Nova Hedwigia, Beiheft 142, 069–085
Stuttgart, September 2013

Article

Silica-scaled chrysophytes from North Tyrol (Austria) including a description of *Mallomonas tirolensis* sp. nov.

Martina Pichrtová[1]*, Yvonne Němcová[1], Pavel Škaloud[1] and Eugen Rott[2]

[1] Department of Botany, Charles University in Prague, Benátská 2, 12801 Prague, Czech Republic

[2] Institute of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria

* Corresponding author: pichrtov@natur.cuni.cz

With 5 figures and 3 tables

Abstract: The silica-scaled chrysophyte flora of the eastern Alpine region of North Tyrol was examined by means of transmission electron microscopy. Altogether, 46 taxa of the genera *Chrysosphaerella*, *Mallomonas*, *Paraphysomonas*, *Spiniferomonas* and *Synura* were recorded including one species new to science, *Mallomonas tirolensis*. The samples were taken from 22 sampling sites that comprised natural lakes, artificial ponds and mires. Moreover, conductivity and pH were measured in situ and major ions and nutrients of water samples were analysed. The pH of the localities ranged from 5.9 to 8.5 with the majority of the sites being slightly alkaline. The most common species in our study were *P. vestita* agg., *C. brevispina*, *M. alpina* and *M. striata* that were recorded in more than 50 % of the sites. On the other hand, several rare species were found, e.g. *M. guttata* and *M. hexagonis*. Furthermore, the presence of *M. pseudocoronata* in the Alps was confirmed. We recorded this species, which was originally described as endemic to North America, in four waterbodies. The species composition of the Tyrolean waterbodies was found to be significantly related to pH, dissolved reactive silica content, lake depth, calcium content and altitude. The biogeographical implications of our findings as well as potential effects of human activities on the flora are also discussed.

Key words: Chrysophyceae, Synurophyceae, North Tyrol, silica scales

Introduction

Silica-scaled chrysophytes are planktonic flagellates belonging to the classes Chrysophyceae and Synurophyceae. The taxonomy of the group is largely based on the ultrastructure of their scales that remain intact in lake sediments for long periods of time. Moreover, many species are believed to occur only within a limited range of environmental conditions. These characteristics make them suitable model organisms for ecological, biogeographical and palaeolimnological research. Therefore, when environmental and geographical data are included in floristic surveys, valuable datasets are created that form a basis for formulating and testing of scientific hypotheses. Databases such as www.chrysophytes.eu (Škaloud et al., this volume) have been established

www.borntraeger-cramer.de
1438-9134/2013/0142-0069 $ 4.25

in order to catalogue the data from individual surveys and make it accessible to scientists all over the world.

Looking at the map of European chrysophyte records (Škaloud et al., this volume), it appears that Central Europe belongs to regions with a long tradition of chrysophyte research and its flora is, in general, very well studied. For example, several studies have been published recently from the Czech Republic (Němcová 2010, Pichrtová et al. 2007, Pichrtová & Veselá 2009) or Hungary (Barreto 2005, Řezáčová & Škaloud 2004). Despite the intensity of chrysophyte research, rare (Němcová & Pichrtová 2009), new-to-Europe (Janatková & Němcová 2009), or even new-to-science (Řezáčová 2006) species can still be found in Central Europe suggesting that the real chrysophyte diversity and biogeography is far from being well explored and understood.

However, even within Central Europe, large areas are still practically neglected with respect to chrysophytes. For instance, strikingly few chrysophyte records have been published from waterbodies within the Alps, Europe's highest mountain range. In 1962, a new species *Mallomonas zellensis* was described from Zeller See, an Austrian lake in the Eastern Alps close to North Tyrol (Fott 1962), and *M. pumilio* var. *silvicola* was found in a high alpine Swiss lake Triebtenseewli (Asmund et al. 1982). Smol (1988) reported *M. pseudocoronata* scales from postglacial sediment of Lake Längsee. Later, Cronberg (2010) published a study concerning the enigmatic recent dispersal of *M. pseudocoronata* in European waterbodies including some lakes in the Eastern Alps. In addition, some palaeolimnological studies of selected high Alpine lake sediments were performed that were based on scales (Guilizzoni et al. 1996) as well as on stomatocyst records (Baumann et al. 2010, Kamenik et al. 2010).

For the Austrian province of North Tyrol, all chrysophyte records published so far have been based solely on light microscopic observations. Ettl (1968) reported several chrysophyte species from various sampling sites including some high alpine lakes. *Mallomonas caudata*, one of the few species whose determination by light microscopy is reliable, was found in Seefelder See and Lanser See. In later years, several species of genera *Chrysosphaerella*, *Mallomonas*, *Spiniferomonas* and *Synura* were reported from other Tyrolean waterbodies (Pipp & Rott 1995, Rott 1983, 1988, Tolotti et al. 2003, Tolotti & Thies 2002). Chrysophytes were found to be important components of phytoplankton in Tyrolean lakes, especially in Piburger See where phytoplankton was regularly collected for long-term monitoring purposes. Moreover, some unpublished transmission electron microscopy (TEM) records exist that include *Mallomonas alpina* from Herzsee, *M. cratis* and *M. striata* from Brenner See and *Synura petersenii* from Seerosenweiher (Rott, unpublished).

In summary, the published chrysophyte records from the Alps are very fragmented and no extensive floristic study has been carried out there so far. On the other hand, the few existing reports indicate that for this region, interesting chrysophyte records could be expected that would contribute to the general knowledge of species' autecology and biogeography of chrysophytes. The main aim of our study was to describe the silica-scaled chrysophyte flora of North Tyrol. We selected various types of waterbodies that vary in their size, environmental conditions and altitude in order to encompass a whole range of species. In addition, the relationship between the species composition and environmental conditions was tested in order to find out which ecological parameters are determining in this habitat type.

Materials and methods

Chrysophytes sampling and investigation

The samples were taken on 20–22 April 2012. At each sampling site, we collected plankton using a plankton net (mesh size 20 µm). In addition, the upper sediment layer was sampled in order to encompass the entire seasonal variability of silica-scaled chrysophyte species. The plankton and sediment samples were mixed and dried on Formvar-coated copper grids at the end of the sampling day; later they were washed in a series of water droplets. The grids were examined using a JEOL 1011 transmission electron microscope. Photomicrographs were obtained using a Veleta CCD camera equipped with image analysis software (Olympus Soft Imaging Solution GmbH). For scanning electron microscopy (SEM), the Formvar-coated grid (already observed in TEM) was mounted onto an SEM stub with double-sided adhesive carbon tape, coated with gold for 5 min (forming a 3-nm layer) with a Bal-Tec SCD 050 sputter coater, and observed with a JEOL 6380LV scanning electron microscope.

Sampling sites characteristics

The samples were taken from 22 waterbodies (Fig. 1) and comprised a majority of natural lakes (13) as well as shallow, natural or hydrologically modified ponds (5) and mires (3). The largest deep natural lake was Walchsee (surface area 95 ha, N. 8 in Table 1), followed by deep lakes Hechtsee and Reintaler See (> 20 ha, Ns. 1 and 11), Piburger See (< 10 ha, N. 19), all contrasting to the shallow depth of the large lake Schwarzsee (16 ha, N. 7). Most of the smaller lakes (< 10 ha) were deep, including Brennersee (N. 12), Lottensee (N. 18), Längsee (N. 5), Egelsee (N. 2) and Pfrillsee (N. 3). The small shallow, still, naturally formed lakes with minor hydrological regulations for swimming use were Seefeldersee (6.7 ha, N. 17), Krummsee (N. 9) and Möserer See (N. 16). The shallow pools and ponds were mostly largely modified for fish hatching (Ns. 10, 14, 21 and 22) with the exception of one natural periodical pool near Pfrillsee (N. 4). Within the mires two were natural – Ambergersee (N. 20) and Seerosenweiher (N. 13) – whereas Gieringer Weiher (N. 6), although still having to a large extent the character of a mire, was modified by a dam to improve swimming facilities. Baggersee Rossau (N. 15) was the only deep artificial gravel pit lake sampled, which is intensively used for swimming in summer. The

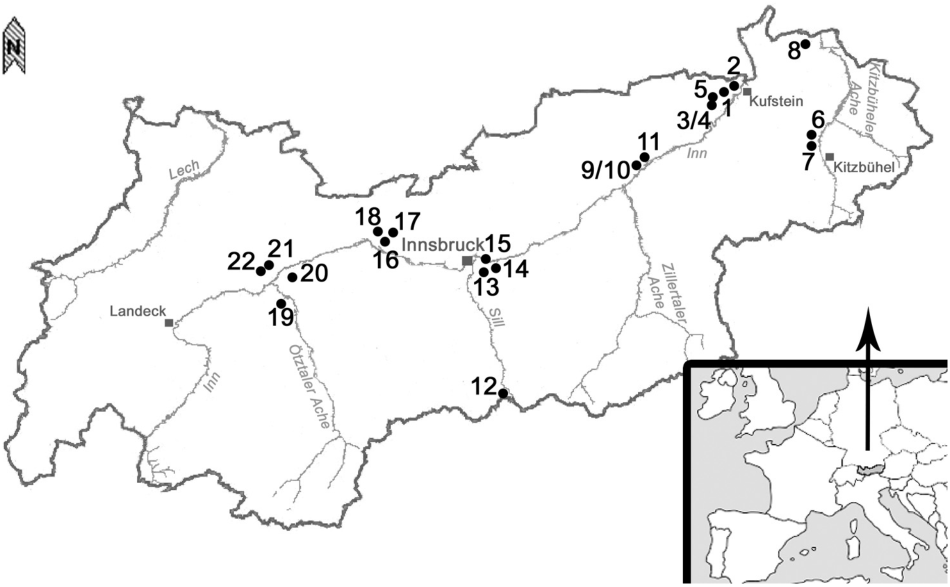

Fig. 1. Sampling sites in the investigated area.

Table 1. List of the investigated sampling sites and values of selected environmental parameters (when available).

	Name of the site	GPS coordinates		Altitude	Size	Max depth	Temp	Cond	pH	Alk/ Gran	SO4	Cl	NO_3 N	NH_4 N	Ptot	PO_4 P	DOC	Mg	Ca	DRSi
		N	E	m	ha	m	°C	µS cm^{-1}		µeq/l	mg/l	mg/l	µg/l	µg/l	µg/l	µg/l	µg/l	mg/l	mg/l	µg/l
1	Hechtsee	47.6103	12.1718	551	28	56	12.0	337	8.49	3340	5.85	7.91	448	9	6.2	3.0	3029	19.37	39.90	277
2	Egelsee	47.6129	12.1668	565	2.8	8	12.6	309	8.14	3290	3.03	0.80	454	79	9.7	3.8	4039	17.14	40.97	1679
3	Pfrillsee	47.5959	12.1463	628	1.8	8	10.5	378	8.28	3750	5.24	6.25	1316	23	6.1	2.0	2675	20.13	47.76	844
4	Pool near Pfrillsee	47.5979	12.1511	635	< 0.5	1	10.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	Längsee	47.599	12.1405	641	4.6	20	11.2	253	8.40	2610	2.81	0.83	233	166	11.7	5.8	5722	10.45	38.21	899
6	Gieringer Weiher	47.4707	12.3626	785	2.9	3	11.9	102	7.57	800	4.28	3.85	187	9	17.8	6.4	5911	3.63	12.19	1253
7	Schwarzsee	47.4558	12.3658	799	16	7	9.5	247	7.69	1760	4.37	20.50	210	13	24.5	7.2	3259	8.79	24.611	2059
8	Walchsee	47.651	12.3252	671	95	20	9.5	343	8.28	3540	7.11	2.71	539	12	7.7	1.9	1663	10.57	57.86	1068
9	Krummsee	47.4566	11.8826	568	4.8	2.5	13.1	461	8.27	4095	32.03	6.68	959	108	14.7	3.9	2939	22.07	62.96	1059
10	Buchsee	47.4554	11.8722	564	2.3	2	12.9	459	8.26	4185	31.98	6.57	951	100	11.8	3.3	2745	21.69	62.95	1047
11	Reintaler See	47.4586	11.8904	568	27.5	10	12.7	451	8.32	4130	29.39	6.62	1037	126	8.7	2.6	2985	22.95	61.11	1726
12	Brennersee	47.0171	11.5037	1316	8.4	11	9	587	8.26	2500	37.07	87.15	675	16	11.9	2.0	945	9.74	58.63	1008
13	Seerosenweiher	47.243	11.419	858	0.4	5	12.1	377	7.73	3670	12.47	4.83	< 1	2	40.7	6.4	6907	14.66	53.36	1714
14	Herzsee	47.2491	11.4543	823	0.8	2	12.8	312	8.15	1780	21.20	21.94	2720	6	79.5	5.4	3020	6.05	36.842	2829
15	Baggersee Rossau	47.2653	11.4458	564	2	15	12.6	673	8.24	3990	49.23	67.91	792	65	7.1	2.2	1943	22.59	77.31	3722
16	Möserer See	47.3144	11.1442	1272	3	5	5.8	371	8.20	3875	2.72	4.20	173	198	5.9	2.1	2661	20.77	43.69	2106
17	Seefelder See (Wildsee)	47.3224	11.192	1179	6.7	5	7.1	414	8.10	4250	3.49	6.46	421	18	16.1	2.1	2904	22.21	49.31	924
18	Lottensee	47.3237	11.1084	1264	5.3	10	4.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19	Piburger See	47.1952	10.8861	920	13.4	24	8.3	70.8	7.54	497	6.53	0.76	261	9	8.5	2.9	1923	0.73	7.91	1131
20	Amberger See	47.226	10.8853	1524	< 0.5	2	6.3	15.9	5.85	67	0.94	0.34	< 1	2	49.3	16.1	9604	2.39	1.36	1574
21	Fishpond near Mauschlhütte	47.2753	10.8098	797	< 0.5	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22	Kropfsee (Strad)	47.273	10.8034	798	1.4	2	14.6	354	8.49	3770	7.30	0.82	7	2	31.3	3.4	3842	21.84	38.18	1956

majority of waterbodies were situated at low and mid altitudes ($17 < 1000$m, $5 > 1000$m) within a mixed landscape of deciduous and evergreen forests of the lower montane vegetation belt and agricultural (pasture) lands.

Most lakes are located in the catchment of the Inn River, the largest river in North Tyrol, and – from W to E – its southern tributaries Ötztaler Ache, Sill and Kitzbühler Ache. Along its lower reach, downstream of Landeck, the Inn valley follows a major fault (Inn valley fault, IVF). Over most of its extent, the IVF separates the Northern Calcareous Alps from the southward adjacent, Central Alps. Whereas the Northern Calcareous Alps consist mainly of Mesozoic carbonate rocks, the Central Alps are composed of a wide suite of metamorphic rocks (mainly ortho- and paragneiss, mica schists, different types of phyllites; Brandner 1985). Most of the lakes formed in morphological depressions excavated by glacial erosion during Quaternary glaciations. In detail, however, additional formative processes of lake basins are varied, and range from: (a) paraglacial sedimentation (e. g. alluvial fans), (b) kettle holes made of decay of dead ice after ice age (c) gravitational rock slope deformation, and (d) chemical weathering and karstification of carbonate rocks. Artificial ponds (3), used mainly for fisheries, were made by construction of dams and flooding of wetland depressions.

Water chemical analyses

Water samples were taken simultaneously with algal samples from 0.5 m depth with a water sampler (Schindler-Patalas Type bottle, sampler volume 4 L, square-shaped plexiglass) or in shallow waters directly from subsurface and filled into pre-rinsed glass bottles. All water samples were stored in a dark cooling box before lab analysis. In situ measurements of temperature and conductivity were made with a portable meter WTW pH/Cond 340 i. In the lab, alkalinity was measured by Gran-Titration. Glass fibre (Whatman GFC) filtrated water was used for analysis of dissolved fractions of the major nutrients: nitrogen (NO_3^-N, NH_4^-N, NO_2^-N), phosphorus (PO_4^-P) and silicon (DRSi). The total available nutrient pool of phosphate (TP) including organic fractions was set free by concentrated sulphuric acid digestion and analysed, as the dissolved phosphorus compounds, with ammonium molybdate detection (detection limit 0.5 µg/L) (Vogler 1966). Nitrogen compounds were analysed by a Total Nitrogen Measurement Unit (Shimadzu TNM-1, detection limit 20 µg/L), for NH_4^-N by indophenol-blue method (dl 1 µg/L) and NO_3^-N by a Dionex ICS 1000/1100 (dl 5 µg/L). Dissolved reactive silicon (DRSi) was measured by a Skalar flow injection analyser with ammonium molybdate (dl 20 µg/L) (Smits & Milne 1981). DOC (dis. organic Carbon) was analysed by a Shimadzu TOC (Vcph, Total Organic Carbon Analyser, dl 25 µg/L). The sulphate and chloride analysis followed standard methods of ion-exchange chromatography (DIONEX, Vienna, Austria) from gradually diluted samples.

Statistical analyses

The relationship between environmental parameters and the species composition was tested with multivariate statistical methods in the program Canoco for Windows (ter Braak & Šmilauer 2002). CCA (canonical correspondence analysis) was selected because of the unimodal character of the data. No data transformations were performed and the Monte Carlo permutation test (5000 permutations) was used to select significant variables.

Results

Chemical status of still waters

In spite of variable bedrock conditions the majority of sites (16) showed well buffered alkaline conditions (pH > 7.5, conductivity > 200 µS cm^{-1}). One mire and a mid-sized lake showed less well-buffered conditions (pH around 7.5, conductivity around 100 µS cm^{-1}), and one mire only showed acidic conditions (pH 5.85, conductivity 15.9 µS cm^{-1}). The concentration of the most limiting nutrient pool (TP) classifies 8 sites as oligotrophic (< 10 µg TP L^{-1}), seven sites as mesotrophic (> 10 < 30 µg TP L^{-1}) and only four (> 30 µg TP L^{-1}) as eutrophic. Six sites were found as nitrate poor (< 250 µg L^{-1}) or extremely poor (not detectable). Whereas several lakes situated within seasonally manured pastureland showed nitrates of > 500 µg L^{-1}, one site – a fertilised fishpond – showed very high nitrates (2720 µg L^{-1}). Moreover, a quite high content of chloride in four of the 22 sites (Baggersee, Brennersee, Herzsee, Schwarzsee) was recorded, which is related to intensive use of road salts in the catchments within winter periods. Silicon, a key element for scale-bearing chrysophytes, was in 15 cases around or > 1000 µg L^{-1}. A complete list of sampling sites together with the summary of selected measured parameters is presented in Table 1.

Silica-scaled chrysophytes

A total of 46 species belonging to five genera (*Chrysosphaerella*, *Mallomonas*, *Paraphysomonas, Spiniferomonas* and *Synura*) were identified during our investigation (Table 2, Figures 2–4). The investigated waterbodies exhibited a large variety in number of recorded taxa that ranged from 4 to 30. Apparently, site 6 – Gieringer Weiher – possessed the richest chrysophyte flora (30 species) including seven species that did not occur anywhere else.

The most abundant species were *P. vestita* agg., *C. brevispina*, *M. alpina* and *M. striata* that were recorded in more than 50 % of the sites. Both varieties of *M. striata* were observed, var. *striata* and var. *serrata*. In our study, *M. striata* var. *striata* was more common whereas *M. striata* var. *serrata* was found in one sampling site only (Gieringer Weiher).

On the other hand, *M. hexagonis*, *M. insignis*, *M. paludosa*, *M. pumilio* var. *dispersa*, *M. punctifera*, *M. schwemmlei*, *P.* cf. *butcherii*, *P. eiffelii*, *Sp. alata*, *Sp. cornuta* and *Sp. serrata* were found in only one single sampling site.

Ecological analyses

Forward selection of variables was applied within the CCA analysis in order to reveal which of the measured parameters were the most important in explaining the species distribution. Altogether, five variables were found to be significant: pH, dissolved reactive silica, lake depth, calcium content and altitude (Table 3). First four CCA axes explained 87 % of the total variance ($p = 0.0002$). The effect of conductivity, though explaining a significant portion of total variance, was largely hidden by the covariation with pH and therefore its effect was not significant.

Separate CCA analyses performed on five above-mentioned significant variables revealed the major effect of pH ($p = 0.0154$) and Ca concentration ($p = 0.0194$) on species composition. *M. acaroides*, *M. tonsurata* and *Sp. abei* preferred localities with a higher concentration of calcium ions (Fig. 5a). On the other hand, *M. papillosa* predominantly occurred in low pH and a low Ca concentration (Fig. 5a, b).

Table 2. List of species and their occurrence.

Species / sampling site No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Chrysosphaerella brevispina Korshikov		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X		
C. coronacircumspina Wujek et Kristiansen		X	X	X		X	X								X							X
Mallomonas acaroides Perty em. Ivanov							X	X	X	X	X					X	X					
M. akrokomos Ruttner in Pascher	X						X						X	X								
M. alata f. *alata* Asmund et al.						X	X			X							X				X	
M. alpina Pascher et Ruttner in Pascher em. Asmund & Kristiansen	X	X	X		X	X	X	X	X	X	X	X		X		X	X				X	X
M. annulata (Bradley) Harris			X	X		X	X		X					X		X						
M. caudata Ivanov em. Krieger		X			X	X	X															
M. crassisquama (Asmund) Fott	X	X				X	X	X											X			
M. cratis Harris et Bradley														X		X						
M. elongata Reverdin	X					X	X															
M. guttata Wujek						X			X							X						X
M. heterospina Lund						X	X	X													X	X
M. hexagonis Nicholls						X																
M. insignis Penard							X															
M. matvienkoae (Matvienko) Asmund & Kristiansen						X										X	X					
M. multisetigera Dürrschmidt						X											X					
M. paludosa Fott																				X		
M. papillosa Harris & Bradley em. Harris						X												X		X	X	
M. parvula Dürrschmidt	X	X																				
M. pillula f. *valdiviana* Dürrschmidt						X			X												X	
M. pseudocoronata Prescott											X			X	X							X
M. pumilio var. *dispersa* Nemcova et al.						X																
M. punctifera Korshikov						X																
M. schwemmlei Glenk em. Glenk & Fott																		X				
M. striata Asmund	X	X	X	X	X	X	X			X	X		X			X	X				X	X
M. tirolensis Pichrtova et al.																X	X					
M. tonsurata Teiling em. Krieger		X	X				X	X		X	X			X	X	X					X	X
Paraphysomonas bandaiensis Takahashi									X									X		X		
P. cf. *butcherii* Pennick & Clarke														X								
P. eiffelii Thomsen						X																
P. imperforata Lucas					X	X	X							X		X			X			
P. takahashii Cronberg & Kristiansen							X									X						
P. vestita agg. (Stokes) De Saedeleer	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X		X	X	X	X
Synura echinulata Korshikov			X	X	X	X			X	X									X	X		X
S. glabra Korshikov em. Kynclova & Skaloud	X					X	X															
S. petersenii Korshikov em. Skaloud & Kynclova				X	X	X	X		X				X			X	X		X	X	X	
S. spinosa Korshikov		X				X	X		X										X	X		
S. uvella Ehrenberg em. Korshikov	X			X		X	X									X						X
Spiniferomonas abei Takahashi									X	X						X					X	
Sp. alata Takahashi						X																
Sp. bilacunosa Takahashi		X	X			X																
Sp. bourrellyi Takahashi		X	X	X			X	X	X		X	X		X					X			X
Sp. cornuta Balonov						X																
Sp. serrata Nicholls																				X		
Sp. trioralis Takahashi		X	X			X	X		X				X						X			X

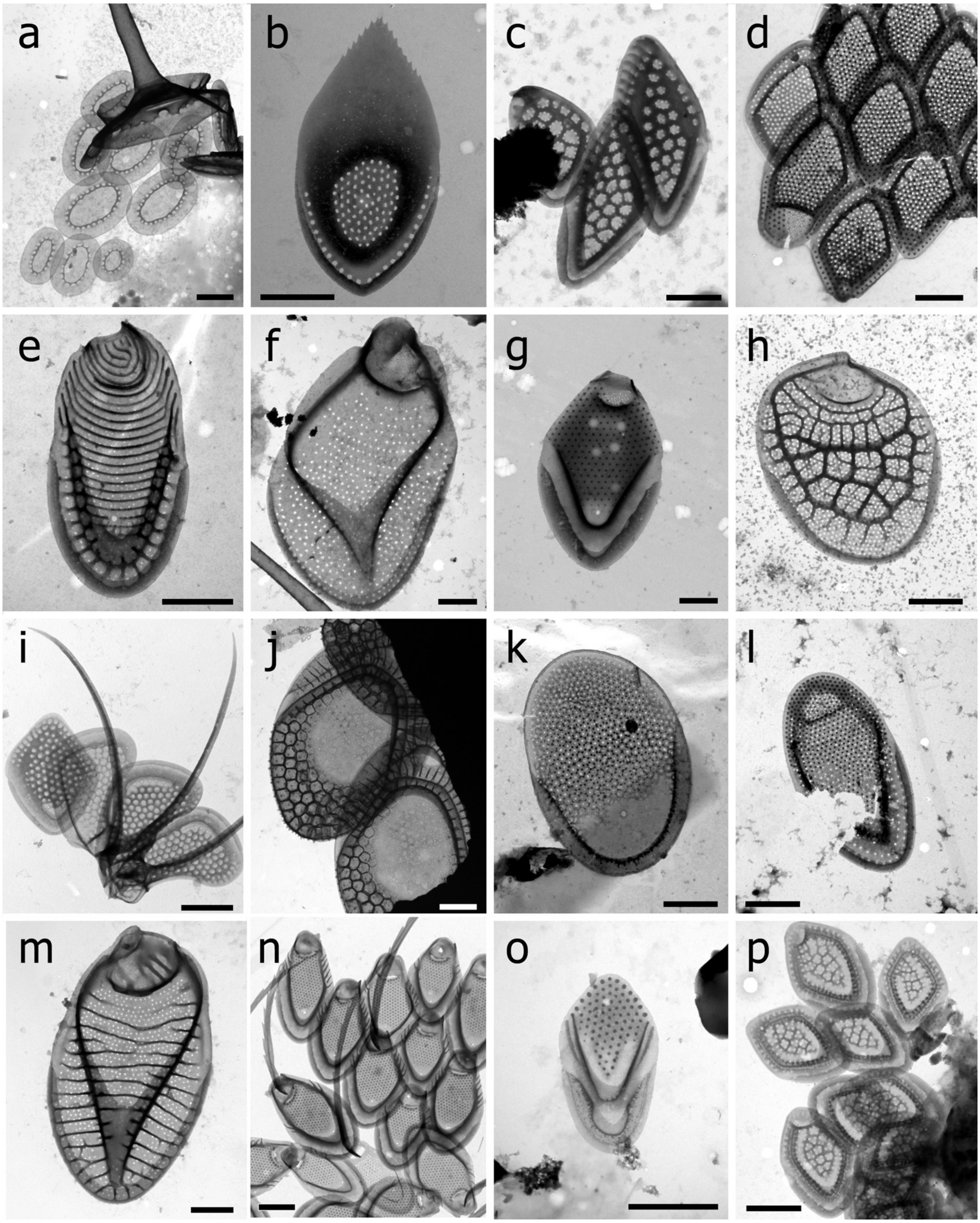

Fig. 2. a: *Chrysosphaerella coronacircumspina*, **b**: *Mallomonas akrokomos*, **c**: *M. alata* f. *alata*, **d**: *M. annulata*, **e**: *M. cratis*, **f**: *M. elongata*, **g**: *M. guttata*, **h**: *M. heterospina*, **i**: *M. hexagonis*, **j**: *M. insignis*, **k**: *M. matvienkoae*, **l**: *M. multisetigera*, **m**: *M. paludosa*, **n**: *M. papillosa*, **o**: *M. parvula*, **p**: *M. pillula* f. *valdiviana*. Scale bars: 1 µm.

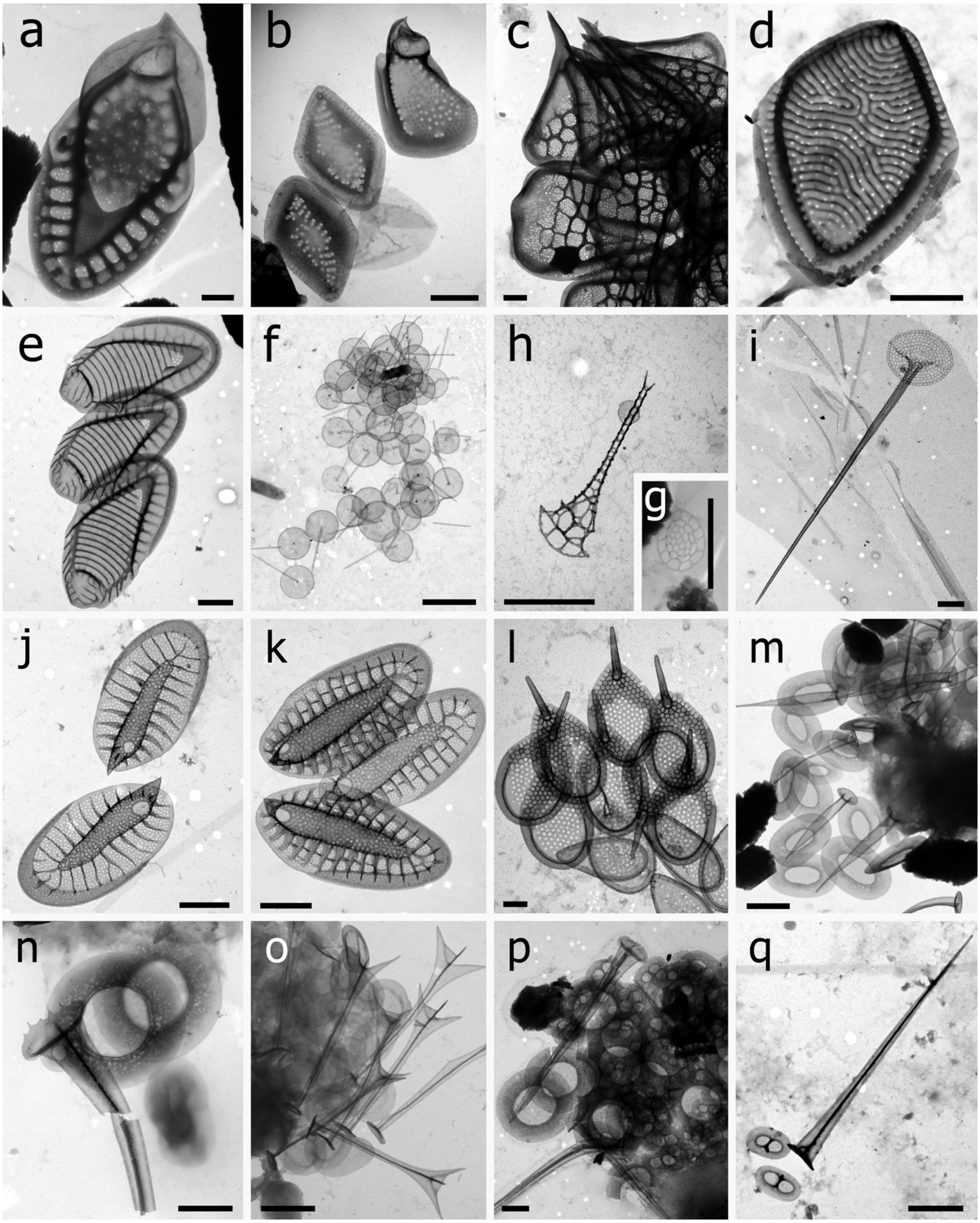

Fig. 3. a: *Mallomonas pseudocoronata,* **b**: *M. pumilio* var. *dispersa*, **c**: *M. punctifera*, **d**: *M. schwemmlei*, **e**: *M. striata*, **f**: *Paraphysomonas bandaiensis*, **g**: *P.* cf. *butcherii*, **h**: *P. eiffelii*, **i**: *P. takahashii*, **j**: *Synura glabra*, **k**: *S. petersenii*, **l**: *S. spinosa*, **m**: *Spiniferomonas abei*, **n**: *Sp. serrata*, **o**: *Sp. alata*, **p**: *Sp. bilacunosa*, **q**: *Sp. cornuta*. Scale bars: 1 µm.

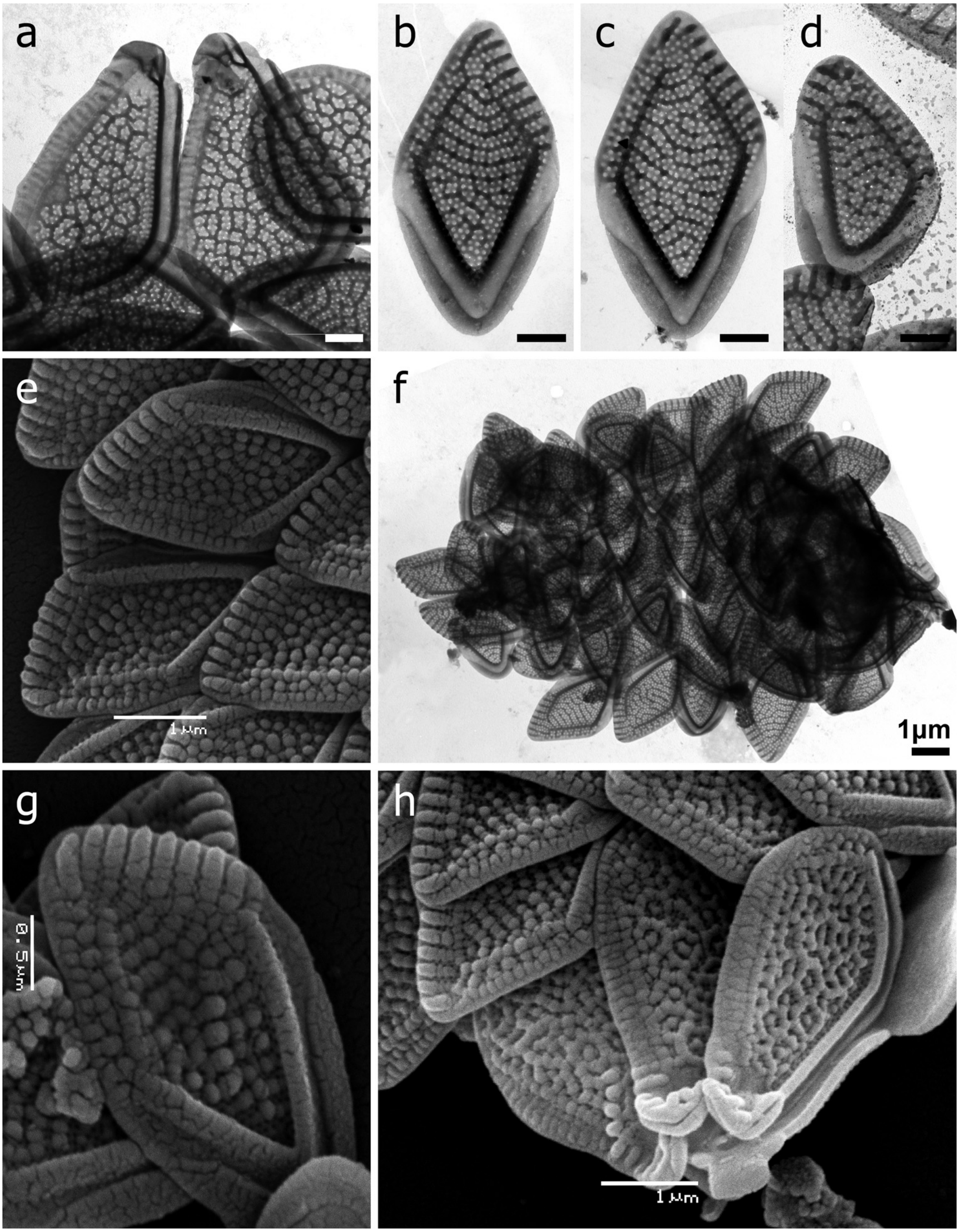

Fig. 4. *Mallomonas tirolensis* Pichrtova, Nemcova, Skaloud & Rott sp. nov. **a**: collar scales observed by transmission electron microscopy (TEM), **b–c**: body scales, TEM, **d**: a rear scale, TEM, **e**: a group of body scales observed by scanning electron microscopy (SEM), **f**: whole cell armour, TEM, **g**: a detail of the body scale, SEM, **h**: four collar scales surrounding the flagellar pore, SEM. Scale bar: 0.5 µm, unless otherwise stated.

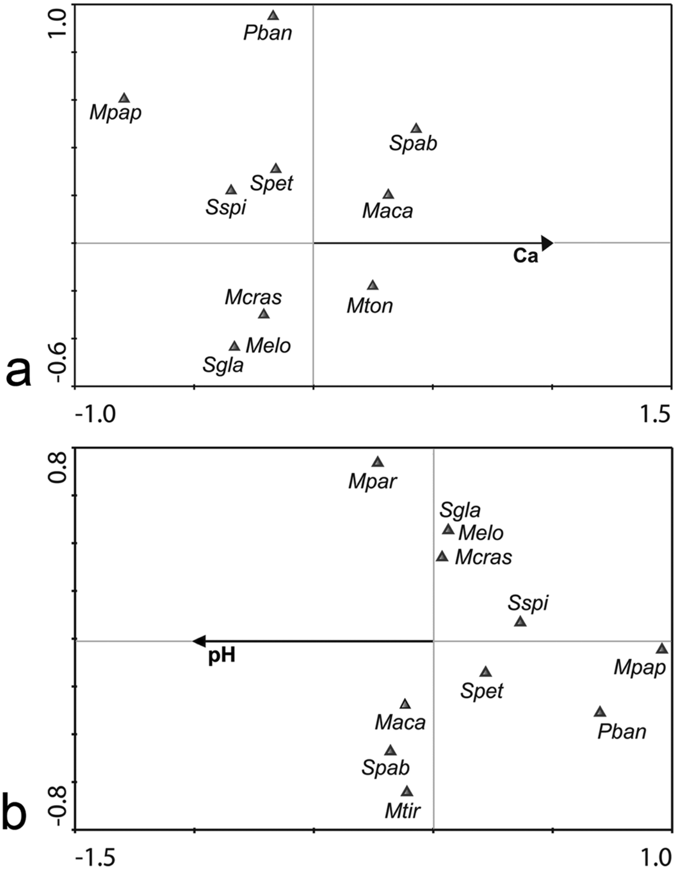

Fig. 5. The CCA ordination plots displaying preferences of the 10 (11) best-fitted species to the a) Ca concentration and b) pH. *Maca*: *Mallomonas acaroides*, *Mcras*: *M. crassisquama*, *Melo*: *M. elongata*, *Mpap*: *M. papillosa*, *Mpar*: *M. parvula*, *Mtir*: *M. tirolensis*, *Pban*: *Paraphysomonas bandaiensis*, *Sgla*: *Synura glabra*, *Spab*: *Spiniferomonas abei*, *Spet*: *Synura petersenii*, *Sspi*: *S. spinosa*.

Mallomonas tirolensis **Pichrtova, Nemcova, Skaloud & Rott sp. nov.** (Fig. 4)

During our investigation unknown *Mallomonas* scales were observed. These scales were quite frequent in two of the samples and the whole variety of scale types could be recorded. Therefore, we decided to formally describe a new species as follows:

Collar, body and rear scales are discerned. Collar scales (3.4–4.0 × 2.1–2.6 µm) are almost triangular (Fig. 4a, h). The submarginal rib is well developed at the dorsal and posterior parts of the scale, but completely reduced along the ventral edge. A dorsal flange is smooth, there are two rows of pores at the proximal ventral corner and pairs of pores are separated by short struts radiating from the submarginal rib. A ventral flange is broad, slightly bent, and ornamented by indistinct ribs. The dome is relatively small, hook-like, continuous with the dorsal submarginal rib and raised above the surface of the rest of the scale. There is no cavity on the inner surface of the scale (adjacent to the plasma membrane, see Fig. 4h). The shield of scale is covered by an irregularly arranged reticulum, and groups of pores (up to 20) are surrounded by short ridges of

Table 3. Forward selection of parameters performed by canonical correspondence analysis (CCA) with 5000 permutations.

Variable	The goodness of fit	p value
pH	0.195	0.0142
DRSi	0.169	0.0236
depth	0.167	0.0348
Ca	0.149	0.0444
altitude	0.148	0.0460

reticulum (looking papillose-like on SEM microphotographs). Body scales (2.7–3.5 × 1.4–2.2 μm) are rhombic and domeless (Fig. 4b, c, e, g). Anterior flanges are asymmetric, one being shorter than the opposite one. The broad flange is marked with regularly spaced ribs and there are two rows of pores between each pair of ribs. The short flange has the same ornamentation as the shield. The posterior flange is smooth. The posterior submarginal rib (V-rib) is prominent, the anterior submarginal rib adjacent to a short flange is more pronounced compared to the one adjacent to the broad flange. The ornamentation of the shield is similar to the collar scales, sometimes more regular, short ridges of reticulum separate two rows of pores (often enclosing four pores). Rear scales (1.8–2.7 × 1.3–1.6 μm) are asymmetric with one anterior flange considerably longer than the other, and the shorter one bears struts (Fig. 4d).

Bristles not observed. Cysts unknown. Dimensions of the cell unknown, and the first row is formed by approximately 4–5 collar scales (Fig. 4h).

Type: Gold-coated grid for electron microscopy containing scales of *M. tirolensis*. Deposited at the Culture Collection of Algae of Charles University in Prague (CAUP, Department of Botany, Charles University in Prague, Benátská 2, 12801 Prague 2, Czech Republic). Item No. CAUP Tir2012/17 I.

Epitype: Figure 4 b

Type locality: Seefelder See (Wildsee), 47.322386 N, 11.192038 E, 1179 m above sea level, sampled on 22.4.2012

Etymology: The epithet "tirolensis" refers to the type locality of the species that is situated in North Tyrol, western Austria.

Distribution: The species was found in the type locality and in one other lake in North Tyrol, Möserer See (sampling sites 16 and 17).

Discussion

To our knowledge, the presented study is the first attempt to describe the silica-scaled chrysophyte flora of North Tyrol using electron microscopic observations. During our survey we were able to find various types of waterbodies that differed in environmental and geographical characteristics as well as in their species composition. Gieringer Weiher, the waterbody richest in chrysophytes (30 species), belongs to the sites with the lower than average measured pH (7.57), conductivity (102 μS cm^{-1}) and nitrate concentration (187 μg L^{-1}), but intermediate total phosphorus (17.8 μg L^{-1}) concentration. Such observations are in agreement with generally accepted chrysophyte ecology: the localities with most diverse chrysophyte flora are usually neutral to slightly acidic, have low conductivity and low nutrient content (Siver 1995).

It is commonly accepted that pH is the key factor affecting the distribution of scaled chrysophytes (Rojackers & Kessels 1986, Siver & Hamer 1989). Other important factors are conductivity, temperature and trophic conditions (Rojackers & Kessels 1986, Siver & Hamer 1989, Suykerbuyk et al. 1995, Tolotti et al. 2003). As well as water-chemistry parameters, altitude and physical parameters of individual lakes turned out to be important for the species distribution in Alpine lakes (Tolotti et al. 2003). In our study, the highly correlated effects of both pH and conductivity were also important as well as altitude and lake depth. Beside those variables, the content of dissolved reactive silica and calcium ions also significantly influenced the species composition. Silicon is the key element for the construction of chrysophyte scales, but it has been experimentally shown that chrysophytes are able to survive even in environments completely lacking silica (Klaveness & Guillard 1975). The content of silica has already been proved to have an impact on species distribution (Forsström et al. 2005, Suykerbuyk et al. 1995) but in those studies it explained only a small proportion of the variation in comparison to other environmental factors. Moreover, the content of both calcium and silica is influenced by bedrock type. As the investigated region comprises both carbonate and metamorphic rocks of the Northern Calcareous Alps and the Central Alps respectively, our results indicate that the geology might also have an effect on distribution pattern of the chrysophyte species.

Selected silica-scaled chrysophytes are also valuable bioindicators because of their preference for specific niches within large environmental gradients (Siver 1995, Smol 1995). With respect to pH, several groups were established that comprise species with different preferred pH-range of occurrence (Siver 1995). The species most common in Tyrolean samples belong to alkaliphilic or pH-indifferent species (Kristiansen & Preisig 2008, Siver 1995). The only species considered as acidophilic to acidobiontic (Škaloud et al., this volume), *M. paludosa*, was found only in Amberger See, which was characterised by the lowest pH measured (5.9). In contrast, *Mallomonas multisetigera* that usually also prefers acidic conditions (Škaloud et al., this volume) was found in two localities with pH 7.6 and 8.2. However, since *M. multisetigera* is not a very common species, its assumed ecological preferences are likely to be modified with increasing number of records.

Certain chrysophyte species can also serve as indicators of pollution and eutrophication (Siver 1995). The increase in chloride in four of the investigated lakes is probably caused by road salts and remnants of earlier domestic discharges. The highest content of both chloride and sodium ions was measured in Brenner See, a lake lying in close vicinity of Brenner highway, one of Europe's most important traffic lines. In Brenner See, four species of chrysophytes were recorded. In addition, *M. striata* and *M. cratis* had been recorded there in 2008 (Rott, unpubl. data). *M. cratis* has already been reported from a high conductivity pond in an area of saline soils in Hortobágy National Park in Hungary (Barreto 2005). Moreover, all other chrysophytes from Brenner See are known to occur even in brackish water of the Baltic Sea (Ikävalko 1994). Therefore, it is likely that the chrysophyte flora of Brenner See consists of species that are able to tolerate higher salinity levels.

It is difficult to compare our present findings with previous floristic data of the region, because those were all based solely on light microscopic observations (Ettl 1968, Pipp & Rott 1995, Rott 1983, 1988, Tolotti et al. 2003, Tolotti & Thies 2002) and only a few species can be reliably determined without an electron microscope. We did not confirm the occurrence of *M. caudata* in the same localities as Ettl (1968), but it was present in four other still waters in 2012. Also *M. akrokomos*, previously reported from Piburger See (Rott 1983, 1988), was reported in four additional different localities in 2012.

From the biogeographical point of view, most of the species presented in this study belonged to cosmopolitan and widely distributed taxa. In addition, several species with northern temperate (*M. hexagonis, M. pseudocoronata, M. punctifera, M. schwemmlei* and *S. glabra*) and bipolar (*M. paludosa*) distribution were found. One of the questions we asked concerned the possible similarities in Arctic and Alpine chrysophyte flora due to similarities in climatic conditions. Although it has been suggested that there is no typical arctic chrysophyte flora (Pichrtová et al.

2011, Siver et al. 2005), some species seem to be restricted to northern regions within Europe, for example, *M. vannigera*, *Synura leptorhabda*, *S. punctulosa*, *Spiniferomonas alata* or *Sp. bilacunosa* (Škaloud et al., this volume). Moreover, *M. zellensis* was described from an Alpine lake (Fott 1968) and since then has been reported only from northern Europe (Hällfors & Hällfors 1988, Ikävalko 1994), which indicates that there may really be parallels between Arctic and Alpine floras. In the present survey, we observed *Sp. bilacunosa* in three sites (2 – Egelsee, 3 – Pfrillsee, 6 – Gieringer Weiher) as the first record south of Denmark so far. Similarly, *Sp. alata*, recorded in Europe from Finland only (Hällfors & Hällfors 1988, Ikävalko 1994) was observed in site 6 (Gieringer Weiher).

During our survey, some species considered as very rare in Europe were also recorded. For example, *Mallomonas hexagonis* has been so far published from North America and Russia only (Kristiansen & Preisig 2007). However, one recent record already exists from the Czech Republic (Němcová, unpublished). Its autecology is still unknown. We found several scales in site 7 (Schwarzsee near Kitzbühel), in pH 7.7 and conductivity 247 µS cm^{-1}. Similarly, *Mallomonas guttata* is a rare species with unknown autecology. The only record from Europe exists from Aquitaine, France (Němcová et al. 2012). Nevertheless, the observed scales differed slightly from the species description in having lower number (3–7) of circular pits on the shield, instead of 9–15 (Kristiansen 2002). Whether this character is just a matter of phenotypic plasticity or of possible taxonomic importance has to be decided in future when more records are reported or molecular methods applied. We found *M. guttata* in four localities where pH ranged from 7.6 to 8.5 and conductivity from 102 to 461 µS cm^{-1}. It should also be mentioned that an only recently described taxon *Mallomonas pumilio* var. *dispersa* (Němcová et al., this volume) was found during our investigation in Gieringer Weiher.

Furthermore, the presence of *M. pseudocoronata* in the Alps was confirmed in our study. It was originally believed to be a North American endemic species. Later, *M. pseudocoronata* was recorded from a sediment core of an Austrian lake Längsee (Smol 1988) and Cronberg (2010) found it living in several Swedish and Austrian localities. Cronberg (2010) concluded that *M. pseudocoronata* must be a new element of the Swedish flora, because the region had been intensively studied for several decades and it could hardly have been overlooked. Its recent distribution might be caused by long distance migrating birds. Since 2005 the number of Swedish records of this species has still been increasing (Cronberg 2010). In Austria, however, it is difficult to decide whether *M. pseudocoronata* is a new migrant or not, due to the scarcity of previous studies. On the contrary, the sediment record (Smol 1988) indicates that *M. pseudocoronata* was present in Austria in the past. This species has already been recorded from 12 lakes in Austrian provinces of Carinthia, Styria and Upper Austria (Cronberg 2010). Here we reported this species from four additional localities in North Tyrol. The pH range of sampling sites with *M. pseudocoronata* was 8.2–8.5, which is in agreement with the known autecology of the species (Siver 1995).

The species described as new in this study, *Mallomonas tirolensis,* was found in two lakes in North Tyrol – Möserer See and Seefelder See (Wildsee) – that are only around three kilometres apart from each other. The conductivity of the sites varied between 371–414 µS cm^{-1}, pH was 8.1–8.2 and according to the concentrations of total phosphorus the lakes can be regarded as oligo- to mesotrophic.

Based on the scale morphology, *M. tirolensis* belongs to the section Torquatae Momeu & Péterfi, series Pumilio. The most similar taxa to *M. tirolensis* are *M. alata* f. *alata* Asmund, Cronberg & Dürrschmidt and *M. alata* f. *hualvensis* Asmund, Cronberg & Dürrschmidt. The scales of these taxa are similar in having asymmetric flanges and reduced hook-like domes. *Mallomonas tirolensis* differs from *M. alata* f. *alata* and *M. alata* f. *hualvensis* by the presence of pronounced ribs alternating with two rows of pores on the broad flange, while the above-mentioned taxa have more extended bended broad flanges with a wavy outlook, perforated by a few pores. Moreover, there is no window (reduced secondary layer) at the base of the posterior submarginal rib (V-rib) in *M. tirolensis* and the rear scales are spineless. The body scales of *M. tirolensis* are distinguishable from those of *M. alata* f. *alata* and *M. alata* f. *hualvensis* in the shield pattern formed by short ridges of reticulum enclosing usually four pores, while in *M. alata* f. *alata* the meshes of reticulum enclose (5)–7–(8) pores and in *M. alata* f. *hualvensis* single pores are regularly spaced over the shield. No bristles were observed in *M. tirolensis*. Correspondingly, no bristles were found in the most similar taxa *Mallomonas alata* f. *alata* and *M. alata* f. *hualvensis* (Asmund et al. 1982). Short and delicate bristles were reported in *M. alata* f. *alata* by Kristiansen (2002) referring to Harris (1970; Fig. 3 of her paper), however no bristles are visible on this microphotograph. Hence, based on published information and our observations (shape of the dome, no cavity on the inner surface of the scale) we presume the absence of bristles in *Mallomonas tirolensis* and related taxa (*M. alata* f. *alata* and *M. alata* f. *hualvensis*).

In conclusion, we showed that waterbodies of North Tyrol are relatively rich in chrysophytes in spite of rather high pH of the majority of the selected sampling sites. The most abundant species were cosmopolitan and pH-neutral to alkaliphilic, but some acidophilic and very rare species were also found and one species new to science was described. In addition, some findings suggest that there may be similarities between the arctic and the Alpine chrysophyte flora. We predict that if more sampling sites with ecological conditions similar to Gieringer Weiher were investigated, additional findings of rare or even new species could be expected for North Tyrol. It would also be desirable to include high-altitude Alpine lakes in future studies since their floras remain largely unexplored although the phytoplankton of these environments was found to be rich in chrysophytes (Tolotti et al. 2006).

Acknowledgements

We thank Josef Franzoi (Limnological lab, University of Innsbruck) for assistance with water chemical analyses, Fabian Martin (Institute of Botany, University of Innsbruck) for the elaboration of Figure 1, Diethard Sanders (Institute of Geology, University of Innsbruck) for help with the texting of the geological settings and Miroslav Hyliš (Electron microscopy lab, Charles University in Prague) for technical help with SEM. The study was supported by the grant no. P506/13-23274 S of the Czech Science Foundation and by Specific University Research project no 263212.

References

Asmund, B., Cronberg, G. & Dürrschmidt, M. (1982): Revision of the *Mallomonas pumilio* group (Chrysophyceae). – Nord. J. Bot. **2**: 383–395.

Barreto, S. (2005): The silica-scaled chrysophyte flora of Hungary. –Nova Hedwigia Beih. **128**: 11–41.

Baumann, E., De Jong, E. & Kamenik, C. (2010): A description of sedimentary chrysophyte stomatocysts from high-Alpine Lake Silvaplana (Switzerland). –Nova Hedwigia Beih. **136**: 71–86.

Brandner, R. (1985): Geologie und Tektonik. – In: Keller, W. (ed.): Tirol-Atlas, Begleittexte IX, pp. 1–12. – Tiroler Heimat, Innsbruck.

Cronberg, G. (2010): *Mallomonas pseudocoronata* Prescott (Synurophyceae), a recent arrival in Scania, southern Sweden? – Nova Hedwigia Beih. **136**: 139–151.

Ettl, H. (1968): Ein Beitrag zur Kenntnis der Algenflora Tirols. – Ber. nat.-med.Verein Innsbruck **56**: 177–354.

Forsström, L., Sorvari, S. & Korhola, A. (2005): The role of the environmental factors in controlling chrysophyte species distribution and biomass structure in Finnish Lapland subarctic lakes. – Nova Hedwigia Beih. **128**: 179–187.

Fott, B. (1962): Taxonomy of *Mallomonas* based on electron micrographs of scales. – Preslia **34**: 69–84.
Guilizzoni, P., Marchetto, A., Lami, A., Cameron, N. G., Appleby, P.G. et al. (1996): The environmental history of a mountain lake (Lago Paione Superiore, Central Alps, Italy) for the last c. 100 years: a multidisciplinary, palaeolimnological study. – J. Paleolimnol. **15**: 245–264.
Hällfors, G. & Hällfors, S. (1988): Records of chrysophytes with siliceous scales (Mallomonadaceae and Paraphysomonadaceae) from Finnish innland waters. – Hydrobiologia **161**: 1–29.
Harris, K. (1970): Species of the Torquata group of *Mallomonas*. – J. Gen. Microbiol. **61**: 77–80.
Ikävalko, J. (1994): Observations on silica-scaled flagellates (Chrysophyceae and Synurophyceae) in the brackish water of Pojo Bay, SW coast of Finland. – Ann. Bot. Fennici **31**: 1–27.
Janatková, K. & Němcová, Y. (2009): Silica-scaled chrysophytes of Southern Bohemian water bodies, including *Mallomonas conspersa* Dürrschmidt with occurrence so far reported from Japan and New Zealand. – Fottea **9**: 93–99.
Kamenik, C., Baumann, E., de Jong, R. & Grosjean, M. (2010): Effects of cold season climate changes versus anthropogenic nutrient inputs on chrysophyte stomatocyst assemblages (AD 1940–2004) in annually laminated sediments of high-Alpine Lake Silvaplana (Switzerland). –Nova Hedwigia Beih. **136**: 103–115.
Klaveness, D. & Guillard, R. R. L. (1975): The requirement for silicon in *Synura petersenii*. – J. Phycol. **11**: 349–355.
Kristiansen, J. (2002): The genus *Mallomonas* (Synurophyceae) – A taxonomic survey based on the ultrastructure of silica scales and bristles. – Opera Botanica **139**: 5–128.
Kristiansen, J. & Preisig, H. R. (2007): Chrysophyte and Haptophyte Algae, 2nd part: Synurophyceae. – In: Büdel, B., Gärtner, G., Krienitz, L., Preisig, H. R. & Schagerl, M. (eds.): Süsswasserflora von Mitteleuropa, vol. 1/2, Springer-Verlag, Berlin.
Němcová, Y. (2010): Diversity and ecology of silica-scaled chrysophytes (Synurophyceae, Chrysophyceae) in the National Nature Monument Swamp and Brehynsky Pond, Czech Republic. – Cryptogamie, Algologie **31**: 229–243.
Němcová, Y., Kreidlová, J., Kosová, A. & Neustupa, J. (2012): Lakes and pools of Aquitaine region (France) – a biodiversity hotspot of Synurales in Europe. – Nova Hedwigia **95**: 1–24.
Němcová, Y. & 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.
Pichrtová, M., Janatková, K. & Němcová, Y. (2011): Silica-scaled chrysophytes from Abisko (Swedish Lapland). – Nord. J. Bot. **29**: 112–118.
Pichrtová, M., Řezáčová-Škaloudová, M. & Škaloud, P. (2007): The silica-scaled chrysophytes of the Czech-Moravian Highlands. – Fottea **7**: 43–48.
Pichrtová, M. & Veselá, J. (2009): The silica-scaled chrysophytes of the Elbe Sandstone Region, Czech Republic. – Fottea **9**: 101–106.
Pipp, E. & Rott, E. (1995): A phytoplankton compartment model for a small meromictic lake with special reference to species-specific niches and long-term changes. – Ecol. Model. **78**: 129–148.
Řezáčová, M. (2006): *Mallomonas kalinae* (Synurophyceae), a new species of alga from northern Bohemia, Czech Republic. – Preslia **78**: 353–358.
Řezáčová, M. & Škaloud, P. (2004): A contribution to the knowledge of the silica-scaled chrysophytes in eastern Hungary. – Czech Phycology **4**: 67–73.
Rojackers, R. M. & Kessels, H. (1986): Ecological characteristics of scale-bearing Chrysophyceae from the Netherlands. – Nord. J. Bot. **6**: 373–383.
Rott, E. (1983): Sind die Veränderungen im Phytoplanktonbild des Piburger Sees Auswirkungen der Tiefenwasserableitung? – Algol. Stud. **34**: 29–80.
Rott, E. (1988): Some aspects of the seasonal distribution of flagellates in mountain lakes. – Hydrobiologia **161**: 159–170.
Siver, P. A. (1995): The distribution of chrysophytes along environmental gradients: their use as biological indicators. – In: Sandgren, C. D., Smol, J. P. & Kristiansen, J. (eds.): Chrysophyte algae, pp. 232–268. – Cambridge Univ. Press, Cambridge.
Siver, P. A. & Hamer, J. S. (1989): Multivariate statistical analysis of the factors controlling the distribution of scaled chrysophytes. – Limnol. Oceanogr. **34**: 368–381.
Siver, P. A., Voloshko, L. N., Gavrilova, O. V. & Getsen, M. V. (2005): The scaled chrysophyte flora of the Bolshezemelskaya tundra (Russia). – Nova Hedwigia Beih. **128**: 125–150.

Škaloud, P., Škaloudová, M., Pichrtová, M., Němcová, Y., Kreidlová, J. & Puzstai, M. (2013): www.chrysophytes.eu – a database on the distribution and ecology of silica–scaled chrysophytes in Europe, this volume.

Smits, J. D. & Milne, P. J. (1981): Spectrophotometric determination of silicate in natural waters by formation of alpha-molybdosilicic acid and reduction with a tin(IV)-ascorbic-acid-oxalic acid mixture. – Anal. Chim. Acta **123**: 263–270.

Smol, J. P. (1988): The North-American endemic *Mallomonas pseudocoronata* (Mallomonadaceae, Chrysophyta) in an Austrian lake. – Phycologia **27**: 427–429.

Smol, J. P. (1995): Application of chrysophytes to problems in paleoecology. – In: Sandgren, C. D., Smol, J. P. & Kristiansen, J. (eds): Chrysophyte algae, pp. 303–329. – Cambridge Univ. Press, Cambridge.

Suykerbuyk, R. E. M., Roijackers, R. M. M. & Houtman, S. S. J. (1995): Ecological characteristics of scale-bearing chrysophytes in a small eutrophic pond in The Netherlands. – Nord. J. Bot. **15**: 665–676.

Ter Braak, C. J. F. & Šmilauer, P. (2002): CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). – Microcomputer Power, Ithaca, NY, USA.

Tolotti, M., Manca, M., Angeli, N., Morabito, G., Thaler, B. et al. (2006): Phytoplankton and zooplankton associations in a set of Alpine high altitude lakes: geographic distribution and ecology. – Hydrobiologia **562**: 99–122.

Tolotti, M. & Thies, H. (2002): Phytoplankton community and limnochemistry of Piburger See (Tyrol, Austria) 28 years of the lake restoration. – J. Limnol. **61**: 77–88.

Tolotti, M., Thies, H., Cantonati, M., Hansen, C. M. E. & Thaler, B. (2003): Flagellate algae (Chrysophyceae, Dinophyceae, Cryptophyceae) in 48 high mountain lakes of the Northern and Southern slope of the Eastern Alps: biodiversity, taxa distribution and their driving variables. – Hydrobiologia **502**: 331–348.

Vogler, P. (1966): Zur Analytik der Phosphorverbindungen in Gewässer. – Limnologica **4**: 437–444.