

Diversity of silica-scaled chrysophytes in high-altitude Alpine sites (North Tyrol, Austria) including a description of *Mallomonas pechlaneri* sp. nov.

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Abstract – To expand on the biodiversity study of the eastern Alpine region of North Tyrol, we investigated silica-scaled chrysophyte flora of unexplored high-altitude Alpine sites. We concentrated mostly on lakes that were 1000-2500 m above sea level; some of them were still partly ice covered. Overall, 27 taxa were recorded at 12 sites, despite 21 sites being sampled altogether. In general, the sites were oligotrophic and species poor. Due to a restricted data set, an extensive analysis was not possible. However, altitude and pH were found to be important variables for explaining species distribution. We described the new species *Mallomonas pechlaneri* from two of the sampled sites. *Mallomonas pechlaneri* most closely resembles *Mallomonas striata* var. *striata* and *M. striata* var. *serrata*; these three taxa are clearly distinguished by bristle morphology and scale shape. The bristles of *M. pechlaneri* terminate in a bifurcated tip consisting of unequal diverging branches. Scale shape was captured by means of landmark-based geometric morphometrics (GM) and evaluated using multivariate statistical analyses. GM methods proved to be an efficient tool to be employed in chrysophycean taxonomy.

Biodiversity / *Mallomonas pechlaneri* / *Mallomonas striata* / North Tyrol / scale shape / silica-scaled chrysophytes

INTRODUCTION

Photosynthetic protists represent key players in nearly all ecosystems. Notably, they participate in energy flux and foodwebs as both food sources and consumers (Cotterill *et al.*, 2008). However, protist diversity is underestimated and largely unexplored. While new species of plants and animals are mostly described from remote and unstudied areas, new protist species are still being revealed at well-investigated regions. The majority of modern protist descriptions are based on molecular data. However, silica-scaled chrysophytes (Chrysophyceae, Stramenopiles, Ochrophyta) are covered with miniature scales (approximately 2-4 µm) that provide sufficient detailed and stable structures for taxonomic classification (Kristiansen & Preisig, 2007). Silica-scaled chrysophytes comprise both heterotrophic and autotrophic flagellates. While all members of the class Chrysophyceae produce silica resting

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stages (stomatocysts), silica scales that form a case around the motile cell occur only in some clades. Scales are studied by means of electron microscopy, and scale designs are diagnostic. The autotrophic Synurales (including the most widely studied genera *Mallomonas* and *Synura*), together with the unrelated autotrophic genera *Chrysosphaerella* and *Spiniferomonas*, represent ecologically successful algae that form rich populations and are considered key primary producers in slightly acidic temperate lakes that are low in nutrient supply (Eloranta, 1986; Siver, 1995; Nemcova *et al.*, 2002). Heterotrophic *Paraphysomonas* has been reported from a wide variety of freshwater, brackish and marine habitats (Scoble & Cavalier-Smith, 2014; Nemcova *et al.*, 2016). More than 300 scale-bearing taxa are currently recognised and accepted taxonomically (Kristiansen & Preisig, 2007; Scoble & Cavalier-Smith, 2014). We suppose significant unrecognised diversity exists within all lineages of silica-scaled chrysophytes. Molecular phylogenetic studies by Scoble and Cavalier-Smith (2014) indicate that the number of known species within *Paraphysomonas* may be double. Based on the unique scale morphology or both the scale morphology and molecular data, new species are continuously being discovered (Jo *et al.*, 2013; Nemcova & Kreidlova, 2013; Nemcova *et al.*, 2015; Siver & Lott, 2016).

Although more than 1300 freshwater bodies have been sampled and investigated in Europe (Skaloud *et al.*, 2013), many 'white spots' on the map of chrysophyte research still exist.

Extensive sampling of the chrysophyte flora of the eastern Alpine region of North Tyrol was accomplished in 2012 (Pichrtova *et al.*, 2013). Altogether, 46 taxa were identified from 22 sites, including natural, hydrologically modified and mire waterbodies. The species composition of the Tyrolean waterbodies was significantly related to pH, dissolved reactive silica content, lake depth, calcium content and altitude. Previous records of silica-scaled chrysophytes are based solely on light microscopy observations (Rott, 1983; Rott, 1988; Tolotti *et al.*, 2006), and only a few species can be reliably determined without an electron microscope. Also, chrysophyte cysts from the Alps, including North Tyrol, have been successfully used as climate indicators of the past (Kamenik *et al.*, 2000; Kamenik & Schmid, 2003).

Landmark-based geometric morphometrics (GM) constitute an extremely efficient tool for the evaluation of silica-scale shape in both laboratory experiments and investigations of natural populations. Landmarks represent discrete loci that are homologous in all objects (scales); each is described by two coordinates. The next step of the analysis is to translate and rotate the landmark configurations into a common position and remove size differences between them (Bookstein, 1991). Each object can then be mapped as a single point into an ordination space. The relative position of landmarks among various objects can be used for the multivariate analysis of shape as well as the quantification and statistical comparison of the amount of shape variation. Shape change can be visualised as a thin-plate spline deformation grid, which provides intuitive visualisation of shape and the spatial localisation of shape variation. In silica-scaled chrysophyte research, landmark-based geometric morphometric methods have been used to study the covariation of scale shape in clonal experimental populations in response to temperature (Rezácova-Skaloudova *et al.*, 2010; Pichrtova & Nemcova, 2011), pH (Nemcova & Pichrtova, 2012) and the combined gradients of light and temperature (Nemcova *et al.*, 2010). Neustupa *et al.* (2010) described scale shape variation in relation to their position within the scale case of *Mallomonas kalinae*, and Siver *et al.* (2013) compared the scale shape of Eocene populations (40 million years old) of *Mallomonas insignis* Penard with the shape of modern populations. In taxonomical studies, landmark-based GM has been used to evaluate slight differences in scale shape in distinct

morphotypes of the *Mallomonas pumilio* group and to help select which morphotype should be identified as a type of *M. pumilio* var. *pumilio* (Nemcova *et al.*, 2013). In that study, the image of the *M. pumilio* var. *pumilio* scale from the type locality was marked by the same set of landmarks, and the similarity of this scale to nine groups of scales belonging to different morphotypes was tested. Neustupa and Nemcova (2007) revealed differences in scale shape between *Mallomonas striata* var. *striata* Asmund and *M. striata* var. *serrata* Harris K. & Bradley D.E., previously separated on the basis of different bristle morphology (smooth vs. serrated).

The goal of this study was i) to investigate the silica-scaled chrysophyte flora of unexplored mountain and high-altitude Alpine lakes and determine the relationship between community structure and environmental variables and ii) to describe the new species *Mallomonas pechlaneri* recorded from two of the sampling sites. Based on studies that combine morphological and molecular data, it is possible to conclude that even slight changes in scale/bristle morphology are sufficient for the delimitation of taxa at the rank of species (Jo *et al.*, 2013; Scoble & Cavalier-Smith, 2014; Skaloud *et al.*, 2014). Here, we describe *M. pechlaneri* on the basis of unique bristle morphology and differences in scale shape.

MATERIALS AND METHODS

Sampling sites (see Fig. 1)

The majority of the lakes were located within a north-south transect extending northward along the Ötztal Ache valley from the highest point at the Timmelsjoch in the south, crossing the Inn valley and part of the Northern Alps toward the Ehrwald basin. In this basin the Loisach River originates which flows to

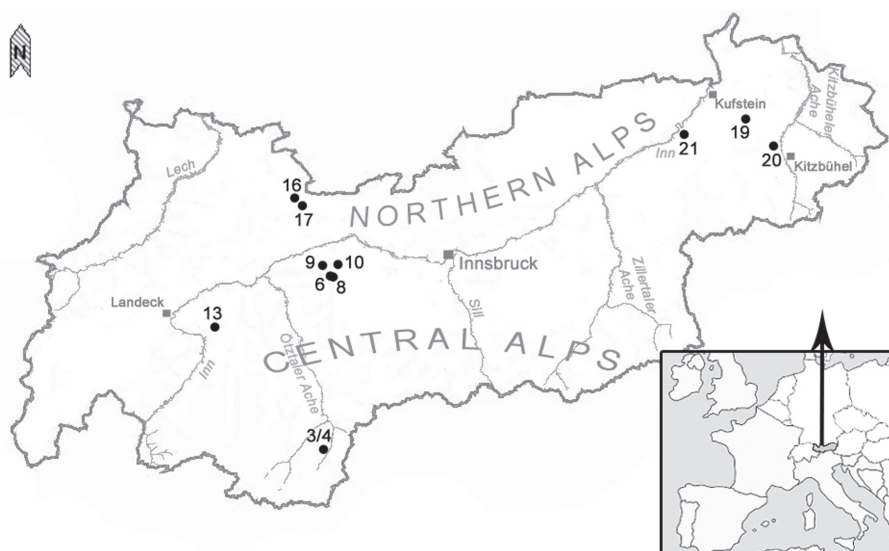


Fig. 1. Location of the study area and sampling sites, where silica scaled chrysophytes were recorded.

the north-east into the Isar River, a southern tributary of the Danube. The Inn valley extending from west to east is crossed in the middle. One extremely western site is situated on a pass near Landeck town, the other three sites are in the eastern most part of North Tyrol close to Kitzbühel town. The Inn valley, situated in the middle of the transect, follows the Inn valley fault which separates the Northern Calcareous Alps from the southern adjacent Central Alps. While the Northern Calcareous Alps consist mainly of Mesozoic carbonate rocks, the Central Alps are composed of a wide array of metamorphic rocks (mainly ortho- and paragneiss, mica schists and different types of phyllites; Brandner, 1985). Most of the lakes formed in morphological depressions excavated by glacial erosion during Quaternary glaciations. In detail, the sites comprised six deep high-altitude lakes (numbers 6, 8, 9, 10, 16 and 19), three bathing mires (13, 20 and 21), and three small and shallow pools (3, 4 and 19). Site 20 has been studied previously (Pichrtova *et al.*, 2013).

Scale chrysophyte sampling and investigation

The sites investigated in this study were sampled 29 June-1 July 2015. The latitude and longitude coordinates as well as altitude were recorded using a Garmin GPS eTrex HC instrument. Plankton samples from each site were taken with a 20 µm net. Samples were concentrated by sedimentation. Unfixed drops of samples were subsequently dried onto Formvar-coated transmission electron microscopy (TEM) grids. The dried material was washed by repeated transfer of the grid into drops of deionised water dispensed onto the hydrophobic surface of a Parafilm strip. Three TEM examinations were performed per sample. The dried 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), Formvar-coated grids (already observed using TEM) were mounted onto SEM stubs with double-sided adhesive carbon tape, coated with platinum for 60 s using a Bal-Tec SCD 050 sputter coater and observed using a JEOL JSM-740 1F FESEM scanning electron microscope.

Water chemical analyses

Surface water temperature, pH and conductivity were measured at the time of collection using a WTW (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) 340 combined pH-conductivity meter. Water samples were collected simultaneously with algal samples into pre-rinsed glass bottles for laboratory analyses. Glass fibre (Whatman GFC)-filtrated water was used to analyse the dissolved fractions of the nutrients. The total available nutrient pool of phosphate (TP), including organic fractions, was liberated by concentrated sulphuric acid digestion. Both TP and dissolved phosphorus compounds (TDP) were analysed using ammonium molybdate detection (detection limit of 0.5 µg L⁻¹) (Vogler, 1966). Total dissolved nitrogen (TDN) was analysed using a total nitrogen measurement unit (Shimadzu TNM-1; detection limit of 20 µg L⁻¹).

Statistical analyses

The relationship between environmental parameters and the species composition was tested using the program Canoco for Windows 4.5 (ter Braak &

Šmilauer, 2002). Canonical correspondence analysis (CCA) was chosen due to the unimodal character of the data. The step-forward selection and the Monte Carlo permutation test (1999 permutations) were used to identify significant variables.

Geometric morphometrics

In total, 67 body scales from the *M. striata* group were used for the length and shape analysis. The lengths of the scales were measured using the TpsDig program (Rohlf, 2006). A non-parametric Kruskal-Wallis test was used in PAST ver. 2.08b to identify significant pairwise differences in length among individual taxa. Results were considered significant if $p < 0.05$.

Mallomonas striata var. *striata* was represented by 20 scales from sampling site N. 17. Only scales from assemblages in which the bristles were visible were used for the analyses. Twenty-two scales of the new species *M. pechlaneri* were imaged from the sampling sites N. 6 and 9. As *M. striata* var. *serrata* was not revealed within this investigation, scales from the strain CCMP 2059 obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) were used. The CCMP 2059 strain was cultivated at 22°C and pH 7.5 under continuous white fluorescent illumination of 20 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Samples were taken from exponentially growing cultures, oxidised in 30% hydrogen peroxide and potassium dichromate to disintegrate the scale case, washed carefully and then transferred to TEM grids. Twenty-five randomly selected scales were analysed. The silica scales represent flat, bilateral, nearly symmetrical objects. The side of the scale adjacent to the plasma membrane is smooth, whereas the upper side is structured. All scales possess a dome, where a slightly curved bristle is attached. A protrusion on the apical portion of the dome enables the left and right sides of the scale to be distinguished and the landmarks to be placed consistently. Twenty-nine landmarks were defined on each scale (Fig. 29) using the TpsDig program (Rohlf, 2006). Twelve landmarks were allowed to slide along the outline of the scale (semi-landmarks). A relative warp analysis (RW; equal to a principal component analysis of the covariance matrix of the partial warp scores) of the shape data, including a shape variable extraction (Bookstein, 1991), was conducted on all 67 silica scales (comprising three taxa). Scores for the objects on the first 10 principal component axes (spanning 93.2% of the total variation) were used for canonical variate analysis (CVA) in PAST ver. 2.08 b (Hammer *et al.*, 2001). The number of PC axes chosen was estimated, so that all the axes with eigenvalues higher than the Jolliffe cut-off value (Jolliffe, 2002) were included. To test for scale shape differences between individual taxa, the scores of the first 10 RWs were used for two-group multivariate permutation tests (10000 permutations) on the Mahalanobis distance (MD) between all group pairs. Shape configurations typical for individual taxa were visualised as deformation grids and generated from the thin-plate spline analysis. Deformation grids demonstrate how the overall consensus configurations must be modified to fit average shapes determined for each taxon.

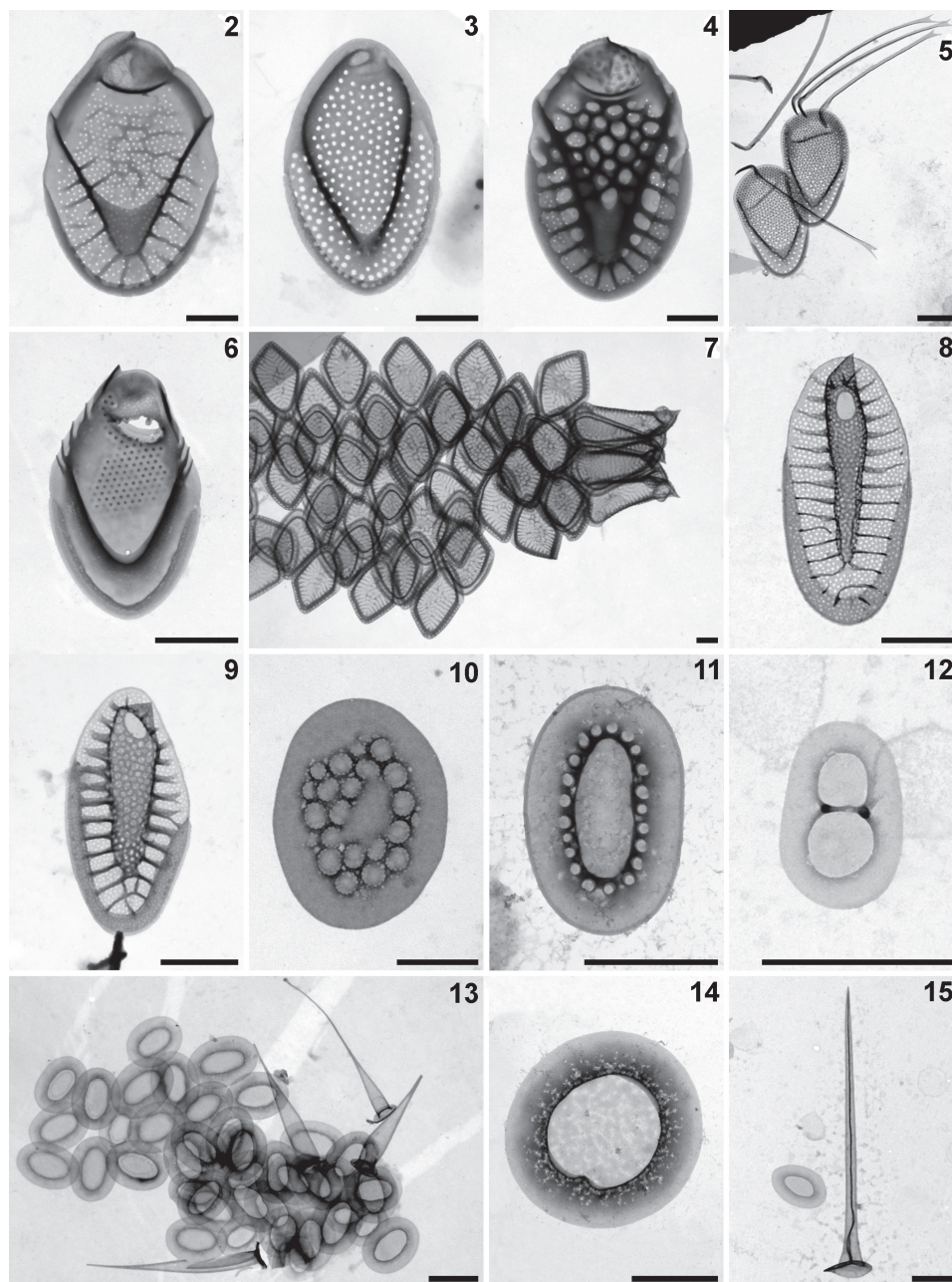
RESULTS

Silica-scaled chrysophytes

Overall, 27 taxa were recorded at 12 sites: 10 *Mallomonas*, 5 *Synura*, 3 *Chrysosphaerella*, 4 *Spiniferomonas* and 5 *Paraphysomonas* (Table 1; Figs 2-39).

Table 1. List of sites and environmental variables

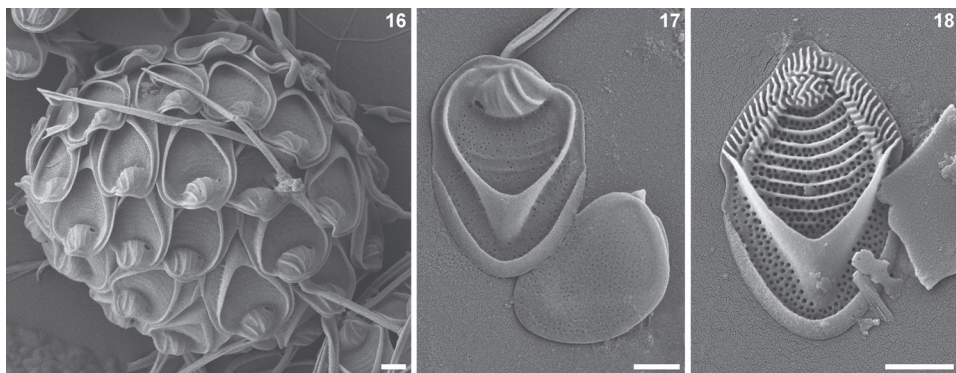
Name of the site		GPS coordinates		Sampling date	Altitude m	Temp °C	Cond $\mu\text{S cm}^{-1}$	pH	P dissolved $\mu\text{g/L}$	N dissolved $\mu\text{g/L}$	Number of taxa
1	Timmelsjoch West	46.90042	11.09812	29.6.2015	2502	0.6	8	6.7	0.9	132	0
2	Schönboden	46.90908	11.09393	29.6.2015	2469	5.9	6	8.0	4.4	96	0
3	Uppermost pool NW ward	46.87731	11.03195	29.6.2015	1973	18.3	15	4.3	16.8	295	1
4	Uppermost pool SW ward	46.87725	11.03227	29.6.2015	1962	17.0	9	5.0	19.4	313	1
5	Lowest pool NE ward	46.88138	11.03353	29.6.2015	1862	12.0	11	5.1	9.7	229	0
6	Oberer Plenderlesee	47.20377	11.03757	29.6.2015	2350	5.0	60	7.3	1.5	232	3
7	Unterer Plenderlesee	47.20457	11.03230	29.6.2015	2295	8.2	70	7.5	2.7	273	0
8	Mittlerer Plenderlesee	47.20950	11.04065	29.6.2015	2331	9.8	22	7.0	1.2	153	4
9	Hirschebensee	47.21459	11.04367	29.6.2015	2180	15.5	30	7.4	2.4	128	6
10	Gossenkoellesee	47.22723	11.01458	29.6.2015	2410	8.6	23	7.3	0.6	470	2
11	Schwarzmoostümpel	47.22723	11.01571	29.6.2015	2364	11.8	22	7.4	2.4	156	0
12	Waldweiher	47.12830	10.64857	30.6.2015	1554	14.5	94	7.7	2.4	115	0
13	Pillersee	47.12978	10.69118	30.6.2015	1368	16.2	128	7.6	3.2	192	6
14	Pond near road to Aifner Alm	47.12193	10.69118	30.6.2015	1602	17.8	54	6.3	16.5	282	0
15	Igelsee	47.37664	10.97239	30.6.2015	1534	22.0	172	8.8	2.4	130	0
16	Seebensee	47.36989	10.93631	30.6.2015	1646	11.4	154	8.5	1.5	311	1
17	Drachensee	47.35941	10.93508	30.6.2015	1862	10.7	149	8.5	0.9	317	3
18	(Ellmau) small pool near the road	47.33510	12.30962	1.7.2015	1020	17.2	28	4.5	3.8	269	0
19	Biedinger Platte (Ellmau) small pool near the road	47.53328	12.30737	1.7.2015	1010	19.0	25	5.5	26.8	524	2
20	Gieringer Weiher	47.47155	12.36263	1.7.2015	786	25.0	141	7.4	11.8	400	9
21	Moorstrandbad Kirchbichl	47.50861	12.08903	1.7.2015	563	n.d.	450	8.3	5.0	1018	5



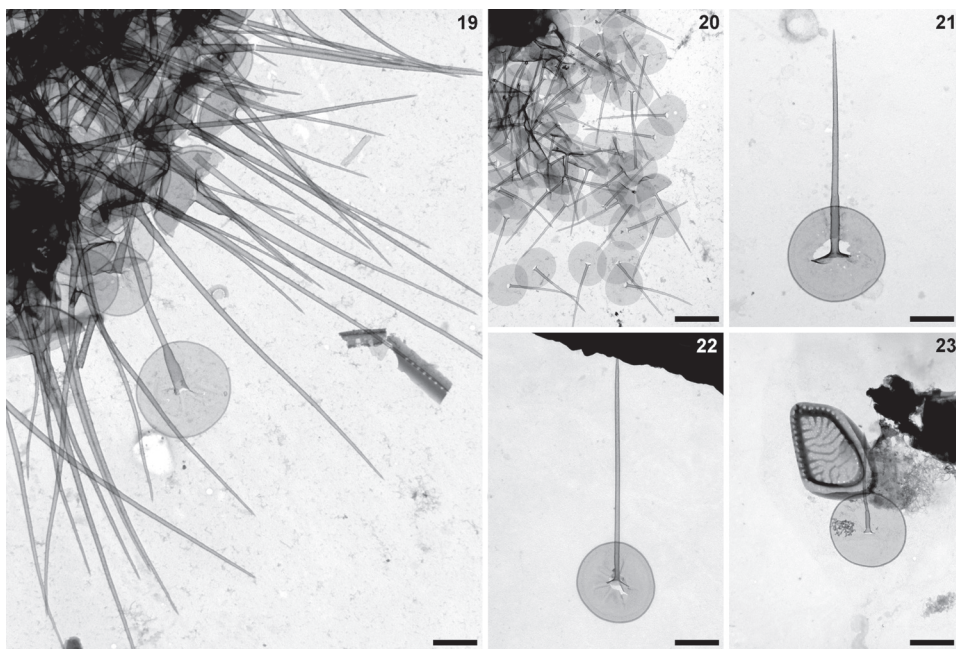
Figs 2-15. Silica-scaled chrysophytes identified in this study – scales in TEM. 2. *Mallomonas acaroides*. 3. *M. alpina*. 4. *M. crassisquama*. 5. *M. multisetigera*. 6. *M. papillosa*. 7. *M. torquata* var. *torquata*. 8. *Synura americana*. 9. *S. conopea*. 10. *Chrysosphaerella annulata*. 11. *C. coronacircumspina*. 12. *Spiniferomonas cornuta*. 13. *Sp. abei*. 14. *Sp. serrata*. 15. *Sp. trioralis*. Bar = 1 μ m.

We sampled 21 sites altogether; however, no silica-scaled chrysophytes were revealed at nine investigated sites.

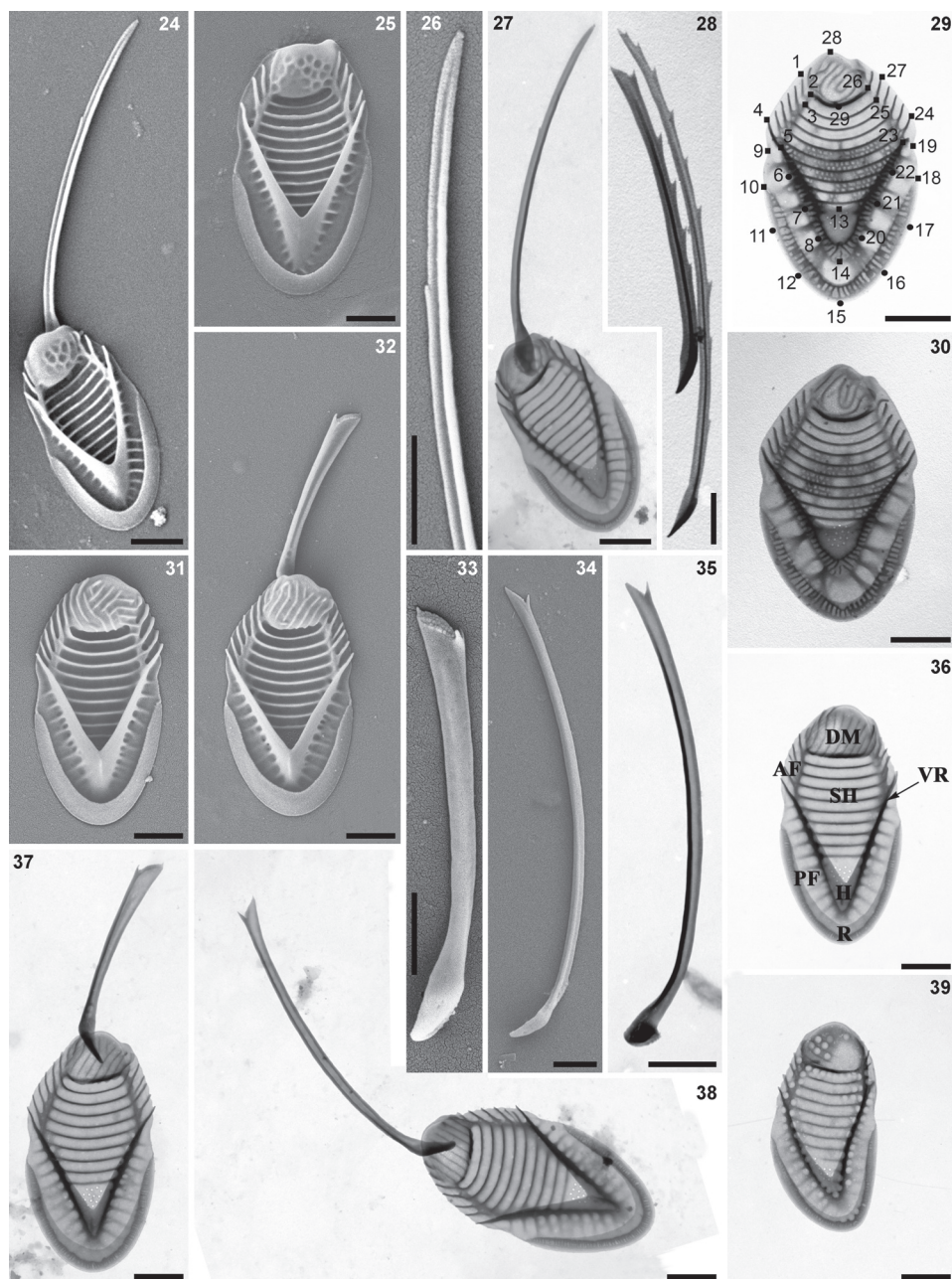
We concentrated mostly on the high-altitude Alpine lakes that were 1000–2500 m above sea level (a.s.l.) (with the exceptions of localities N. 20 and 21, which were 786 m a.s.l. and 563 m a.s.l., respectively); some were still partly ice covered. Ultra-oligotrophic high-altitude lakes (e.g. N. 1, N. 2 or N. 5), were free of scaled chrysophytes at the time of sampling. Lower temperature and a fewer number of days since ice-out may contribute to the lack of chrysophytes.



Figs 16–18. Silica-scaled chrysophytes in SEM. **16–17.** *Mallomonas costata*. **16.** the whole cell armor. **17.** a body scale and a rear scale with a spine. **18.** *M. actinoloma* var. *maramuresensis*. Bar = 1 μ m.



Figs 19–23. *Paraphysomonas* – TEM of scales. **19.** *Paraphysomonas longispina*. **20.** *P. cf. imperforata*. **21.** *Paraphysomonas* sp. **22.** *Paraphysomonas uniformis hemiradia*. **23.** *Paraphysomonas vulgaris brevispina*. Bar = 1 μ m.



Figs 24-39. Species of *Mallomonas striata* group. 24-27. *M. striata* var. *striata*. 28-30. *M. striata* var. *serrata*. 29. position of landmarks (circles) and semi landmarks (squares) on a body scale. 31-39. *M. pechlaneri*. 36. scale morphology: (AF) anterior flange, (DM) dome, (H) hood, (PF) posterior flange, (R) rim, (SH) shield, (VR) V-rib. Bar = 1 μ m.

The average number of species per site was 3.58 (alpha diversity). In general, there were fewer species at localities with a pH greater than 8 (e.g., in the Northern Calcareous Alps; a single species at N. 16 (Seebensee)) and at sites with a low pH (a single species of *Mallomonas costata* in small acidic pools at N. 3 and 4 (Ochsenkopf Obergurgl) with pH values of 4.3. and 5.0, respectively). *Mallomonas costata* formed extremely dense populations in these two pools (Figs 16-17). Site N. 20 (Gieringer Weiher) had the richest chrysophyte flora (nine species), including six species that did not occur anywhere else. *Chrysosphaerella brevispina* was the most frequent species with four records.

Water chemical analyses

The chemical conditions of the water showed a broad range, from low conductivity (4-5 $\mu\text{S cm}^{-1}$) and low pH in the little ponds in the Central Alps near Obergurgl to moderate conductivity (20-60 $\mu\text{S cm}^{-1}$) and slightly alkaline pH in the high mountain lakes in the K  t  i area, as well as higher conductivity ($> 300 \mu\text{S cm}^{-1}$) in the high mountain lakes in the Northern Calcareous Alps (Table 1). With respect to nutrients, the high mountain lakes ($> 2000 \text{ m}$ in the K  t  i area and $> 1500 \text{ m}$ in the Ehrwald region) were oligotrophic (TDP $< 2.4 \mu\text{g L}^{-1}$); however, slightly elevated nitrate concentrations (TDN $> 300 \mu\text{g L}^{-1}$) were observed in three of the six cases. Both bathing mires (Gieringer and Kirchbichl) should be classified as mesotrophic with alternatively elevated TP and/or nitrates. All small pools (N. 3, 4 and 19) should be classified as meso-eutrophic and showed some humic influence and lowered pH. Water temperature in deeper lakes at the time of collection was mainly influenced by the number of days since ice-out (related to altitude), while shallow pools were earlier ice free. Moreover, dark humic water absorbed more solar radiation causing warming of the water column. Localities accompanied by geographical location, sampling date and environmental parameters are listed in Table 1.

Ecological analyses

The 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.

Altitude and pH were the only significant variables and explained 14.7% ($p = 0.0004$) and 14.6% of variation in the data, respectively (Fig. 40). Altitude was significantly negatively correlated with TDN ($r = -0.663$; $p = 0.019$) and conductivity ($r = -0.684$; $p = 0.048$). TDP was highly negatively correlated with pH ($r = -0.781$; $p = 0.003$), and conductivity was positively correlated with TDN ($r = 0.774$; $p = 0.003$). *Mallomonas crassisquama*, *Mallomonas actinoloma* var. *maramuresensis*, *Mallomonas torquata* f. *torquata* and the newly described species *M. pechlaneri* were sampled preferentially at high-altitude localities.

***Mallomonas pechlaneri* Nemcova & Rott sp. nov.**

Figs 31-39

The scales ($3.6\text{-}4.7 \times 1.9\text{-}2.4 \mu\text{m}$) are oval with lateral incurvings. All scales share a sub-circular dome with ribs or reticulum (Figs 31-32). The anterior scales are narrower and slightly asymmetric (Fig. 39). The V-rib is rounded and acutely angled, with a smooth lower edge. Anterior submarginal ribs are well developed. The anterior flange is marked with 4-6 closely spaced struts; the posterior

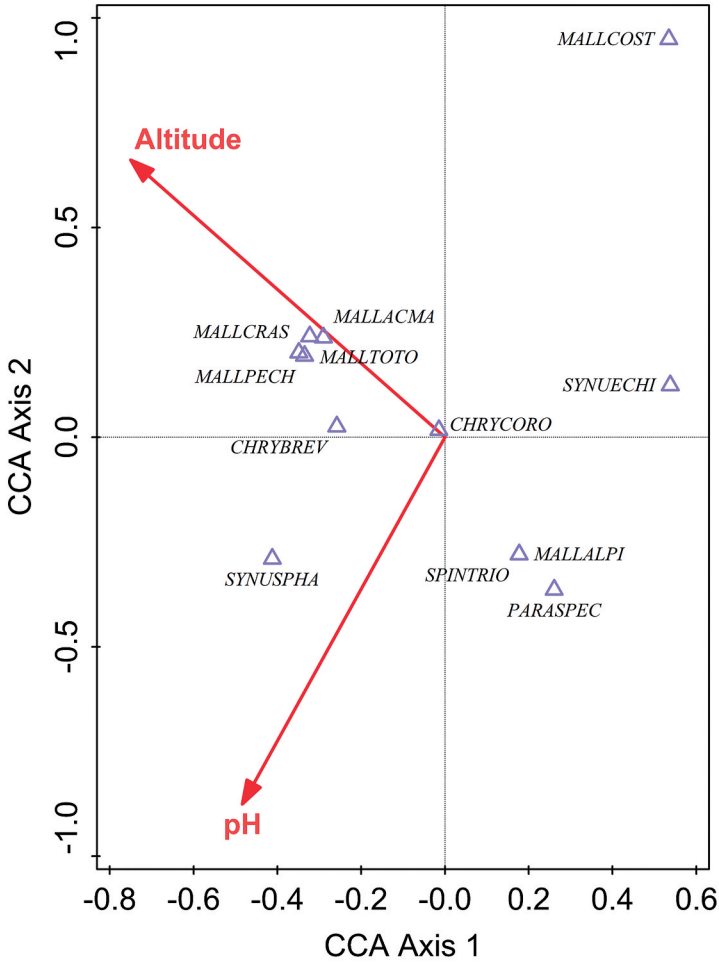


Fig. 40. CCA ordination diagram displaying preferences of species to the altitude and pH. Only species recorded more than once are displayed. CHRYBREV = *Chrysosphaerella brevispina*, CHRYCORO = *C. coronacircumspina*, MALLACMA = *Mallomonas actinoloma* var. *maramuresensis*, MALLALPI = *M. alpina*, MALLCOST = *M. costata*, MALLCRAS = *M. crassisquama*, MALLPECH = *M. pechlaneri*, MALLTOTO = *M. torquata* f. *torquata*, PARASPEC = *Paraphysomonas* sp., SPINTRIO = *Spiniferomonas trionalis*, SYNUECHI = *Synura echinulata*, SYNUSPHA = *S. sphagnicola*.

flange bears approximately 18 struts. The shield is marked with approximately nine regularly-spaced transverse ribs. A group of 10-16 pores is located on the posterior part of the shield (Figs 37-39). The proximal border (rim) bears internal struts but is smooth on the surface (Figs 36-38). Bristles (4.3-8 μ m) are slightly curved (Figs 33-35). The distal end is often rather wide (Figs 33, 37). The tip is bifurcated with unequal diverging branches. One branch is short and sharp (reminiscent of subapical teeth), whereas the other is wide with an acute tip and flat dorsal side, sometimes containing papillae (Fig. 33).

Cyst unknown. Cell shape and dimensions unknown.

Holotype specimen: Single gathering of cells on an SEM stub deposited at the Culture Collection of Algae of Charles University in Prague, H2016/1. Fig. 32 shows a representative scale and bristle from the specimen.

Type locality: Hirschebensee, North Tyrol, Austria (47.21459N, 11.04367E, 2180 m a.s.l.), sampled by Nemcova and Rott on 29 June 2015.

Etymology: The epithet is in honour of the limnologist Roland Pechlaner, Innsbruck University, Austria, who engaged in nature protection with special reference to the high mountain lakes in Kühtai area (North Tyrol).

Distribution: This species was found at the type locality (N. 9) and at locality N. 6 (Oberer Plenderlesee).

Scale size and shape analysis

All pairs of taxa differed significantly in scale size ($p < 0.05$ in all pairwise comparisons). The largest were the body scales of *M. striata* var. *striata*, and the smallest were the scales of *M. pechlaneri*. RW analysis revealed the most important trends in the overall shape variation for the entire data set (ordination diagram not shown). RWs 1-4 accounted for 74.8% of the total variation in shape (RW1, 38.1%; RW2, 16.3%; RW3, 12.5%; and RW4, 7.9%). The CVA indicated significant separation among all three investigated taxa (Wilk's $\lambda = 0.0052$; $p < 0.0001$). The first two canonical axes explained 75.2% and 24.8% of the shape variation, respectively (Fig. 41). Subsequent two-group tests indicated that all the investigated groups significantly differed in shape ($p < 0.001$). The scale shape similarity between the pairs of taxa was compared using the MD values. The smaller the value, the more similar in scale shape were the compared taxa. The most similar were *M. striata* var. *striata* and *M. pechlaneri* (MD = 52.6). On the other hand, the most dissimilar with respect to shape were *M. pechlaneri* and *M. striata* var. *serrata* (MD = 77.4). The deformation grids demonstrating how the overall consensus configurations must be transformed to fit average shapes determined for each taxon are shown in Fig. 41 (enclosed pictures). The scales of *M. striata* var. *serrata* are characterised by a relatively small shield and sub-circular dome as well as a thicker V-rib that forms a prominent hood. The scales of *M. striata* var. *striata* have an expanded shield, a non-protruding wider dome and a thin V-rib. *Mallomonas pechlaneri* is characterised by an expanded shield, a higher sub-circular dome and a thin V-rib.

DISCUSSION

Our silica-scaled chrysophyte investigation of high-altitude Alpine lakes and mountain ponds represents an expansion of a study (Pichrtova *et al.*, 2013) on the chrysophytes of the lower lakes and water bodies of the eastern Alpine region of North Tyrol. Site N. 20 (Gieringer Weiher; 786 m a.s.l.) was the only site investigated in both studies. In both cases, this site had the highest species diversity. In 2012, the Gieringer Weiher lake was sampled at the end of April and revealed 30 taxa. However, in 2015, the same lake was sampled at the end of June but only nine taxa were recorded. These findings agree with the idea that silica-scaled chrysophytes in mesotrophic water bodies contribute to the spring phytoplankton maximum and are replaced by other groups (*i.e.*, green algae) when the water temperature increases (Kristiansen, 1988). The most striking difference in environmental variables during different sampling episodes in the Gieringer Weiher

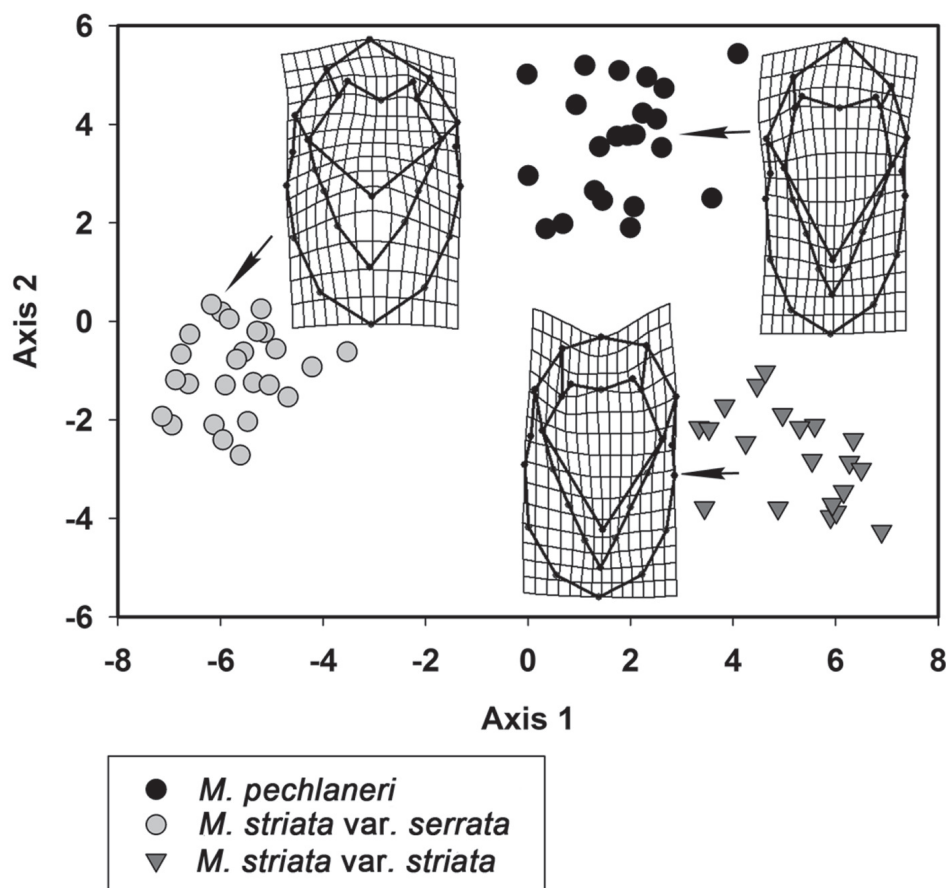


Fig. 41. Scale-shape discrimination in three varieties within the *Mallomonas striata* group. CVA ordination plot shows individual scale scores along canonical axes. First and second canonical axes describe 75.2% and 24.8% of total variation, respectively. Deformation grids within the ordination diagram demonstrate how consensus configuration must be warped to fit mean shapes determined for each variety. Scale factor: twice exaggerated to emphasize differences.

lake involved water temperature and dissolved nitrogen (11.9°C and 25°C as well as 187 $\mu\text{g L}^{-1}$ and 400 $\mu\text{g L}^{-1}$ in April 2012 and June 2015, respectively). In general, much lower alpha diversity was recorded in the high-altitude Alpine lakes than in the lower-situated lakes in April 2012 (3.58 and 10.72, respectively). Only five taxa were common to both data sets. The generally accepted hypothesis in chrysophyte ecology is that the most diverse community occurs in neutral to slightly acidic localities that have low conductivity and low nutrient contents (Siver & Hamer, 1989; Siver, 1995). This hypothesis is true for the lower Alpine lakes (Pichrtova *et al.*, 2013). However, high-altitude lakes are too oligotrophic and have very low nutrient (mostly TDP) contents (Table 1). These lakes are unable to support an abundant chrysophyte community. This observation is in agreement with findings of Siver (1995) and Kristiansen (2005) who found only a few, if any, scaled chrysophytes in ultra-oligotrophic lakes of North America and Newfoundland, respectively.

In addition to lakes, we also investigated several small acidic shallow pools that contained dark water coloured by humic acids (sites N. 3, 4 and 19). In the neighbouring pools (Ochsenkopf Obergurgl N. 3 and N. 4), *M. costata* formed extremely dense populations (more than 10^3 cells mL⁻¹). Monospecific blooms of silica-scaled chrysophytes in small pools are observed relatively often. Blooms of *Mallomonas annulata* were recorded in alluvial pools of the Blanice River, Czech Republic (Nemcova, unpublished) and blooms of *Mallomonas adamas* in a small, peat-rich Ostrov pool, Bohemian Switzerland National Park, Czech Republic (Novakova *et al.*, 2004). This pattern could be underlined by the founder effect combined with the rapid local adaptation of the first originating species when colonising a new habitat. The effective monopolisation of resources can yield a strong priority effect (De Meester *et al.*, 2015). The presence of a large resting propagule bank (siliceous stomatocysts in the case of *M. costata*) provides a powerful buffer against newly invading species that have similar environmental requirements. However, to confirm the priority effect of *M. costata* in these shallow pools repeated sampling throughout the year is needed.

pH is generally accepted to represent one of the key factors underlining the distribution of silica-scaled chrysophytes (for review, see Siver (1995)). The low abundance/absence of chrysophytes in lakes in the Northern Calcareous Alps (N. 16 (Seebensee) and N. 17 (Drachensee)) can be explained by higher pH caused by carbonate bedrock. In water, carbon dioxide equilibrates with bicarbonate on a pH-dependent basis, with carbon dioxide decreasing with increasing pH. Chrysophytes lack a carbon-concentrating mechanism to concentrate carbon dioxide around the carbon-fixing enzyme RuBisCO, preventing RuBisCO from operating at a higher efficiency. Chrysophytes therefore rely only on the diffusion of water-dissolved carbon dioxide (Saxby-Rouen *et al.*, 1998; Maberly *et al.*, 2009), which at high pH is very low.

Altitude and pH were the most important variables influencing the community of silica-scaled chrysophytes and were the only significant variables. More than 14% of variation in the data was explained by altitude. However, the species recorded in the high-alpine lakes represent the common and most widely distributed cosmopolitan species. In addition, *M. actinoloma* var. *maramuresensis* and *M. torquata* f. *torquata* have northern temperate distributions (Kristiansen, 2002; Skaloud *et al.*, 2013). The newly described *M. pechlaneri* retains its endemic status. It is debated whether the same pools of species thrive in arctic and Alpine waterbodies due to similarities in climatic conditions. Although it has been suggested that no typical arctic chrysophyte flora exists (Siver *et al.*, 2005; Pichrtova *et al.*, 2011), some species seem to be restricted to northern regions within Europe, probably to avoid warmer climate. These are, for example, *Mallomonas vannigera*, *Synura leptorhabda*, *S. punctulosa*, *Spiniferomonas alata* or *Sp. bilacunosa* (Skaloud *et al.*, 2013). The highest-situated lake studied for chrysophytes thus far (glacial lake on Haba Snow Mountain, China at 4192 m a.s.l.) also hosts several widely distributed species (e.g., *Synura conopea*, *M. annulata*, *Mallomonas papillosa* and *Mallomonas akrokomos*) together with the recently described *Mallomonas alpestrina* (Nemcova *et al.*, 2015). Similarly, in the present study, the species correlated with high altitude were also known from lower regions (Skaloud *et al.*, 2013). We are aware of the limits of the obtained data, which is why we only concentrated on the putative distribution of the most frequent species on altitudinal and pH gradients.

Mallomonas alpina was recorded in three bathing ponds that had high conductivity values (Table 2; N. 13 (Pillersee), N. 20 (Gieringer Weiher) and N. 21 (Moorstrandbad Kirchbichl), which had conductivity values of 125 $\mu\text{S cm}^{-1}$,

Table 2. List of species, only sites where silica-sealed chrysophytes have been recorded are listed

Species / sampling site No.	Fig.	3	4	6	8	9	10	13	16	17	19	20	21
<i>Mallomonas acaroides</i> Perty em. Ivanov	2							X					
<i>M. actinoloma</i> var. <i>maramuresensis</i> Peterfi & Momeu	18				X	X							
<i>M. alpina</i> Pascher & Ruttner in Pascher	3							X				X	X
<i>M. costata</i> Dürschmidt	16-17	X	X										
<i>M. crassisquama</i> (Asmund) Fott	4					X	X						
<i>M. multisetigera</i> Dürschmidt	5										X		
<i>M. papillosa</i> Harris & Bradley	6			X									
<i>M. pechlaneri</i> Nemcova & Rott	31-39			X		X							
<i>M. striata</i> var. <i>striata</i> Asmund	24-27								X				
<i>M. torquata</i> f. <i>torquata</i> Asmund & Cronberg	7				X	X	X						
<i>Synura americana</i> Kynčlová & Škaloud	8											X	
<i>S. conopea</i> Kynčlová & Škaloud	9							X					
<i>S. curispina</i> (Petersen & Hansen) Asmund								X					
<i>S. echinulata</i> Korshikov										X	X		
<i>S. sphagnicola</i> (Korshikov) Korshikov									X				
<i>Chrysosphaerella annulata</i> Kristiansen & Tong	10												X
<i>C. brevispina</i> Korshikov em. Harris & Bradley					X	X		X		X			
<i>C. coronacircumpina</i> Wujek & Kristiansen	11			X								X	
<i>Spiniferomonas abei</i> Takahashi	13								X				
<i>Sp. cornuta</i> Balonov	12												X
<i>Sp. serrata</i> Nicholls	14											X	
<i>Sp. triordalis</i> Takahashi	15							X				X	
<i>Paraphysomonas</i> cf. <i>imperfurata</i> Lucas	20												
<i>P. longispina</i> Scoble & Cavalier-Smith	19											X	
<i>Paraphysomonas</i> sp. – closest to <i>P. vulgaris</i> Scoble & Cavalier-Smith	21											X	X
<i>P. uniformis</i> hemiradia Scoble & Cavalier-Smith	22					X							
<i>P. vulgaris</i> brevispina Scoble & Cavalier-Smith	23				X								

141 $\mu\text{S cm}^{-1}$ and 450 $\mu\text{S cm}^{-1}$, respectively). *Mallomonas alpina* has a strong ecological tolerance to conductivity levels. This species was one of the most frequently sampled species in the brackish waters of Pojo Bay, which is in the Baltic Sea (Ikävalko, 1994). Moreover, during the study of chrysophytes along the salinity gradient in the Gulf of Bothnia in the Baltic Sea, *M. alpina* was found to prefer brackish habitats that had high conductivity rather than freshwater habitats (Nemcova *et al.*, 2016). *Synura americana* predominately occurs in the USA and Canada and is relatively rare in Europe (Skaloud *et al.*, 2014). Within the present investigation, *S. americana* was recorded at N. 20 (Gieringer Weiher).

The second part of this study involves the delineation and description of the new species *M. pechlaneri*. Based on scale morphology, *M. pechlaneri* could be placed in the section Striatae (Asmund & Kristiansen, 1986). However, based on molecular data, the taxa currently assigned to that section are not monophyletic (Jo *et al.*, 2013). *Mallomonas pechlaneri* most closely resembles *M. striata* var. *striata* (Asmund, 1959) and *M. striata* var. *serrata* (Harris & Bradley, 1960). We decided to describe the new taxon at the species level because i) in the era of molecular phylogenetic data, there is no suitable definition for a variety and ii) studies that combine morphological and molecular data clearly show that even slight differences in scale/bristle morphology are sufficient for the delimitation of species. On the other hand we kept both previously described varieties to avoid confusion. The variety *serrata* of *M. striata* cannot be easily erected to the species level, because *M. serrata* (Nicholls) is validly described taxon within the Eoae Series. Molecular data are needed to evaluate whether these taxonomic units should be treated as independent species. However, considering the present state of knowledge, we believe that treating *M. pechlaneri* as a distinct species, is the best working hypothesis. The diagnostic features of silicate structures are listed in Table 3. These three taxa are clearly distinguished by bristle morphology. *Mallomonas pechlaneri* has bristles that are terminated by a bifurcated tip consisting of unequal diverging branches (Figs 33-35). The bristles of *M. striata* var. *striata* terminate with a blunt tip and a single small subapical tooth (Figs 26, 27), and *M. striata* var. *serrata* bristles are serrated with short pointed teeth along the whole length (Fig. 28). The scales of all three taxa are similar in overall appearance. These scales have a comparable number of ribs on the shield and also a comparable number of struts on both posterior and anterior flanges. A special group of pores located on the posterior of the shield is also a common feature. The scales of *M. pechlaneri* are similar to those of *M. striata* var. *striata*; both have an acutely angled V-rib with a smooth lower edge. *Mallomonas striata* var. *serrata* scales are relatively wider, the acutely angled V-rib is strongly hooded, and the lower edge is supported by internal radial struts (visible only in TEM microphotographs). The dome of *M. striata* var. *striata* is oval, relatively low and has a protrusion that can exceed the perimeter of the dome (Figs 24, 25). On the other hand, the domes of *M. pechlaneri* (Fig. 37) and *M. striata* var. *serrata* (Fig. 30) have a sub-circular appearance with a protrusion not exceeding the top of the dome. The ornamentation of the dome appears to be a relatively variable feature of *M. pechlaneri* and *M. striata* var. *striata*; both ribs and reticulation were observed. The domes of *M. striata* var. *serrata* are ornamented consistently by U-shaped ribs (Figs 29, 30). The scales of *Mallomonas siveri* (Nemcova *et al.*, 2011) differ from those of *M. pechlaneri* by having a roundly angled V-rib. Moreover, the arms of the V-rib curve become continuous with the anterior submarginal ribs, unlike those of *M. pechlaneri*, which extend almost to the margins of the scale and are not continuous with submarginal ribs. Unlike those of *M. pechlaneri*, the scales of both *M. striata* var. *getseniae* and *M. striata* var. *balonovii* (Voloshko, 2009) are larger

Table 3. A survey of diagnostic features on silicate structures in *Mallomonas pechlaneri* and related taxa *M. striata* var. *striata* and *M. striata* var. *serrata*. * represents our own measurements; all other measurements are based on Kristiansen (2002)

	<i>M. striata</i> var. <i>striata</i>	<i>M. striata</i> var. <i>serrata</i>	<i>M. pechlaneri</i>
Scale	(4.3)-4.4-(4.9) × (2.2)-2.4-(2.6)*	(3.9)-4.1-(4.4) × (2.2)-2.3-(2.5)*	(3.6)-4.3 -(4.7) × (1.9) -2.1-(2.4)*
Dome	4-6.5 × 2-4µm rounded with ribs or reticulum	3-4.8 × 1.5-3 µm sub-circular, ribs mostly U-shaped	— sub-circular with ribs or reticulum
V-rib	acutely angled, lower edge smooth	acutely angled, lower edge supported by internal radial struts in well silicified scales	acutely angled, lower edge smooth
Hood	well developed	well developed, strongly hooded	well developed
Proximal border (rim)	bearing internal struts	bearing distinctive internal struts	bearing internal struts
Number of ribs on the shield	10-12*; about 12	9-11*	8-10*
Struts on anterior flange	3-4*	5-7*, sometimes connected with net like reticulum	4-6*
Struts on posterior flange	16-20*; about 16	7-13*	16-20*
Bristle	with a blunt tip and a single small subapical tooth (8.1)-9.5-(10.7) *; 9-13 µm	serrated with short pointed teeth (5.7)- 9.1-(14.1)*; 6-13 µm	bifurcate tip with unequal diverging branches (4.3) -6-(8)*µm

with expanded posterior flanges, less struts and higher numbers of ribs on the shield (more than 15). The dome of *M. striata* var. *getseniae* possesses a labyrinth-like reticulum, whereas the dome of *M. striata* var. *balonovii* is smooth.

Closely related to this study is the investigation by Neustupa and Nemcova (2007), who used landmark-based geometric morphometry to emphasise differences in scale shape between *M. striata* var. *striata* and *M. striata* var. *serrata*. These authors used a slightly different set of landmarks, and the area of the dome was excluded from the analysis. The scales of *M. striata* var. *striata* were characterised by a narrower and longer anterior flange connected to a wider shield area and a thinner V-rib. On the other hand, *M. striata* var. *serrata* was characterised by a wide and short anterior flange, a narrower shield and a thicker V-rib, particularly in the hood area (Neustupa & Nemcova, 2007). In the present study, a third group of scales (*M. pechlaneri*) was added to the shape analysis. When all three groups of scales were analysed together, the length and width of the anterior flange failed to distinguish between *M. striata* var. *striata* and *M. striata* var. *serrata* (Fig. 41, deformation grids). The appearance of the anterior flanges is probably associated with the position of the scale on the cell body. Because scales are usually individually dispersed on TEM grids, their original position on the cell cannot be discerned. However, the overall scale width and thickness of the V-rib seem to be stable characters for distinguishing these two varieties. However, only single population for both *M. striata* var. *striata* and var. *serrata* were subjected to shape analyses and we are aware that more populations from other geographic regions are needed to verify these distinguishing characters. On the other hand, *M. striata* var. *striata* and *M. pechlaneri* differ especially by the shape of their dome. When investigating scale shape, ecologically induced variation should also be considered. After studying the clonal variation of *M. striata* var. *serrata* scale shape, Nemcova and Pichrtova (2012) concluded that the pH of the cultivation medium affects scale biogenesis. Cells from higher-pH treatments unmistakably economised on the silicon, thus producing less silicified scales that have reduced V-rib hoods. However, this was not the case in the present study, as the *M. striata* var. *serrata* strain was cultivated at an optimum pH of 7.5 and excessive Si concentration. Moreover, two other taxa (*M. striata* var. *striata* and *M. pechlaneri*) were sampled from natural waters, where the decreased availability of dissolved reactive silicon for chrysophytes can occur in environments that have pH values as high as 9 (Reynolds, 2006).

The high-alpine lakes of the eastern Alpine region of North Tyrol have a considerably lower silica-scaled chrysophyte diversity compared with the lower-situated lakes that have higher amounts of nutrients and higher conductivity (Pichrtova *et al.*, 2013). This region represents an excellent suite of waterbodies along an elevation gradient that would be valuable to monitor impacts of future climate change. Although the phytoplankton of these lakes has been thoroughly studied (Rott, 1983), it is still possible to identify new species in those locations. Even in light of fast-developing molecular approaches, we expect that the scale design will continue to define the morphology and will continue to be used for chrysophycean taxonomy.

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