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CHRYSOPHYTE STOMATOCYSTS OF THE SULPHURIC SALT MARSH IN THE OWCZARY RESERVE (CENTRAL POLAND)

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Abstract: Eleven chrysophycean stomatocysts are reported from sulphuric saline waters in the Owczary Reserve in Central Poland. Of these, two morphotypes are new to science, one is new to Europe, and one is new to Poland. All specimens were represented only by unornamented stomatocysts without a collar. These studies provide the first documentation of an assemblage of extant chrysophycean stomatocysts in sulphuric saline waters of Poland. All cysts are described according to International Statospore Working Group (ISWG) guidelines and illustrated with SEM micrographs.

Key words: Stomatocysts, chrysophytes, taxonomy, sulphuric saline waters, Owczary Reserve, Poland

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INTRODUCTION

Chrysophycean stomatocysts have been studied mainly in freshwater habitats so far, mostly in arctic and alpine environments (e.g., Duff *et al.* 1995; Pla 2001; Wilkinson *et al.* 2001). The only available work presenting the results of an investigation of stomatocysts occurring in brackish waters is a paper by Rull and Vegas-Vilarrúbia (2000), which describes and discusses several chrysophycean stomatocysts found in mangrove habitats of the Caribbean Sea, and also cites several older studies on stomatocysts found in pre-Cretaceous and Tertiary fossils collected in marine sediments from the Gulf of Mexico, the Caribbean and the Atlantic.

In 2004 we collected water samples to study chrysophycean stomatocysts occurring in sulphuric saline waters of the Owczary Reserve in Central Poland. The Owczary Reserve is a unique habitat, with several halophytic plant species (e.g., Wóycicki 1915; Dziubałtowski 1916; Piech 1934; Trzcińska-Tacik 1988, 1995), interesting halophilous algae such as *Vaucheria dichotoma* (L.) C. Agardh and sulphuric or halophilous bacteria (Topińska-Luchter 1951; Piątek & Piątek 2005). The present study documents an assemblage of chrysophycean stomatocysts in sulphuric saline waters of Poland for the first time. It is probably only the second study on extant stomatocysts known from brackish habitats. All stomatocysts are fully described and illustrated with SEM micrographs.

MATERIAL AND METHODS

Material was collected on 3 May and 12 July 2004 from the Owczary Reserve. The reserve is one of the most interesting places in Central Poland, harboring halophyte flora. The reserve covers 0.62 ha and includes a small fragment of marshy meadow with a sulphuric saline spring. The Owczary Reserve is described in more detail in Piątek & Piątek (2005). The materials were collected in 120 ml plastic containers from the sulphuric salt marsh in the central part of the reserve and directly from the sulphuric saline spring. The collections included (i) water from the spring, (ii) sediment from the bottom of the sulphuric salt marsh (including a drainage ditch) in the central part of the reserve, at 0–10 cm depth, and (iii) water squeezed from *Vaucheria dichotoma* from the central part of the reserve.

The chemical and physical parameters of the water from the sulphuric saline spring and drainage ditch were analyzed on 10 July 2004 immediately after sampling, using the Aquamerck® Compact Laboratory for Water Testing (Merck). This equipment enables determination of ammonium, carbonate hardness (acid-binding capacity), total hardness, nitrates, nitrites, pH and oxygen. The tests were based on colorimetric and titrimetric methods described in the manufacturer's instructions, available from the authors upon request. Water temperature and conductivity ($\mu S \cdot cm^{-1}$) were measured immediately after collection using a CC-102 conductivity meter (Elmetron). The chemical and physical parameters of the sulphuric saline spring and drainage ditch are presented in Table 1.

Table 1. Chemical and physical parameters of water in the central part of the sulphuric salt marsh in the Owczary Reserve.

Chemical and physical parameters	Measurements
Ammonium (NH_4^+) mg $\cdot l^{-1}$	4.0
Nitrite (NO_2^-) mg $\cdot l^{-1}$	0.025
Nitrate (NO_3^-) mg $\cdot l^{-1}$	below detection
pH	7.8
Total hardness °d	142.24
Carbonate hardness (acid-binding capacity) °d	22.96
Oxygen (O_2) mg $\cdot l^{-1}$	9.1
Temperature of water °C	29
Conductivity $\mu S \cdot cm^{-1}$	19 990

Scanning electron microscopy (SEM) micrographs were taken in the Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences of the Jagiellonian University. SEM preparations were made as described in Cabała & Piątek (2004). Cysts were measured and described from SEM micrographs according to International Statospore Working Group (ISWG) guidelines (Cronberg & Sandgren 1986). Taxonomy follows Duff *et al.* (1995), Facher and Schmidt (1996), Vorobyova *et al.* (1996), van de Vijver & Beyens (1997a, b, 2000), Mrozińska *et al.* (1998), Rull and Vegas-Vilarrúbia (2000), Hansen (2001), Kamenik *et al.* (2001), Pla (2001), Wilkinson *et al.* (2001), Cabała (2002, 2003a, b, 2005a), Cabała and Piątek (2004) and Wołowski *et al.* (2004).

New stomatocysts not previously published were assigned numbers from Piątek J., beginning with stomatocyst #31, Piątek J., and are cited as 'this paper'. The numbering system is continuation of numbering of stomatocysts under the name Cabała J. (Cabała 2002, 2003a, b, 2005a, b; Cabała & Piątek 2004; Wołowski *et al.* 2004) with recent described stomatocyst 30, Cabała J. (Cabała 2005b), and is due to the change of the author's name to Piątek J. Such decision was made after suggestions of Professors John P. Smol and Barbara A. Zeeb, who proposed some of guidelines for descriptions of stomatocysts, concluding that it is better to continue a numbering sequence if the author remains the same, instead of starting a new numbering scheme with an author's new name.

'Number of specimens' refers to the number of scanning electron micrographs used for the description of the stomatocysts. Negatives are deposited in the Iconotheca of Algae (KRAM) in the Department of Phycology of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

RESULTS

The chrysophycean stomatocysts were found only in the sulphuric salt marsh, that is, in samples from bottom sediment and in water squeezed from *Vaucheria dichotoma*. None were obtained from the sulphuric saline spring. All stomatocysts were viewed by scanning electron microscopy (SEM). The recorded cysts are grouped by common morphological characteristics following Duff *et al.* (1995), Pla (2001) and Wilkinson *et al.* (2001). Asterisks indicate morphotypes new to Poland (*), new to Europe (**), and described here for the first time (***).

UNORNAMENTED STOMATOCYSTS

SPHERICAL, NO COLLAR, NO ORNAMENTATION

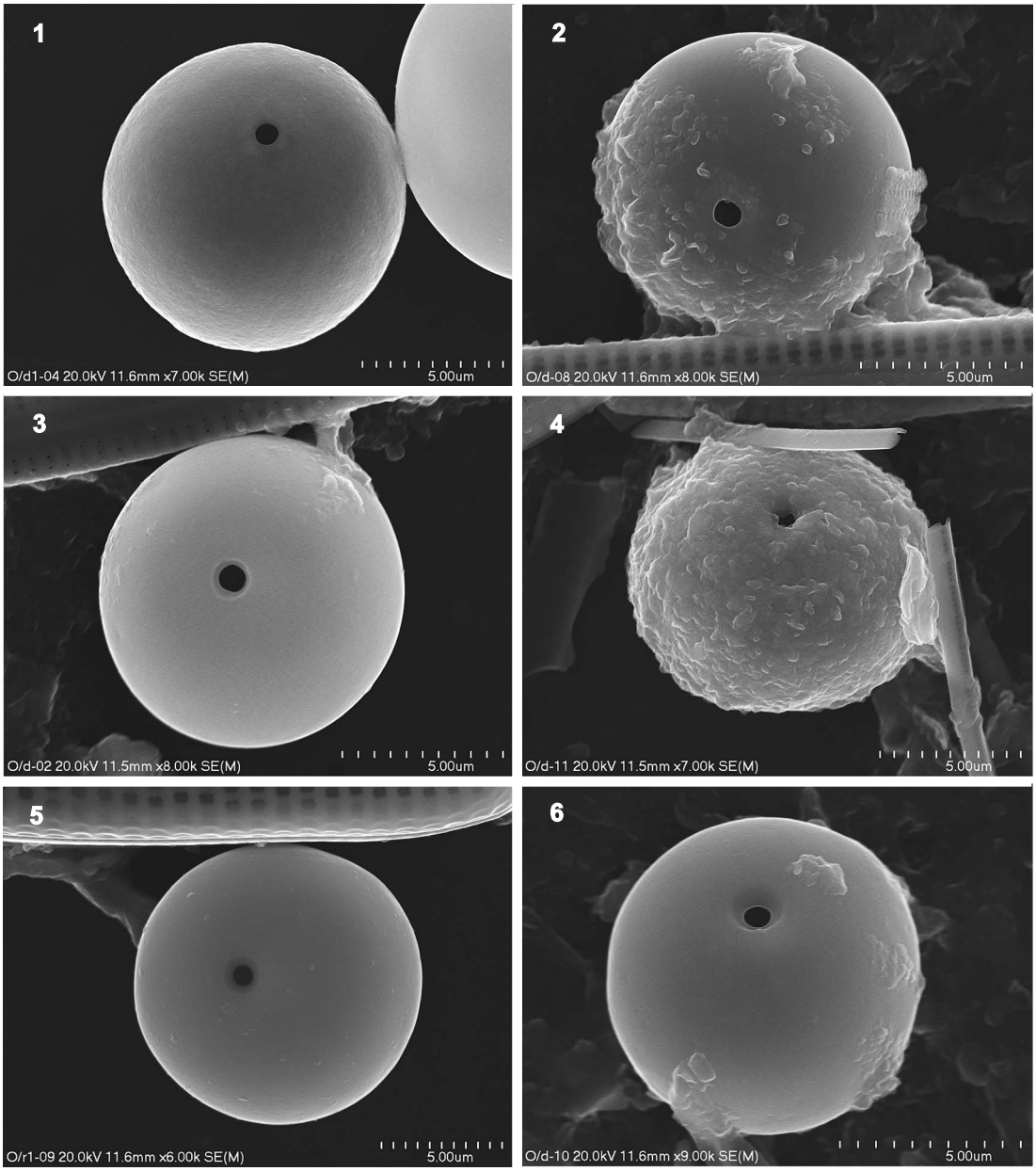
Stomatocyst 15, Duff & Smol 1988 *emend.* Zeeb & Smol 1993 (Figs 1 & 2)

NEGATIVE NUMBER. O/d1-04.

NUMBER OF SPECIMENS. 4.

LOCALITY. Owczary Reserve (50°27'N/20°45'E), bottom sediment.

BIOLOGICAL AFFINITY. This cyst probably is



Figs 1–6. 1 – Stomatocyst 15; 2 – Stomatocyst 15 with crystals of salt on part of the cyst body; 3 – Stomatocyst 16; 4 – Stomatocyst 16 with crystals of salt on all of the cyst body; 5 – Stomatocyst 42; 6 – Stomatocyst 120.

produced by several species, including *Chrysosphaerella brevispina* Korsikov (according to Duff *et al.* 1995).

DESCRIPTION. This is a smooth, spherical stomatocyst, 9.6–11.8 μm in diameter. The pore is regular, 0.8–0.9 μm in diameter, and no collar is present.

NOTES. The specimen shown in Figure 2 is

covered by crystals of salt (for more remarks, see Discussion).

DISTRIBUTION. This stomatocyst has been found in Canada, the U.S.A., Greenland (Duff *et al.* 1995), the Spanish Pyrenees (Pla 2001), and in Poland in the Staszów sink-hole (Wołowski *et al.* 2004) and the Staw Toporowy Wyżni peat bog in the Tatra National Park (Cabała 2005a).

** **Stomatocyst 16**, Hansen 2001 (Figs 3 & 4)

NEGATIVE NUMBER. O/d-02.

NUMBER OF SPECIMENS. 3.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), bottom sediment and water squeezed from *Vaucheria dichotoma*.

BIOLOGICAL AFFINITY. Unknown.

DESCRIPTION. This is a smooth, spherical stomatocyst, 8.9–12.6 μm in diameter. The regular pore is 0.5–0.8 μm in diameter, with a planar pseudoannulus.

NOTES. This stomatocyst is distinguished from stomatocyst 42, Duff & Smol 1989 on the basis of pore morphology, and from stomatocyst 120, Duff & Smol *emend.* Zeeb & Smol 1993 on the basis of size and pore morphology. The specimen shown in Figure 4 is covered by crystals of salt (for more remarks, see discussion).

DISTRIBUTION. This stomatocyst has been found in the Azores (Hansen 2001).

Stomatocyst 42, Duff & Smol 1989 (Fig. 5)

NEGATIVE NUMBER. O/r1-09.

NUMBER OF SPECIMENS. 8.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), bottom sediment and water squeezed from *Vaucheria dichotoma*.

BIOLOGICAL AFFINITY. This stomatocyst is produced by more than one species (according to Duff *et al.* 1995).

DESCRIPTION. This is a smooth, spherical stomatocyst, 10.0–11.8 μm in diameter. The pore is concave, 0.4–0.8 μm (usually 0.5 μm) in diameter.

DISTRIBUTION. This stomatocyst has been found in Canada, the U.S.A. (Duff *et al.* 1995), South Georgia (van de Vijver & Beyens 1997b), the Spanish Pyrenees (Pla 2001) and Poland (Duff *et al.* 1995; Wołowski *et al.* 2004).

Stomatocyst 120, Duff & Smol *in* Duff *et al.* 1992, *emend.* Zeeb & Smol 1993 (Fig. 6)

NEGATIVE NUMBER. O/d-10.

NUMBER OF SPECIMENS. 3.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), bottom sediment.

BIOLOGICAL AFFINITY. This cyst probably is produced by more than one species, such as *Chrysosphaerella longispina* Lauterborn *emend.* K. H. Nicholls (Sandgren 1989).

DESCRIPTION. This is a smooth, spherical stomatocyst, 6.8–8.5 μm in diameter. The pore is concave, 0.5–0.7 μm in diameter, and no collar is present.

NOTES. This stomatocyst is distinguished from stomatocyst 16, Hansen 2001 on the basis of size and pore morphology.

DISTRIBUTION. This stomatocyst has been found in Canada, the U.S.A. (Duff *et al.* 1995), Central Europe (Facher & Schmidt 1996), South Georgia (van de Vijver & Beyens 1997b, 2000), the Spanish Pyrenees (Pla 2001), Austria (Kamenik *et al.* 2001) and several places in Poland (Cabała 2002, 2004, 2005a).

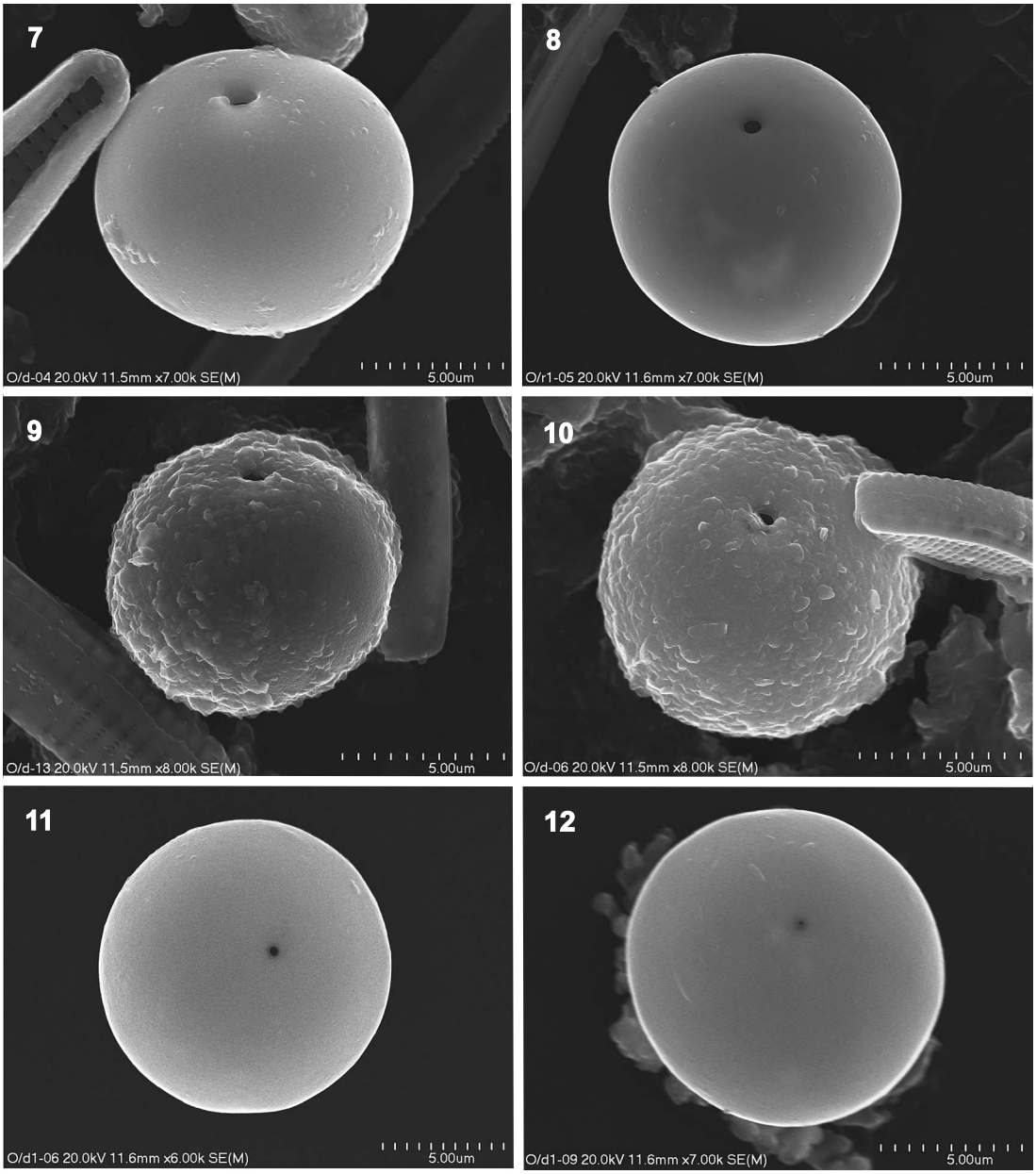
* **Stomatocyst 150**, Zeeb & Smol 1993 (Fig. 7)

NEGATIVE NUMBER. O/d-04.

NUMBER OF SPECIMENS. 4.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), bottom sediment and water squeezed from *Vaucheria dichotoma*.

BIOLOGICAL AFFINITY. Unknown.



Figs 7–12. 7 – Stomatocyst 150; 8 – Stomatocyst cf. 189; 9–10 – Stomatocyst cf. 189 with crystals of salt on part and on all of the cyst body; 11–12 – Stomatocyst #31.

DESCRIPTION. This is a smooth, spherical stomatocyst, 8.2–11.4 μm in diameter. The pore is regular, 0.7–0.8 μm in diameter, and no collar is present.

DISTRIBUTION. This stomatocyst has been found in Canada, the U.S.A. (Duff *et al.* 1995) and the Spanish Pyrenees (Pla 2001).

Stomatocyst cf. 189, Zeeb & Smol *in* Zeeb *et al.* 1996 (Duff *et al.* 1995) (Figs 8–10)

NEGATIVE NUMBER. O/r1-05.

NUMBER OF SPECIMENS. 5.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), bottom sediment and water squeezed from *Vaucheria dichotoma*.

BIOLOGICAL AFFINITY. Unknown, but may be produced by a cold-tolerant species (according to Duff *et al.* 1995).

DESCRIPTION. This is a smooth, spherical stomatocyst, 8.9–10.4 μm in diameter. The pore is regular to slightly conical, 0.6–0.7 μm in diameter, and no collar is present.

NOTES. The specimens found in the Owczary Reserve are larger then in the original description (6.0–8.9), but otherwise match well with the concept of this morphotype. The specimens shown in Figures 9 and 10 are covered by crystals of salt (for more remarks, see discussion).

DISTRIBUTION. This stomatocyst has been found in Canada, the U.S.A., Greenland (Duff *et al.* 1995), the Spanish Pyrenees (Pla 2001) and two places in Poland (Cabała 2002; Wołowski *et al.* 2004).

*** **Stomatocyst #31**, Piątek J., this paper (Figs 11 & 12)

NEGATIVE NUMBER. Piątek J., negative O/d1-06, Fig. 11.

NUMBER OF SPECIMENS. 3.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), bottom sediment.

BIOLOGICAL AFFINITY. Unknown.

DESCRIPTION. This is a smooth, spherical stomatocyst, 10.5–12.2 μm in diameter. The pore is small and concave, 0.2–0.3 μm in diameter.

NOTES. This stomatocyst differs in pore morphology from the original description given for stomatocyst 64, Rull & Vegas-Vilarrúbia 2000, and in pore morphology and cyst body shape from the description of stomatocyst 67, Rull & Vegas-Vilarrúbia 2000. However, both of those stomatocysts were described only on the basis of LM micrographs that do not show many important details of pore and collar morphology. Stomatocyst #31, Piątek J. is distinguished from stomatocyst #32, Piątek J. on the basis of cyst body shape (#31 is spherical, #32 is obovate) and mainly pore morphology. Stomatocyst #31 has a concave pore, whereas #32 has a planar pseudoannulus with a regular pore, visible in Figure 17. Stomatocyst #31 is characterized mostly on the basis of pore morphology, which is only visible by SEM. It cannot be determined by light microscopy.

DISTRIBUTION. We have not definitively identified this stomatocyst elsewhere, and tentatively consider this cyst to be connected with sulphuric or, more probably, saline waters.

OVAL, NO COLLAR, NO ORNAMENTATION

Stomatocyst 19, Duff & Smol 1988 (Fig. 13)

NEGATIVE NUMBER. O/r-03.

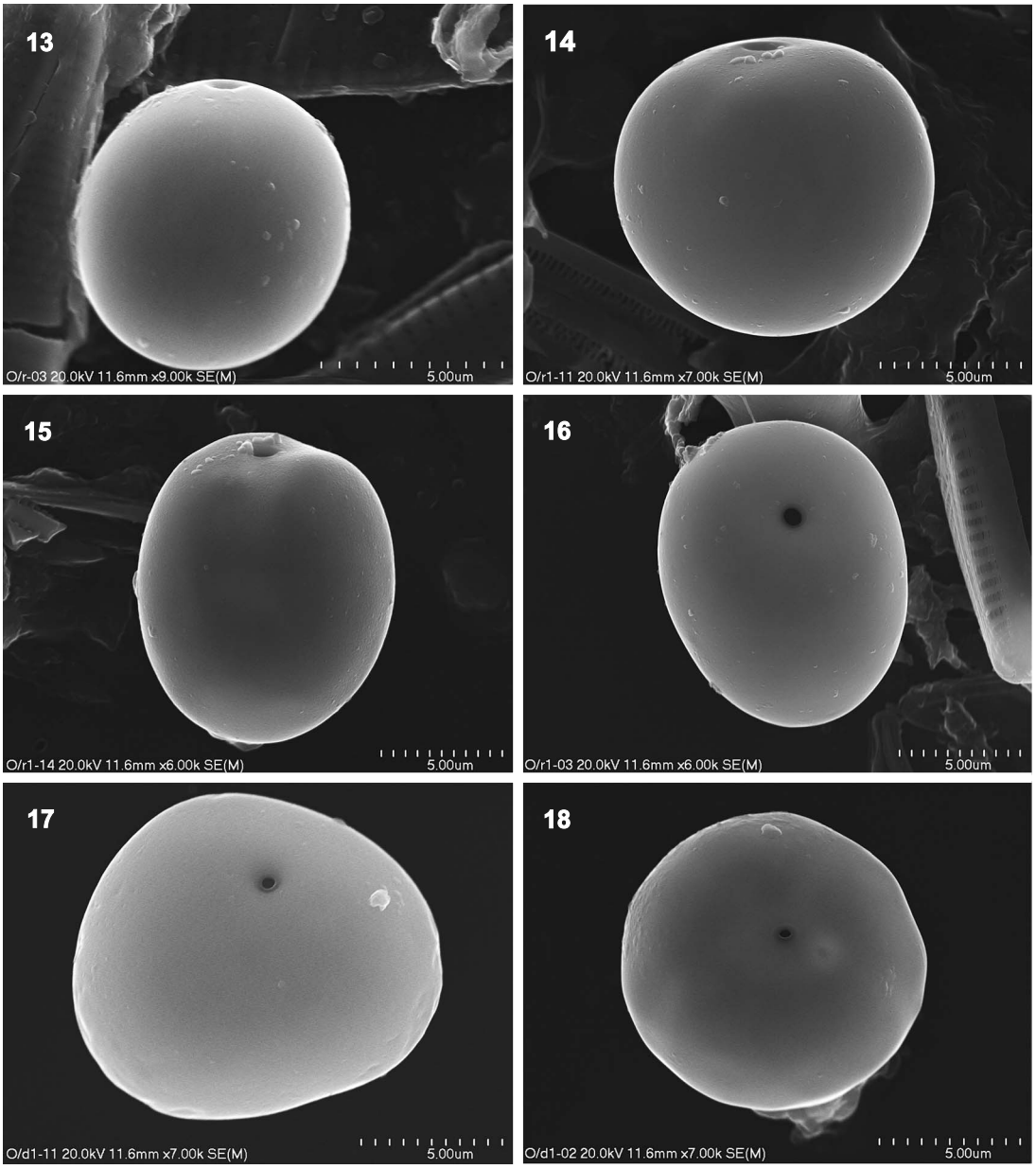
NUMBER OF SPECIMENS. 1.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), water squeezed from *Vaucheria dichotoma*.

BIOLOGICAL AFFINITY. This cyst is similar to the cyst produced by several *Epipyxis* species (according to Duff *et al.* 1995), perhaps *Epipyxis tubulosa* (Mack) D. K. Hilliard & Asmud (Hilliard & Asmund 1963).

DESCRIPTION. This is a smooth, oval stomatocyst, 7.9 μm long, 7.4 μm wide. The pore is not visible, and no collar is present.

DISTRIBUTION. This stomatocyst has been found in Canada (Duff *et al.* 1995), Central Europe (Facher & Schmidt 1996) and in Poland in the Budzyń peat bog near Kraków (Cabała 2002) and the Staw Toporowy Wyżni peat bog in Tatra National Park (Cabała 2005a).



Figs 13–18. 13 – Stomatocyst 19; 14 – Stomatocyst 49; 15–16 – Stomatocyst 196; 17–18 – Stomatocyst #32.

OBLATE, NO COLLAR, NO ORNAMENTATION

Stomatocyst 49, Duff & Smol 1991 *emend*. Zeeb & Smol 1993 (Fig. 14)

NEGATIVE NUMBER. O/r1-11.

NUMBER OF SPECIMENS. 3.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), bottom sediment and water squeezed from *Vaucheria dichotoma*.

BIOLOGICAL AFFINITY. This cyst probably is

produced by *Chrysosphaerella longispina* Lauterborn *emend.* Nichols (Sandgren 1989).

DESCRIPTION. This is a smooth, oblate stomatocyst, 9.2–10.6 μm long, 10.5–11.4 μm wide. The pore is not visible, and no collar is present.

DISTRIBUTION. This stomatocyst has been found in Canada, the U.S.A. (Duff *et al.* 1995), Central Europe (Facher & Schmidt 1996), South Georgia (van de Vijver & Beyens 1997, 2000), Austria (Kamenik *et al.* 2000), the Spanish Pyrenees (Pla 2001) and Poland in the Budzyń peat bog near Kraków (Cabała 2002).

OBOVATE, NO COLLAR, NO ORNAMENTATION

Stomatocyst 196, Duff & Smol 1994 *emend.* Wilkinson & Smol 2001 (Figs 15 & 16)

NEGATIVE NUMBER. O/r1-03.

NUMBER OF SPECIMENS. 3.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), water squeezed from *Vaucheria dichotoma*.

BIOLOGICAL AFFINITY. This cyst may be produced by *Chrysoxys major* Skuja (according to Wilkinson *et al.* 2001).

DESCRIPTION. This is a smooth, obovate stomatocyst, 12.4–13.0 μm long, 10.6–12.7 μm wide. The pore is conical or slightly concave, and no collar is present.

NOTES. This stomatocyst is very similar to stomatocyst 67, Rull & Vegas-Vilarrúbia 2000, which according to the original description has a regular or conical pore, but this character is not visible in the LM micrograph given. Because Rull and Vegas-Vilarrúbia (2000) did not include any SEM micrograph of their new morphotype, we consider stomatocyst 67, Rull & Vegas-Vilarrúbia 2000 to be open to question.

DISTRIBUTION. This stomatocyst has been found in Denmark, the U.S.A., Canada (Wilkinson *et al.* 2001), and Poland in the Staszów sink-hole (Wołowski *et al.* 2004).

*** **Stomatocyst #32**, Piątek J., this paper (Figs 17 & 18)

NEGATIVE NUMBER. Piątek J., negative O/d1-11, Fig. 17.

NUMBER OF SPECIMENS. 2.

LOCALITY. Owczary Reserve (50°27′N/20°45′E), bottom sediment.

BIOLOGICAL AFFINITY. Unknown.

DESCRIPTION. This is a smooth, obovate stomatocyst, 11.0–13.0 μm in diameter. The pore is regular, 0.4 μm in diameter, with a planar pseudoannulus.

NOTES. This stomatocyst differs in size, pore morphology and cyst body shape from stomatocyst 15, Duff and Smol 1988 *emend.* Zeeb and Smol 1993; it differs in cyst body size and shape from stomatocyst 120, Duff & Smol *emend.* Zeeb & Smol 1993; it differs in pore morphology from the original descriptions of stomatocysts 66 and 67, Rull & Vegas-Vilarrúbia 2000. Stomatocyst #32, Piątek J. differs in cyst body shape and pore morphology from stomatocyst #31, Piątek J. It is characterized mainly on the basis of pore morphology, and therefore cannot be determined without SEM studies (see also discussion under stomatocyst #31).

DISTRIBUTION. We have not definitively identified this stomatocyst elsewhere, and tentatively consider it to be connected with sulphuric or, more probably, saline waters.

DISCUSSION

The present studies document an assemblage of chrysophycean stomatocysts in sulphuric saline waters of Poland for the first time. In the collected material we identified 11 stomatocysts. Of these, two morphotypes are new to science, one is new to Europe, and one is new to Poland. It was difficult or even impossible to identify some stomatocysts because untypical, irregular 'ornamentation' resembling verrucae were visible on all or part of the cyst body (Figs 2–4, 9–10). They

may be remnants of reagents after chemical reactions during preparation of the material or, more probably, salt crystals present on the cyst bodies in the natural habitat. In previous studies (e.g., Cabała 2002, 2003a–b, 2005a; Cabała & Piątek 2004) in which the material was prepared by the same method as here, such 'ornamentation' has not been observed, so we conclude that they were formed by salt crystals.

The assemblage of stomatocysts in the Owczary Reserve has low diversity of morphotypes but is not dominated by one stomatocyst as in the tropical brackish water studied in the Caribbean mangrove, in which stomatocyst 64, Rull & Vegas-Vilarrúbia 2000 constituted 90% of the assemblage (Rull & Vegas-Vilarrúbia 2000). The most interesting character of the community of stomatocysts in the reserve is the presence of only unornamented stomatocysts without a collar. Similar observations were made by Rull and Vegas-Vilarrúbia (2000) during their studies in the Caribbean mangrove. They also found only smooth stomatocysts, but unlike in our observations, some percentage of them had a collar.

Another striking feature of the community of stomatocysts in the Owczary Reserve is the presence of morphotypes with similar biometric characters. Seven morphotypes are spherical in shape and range from (6.8–)8.5–12.2 μm, with small pores usually 0.5–0.8 μm in diameter (whole range 0.2–0.9 μm). Notably, the pores are always less than 1 μm in diameter. Two stomatocysts are obovate, 10.6–13.0 μm in diameter, and have small pores 0.4–0.7 μm in diameter. One is oval and another is oblate, ranging 7.4–7.9 μm and 9.2–10.6 × 10.5–11.4 μm, respectively. These data suggest that relatively large cyst body size combined with small pore size may be a characteristic feature of stomatocysts connected with or tolerant to saline waters. The combination of large cyst body size (10.5–13.0 μm in diameter) and small pore size is evident especially in two new stomatocysts, which are probably halophilous: stomatocyst #31, Piątek J. (pore diameter 0.2–0.3 μm) and stomatocyst #32, Piątek J. (pore diameter 0.4 μm).

These results suggest that some of the stomatocysts found in the Owczary Reserve are probably produced by freshwater chrysophytes widely distributed and tolerant to salinity, and that some of them may be formed by taxa associated with saline habitats. The low morphotype diversity of the stomatocysts can be explained by the probability that saline waters are not favorable habitats for chrysophytes. According to Round (1981) the two major microscopic algal groups colonizing salt marshes are blue-green algae and diatoms.

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