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REGULAR PAPER

Extremely high diversity of euglenophytes in a small pond in eastern Poland

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Background and aims – Phytoplankton samples were taken from a periodic, small and very shallow former clay-pit pond in eastern Poland near Lublin city. Diversity of the euglenophyte community was assessed during the period 2002–2004 and in 2014.
Methods – Water samples were collected with a 20 µm plankton net and with a slime aspirator (20 ml capacity) from the surface of the bottom. One aliquot of each sample was fixed for SEM observation; the fresh part of the sample was analysed using light microscope. Physical and chemical properties of the water (pH, temperature, conductivity, phosphates and ammonium salt contents) were measured. Diversity indices were calculated (Shannon-Wiener, evenness, Margalef and Simpson) and UPGMA cluster analysis was applied to discern differences among euglenoid assemblages.
Key results – In total, 63 euglenophyte taxa were found. The euglenophyte community was dominated by species belonging to the *Trachelomonas* genus (29 taxa). The most abundant and constant component were widespread and common species such as *T. caudata*, *T. hispida*, *T. intermedia*, *T. volvocina* and *T. volvocinopsis*. They usually occurred in very high densities. We also found some rarely reported euglenophytes including *Euglena granulata*, *Trachelomonas lemmermannii*, and *T. sydneyensis*.
Conclusions – Euglenophytes were, with a few exceptions, the only group inhabiting the pond. Representatives of other groups such as diatoms or *Scenedesmus* species were recorded only occasionally. Nearly half of the taxa that were recorded in the first period (2002–2004) were found again after ten years. Of special concern was taxa belonging to the genus *Trachelomonas*. Trachelomonads, although known to represent taxa preferring waters moderately rich in nutrients, were very numerous in the investigated pond, which was enriched in phosphorus and nitrogen compounds.

Key words – Diversity, euglenoids, small water bodies, *Trachelomonas*.

INTRODUCTION

Euglenoids are known to be constant organisms in freshwater ecosystems (Wołowski & Hindák 2005). They are very sensitive to changes in environmental conditions, and populations may appear or disappear very rapidly (Heckmann et al. 1996). Rapid environmental changes may be caused by a heavy rainstorm, which can be sufficient to cause a complete dispersion of an euglenoid bloom, such as *Trachelomonas* species (Heckmann et al. 1996). Rapid changes are observed mainly in small and shallow water bodies because of their hydromorphological character. These kinds of water bodies are ideal sites for establishment of euglenoids. There is a lot of information from all over the world about euglenoids in small ponds, swamps and oxbow lakes where they often form blooms (e.g. Wołowski 1998, Wołowski & Hindák 2004, Wołowski & Walne 2007, Poniewozik 2009, Duangjan & Wołowski 2013, Duangjan et al. 2014, Pęczuła et al. 2014). Conversely, little is known about blooms of euglenoids in large lakes or reservoirs, but some data are available (Conforti & Ruiz 2001, Wołowski & Grabowska 2007). Rarity of euglenophyte blooms may also be due to specific demands such as dissolved or particulate organic matter amount, water temperature or water reaction and also water motion and circulation. These organisms cannot meet their requirements in large water bodies.

There is also scarce information on how euglenoids survive unfavourable life conditions during short- or long-term weather-related or environmental stress. Euglenoids, espe-

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cially representatives of the genus *Euglena*, can form palmelloid stages of three different types: protective, reproductive and temporary (Gojdics 1953) that can help them to survive these unfavourable conditions or to overwinter. However, in recent years, only a few papers about resting cysts formed by euglenoids have been published. The process of cyst formation was bserved in some species of freshwater *Euglena* (Hindák et al. 2000) and marine *Eutreptiella gymnastica* (Olli 1996). Undoubtedly, the rest of the euglenoid representatives have also strategies to survive in a water body, since many species appear in freshwaters in subsequent years (e.g. Nixdorf et al. 2003, Poniewozik 2007, Salmaso & Cerasino 2012, Naselli-Flores et al. 2016).

The aim of our study was to assess how many taxa of euglenoids persist in a small, regularly drying and overgrowing water body over a period of ten years. Our results provide indirect indications on the formation process of resting cysts. We also focused on detailed morphological characteristics of the species, especially the taxa that we found after the ten year period, as they may represent a constant component of the pond.

MATERIAL AND METHODS

The study was conducted from May to September, from 2002 to 2004 and in 2014, in monthly intervals. The samples were taken from a periodic, small and very shallow (with maximum depth about 0.7 m) pond (figs 1 & 2), which is a former clay-pit situated near Lublin city (51°22.19′N, 23°1.79′E) in eastern Poland. The pond is supplied mainly by rainwater and because of its shallowness is overgrown by macrophytes such as: *Juncus effusus* L., *Glyceria fluitans* (L.) R.Br. and *Alisma plantago-aquatica* L. The pond usually completely dries up during or after rainless, hot summers. Water samples were collected with a 20 µm plankton net and with a slime aspirator (20 ml capacity) from the surface of the bottom. One part of each sample was fixed for SEM observation; the other, fresh one, was analysed using a Nikon Eclipse E600 light microscope. Samples for scanning electron microscope were prepared according to the procedures proposed by Bozzola & Russel (1995). SEM observations of carbon-coated material were made with either a Leo 1430VP, Hitachi S-4700 or JEOL JSM-7401F microscopes. Physical and chemical properties of water (pH, temperature and conductivity, phosphates and ammonium salt contents) were also measured. The measurements were conducted using an Elmetron CP-401 pH-meter and a CC-411 conductivity-meter.

The concentration of phosphates (ammonium molybdate method according to Hermanowicz et al. 1999) and ammonium salts (direct nesslerization method by Hermanowicz et al. 1999) were determined in the laboratory. Species abundance was estimated from three slides (20 × 20 mm) of each sample. For the display of abundance, the modified Braun-Blanquet scale was used, as follows: r, rare, species that do not occur on each slide; +, single, the occurrence of 1–5 specimens on a slide; 1, sparse (5–10 specimens on a slide); 2, frequent (10–15); 3, very frequent (about 20); 4, abundant (more than 20); 5, in masses. In order to display quantitative relationships in the euglenoid community, the primary scale was transformed into van der Maarel's numerical scale (van der Maarel 2007). Species diversity (*H'*), evenness (*J'*), Simpson's index (*d1*) and Margalef Richness Index (*d2*) were calculated (Shannon & Wiener 1963, Pielou 1975, Ludwig & Reynolds 1988).

A cluster analysis of unweighted pair group mean averages (UPGMA) with Manhattan distance was applied to discern differences among euglenoid assemblages in the following months of study. All statistical analyses were conducted using the Multi-Variate Statistical Package (MVSP) program version 3.01 (Kovach 1998).

RESULTS

Physical and chemical conditions

The physical chemical conditions in the pond during the study periods were characterized as typical of high-nutrient water, especially rich in ammonium salts (table 1). Water reaction was from slightly acid to alkaline, conductivity values were on a low level. Water temperature was typical of the season in the temperate zone to extremely high in August 2003, when it reached almost 34°C (table 1).

Figure 1 – The pond overgrown with *Juncus effusus*.

Figure 2 – Densely overgrown site of this study.

Table 1 – **Basic physical chemical properties of pond water.**

	2002–2004	2014
pH	7.16–9.68	6.23–6.82
conductivity [µS.cm^{-1}]	164–285	169–183
T [°C]	18.3–33.9	17.2–18.3
P-PO_4 [mg.dm^{-3}]	0.058–0.153	0.182
N-NH_4 [mg.dm^{-3}]	1.210–3.110	2.225
N-NO_3 [mg.dm^{-3}]	0.160–0.840	0.187

Taxa identified

During the study period, we found 63 taxa of euglenoids (table 2). They were composed mainly of representatives of the *Trachelomonas* genus (29 taxa), whereas less diversity was observed in case of species belonging to the genera *Phacus* (11), *Euglena* (7), *Lepocinclis* (7), *Strombomonas* (4), *Monomorphina* (3), *Euglenaformis* (1) and *Euglenaria* (1). In 2002–2004, we found 48 taxa and some data concerning the trachelomonads (Poniewozik 2009). In 2014, we found 42 taxa, mostly belonging to the genus *Trachelomonas*. In 2014, the euglenophyte community consisted of a high percentage of species (43%), which was observed in the study conducted ten years earlier. Most of these taxa are widespread globally and are commonly known species. The most often found taxa, which were occurring in enormous densities in 2014, were *T. volvocina*, *T. volvocinopsis*, *T. hispida* var. *hispida*, *T. intermedia* and *T. caudata*. *Phacus monilatus* and *Lepocinclis texta* also formed very dense populations, especially during 2002–2004. In that period, the euglenophyte community was more diverse and variable than in 2014. Usually it consisted of representatives of the main euglenoid genera; many of them developed into high densities, although the dominance of trachelomonad representatives was almost always observed. After ten years, the community of trachelomonads was dominant over the other taxa, which were secondary to the trachelomonads.

Diversity indices

The diversity indices (Shannon-Wiener index, Margalef Richness Index, evenness) calculated for both periods proved that although a very close number of species was determined (48 in 2002–2004 and 42 in 2014), the euglenoid community was more diverse in the first period (table 3). In 2014, euglenophytes were dominant (mainly by *T. volvocinopsis*, *T. volvocina* or *T. hispida*). In the earlier period, the dominance was shared between several species also from different genera (*Lepocinclis* or *Phacus*), which was reflected in the values of the calculated indices (table 3).

It was very unusual that different algal or cyanobacterial species, which are common in pond phytoplankton, were almost absent in the planktonic community in the pond during the entire study period. There were only some species representing green algae, diatoms and blue-green algae that we determined during the research (table 3). The most frequent were green algae such as *Desmodesmus quadricauda*, *Tetradesmus obliquus*, *Tetraedron minimum*, and a cyanophyte, *Cylindrospermum* sp. As the density of these species was very limited (compared to densities of euglenophytes, see figs 3 & 4), they should not be considered as an essential component of the algae composition of the ponds.

UPGMA dendrogram

The UPGMA dendrogram of samples (fig. 5) indicated a close relationship among sampling data gathered during the same season (late spring, summer, early autumn) from the perspective of the euglenoids diversity and abundance. As shown in figure 5, sampling periods for one season were often gathered in the same cluster, except for sample of May 2004 and June 2014, which were distributed in separate clusters (groups I and III). June 2014 (III) was very specific against the background of the whole period, due to the fact that the number of taxa found was really high (29), but none

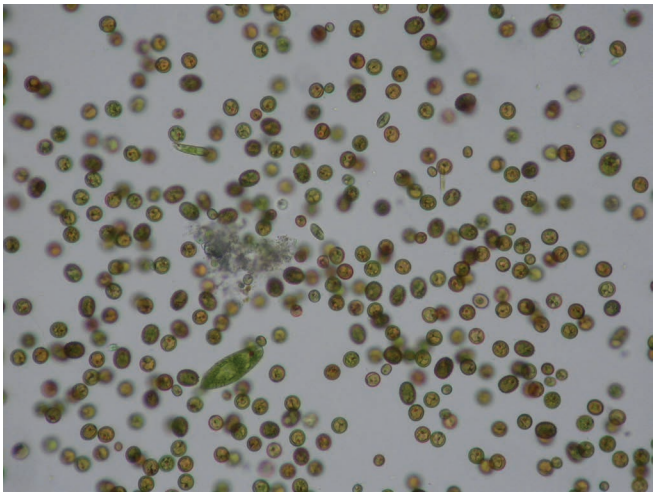

Figure 3 – Density of trachelomonad community represented mainly by *T. volvocina* and *T. hispida*.

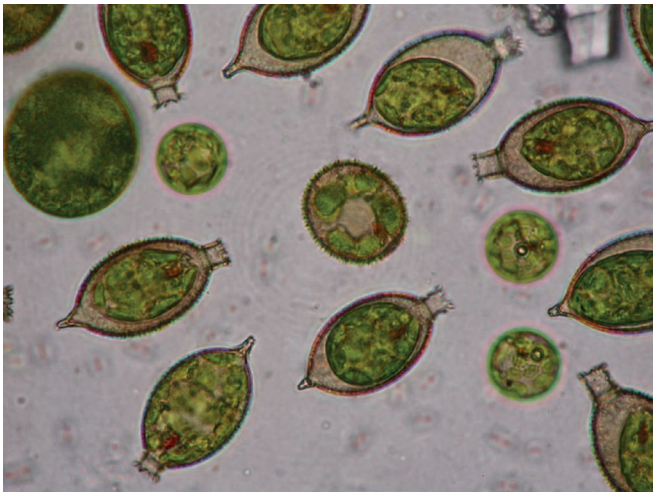

Figure 4 – Density of trachelomonad community represented mainly by *T. caudata*.

Table 2 – Occurrence of euglenoids in the pond during studied vegetation seasons.

	2002–2004	2014		2002–2004	2014
Euglena			***Strombomonas***		
E. ehrenbergii	+	+	*S acuminata*	+	+
E. geniculata		+	*S. costata*		+
E. granulata	+		*S. eurystoma*	+	+
E. sanguinea	+		*S. urceolata*		+
E. sociabilis	+		***Trachelomonas***		
E. splendens	+	+	*T. abrupta*	+	+
E. viridis	+		*T. acanthostoma*		+
Euglenaformis			*T. armata* var. *echinata*		+
E. proxima	+		*T. armata* var. *heterospina*		+
Euglenaria			*T. caudata*	+	+
E. anabaena	+		*T. cervicula* var. *heterocollis*	+	+
Lepocinclis			*T. drezepolskiana*	+	
L. acus	+	+	*T. dubia*	+	+
L. cylindrica	+		*T. hispida* var. *hispida*	+	+
L. fusiformis	+	+	*T. hispida* var. *coronata*	+	
L. ovum		+	*T. hispida* var. *crenulatocollis*		+
L. oxyuris	+	+	*T. intermedia*	+	+
L. spirogyroides	+		*T. lacustris*	+	
L. texta	+	+	*T. lemmermannii*		+
Monomorphina			*T. manginii*		+
M. mirabilis	+		*T. mirabilis* var. *obesa*		+
M. pyrum	+	+	*T. ornata*	+	
M. splendens		+	*T. pavlovskoensis*		+
Phacus			*T. planctonica*	+	+
P. acuminatus	+		*T. pulchella*	+	
P. alatus	+		*T. pulchra*	+	
P. curvicauda	+	+	*T. sarmatica*	+	
P. limnophilus	+	+	*T. scabra*	+	
P. longicauda	+	+	*T. similis*	+	+
P. monilatus	+	+	*T. superba*	+	+
P. orbicularis	+	+	*T. sydneyensis*	+	+
P. pleuronectes	+		*T. verrucosa*		+
P. tortus	+	+	*T. volvocina*	+	+
P. triqueter		+	*T. volvocinopsis*	+	+
P. undulatus	+				

Table 3 – **Diversity of euglenophytes in the pond studied.**

	2002–2004	2014
Shannon-Wiener Index (H')	4.06	3.52
Margalef Diversity Index	14.56	9.23
Simpson Index	0.98	0.96
Evenness Index (J')	0.97	0.92
taxa of the greatest density	*Lepocinclis texta*, *Phacus alatus* *Phacus limnophilus*, *Trachelomonas volvocinopsis* *Trachelomonas volvocina*	*Trachelomonas volvocinopsis* *Trachelomonas volvocina*
number of taxa	48	42
accompanying species (occurring occasionally)	*Scenedesmus quadricauda*, *Tetraëdron minimum*, *Navicula* sp., *Closterium attenuatum*, *Closterium dianae*	*Desmodesmus magnus*, *Coelastrum microporum*, *Tetradesmus obliquus*, *Cylindrospermum* sp.

of them seemed to be a dominant taxon. The first group of the UPGMA dendogram includes one sampling period (May 2004) when 24 taxa were found, belonging mainly to the genus *Trachelomonas*; five of them occurred in a high abundance (*Trachelomonas volvocina*, *T. volvocinopsis*, *T. hispida*, *T. ornata* and *T. abrupta*). Group II was composed of two sampling periods: July and August 2014. The community of euglenophytes was similar in both periods and was represented mainly by *Trachelomonas* species. The community also reached a high density with *T. volvocina* and *T. volvocinopsis* as the most numerous species. Group IV includes the remaining periods of sampling, showing that the euglenophyte community was similar to some extent in almost the whole period of study of 2002–2004.

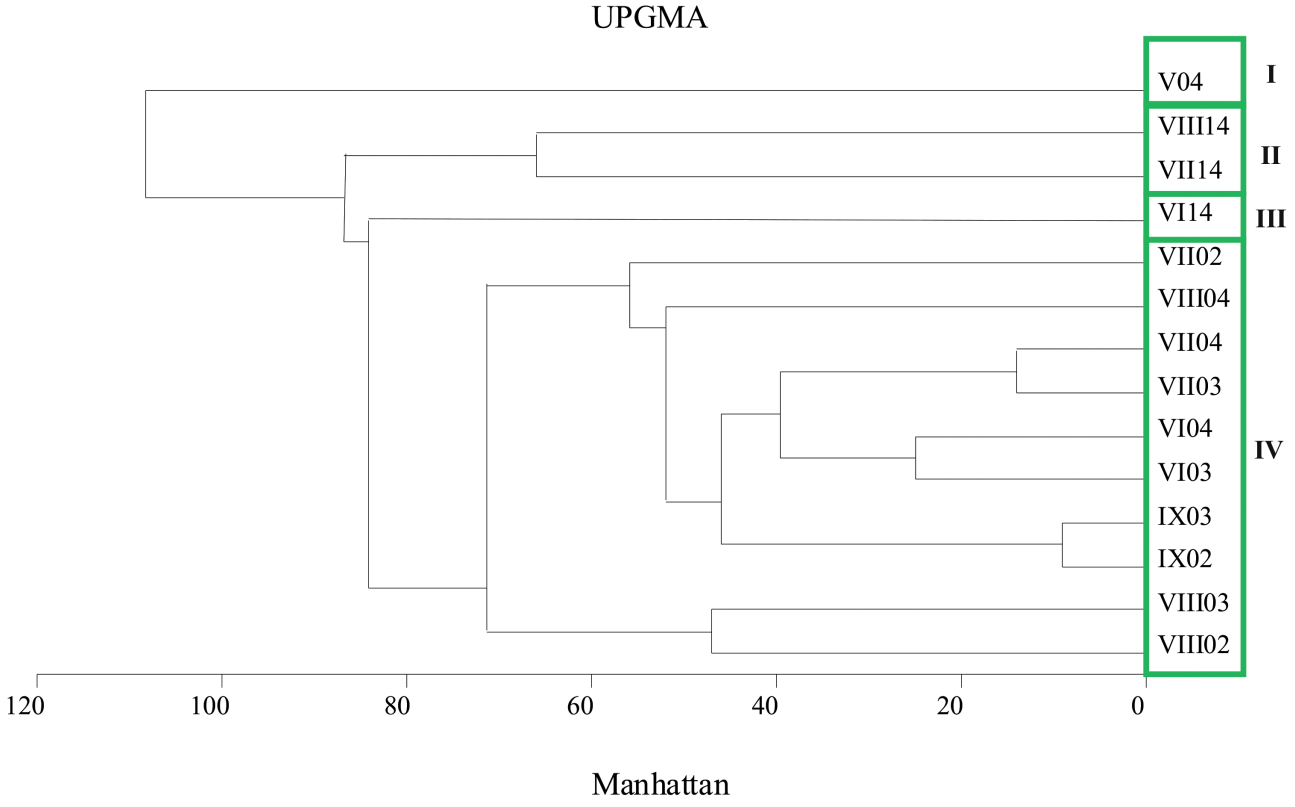

Figure 5 – UPGMA dendrogram shows four major clustering groups. V, May; VI, June; VII, July; VIII, August; IX, September; 02, 2002; 03, 2003; 04, 2004; 14, 2014.

Taxa identified during the investigation

We list below taxa that were observed in the pond during our study; taxa that were observed during the whole period of the study are indicated with an asterisk. The descriptions are provided for all species found during the entire study with exclusion of some *Trachelomonas* species found only in 2002–2004 and described in an earlier work (Poniewozik 2009).

Abundance information is given in square brackets after the name.

Euglena Ehrenb.

****Euglena ehrenbergii*** G.A.Klebs
Fig. 6A [+ - 2]
Cells 116.1–234.6 µm long and 22.2–50.7 µm wide, cylindrical, both ends broadly rounded. Periplast very flexible allowing the cell to change its shape to large extent. Chloroplasts small and discoid.

Euglena geniculata Dujard.
Fig. 6B [r - +]
Cells 67.5–91.4 µm long and 12.0–20.0 µm wide, cylindrical with short cauda at the end. Chloroplasts two or three, star-shaped.

Euglena granulata (G.A.Klebs) F.Schmitz
Fig. 6C [r]
Cells 67.5–106.2 µm long and 13.8–27.2 µm wide, broadly fusiform with rounded apical end. Periplast spirally striated with large muciferous bodies. Chloroplasts elongated with double-sheathed pyrenoids.

Euglena sanguinea Ehrenb.
Fig. 6D [+]
Cells 36.8–89.2 µm long and 24.7–32.1 µm wide, broadly spindle-shaped, rounded at the anterior end and tapering at the posterior end. Cells red or red-brown due to hematochrome production. Chloroplasts small, numerous with pyrenoids.

Euglena sociabilis P.A.Dang.
Fig. 6E [r]
Cells 66.8–93.2 µm long and 14.7–25.0 µm wide, longitudinally oval ended with a small hyaline projection at the posterior end. Chloroplasts as thin lobes protruding from the double-sheathed pyrenoids to the cell's edge.

****Euglena splendens*** P.A.Dang.
Fig. 6F [+ - 2]
Cells 49.8–88.2 µm long and 20.0–24.2 µm wide, pear-shaped, attenuated at the posterior end. Chloroplasts with thin lobes and small paramylon grains.

Euglena viridis (O.F.Müll.) Ehrenb.
Fig. 6G [+ - 2]
Cells 27.8–69.2 µm long and 10.0–19.2 µm wide, fusiform. Chloroplast star-shaped located in the centre of the cell.

Euglenaformis M.S.Bennett & Triemer

Euglenaformis proxima (P.A.Dang.) M.S.Bennett & Triemer
Fig. 6H [+ - 1]
Cells 52.5–72.6 µm long and 9.5–18.8 µm wide, cylindrically fusiform ended with small, hyaline projection. Chloroplasts small and numerous, without pyrenoids.

Euglenaria A.Karnkowska, E.W.Linton & Kwiat.

Euglenaria anabaena (Mainx) A.Karnkowska-Ishikawa & E.W.Linton
Fig. 6I [+ - 1]
Cells 32.8–59.8 µm long and 11.0–18.5 µm wide, spindle-shaped with flexible periplast. Chloroplasts several, lobate with very distinct, small pyrenoids.

Lepocinclis Perty

****Lepocinclis acus*** (O.F.Müll.) B.Marin & Melkonian
Fig. 6J [+ - 1]
Cells 96.3–163.4 µm long and 6.8–11.1 µm wide, acicular, gradually tapering to the posterior end. Chloroplasts small and numerous.

Lepocinclis cylindrica (Korshikov) W.Conrad
Fig. 6K [+]
Cells 23.5–33.6 µm long and 9.9–12.5 µm wide, cylindrical, truncated at the anterior end and with approx. 2–2.5 µm long extension at the posterior end. Periplast with spiral striae. Chloroplasts small and numerous.

****Lepocinclis fusiformis*** (H.J.Carter) Lemmerm.
Fig. 6L [+]
Cells 32.1–37.1 µm long and 21.0–21.5 µm wide, broadly fusiform, conically ended at the both sides. Chloroplasts small and discoid. Two large, ring-shaped paramylon grains in a cell.

Lepocinclis ovum (Ehrenb.) Lemmerm.
Fig. 6M [+]
Cells 16.9–35.3 µm long and 10.1–22.2 µm wide, oval ended with short cauda at the posterior end; anterior end conical with small depression at the top. Chloroplasts small and discoid. Two large ring-like paramylon bodies.

****Lepocinclis oxyuris*** (Schmarda) B.Marin & Melkonian
Fig. 6N [+ - 1]
Cells 111.2–190.0 µm long and 17.3–30.0 µm wide, cylindrical ended with sharp process at the posterior end, two big and ring-shaped paramylon bodies at both sides of the nucleus. Periplast longitudinally striated. Chloroplasts small and discoid.

Lepocinclis spirogyroides B.Marin & Melkonian
Fig. 6O [+ - 1]
Cells 86.5–172.3 µm long and 12.2–27.2 µm wide, cylindrical, slightly flattened, ended with sharp, hyaline cauda 8.0-

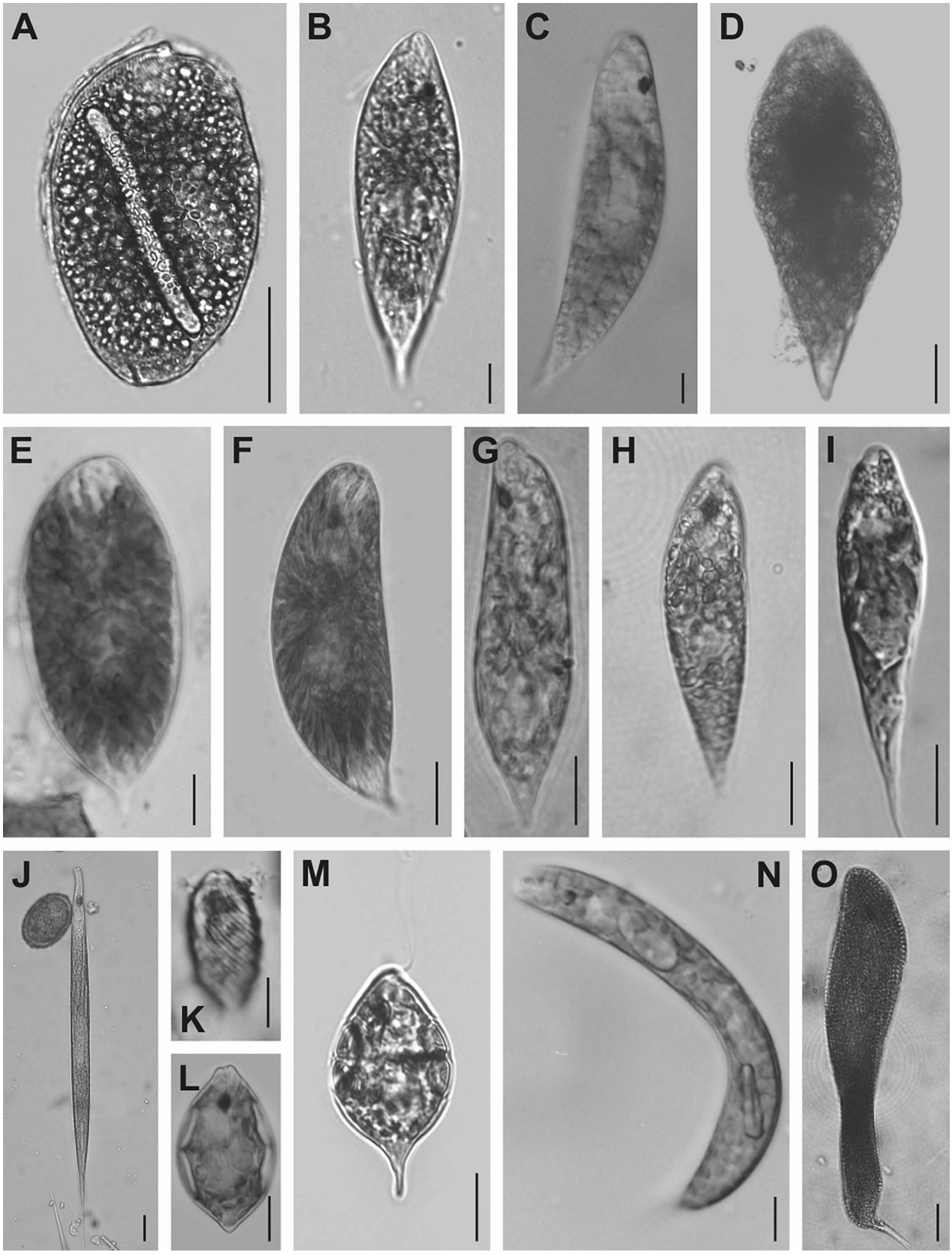

Figure 6 – Observed species of *Euglena*, *Euglenaformis*, *Euglenaria* and *Lepocinclis*. A, *Euglena ehrenbergii*; B, *E. geniculata*; C, *E. granulata*; D, *E. sanguinea*; E, *E. sociabilis*; F, *E. splendens*; G, *E. viridis*; H, *Euglenaformis proxima*; I, *Euglenaria anabaena*; J, *Lepocinclis acus*; K, *L. cylindrica*; L, *L. fusiformis*; M, *L. ovum*; N, *L. oxyuris*; O, *L. spirogyroides*. Scale bars = 10 µm.

18.5 µm long. Periplast with longitudinally spiral rows of cubic warts. Chloroplasts small and discoid.

***Lepocinclis texta** (Dujard.) Lemmerm.
Figs 7A & 9A [+ - 4]
Cells 37.1–59.4 µm long and 24.7–53.2 µm wide, egg-shaped. Periplast obliquely striped, inflexible. Chloroplasts numerous and discoid, without pyrenoids.

Monomorphina Mereschk.

Monomorphina mirabilis (Pochm.) Safonova
Fig. 7B [r - +]
Cells 34.1–49.3 µm long and 17.1–22.2 µm wide, oval ended with long (10.0–15.5 µm length) cauda. Periplast with spirally arranged stripes and ribs. Chloroplasts numerous.

***Monomorphina pyrum** (Ehrenb.) Mereschk.
Fig. 7C & D [r - 2]
Cells 27.2–44.7 µm long and 13.6–19.6 µm wide, oval and flattened ended with long, hyaline cauda. Periplast spirally striated. Chloroplasts discoid, not very numerous.

Monomorphina splendens (Pochm.) T.G.Popova
Fig. 7E [r]
Cells 27.2–33.4 µm long and 15.1–16.3 µm wide, oval with long cauda at the posterior end. Periplast spirally ribbed; two large, laterally located bowl-shaped paramylon grains. Chloroplasts numerous.

Phacus Dujard.

Phacus acuminatus A.Stokes
Fig. 7F [+ - 2]
Cells 32.1–45.0 µm long and 19.0–33.6 µm wide, broadly oval with extension at the posterior end with one ring-shaped paramylon grain. Chloroplasts small and numerous.

Phacus alatus G.A.Klebs
Fig. 7G [+ - 4]
Cells 34.2–39.5 µm long and 22.8–32.1 µm wide, oval and twisted with curved cauda at the posterior end with two big lateral grains of paramylon. Chloroplasts numerous.

Phacus curvicauda Svirenko
Fig. 7H [+]
Cells 32.1–39.5 µm long and 22.2–34.6 µm wide, broadly oval to almost round with short, curved process at the posterior end with two large, parietal paramylon grains. Chloroplasts small and discoid.

***Phacus limnophilus** (Lemmerm.) E.W.Linton & A.Karnkowska-Ishikawa
Fig. 7I [+ - 3]
Cells 49.4–76.6 µm long and 8.0–14.8 µm wide, spindle-shaped with long tail at the posterior. Periplast flexible, finely spirally striated. Chloroplasts small and discoid.

***Phacus longicauda** (Ehrenb.) Dujard.
Fig. 7J [r – 3]
Cells 74.4–145.7 µm long and 37.2–69.2 µm wide, oval or broadly oval, ended with long (26.4-56.8 µm), thin and sharp cauda with one large paramylon grain in the centre of the cell. Periplast longitudinally striated. Chloroplasts numerous, small.

***Phacus monilatus** (A.Stokes) Lemmerm.
Figs 7K & 9B [+ - 2]
Cells 34.5–37.5 µm long and 21.7–29.6 µm wide, broadly oval with cauda at the posterior end. Periplast with longitudinally arranged rows of small granules. Chloroplasts small and numerous.

***Phacus orbicularis** K.Hübner
Figs 7L & 9C [+ - 1]
Cells 57.2–67.8 µm long and 27.4–48.5 µm wide, broadly oval with sharp, curved cauda with one big paramylon body in the center of the cell. Periplast longitudinally striated with connections between stripes. Chloroplasts numerous, small.

Phacus pleuronectes (O.F.Müll.) Nitzsch ex Dujard.
Fig. 7M [+]
Cells 37.1–50.0 µm long and 29.6–33.8 µm wide, broadly oval to pear-shaped ended with short cauda. Chloroplasts numerous and small.

***Phacus tortus** (Lemmerm.) Skvortzov
Fig. 7N [+ - 2]
Cells 71.6–99.0 µm long and 37.1–54.5 µm wide, once spirally twisted in the lower part with long cauda at the posterior end with one large paramylon body in the cell. Periplast longitudinally striated. Chloroplasts numerous.

Phacus triqueter (Ehrenb.) Dujard.
Fig. 7O [r - +]
Cells 32.0–42.0 µm long and 16.0–32.1 µm wide, broadly oval to even round ended with sharp, thin cauda at the posterior end. Distinct keel on the back that makes cell's cross-section triangular. Chloroplasts numerous and discoid.

Phacus undulatus (Skvortzov) Pochm.
Fig. 8A [+]
Cells 54.8–62.0 µm long and 36.0–42.5 µm wide, oval with corrugated rims and curved cauda at the posterior end. Chloroplasts numerous and small.

Strombomonas Deflandre

***Strombomonas acuminata** (Schmarda) Deflandre
Fig. 8B [+]
Loricae 27.2–45.6 µm long and 20.1–26.4 µm wide, rectangular in the outline with concavity in the middle part, smooth or finely verrucose. The anterior end with obliquely or straight truncated collar, smooth at the rim. The posterior end with long, conical spine.

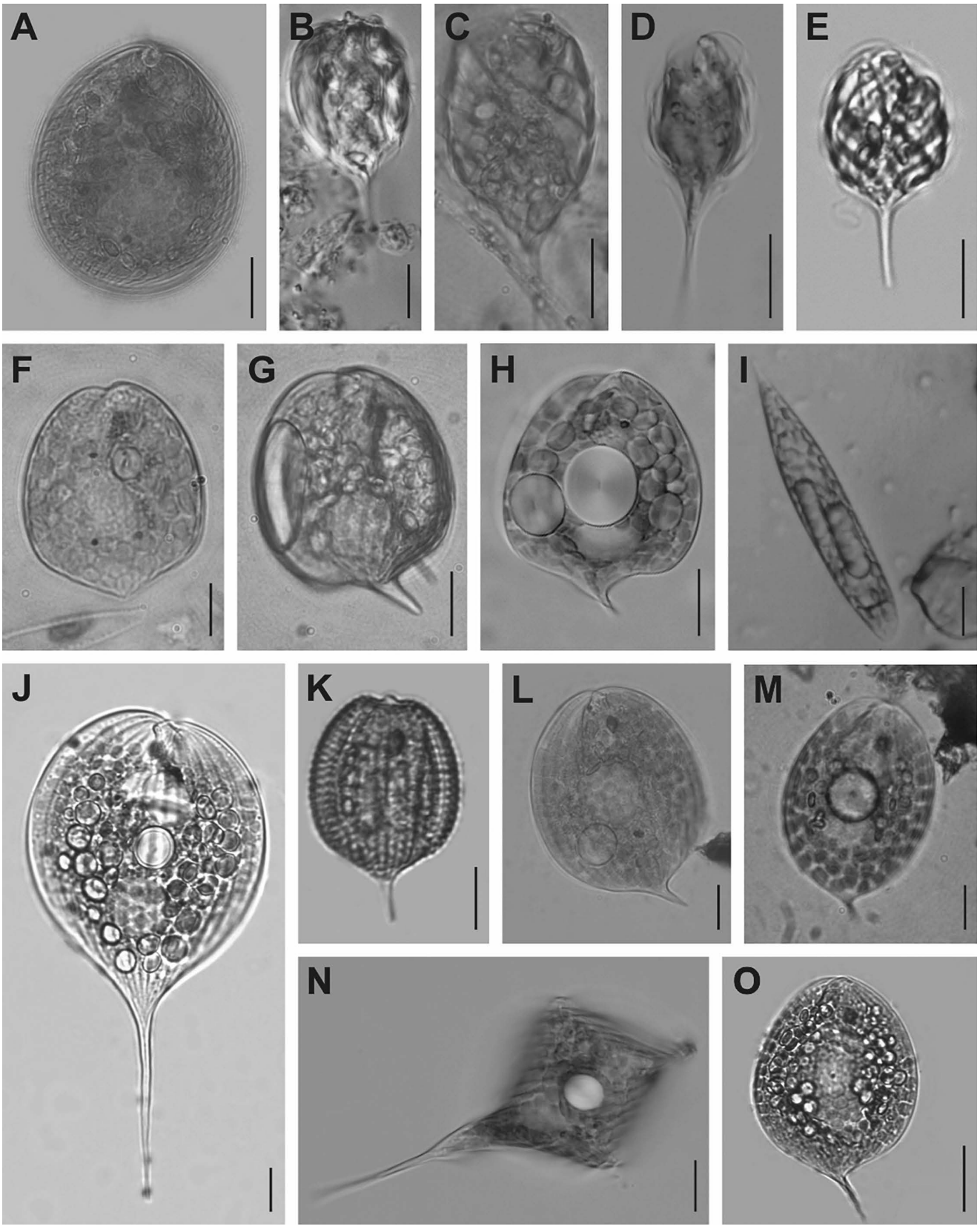

Figure 7 – Observed species of *Lepocinclis*, *Monomorphina* and *Phacus*. A, *Lepocinclis texta*; B, *Monomorphina mirabilis*; C & D, *M. pyrum*; E, *M. splendens*; F, *Phacus acuminatus*; G, *P. alatus*; H, *P. curvicauda*; I, *P. limnophilus*; J, *P. longicauda*; K, *P. monilatus*; L, *P. orbicularis*; M, *P. pleuronectes*; N, *P. tortus*; O, *P. triqueter*. Scale bars = 10 µm.

Stromobomonas costata Deflandre
Fig. 8C [r - +]
Loricae 62.0–88.0 µm long and 33.0–41.0 µm wide, broadly spindle-shaped, ended with long and thick spine at the posterior end. The anterior end with long, obliquely truncated collar. The wall of lorica wrinkled with thin, longitudinal furrows.

****Strombomonas eurystoma*** (F.Stein) T.G.Popova
Fig. 8D [+ - 1]
Loricae 25.9–35.0 µm long and 17.3–22.5 µm wide, obovoid in outline, narrowed at the apical end with a collar obliquely or straight truncated at the rim. The wall of lorica slightly wrinkled.

Strombomonas urceolata (A.Stokes) Deflandre
Fig. 8E [r - +]
Loricae 39.5–44.0 µm long and 22.5–27.8 µm wide, longitudinally oval, gradually tapering to a thin, conical cauda at the posterior. The anterior ended with a long collar, smooth at the rim and straight or obliquely truncated.

Trachelomonas Ehrenb.

****Trachelomonas abrupta*** Svirenko
Fig. 8F & G [+ - 4]
Loricae 23.5–29.0 µm long and 11.3–16.0 µm wide, cylindrical, covered with fine spines. Apical pore without collar. Chloroplasts small, discoid, without pyrenoids.

Trachelomonas acanthostoma A.Stokes
Fig. 8H [+]
Loricae 28.3–37.2 µm long and 21.3–34.6 µm wide, broadly ellipsoidal with granules on the surface. Apical pore surrounded with the sharp spines.

Trachelomonas armata (Ehrenb.) F.Stein var. ***echinata*** (A.M.Cunha) T.G.Popova
Figs 8I & 9D [+]
Loricae 37.8–40.3 µm long and 25.2–27.6 µm wide, broadly oval, dark brown. Long and sharp spines covered the lorica, several longer spines at the posterior end. Chloroplasts small, discoid, without pyrenoids.

Trachelomonas armata (Ehrenb.) F.Stein var. ***heterospina*** Svirenko
Figs 8J & 9E [+]
Loricae 33.2–42.3 µm long and 23.8–29.6 µm wide, broadly oval to egg-shaped, dark brown. Small, not numerous spines surround the apical pore, several long, sharp and curved form wreath around the posterior end. Chloroplasts small, discoid, without pyrenoids.

****Trachelomonas caudata*** (Ehrenb.) F.Stein
Figs 8K & 9F [1 - 3]
Loricae 34.0–44.5 µm long and 19.8–23.5 µm wide, lemon-shaped with blunt process at the posterior end, punctuated, without spines. Collar long and wide, extended and denticulated at the rim. Chloroplasts numerous, without pyrenoids.

****Trachelomonas cervicula*** (A.Stokes) var. ***heterocollis*** Svirenko
Fig. 8L [+ - 2]
Loricae 22.0–25.9 µm long and 19.8–20.5 µm wide, round, rather small, smooth, yellow-green. Collar short, its part is directed inside the lorica. Chloroplasts numerous, small, without pyrenoids.

Trachelomonas drezepolskiana W.Conrad
Poniewozik (2009: fig. 2:3) [+]

****Trachelomonas dubia*** Svirenko
Figs 8M & 9G [+ - 1]
Loricae 26.7–30.9 µm long and 13.2–16.9 µm wide, elliptical cylindrical with lower part slightly conical, smooth, bright in colour. Collar narrow, smooth at the rim. Chloroplasts small and discoid.

****Trachelomonas hispida*** (Perty) F.Stein var. ***hispida***
Figs 8N & 9H [+ - 4]
Loricae 27.2–39.5 µm long and 17.0–29.6 µm wide, broadly oval, covered with sharp, short spines, apical pore without collar. Chloroplasts not numerous with double-sheathed pyrenoids.

Trachelomonas hispida (Perty) F.Stein var. ***coronata*** Lemmerm.
Poniewozik (2009: fig. 2:1) [1]

Trachelomonas hispida (Perty) F.Stein var. ***crenulatocollis*** (Maskell) Lemmerm.
Figs 8O & P [1]
Loricae 32.1–33.5 µm long and 22.0–23.5 µm wide, longitudinally oval densely covered with sharp spines. Collar short and surrounded with the spines. Chloroplasts not numerous with double-sheathed pyrenoids.

****Trachelomonas intermedia*** P.A.Dang.
Fig. 9I [+ - 3]
Loricae 14.8–24.0 µm long and 13.6–20.9 µm wide, brown or reddish-brown, oval with small pores and thin and sparsely distributed blunt spines. Apical pore surrounded by low collar denticulated at the edge. Chloroplasts with small double-sheathed pyrenoids.

Trachelomonas lacustris Drezep.
Poniewozik (2009: fig. 1:12) [2]

Trachelomonas lemmermannii Wołosz.
Fig. 9J [+]
Loricae 29.6–30.1 µm long and 19.8–20.5 µm wide, elliptical in the outline with conical posterior end, covered with small, densely arranged spines. Apical pore surrounded by spines. Chloroplasts without pyrenoids, small and numerous.

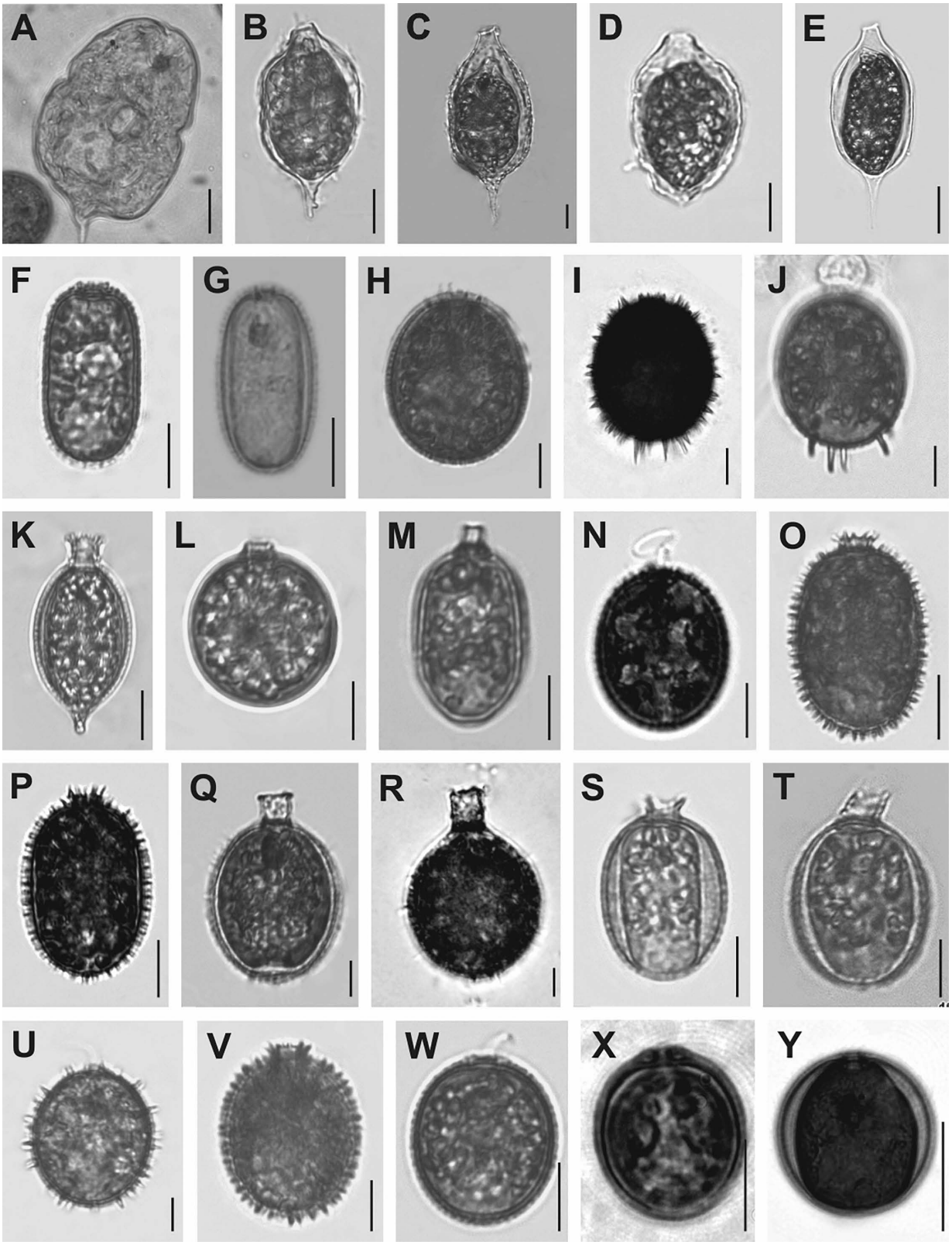

Figure 8 – Observed species of *Phacus*, *Strombomonas* and *Trachelomonas*. A, *Phacus undulatus*; B, *Strombomonas acuminata*; C, *S. costata*; D, *S. eurystoma*; E, *S. urceolata*; F & G, *Trachelomonas abrupta*; H, *T. acanthostoma*; I, *T. armata* var. *echinata*; J, *T. armata* var. *heterospina*; K, *T. caudata*; L, *T. cervicula* var. *heterocollis*; M, *T. dubia*; N, *T. hispida* var. *hispida*; O & P, *T. hispida* var. *crenulatocollis*; Q & R, *T. pavlovskoensis*; S, *T. planctonica*; T, *T. similis*; U, *T. superba*; V, *T. sydneyensis*; W, *T. verrucosa*; X, *T. volvocina*; Y, *T. volvocinopsis*. Scale bars=10µm.

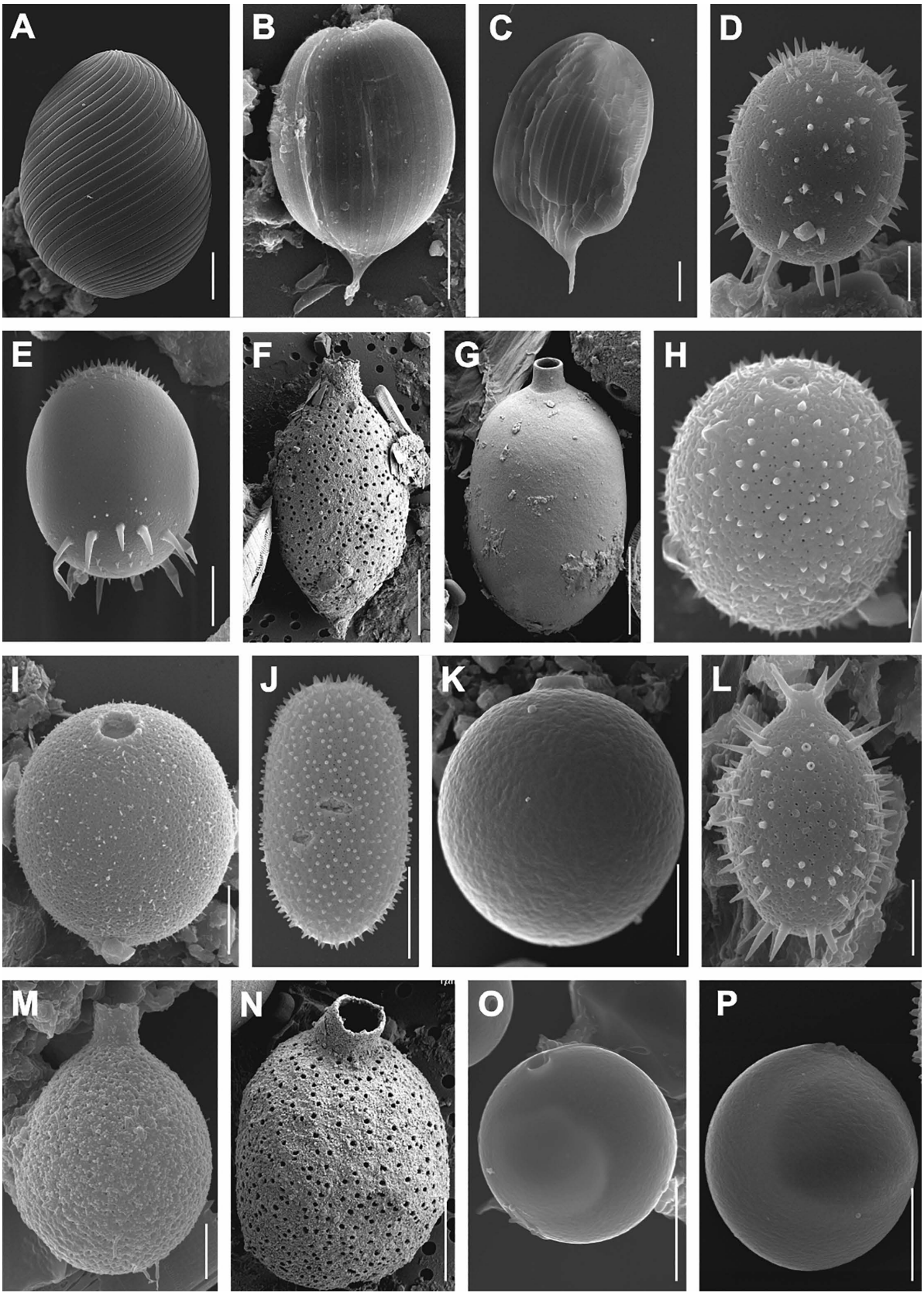

Figure 9 – Scanning electron micrographs of euglenoid taxa. A, *Lepocinclis texta*; B, *Phacus monilatus*; C, *P. orbicularis*; D, *Trachelomonas armata* var. *echinata*; E, *T. armata* var. *heterospina*; F, *T. caudata*; G, *T. dubia*; H, *T. hispida*; I, *T. intermedia*; J, *T. lemmermannii*; K, *T. manginii*; L, *T. mirabilis* var. *obesa*; M, *T. pavlovskoensis*; N, *T. similis*; O, *T. volvocina*; P, *T. volvocinopsis*. Scale bars = 10 µm.

Trachelomonas manginii Deflandre
Fig. 9K [1]
Loricae 29.6–30.1 µm long and 19.8–20.5 µm wide, round, small and smooth at the surface. Collar short, narrowing to the rim, straight truncated at the top. Chloroplasts small, without pyrenoids.

Trachelomonas mirabilis Svirenko var. ***obesa*** (Messik.) W.Conrad
Fig. 9L [+]
Loricae 34.1–33.8 µm long and 21.2–24.3 µm wide, elliptical with narrow collar equipped with long spines at the rim. The surface of the loricae regularly covered with long, sharp thorns. Chloroplasts discoid.

Trachelomonas ornata Skvortzov
Poniewozik (2009: fig. 2:10) [2]

Trachelomonas pavlovskoensis (Poljansky) T.G.Popova
Figs 8Q & R, 9M [+]
Loricae 47.9–55.2 µm long and 20.2–26.3 µm wide, round to oval with surface coated by granules and covered densely with pores. Collar long, crenulated at the rim. Chloroplasts without pyrenoids.

****Trachelomonas planctonica*** Svirenko
Fig. 8S [+ - 1]
Loricae 24.9–30.9 µm long and 20.9–24.3 µm wide, oval, thin, then bright in colour, with small pores. Apical pore with collar straight truncated or denticulated at the rim. Chloroplasts not numerous, without pyrenoids.

Trachelomonas pulchella Drezep.
Poniewozik (2009: fig. 2:6) [+]

Trachelomonas pulchra Svirenko
Poniewozik (2009: fig. 1:6) [1]

Trachelomonas sarmatica Drezep.
Poniewozik (2009: fig. 2:9) [+]

Trachelomonas scabra Playfair
Poniewozik (2009: fig. 1:14) [+ - 1]

****Trachelomonas similis*** A.Stokes
Figs 8T & 9N [+]
Loricae 23.9–32.2 µm long and 15.1–22.2 µm wide, oval, smooth or covered with pores. Collar long and bent, slightly jagged at the rim. Chloroplasts numerous, discoid.

****Trachelomonas superba*** Svirenko
Fig. 8U [r - 1]
Loricae 39.5–46.9 µm long and 21.0–35.8 µm wide, broadly oval with sharp spines on the lorica. Apical pore without collar. Chloroplasts discoid, without pyrenoids.

****Trachelomonas sydneyensis*** Playfair
Fig. 8V [r]
Loricae 32.0–34.6 µm long and 23.5–25.0 µm wide, oval to round, covered with strong spines, collar short and ragged at the edge. Chloroplasts without pyrenoids.

Trachelomonas verrucosa A.Stokes
Fig. 8W [r - 1]
Loricae 18.5–25.0 µm long and 18.5–22.2 µm wide, round to oval, covered with small spines or rather small verrucae. Apical pore surrounding by thickening. Chloroplasts large with double-sheathed pyrenoids.

****Trachelomonas volvocina*** Ehrenb.
Figs 8X & 9O [1 - 5]
Loricae 13.4–24.0 µm in diameter, small, smooth on the surface and round to slightly oval. Apical pore without thickening and collar. Chloroplasts two, parietally located with double-sheathed pyrenoids.

****Trachelomonas volvocinopsis*** Svirenko
Figs 8Y & 9P [1 - 5]
Loricae 14.1–25.2 µm in diameter, small, round and smooth at the surface, apical pore without collar and thickening. Chloroplasts small, numerous, without pyrenoids.

DISCUSSION

Euglenophyte diversity

An exceptional high diversity and high abundance of euglenoids were found in the small periodic pond, which we investigated. Representatives of the genus *Trachelomonas* were the most numerous and diverse, although other genera were also frequently found. During the whole period of this study, we mainly found cosmopolitan taxa, including *Euglena ehrenbergii*, *E. splendens*, *Lepocinclis acus*, *L. texta*, *Phacus longicauda*, *P. orbicularis*, *P. tortus*, *Trachelomonas hispida* var. *hispida*, *T. hispida* var. *coronata*, *T. hispida* var. *crenulatocollis*, *T. intermedia*. However, some interesting, rarely occurring species such as *T. cervicula* var. *heterocollis* or *T. sydneyensis* were also observed. The highest density was observed for trachelomonads. This observation is unexpected because for such fertile, turbid waters representatives of the genera *Euglena* and *Phacus* are more characteristic, whereas according to the literature, *Trachelomonas* prefer less fertile and less polluted conditions – usually from oligosaprobic to β-mesosaprobic (Cyrus & Sládeček 1973). High diversity of aquatic microorganisms was also observed in small ponds and a stream and its oxbow within the Dęby Boruszowickie forest complex in Upper Silesia, Poland (Płachno et al. 2015). In their studies, the unexpected diversity was noted for euglenophytes, and species of *Phacus* were the most diverse. It was interesting to note that the habitats were highly contaminated by heavy metals (especially thallium). It is known that heavy metals or other kinds of pollution, e.g. cyanobacterial toxins, usually alter algal diversity, and Płachno et al. (2015) suggested that euglenoids were remarkably tolerant to various kinds of pollution. They pointed out the features that may facilitate euglenoids to survive in an unfavourable envi-

ronment: fast reproduction, cysts formation and mixotrophy as a mode of nutrition (Płachno et al. 2015). It may also explain their abundant development in very fertile water bodies extremely susceptible to environmental changes such as the pond where the studies were conducted.

UPGMA dendrogram

The UPGMA dendrogram showed four distinct groups (fig. 5). The first group included only one sampling period (May 2004). The euglenoid community was very diverse, since 24 taxa were found and several of them occurred in high abundance. It is well known that the diversity of algae, including euglenoids, is higher in spring than during summer when the bloom of one or two distinct dominants is often found (Wołowski 1998, Wojciechowska et al. 2000, Gligora et al. 2007). The dominance of species from *Trachelomonas* may prove that there was a preference of these organisms to lower water temperature, especially *T. volvocinopsis* (Poniewozik 2016), which was very abundant in the spring. This species was also co-dominant in the summer of 2014; however, the summer that year was cool (about 17–18°C) during July and August (table 1). It should be noted that among trachelomonads many species are considered cosmopolitan, ubiquitous and have high tolerance to different environmental factors (Heckmann et al. 1996, Płachno et al. 2015). *Trachelomonas volvocina* and *T. hispida* are the best known. Little is known about *Lepocinclis* dominance or co-dominance. In our study, they formed very dense populations, especially during summer throughout the investigation. The most abundant was *Lepocinclis texta* (previously named *Euglena texta*), but the data about its rich development and dominance in water bodies are very scarce. Padisák et al. (2003) found the species was dominant in some small and shallow lakes in Hungary during the summer. We observed a similar situation in our study, where *L. texta* co-dominated with *Trachelomonas* species.

Ecological preferences of euglenophytes

Development of *Trachelomonas* species is known to be particularly luxuriant in case of an abundant supply of phosphorus and nitrogen (Starmach 1983). Generally, euglenophytes are known as a group of microorganisms typical of small and shallow water bodies enriched with organic matter (Reynolds et al. 2002). They are observed in waters that are characterized by a very high concentration of ammonium nitrogen. This observation was also confirmed by the results of our study, since the water in the pond was rich in biogenic elements, especially ammonium nitrogen. Duangjan et al. (2014) identified 64 taxa of *Phacus* and two taxa of *Monomorphina* in small and shallow ponds and ditches in Thailand that were heavily loaded by ammonium salts. Similar observations were made by Chaimongkhon et al. (2014) from moderate to eutrophic and polluted sites rich in nutrient content in different regions of Thailand, where the dominant taxa belonged to *Euglena*, *Lepocinclis* and *Phacus*. Many authors (Conforti & Joo 1994, Wołowski 1998, Kim et al. 2000, Wołowski & Hindák 2004, Valadez et al. 2010) have reported rich development of euglenoids in small water bodies, which probably had a high concentration of phosphorus and nitrogen compounds, including ammonium salts. Unfortunately, detailed research on nutrients was not conducted. It is obvious; however, that the composition and the biomass of phytoplankton mainly depend on growth stimulating nutrient concentrations, but also on turbulent mixing and food web effects (Rhew et al. 1999). The strong relationship between total numbers of phytoplankton and ammonium nitrogen is probably related to nitrogen limitation and indicates preference of ammonium as a source of nitrogen by different groups of phytoplankton (Beshkova & Botev 2004). Kalchev & Tsvetkova (1996) have found a relationship between the preference of primary producers of one of the forms of nitrogen and the stage of successional development of water basins, but the question why some planktonic organisms prefer ammonium salts, whereas the others prefer nitrates remains elusive (Beshkova & Botev 2004).

Small water bodies, including high fertility ponds and overgrown aquatic habitats, are very often sites in which a great diversity and species richness of algae are observed (Figueredo & Giani 2001, Padisák et al. 2003, Burchardt et al. 2006, Peretyatko et al. 2007), although the succession patterns of phytoplankton are poorly known in such hypertrophic ecosystems (Figueredo & Giani 2001). Scheffer et al. (2006) suggested that shallow lakes and ponds can offer good conditions for some groups of organisms due the low fish biomass and high vegetation abundance. Williams et al. (2004) claimed that ponds contributed to biodiversity more than other water body types, supporting considerably more species that are rare and unique. This was stated for macrophytes and invertebrates in shallow ponds, but the paper lacked specific details on phytoplankton. We could assume, however, that the statement could be applicable for phytoplankton. Additionally, we could link the diversity of phytoplankton with the higher biomass and diversity of macrophytes as shown in several studies, e.g. Medeiros Fonseca & de Mattos Bicudo (2010). The latter authors claimed that the presence of macrophytes in a shallow lake also seems to qualitatively influence the phytoplankton community, particularly favouring flagellated species. Species richness of phytoplankton in ponds results from characteristics of that water body, that is, they are usually shallow, water temperature is high, and vegetation growing on the banks provides cover against wave action. Some of these statements were supported by the work of Kruk et al. (2009) on subtropical shallow lakes. These authors stated that shallow lakes and ponds contribute disproportionately to species richness compared to other aquatic ecosystems and that the extremely high plant cover could potentially lead to different richness patterns in some groups, including phytoplankton. Their results showed some tendency for total phytoplankton richness to decrease with increasing lake area and with increasing submerged vegetation. Due to usually dense aquatic macrophyte cover, the amount of decaying organic matter is high, water colour is yellow or even brownish, and water transparency is low. This kind of conditions were also observed in the locality that we studied, and are common for localities where euglenoid studies have been conducted. It creates favourable conditions for phytoplankton development such as physical factors; especially temperature and light intensity do seem to be the key factors for its seasonal appearance (Reynolds 1984, Heckman et al. 1996, Reynolds

et al. 2002, Duangjan et al. 2014). In addition, being rich in nutrients, waters have the potential to become turbid due to high phytoplankton growth, which is their normal stable state (Peretyatko et al. 2007). Conditions of warm water combined with high nutrient concentrations are conducive to development of different groups of microorganisms, such as coccal or filamentous green algae and especially cyanobacteria. There are many records about these groups of photoautotrophic organisms growing in shallow or/and small water bodies including lakes and ponds (e.g. Briand et al. 2002, Kuczyńska-Kippen et al. 2003, Nixdorf et al. 2003, Beshkova & Botev 2004, Burchardt et al. 2006, Yamamoto & Nakahara 2009). The physical properties of small water bodies are often similar to those occurring in brown water humic lakes, where the phytoplankton community has a higher share of mixotrophic species which can ingest bacteria to obtain nutrients (Caron et al. 1990). Small, nutrient rich and/or coloured freshwater is also preferred by euglenophytes, which develop blooms in warm, fertile waters with poor light conditions (Wołowski & Walne 2007, Duangjan & Wołowski 2013, Wołowski et al. 2013, Duangjan et al. 2014). Apart from other flagellates belonging to cryptophytes, dinoflagellates, chrysophytes, raphidophytes and chlorophytes, the majority of photosynthetic euglenophytes are mixotrophic (Willey et al. 1988, Olrik 1998, Płachno et al. 2015) and develop very well in small ponds rich in organic matter. High growth of euglenoids in coloured waters may result from high loads of particulate and dissolved organic matter, which is used as a source of food (Borics et al. 2012). In our pond, the water colour was yellow-brown which was probably due to high amount of organic matter from vegetation densely overgrowing the pond. Similar results were observed by Stević et al. (2013) who recorded a rich diversity of *Trachelomonas* species in conditions of high amounts of organic matter and consequent high concentration of phosphorus and nitrogen due to decaying vegetation.

Succession pattern in small water bodies

Little is known about the phenomenon of euglenoids inhabiting an aquatic ecosystem and forming the only group of phytoplankton during the growing season. In water bodies, the process of regular succession has been well documented (Nixdorf et al. 2003). Usually, within natural seasonal succession, there may be a dominant group of organisms that is replaced by the others in the succession process (Padisák et al. 2003, Poniewozik & Płaska 2013). In our pond, the succession encompassed only one group of phytoplankton, in which representatives of *Trachelomonas* were dominant. Our study shows occurrence of euglenoids in the pond during subsequent years and additionally many species occurred annually (e.g. *Trachelomonas volvocina* var. *volvocina*, *T. volvocinopsis*, *T. hispida* var. *hispida*, *Lepocinclis texta*, *L. spirogyroides*, *Phacus limnophilus*, *P. longicauda*, *P. orbicularis*). More than 40% of all taxa identified during 2002–2004 were also found in the investigation conducted ten years later. There are at least two ideas how to explain this observation. Firstly, we could assume that these species form resting stages and in some cases, these are formed very quickly, because the pond is exposed to intense water fluctuation depending on air temperature and rainfall supply. In this situation, euglenophytes, particularly taxa belonging to *Trachelomonas*, have to be able to form resting stages to outlive unfavourable conditions in bottom sediments of the pond. Heckman et al. (1996) observed very rapid reaction to changing weather conditions and in that connection relatively quick appearance or disappearance of euglenophyte blooms in water bodies in tropical microhabitats of Mato Grosso, Brazil. They described a situation when a short, heavy rainstorm completely dispersed a bloom of *Euglena sanguinea* or *E. gracilis*, or appearance of members of *Trachelomonas* in enormous numbers a few hours after a rainfall. No research describes in a direct way on this process. It is known, however, that representatives of the genus *Euglena* form resting stages, called cysts. They are mentioned by Hindák (1986), as *Trachelomonas*-like resting cysts, and by Hindák et al. (2000), who documented for the first-time resting cysts of *Euglena agilis* and *Euglenaria anabaena* (formerly *Euglena anabaena*). Since *Euglena* and *Euglenaria* spp. form such stages, *Trachelomonas* also forms cysts. A second explanation of this phenomenon could be based on the possible ways of spreading euglenophytes, followed by reintroduction to the locality. Algae and cyanobacteria are distributed between freshwater bodies by connection through running waters or through different kinds of vectors such as air, animals (e.g. birds) or humans and their activities (e.g. Kristiansen 1996, Sahu & Tangutur 2015, Naselli-Flores & Padisák 2016, Padisák et al. 2016). Padisák et al. (2016) commented that algal propagules could move from an aquatic ecosystem to another over land, exposing risk of desiccation, as well as some groups of algae have specialized forms (e.g. akinetes, cysts, spores), which are resistant to terrestrial conditions. Animals seem to be the main dispersers of euglenophytes, especially birds are effective vectors (Kristiansen 1996, Naselli-Flores & Padisák 2016); in addition, several works listed freshwater invertebrates as dispersers, e.g. dragonflies or water beetles (Stewart & Schlichting 1966, Kristiansen 1996). Stewart & Schlichting (1966) cultivated algae from the surface of 26 species of aquatic insects and found forty algae including three euglenoid genera (*Euglena*, *Peranema* and *Anisonema*). Euglenophytes could survive air transport in the form of resting stages. This needs further study, since there is no published data on this mode of introduction of euglenoids.

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