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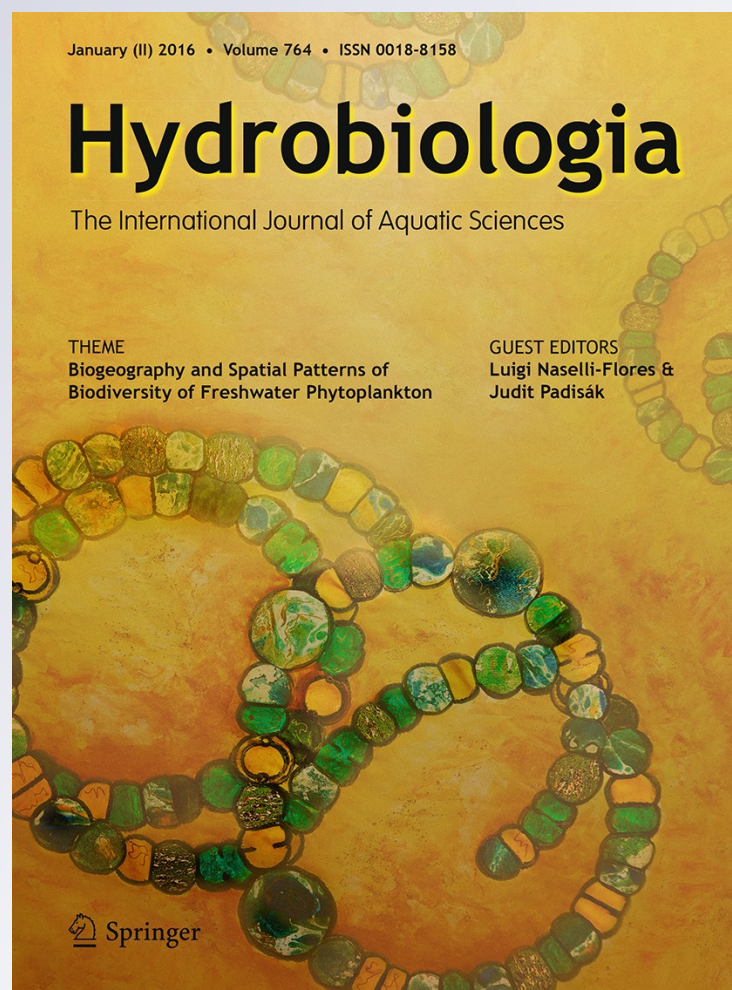
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Silica-scaled chrysophytes (Stramenopiles, Ochrophyta) along a salinity gradient: a case study from the Gulf of Bothnia western shore (northern Europe)

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Abstract Silica-scaled chrysophytes (Stramenopiles, Ochrophyta) are represented predominantly by freshwater flagellates. Diverse-scaled chrysophyte communities were previously reported from brackish waters of the Baltic Sea. However, it was clear that part of the community was delivered by freshwater river inputs. To investigate the effect of salinity on species diversity and community structure, we sampled the lakes in the close proximity to the shoreline (up to 5 km) and in almost separated deep bays (salinity values of 0.01–3.08 practical salinity units) of the Gulf of Bothnia, Baltic Sea. We excluded exposed seashore sites to minimize river flow impact. Species determination was based on scale morphology, which was investigated with a transmission electron microscope. Salinity was the important predictor of diversity; the Simpson index significantly declined with increasing salinity. Community structure of silica-scaled chrysophytes was

influenced most strongly by pH. The effect of salinity was also important; however, the net effect of salinity was overridden by pH which was highly correlated to salinity. The occurrence of twelve recorded silica-scaled chrysophytes (out of 83 taxa) was significantly negatively correlated to salinity. Conversely, six species incl. *Mallomonas tonsurata* and *M. alpina* favored more saline habitats.

Keywords Baltic Sea · Community structure · Diversity · Salinity gradient · Silica-scaled chrysophytes

Introduction

Silica-scaled chrysophytes represent a relatively species-poor group of flagellates within the class Chrysophyceae (Stramenopiles, Ochrophyta; Cavalier-Smith, 2015). Species bearing silica scales occur in at least three positions on the phylogenetic tree. The order Synurales contains the scale-bearing genera *Synura*, *Mallomonas*, *Chrysodidymus*, and *Tessellaria*. Recent work reported that the genus *Chrysosphaerella* is unrelated to *Paraphysomonas* (Škaloud et al. 2013a). The phylogenetic position of the scale-bearing genus *Spiniferomonas* is unknown. Currently, more than 300 scale-bearing taxa have been described (Kristiansen & Preisig, 2007; Scoble & Cavalier-Smith, 2014). However, species diversity is probably strongly underestimated, and new species are continuously discovered

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(Ma & Wei, 2013; Nemcová & Kreidlová, 2013; Němcová et al., 2013; Pang & Wang, 2013). Extensive analysis of molecular data revealed hidden diversity within several species complexes, such as that in *Synura petersenii* s.l. (Škaloud et al., 2014), *Mallomonas matvienkoeae* s.l. (Jo et al., 2013), and *Paraphysomonas vestita* s.l. (Scoble & Cavalier-Smith, 2014). These species have very similar scale morphology. However, classification of species based on scale morphology is still widely used in diversity surveys, ecological analyses, and paleolimnological studies. The scales detach when the organism dies, and they may be preserved in sediments for prolonged periods of time, up to millions of years under optimal conditions (Siver & Wolfe, 2005). Sediment in the uppermost surface records the identity of species that recently inhabited a waterbody. The scales revealed in stratigraphic profiles along with other siliceous microfossils (diatom frustules and stomatocysts) provide evidence about past chrysophyte assemblages, and can be used to reconstruct past environments (Smol, 2005).

Several recent papers on community ecology and dispersal dynamics of lake phytoplankton suggest that these microcommunities are structured by both environmental and spatial variables (Ptacnik et al., 2010; Soininen et al., 2011). At large scales, microbial communities may be structured by dispersal-related processes, evolution, and historical factors in a similar way as macroorganisms (Martiny et al., 2006; Vyverman et al., 2007; Evans et al., 2009). Siver & Lott (2012) assessed the distribution of scaled chrysophytes in fresh waterbodies along 3200 km long transect on the east coast of North America to determine any biogeographic patterns in relation to the environmental and spatial variables. Environmental variables clearly played a significant role in determining whether species will occupy a given site. However, species were not always found in waterbodies likely to support their growth, which implies also a space-driven pattern (Siver & Lott, 2012). Similarly, Soininen et al. (2011) concluded that the local environment appeared to influence phytoplankton communities of 100 small lakes in Finland more strongly than dispersal-driven assembly. However, the balance between these two forces depended on the study scale.

The shift of species composition of silica-scaled chrysophytes along environmental gradients has been well documented (for review, see Siver, 1995). The values of pH, conductivity/salinity, nutrient content,

temperature, and irradiance are primary environmental factors controlling species occurrence and distribution in natural biotopes. Silica-scaled chrysophytes appear to prefer localities that have low conductivity (Sandgren, 1988; Siver & Hamer, 1989; Siver, 1991; Němcová et al., 2002). Diverse chrysophyte communities were observed in oligotrophic lakes of continental Fennoscandia (e.g., Cronberg & Kristiansen, 1980; Eloranta, 1985; Hällfors & Hällfors, 1988; Eloranta, 1989; Ikävalko, 1994a). Silica-scaled chrysophytes often represent a dominant phytoplankton group in these boreal, nutrient-poor lakes. Hansen & Kristiansen (1997) investigated chrysophytes in lakes and waterbodies of the Åland archipelago at the entrance to the Gulf of Bothnia, which consists of approximately 6500 islands. Land upheaval separated brackish bays from the sea, and these were transformed into freshwater lakes. Åland provides a unique system of bay-lake transition with distinct salinity gradient. Although silica-scaled chrysophytes are predominantly freshwater protists, some species thrive in high-conductivity/brackish localities. Gutowski (1989) observed 23 taxa during the course of year in a eutrophic pond with conductivity ranging from 345 to 800 $\mu\text{S cm}^{-1}$. Two studies reported scale-covered chrysophytes from the Baltic Sea (Ikävalko, 1994b; Ikävalko & Thomsen, 1996). During the year-long sampling period, 51 taxa were identified at five stations located in Pojo Bay (southwestern coast of Finland). Ikävalko & Thomsen (1996) studied flagellates from different structural and developmental stages of the Baltic Sea ice cover. At least seven taxa were preserved as whole cells and readily identified, indicating that these species actually lived within the ice. Ten more species were identified based on loose scales observed in transmission electron microscope preparations. However, information on ecological responses of silica-scaled chrysophyte community composition along salinity gradient is still very limited. In this study, we aimed to (1) investigate the effect of salinity on species diversity and community structure, and (2) study species preferences with respect to salinity.

Materials and methods

Study area and sites

The Gulf of Bothnia is the northernmost extension of the Baltic Sea. The water in open areas is brackish with

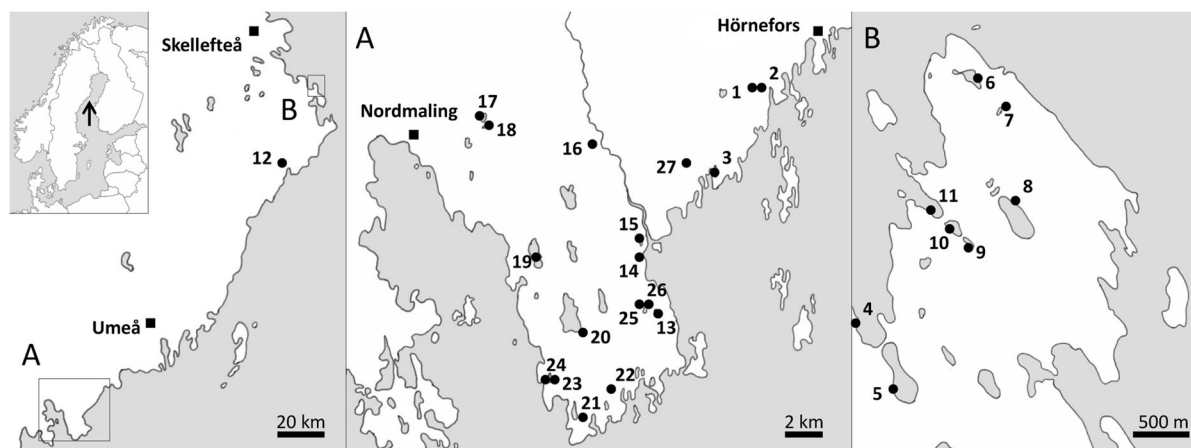


Fig. 1 Location of the study area and sampling sites on the western shore of a Gulf of Bothnia. **A** Detailed location of sampling sites 13–27, The Quark region, **B** detailed location of

sampling sites 4–12, Bothnian Bay shore. The limit distance from the seashore was 5 km

salinity ranging from 5 to 7 practical salinity units (psu) in the Bothnian Sea and 3–4 psu in the Bothnian Bay, whereas coastal areas near the river mouths are almost freshwater (Håkansson et al., 1996). The study area covered part of the western shore of the Gulf of Bothnia from $64^{\circ}33'19.66''\text{N}$ to $63^{\circ}28'57.18''\text{N}$ (Fig. 1). Sampling sites 4–12 were located near the Bothnian Bay shore ($64^{\circ}33'19.66''\text{N}$ to $64^{\circ}19'26.97''\text{N}$), whereas the remaining sites were situated near the Quark region (northern part of the Bothnian Sea; $63^{\circ}35'36.47''\text{N}$ to $63^{\circ}28'57.18''\text{N}$). Localities were selected to maximally cover the salinity gradient. The study area was restricted to within 5 km from the seashore. Most of our investigated sites were lakes completely separated from the sea (20 sites). Source for the observed salinity gradient was the distance and connectivity of sampled lakes with the sea. The distance reflected the time period since brackish bays have been separated and transformed into the lakes. We excluded the most exposed seashore sites to minimize river flow impact. Only sites located in deep almost separated bays and fjords (bay-lake transition) were used. In total, 27 localities with surface water salinity ranging from 0.01–3.08 psu were sampled in May/June 2012.

Sampling and sample processing

The localities were sampled from May 30 until June 3, 2012. Water temperature, pH, and salinity were measured at the time of collection using a combined pH/conductometer WTW 340 (Wissenschaftlich-

Technische Werkstätten GmbH, Germany). Distance of the sampling site to the nearest seashore was measured later based on the sampling site coordinates. The plankton samples (20 μm mesh net) were combined with water squeezed from the submerged vegetation and the surface sediment layer (the upper surface layer of approximately 2 mm). Water samples were concentrated by sedimentation. Subsequently, unfixed drops of the sample were dried onto Formvar-coated transmission electron microscopy (TEM) grids. Dried material was washed by repeated transfer of the grid into a drop of deionized water on a Parafilm strip (hydrophobic material). Three TEM grids were carefully observed, and scales were photographed for each sample using a JEOL 1011 TEM.

Species data analyses

A principal coordinates analysis (PCoA) based on the distance matrix of Manhattan distances among sites was used to ordinate samples by their species composition. Symbol size was used to show relative values of salinity (Fig. 2). The statistical software package PAST, version 2.08, was used for PCoA analysis (Hammer et al., 2001). Variance in species diversity (represented by Simpson index) and community structure was partitioned into components attributable to individual environmental factors (pH, salinity, and distance from the seashore). Permutational multivariate analysis of variance (Per-MANOVA) using distance matrices was conducted with Euclidean distance

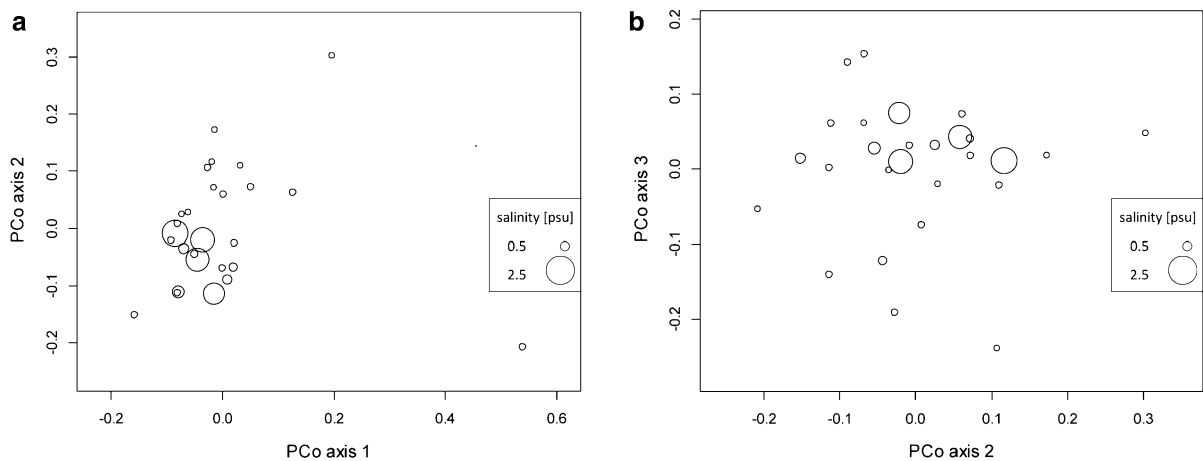


Fig. 2 The Principal coordinates analysis (PCoA) plots based on the distance matrix of Manhattan distances among sites show ordination of samples by their species composition. Symbol size is used to show relative values of salinity. **a** Visualization of the

first and the second PCo axes, **b** visualization of the second and the third PCo axes. The PCo1 axis accounted for 25.8%, the PCo2 for 18.0% and the PCo3 for 12.5% of the variability

measure to partition variance in species diversity. Bray–Curtis and Jaccard index-based partitions were used to evaluate the effect of individual factors on community structure. Species abundance was estimated based on average number of scales on three investigated TEM grids (1, <5 scales per grid; 2, 5–30 scales per grid; 3, >30 scales per grid). The function *adonis* of the package *vegan* in R, version 3.0.2 (2013), was used for Per-MANOVA analysis. *Adonis* partitions distance matrices among sources of variation and fits linear models (e.g., factors) to distance matrices; it uses a permutation test (9999 permutations) with pseudo-F ratios to inspect the significances of those partitions. The tests are sequential, and individual factors are evaluated in the order they appear in the formula. Therefore, the net effect of an individual factor appearing at the last position in the predictor part of the formula can be determined (Oksanen et al., 2014). The R^2 values corresponding to individual factors are shown in Table 1.

Correlations between pairs of variables were tested in PAST, version 2.08 (Hammer et al., 2001). Salinity values were log-transformed. Non-parametric rank-order correlation with Kendall tau coefficient was used to investigate correlation between occurrence of individual species and salinity. The Kendall tau rank correlation coefficient evaluates the degree of similarity between two sets of ranks (localities with corresponding salinity) given to a same sets of objects (species; Abdi, 2007).

Results

A total of 83 species of silica-scaled chrysophytes (Supplementary Table 1) were recorded in 27 localities spanning a salinity gradient of 0.01–3.08 psu (Supplementary Table 2).

The PCoA plot showed that more saline sites were ordinated within the space spanned by less saline localities (Fig. 2). The first PCo axis accounted for 25.8%, the second for 18.0%, and the third for 12.5% of the variability. A small subset of species that thrived in low salinity, oligotrophic sites was also found in more saline localities. The Per-MANOVA model revealed that the net effects of salinity, pH, and distance of the sampling site to the sea on species diversity were not significant (data not shown). All three investigated factors were highly correlated (pH versus log-transformed salinity, $r = 0.69$, $P < 0.001$; distance versus log-transformed salinity, $r = -0.48$, $P < 0.01$), so it was difficult to evaluate a net effect of the individual factor. However, salinity was the important predictor of diversity. Species diversity significantly decreased with increasing salinity ($r = -0.459$, $P = 0.016$; Fig. 3). The Ängersjärden Lake (locality no. 2) contained 41 species and was the richest locality. The lake had low salinity (0.05 psu) and pH (5.8), and the distance of the sampling site to the sea was 1.5 km. By contrast, locality no. 23 (Slädan fjord) was the most saline sampling site (3.08 psu; pH 7.2). This locality represented transitional lake partly connected to the sea

Table 1 Results of permutational multivariate analyses of variance to evaluate effects of salinity, pH, and distance of the sampling site to the sea shore on the community structure

Factor	Df	Jaccard index				Bray–Curtis index			
		Sums of squares	Pseudo-F ratios	R ²	P value	Sums of squares	Pseudo-F ratios	R ²	P value
<i>Adonis A</i>									
pH	1	0916	2803	0099	***	0974	4027	0135	***
Salinity	1	0392	1195	0042	n.s.	0309	1277	0043	n.s.
Distance	1	0438	1338	0047	n.s.	0368	1522	0051	n.s.
Residuals	23	7536	–	0812	–	5562	–	0771	–
<i>Adonis B</i>									
Salinity	1	0652	1990	0070	***	0629	2603	0087	**
Distance	1	0389	1186	0042	n.s.	0303	1253	0042	n.s.
pH	1	0708	2160	0076	***	0718	2970	0100	***
Residuals	23	7536	–	0812	–	5562	–	0771	–
<i>Adonis C</i>									
Distance	1	0688	2098	0074	***	0690	2852	0096	***
pH	1	0791	2413	0085	***	0821	3396	0114	***
Salinity	1	0270	0825	0029	n.s.	0140	0578	0019	n.s.
Residuals	23	7536	–	0812	–	5562	–	0771	–

Significant values are marked in bold

The effects of individual factors were evaluated sequentially following the stepwise forward model selection. Three *adonis* models (A, B, C) with a different order of factors were conducted using the Jaccard and the Bray–Curtis indices and 9999 permutations. Salinity and distance values were log-transformed.

Significance values are represented as *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and n.s. $P > 0.05$

where three silica-scaled chrysophyte species were recorded.

Besides the effect of salinity, pH, and distance on species diversity, we also evaluated how these factors influence the community structure. Per-MANOVA-based variance partitions are presented in Table 1. The model using binary Jaccard similarity index did not substantially differ from that based on quantitative Bray–Curtis index. The relative proportion among individual factors remained constant, whereas the proportion of unexplained variance with Jaccard index was slightly higher. Community structure was mainly driven by pH (net effect, 7.6 and 10.0% of explained variance, Jaccard and Bray–Curtis, respectively). The net effect of distance and salinity was not significant in either analysis. The effect of salinity was important, but the net effect of this factor was masked by the highly correlated variable, pH (Table 1). High proportions of unexplained variance indicated that other than analyzed factors may play an important role in structuring the silica-scaled chrysophyte communities.

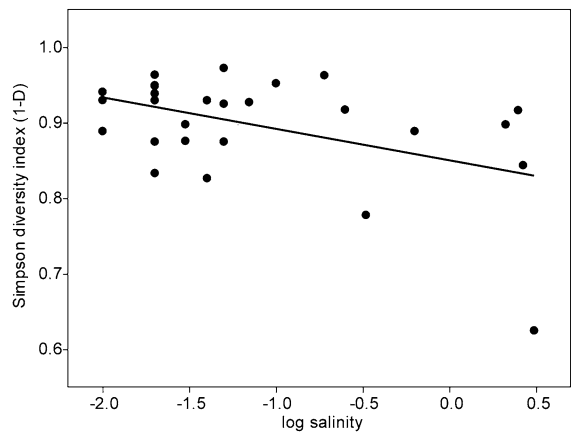


Fig. 3 Correlation between species diversity and salinity. Salinity values were log-transformed. Species diversity (expressed by the Simpson index 1-D) significantly decreased with increasing salinity ($r = -0.459$, $P = 0.016$)

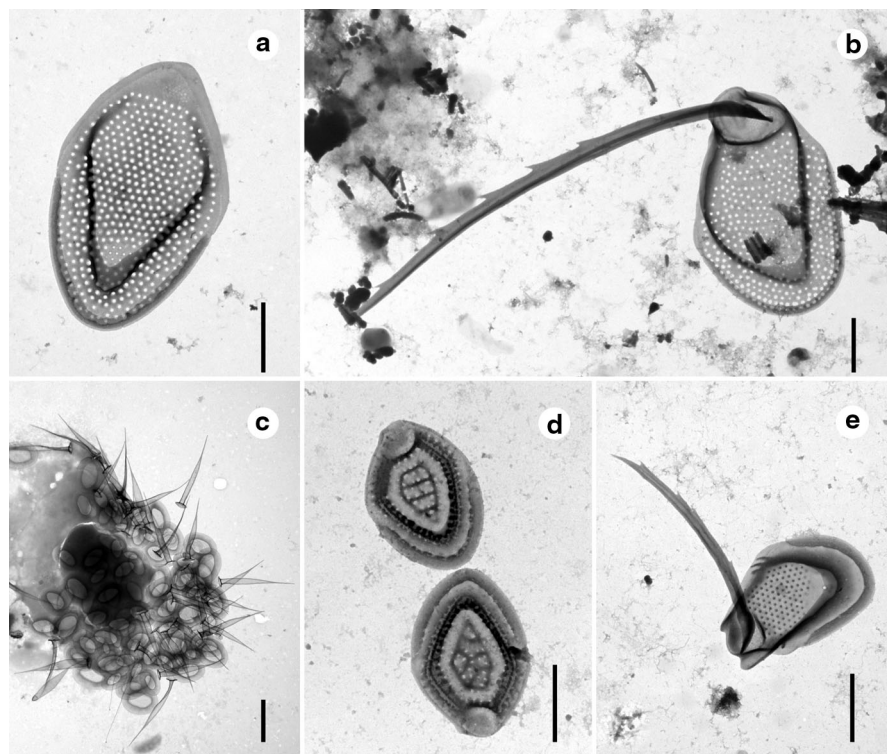
Significant positive correlation between species occurrence and salinity was identified for six species (Kendall tau coefficient T and probability P are listed with individual species in Supplementary Table 1).

The highest correlation was revealed for *Mallomonas tonsurata* Teiling emend. Krieger and *M. alpina* Pascher & Ruttner in Pascher ($T = 0.39$ and 0.32 , respectively; Fig. 4a, b). By contrast, 12 species occurred preferentially in low saline habitats. The highest significant negative correlation was observed for *Spiniferomonas abei* Takahashi, *Mallomonas pillula* f. *valdiviana* Dürschmidt, and *M. papillosa* Harris & Bradley ($T = -0.48$, -0.44 , and -0.43 , respectively; Fig. 4c–e).

Several potentially new or rare species were observed during this study (Fig. 5). *Mallomonas* sp. (Fig. 5a) was identified from two scales observed in peat-bog localities no. 14 and 25 (distance between the sites was approximately 2 km). This species belongs to the section Heterospinae. According to the longitudinal apical rib connecting the lateral parts of the submarginal rib, the scale most resembles *M. pugio* Bradley or recently described *M. temonis* Němcová (Němcová & Kreidlová, 2013). However, the distal portion of *Mallomonas* sp. scale, above the transversal rib, bears a system of 10–12 thin closely spaced parallel ribs, with some lateral struts connecting adjacent ribs. The dome resembles that of *M. pugio*, but it is smooth. We believe that *Mallomonas* sp.

represents a new species; however, more scales and whole-cell samples must be examined to completely describe and characterize this species. *Mallomonas pumilio* var. *dispersa* Němcová et al. (Fig. 5b) was recently separated from *M. pumilio* Harris & Bradley emend. Němcová et al. (2013). This species was found only in the Ängerfjärden Lake (locality 2), which represented the richest locality. The shield is covered with dispersed single pores, sometimes arranged in rows. Distribution of this taxon is probably scattered, and it may be overlooked because of its small size (cells reach approximately $10\ \mu\text{m}$). *Mallomonas pumilio* var. *dispersa* was previously recorded in the Czech Republic (Němcová et al., 2013), France (Němcová et al., 2012), Sweden (Asmund et al., 1982), and North and Central America (Jacobsen, 1985). *Synura borealis* Škaloud & Škaloudová (Fig. 5c) belongs to the *Synura petersenii* species complex. We found the species in six localities (0.02–0.63 psu; pH 5.6–8.1). *Synura borealis* seems to be acclimated to colder environments, and its distribution is probably limited by higher summer temperatures. This species was previously recorded from Fennoscandia and Greenland (Škaloud et al., 2014). *Chryso-sphaerella* sp. (Fig. 5d) was found in

Fig. 4 a, b Species with occurrence significantly positively correlated to salinity, a *Mallomonas tonsurata*, b *M. alpina*, c–e Taxa showing the highest significant negative correlation between occurrence and salinity, c *Spiniferomonas abei*, d *M. pillula* f. *valdiviana*, e *M. papillosa*



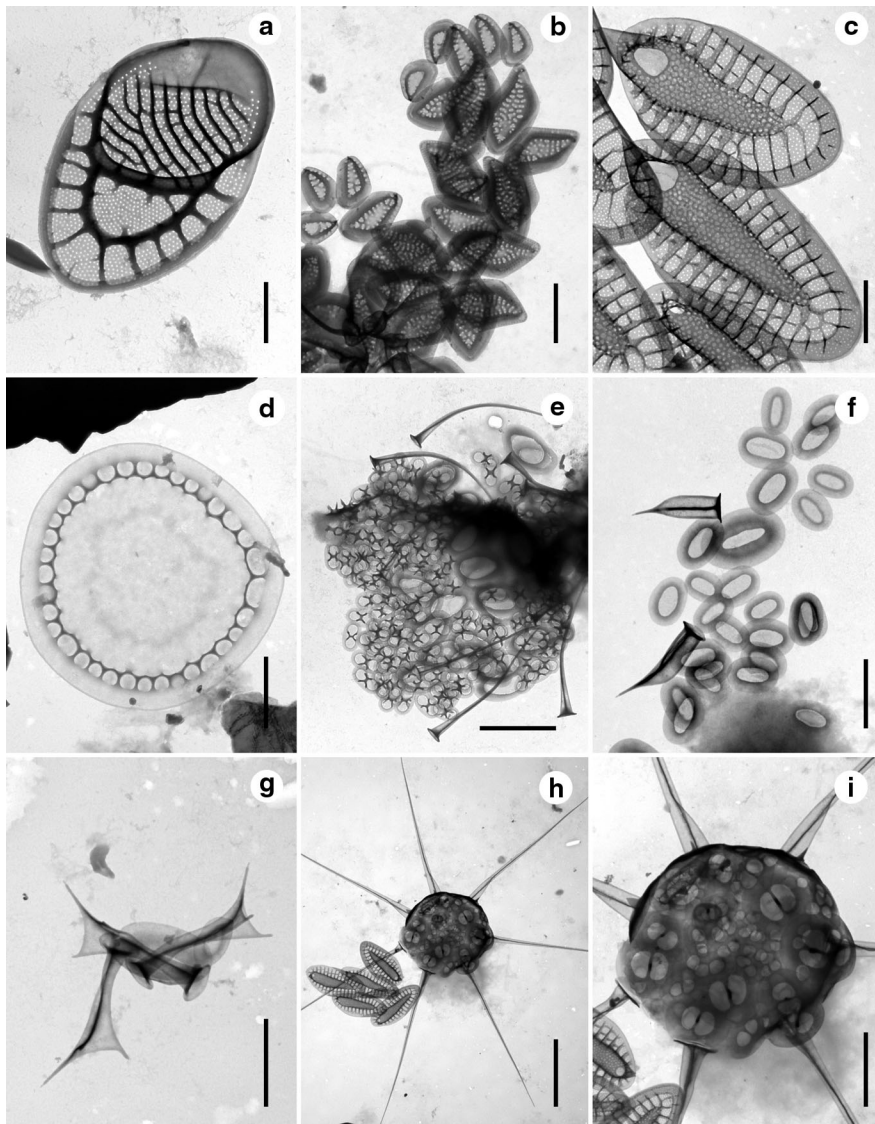


Fig. 5 Potentially new or rare species identified in this study **a** *Mallomonas* sp., **b** *Mallomonas pumilio* var. *dispersa*, **c** *Synura borealis*, **d** *Chryso-sphaerella* sp., **e** *Spiniferomonas crucigera*, **f** *Sp. minuta*, **g** *Sp. takahashii*, **h**, **i** *Sp. triangularis*

locality no. 2 (0.05 psu; pH 5.8). The large circular scales resembled the recently described *C. rotundata* (Škaloudová & Škaloud, 2013). The scales of both species are similar in shape and ornamentation; however, *C. rotundata* scales are much smaller than those of *Chryso-sphaerella* sp. ($3.0\text{--}3.5 \times 2.2\text{--}3.1 \mu\text{m}$ vs. $3.3\text{--}4.0 \times 2.2\text{--}3.6 \mu\text{m}$). Our material also resembled the large circular scales determined as *Chryso-sphaerella brevispina* Korshikov (Nicholls, 1984, p. 113, Fig. 17). The diversity of this genus is largely undescribed; therefore, it is possible that these scales

belong to a currently undescribed species. *Spiniferomonas crucigera* Takahashi (Fig. 5e) was recorded in six localities (0.01–0.1 psu; pH 4.3–7.4). This species was identified in inland Finland (Ikävalko, 1994a; Eloranta, 1989; Hällfors & Hällfors, 1988), Connecticut (Siver, 1988), and Texas (Wujek et al., 2002). *Spiniferomonas minuta* Nicholls (Fig. 5f) was found in five localities (0.02–0.1 psu; pH 5.6–6.3). Two types of scales (elliptical plate scales and thick broadly bent spine scales) were discerned. There are only three records from Finnish lakes published in Europe

(Ikävalko, 1994a) and two records in Connecticut (USA, Siver, 1988). *Spiniferomonas takahashii* Nicholls (the species with elliptical plate scales and three-winged spines; Fig. 5g) was recorded in locality no. 2 (0.05 psu; pH 5.8). The previous European record was from brackish water of Pojo Bay (max. 3.12 psu; Ikävalko, 1994b). This species appears to be adapted to brackish environments. We also observed the rarely reported taxon *Sp. triangularis* Siver (Fig. 5h, i) in localities no. 15 and 16 (0.1 and 0.02 psu, respectively; pH 6.7 and 6.3, respectively). *Spiniferomonas triangularis* possesses both large and small bilacunate scales. The species was originally described from Connecticut (USA) by Siver (1988), and subsequently reported from Ontario (Canada; Kling & Findlay, 2001) and the Bolshezemelskaya tundra (Russia, Siver et al., 2005).

Discussion

An important aspect of ecology of any group of organisms is an understanding of the relative importance of each factor in driving diversity and community structure of the group. The sampling design of the present study obtained data from low saline (lakes 5 km from the shore) and brackish (deep almost separated fjords and bays) sites to cover a wide salinity gradient in natural biotopes inhabited by silica-scaled chrysophytes. The Baltic Sea is a young ecosystem, continuously undergoing post-glacial successional changes. Coastal lakes emerged due to separation of brackish embayments by post-glacial isostatic land uplift, and some of these lakes are still connected with the sea (Kautsky & Kautsky, 2000). This unique system of sea-lake transition enabled an investigation of salinity effects on species diversity and community structure of silica-scaled chrysophytes, and a study of species preferences with respect to salinity.

In addition to pH, conductivity/salinity was documented as a major environmental factor structuring silica-scaled communities in natural habitats (Siver & Hamer, 1989). Siver (1993) used extant population of scaled chrysophytes in 28 Connecticut lakes (USA) spanning a relatively short conductivity gradient of 28–249 $\mu\text{S cm}^{-1}$ (approximately 0.02–0.17 psu) to develop a conductivity inference model that was used to reconstruct historical conductivity in a small soft-water lake. The present study was inspired by two

published papers of Ikävalko (1994b) and Ikävalko & Thomsen (1996), who reported rich populations of scaled chrysophytes (including Synurales) from the Baltic Sea (0.46–3.14 psu). This study included a broader salinity gradient (0.01–3.08 psu) shifted towards low saline sites. A diverse community of silica-scaled chrysophytes (83 taxa in total) was revealed in the investigated area. However, most of the observed diversity was concentrated in low saline localities separated from brackish Bothnian Bay. Although salinity was significantly correlated with pH, salinity proved to be important predictor of species diversity (i.e., Simpson index was negatively correlated to local salinity levels; Fig. 3). Our observation is consistent with that of Siver & Hamer (1989), who reported greater abundance of chrysophyte species in low-conductivity freshwater ($<40 \mu\text{S cm}^{-1}$) compared to waterbodies of conductivity above $160 \mu\text{S cm}^{-1}$. Diverse communities of silica-scaled chrysophytes were observed in Pojo Bay (Baltic Sea) at the time of, or shortly after, the ice break-up in conditions of low water temperature and low salinity. Increasing salinity in Pojo Bay water during the summer may have caused the abrupt species decline. Ikävalko (1994b) discussed in detail the hydrography of Pojo Bay and emphasized that part of the phytoplankton community was washed in with freshwater inflow of the Mustio River. This is an important consideration also for this study as the rivers Öre and Bure discharge their selves to the Gulf of Bothnia near the investigated area. To minimize the river impact, we sampled sites further than 5 km from the river mouth. It was shown that Öre River supplied material rapidly sedimented out of the water column (Forsgren & Jansson, 1992). Moreover, we excluded the most exposed seashore sites and concentrated on deep almost separated bays and fjords. To record autochthonous communities, we drown plankton net hauls also through the submerged vegetation and squeezed the metaphyton. Silica-scaled chrysophytes are known to prefer sheltered sites to open water and their association with the metaphyton was frequently observed during our previous studies (e.g., Němcová et al., 2002). However, it is not possible to exclude completely the possibility that the most saline localities may have been influenced by a random drift of cells or scales. Laboratory experiments are necessary to test the ability of revealed species to grow and divide under environmental conditions recorded in these sites.

A decrease in diversity with increasing salinity also was observed by Ulanova & Snoeijs (2006), who investigated diatom communities living on submerged stones along 2000 km of Baltic Sea coastline. Low species richness of epilithic diatoms was explained by either the large dominance of certain species or by increased abundance of small macroalgae (with thallus <1 mm). Small macroalgae are capable of rapid nutrient uptake and growth, and may compete for space, light, and nutrients with epilithic diatoms (Snoeijs, 1999; Ulanova & Snoeijs, 2006). In contrast to diatoms where a gradually change of species composition on salinity gradient was recorded (the number of freshwater species decreased and the number of brackish to marine species increased; Ulanova et al., 2009), in predominantly freshwater scaled chrysophytes the diversity declined with increasing salinity.

In contrast to alpha-diversity patterns, silica-scaled chrysophyte community structure was driven primarily by pH; however, the effect of salinity also was important (Table 1). Although pH appears to be a primary driver, the influence of other factors will need to be understood to reduce high proportions of unexplained variance. Snoeijs (1994) and Ulanova et al. (2009) found that salinity was the overriding environmental factor structuring epiphytic diatom communities in the brackish Baltic Sea, whereas exposure to wave action and nutrient concentrations were of secondary importance. However, pH was not measured in those studies. Snoeijs (1994) reported a sharp transition in community structure at salinity levels 5–6 psu. Below this value, communities primarily contained diatoms with freshwater affinities (>95%); above this value, diatoms with marine affinities were predominant (>95%). In the presented study, a silica-scaled species pool inhabiting the more saline environment represented a subset of the species pool thriving in low saline waters (Fig. 2). Occurrence of six species was positively correlated to salinity; *Mallomonas tonsurata* and *M. alpina* exhibited the highest correlation. Both species have a wide ecological tolerance of conductivity levels. It is well documented that *Mallomonas tonsurata* is an alkaliphilous to alkalibiontic species with only scarce occurrence in softwater localities with values below $40 \mu\text{S cm}^{-1}$ (Siver, 1991; Škaloud et al., 2013b). *Mallomonas alpina* was sampled in a eutrophic high-conductivity

lake ($800 \mu\text{S cm}^{-1}$; Němcová et al., 2013) and in a tundra pond ($20 \mu\text{S cm}^{-1}$; Siver et al., 2005). This species was one of the most frequently sampled species in Pojo Bay, Baltic Sea (Ikävalko, 1994b), with salinity ranging from 0.21 to 3.15 psu. Conversely, a total of 12 species significantly preferred low saline environment. However, two taxa of this group (*Chryso-sphaerella brevispina* and *Synura sphagnicola* Korshikov) were detected in the phytoplankton of Pojo Bay, Baltic Sea (Ikävalko, 1994b) and are listed also in the Checklist of Baltic Sea Phytoplankton Species (Hällfors, 2004). We speculate that these species were most probably washed into Pojo Bay from freshwater localities. Occurrence of silica-scaled chrysophytes in saline sites could have been restricted due to low partial pressure of CO_2 ($p\text{CO}_2$) in water. Partial pressure of CO_2 has large seasonal variability in the Baltic Sea and is strongly influenced by salinity of the surface water (Wesslander et al., 2011). A conspicuous trait of chrysophyte physiology is the lack of carbon concentrating mechanism (CCM) that concentrates carbon dioxide around the carbon fixing enzyme RuBisCO and chrysophytes therefore rely only on diffusive entry of CO_2 during photosynthesis (Saxby-Rouen et al., 1998; Maberly et al., 2009; Wolfe & Siver 2013). However, this generalization is based on only a handful of species. As some species (e.g., *Mallomonas tonsurata* and *M. alpina*) were repeatedly recorded from high saline/conductivity/pH environments, there is space to consider some exceptions. Among silica-scaled chrysophytes, only heterotrophic flagellates of the genus *Paraphysomonas* belong to a phylogenetic lineage that is equally distributed in freshwater and marine environments. The genus *Chryso-sphaerella* and the order Synurales probably diversified in freshwater (Massana et al., 2006; del Campo & Massana, 2011). Conversely, modern hypotheses of diatom origin tend to agree that they developed from a scaly ancestor in a shallow marine environment, and repeatedly invaded and radiated into freshwater (Sims et al., 2006). Different evolutionary origins of these stramenopile groups correspond to the observed patterns that the silica-scaled chrysophytes predominantly preferred freshwater conditions, whereas the diatoms were present in both freshwater and brackish localities with a gradual increase of marine species and decrease of freshwater ones along the salinity gradient (Ulanova et al., 2009).

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References

- Abdi, H., 2007. Kendall rank correlation. In Salkind, N. J. (ed.), Encyclopedia of Measurement and Statistics. Sage, Thousand Oaks, CA.
- Asmund, B., G. Cronberg & M. Dürrschmidt, 1982. Revision of the *Mallomonas pumilio* group (Chrysophyceae). Nordic Journal of Botany 2: 383–395.
- Cavalier-Smith, T., 2015. Heterokontophyta/Ochrophyta. In Frey, W. (ed.), Syllabus of Plant Families, Volume 2/1: Photoautotrophic Eukaryotic Algae. Borntraeger, Stuttgart.
- Cronberg, G. & J. Kristiansen, 1980. Synuraceae and other Chrysophyceae from central Smaland. Sweden Botaniska Notiser 133: 595–618.
- del Campo, J. & R. Massana, 2011. Emerging diversity within chrysophytes, choanoflagellates and bicosoecids based on molecular surveys. Protist 162: 435–448.
- Eloranta, P., 1985. Notes on the scaled chrysophytes (Synuraceae, Chrysophyceae) in small lakes in and near Salamajärvi National Park, western Finland. Memoranda Societatis pro Fauna et Flora Fennica 61: 77–83.
- Eloranta, P., 1989. Scaled chrysophytes (Chrysophyceae and Synurophyceae) from the national park lakes in southern and central Finland. Nordic Journal of Botany 8: 671–681.
- Evans, K. M., V. A. Chepurinov, H. J. Sluiman, S. J. Thomas, B. M. Spears & D. G. Mann, 2009. Highly differentiated populations of the freshwater diatom *Sellaphoracapitata* suggest limited dispersal and opportunities for allopatric speciation. Protist 160: 386–396.
- Forsgren, G. & M. Jansson, 1992. The turnover of river-transported iron, phosphorus and organic carbon in the Öre estuary, northern Sweden. Hydrobiologia 235(236): 585–596.
- Gutowski, A., 1989. Seasonal succession of scaled chrysophytes in a small lake in Berlin. Nova Hedwigia, Beiheft 95: 159–177.
- Håkansson, B., P. Alenius & L. Brydsten, 1996. Physical environment in the Gulf of Bothnia. Ambio Special Report 8: 5–12.
- Hammer, Ø., D. A. T. Harper & P. D. Ryan, 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1–9.
- Hällfors, G., 2004. Checklist of Baltic Sea phytoplankton species (including some heterotrophic protistan groups). Baltic Sea Environment Proceedings 95: 1–208.
- Hällfors, G. & S. Hällfors, 1988. Records of chrysophytes with siliceous scales (Mallomonadaceae and Paraphysomonadaceae) from Finnish inland waters. Hydrobiologia 161: 1–29.
- Hansen, P. & J. Kristiansen, 1997. Silica-scaled chrysophytes from Åland. Memoranda Societatis pro Fauna et Flora Fennica 73: 45–52.
- Ikävalko, J., 1994a. Contribution to the flora of silica-scaled flagellates in Mikkeli, central Finland. Nova Hedwigia 58: 475–505.
- Ikävalko, J., 1994b. Observations on silica-scaled flagellates (Chrysophyceae and Synurophyceae) in the brackish water of Pojo Bay, SW coast of Finland. Annales Botanici Fennici 31: 1–27.
- Ikävalko, J. & H. A. Thomsen, 1996. Scale-covered and loricate flagellates (Chrysophyceae and Synurophyceae) from Baltic Sea ice. Nova Hedwigia, Beiheft 114: 147–160.
- Jacobsen, B. A., 1985. Scale-bearing Chrysophyceae (Mallomonadaceae and Paraphysomonadaceae) from West Greenland. Nordic Journal of Botany 5: 381–398.
- Jo, B. Y., W. Shin, H. S. Kim, P. A. Siver & R. A. Andersen, 2013. Phylogeny of the genus *Mallomonas* (Synurophyceae) and descriptions of five new species on the basis of morphological evidence. Phycologia 52: 266–278.
- Kautsky, L. & N. Kautsky, 2000. The Baltic Sea, including Bothnian Sea and Bothnian Bay. In Sheppard, C. (ed.), Seas at the Millennium: An Environmental Evaluation. Elsevier, Amsterdam: 121–133.
- Kling, H. J. & D. L. Findlay, 2001. Dominant scaled and non-scaled chrysophytes in northwestern Ontario Shield lakes. Nova Hedwigia, Beiheft 122: 75–79.
- Kristiansen, J. & H. R. Preisig, 2007. Chrysophyte and Haptophyte algae, 2nd part: Synurophyceae. In Büdel, B., G. Gärtner, L. Krienitz, H. R. Preisig & M. Schagerl (eds), Süßwasserflora von Mitteleuropa, Vol. 1/2. Springer, Berlin.
- Ma, C.-X. & Y.-X. Wei, 2013. A new species of the genus *Mallomonas* found in the national wetland preserve in Zhenbaodao, Heilongjiang, northeast China. Nova Hedwigia 96: 457–462.
- Maberly, S. C., L. A. Ball, J. A. Raven & D. Sueltemeyer, 2009. Inorganic carbon acquisition by chrysophytes. Journal of Phycology 45: 1052–1061.
- Martiny, J. B. H., B. J. M. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Øvreås, A. L. Reysenbach, V. H. Smith & J. T. Staley, 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4: 102–112.
- Massana, R., R. Terrado, I. Forn, C. Lovejoy & C. Pedros-Alio, 2006. Distribution and abundance of uncultured heterotrophic flagellates in the world oceans. Environmental Microbiology 8: 1515–1522.
- Němcová, Y., J. Neustupa, S. Nováková & T. Kalina, 2002. Silica-scaled chrysophytes of the Sumava National Park and the Trebnsko UNESCO Biosphere Reserve (Southern Bohemia, Czech Republic). Nordic Journal of Botany 22: 375–383.
- Němcová, Y., J. Kreidlová, A. Kosová & J. Neustupa, 2012. Lakes and pools of Aquitaine region (France): a biodiversity hotspot of Synurales in Europe. Nova Hedwigia 95: 1–24.
- Němcová, Y. & J. Kreidlová, 2013. Two new species of *Mallomonas* (Chrysophyceae: synurales): *Mallomonas temonis* and *Mallomonas divida*. Phytotaxa 87: 11–18.
- Němcová, Y., J. Kreidlová, M. Pusztai & J. Neustupa, 2013. *Mallomonas pumilio* group (Chrysophyceae/Stramenopiles) – a revision based on the scale/scale-case

- morphology and analysis of scale shape. *Nova Hedwigia*, Beiheft 142: 27–49.
- Nicholls, K. H., 1984. *Spiniferomonas septispina* and *S. enigmata*, two new algal species confusing the distinction between *Spiniferomonas* and *Chryso-sphaerella* (Chryso-phyceae). *Plant Systematics and Evolution* 148: 103–117.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens & H. Wagner, 2014. Package 'vegan' Community Ecology Package. Version 2.0–10. Available on internet at <http://cran.r-project.org/web/packages/vegan/vegan.pdf>.
- Pang, W. & Q. Wang, 2013. A new species, *Synuramorusimila* sp. nov. (Chrysophyta), from Great Xing'an Mountains, China. *Phytotaxa* 88: 55–60.
- Ptácnik, R., T. Andersen, P. Brettum, L. Lepistö & E. Willén, 2010. Regional species pools control community saturation in lake phytoplankton. *Proceedings of the Royal Society B277*: 3755–3764.
- R Development Core Team, 2013. R: A Language and Environment for Statistical Computing, Ver. 3.0.2. R Foundation for Statistical Computing, Vienna.
- Saxby-Rouen, K. J., B. S. C. Leadbeater & C. S. Reynolds, 1998. The relationship between the growth of *Synura petersenii* (Synurophyceae) and components of the dissolved inorganic carbon system. *Phycologia* 37: 467–477.
- Sandgren, C. D., 1988. The ecology of chrysophyte flagellates: their growth and perennation strategies as freshwater phytoplankton. In Sandgren, C. D. (ed.), *Growth and Reproductive Strategies of Freshwater Phytoplankton*. Cambridge University Press, Cambridge: 9–104.
- Scoble, J. M. & T. Cavalier-Smith, 2014. Scale evolution in Paraphysomonadida (Chrysophyceae): sequence phylogeny and revised taxonomy of *Paraphysomonas*, new genus *Clathromonas*, and 25 new species. *European Journal of Protistology*. doi:10.1016/j.ejop.2014.08.001.
- Sims, P. A., D. G. Mann & L. K. Medlin, 2006. Evolution of the diatoms: insights from fossil, biological and molecular data. *Phycologia* 45: 361–402.
- Siver, P. A., 1988. The distribution and ecology of *Spiniferomonas* (Chrysophyceae) in Connecticut (USA). *Nordic Journal of Botany* 8: 205–212.
- Siver, P. A., 1991. *The Biology of Mallomonas*. Morphology, Taxonomy, Ecology. Kluwer Academic Publishers, Dordrecht.
- Siver, P. A., 1993. Inferring lakewater specific conductivity with scaled chrysophytes. *Limnology and Oceanography* 38: 1480–1492.
- Siver, P. A., 1995. The distribution of chrysophytes along environmental gradients: their use as biological indicators. In Sandgren, C. D., J. P. Smol & J. Kristiansen (eds), *Chrysophyte Algae*. Cambridge University Press, Cambridge: 232–268.
- Siver, P. A. & J. S. Hamer, 1989. Multivariate statistical analysis of the factors controlling the distribution of scaled chrysophytes. *Limnology and Oceanography* 34: 368–381.
- Siver, P. A. & A. M. Lott, 2012. Biogeographic patterns in scaled chrysophytes from the east coast of North America. *Freshwater Biology* 57: 451–466.
- Siver, P. A. & A. P. Wolfe, 2005. Eocene scaled chrysophytes with pronounced modern affinities. *International Journal of Plant Sciences* 166: 533–536.
- Siver, P. A., L. N. Voloshko, O. V. Gavrilova & M. V. Getsen, 2005. The scaled chrysophyte flora of the Bolshezemel'skaya tundra (Russia). *Nova Hedwigia*, Beiheft 128: 125–150.
- Škaloudová, M. & P. Škaloud, 2013. A new species of *Chryso-sphaerella* (Chrysophyceae: Chromulinales), *Chryso-sphaerella rotundata*, sp. nov., from Finland. *Phytotaxa* 130: 34–42.
- Škaloud, P., J. Kristiansen & M. Škaloudová, 2013a. Developments in the taxonomy of silica-scaled chrysophytes – from morphological and ultrastructural to molecular approaches. *Nordic Journal of Botany* 31: 385–402.
- Škaloud, P., M. Škaloudová, M. Pichrtová, Y. Němcová, J. Kreidlová & M. Pusztai, 2013b. A database on distribution and ecology of silica-scaled chrysophytes in Europe. *Nova Hedwigia*, Beiheft [available on internet at www.chrysophytes.eu] 142: 141–146.
- Škaloud, P., M. Škaloudová, A. Procházková & Y. Němcová, 2014. Morphological delineation and distribution patterns of four newly described species within the *Synurapetersenii* species complex (Chrysophyceae, Stramenopiles). *European Journal of Phycology* 49: 213–229.
- Smol, J. P., 2005. Applications of chrysophytes to problems in paleoecology. In Sandgren, C. D., J. P. Smol & J. Kristiansen (eds), *Chrysophyte Algae*. Cambridge University Press, Cambridge: 232–268.
- Snoeijs, P., 1994. Distribution of epiphytic diatom species composition, diversity and biomass on different macroalgal hosts along seasonal and salinity gradients in the Baltic Sea. *Diatom Research* 9: 189–211.
- Snoeijs, P., 1999. Marine and brackish waters. In Rydin, H., P. Snoeijs, & M. Diekmann (eds), *Swedish Plant Geography*. Acta Phytogeographica Suecica 84: 187–212.
- Soininen, J., J. J. Korhonen, J. Karhu & A. Vetterli, 2011. Disentangling the spatial patterns in community composition of prokaryotic and eukaryotic lake plankton. *Limnology and Oceanography* 56: 508–520.
- Ulanova, A. & P. Snoeijs, 2006. Gradient responses of epilithic diatom communities in the Baltic Sea proper. *Estuarine, Coastal and Shelf Science* 68: 661–674.
- Ulanova, A., S. Busse & P. Snoeijs, 2009. Coastal diatom–environment relationships in the brackish Baltic sea. *Journal of Phycology* 45: 54–68.
- Vyverman, W., E. Verleyen, K. Sabbe, K. Vanhoutte, M. Sterken, D. A. Hodgson, D. G. Mann, S. Juggins, B. Van de Vijver, V. Jones, R. Flower, D. Roberts, V. A. Chepurnov, C. Kilroy, P. Vanormelingen & A. De Wever, 2007. Historical processes constrain patterns in global diatom diversity. *Ecology* 88: 1924–1931.
- Wesslander, K., P. Hall, S. Hjalmarsson, D. Lefevre, A. Omstedt, A. Rutgersson, E. Sahlée & A. Tengberg, 2011. Observed carbon dioxide and oxygen dynamics in a Baltic Sea coastal region. *Journal of Marine Systems* 86: 1–9.
- Wolfe, A. P. & P. A. Siver, 2013. A hypothesis linking chrysophyte microfossils to lake carbon dynamics on ecological and evolutionary time scales. *Global and Planetary Change* 111: 189–198.
- Wujek, D. E., J. L. Wee & J. E. Van Kley, 2002. Silica-scaled chrysophytes and synurophytes from east Texas. *Journal of Science* 54: 27–36.