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Morphology of *Reimeria ovata* (Hust.) Levkov & Ector in comparison with similar *Reimeria* species

by

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Abstract

Reimeria ovata (Hust.) Levkov & Ector was found during field research conducted from 2013 to 2015 on the Wołosaty stream and its selected tributaries. Valves measured in the studied material are narrower than valves described in the currently available literature and have more striae per 10 µm. Specimens of *R. ovata* observed under a light microscope were morphologically very similar to those of *R. sinuata*, particularly in the case of small individuals. The precise distinction between these two species was only possible under SEM. *R. ovata* has been found in cold, oligotrophic waters, highly saturated with oxygen, with alkaline or circumneutral pH, but never in large numbers. The aim of this work was to identify the new locations of *R. ovata* and to determine morphological and ecological characteristics of this species.

Key words: *Reimeria ovata*, morphology, ecology, SEM observations, SE Poland

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Introduction

The small genus *Reimeria* was separated from the genus *Cymbella* by Kociolek and Stoermer (1987) and comprises 13 species, including 9 flagged as currently taxonomically accepted (Guiry, Guiry 2016). *Reimeria sinuata* (Gregory) Kociolek & Stoermer is a type species to this genus. It is a cosmopolitan species, widely distributed in freshwater and aerial habitats, among mosses, etc. (Krammer, Lange-Bertalot 1986; Round et al. 1990; Hofmann et al. 2011).

Two *Reimeria* species are known in Poland, of which *Reimeria sinuata* is more common. It occurs throughout the country (Rakowska 2001; Siemińska, Wołowski 2003; Wojtal 2009; 2013; Bąk et al. 2012; Noga et al. 2014). *Reimeria uniseriata* Hall, Guerrero & Ferrario is less common and was described for the first time from the Kobylanka stream (Wojtal, Sobczyk 2006). It occurs in many rivers and streams in SE Poland, but only in the form of single cells (Żelazna-Wieczorek 2012; Noga et al. 2014).

Reimeria ovata (Hust.) Levkov & Ector, formerly known as *Cymbella sinuata* var. *ovata* Hust. 1922, was distinguished as a new species in 2010 (Levkov, Ector 2010). To date, the species has been reported from Poland as *Cymbella sinuata* f. *ovata* and *C. sinuata* var. *ovata*. Detailed information on the distribution of these species is included in the work published by Siemińska and Wołowski (2003).

The species *Reimeria sinuata* and *Reimeria ovata* are almost morphologically identical under a light microscope. They can be distinguished one from another only when using scanning electron microscopy – SEM (Levkov, Ector 2010).

The aim of the study was to show the new records of *Reimeria ovata* in SE Poland based on the morphological and ecological characteristics of the species. The authors have also attempted to distinguish the two taxa using a light microscope.

Study area

The study area was located mainly within the Bieszczady National Park – only the lower section of the Wołosaty stream flows through the territory of the San Valley Landscape Park. The bedrock of this area is built of sedimentary rocks such as sandstones and siltstones, rarely marl and conglomerates referred to as Carpathian flysch (Winnicki, Zemanek 2009).

The Wołosaty stream, known as "Wołosatka" in its upper section, is a left tributary of the San River. The source of the stream is located at an altitude of 1200 m a.s.l. At first, the Wołosatka flows from the east to the west in the depression between the ridges of the mountains. Near Ustrzyki Górne, the stream changes its course to the north and from here it is referred to as the "Wołosaty" stream. It flows into the River San behind the village of Stuposiany at an altitude of 550 m a.s.l. (Kukuła 2002; the Bieszczady and Sanocko-Turczańskie Mountains – tourist map).

The Terebowiec stream is 8.01 km long. The mouth of the creek is located at an altitude of 650 m a.s.l., while the source – at an altitude of 1225 m a.s.l. The Terebowiec stream flows into the Wołosatka stream in Ustrzyki Górne and the two streams together form the Wołosaty stream. The slopes of the valley are covered with old trees with a dominance of *Fagus sylvatica* L., a large contribution of *Acer pseudoplatanus* L. and a small admixture of *Picea abies* (L.) H. Karst. The valley is located in a strictly-protected area and is one of the best preserved natural parts of the park (Przybylska, Kucharzyk 1999; Żarnowiec 2010).

The Rzeczyca stream is a left tributary of the Wołosaty stream, with a length of 13.17 km. The sources of the stream are located at the foot of Połonina Caryńska at an elevation of 1030 m a.s.l., while the mouth of the stream is located at an altitude of 660 m a.s.l. (Czarnecka 2005).

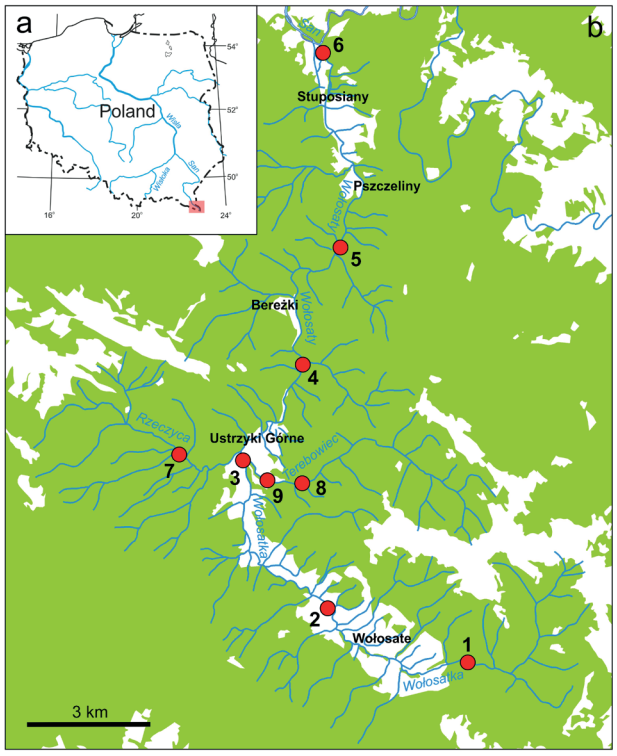

Figure 1

Location of the study area (a) and sampling sites (1-9) distribution at Wołosaty stream and tributaries (b)

Materials and methods

Studies were conducted at nine sampling sites in the Wołosatka, Wołosaty, Rzeczyca and Terebowiec streams between 2013 and 2015 (Fig. 1).

Samples were collected in September 2013, May and October 2014, and October 2015 from available habitats, such as stones (at all sampling sites) and aquatic macrophytes (mainly from mosses and green algae). The temperature, pH, dissolved oxygen and electrolytic conductivity were measured in situ. Water samples for chemical analysis were collected at the same time. Algae samples were preserved in 4% solution of formaldehyde.

Chemical analysis of the water was carried out in the Departmental Laboratory of Analysis of Environmental Health and Materials of Agricultural Origin of the University of Rzeszów by means of DIONEX ICS–5000+DC liquid chromatography equipment.

Diatom samples were prepared according to methods used by Kawecka (1980) for river algae. A portion of each sample was cleaned with a mixture of sulfuric acid and potassium dichromate at a 3:1 ratio, and then washed in a centrifuge (at 2500 rpm). Diatoms were mounted in synthetic resin (Pleurax; refractive index 1.75).

Diatoms were identified and counted under a Carl Zeiss Axio Imager A2 microscope equipped with a 100× Plan Apochromatic objective with differential interference contrast (DIC) for oil immersion (NA 1.4).

Diatom identification was supported by the following references: Krammer & Lange-Bertalot (1986), Sala et al. (1993), Levkov and Ector (2010), Hofmann et al. (2011) and Bąk et al. (2012).

Species composition was determined in the collected samples by counting 400 specimens in randomly selected fields under a light microscope. Species with a content above 5% in a given diatom assemblage were defined as the most abundant ones.

For SEM observations, samples were coated using a Quorum Q 150OT ES Turbo-Pumped Sputter Coater with 20 nm of gold, and observed under a Hitachi SU 8010 scanning electron microscope (SEM) at the Subcarpathian Center for Environmental Research and Innovation at the University of Rzeszów.

Results and discussion

Water in the studied streams was mostly alkaline; pH values were lower (near to neutral) only in September 2013. Electrolytic conductivity ranged from 78 to 284 µS cm^{-1}. The oxygen saturation level was high in each sampling season. Furthermore, the water was characterized by a low nutrient content and average calcium content (30.3-41.6 mg l^{-1}) (Table 1).

Table 1

Values of water physicochemical parameters in the Wołosaty stream and tributaries in 2013-2015, and description of sampling sites together with the most frequent accompanying diatom taxa (* below the limit of detection)

Streams		Wołosatka	Wołosaty	Rzeczyca	Terebowiec
Total number of sampling sites		2	4	1	2
Width	m	3-6	4-20	1.5-3	5-7
Depth	m	0.1-0.3	0.2-0.4	0.1-0.3	0.2-0.4
Type of the bottom		stones, at the first site stones covered with mosses in 30-40%	stones, at sites 5-6 stones covered with mosses and other algae from the genus Chlorophyta in 20-30%	stones	stones, at site 8, larger stones covered with mosses
Insolation		site 1 – low site 2 – high	high at all sites	low	site 8 – low site 9 – average
physicochemical parameters					
Temperature	°C	8.0-12.2	5.6-10.8	7.4-10.0	6.1-9.3
pH		6.4-8.8	6.5-8.6	6.4-8.4	6.4-8.4
Conductivity	µS cm^{-1}	78-254	146-278	97-246	86-284
O_2	mg l^{-1}	10.16-10.67	10.65-11.44	10.6-10.24	10.67-11.06
Cl^-	mg l^{-1}	0.43-5.33	0.65-5.35	0.60-5.31	0.41-5.59
SO_4^{2-}	mg l^{-1}	11.05-20.69	12.79-26.77	16.31-33.34	12.53-27.19
NO_3^-	mg l^{-1}	1.67-2.46	1.94-3.15	1.78-3.20	2.01-2.86
PO_4^{2-}	mg l^{-1}	*	*-0.64	*	*
NH_4^+	mg l^{-1}	0.06-0.71	0.14-0.88	0.04-0.06	0.06-0.13
Mg^{2+}	mg l^{-1}	5.00-7.82	5.26-10.12	5.33-9.32	5.62-10.10
Ca^{2+}	mg l^{-1}	30.31-34.25	33.72-41.55	38.75-39.72	32.42-35.31
The most frequent taxa		*Achnanthidium pyrenaicum*, *Cocconeis placentula* var. *lineata*, *C. placentula* var. *euglypta*, *Diatoma ehrenbergii*	*Achnanthidium pyrenaicum*, *A. minutissimum* var. *minutissimum*, *Cymbella parva*, *Diatoma ehrenbergii*	*Achnanthidium pyrenaicum*, *A. minutissimum* var. *minutissimum*, *A. thienemannii*, *Cymbella parva*	*Achnanthidium pyrenaicum*, *Diatoma ehrenbergii*

Reimeria ovata

(Hust.) Levkov & Ector (Fig. 2 m-u, Fig. 3)

Basionym: *Cymbella sinuata* var. *ovata* Hustedt (1922)

Our dimensions, valves: 12.4-31.0 µm long, 4.6-6.3 µm wide with 9-13 striae per 10 µm.

According to Levkov & Ector (2010), valves: 14-30 µm long, 6-8 µm wide with 9-10 striae per 10 µm.

Morphology. The precise morphological description of the *R. ovata* cell, containing light microscopy and SEM observations, was presented by Levkov and Ector in 2010. Cells taken from the studied samples and observed under SEM were very similar to those described in the work of Levkov and Ector (2010). The studied cells were narrower and slightly longer. The single cells are narrower, longer and have more striae per 10 µm than those described by Levkov and Ector.

Under a light microscope, valves of *R. ovata* were morphologically very similar to those of *R. sinuata* (Fig. 2a-u). However, most of the observed *R. ovata* cells were larger and wider in comparison with *R. sinuata* (Table 2). Small valves looked identical under a light microscope. Detailed SEM analysis has shown that all observed cells wider than 4.6 µm belong to *R. ovata*. Based on this, the authors distinguished both species under a light microscope (Fig. 2a-u). Although there was a difference in the width of *R. ovata* and *R. sinuata* cells, the width cannot be used as a distinguishing feature in the case of other populations. Therefore, precise distinction between these species was only possible using SEM. *R. ovata* has C-shaped areolae on the valve face and the striae are slightly depressed (Fig. 3d). In the case of *R. sinuata,* many researchers have stated that the areolae on the valve face and the valve mantle have small, round foramina which are alternately biseriate (Krammer, Lange-Bertalot 1986; Kociolek, Stoermer 1987; Sala et al. 1993; Levkov, Ector 2010). In our samples, valves of *R. sinuata* looked similar (Fig. 4a-c). The third examined species, *R. uniseriata,* is clearly distinguishable from the two described above (Fig. 2v-ad). Striae are uniseriate and extend onto the valve mantle. Areolae have C-shaped foramina. The interstriae are much wider than the striae (Fig. 4d-e) (Sala et al. 1993; Levkov, Ector 2010). According to Levkov and Ector (2010), *R. ovata* is very similar to *R. fontinalis* Levkov, but was not recorded in our material.

Distribution. There is no detailed information on the occurrence of this species, because it was only recently distinguished from *Reimeria sinuata* (*Cymbella sinuata*). *C. sinuata* f. *ovata* was often considered a synonym of *C. sinuata* (Kociolek and Stoermer 1987). The species was initially described as *Cymbella sinuata* var. *ovata* by Hustedt (1922). The species was separated from *Reimeria sinuata* in 2010 by Levkov and Ector and described as *R. ovata*. In Poland, the species was often described as *Cymbella sinuata* f. *ovata*, and less commonly as *Cymbella sinuata* var. *ovata* (Siemińska 1964; Siemińska, Wołowski 2003).

Ecology. According to Levkov and Ector (2010), *R. ovata* occurs often in oligotrophic cold waters, but never in large numbers. Our studies confirm this information. The streams, in which *R. ovata* was recorded in the course of our study, were characterized by alkaline or close to neutral pH, very high saturation of oxygen (always >10 mg l^{-1}) and a low nutrient content. As the study was conducted only in spring and autumn, the water temperature did not usually exceed 12°C. Electrolytic conductivity values at most sites were at moderate levels, except for autumn 2013 and 2015 when values exceeded 200 µS cm^{-1} (Table 1). In the collected material we always observed only single cells. (Table 2). *Achnanthidium pyrenaicum* (Hust.) Kobayasi was the main dominant species at each site in all the studied streams and often exceeded 50% of the species composition of diatom assemblages (mainly in the Rzeczyca and Wołosaty streams). *Achnanthidium*

Table 2

Comparison of the valve morphology and occurrence of *Reimeria sinuata* with the other taxa from the genus *Reimeria*, recorded in the Wołosaty stream and tributaries

Taxa	*Reimeria ovata* (n=30)	*Reimeria sinuata* (n=30)	*Reimeria uniseriata* (n=10)
Length (µm)	12.4-31.0	8.9-14.2	16.5-26.5
Width (µm)	4.6-6.3	3.8-4.3	4.2-6.4
Number of striae in 10 µm	9-13 most commonly 10-11	12-15	7-9
Occurrence	Always as single cells, found on stones and most frequent on mosses.	Very rare, found on stones and mosses.	Very rare, found mostly on stones. The rarest of the three taxa found in the analyzed samples.

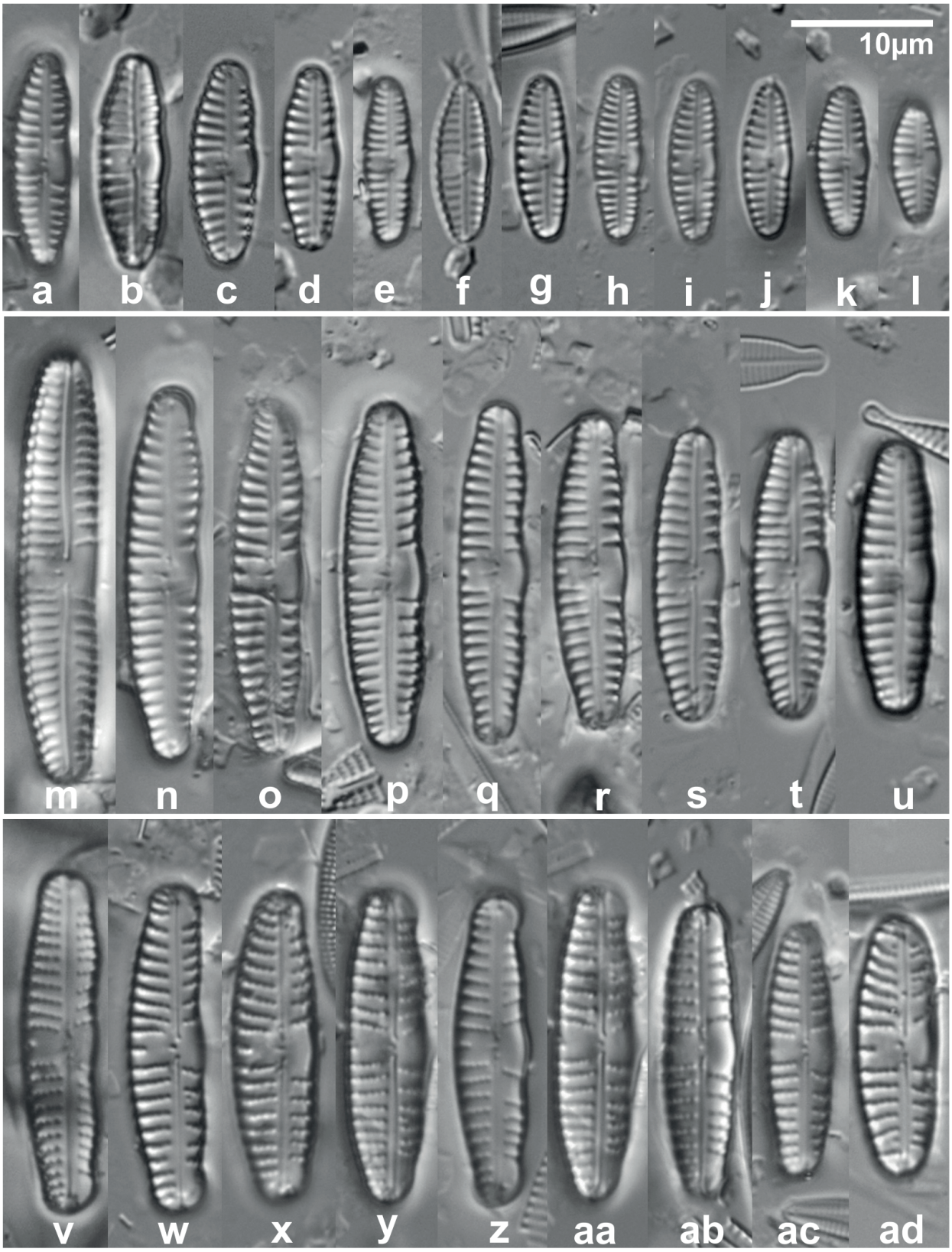

Figure 2

Light microscope (LM) images of *Reimeria sinuata* (a-l), *Reimeria ovata* (m-u) and *Reimeria uniseriata* (v-ad)

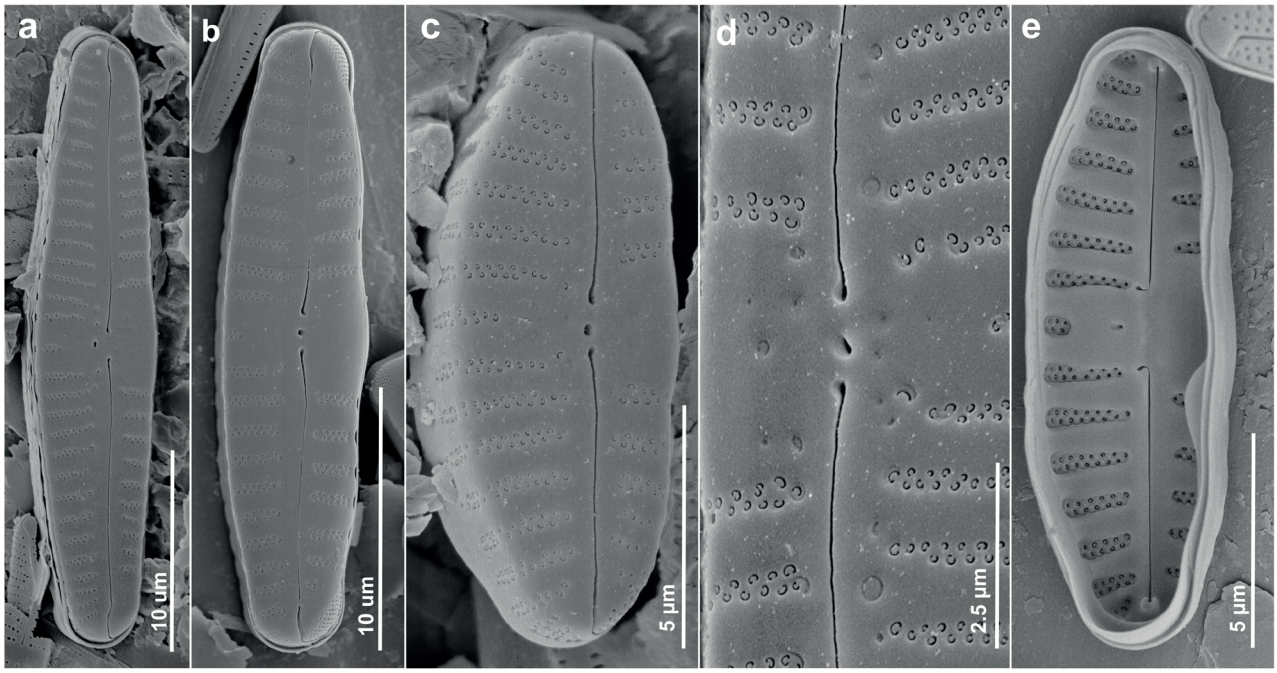

Figure 3

Scanning electron microscope (SEM) images of *Reimeria ovata*. External view of the entire valve (a-c), external detail of the central area of the valve (s) and internal view of the valve (e)

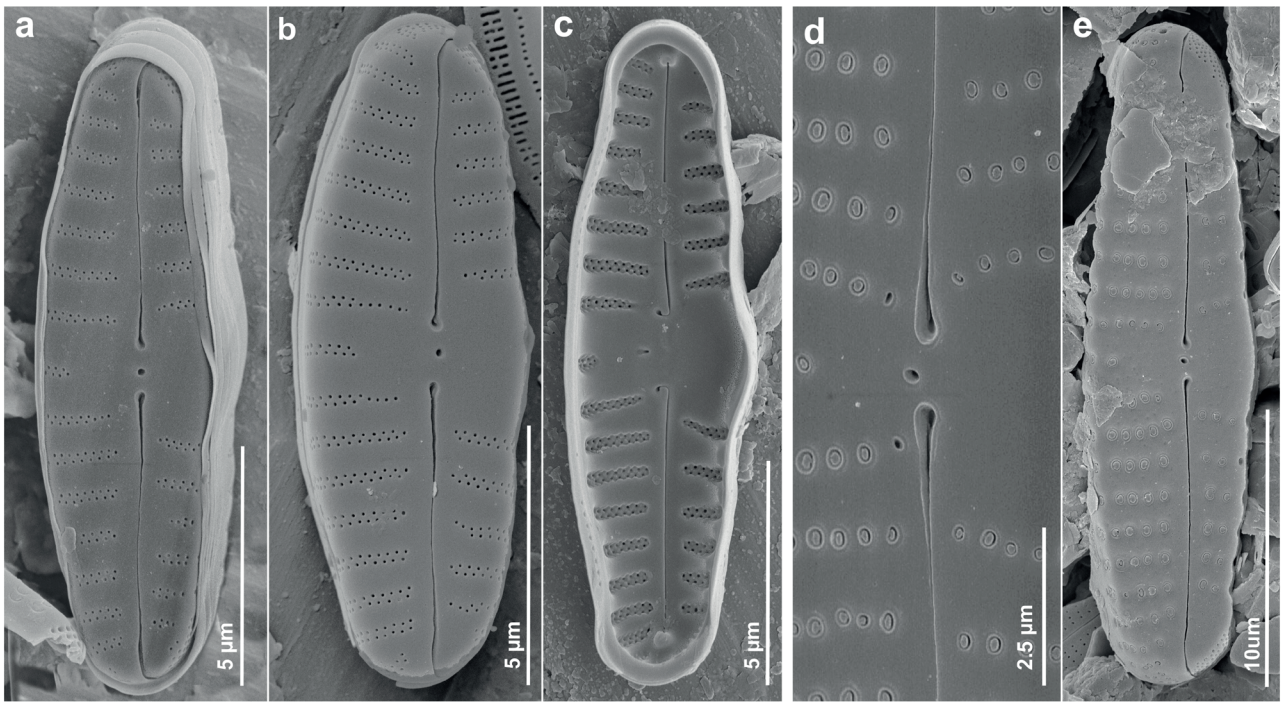

Figure 4

Scanning electron microscope (SEM) images; *Reimeria sinuata*: external view of the entire valve of (a-b), internal view of the valve (c). *Reimeria uniseriata*: external detail of the central area of the valve (d), external view of the entire valve (e)

minutissimum (Kütz.) Czarnecki var. *minutissimum* and *Diatoma ehrenbergii* Kütz were less numerous (up to 30% of the species composition). All taxa are common in Europe. *Achnanthidium pyrenaicum* and *Diatoma ehrenbergii* thrive best in the calcium-rich waters of mountain regions and uplands where they often occur in large numbers (Krammer, Lange-Bertalot 1986, 1991; Hofman et al. 2011).

Reimeria sinuata is a common species in Poland, occurring all over the country (Siemińska 1964; Rakowska 2001; Siemińska, Wołowski 2003; Wojtal, Sobczyk 2006; Wojtal 2009, 2013; Bąk et al. 2012; Kawecka 2012; Noga et al. 2014). It is a cosmopolitan species, widespread throughout Central Europe in different types of waters, particularly in alpine and subalpine areas (Krammer, Lange-Bertalot 1986; Hofmann et al. 2011). It is also common throughout the Czech Republic (Fránková et al. 2009). It also develops in aerial biotopes, mainly on wet rocks, among mosses (Ettl, Gärtner 1995) and very rarely on soils (Noga et al. 2014).

Reimeria uniseriata was noted in the Kobylanka stream (Kraków-Częstochowa Upland) in 2006. It was the first information on this species from Poland (Wojtal, Sobczyk 2006; Wojtal 2009). Currently, it is frequently found in south-eastern Poland where it develops individually in different types of flowing waters (Żelazna-Wieczorek 2012; Noga 2012; Pajączek et al. 2012; Noga et al. 2014, 2015). A precise morphological description was made by Sala et al. (1993) and Levkov & Ector (2010) based on observations under both a light microscope and an SEM, but still little is known about the ecology of this species. It is probably a cosmopolitan species, common in benthic habitats (Wojtal, Sobczyk 2006; Wojtal 2009; Potapowa 2010). According to Bąk et al. (2012), it grows in mesotrophic rivers and lakes. Considering the presence of *R. uniseriata* in the studied rivers and streams of SE Poland (Noga 2012; Pajączek 2012; Noga et al. 2013; 2014; 2015) as well as the Wołosaty stream and its tributaries, it can be concluded that the species has a wide range of occurrence in various types of flowing waters. It develops in alkaline or neutral waters (usually pH 7-8), oligo-, meso- and eutrophic waters, at the source or in the middle and lower sections, and always in the form of individual specimens (<0.5%). It was often found in mesotrophic waters, but also as single cells.

Reimeria ovata has not been previously reported as *Cymbella sinuata* f. *ovata* Hust., but the morphological description of this species presented by Siemińska (1964) ("small valves, short and wide, almost elliptical, 10-12 µm long and 6 µm wide") does not correspond to the cell morphology recently described as *Reimeria ovata*. All specimens in this study were bigger than reported by Siemińska (1964).

The species was identified in each of the streams surveyed in this study, at all the selected sites: the Wołosatka, Wołosaty, Rzeczyca and Terebowiec streams. The species was always present as individual cells. The species was observed more frequently only in the Terebowiec stream, but with the maximum abundance of only 0.5% in the total species composition of diatom assemblages. At each study site, it was associated with *Reimeria sinuata*, which was also very rare (<1%). The third species, *Reimeria uniseriata,* occurred least often in the studied material and only single specimens were found on slides.

The studies carried out in the Wołosaty stream and its tributaries suggest that *R. ovata* could also develop in other oligotrophic rivers and streams, either in SE Europe or in other regions of the country. However, it could easily be confused with *R. simuata,* due to their similarity under a light microscope. Therefore, to properly determine whether the species is present or not in a given material, it is advisable to perform SEM observations in order to dispel doubts in the identification of *R. sinuata* and *R. ovata*.

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