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Chrysophycean stomatocysts associated with the carnivorous plants (genus *Utricularia*) from Jeleniak-Mikuliny Nature Reserve

Konrad Wołowski[1,*], Jolanta Piątek[1], Bartosz J. Płachno[2]

[1]*Department of Phycology, W. Szafer Institute of Botany, Polish Academy of Science. Lubicz 46, PL–31–512 Kraków, Poland*
[2]*Department of Plant Cytology and Embryology, Jagiellonian University in Kraków, Gronstajowa 9, PL–30–387 Kraków, Poland*

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Abstract

An account is given of chrysophycean stomatocysts and algae occurring together with the carnivorous plants known as bladderworts, *Utricularia intermedia*, *U. minor* and *U. australis*, in the peat bog of Jeleniak-Mikuliny Nature Reserve. Eleven chrysophycean stomatocyst morphotypes were found, all reported for the first time from this nature reserve. Among them, two are new records for Europe (stomatocysts 330 and 208) and another two are new for Poland (stomatocysts 112 and 387). Descriptions are provided together with SEM illustrations. General data about cyanobacteria and eukaryotic algae occurring with these stomatocysts are enumerated.

* Corresponding author e-mail: *k.wolowski@botany.pl*

INTRODUCTION

Carnivorous plants form an ecological group of plants that trap and consume invertebrates (mainly insects and crustaceans) as well as small vertebrates, protozoa and algae (Juniper et al. 1989, Peroutka et al. 2008, Król et al. 2012). Studies continue to extend the range of angiosperm families where the carnivorous syndrome has been reported (e.g. Darnowski et al. 2006, Płachno et al. 2009). For instance, the syndrome has recently been confirmed in the Plantaginaceae genus *Philcoxia* (Pereira et al. 2012). Increasingly the role of organisms such as bacteria, fungi, protozoa and invertebrates in grinding and digesting the victims of carnivorous plants has been recognized (e.g. Sirová et al. 2009, Koopman et al. 2010, Adlassnig et al. 2011, Bazile et al. 2012).

The genus *Utricularia* (Lentibulariaceae), with over two hundred described species (Taylor 1989, Fleischmann 2012, Jobson 2012), is the largest genus of carnivorous plants. *Utricularia* traps are not only lethal to some organisms (Adamec 2007, 2011), but also form micro-environments in which other organisms can function and proliferate (Richards 2001, Sirova et al. 2009, Płachno et al. 2012). Algae are among such organisms often occurring in bladderwort traps (Gordon and Pacheco 2007; Alkhalaf et al. 2009, 2011; Wołowski et al. 2011; Płachno et al. 2012). Some algae living inside *Utricularia* traps release digestive enzymes into the trap (e.g. Płachno 2006, Płachno et al. 2006) and a similar phenomenon has also been found in traps of *Genlisea* (Płachno and Wołowski 2008), a genus related to *Utricularia*. In *Utricularia* not only traps but also the surfaces of the organs of these plants can provide a habitat for various species of algae (Diaz-Olarte et al. 2007). Among the algal groups many taxa of chrysophycean stomatocysts were reported to

co-exist with the species of *Utricularia* in Virginia, USA (Wołowski et al. 2011), but these studies were limited to one location.

The aim of the present study was to establish the extent to which stomatocysts co-exist with *Utricularia* elsewhere. The Jeleniak-Mikuliny Nature Reserve was chosen for the study area, since a broader study of the algal flora is already underway in this extremely valuable natural area, where the common crane (*Grus grus*) and rare dragonflies are protected.

MATERIALS AND METHODS

Material was obtained from the Jeleniak-Mikuliny Nature Reserve near the town of Lubliniec in the northern part of the Silesian Upland (Klama et al. 1991; see also Plachno & Świątek 2008). The area of the Jeleniak-Mikuliny reserve is created by two shallow, overgrown water reservoirs lying in the lowland between two sand dunes. About 177 species of vascular plants and 32 bryophytes were reported by Klama et al. (1991).

The fieldwork was conducted during the main growing seasons of 2009, 2010 and 2011. Samples included plankton, peat, water squeezed from plants, and traps of *Utricularia* (*U. intermedia*, *U. minor*, *U. australis*) taken from the communities of *Utricularietum australis* (Spałek 2006) and *Utricularietea intermedia-minoris* (Spałek 2002). All studies on the protected area and on protected plants were done in compliance with Polish law after obtaining permissions (DLOPiK-op/ogiz-4211/I-29.2/8052/06/msz, DLOPiK-op/ogiz-4211/I-29.3/8052/06/msz and DLOPiK-op/ogiz-4211/I\66/7000/07/msz) from the Ministry of the Environment of the Republic of Poland.

In the laboratory, part of each sample was prepared for SEM observations. The material was first placed in a glass scintillation vial, then poured over 10% HCl and allowed to sit for 24 h. The samples were boiled for 15 min, clined several times with distilled water, and poured over 30% H_2O_2. This was allowed to stand for 24 h, then boiled for 30 min with a pinch of $KClO_3$ and cleaned several times with distilled water. Finally, the sample was slurried in a glass vial and covered with 95% ethanol. For SEM analysis, a few drops of the prepared sample were placed on cleaned glass, air-dried and fixed to an aluminium stub with double-sided transparent tape. The stubs were sputter-coated with gold and investigated with a Hitachi S-4700 scanning electron microscope (Scanning Microscopy Laboratory of Biological and Geological Sciences, Jagiellonian University). The stomatocysts were measured from the SEM micrographs and described according to International Statospore Working Group (ISWG) guidelines (Cronberg & Sandgren 1986). The stubs with algal material are deposited in the Department of Phycology, Institute of Botany, Polish Academy of Sciences (KRAM). To identify stomatocysts, the cyst morphotypes from Jeleniak-Mikuliny Nature Reserve were compared with descriptions of stomatocysts in relevant publications (e.g. Cabała 2003a, b, 2005a, b; Cabała & Piątek 2004; Duff et al. 1995; Hansen 2001; Piątek 2007; Piątek et al. 2009; Pla 2001; Wilkinson et al. 2001; and Wołowski et al. 2004).

RESULTS AND DISCUSSION

The recorded stomatocysts are presented in groups based on shared morphological characteristics, following Wilkinson et al. (2001). For each morphotype a short description and illustration (SEM micrograph) is given.

Unornamented stomatocysts

Spherical, without collar

Stomatocyst 9, Duff & Smol 1988 *emend.* Zeeb & Smol 1993 Fig. 1.1
Picture-file number: Mik6–19.
Number of specimens: 1.

Biological affinity: This morphotype may be produced by more than one species, e.g. *Chrysolepidomonas dendrolepidota* Peters & Andersen (according to Duff et al. 1995).

SEM description: This stomatocyst is spherical, 7.3 µm in diameter. The pore is regular, 0.5 µm in diameter. The cyst body is unornamented.

Comments: Stomatocyst 9 is a widely distributed morphotype, reported from many lakes, ponds, peat bogs and karstic sinkholes in Europe (including Svalbard), North America (Canada, U.S.A.) and Greenland.

Stomatocyst 120, Duff & Smol in Duff et al. 1992 *emend.* Zeeb & Smol 1993 Fig. 1.2
Picture-file number: JM09–14.
Number of specimens: 1.

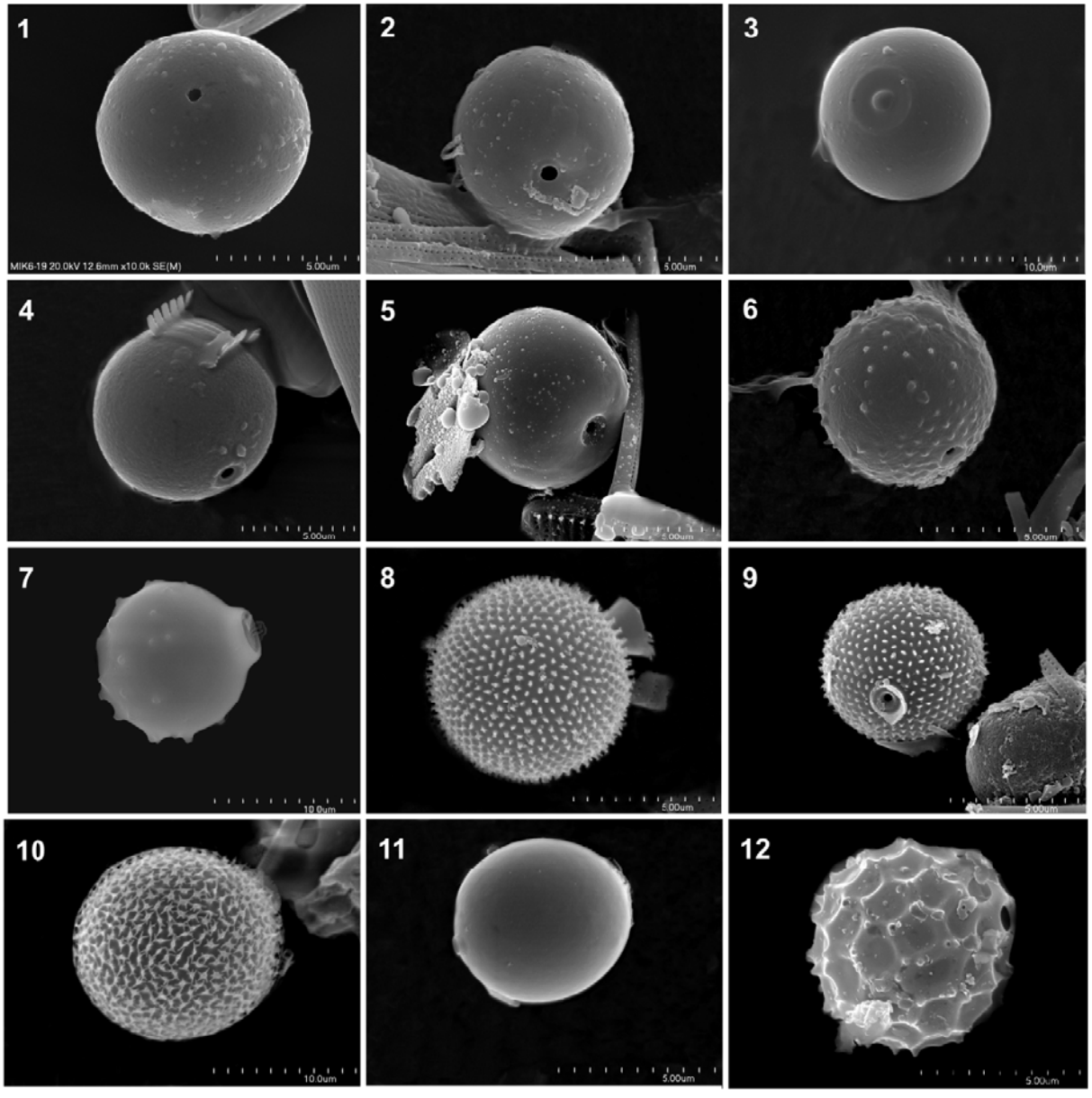

Fig. 1. SEM micrographs (**1.** Stomatocyst 9, Duff & Smol 1988 *emend.* Zeeb & Smol 1993; **2.** Stomatocyst 120, Duff & Smol in Duff et al. 1992 *emend.* Zeeb & Smol 1993; **3.** Stomatocyst 112, Zeeb et al. 1990 *emend.* Duff & Smol 1994; **4.** Stomatocyst 234, Duff et al. 1995; **5.** Stomatocyst 387, Pla 2001; **6.** Stomatocyst 330, Wilkinson & Smol 1998; **7.** Stomatocyst 208, Duff & Smol 1994; **8–9.** Stomatocyst 73, Duff & Smol 1991; **10.** Stomatocyst 214, Duff & Smol 1994 *emend.* Wilkinson & Smol 1998; **11.** Stomatocyst cf. 57, Duff & Smol 1991; **12.** Stomatocyst 86, Duff & Smol 1991.

Biological affinity: This morphotype may be produced by more than one species, e.g. *Chrysosphaerella longispina* Lauterborn *emend.* Nichols (according to Duff et al. 1995).

SEM description: This stomatocyst is spherical, 5.9 µm in diameter. The pore (outer diameter 1.0 µm, inner diameter 0.5 µm) is regular with a planar pseudoannulus. The cyst body is unornamented.

Comments: Stomatocyst 120 is a widely distributed morphotype, reported from many lakes, ponds, peat bogs, karstic sinkholes, and highly mineralized, saline habitats with *Vaucheria dichotoma* (L.) C. Agardh (Piątek & Piątek 2005) in Europe (including

Svalbard) and North America (Canada, U.S.A.), South America, Greenland and Antarctica.

Stomatocysts smooth with a simple cylindrical or conical collar

Stomatocyst 112, Zeeb et al. 1990 *emend.* Duff & Smol 1994 Fig. 1.3
Picture-file number: JM09–2.
Number of specimens: 4.

Biological affinity: This morphotype is probably produced by *Ochromonas globosa* Skuja (according to Duff et al. 1995).

SEM description: This morphotype is smooth and spherical, 13.5–14.8 µm in diameter. The collar is low and cylindrical, 4.8–5.8 µm in diameter and 0.3 µm high, collar diameter:stomatocyst diameter ratio 0.35–0.40. The pore is not obscured on investigated SEM-micrographs, because it is covered the siliceous plug.

Comments: Stomatocyst from Jeliniak-Mikuliny Nature Reserve has smaller diameter and smaller high of collar than those in the original description (6.8–8.0 µm in diameter and 1.0–1.3 µm high). However, we identified these specimens as cyst 112 because we believe that they represent the same morphotype. This stomatocyst was reported from lakes in Europe and North America (Canada, U.S.A.). This is the first record for Poland.

Stomatocyst 234, Duff et al. 1995 Fig. 1.4
Picture-file number: JM09–3.
Number of specimens: 2.

Biological affinity: This morphotype may be produced by more than one species, especially by several species of *Paraphysomonas* (Duff et al. 1995).

SEM description: This stomatocyst is smooth and spherical (sometimes oval), 7.7–8.1 µm in diameter. The collar is cylindrical to conical, 1.7–1.8 µm in diameter and 0.2–0.3 µm high, collar diameter: stomatocyst diameter ratio 0.21–0.25. The pore is regular, 0.6–0.7 µm in diameter, surrounded by a planar pseudoannulus.

Comments: Stomatocyst 234 is a widely distributed morphotype, reported from lakes in Europe (including Svalbard), North America (U.S.A.), Greenland, Antarctica and in two high-altitude volcanic lakes in Central Mexico (Vilaclara et al. 2005). Previously reported among carnivorous plants (*Utricularia* and *Aldrovanda*) by Wołowski et al. (2011).

Stomatocyst 387, Pla 2001 Fig. 1.5
Picture-file number: JM09–10.
Number of specimens: 1.
Biological affinity: Unknown.

SEM description: This morphotype is spherical, 7.7 µm in diameter. The pore is concave, 0.5 µm in diameter. The collar is low, 1.5 µm in diameter. The cyst body is smooth (small granules on the cyst body are contamination).

Comments: Stomatocyst 387 has only been reported from Europe (Pla 2001). First record for Poland.

Ornamented stomatocysts

With scabrae

Stomatocyst 330, Wilkinson & Smol 1998 Fig. 1.6
Picture-file number: JM09–20.
Number of specimens: 1.
Biological affinity: Unknown.

SEM description: This morphotype is spherical, 5.7 µm in diameter. The pore is regular, 0.4 µm in diameter and surrounded by the planar pseudoannulus. The collar is low, 0.9 µm in diameter and 0.1 µm high. The cyst body surface is ornamented with scabrae, 0.1–0.2 µm in diameter and 0.1–0.2 µm high.

Comments: Stomatocyst 330 has previously been reported from North America (Canada). This is the first time it has been reported from Poland and Europe.

With conula

Stomatocyst 208, Duff & Smol 1994 Fig. 1.7
Picture-file number: JM09–27.
Number of specimens: 1.
Biological affinity: Unknown.

SEM description: This morphotype is spherical, 12.1 µm in diameter. The pore is not visible on our SEM-micrograph. The collar is conical, basal diameter 4.4 µm, apical diameter 3.3 µm and 1.0 µm high. The

cyst body surface (mainly posterior hemisphere) is ornamented with conula, 0.8–1.0 μm in diameter and 0.5–0.6 μm high.

Comments: Stomatocyst 208 has previously been reported from North America (Canada). This is not only the first record for Poland, but also for Europe.

With spines

Stomatocyst 73, Duff & Smol 1991 Figs 1.8–1.9
Picture-file number: JM09–6.
Number of specimens: 5.
Biological affinity: Unknown.

SEM description: This morphotype is spherical, 6.3–7.6 μm in diameter. The pore is regular, 0.3 μm in diameter, with a planar pseudoannulus. The collar is obconical, 1.3–2.3 μm in diameter and 0.4–1.2 μm high. The whole cyst body surface is ornamented with short, echinate, bifurcating and bacculate spines, 0.1–0.2 μm in diameter and 0.1–0.3 μm high.

Comments: Stomatocyst 73 is a widely distributed morphotype, reported from lakes, ponds, and peat bogs in Europe (including Svalbard), North America (Canada, U.S.A.) and Antarctica.

Stomatocyst 214, Duff & Smol 1994 *emend.* Wilkinson & Smol 1998 Fig. 1.10
Picture-file number: JM09–26.
Number of specimens: 1.

Biological affinity: Stomatocyst 214 is probably produced by *Mallomonas torquata* Asmund & Cronberg (Wilkinson et al. 2001).

SEM description: This morphotype is oval, 15.0 μm long and 13.8 μm wide. The pore and collar were not observed on our single specimen, but the cyst body has characteristic ornamentation with spines, which are bifurcating, 0.6 μm in diameter and 0.5 μm high. These details were needed to determine the morphotype.

Comments: This stomatocyst was reported from Europe (Cronberg 1980) and North America (Canada, U.S.A.). Previously reported among carnivorous plants (*Utricularia* and *Aldrovanda*) by Wołowski et al. (2011).

With ridges

Stomatocyst cf. 57, Duff & Smol 1991 Fig. 1.11
Picture-file number: JM09–25.
Number of specimens: 3.
Biological affinity: Unknown.

SEM description: This morphotype is smooth and slightly obovate, 5.9–7.4 μm long and 5.3–7.5 μm wide. The collar is cylindrical, 0.7 μm in diameter and 0.6 μm high. The pore is not visible. The posterior hemisphere of the cyst body is ornamented with a network of small curved and irregular ridges, which are partially visible in Fig. 11. In the original description stomatocyst 57 is smaller (4.9–6.0 × 5.4–6.4 μm in diameter) and has a higher collar (0.8–1.4 μm) (Duff et al. 1995), therefore we identified it with cf. However, we believe that these specimens represent morphotype 57.

Comments: Stomatocyst 57 is a widely distributed morphotype, reported from lakes and ponds in Europe and North America (U.S.A.). Previously reported among carnivorous plants (*Utricularia* and *Aldrovanda*) by Wołowski et al. (2011).

With spines and reticulum

Stomatocyst 86, Duff & Smol 1991 Fig. 1.12
Picture-file number: JM09–9.
Number of specimens: 1.
Biological affinity: Unknown.

SEM description: This morphotype is spherical, 6.7 μm in diameter. The pore is regular, 0.8 μm in diameter. The short primary collar is not observed, the secondary collar is polygonal, 2.4 μm in basal diameter, 1.7 μm in apical diameter and 0.5 μm high, with 7–8 ridges radiating from each corner. The cyst body surface is covered with a low variable, polygonal reticulum, with lacunae, and short spines, which are located at each reticular interstice.

Comments: We found only one specimen, which is not fully developed. Stomatocyst 86 is a widely distributed morphotype, reported from lakes and peat bogs in Europe (including Svalbard) and North America (U.S.A.). Previously reported among carnivorous plants (*Utricularia* and *Aldrovanda*) by Wołowski et al. (2011).

CONCLUSIONS

In this study eleven chrysophycean stomatocysts from Jeleniak-Mikuliny Nature Reserve were described. Five morphotypes were unornamented and six were ornamented with scabrae, conula, spines, ridges and compound ornamentation. Most of the determined morphotypes are common and have been found previously in other parts of the world, including Poland (stomatocysts 9, 120, 234, 73, cf. 57 and 86). Two are reported for the first time from Europe (stomatocysts 330 and 208) and another two from Poland (stomatocysts 112 and 387). All stomatocysts occurred singly with 1 to 5 specimens of each morphotype in a sample. In the one previous study of stomatocysts occurring in habitats with carnivorous plant material from Virginia, U.S.A, twelve morphotypes were reported, of which two were described as new to science (stomatocysts 2 and 3) (Wołowski et al. 2011). Two were new to the U.S.A. and another two were reported for the first time from Virginia. In the two similar habitats with carnivorous plants, on two different continents (North America and Europe), the richness of chrysophycean stomatocysts was very similar. In the U.S.A. twelve cysts were identified, and in Poland eleven cysts were identified. Four stomatocysts were observed in both studied areas: 234, 214, 57 and 86. As in our previous study (Wołowski et al. 2011), we did not find stomatocysts inside *Utricularia* traps. Other authors who also studied algae in *Utricularia* traps likewise did not record stomatocysts inside them (e.g. Peroutka et al. 2008; Sirová et al. 2009; Alkhalaf et al. 2009, 2011; Płachno et al. 2012). However, stomatocysts were recorded inside traps of other carnivorous plants from the genus *Genlisea* (Płachno & Wołowski, 2008).

The communities of algae in Jeleniak Mikuliny Nature Reserve are typical for low pH peat-bog habitats. All the morphotypes described occurred among 96 taxa of algae belonging to Cyanophyceae (4), Bacillariophyceae (20), Chrysophyceae (1), Raphidiophyceae (1), Xantophyceae (1), Cryptophyceae (2), Dinophyceae (1), Euglenophyceae (24), Chlorophyceae (19), and Zygnematophyceae (27). We observed the dominance of desmids, euglenophytes and diatoms in the company of *Utricularia* sp.

The large number of euglenophytes indicated the high concentration of nutrients (Wołowski 2003) in the water surrounding the traps. On the surface of the trap entrance there are glandular hairs, which produce mucilage. These substances may attract euglenophytes, desmids and diatoms. Thus, this would explain the high density of the algae near bladderwort traps. It is very interesting that we did not find loricate taxa such as *Strombomonas* and *Trachelomonas* (Wołowski & Hindák 2004, Wołowski & Walne 2007). We mostly found representatives of *Euglena* and *Phacus* and colourless taxa such as *Anisonema dexiotaxum*, *Enthosiphon sulcatus*, *E. ovatus*, *Menoidium pellucidum*, *Peranema macromastix* and *Petalomonas mediocanellata*. *P. praegnans* and *P. sphagnophila* were rarely reported.

Thus, our study contributes to the understanding of the biodiversity of a valuable natural area, and also contributes to a better understanding of the microhabitats of carnivorous bladderworts.

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