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New and rare species of Volvocaceae (Chlorophyta) in the Polish phycoflora

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Abstract

Seven species of Volvocaceae were recorded in the lower Vistula River and its oxbow lakes, including *Pleodorina californica* for the first time in Poland. Three species – *Eudorina cylindrica*, *E. illinoisensis* and *E. unicocca* – were found in the Polish Vistula River in the 1960s and 1970s, as well as at present. They are rare species in the Polish aquatic ecosystems. Three species are common both in the oxbow lakes and in the Vistula River: *Eudorina elegans, Pandorina morum* and *Volvox aureus*. New and rare Volvocaceae species were described in terms of morphology and ecology; also photographic documentation (light microscope microphotographs) was completed.

Keywords: Pleodorina, Pandorina, Eudorina, biodiversity, phytoplankton, oxbow lake, lower Vistula

Introduction

The family of Volvocaceae (Chlorophyta, Volvocales) comprises 7 genera: *Eudorina, Pandorina, Platydorina, Pleodorina, Volvox, Volvulina* and *Yamagishiella* [1]. The genera *Astrephomene* and *Gonium* were excluded from Volvocaceae and they form new families: Goniaceae – based on the ultrastructure of the gelatinous matrix [2] and Tetrabaenaceae (*Gonium sociale*) – based on the cladistic analysis of morphological data [3]. Over the last decades, several new species were identified [4,5] and phylogenetic relationships within this group were determined [1,6–8] owing to modern technologies, such as genetic and molecular analysis.

Volvocaceae is a group of multicellular colonial algae with the number of cells ranging from 8–16 cells in *Pandorina*, *Volvulina*, 16–32 cells in *Eudorina*, *Platydorina*, *Yamagishiella* to 32–64, or even 128 cells in the genus *Pleodorina*. Colonies consisting of 200 to over 50000 cells represent the genus *Volvox*. Colonies from the genus *Platydorina* are flattened, and spherical or oval from other genera. There are no differences between somatic and reproductive cells in the genera of *Eudorina* and *Pandorina*, while in *Pleodorina*, somatic cells are smaller and reproductive cells are larger. In *Volvox* colonies, somatic functions are separated from reproductive ones, however most of the cells (>99%) perform somatic functions [1]. Green algae from the family of Volvocaceae are frequently encountered in eutrophic waters. All species from this family live in fresh waters: lakes, ponds, rivers, and even puddles. Coleman [1] reports that out of ca. 200 colonial Volvocaceae in culture collections, $\sim\frac{1}{3}$ came from puddles, $\sim\frac{1}{3}$ – from lakes and rice fields, and $\sim\frac{1}{3}$ – from zygotes in soil samples from watersides.

Most of the Volvocales taxa have a limited geographical range; some occur at only few sites. In recent years, in aquatic ecosystems of the temperate zone, one can observe unexpected expansion of species previously considered to be tropical. *Pleodorina indica* found in the Czech Republic is one of the examples of this phenomenon [9]. *Pandorina morum, Eudorina elegans, Volvox aureus* and *V. globator* have been found relatively frequently in Poland [10].

Ecology and environmental requirements of many Volvocaceae species are still poorly researched. The knowledge about the requirements of these species can be helpful in the assessment of aquatic ecosystems, the rate of the eutrophication process or climate changes on the Earth. Insufficient knowledge about Volvocaceae in Poland results mainly from their low abundance and incidence.

The present studies were undertaken in order to identify Volvocaceae in six eutrophic, shallow oxbow lakes of the Vistula River, in the main channel of the river and in Włocławek Dam Reservoir. All taxa were documented on light microscope (LM) microphotographs.

Material and methods

In 1994–2000, the research on phytoplankton of the Lower Vistula was conducted between the towns of Wyszogród and Toruń (on the 163th km of the river). The largest reservoir in Poland is located in this area. More details about the study area, water quality, chemical composition and seasonal changes are presented in the paper by Kentzer et al. [11].

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Tab. 1 Morphometric and physicochemical (min-max range) parameters of the investigated oxbow l	Tab. 1	5.1 Morphometric and phy	/sicochemical (r	min–max range)	parameters of the investi	gated oxbow lak
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	W1	W2	W3	Ma	Pr	P.D.
Geographical location	N 53°01' E 18°39'	N 53°01' E 18°38'	N 53°01' E 18°39'	N 53°00' E 18°34'	N 53°00' E 18°33'	N 53°01' E 18°30'
Area (ha)	1.5	2.0	2.5	2.8	1.0	71
Maximum depth (m)	1.8	1.8	2.5	2.5	2.0	1.8
Maximum length (m)	140	172	220	640	160	1800
Maximum width (m)	67	103	40	61	100	390
SD visibility (m)	0.45-1.8	0.6-1.8	0.5-2.0	0.4-2.5	0.4-1.3	0.3-1.8
T (°C)	7.6-22.3	7.6-23.2	7.6-22.2	7.9-24.8	7.4-23.1	7.6-25.4
pН	7.5-8.7	7.6-9.9	7.6-8.6	7.9-9.0	7.7-10.5	7.8-9.2
<i>EC</i> (μS cm ⁻¹)	598-950	641-1130	56-844	439-1072	413-708	473-775
$N-NH_4$ (mg dm ⁻³)	0.13-0.95	0.13-0.75	0.16-0.45	0.12-0.42	0.16-0.45	0.01-0.75
$N-NO_3$ (mg dm ⁻³)	0.09-0.3	0.09-0.88	0.8-0.25	0.8-0.23	0.11-1.0	0.6-1.2
$P-PO_4 (mg dm^{-3})$	0-0.23	0-0.25	0.02-0.25	0.01-0.12	0.01-0.18	0-0.08
Chl a (µg dm ⁻³)	2.68-58.52	3.97-70.86	3.12-35-24	1.44-73.90	4.48-210.93	4.05-184.14

Tab. 2 Volvocaceae taxa found at the investigated sites.

	WDR	Vist.	W1	W2	W3	Ma	Pr	P.D.
Eudorina illinoisensis (Kofoid) Pascher*	+	+	+	-	+	-	-	-
<i>Eudorina cylindrica</i> Korschikoff*	+	-	-	-	+	+	+	+
Eudorina elegans Ehrenberg	+	+	+	+	-	+	+	+
Eudorina peripheralis (Goldstein) Yamada and/or	+	+	+	-	+	+	+	-
<i>Yamagishiella unicocca</i> (Rayburn et Starr) Nozaki*								
Pleodorina californica Shaw*	+	-	+	-	+	+	+	-
Pandorina morum Bory	+	+	+	+	+	+	+	+
Volvox aureus Ehrenberg	-	+	+	-	-	+	-	-

"+" - present; "-" - absent. * rare species described in the text.

In 2006–2010, the research covered the Vistula River and six oxbow lakes located in the city of Toruń (N 53°00'–53°01'; E 18°30'–18°39') between the 734th and 745th km. Four reservoirs are isolated from the river; two of them are in constant contact with waters of the Vistula River. All reservoirs are shallow – max. depth 2.5 m. Tab. 1. presents morphological and physicochemical parameters of the research sites. The sites are listed in the species description.

A total of 312 phytoplankton samples were collected in the Vistula River and in the Włocławek Reservoir, and 208 samples in the oxbow lakes. Miscellaneous species from the researched family of Volvocaceae were found in the phytoplankton. Phytoplankton was collected from the entire water column with a plankton net No. 25. Samples were preserved with formaldehyde or with Lugol's solution. Live or preserved algae were observed under LM. To examine gelatinous matrix of the colonies methylene blue was used [4,5]. Together with phytoplankton sampling, selected physicochemical water parameters were determined: transparency (SD), temperature (T), pH and electrolytic conductivity (*EC*). The content of chlorophyll *a* (Chl *a*) and mineral forms of nitrogen and phosphorus were analyzed in the oxbow lakes.

Results

In the studied reservoirs seven Volvocaceae taxa were recorded (Tab. 2). All taxa were documented using LM microphotographs (Fig. 1–Fig. 7). More detailed accounts were prepared for the rare species: *Eudorina cylindrica*, *E. illinoisensis*, *E. peripheralis* and/or *Yamagishiella unicocca* and *Pleodorina californica*.

Eudorina cylindrica Korschikoff

Cylindrical coenobia consist of 16 cells arranged in four rows with four cells in each row. Coenobia are $65-70 \mu m \log and 45-50 \mu m$ wide. Cells are $12-15 \mu m$ in diameter (Fig. 1).

The species is known from North and South America [1], as well as from Europe: Southern Russia [12], Hungary [13–15] and Romania [16]. In the Vistula River, *E. cylindrica* was found for the first time in 1968 by Uherkovich [17].

In the phytoplankton of the Lower Vistula researched since 1994, this species was recorded occasionally during spring and summer months, i.e. from May to September 1997, near the dam and at the site in Nieszawa, downstream from the dam [18–20].

In the P.D. oxbow lake, *E. cylindrica* occurred only on 05 August 2008, when the temperature was 20.8° C and the concentration of biogenic elements was as follows: N-NH₄ 0.75 mg dm⁻³, N-NO₃ 0.2 mg dm⁻³ and P-PO₄ 0.08 mg dm⁻³. In the Prz. reservoir, at the time of isolation from the Vistula River, the species occurred in late August in 2008 and 2009 at a temperature of (respectively) 19.9 and 18.8°C, and the concentration of N-NH₄ 0.25 mg dm⁻³ and 1.6 mg dm⁻³, N-NO₃ 0.24 mg dm⁻³, and P-PO₄ 0.18 mg dm⁻³ and 0.3 mg dm⁻³. In the Ma reservoir, this species occurred in July and August 2010 (2–3)

months after flooding) – at a temperature exceeding 20.5°C. In the W3 reservoir, *E. cylindrica* occurred in August 2010 when the temperature fluctuated within the range of 19.6–20.5°C.

Eudorina illinoisensis (Kofoid) Pascher

Coenobia are oval shaped, $100-115 \,\mu m \log and 95-100 \,\mu m$ wide, with 16–64 cells. Large cells are up to 15 μm in diameter, small ones – up to 8–11 μm . There are always 4 small cells at one pole. Cells are facultatively somatic, but they also participate in asexual and sexual reproduction [4] (Fig. 3).



Fig. 1 Eudorina cylindrica Korschikoff. Scale bars: 10 µm.



Fig. 2 Eudorina elegans Ehrenberg. Scale bars: 10 µm.



Fig. 3 Eudorina illinoisensis (Kofoid) Pascher. Scale bars: 10 µm.



Fig. 4 a *Eudorina peripheralis* (Goldstein) Yamada and/or *Yamagishiella unicocca* (Rayburn et Starr) Nozaki. b *Eudorina peripheralis* (Goldstein) Yamada and/or *Yamagishiella unicocca* (Rayburn et Starr) Nozaki; picture below after staining with blue methylene. Scale bars: 10 μm.

The species was recorded in Asia and Europe: in Great Britain [21], Poland [17] and Romania [16], as well as in North and South America: in the region of Great Lakes [22] and in Brazil [23].

The subsequent research in the Vistula River and in the Włocławek Reservoir [18–20] confirmed the presence of this taxon. The species was recorded in spring and summer months, i.e. between May and September every year from 1995 to 2000, at the sites located in the free-flowing river and in WDR. The species occurred at a water temperature exceeding 21°C and remained till it dropped to 9.7°C. In July, the presence of *E. illinoisensis* in the Włocławek Reservoir was accompanied by a water bloom of *Microcystis*. In reservoir W1, *E. illinoisensis* was found in July 2010 after the flooding when the temperature fluctuated within the range of 20.6–21.7°C. In W3, the species was recorded on 11 August 2010 at a temperature of 19.8°C.

Eudorina peripheralis (Goldstein) Yamada and/or Yamagishiella unicocca (Rayburn et Starr) Nozaki

Syn. *Pandorina charkowiensis* Korschikoff/*Pandorina unicocca* Rayburn et Starr./*Eudorina unicocca* G. M. Smith (Fig. 4a,b).

Coenobia are oval shaped, 32-celled, 90–115 μ m long and 60–80 μ m wide. Spherical cells are 12–18 μ m long and are arranged in five rows: three central rows consist of 8 cells, two outermost – of 4 cells. Gelatinous matrix is homogeneous. Distinction between species *Eudorina peripheralis* and *Yamagishiella unicocca* is possible after determine the number and location of contractile vacuoles in the cell [24]. The publication appeared in 2008 year, when most of the presented material was preserved and observation of the vacuoles was impossible. Earlier Nozaki [25] wrote that it seems impossible to distinguish these two species based on observations of vegetative colonies.



Fig. 5 Pandorina morum Bory. Scale bars 10 µm.



 $\label{eq:Fig.6} Fig. 6 \quad \textit{Pleodorina californica Shaw; pictures at the bottom after staining with blue methylene. Scale bars: 10 \ \mu m.$

The species is known from Asia, Europe, as well as North and South America [1]. *Yamagishiella unicocca* occurs near Kharkiv [12,26], in Siberia [27], in the U. S. states of Indiana, Massachusetts and Oregon [28], in southern Brazil [23], in Singapore [29], in Germany in the Elde River [30], in Hungary in the Tisza River [15] and in Spain [31]. These taxa, originally described as *Pandorina charkowiensis* and *E. unicocca*, were recorded in the Vistula River in Poland by Uherkovich [17].

In the Vistula (along the distance from the town of Wyszogród up to the town of Toruń) and in WDR (in the main stream and in the marsh part), the species occurred between late March and November in 1996–2000 and was present at all sites [18–20]. At that time the temperature ranged from 10.6 to 24.0°C. In the Ma reservoir, the species occurred in August 2010 (3 months after the flooding) – at a temperature ranging from 20.7 to 22.0°C. In reservoir W1, it occurred in July and August

2010 when the temperature fluctuated within the range of 19.6–21.7°C. In reservoir W3, it occurred in August 2010 when the temperature fluctuated within the range of 19.8–20.1°C.

Pleodorina californica Shaw

Syn. Eudorina californica (Starr) Goldstein (Fig. 6)

Pleodorina californica is a coenobial green alga with oval or spherical colonies and the diameter of $50-190 \ \mu m$ with 128, 64 or 32 cells. Gelatinous cover is homogeneous. Biflagellate cells are differentiated into somatic (small) cells of $7-9 \ \mu m$ and reproductive (large) cells of $12-20 \ \mu m$. In the colonies from the Vistula River and the oxbow lakes, somatic cells accounted for 32 to 50% of all cells.

P. californica was recorded in Africa, Asia, Europe, North and South America [1]. *P. californica* occurs at few sites but is relatively common in the USA [3,22,32]. The species was also



Fig. 7 Volvox aureus Ehrenberg. Scale bars: 10 μ m.

found on Java [33], in Venezuela [34] and in Ontario (Canada) [35], as well as in Australia and New Zealand [36]. In Europe, it was found in Ukraine [37], on the Iberian Peninsula [38], and in Romania [16,39].

This is the first information about the species occurrence in Poland. In the Włocławek Reservoir, P. californica occurred only once - on 07 August 1998. Later, it was recorded several times in the Vistula oxbow lakes in Toruń. In the Prz. oxbow lake, which at that time was isolated from the Vistula River because of the very low water level in the river, the species occurred several times in August, September and October 2009. It occurred at a temperature of 22.3°C, the concentration of N-NH₄ 1.6 mg dm⁻³ and N-NO₃ 0.24 mg dm⁻³, and P-PO₄ 0.3 mg dm⁻³ and was present till October when the temperature dropped to 11.5°C, and the concentrations of biogenic elements were as follows: N-NH, $0.4 \text{ mg} \text{ dm}^{-3}$, N-NO₂ $0.48 \text{ mg} \text{ dm}^{-3}$, and P-PO₄ $0.04 \text{ mg} \text{ dm}^{-3}$. In the Ma reservoir, the species occurred only once - on 25 August 2009 in similar thermal and chemical conditions - 20.8°C and the concentration of N-NH, 0.34 mg dm⁻³, N-NO₂ 0.18 mg dm⁻³ and P-PO₄ 0.04 mg dm⁻³. In W1 and W3 reservoirs, P. californica occurred in July 2010, when the temperature fluctuated within the range of 20.8-21.2°C. Biocoenoses of these reservoirs were in the process of regeneration after May inundation.

Discussion

In 1968, a total of 8 taxa were found in the seston of the Vistula River [17], which at present are included in Volvocaceae. Those are: *Eudorina charkowiensis* (syn. *Pandorina charkowiensis* [26]), *E. cylindrica, E. elegans, E. illinoisensis, E. unicocca, Pandorina morum, Volvox aureus, V. globator.*

Several of the aforementioned species were found during the research conducted in 1994–2010 in the lower Vistula and the oxbow lakes. Those were: *Eudorina cylindrica*, *E. elegans*, *E. illinoisensis*, *E. unicocca* (it is probably *E. peripheralis* and/ or *Yamagishiella unicocca*), *Pandorina morum*, *Volvox aureus*.

According to Nozaki et al. [25], it is impossible to distinguish between *E. unicocca* and *Yamagishiella unicocca* without genetic analysis (*rbcL* gene sequence) or observations of sexual reproduction (isogamy/anisogamy). Both species are identical in morphological terms. However, in further work Yamada et al. [24] report that number and distribution of contractile vacuoles, in all strains of *Eudorina* and *Yamagishiella* can be clearly distinguished two genera. *Eudorina* strains had several contractile vacuoles distributed over the entire surface of vegetative colony protoplasts, whereas *Yamagishiella* strains had only two contractile vacuoles near the base of the flagella.

Two species, *E. unicocca* and *E. peripheralis* have been separated from the species *Eudorina unicocca*. *E. unicocca* has characteristic gelatinous matrix divided into individual sheath covers of each cell. *E. peripheralis* differs from *E. unicocca* in lacking individual sheaths [24]. In disputed species, the gelatinous matrix is not divided, but unfortunately, contractile vacuoles are not visible in the preserved material. This makes it impossible to distinguish these two taxa – *E. peripheralis* and/ or *Yamagishiella unicocca* of material collected earlier.

Eudorina cylindrica, E. Illinoisensis and *E. peripheralis* and/ or *Yamagishiella unicocca* [10] are rare in Poland. *P. californica* from the genus *Pleodorina* is new to Poland – the species was first recorded in 1998 in the Włocławek Reservoir and then several times during the subsequent research in the Vistula oxbow lakes. So far, six species from the genus *Pleodorina* were described: *P. californica* Shaw, *P. japonica* Nozaki, *P. indica* (Iyengar) Nozaki, *P. sphaerica* Iyengar [4] and *P. starrii* H. Nozaki et al. and *P. thompsonii* F. D. Ott et al. [5]. *P. japonica*, *P. sphaerica*, *P. starrii* and *P. thompsonii* were found only outside Europe. *P. indica*, originally described from India, Mexico and Argentina [9], was also found in Europe in the oxbow lakes of the Danube River [8], in the Malše River where in 2003 it caused a large-scale water bloom [32], and a year later, the species was reported from eutrophic ponds in the Czech Republic. *Pleodorina californica*, known mainly from the southern U. S. states and single sites in Europe, was also found in Poland in the Włocławek Reservoir and the Vistula oxbow lakes in Toruń. This is the first publication about the presence of this species in Poland.

In order to confirm the identification of *Eudorina peripheralis/Yamagishiella unicocca* and *Pleodorina californica* found in the Vistula River and this oxbow lakes, a further morphological observations and genetic studies are required. Because of the small number of specimens and low incidence, genetic studies have not yet been carried out.

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