. Nuclear SSU and plastid rbcL genes and ultrastructure of *Mallomonas caudata* (Synurophyceae) from Korea. – *Korean J. Limnol.* 40: 387–394.
- Klaveness, D. et al. 2011. The 18S and 28S rDNA identity and phylogeny of the common lotic chrysophyte *Hydrurus foetidus*. – *Eur. J. Phycol.* 46: 282–291.
- Klebs, A. 1892. Flagellatenstudien I–II. – *Z. Wiss. Zool.* 55: 265–351, 353–444.
- Korshikov, A. A. 1927. *Skadovskiella sphagnicola*, a new colonial chrysomonad. – *Arch. Protistenk.* 58: 450–455.
- Korshikov, A. A. 1929. Studies on the Chrysomonads. I. – *Arch. Protistenk.* 67: 253–290.
- Korshikov, A. A. 1942. On some new or little known flagellates. – *Arch. Protistenk.* 95: 22–44.
- Krieger, W. 1930. Untersuchungen über Plankton-Chrysomonaden. – *Bot. Arch.* 29: 258–329.
- Kristiansen, J. 1971. A *Mallomonas* bloom in a Bulgarian mountain lake. – *Nova Hedwigia* 21: 877–882.
- Kristiansen, J. 1975a. On the occurrence of the species of *Synura*. – *Verhandl. Intern. Verein. Limnol.* 19: 2709–2715.
- Kristiansen, J. 1975b. Studies on the Chrysophyceae of Bornholm. – *Bot. Tidsskr.* 70: 126–142.
- Kristiansen, J. 1986a. The ultrastructural bases of chrysophyte systematics and phylogeny. – *CRC Crit. Rev. Plant Sci.* 4: 149–211.
- Kristiansen, J. 1986b. Silica-scale bearing Chrysophytes as environmental indicators. – *Br. Phycol. J.* 21: 425–436.
- Kristiansen, J. 1988. The problem of ‘enigmatic chrysophytes’. – *Arch. Protistenk.* 135: 9–15.
- Kristiansen, J. 2001. Biogeography of silica-scaled chrysophytes. – *Nova Hedwigia Beih.* 122: 23–39.
- Kristiansen, J. 2002. The genus *Mallomonas* (Synurophyceae). – *Opera Bot.* 139: 1–218.
- Kristiansen, J. 2008. Dispersal and biogeography of silica-scaled chrysophytes. – In: Foissner, W. (ed.), *Protist biodiversity and geographical distribution. Biodivers. Conserv.* 17: 419–426.
- Kristiansen, J. and Tong, D. 1989. *Chryso-sphaerella annulata* n. sp., a new scale-bearing chrysophyte. – *Nord. J. Bot.* 9: 329–332.
- Kristiansen, J. and Preisig, H. R. 2001. Encyclopedia of Chrysophyte genera. – *Bibl. Phycol.* 110.
- Kristiansen, J. and Preisig, H. R. 2007. Chrysophyte and haptophyte algae, part 2: Synurophyceae. – In: Büdel, B. et al. (eds), *Süßwasserflora von Mitteleuropa*. Vol. 1–2. Spektrum Akad. Verlag, Springer, pp. 1–252.
- Kynčlová, A. et al. 2010. Unveiling hidden diversity in the *Synura petersenii* species complex (Synurophyceae, Heterokontophyta). – *Nova Hedw. Beih.* 136: 283–298.
- Lauterborn, R. 1899. Protozoen-Studien IV. Flagellaten aus dem Gebiete des Oberrheins. – *Z. Wiss. Zool.* 65: 369–391.
- Lavau, S. et al. 1997. A phylogenetic analysis of the Synurophyceae using molecular data and scale case morphology. – *J. Phycol.* 33: 135–151.
- Lemmermann, E. 1899. Das Phytoplankton sächsischer Teiche. – *Forschungsber. Biol. Stat. Plön* 7: 96–135.
- Lemmermann, E. 1904. Das Phytoplankton schwedischer Gewässer. – *Ark. Bot.* 2: 1–210.
- Lemmermann, E. 1910. Algen. Kryptogamenflora von Mark Brandenburg III. – *Bornträger*, Leipzig.
- Manton, I. 1955. Observations with the electron microscope on *Synura caroliniana* Whitford. – *Proc. Leeds Phil. Soc.* VI(V): 306–316.
- Miller, S. R. 2003. Evidence for the adaptive evolution of the carbon fixation gene *rbcL* during diversification in temperature tolerance of a clade of hot spring cyanobacteria. – *Mol. Ecol.* 12: 1237–1246.
- Müller, O. F. 1786. *Animalcula infusoria fluviatilia et marina*. – Hafnia (Copenhagen).
- Němcová, Y. and Pichrtová, M. 2009. The rare species *Synura lapponica* (Synurophyceae) new to the Czech Republic, local vs global diversity in colonial synurophytes. – *Biologia* 64: 1070–1075.
- Nicholls, K. H. 1984. *Spiniferomonas septispina* and *S. enigmata*. Two new algal species confusing the distinction between *Spiniferomonas* and *Chryso-sphaerella* (Chrysophyceae). – *Plant Syst. Evol.* 148: 103–117.
- Pascher, A. 1910. Der Grossteich bei Hirschberg in Nord-Böhmen. I. Chrysomonaden. – *Monogr. Abh. Int. Rev. Hydrobiol. Hydrogr.* 1: 1–66.
- Pascher, A. 1912. Über Rhizopoden und Palmellastadien bei Flagellaten (Chrysomonaden), nebst einem Übersicht über die braunen Flagellaten. – *Arch. Protistenk.* 25: 153–200.
- Pascher, A. 1913. Chrysomonadinae. – In: Pascher, A. (ed.), *Die Süßwasserflora Deutschlands, Österreichs und der Schweiz* 2: 7–95.
- Pascher, A. 1914. Über Flagellaten und Algen. – *Ber. Deutsch. Bot. Ges.* 32: 136–160.
- Pascher, A. 1925. Neue und wenig bekannte Protisten. XVIII. Neue und wenig bekannte Flagellaten. XVI. – *Arch. Protistenk.* 52: 565–584.
- Perty, M. 1852. Zur Kenntnis kleinster Lebensformen. – Bern.
- Petersen, J. B. 1918. Om *Synura uella* Stein og nogle andre Chrysomonadiner. – *Vid. Medd. Dansk Naturhist. Forening* 69: 345–357.

- Petersen, J. B. and Hansen, J. B. 1956. On the scales of some *Synura* species. – Biol. Medd. Kgl. Dan. Vid. Selsk. 23: 1–27.
- Pipes, L. D. et al. 1991. Ultrastructure of *Tessellaria volvocina* (Synurophyceae). – Br. Phycol. J. 26: 259–278.
- Phillips, F. W. 1884. On *Chlorodesmos hispida*, a new flagellate animalcule. – Trans. Herts. Nat. Hist. Soc. 2: 92–94.
- Pond, S. L. K. et al. 2005. HyPhy: hypothesis testing using Phylogenies. – Bioinformatics 21: 676–679.
- Preisig, H. R. 1995. A modern concept of chrysophyte classification. – In: Sandgren, C. D. et al. (eds), Chrysophyte algae. Ecology, phylogeny and development. Cambridge Univ. Press, pp. 46–74.
- Preisig, H. R. and Hibberd, D. J. 1982. Ultrastructure and taxonomy of *Paraphysomonas* and related genera. 1. – Nord. J. Bot. 2: 397–420.
- Preisig, H. R. and Hibberd, D. J. 1983. Ultrastructure and taxonomy of *Paraphysomonas* and related genera. 2–3. – Nord. J. Bot. 2: 601–638, 3: 695–723.
- Řezáčová, M. 2006. *Mallomonas kalinae* (Synurophyceae), a new species of alga from northern Bohemia, Czech Republic. – Preslia 78: 353–358.
- Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics 19: 1572–1574.
- Rydin, C. and Källersjö, M. 2002. Taxon sampling and seed plant phylogeny. – Cladistics 18: 485–513.
- Schiller, J. 1954. Über winterliche pflanzliche Bewohner des Wassers, Eises und des darauf liegenden Schneegebietes. I. – Österr. Bot. Zhürn. 101: 236–284.
- Seligo, A. 1893. Über einige Flagellaten des Süßwasserplanktons. – Festgabe Westpreuss. Fischereivereins Danzig: 1–8.
- Silva, P. C. 1980. Names of classes and families of living algae. – Regnum Veg. 103: 1–156.
- Siver, P. 1991. The Biology of *Mallomonas*. – Dev. Hydrobiol. 63: 1–228.
- Škaloud, P. et al. 2012. Toward a revision of the genus *Synura*, section Petersenianae (Synurophyceae, Heterokontophyta): morphological characterization of six pseudo-cryptic species. – Phycologia 51: 303–329.
- Skuja, H. 1956. Taxonomische und biologische Studien über das Phytoplankton schwedischer binnengewässer. – Nov. Act. Reg. Soc. Sci. Upsal. IV 16: 1–404.
- Skvortzow, B. V. 1961. Harbin Chrysophyta, China boreali-orientalis. – Bull. Herb. N. E. For. Acad. 12: 1–70.
- Smith, G. M. 1938. Cryptogamic Botany. I. – McGraw Hill Book Comp.
- Starmach, K. 1968. Chrysophyceae – Złotowiciowce. – Flora Ślōdkowodna Pol. 5: 1–598.
- Starmach, K. 1985. Chrysophyceae und Haptophyceae. – In: Ettl, H. et al. (eds), Süßwasserflora von Mitteleuropa 1. – VEB Gustav Fischer.
- Stein, F. R. 1878. Der Organismus der Infusionsthiere III: Der Organismus der Flagellaten I. – Leipzig.
- Stokes, A. C. 1885. Notices of new freshwater Infusoria. III. – Am. Monthly Microsc. J. 6: 121–127.
- Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Ver. 4. – Sinauer.
- Takahashi, E. 1973. Studies on genera *Mallomonas* and *Synura*, and other plankton in freshwater with the electron microscope. VII. New genus *Spiniferomonas* of the Synuraceae (Chrysophyceae). – Bot. Mag. Tokyo 86: 75–88.
- Takishita, K. et al. 2009. A hypothesis for the evolution of nuclear-encoded, plastid-targeted Glyceraldehyde-3-phosphate dehydrogenase genes in ‘chromalveolate’ members. – PLoS One 4: e4737.
- Talavera, G. and Castresana, J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. – Syst. Biol. 56: 564–577.
- Tamura, K. et al. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. – Mol. Biol. Evol. 28: 2731–2739.
- Tyler, P. A. et al. 1989. *Tessellaria volvocina* rediscovered. – Br. Phycol. J. 24: 329–337.
- Verbruggen, H. 2012. SiteStripper, ver. 1.01. – <www.phycoweb.net/software/SiteStripper/index.html>.
- Waddell, P. J. et al. 1999. Using novel phylogenetic methods to evaluate mammalian mtDNA, including amino acid-invariant sites-LogDet plus site stripping, to detect internal conflicts in the data, with special reference to the positions of hedgehog, armadillo and elephant. – Syst. Biol. 48: 31–53.
- Wee, J. L. 1997. Scale biogenesis in synurophycean protists: phylogenetic implications. – Crit. Rev. Plant Sci. 16: 497–534.
- Wee, J. L. et al. 2001. ITS/5.8S DNA sequence variation in 14 isolates of *Synura petersenii* Korshikov (Synurophyceae). – Nova Hedw. Beih. 122: 245–258.
- Yang, E. C. et al. 2012. Supermatrix data highlight the phylogenetic relationships of photosynthetic Stramenopiles. – Protist 163: 217–231.
- Young, J. N. et al. 2012. Adaptive signals in algal Rubisco reveal a history of ancient atmospheric carbon dioxide. – Phil. Trans. R. Soc. B 367: 483–492.
- Zacharias, O. 1897. Neue Beiträge zur Kenntnis des Süßwasserplanktons. – Forschungsber. Biol. Stat. Plön 5: 1–9.
- Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. – PhD thesis, Univ. of Texas at Austin.

Supplementary material (Appendix NJB-00119 at <www.oikosoffice.lu.se/appendix>). Appendix 1.