Sphagnum-associated chrysophytes from the "Dermansko-Ostrozsky" National Nature Park (Ukraine)

Dmitry Kapustin, Maxim Kulikovskiy

Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia

SUMMARY

Eight chrysophyte species and eight chrysophycean stomatocyst morphotypes associated with *Sphagnum* were recorded from a peatland (fen) within the "Dermansko-Ostrozsky" National Nature Park. The morphology of siliceous scales and stomatocysts was studied using scanning electron microscopy (SEM). *Poterioochromonas malhamensis* was recorded for the first time in Ukraine, and one of the chrysophycean stomatocyst morphotypes described was new to science.

KEY WORDS: golden-brown algae, morphology, resting stage, SEM, siliceous cysts, siliceous scales

INTRODUCTION

Chrysophytes, or golden-brown algae, compose one of the most diverse groups of microeukaryotes inhabiting peatlands. Their ecological preferences arise from the absence of carbonic anhydrase enzymes in most chrysophytes (Andersen 2021). In acidic environments, dissolved carbon dioxide is relatively abundant and potential pH shifts are moderated by organic buffers, so chrysophytes can take up inorganic carbon.

The resting stages of chrysophytes, called stomatocysts, may be formed as a result of either asexual or sexual processes (Sandgren 1983). Stomatocysts are siliceous and have a rather diverse external morphology.

The species composition of chrysophytes and the morphological diversity of chrysophycean stomatocysts from peat bogs around the world have been the subject of many studies (e.g. Matvienko 1952, Ellis-Adam 1983, Piątek 2007, Cambra 2010, Pang *et al.* 2012, Pang & Wang 2014, Bai *et al.* 2018, 2020). However, the only data on chrysophytes from the the "Dermansko-Ostrozsky" National Nature Park (Ukraine) that are available in the literature relate to the single species *Synura synuroidea* (=*Chrysodidymus synuroideus*) (Kapustin & Gusev 2016, Siver *et al*. 2018).

The aim of this study is to explore the diversity of chrysophytes and their stomatocysts from a *Sphagnum* site within the territory of the "Dermansko-Ostrozsky" National Nature Park.

METHODS

National Nature Park "Dermansko-Ostrozsky" is situated within the Ostrog and Zdolbuniv districts of Rivne Region (Ukraine). Its total area is 5448.3 ha and it has a warm-summer humid continental climate according to the Köppen-Geiger climate classification (Peel *et al.* 2007). The average annual temperature is +18 °C in July and -5 °C in January, and the average annual precipitation is 600–650 mm (Andrienko *et. al.* 2012). The vegetation cover is dominated by forests, but fens and marshy meadows are also present. The flora and vegetation are described in detail by Andrienko *et al.* (2012).

A single sample was collected on 09 September 2014 from a *Sphagnum* site on the Bushchanske fen (50° 17' 54.6" N, 26° 11' 54.9" E), which belongs to the Important Plant Areas of Ukraine (Holovko & Onyshchenko 2017). The sample was obtained by squeezing water from *Sphagnum*. Environmental variables were not measured.

Light microscopical (LM) observations were made using a Carl Zeiss AxioScope A1 microscope at ×100 magnification with a Carl Zeiss AxioCam ERc 5s camera. For scanning electron microscopical (SEM) studies, an aliquot of the sample was digested for 4–5 minutes in sulphuric acid with potassium dichromate, then washed by repeated centrifugation in deionised water. A few drops of the washed sample were air-dried on aluminum stubs. The stubs were sputter coated with gold for ten minutes, then examined with a JEOL JSM-6510 LV scanning

electron microscope (Papanin Institute for Biology of Inland Water, Russian Academy of Sciences, Borok, Russia).

For stomatocyst descriptions we used the terminology according to Duff *et al.* (1995) and Wilkinson *et al.* (2001). The new morphotype was assigned a number from Kapustin *et al.* (2016), beginning with Stomatocyst 5.

RESULTS

In total, eight chrysophyte species and eight stomatocyst morphotypes were recorded from the *Sphagnum* site on the Bushchanske fen. The species belong to four genera, namely *Poterioochromonas*, *Paraphysomonas*, *Mallomonas* and *Synura*. The biological affinities of the eight stomatocysts are unknown. The scales of two taxa, *Mallomonas paludosa* and *Synura sphagnicola*, were the most frequently observed. *Poterioochromonas malhamensis* was recorded for the first time in Ukraine. Also, we found stomatocysts of this species. Among the stomatocyst morphotypes, Stomatocysts 86 and 135 were the most abundant. We could not identify one morphotype using available literature and described it as new to science. All taxa and stomatocysts are briefly characterised below.

Poterioochromonas malhamensis (E.G. Pringsh.) L.Ş. Péterfi ex R.A. Andersen *et al.* **(Figure 1)**

This is a new generic record for Ukraine. A golden neuston film consisting of *P. malhamensis* appeared on the water surface after the sample had been stored on a windowsill for a few weeks. Pyriform to spherical cells were attached to a goblet-shaped hyaline lorica (7.85–7.94 µm wide, 6.6–7.21 µm long) with a long (20.8–29.9 µm) stalk. Some specimens were encysting so we could observe stomatocysts of this species (see below).

Paraphysomonas sp. **(Figure 2)**

Only a single scale with a broken spine was observed, so its identification was impossible.

Mallomonas paludosa Fott **(Figures 3, 4)**

This is a typical acidobiontic species. In Ukraine it has previously been recorded from water bodies in the Polessian Nature Reserve (Kapustin *et al.* 2020).

Mallomonas papillosa K. Harris & D.E. Bradley emend. K. Harris **(Figures 5, 6)**

We observed either scales covered with scattered papillae (see Figure 5) or scales with a smooth shield (see Figure 6). Similar scales have been observed in samples from the water bodies of the Polessian Nature Reserve (Kapustin *et al.* 2020).

Synura echinulata Korshikov **(Figures 7, 8)**

This is a typical acidobiontic species. The only previous Ukrainian records confirmed with EM were from the water bodies of the Polessian Nature Reserve (Kapustin *et al.* 2020).

Synura petersenii Korshikov **(Figure 9)**

This is a widespread species; however, EM or even molecular methods are required for its correct identification.

Synura sphagnicola (Korshikov) Korshikov **(Figure 10)**

This is a typical acidobiontic species. The only previous records from Ukraine that were confirmed with EM originate from the water bodies of the Polessian Nature Reserve (Kapustin *et al.* 2020).

Synura synuroidea (Prowse) Pusztai *et al.* **(Figure 11)**

This record (as *Chrysodidymus synuroideus* Prowse) was the subject of a separate publication (Kapustin & Gusev 2016).

Stomatocyst of *Poterioochromonas malhamensis* **(Figures 12, 13)**

This stomatocyst is spherical (8.6–10.6 µm in diameter) with a regular pore (0.6–0.78 µm in diameter) surrounded by a cylindrical primary collar (0.9–1.8 µm in diameter); the secondary collar (4.7–6.1 µm in diameter) consists of several wide lobes deflected inwards.

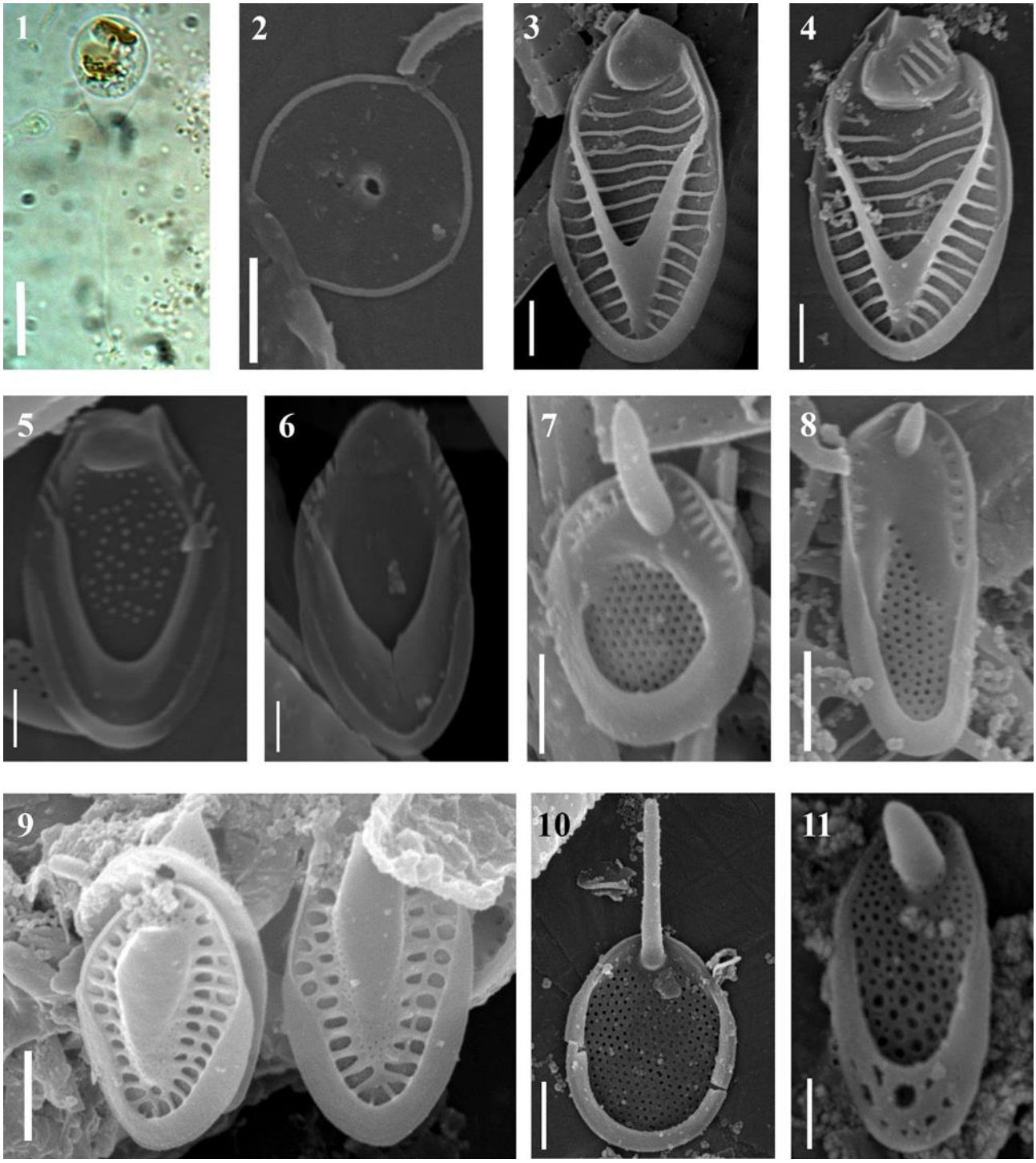
Several similar morphotypes have been described in the literature, e.g. Stomatocysts 136 *forma A* Duff & Smol emend. Gilbert & Smol (Gilbert *et al.* 1997), 47 and 51 (Hansen 2001). Stomatocyst 136 is oval with 4–5 inwardly flexed and flattened projections around the collar. Stomatocyst 47 is characterised by having collars consisting of three (rarely two) lobes, and Ignatenko *et al.* (2022) have suggested it is identical to the stomatocyst of *P. malhamensis*. Stomatocyst 51 has 3–4 inwardly flexed and flattened projections around the collar.

Stomatocyst 5 Kapustin, this paper **(Figure 14)**

Stomatocyst is spherical (15.3 µm in diameter) with a smooth surface; a regular pore (0.9 µm in diameter) is surrounded by a low cylindrical primary collar (height 0.4 µm, diameter 1.12 µm); the interannulus is very narrow and almost inconspicuous; the secondary collar is thick and cylindrical (height 1.4 µm, diameter 5.2 µm).

Locality: Ukraine, Rivne Region, Zdolbuniv District, env. of Mosty, Bushchanske fen, *Sphagnum* site. 09 September 2014. *leg. D. Kapustin.*
Picture-file number: 18MP-0240
Number of specimens: 1.

Biological affinity: unknown.
Comment: our morphotype is similar to Stomatocyst 16 (Duff & Smol 1988) but differs in having a significantly thicker secondary collar and larger size. Our morphotype can be differentiated from

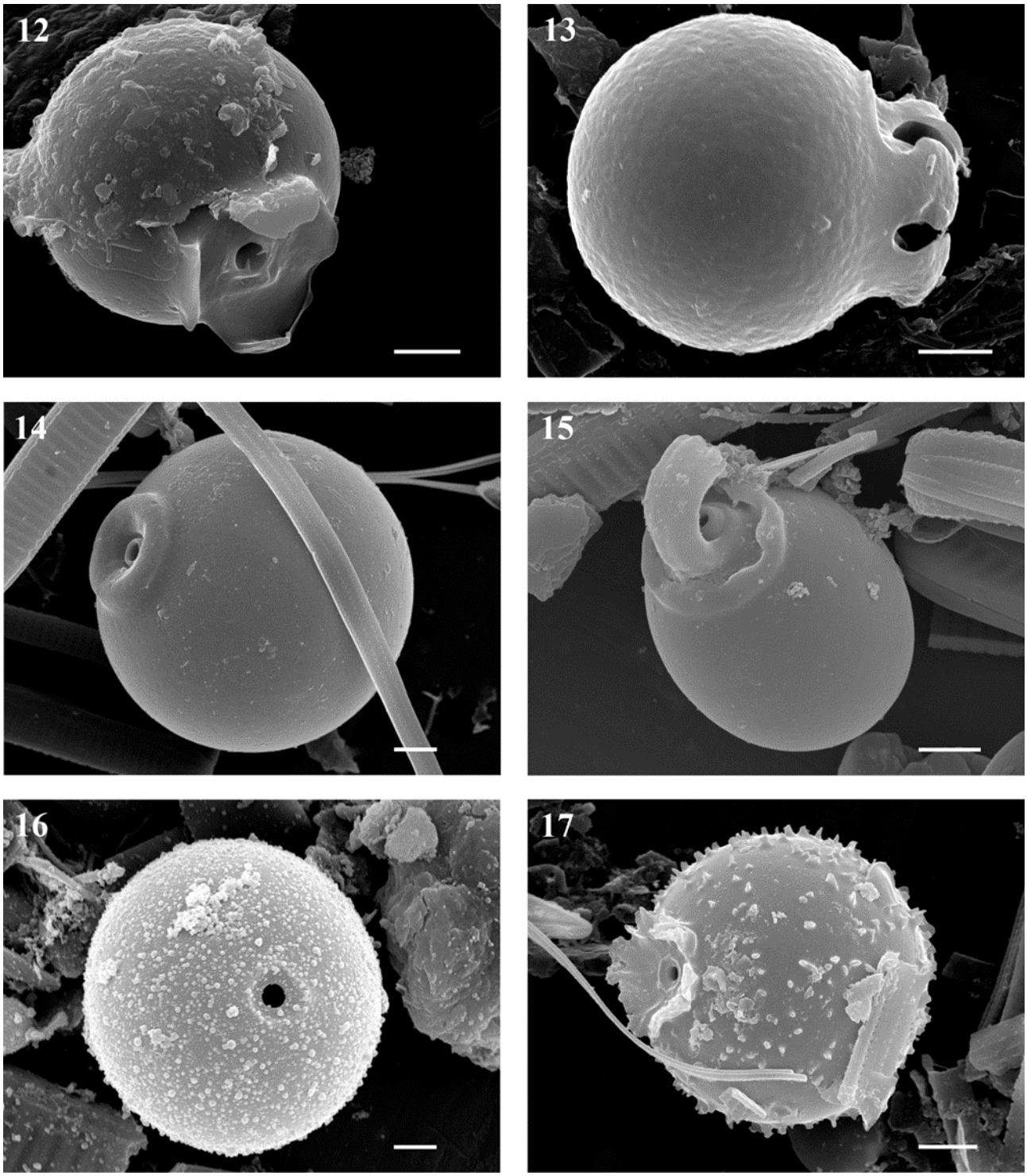


Figures 1–11. *Sphagnum*-associated chrysophytes from the National Nature Park "Dermansko-Ostrozsky". 1: *Poterioochromonas malhamensis*; 2: *Paraphysomonas* sp.; 3, 4: *Mallomonas paludosa*; 5, 6: *Mallomonas papillosa*; 7, 8: *Synura echinulata*, body scale (7) and transition scale (8); 9: *Synura petersenii*; 10: *Synura sphagnicola*; 11: *Synura synuroidea*. Scale bars: 5 µm (1); 1 µm (2–4 and 6–10); 0.5 µm (5, 11).

Stomatocyst 14 W. Pang & Q. Wang (2013) by the structure of the collar complex. Stomatocyst 14 has a simple conical collar which is lower (0.6 µm *vs.* 1.4 µm), narrower (2.7–3.8 µm *vs.* 5.2 µm) and thinner than in Stomatocyst 5, and the pore is surrounded by a swollen annulus.

Stomatocyst 135 Duff & Smol in Duff *et al*. 1992 **(Figure 15)**

This stomatocyst is oval (8.5 µm long, 8.1 µm wide) with a smooth surface; a regular pore (0.5 µm in diameter) surrounded by a conical primary collar (0.76 µm in diameter); the conical secondary collar

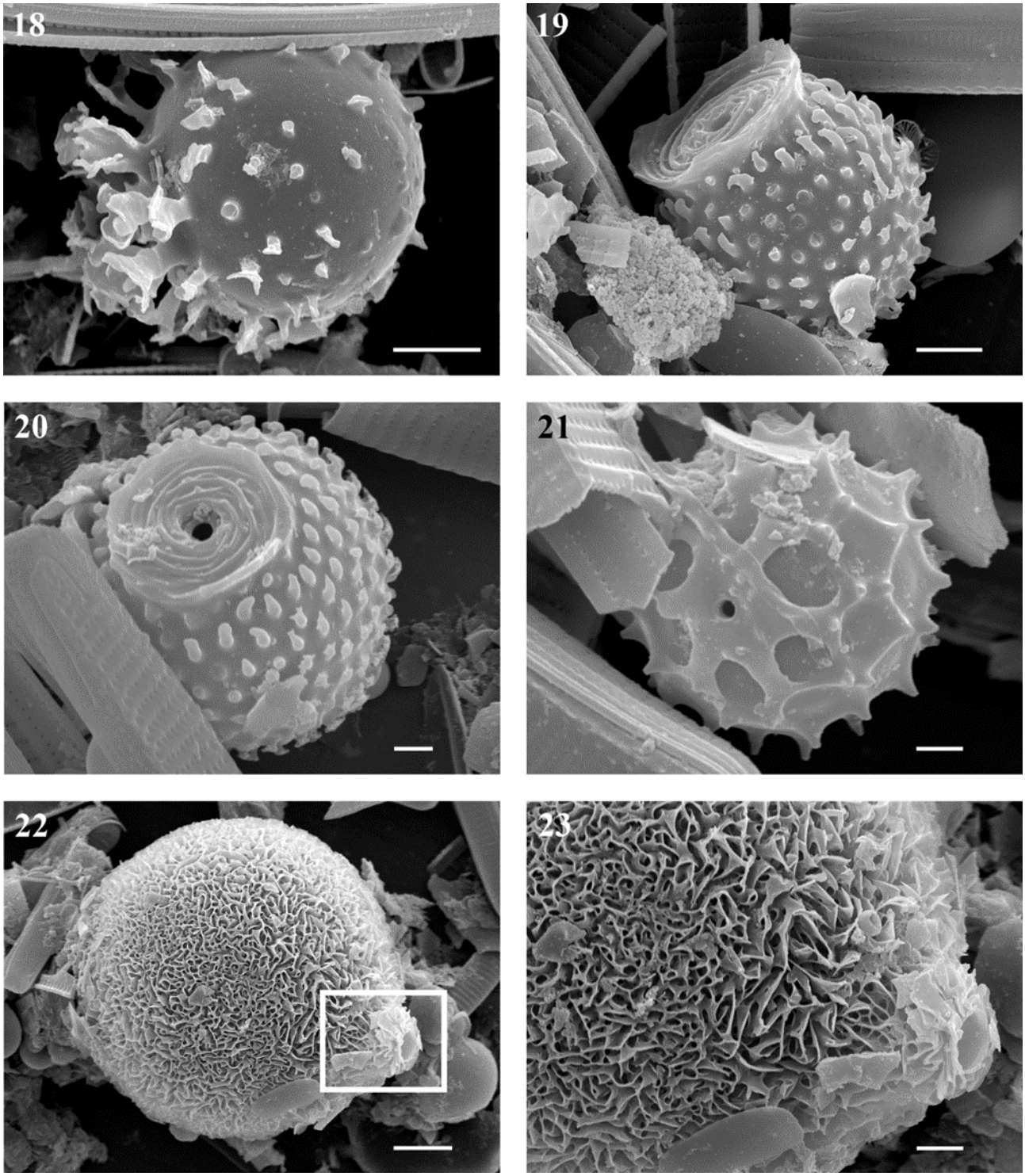


Figures 12–17. Chrysophycean stomatocysts from the "Dermansko-Ostrozsky" National Nature Park. 12, 13: stomatocysts of *Poterioochromonas* cf. *malhamensis*; 14: Stomatocyst 5 Kapustin; 15: Stomatocyst 135 Duff & Smol; 16: Stomatocyst 42 Facher & Schmidt; 17: Stomatocyst 76 Pang & Wang. Scale bars: 2 µm (12–15 and 17); 1 µm (16).

has an inwardly hooked projection; the tertiary collar is cylindrical (4.5 µm in diameter).

In contrast, Stomatocyst 134 Duff & Smol (Duff *et al.* 1992) has a simple conical collar with a hooked projection. Stomatocyst 43 Piątek (Piątek & Piątek 2008) differs by having a reticulate cyst body.

Stomatocyst 42 Facher & Schmidt 1996 **(Figure 16)** This stomatocyst is spherical (7.13 µm in diameter) with a concave pore (0.57 µm in diameter) surrounded by a low collar (1.28 µm in diameter). The cyst surface is covered with irregularly scattered verrucae.



Figures 18–23. Chrysophycean stomatocysts from the "Dermansko-Ostrozsky" National Nature Park. 18: Stomatocyst 80 W. Pang & Q. Wang; 19, 20: Stomatocyst 1 W. Pang & Q. Wang; 21: Stomatocyst 86 Duff & Smol; 22, 23: Stomatocyst 29 Pang & Wang. Scale bars: 5 µm (18, 22); 2 µm (19, 23); 1 µm (20, 21).

Stomatocyst 42 is similar to Stomatocyst 67 (Duff & Smol 1991) which, however, lacks the collar and has larger and less numerous verrucae.

Stomatocyst 76 W. Pang & Q. Wang 2014 **(Figure 17)**

This stomatocyst is spherical (10 µm in diameter); a flat planar annulus surrounds the conical pore (outer diameter 1.12 µm, inner diameter 0.46 µm); the collar is obconical (apical diameter 4.2 µm) with irregular margin. The cyst surface is ornamented with small ridges and spines.

Stomatocyst 80 W. Pang & Q. Wang 2014 **(Figure 18)**

This stomatocyst is spherical (16.4 µm in diameter) with a cylindrical collar (3.4 µm in diameter) surrounded by numerous baculate spines with foliaceous apices; the cyst surface is covered with baculate spines.

Stomatocyst 1 W. Pang & Q. Wang 2012 **(Figures 19, 20)**

This stomatocyst is spherical (7.6–8.4 µm in diameter) and ornamented with baculate spines; the immature spines have flattened apices whereas the mature ones have finger-like projections; a regular pore (0.5 µm in diameter) is surrounded by a low primary collar (0.9–1.0 µm in diameter); the interannulus has a series of concentric rings; the secondary collar is obconical (4.7–6.0 µm in diameter).

Stomatocyst 86 Duff & Smol 1991 **(Figure 21)**

This stomatocyst is spherical (6.3–7.2 µm in diameter) with a regular pore (0.46 in diameter); the collar is polygonal with a straight ridge radiating from each corner; the cyst surface is ornamented by a polygonal reticulum with an echinate spine located at each reticular interstice.

Stomatocyst 6 (Duff & Smol 1988) is similar to Stomatocyst 86 but has a different collar structure and lacks the echinate spines.

Stomatocyst 29 W. Pang & Q. Wang 2014 **(Figures 22, 23)**

This stomatocyst is spherical (diameter 28.7 µm) with a conical collar (apical diameter 2.75 µm, basal diameter 6.2 µm). The stomatocyst surface is covered by a complex reticulum consisting of laminal ridges. Our specimen did not have a long cylindrical collar, unlike Chinese specimens (Pang & Wang 2014). Probably, this long collar was broken or our specimen represents an immature stage of Stomatocyst 29.

DISCUSSION

From our single sample we uncovered eight chrysophyte species and eight chrysophycean stomatocysts associated with *Sphagnum* sp., and this number is rather significant. Almost all silica-scaled chrysophytes (i.e. taxa belonging to the genera *Paraphysomonas*, *Mallomonas* and *Synura*) have previously been reported from water bodies in the Ukrainian Polissia (Kapustin *et al.* 2020). However, the only Ukrainian record for *Synura synuroidea* (formerly *Chrysodidymus synuroideus*) is the *Sphagnum* site on the Bushchanske fen (Kapustin & Gusev 2016, Siver *et al.* 2018).

In the study reported here we recorded *Poterioochromonas malhamensis*, a typical myxotrophic loricate chrysophyte, for the first time in Ukraine. Additionally, we were able to observe its stomatocysts. Morphologically, these correspond well with the stomatocysts described by Andersen *et al.* (2017) and Chen *et al.* (2020).

One chrysophycean stomatocyst was described as new to science and seven morphotypes were identified. Although the biological affinities of these stomatocysts are unknown, they help to estimate the hidden diversity of chrysophytes. The record of Stomatocyst 1 is interesting in terms of biogeography. It was described from peatlands near Da'erbin Lake in China (Pang *et al.* 2012) and those authors supposed that it might be produced by an endemic species. The present record expands the distributional range of Stomatocyst 1. Moreover, this morphotype has also been observed in peatlands located in the Ukrainian Polissia, Moscow Region and Subpolar Urals (D. Kapustin, unpublished). It would be interesting to link this remarkable stomatocyst to the species that produce it. Other stomatocysts described from peaty localities in China, e.g. Stomatocysts 29, 76 and 80 (Pang & Wang 2014), have been recorded here for the first time since they were described.

Further studies are needed to expand our knowledge about the diversity of chrysophytes and their stomatocysts from Ukraine.

ACKNOWLEDGEMENTS

The authors are grateful to Dr Oxana Holovko (Ukraine) for her assistance during sampling; to the staff of the Interlaboratory Centre of Electron Microscopy of the Papanin Institute for Biology of Inland Waters, RAS, for technical assistance; to the two anonymous reviewers for their critical comments and valuable suggestions; and to the Editor-in-Chief,

Dr Olivia Bragg, for editing the final version of the manuscript and improving English. This study was supported by a grant from the Russian Science Foundation (Project No. 22–24–00662).

AUTHOR CONTRIBUTIONS

DK conducted the fieldwork and microscopic investigations, and wrote the first draft of the manuscript; MK wrote and edited the final draft.

REFERENCES

Andersen, R.A. (2021) *Lepochromulina bursa* Scherffel (Chrysophyceae) from Michigan, with ultrastructural observations on the flagellar apparatus and lorica. *Nova Hedwigia*, Beiheft 151, 9–26.

Andersen, R.A., Graf, L., Malakhov, Y., Yoon, H.S. (2017) Rediscovery of the *Ochromonas* type species *Ochromonas triangulata* (Chrysophyceae) from its type locality (Lake Veysove, Donetsk region, Ukraine). *Phycologia*, 56, 591–604.

Andrienko, T.L., Onyshchenko, V.A., Datsyuk, V.V. (2012) "Dermansko-Ostrozsky" National Nature Park. In: Onyshchenko, V.A., Andrienko, T.L. (eds.) *Фіторізноманіття заповідників і національних природних парків України* (*Phytodiversity of Nature Reserves and National Nature Parks of Ukraine*), Part 2, Phytosociocentre, Kyiv, 206–214 (in Ukrainian).

Bai, X., Bu, Z.J., Chen, X. (2018) Morphology of Chrysophycean stomatocysts in three peatlands in central China. *Mires and Peat*, 21, 19, 16 pp.

Bai, X., Piątek, J., Wołowski, K., Bu, Z., Chen, X. (2020) New stomatocysts discovered in *Sphagnum* peatlands, central China. *Phytotaxa*, 477, 151–170.

Cambra, J. (2010) Chrysophytes from some lakes and peat-bogs in the eastern Pyrenees, Catalonia (Spain). *Biologia*, 65/4, 577–586.

Chen, M., Ma, M.-Y., Wang, H.-X., Hu, Q., Gong, Y.-C. (2020) Redescription of mixotrophic *Poterioochromonas malhamensis* and its phylogenetic analysis. *Acta Hydrobiologica Sinica*, 44, 1130–1142 (in Chinese with English abstract).

Duff, K.E., Smol, J.P. (1988) Chrysophycean stomatocysts from postglacial sediments of a High Arctic lake. *Canadian Journal of Botany*, 66, 1117–1128.

Duff, K.E., Smol, J.P. (1991) Morphological descriptions and stratigraphic distributions of the chrysophycean stomatocysts from a recently acidified lake (Adirondack Park, N.Y.). *Journal of Paleolimnology*, 5, 73–113.

Duff, K.E., Douglas, M.S.V., Smol, J.P. (1992) Chrysophyte cysts in 36 Canadian high arctic ponds. *Nordic Journal of Botany*, 12, 471–499.

Duff, K.E., Zeeb, B.A., Smol, J.P. (1995) *Atlas of Chrysophycean Cysts*. Kluwer Academic Publishers, Dordrecht, 189 pp.

Ellis-Adam, A.C. (1983) Some new and interesting benthic Chrysophyceae from a Dutch moorland pool complex. *Acta Botanica Neerlandica*, 32(1–2), 1–23.

Gilbert, S., Zeeb, B.A., Smol, J.P. (1997) Chrysophyte stomatocyst flora from a forest peat core in the Lena River Region, northeastern Siberia. *Nova Hedwigia*, Beiheft 64, 311–352.

Hansen, P. (2001) Chrysophyte stomatocysts in the Azores - Biogeographical Implications and 110 New Morphotypes. Opera Botanica 138, Lunds botaniska forening / Carl Blom, Lund, 96 pp.

Holovko, O.V., Onyshchenko, V.A. (2017) Bushchanske boloto. In: Onyshchenko, V.A. (ed.) *Important Plant Areas of Ukraine*, Alterpress, Kyiv, 48–49.

Ignatenko, M., Yatsenko-Stepanova, T., Kapustin, D. (2022) Additions to chrysophycean stomatocyst flora from South Urals shallow lake including descriptions of three new morphotypes. *Phytotaxa*, 561, 14–26.

Kapustin, D., Gusev, E. (2016) *Chrysodidymus* Prowse (*Chrysophyceae*, *Synurales*), a new genus *Chrysophyta* for the Ukrainian algal flora. *International Journal on Algae*, 18(2), 105–110.

Kapustin, D.A., Philippov, D.A., Gusev, E.S. (2016) Four new chrysophycean stomatocysts with true complex collar from the Shichengskoe raised bog in Central Russia. *Phytotaxa*, 288, 285–290.

Kapustin, D.A., Gusev, E.S., Lilitskaya, G.G., Kulikovskiy, M.S. (2020) Silica-scaled chrysophytes from the Ukrainian Polissia. *Cryptogamie, Algologie*, 41(12), 121–135.

Matvienko, A.M. (1952) Chrysomonadineae e viciniis urbis Charkov (Chrysomonadineae from the vicinity of the city of Charkov). *Botanicheskie Materialy Otdela Sporovykh Rasteniy*, 8, 16–33 (in Russian).

Pang, W., Wang, Q. (2013) Chrysophycean stomatocysts from the Stone Ponds in the Aershan National Geological Park, China. *Nova Hedwigia*, Beiheft 142, 51–67.

Pang, W., Wang, Q. (2014) Chrysophycean stomatocysts from the Aershan Geological Park (Inner Mongolia), China. *Phytotaxa*, 187, 1–92.

Pang, W., Wang, Y., Wang, Q. (2012) Ten new

chrysophycean stomatocysts ornamented with spines from bogs near Da'erbin Lake, China. *Nova Hedwigia*, 94, 193–207.

Peel, M.C., Finlayson, B.L., McMahon, T.A. (2007) Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, 11, 1633–1644.

Piątek, J. (2007) *Algae of the Peat Bog in Modlniczka near Kraków (Wyżyna Krakowsko-Częstochowska Upland, S Poland)*. Polish Botanical Studies 24, Polish Academy of Sciences W. Szafer Institute of Botany, Kraków, 74 pp.

Piątek, J., Piątek, M. (2008) Chrysophyte stomatocysts from gypsum damp vegetation in Southern Poland. *Polish Botanical Journal*, 53(1), 57–67.

Sandgren, C.D. (1983) Survival strategies of chrysophycean flagellates: reproduction and the formation of resistant resting cysts. In: Fryxell, G.A. (ed.) *Survival Strategies of the Algae*, Cambridge University Press, UK, 23–48.

Siver, P.A., Kapustin, D., Gusev, E. (2018) Investigations of two-celled colonies of *Synura* formerly described as *Chrysodidymus* with descriptions of two new species. *European Journal of Phycology*, 53(3), 245–255.

Wilkinson, A.N., Zeeb, B.A., Smol, J.P. (2001) *Atlas of Chrysophycean Cysts II*. Kluwer Academic Publishers, Dordrecht, 169 pp.

Submitted 16 Aug 2023, revision 07 Oct 2023
Editor: Olivia Bragg

Author for correspondence: Dr Dmitry Kapustin, Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, 127276, Moscow, Russia. E-mail: dima_kapustin@outlook.com