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Silica-scaled chrysophytes (Chrysophyceae, Stramenopiles) of the Istrian Peninsula (Slovenia, Croatia) – species flourishing in humid subtropical climate

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There are still blank areas on the map of distribution of silica-scaled chrysophytes in Europe, the subtropical regions are especially understudied. Here, a survey of species from nine sampling sites from the Istrian Peninsula is provided. In total, only 17 mostly cosmopolitan or widely distributed species were recorded. A relatively low number of species per locality (3.7 on average) might be a consequence of low connectivity of freshwater bodies in the Istrian Peninsula, and higher levels of conductivity and pH, caused by carbonate and flysch bedrock. Species known to prefer or tolerate higher levels of those factors were especially prevalent in the investigated sites (e.g. *Mallomonas heterospina* and *M. alata*). Resistant silica scales make silica-scaled chrysophytes a flagship group of organisms to study the biogeography of protists. Records of species from European humid subtropical climate regions have not been published to date, and the current paper may help to fill in the missing information.

Silica-scaled chrysophytes comprise two separate lineages: the Synurales and Paraphysomonadaceae, within the class Chrysophyceae (Stramenopiles), in which cells are covered with an armour of silica scales attached to the plasma membrane in a more or less organized way. Chrysophytes represent free-living or colonial flagellates that reside predominantly in fresh waters. However, some species have also been reported from brackish waters (Ikävalko 1994). Silica-scaled chrysophytes form rich populations and represent key primary producers in slightly acidic temperate lakes that are low in nutrient supply, (Eloranta 1986, Němcová et al. 2008). European investigations have largely targeted the temperate and subarctic/arctic zones (Škaloud et al. 2013).

According to the Köppen-Geiger climate classification, the Istrian Peninsula has humid subtropical climate (Peel et al. 2007). This climate type is characterized by warm, moist summers and mild to cool winters. Mean annual precipitation is evenly distributed throughout the year. There are no published records of silica-scaled chrysophytes from European regions with humid subtropical climate, however, several studies have analyzed dry-summer subtropical (or Mediterranean) climate regions in Greece and Portugal (Kristiansen 1980, 1983, Santos and Leedale 1993, Calado and Craveiro 1995). Greek studies have summarized the investigation of silica-scaled chrysophytes from plankton and whole-water samples in six lakes in northwestern Greece. In total, only 15 synuracean, mostly cosmopolitan species were recorded. Only *Mallomonas portae-ferreae* Péterfi and Asmund and *Synura glabra* appear to thrive in warmer habitats (Kristiansen 2002, Škaloud et al. 2012). Portuguese studies mostly revealed widely distributed common species, except for *M. peronoides* var. *bangladeshica* (E. Takahashi and T. Hayakawa) Kristiansen and Preisig, which mainly has a tropical distribution.

Humid subtropical climate is commonly found on the southeast side of all continents, generally between latitudes 25° and 40° and tends to be located in coastal or near coastal regions. However, in some cases this climate extends well inland, most notably in China and the USA (Peel et al. 2007). Extended research of silica-scaled chrysophytes in humid subtropical climates has been performed in North and South Carolina and in Florida, USA (Siver and Wujek 1999, Wujek et al. 2004, Lott and Siver 2005) and also in China (Kristiansen and Tong 1989, Wei and Kristiansen 1994, 1998, Wei and Yuan 2001). Several investigated localities in east Australia also match this climate type (Furlotte et al. 2000).

The aim the present study is to investigate the silica-scaled chrysophytes of different freshwater habitats of the Istrian Peninsula and to extend our knowledge on distribution of species in the European humid subtropical climate zone.

Material and methods

Istria is the largest peninsula in the Adriatic Sea. According to the geological and geomorphological structure, it can be divided into three different areas: the Red Istria, located in the southern and western Istrian erosional plain, is named after the 'terra rossa'; the Grey Istria, a Paleogene flysch basin of central Istria; and the White Istria, in the eastern, northeastern and northern Istria, represented by mountain ridges with exposed 'white' Cretaceous–Paleogene limestones (Faivre et al. 2011). Sampling sites 1 and 2 were located on a carbonate plain covered with red soil: the Red Istria part (Fig. 1). Red soil or terra rossa is the most common soil type in the western part of Istria. All other investigated sites were situated in the flysch basin. Sites 3–5 and 8 represented alluvial pools/oxbow lakes of the Mirna and Dragonja Rivers, respectively. Both rivers originate in the flysch area, further downstream the Mirna River passes through karst areas. While the Dragonja River does not flow through settlements, the Mirna River valley is used mostly for agriculture and pasture. Most of the course of the Mirna River has been straightened and an artificial river bed has been constructed. Site 6, however, was located on the old river bed. There are two lakes in the vicinity of Fiesa Bay; the larger one lies closer to the sea shore. This lake resulted from the infilling of a former clay pit next to a brick factory in the first half of the twentieth century (Krivograd Klemenčič et al. 2007) and represents the only brackish lake in Slovenia (conductivity of 3220–4000 µS cm^{-1}). The smaller Fiesa Lake is of natural origin.

The sites investigated in this study were sampled in March 2012. Water temperature, pH and conductivity were measured at the time of collection, with a combined pH–conductometer WTW 340. Plankton samples (20 µm mesh net) were combined with water squeezed from the submerged vegetation. In deeper water body (site 5), the surface layer of the sediment (approximately the upper 2 mm) was also investigated, whereas in shallow sites, the sediment was included in the plankton hauls. A single sample per locality (except for site 5) was collected. Water samples were concentrated by sedimentation. Subsequently, unfixed drops of the sample were dried onto Formvar-coated transmission electron microscopy (TEM) grids. Dried material was washed by repeated transfer of the grid into drops of deionized water dispensed onto the hydrophobic surface of a Parafilm strip. Three TEM examinations were performed per sample (three TEM grids were carefully observed and scales photographed). Dried grids were examined with a JEOL 1011 TEM.

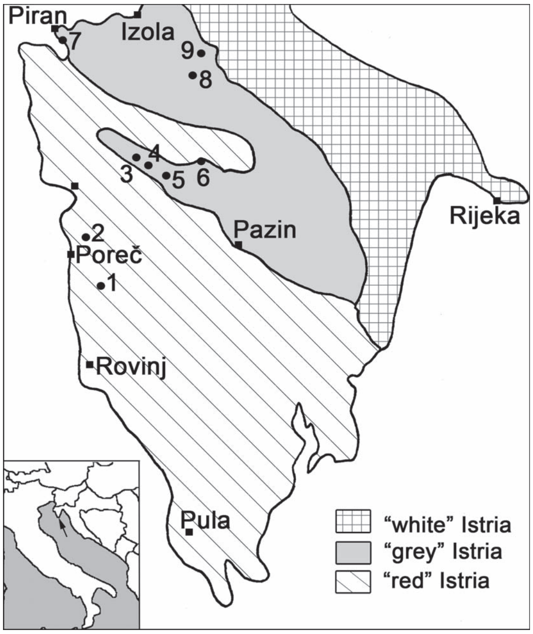

Figure 1. Map of the Istrian Peninsula. Inserted image shows position of the Istria Peninsula on the map of Europe.

Results and discussion

Fifteen species of Synurales and two species of Paraphysomonadaceae were revealed (Table 2). Generally, the number of species per locality was relatively low (mean 3.7), except for site 5 (Mirna River alluvial pool, near Trombar), with 13 taxa. Sampling sites 1 and 2 belonging to Red Istria did not differ substantially in pH or conductivity from the sites located in the flysch area (Grey Istria; Table 1). Conductivity and pH of the

Table 1. List of localities including geographical location and environmental variables. n.m. = not measured, Cond. = conductivity; T = temperature.

Locality	pH	Cond. (µS cm $^{-1}$)	T (°C)	Latitude (°N)	Longitude (°E)	Sampling date
1. a small shallow pool Grdenica, near the Dracevac village	7.7	195	6.0	45.193379	13.668620	13 Mar 2012
2. a small shallow pool Camper south of Kukci	8.6	340	n.m.	45.253097	13.636398	13 Mar 2012
3. an oxbow lake close to the Mirna River, near the Zudetici village	7.6	649	7.1	45.357227	13.736116	14 Mar 2012
4. a Mirna River alluvial pool, near the Zudetici village	8.7	399	15.0	45.353298	13.750823	14 Mar 2012
5. Mirna River alluvial pool, near Trombar, where a Krvar stream joins the river	7.6	395	15.0	45.345583	13.787532	14 Mar 2012
6. an old non-regulated part of the Mirna River, near Golubici village	7.8	618	12.3	45.360833	13.856125	14 Mar 2012
7. a smaller lake of Fiesa, near Portoroz	7.5	606	8.2	45.522523	13.582815	15 Mar 2012
8. Dragonja River alluvial pool, near Trsek village	7.8	606	11.1	45.478096	13.835560	15 Mar 2012
9. an unnamed pond next to the road to Popetre	7.7	368	14.1	45.505165	13.855443	15 Mar 2012

examined sites ranged from 195 to 649 and from 7.5 to 8.6, respectively. Sites with conductivity over 600 µS cm^{-1} usually accommodated fewer species (sites 3, 6–8). Mirna River alluvial pool, near Trombar (site 5) was the only locality with high chrysophyte diversity. It differed from the rest of the sampling sites in several aspects. It was the only locality where large surface area (900 m^2), relative deepness and lower conductivity were combined. Moreover, the littoral was overgrown with *Phragmites* sp., providing diversity of suitable habitats. Association of silica scaled chrysophytes with the methaphyton was frequently observed during our previous studies (Němcová et al. 2002).

The relatively low number of species per locality might be a consequence of low connectivity of freshwater bodies in the Istrian Peninsula (especially sites 1, 2, 7 were isolated, inverted islands on the land). The importance of proximate geographical distance and connectivity between lakes has been demonstrated to impact microbial species richness, together with local environmental factors (Crump et al. 2007, Ptacnik et al. 2010). Alluvial pools of the Mirna and Dragonia Rivers were also affected by an extremely dry season (spring 2012 with almost no precipitation was the driest for several years; long-term March mean precipitation amount in Portoroz is 71.348 mm; <http://www.tutiempo.net>), which caused high evaporation, a decrease in the water level and consequently, severe conductivity fluctuations.

Species thriving at higher conductivity and pH or species indifferent to these environmental variables were frequent in the investigated sites. Silica scales of *M. akrokomos*, *M. heterospina* and *M. alpina* were have even been reported from brackish waters (conductivity of 5600 µS cm^{-1}) of the Pojo Bay, the Gulf of Finland, Baltic Sea (Ikävalko 1994). However, part of the phytoplankton communities might have been washed in from surrounding freshwater bodies, and were thus not indigenous to Pojo Bay. *Mallomonas alata* and *M. cratis* are both pH-indifferent alkaliphilic species and are frequently reported at pH above 8.0 (Kristiansen 1988, Siver and Hamer 1989, Siver 1991). Moreover, *M. cratis* is a species that prefers high conductivity (Siver and Hamer 1989) and was reported from a small pond in eastern Hungary with conductivity of 2540 µS cm^{-1} (Barreto 2005). *Mallomonas striata* has been classified as a medium conductivity, pH-indifferent species (Siver 1991). In laboratory experiments a positively growing culture was obtained within a pH range between 4.5–8.7 (Němcová and Pichrtová 2012). *Mallomonas heterospina* and *M. akrokomos* have been reported from both acidic (Takahashi 1978, Green 1979) and alkaline localities (Němcová et al. 2002). Contrary to the above-mentioned taxa, *M. akrokomos* has been found to commonly inhabit oligotrophic, low-conductivity water-bodies (< 40 µS cm^{-1}; Siver 1991). All species found are cosmopolitan or widely distributed taxa, which are known also from temperate

Table 2. Species list and distribution of silica-scaled chrysophytes found here.

Taxon	Figure	1	2	3	4	5	6	7	8	9
Mallomonas acaroides Perty em. Ivanov	2A					x				
M. akrokomos Ruttner in Pascher	2B	x				x		x		
M. alata Asmund, Cronberg and Dürrschmidt	2C–D	x		x		x	x			
M. alpina Pascher and Ruttner in Pascher em. Asmund and Kristiansen	2E							x		x
M. annulata (D. E. Bradley) K. Harris	2F	x				x				
M. cratis K. Harris and D. E. Bradley	2G–H					x	x			
M. heterospina J. W. G. Lund	2I	x	x			x	x			
M. parvula Dürrschmidt	2J					x				
M. paxillata (D. E. Bradley) L. S. Péterfi and Momeu	2K					x				
M. peronoides (K. Harris) Momeu and L. S. Péterfi	2L					x				
M. striata Asmund	2M	x	x						x	
M. tonsurata Teiling em. Willi Krieger	3A–B				x	x				
Synura curtispina (J. B. Petersen and J. B. Hansen) Asmund	3E		x							
S. glabra Korshikov em. Kynčlová and Škaloud	3G					x	x			
S. petersenii Korshikov em. Škaloud and Kynčlová	3F				x				x	
Paraphysomonas vestita (A. C. Stokes) De Saedeleer	3C					x				
Spiniferomonas trioralis E. Takahashi	3D					x				

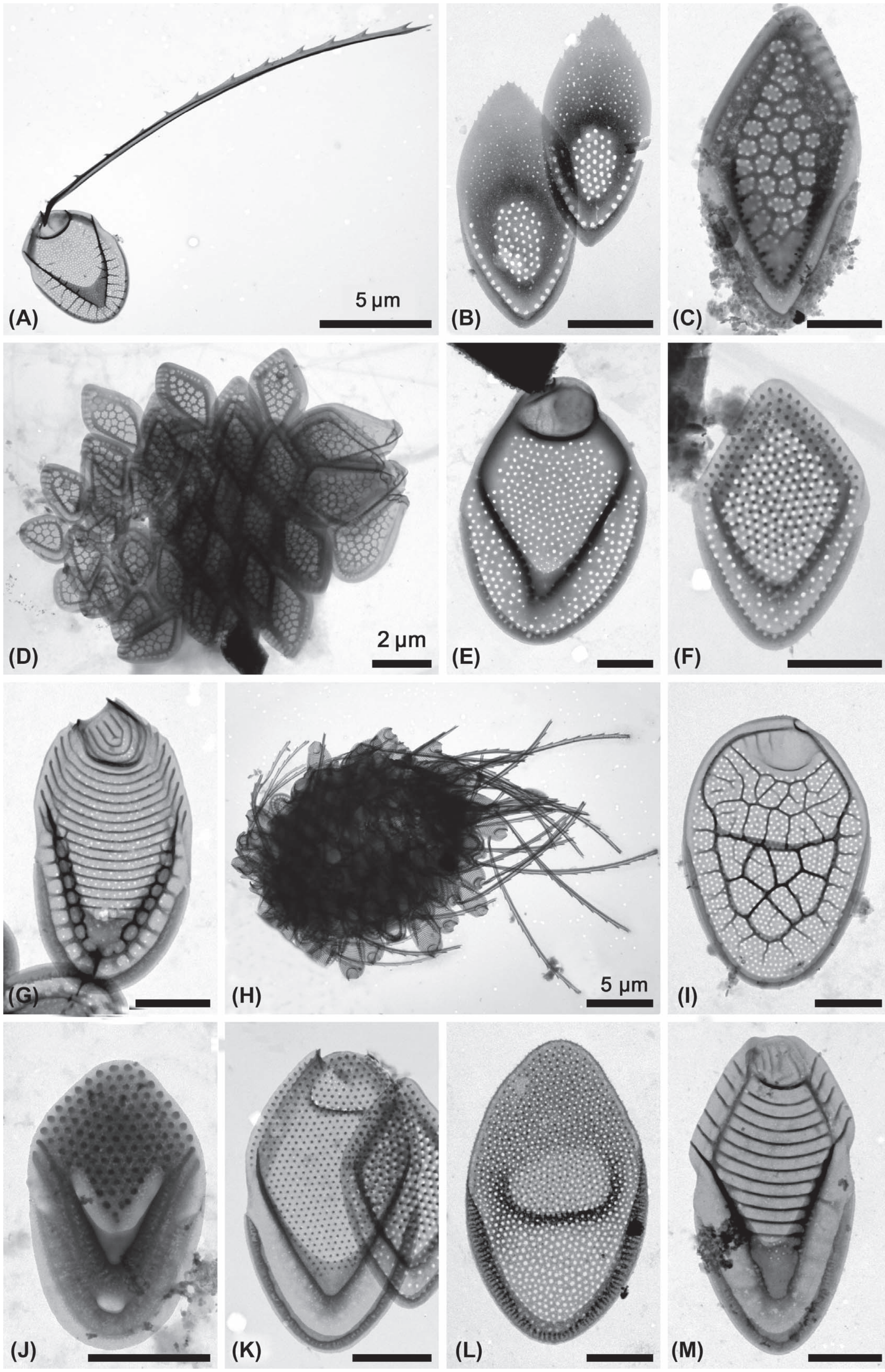

Figure 2. Silica-scaled chrysophytes from the Istrian Peninsula: (A) *Mallomonas acaroides*, a body scale with an attached bristle, (B) *M. akrokomos*, (C)–(D) *M. alata*: (C) a body scale with a wing-like anterior flange, (D) a whole cell, (E) *M. alpina*, (F) *M. annulata*, (G)–(H) *M. cratis*: (G) a body scale, (H) a whole cell, (I) *M. heterospina*, (J) *M. parvula*, (K) *M. paxillata*, (L) *M. peronoides*, (M) *M. striata.* Scale bar = 0.5 µm, if not stated otherwise.

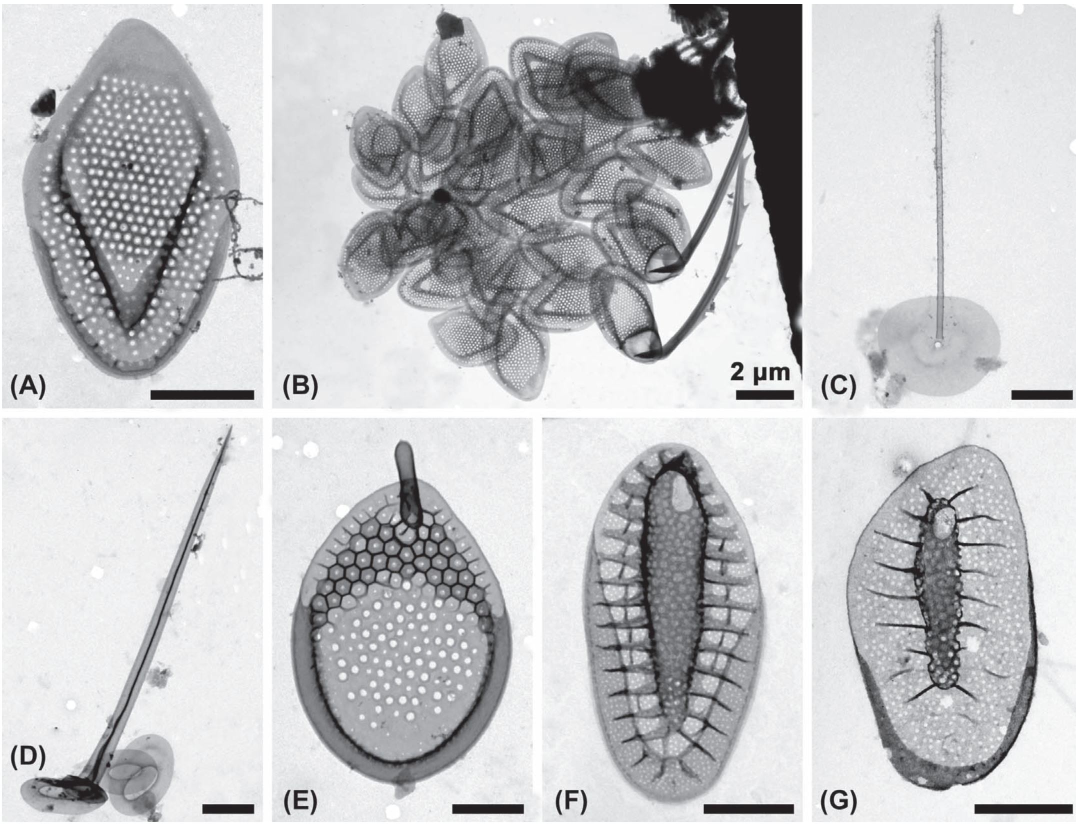

Figure 3. Silica-scaled chrysophytes from the Istrian Peninsula: (A)–(B) *Mallomonas tonsurata*: (A) a body scale, (B) a whole cell, note that only apical scales are provided with a dome and bristles, (C) *Paraphysomonas vestita*, (D) *Spiniferomonas trioralis*, (E) *Synura curtispina*, (F) *S. petersenii*, (G) *S. glabra.* Scale bar = 0.5 µm, if not stated otherwise.

regions. No species endemic to the region were observed, as was the case for the western Cape (South Africa, Němcová et al. 2011, Němcová and Kreidlová 2013) and the southeastern Cape (Tasmania, Australia, Croome and Tyler 1986, Croome et al. 1998).

As silica scales remain in sediments for prolonged periods of time, silica-scaled chrysophytes provide an ideal experimental group to study biogeography and dispersal of protists. This study contributes data on the occurrence of silica-scaled chrysophytes from regions where no information previously was available.

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