New European locality of three rare taxa (Zygnematophyceae, Streptophyta): *Cosmarium pseudoprotuberans* var. *sulcatum* (Nordstedt) Coesel, *Gonatozygon aculeatum* W.N.Hastings and *Pleurotaenium simplicissimum* Grönblad

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Abstract. In the current article the new Ukrainian desmid material: three species new for Ukraine and their location are examined. The discussion focuses on comparing newly found species in Ukraine to their previous published descriptions in Europe. The reported distribution of these taxa in Europe: *Cosmarium pseudoprotuberans* var. *sulcatum* (Nordstedt) Coesel was reported for five countries, *Gonatozygon aculeatum* W.N.Hastings was found in eight countries and *Pleurotaenium simplicissimum* Grönblad was previously revealed only in four European countries. We provide a detailed description of the morphological characters of the Ukrainian cells and supply it with the drawings and pictures. The ecological aspects of the new habitat important for the European flora of desmids are discussed. This article provides information for better understanding of desmids ecology and correct identification of the described rare and sometimes poorly known species.

Key words: Chernihiv Polesia, morphology, plant communities, quarry pond, Zygnematophyceae.

1. Introduction

We are faced with a dilemma: should we devote more efforts to completing the global inventory (and if so, how and which classes of organism?) or should we focus our efforts on those classes of organisms that are known to contain species that are important in human activities (such as food crops, timber trees, domesticated animals, etc and their wild relatives) and if so how do we determine priorities in terms of species? (Heywood, 1995). We believe that it is equally important to study and conserve plant genetic resources for food and agriculture, and the global diversity inventory. Phytobiota inventory is the basis for developing a plant conservation strategy. A widely accessible Flora of all known plant species is a fundamental requirement for plant conservation and provides a baseline for the achievement and monitoring of targets of the Global Strategy for Plant Conservation 2011-2020 (Sharrock, 2020). Particular attention is paid to the protection of macroscopic plant species (e.g. vascular plants and bryophytes), which are

more vulnerable to anthropogenic influence. However, there is evidence that there are endangered algae and that some have become extinct in recent years. The concept of microalgae that '*everything is everywhere*' is challenged. Evidence that some species have restricted ranges means that their biogeography has to be taken into account in their conservation. Taxonomic knowledge and a global approach are also vital to the conservation of the algae. (Brodie et al., 2009).

Desmids are known for their demands on environmental conditions; desmid algae diversity is dependent on habitat and location. A detailed description of both desmid species and their locality is a prerequisite for an algal flora inventory on a sound scientific basis.

Chernihiv Polesia is an understudied region from the phycological point of view: only isolated information is known about algae found in this area. The northwestern part of Chernihiv Polesia is particularly interesting for research, as this territory is a system of dead aqua-glacial valleys of the Zamhlai, Smyach and Ubid rivers, which affects the formation of vegetation, flora and algal composition of ecotopes.

2. Study area

Three remarkable desmid species (Zygnematophyceae, Streptophyta) new for Ukraine and rare and extra rare for the European flora, were found in in the northwestern part of Chernihiv Polesia (north of Ukraine) in the sand quarry Zavodske pond (Fig. 1). Zavodske is a small pond (8625 m^2, depth 1.5–2 m), located two hundred meters east of the Zavodske village, Chernihiv region, Ukraine (N 51°96'3"; E 31°18'6"). It is one of the smallest ponds of a group of technogenic ponds known as "Blue Lakes". They are located among the massif of pine and oak-pine forests. Former quartz sand quarries (second half of the 20^{th} century) now they are ponds filled with spring water.

The territory of the northwestern part of Chernihiv Polesia is an accumulative terrace plain, composed of aqua-glacial and ancient alluvial deposits. The Antropocene deposits are represented by aqua-glacial and ancient alluvial sands and sandy clays. In some places, a significantly eroded moraine comes to the surface (Marinich, 1963).

The study area is dominated by green-moss and blueberry pine forests, oak-pine forests and birch forests instead of pine forests. There are also areas of pine-oak-hornbeam and oak-hornbeam forests in those areas where sandy deposits are underlain by moraine loams and variegated clays. The border-areal and relict species of vascular plants, in particular, aquatic ones are widely present in the north-western part of the Chernihiv Polesia, e.g.: *Najas major* All., *Potamogeton friesii* Rupr., and *Sparganium glomeratum* L. (Lukash, 2009).

Figure 1. Photograph of Zavodske pond. Photo by I. Shyndanovina.

3. Materials and methods

Twenty algological samples were collected from Zavodske pond during the field research of the Chernihiv Polesia flora and vegetation in 2018–2021. *C. pseudoprotuberans* var. *sulcatum* was found in 7 of 20 collected samples, *G. aculeatum* and *P. simplicissimum* were found in 5 of 20 samples (see Table 1).

The samples of Zavodske pond were taken from the euphotic zone of the pond by scraping off periphyton and by squeezing water plants floating on the water surface.

The samples were fixed with 4% formalin. We studied live and fixed samples. Olympus BX-51 microscope (samples of 2019–2021) and Zeiss Imager A2 (samples of 2021) were used for examination. Photos were made with Canon cameras EOS 1000D, and EOS R6. The cells were measured using ocular micrometre. To measure pH and conductivity, the EZODO 8200M, H&M COM-100 and H&M PH-200 were used.

Table 1. Algological samples collected in Zavodske pond within 2018–2021.

	Winter		**Spring**		**Summer**		**Autumn**	
	Date	Q-ty of samples	Date	Q-ty of samples	Date	Q-ty of samples	Date	Q-ty of samples
Quantity of field trips within 2018–2021	Dec.–Feb.	1	March–May	-	June–Aug.	5	Sept.–Nov.	4
Quantity of samples within 2018–2021		1		-		12		7
Cosmarium pseudoprotuberans var. *sulcatum* found in the following samples:					Aug.04, 2018	2		
					June 25, 2019	2		
					Aug.28, 2021	1		
							Sept.12, 2021	1
							Oct.03, 2021	1
Total samples:						**5**		**2**
Gonatozygon aculeatum found in the following samples:					Aug.04, 2018	1		
					June 25, 2019	2		
					Aug.28, 2021	1		
							Sept.12, 2021	1
Total samples:						**4**		**1**
Pleurotaenium simplicissimum found in the following samples:					Aug.04, 2018	1		
					June 25, 2019	2		
					Aug.28, 2021	1		
							Sept.12, 2021	1
Total samples:						**4**		**1**

The field study of the vegetation was carried out by geobotanical methods (Korchagin & Lavrenko, 2012). The syntaxa vegetation was identified on an ecological-floristic basis (Matuszkiewicz, 2019). Syntax names (classes, orders and alliances) are ordered according to L. Mucina et al. (2016).

The hydrochemical data are from one-time measurements. Pond water samples for chemical analysis were taken in October 2021.

The content of ions in water was determined by colourimetric estimation method using a portable photocolorimeter AQ4000. The photocolor imetric analysis was done by Dr O. Kupcyk (T. H. Shevchenko National University “Chernihiv Collehium”).

4. Results and discussion

Hydrochemical indicators of Zavodske pond

The hydrochemical indicators of the algae habitat are: pH – 7.5–7.7, EC - 36–38 μS.cm^{-1}, ion concentrations (mg/l) NO_3^- – 0.017, NH_4^+ – 0.018, PO_4^{3-} – 9.283, Cu^{2+} – 0.057, Zn^{2+} – 0.036, $Fe^{(2+,3+)}$ – 0.016, Mn^{2+} – 0.263. Attention should be paid to the content of nitrogen and phosphorus compounds.

The presence of inorganic nitrogen compounds in oxidized forms in natural waters indicates that the processes of “self-cleaning” are taking place in the aquatic environment through nitrification - reactions of the nitrogen cycle in the biosphere, which are carried out by chemolithoautotrophic nitrifying bacteria (Gołaś et al., 2008; Carini & Joye, 2008). Probably as a result of ammonification, the bottom sediments (which have a thickness of 10 to 30 cm) and water (maximum depth of 2 m) are enriched with easily digestible forms of nitrogen in the studied pond. Nitrification completes the process of mineralization of organic substances in the aquatic ecosystem.

Phosphorus, like nitrogen, is a critical nutrient required for algae growth. Phosphorus has a major impact on algal food quality (Li et al., 2022). The most common form of phosphorus used by biological organisms is phosphate. The high content of PO_4^{3-} ions was detected in water samples from the Zavodskoe pond, as well as in samples from other “Blue Lakes” reservoirs. It is likely that orthophosphates enter surface water during rain or snowmelt from a nearby forest nursery. In addition, the Zavodskoe pond is located within the recreation complex “Blue Lakes”, where recreationists use water from the lakes for washing dishes and washing clothes.

Phytocenotic conditions of the algae habitat

The vegetation of Zavodske pond, which occupies a third of its surface, was formed in the coastal waters and on two “islands”. In the along shore waters, the vegetation is formed by communities of *Phragmition communis* Koch 1926 (associations of *Typhetum angustifoliae* (Allorge 1922) Soó 1927, *Typhetum latifoliae* Soó 1927, *Scirpetum lacustris* Schmale 1939, *Sparganietum erecti* Roll 1938) and *Magnocaricion elatae* Koch 1926 (associations of *Caricetum ripariae* Soó 1928, *Caricetum acutiformis* Sauer 1937, *Caricetum elatae* Koch 1926).

These communities alternate along the coast in water belt (up to 1.5 m wide). The area is dominated by coenoses with monodominance of *Typha angustifolia* L.

At 2–3 meters distance from the shore the phenomenon of spatial change of ecosystems can be observed in water layer, namely the formation of an island ecocline of loose soils. It was formed in the pond during the rise of the water level: small (2-2.5 square meters) fragments overgrown with *Typha angustifolia* L. separated from the coastal communities of *Typhetum angustifoliae* (Allorge 1922) Soó 1927. These fragments moved slightly to the center of the pond during the decrease of the water level in it. After the separation these ecoclines kept the characters of the phytocenosis of *Typhetum angustifoliae* (Allorge 1922) Soó 1927 but later they were populated by species of the *Carici-Rumicion hydrolapathi* Passarge 1964 union – *Thelypteris palustris* Schott (80%), *Solanum dulcamara* L., *Lycopus europaeus* L., *Carex pseudocyperus* L., *Lysimachia thyrsiflora* L., *Alnus glutinosa* (L.) P. Gaertn.

It should be noted that the *Carici-Rumicion hydrolapathi* Passarge 1964 alliance is the vegetation of wetland herbs on organic muddy sediments. This alliance includes vegetation of tall to short wetland graminoids or broad-leaved herbs occurring on wet, organic muddy sediments. They produce creeping stolons, rhizomes and adventitious roots, which ensure their stability in the unstable substrate, and also allow formation of carpet-like stands floating on the water surface. The vegetation types treated within this alliance occur in mesotrophic or dystrophic, rarely also eutrophic habitats, in an advanced stage of terrestrialization. They are typical of fishpond littoral zones, especially on peat accumulations, oxbows, alluvial pools, ditches and peatlands. These habitats have a relatively stable water level, without floods that bring in mineral sediment accumulation. The water table is usually up to 50 cm high though it can recede far enough to expose the bottom, but the substrate never dries up. *Carici-Rumicion hydrolapathi* is typical of the boreal zone of Eurasia and of oceanic north-western Europe (Šumberová et al., 2011). In Chernihiv Polesia that is located in the temperate zone, such biotopes are rare.

The third part of the plant cover of the pond consists of phytocenoses of unrooted free-floating plants on the surface and inside the water column – pleistophytes: *Hydrocharitetum morsus-ranae* van Langendonck 1935, *Ceratophyllo-Hydrocharitetum* Pop 1962. They belong to the class *Lemnetea* O. de Bolòs et Masclans 1955, in particular the union *Stratiotion* Den Hartog et Segal 1964. Communities of attached and free-floating hydatophytes with leaves floating on the surface or submerged in the water column (*Potamogetonetea* Klika in Klika et Novák 1941) are represented by phytocenoses of the union *Potamogetonion* Libbert 1931: *Potametum natantis*

Hild 1959, *Elodeetum canadensis* Nedelcu 1967. The three algae described in the article were found on submerged roots and leaves of *Sparganium erectum* L.

Morphological characteristics of the algae

We provide detailed diagnoses of three rare and extra rare taxa newly found in Ukraine.

Cosmarium pseudoprotuberans var. *sulcatum* (Nordstedt) Coesel (1991). Cells 1.13–1.19 times longer than broad, semicell is hexagonal in outline, apex truncate straight or barely concave, sometimes slightly inclined, upper lateral sides are straight sometimes concave, lower lateral sides are slightly convex. The sinus is deep; it is often different on two sides of the cell. Close to its apex the sinus is slightly undulated and narrowly open, and then it is widely rounded and open. In apical view it is elliptic the lateral sides of the cell are undulate due to three papilliform thickenings. The thickening is also present on the angles. Circular semicells in the side view are furnished with lateral extensions that are just above the center of the semicell. Every semicell has one chloroplast with one pyrenoid. Length is 36-37µm, breadth is 30–32.5 µm, thickness 21–22 µm, and isthmus is 7.5–9 µm (Figs 2 and 3). In the following text of the article, the algae sample collected by us will be called the “Ukrainian cell”.

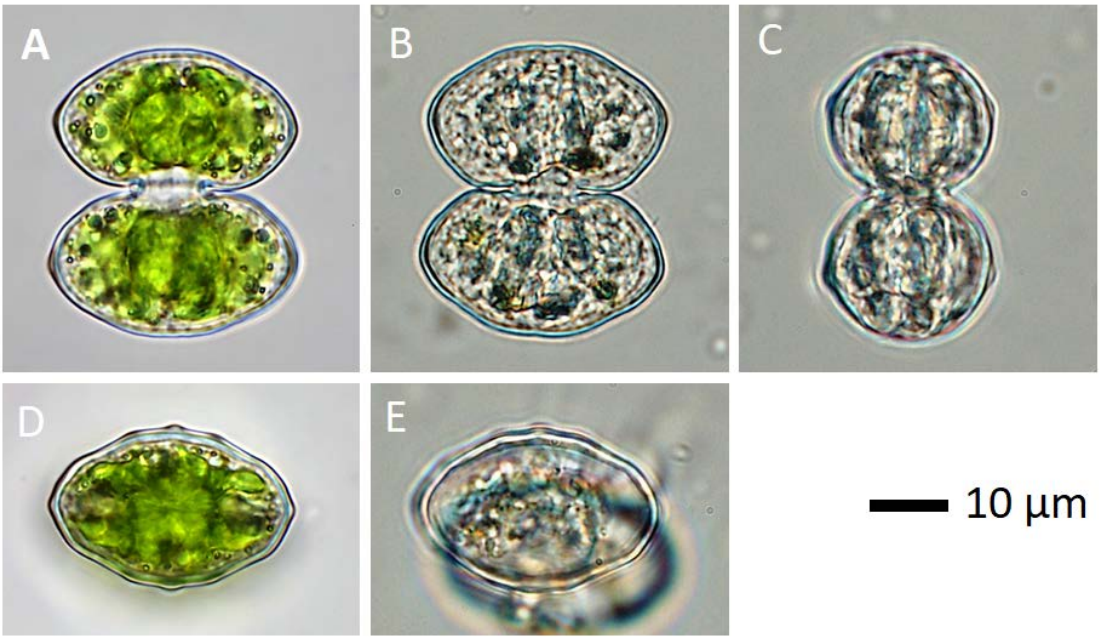

Figure 2. Photographs of *Cosmarium pseudoprotuberans* var. *sulcatum*: face view (A, B, C), side view (C), apical view (D, E). Scale bar: 10 µm (A–E). Photos by I. Shyndanovina.

The first and the only published finding of this cell in Ukraine was done by the Polish algologist R. Gutwiński (1892). *Cosmarium sulcatum* Nordstedt 1878 was found in the valley of the hilly part of Kiliński (now Stryjski) park in Lviv (former Kingdom of Galicia and Lodomeria

within Austro-Hungarian Monarchy – now Ukraine). The author indicated that locality is situated on Miocene sands. The size of the cell found by. Gutwiński is nearly the same compared to the one found by us: length 36 µm, breadth is 29 µm, thickness is 19 µm, and isthmus is 7 µm. This publication does not contain drawing of this cell. Palamar-Mordvintseva (2005) included this species in the flora of Ukraine also without any drawing and indicated that it is poorly known and insufficiently studied. Indeed occurrence of this cell in Europe is reported only in 5 countries. Capdevielle (1978) was the first in Europe to give the drawing with side view *C. sulcatum* collected in France (French cell). The size of the French cell *C. sulcatum* is somewhat bigger than the Ukrainian one: length is 36.5–40 µm, breadth is 29–31 µm, and isthmus is 8–9 µm. Coesel (1991), and Coesel and Meesters (2007) moved *C. sulcatum* collected in Netherlands to *Cosmarium pseudoprotuberans* as var. *sucatum*. The author underlines its very rare occurrence. The size of the Ukrainian cell is within the range given by Coesel (1991). The *Cosmarium pseudoprotuberans* var. *sucatum* was also found in Austria in the similar habitat (Lenzenweger, 1999). The specified size range is by 2–3 µm smaller than the dimensions of the Ukrainian cell. The size of the Ukrainian cell also complies with the Czech material: length is 36.5–40 µm, breadth is 29–31 µm, and isthmus is 8–9 µm (Šťastný, 2010). The following aspects of this species ecology are specified: this cell occurring at pH < 6.5, mesotrophic habitat, very rare for the Czech Republic with high degree of ecological sensivity. The species in question seems to be characteristic of highly structured, finely balanced ecosystems (Šťastný, 2010).

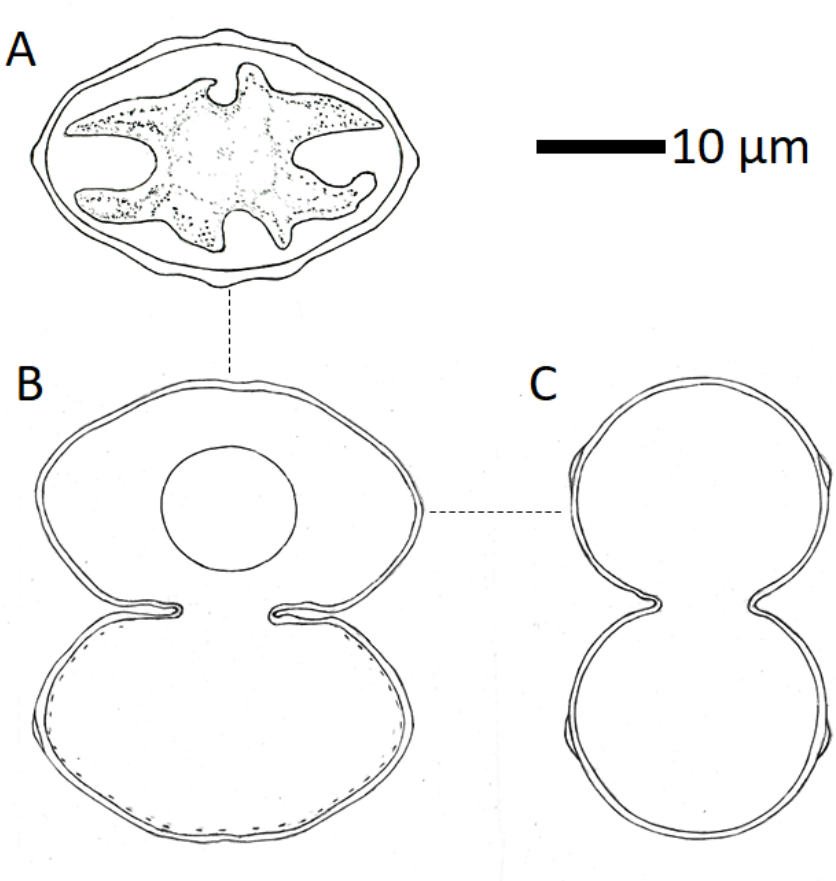

Figure 3. Drawing of *Cosmarium pseudoprotuberans* var. *sulcatum*: face view (A), apical view (B), side view (C). Scale bar: 10 µm (A–E). Drawing by I. Shyndanovina.

Gonatozygon aculeatum W.N. Hastings (1892). L: 64–67 µm, B (not apex): 7–8 µm, B (apices): 8–9 µm, L/B: 6.8–7.7. Cylinder shaped cell, length is 7 times breadth without spines (3 cells observed in samples of two different years), apices dilated and truncate, and at the edge of apex the cell wall creates a thickened ring because the bottom of apex is somewhat indented. Ends are irregularly ornamented with rare granules that look like undeveloped spines (present only on apices), they are often visible on face view and present on some drawings of this taxon (Růžička, 1977; Kouwets, 1987; Lezenweger, 1999). Spines are rather short (2.5 µm) but making over 30% of the cell diameter (7–8 µm) it seems worth considering that ratio in taxon identification knowing that cells size varies largely (in a similar way cell length to breadth ratio is indicated in most publications). Spines are scattered moderately densely, they are quite strong (broader at the basis) and have regular not very much variable length. Ukrainian specimen (Fig. 4) is very small comparing to other reports on this taxon and is closer in dimensions to report from Check Republic (Šťastný, 2010).

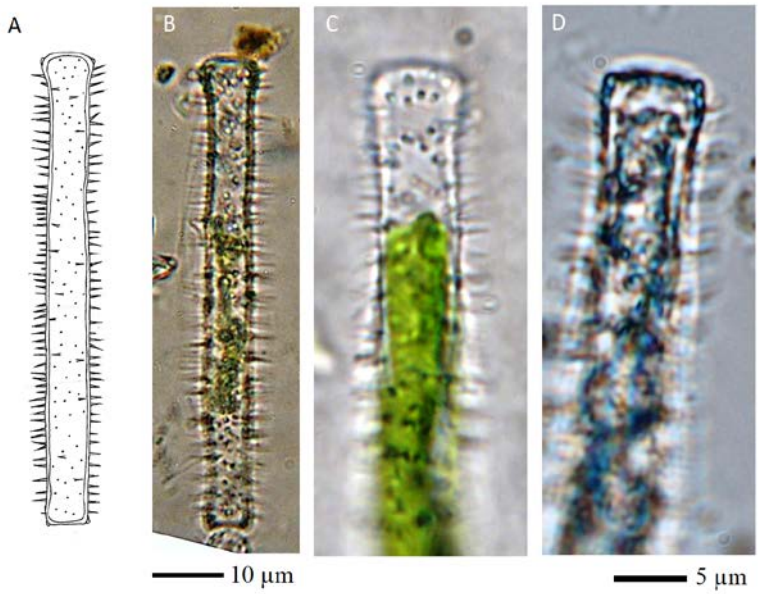

Figure 4. Drawing of *Gonatozygon aculeatum*: drawing (A), photos (B-D). Scale bar: 10 µm (A–B), 5 µm (C–D). Drawing and photos by I. Shyndanovina.

Pleurotaenium simplicissimum Grönblad (1920: 27) (L: 600–625 µm, B (basal): 31.5–34.0 µm, B (ends): 25.5–29.7 µm, L/B: 17.8–18.7 (measured 2 cells so far). It is a very rare taxon (Růžička, 1977; Šťastný, 2010). Long, rod shape cells with slightly marked basal swelling and nearly unwaved side lines. Truncate apices slightly expanded or straight with rounded angles and the crown of granules on the top, 8–10 small granules can be observed on face view. Cell walls are covered with small pores and gently punctate (Figs 5 and 6).

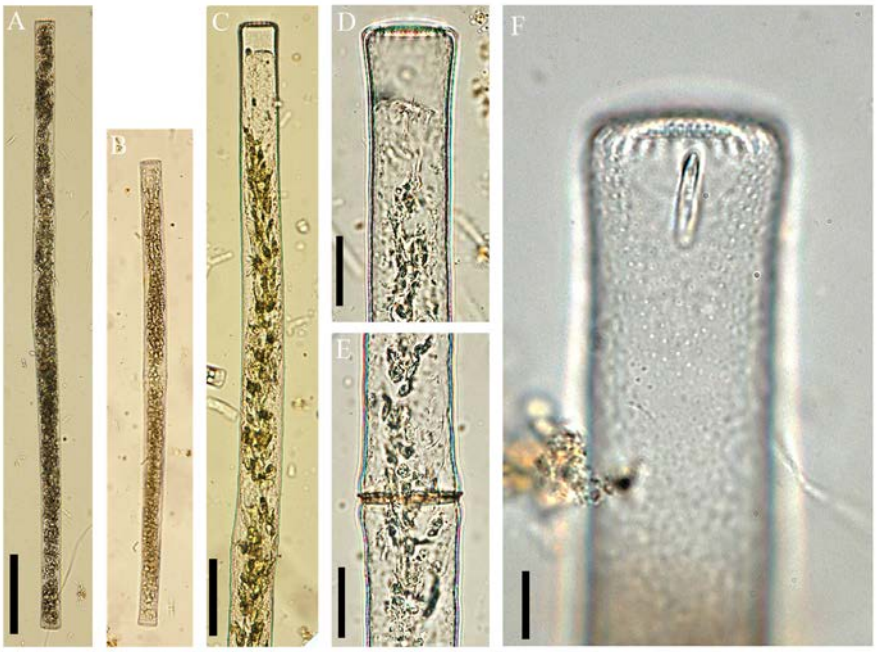

Figure 5. *Pleurotaenium simplicissimum*: Scale bar: 100 µm (A,B), 50 µm (C), 20 µm (D, E), 10 µm (F). Photos by I. Shyndanovina.

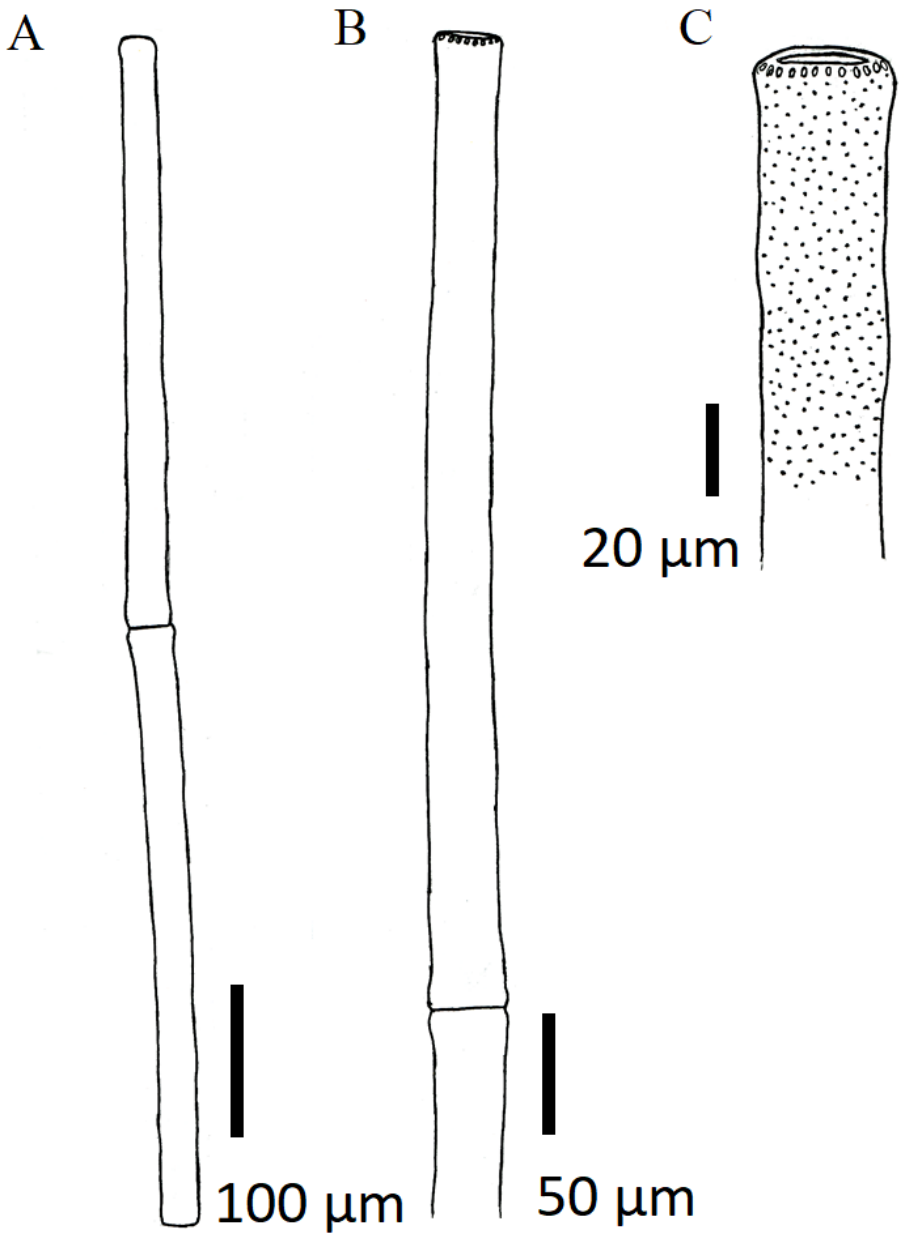

Figure 6. *Pleurotaenium simplicissimum.* Drawings by I. Shyndanovina.

In Europe the first *P. simplicissimum* was found in Finland (Grönblad, 1920). Roll (1923) reports on *Pleurotaenium baculiferum* Roll found in Karelia (part of Russia bordering Finland) that was indicated as synonym *P. simplicissimum*. The species has also been found in Germany (Krieger, 1937), Netherlands (Coesel, 1985), Czech Republic (Šťastný, 2009, 2010). Ukrainian specimen does not comply in size (smaller) with Finish, Russian and German cells. And it is within size limits reported from Netherlands and Czech Republic. J. Šťastný (2009) supposed

that *P. simplicissimum* is extra rare in Europe taxon is close to extinction. Fortunately our *P. simplicissimum* finding in Ukraine gives ground for optimism in this respect.

Conclusions

New European locality of three rare taxa (Zygnematophyceae, Streptophyta): *Cosmarium pseudoprotuberans* var. *sulcatum* (Nordstedt) Coesel, *Gonatozygon aculeatum* W.N.Hastings, *Pleurotaenium simplicissimum* Grönblad was found in Zavodske sand quarry pond (mesotrophic reservoir with pronounced processes of ammonification and nitrification) situated in the northwestern part of Chernihiv Polesia (north of Ukraine). These species are new for the Ukrainian flora.

Detailed description of locality, diagnoses, photos and drawings of the species contributes to Phytobiota inventory that is the basis for developing a plant conservation strategy. Locations important to algal flora deserve protection to prevent rare species and algal diversity from extinction.

We hope that this article will help to algologists to find new localities of these rare taxa in Europe.

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