

Luticola frequentissima Levkov, Metzeltin & Pavlov – morphological and ecological characteristics of a population from Southern Poland

by

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Abstract

This paper presents first polish site of *Luticola frequentissima* Levkov, Metzeltin & Pavlov, with morphological and ecological characteristics. The species was found in samples collected from flowing waters and soils within the area of the Bieszczady National Park and the Magura National Park. In south-eastern Poland a low number of *Luticola frequentissima* cells occurred in small shaded streams. It was most frequently observed in aerophilic places (shallow and drying watercourses) and in soils. In the studied samples, apart from those cells with typical dimensions, much smaller and narrower cells with a higher number of striae were found in comparison to those in the currently available literature.

Key words: *Luticola frequentissima*, new records, Poland, taxonomy, ecology, SEM images

Introduction

The genus *Luticola* differs from *Navicula* sensu stricto in nearly every aspect of valve morphology and cell organization. It is a large genus with around 200 different species (Levkov et al. 2013). *Luticola mutica* D.G. Mann in Round et al. (1990) is *typus generis* for the genus *Luticola* D.G. Mann. Cells are usually lanceolate, linear or elliptic, with differently developed ends. The central area is transversely rectangular with one single stigma on one site. In the external view, the stigma is rounded or elongated, whereas in the internal view, in most of the taxa, it is covered by a circular and lipped structure. Striae are composed of a few areolae. Diatoms from the genus *Luticola* occur most frequently in freshwater or slightly saline waters, in soils or subaerial habitats and in estuaries (Round et al. 1990; Hofmann et al. 2011; Bąk et al. 2012).

Over a dozen species from the *Luticola* genus (previously reported as *Navicula*) are known in Poland, the most common of which are: *L. acidoclinata*, Lange-Bert., *L. cohnii* (Hilse) D.G. Mann, *L. goeppertiana* (Bleisch) D.G. Mann, *L. mutica* (Kütz.) D.G. Mann, *L. nivalis* (Ehrenb.) D.G. Mann and *L. ventricosa* (Kütz.) D.G. Mann (Rakowska 2001; Siemińska, Wołowski 2003; Wojtal 2009; Bąk et al. 2012; Noga et al. 2014).

Luticola frequentissima Levkov, Metzeltin & Pavlov was described from the Vardar River in Macedonia and is widespread in freshwater, but can be confused with *L. mutica* (Kütz.) D.G. Mann. Both species can be distinguished by the shape of the central area, the striae morphology and in the case of *L. mutica* – by the presence of ornamented cribrum in areolae. In *L. mutica*, proximal endings are close to one another, while distal endings are hooked on to the valve mantle (Levkov et al. 2013). So far, the species has not been recorded in Poland.

This paper presents new sites as well as ecological and morphological (including SEM) characteristics of *Luticola frequentissima* Levkov, Metzeltin & Pavlov in south-eastern Poland.

Study area

The study was conducted in the catchment areas of two large rivers: the Wisłoka River, which flows through the Magura National Park and the Wołosaty River, which flows through the Bieszczady National Park (Fig. 1).

The Wisłoka, which is a right-bank tributary of the Vistula River, begins its course at an elevation of 664 m a.s.l. The length of the Wisłoka River is 164 km, and the catchment area is 4 110 km². The upper and middle

parts of the Wisłoka catchment area are composed of Tertiary and Quaternary flysch strata, i.e. sandstone and siltstone, and rarely marl and conglomerates (Buszko & Kiryk 1995; Boratyn & Brud 1996). Rock layers of varying thickness are arranged alternately and they make up the so-called Carpathian flysch (Klimaszewski & Starkel 1972; Winnicki & Zemanek 2014). This area is characterized by low- and medium-height mountains (650-700 m a.s.l.), and is covered by forests and scrubs in 90%, mostly within the territory of the Magura National Park. The analyzed streams show large changes in the water level, which is typical for mountain rivers. During rainless periods, the stream flow is very small and in summer, the water temperature is high (Soja 2009). The study area is sparsely populated with a small amount of tourist traffic, while single small farms are located within the buffer zone of the park.

The Wołosaty stream (the upper section of which is called 'Wołosatka') is a right-bank tributary of the San River. The upper part of the Wołosaty catchment area is 8.32 km² and is located between the peaks of Tarnica, Halicz and Rozsypaniec (Siwek et al. 2009). The San is the richest river in terms of water and the largest river in the Carpathians (443.4 km length), the sources of which are located in Ukraine. Much of the San catchment area is covered by protected areas, including the Bieszczady National Park. The study area is part of the Western Bieszczady, also called the High Bieszczady (900-1300 m a.s.l.) and is composed of the Carpathian flysch. The area consists mainly of sparsely populated, natural and slightly transformed ecosystems. Some parts of this area are preserved in almost primeval conditions.

Materials and methods

Samples were collected between 2013 and 2015 (September 2013, March and October 2014 and October 2015) in the catchment area of the Wołosaty within the Bieszczady National Park, and between 2013 and 2014 (April, July, August, October 2013 and April and October 2014) in the upper Wisłoka River within the Magura National Park (Fig. 1). Nine sampling sites were designated in the Wołosaty catchment area and 26 sampling sites in the catchment area of the Wisłoka. In total, the research covered 12 rivers and streams in the Magura National Park and 3 streams in the Bieszczady National Park (Table 1).

Samples were collected and prepared according to methods used by Kawecka (1980) for river algae. Diatom samples were preserved in 4% solution of formaldehyde. A portion of each sample was cleaned

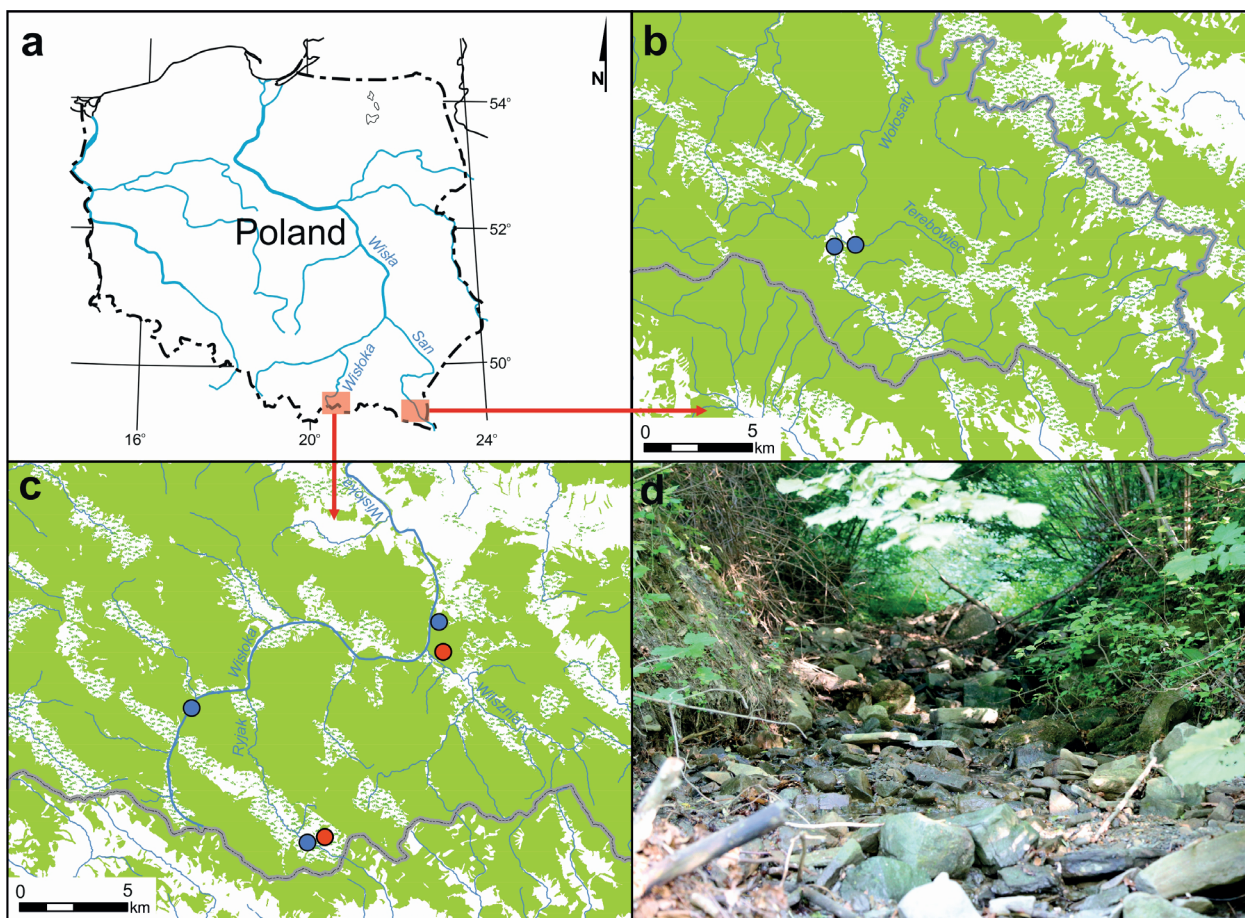


Figure 1

Location of the study area (a), location of the sampling sites at the Wołosaty and Terebowiec streams (b) and at the Wisłoka with its tributary and the Ryjak (c), the Wisłoka's tributary (d); red dots represent sampling sites on the soil, blue dots at the watercourses

Table 1

Characteristics of the studied watercourses in Bieszczady and Magura National Park

Rivers and streams	Number of sites	Watercourse length (km)	Short description
Bieszczady National Park			
Wołosaty	6	27.8	right-bank tributary of the San River in the upper section called Wołosatka
Terebowiec	2	8	right-bank tributary of the Wołosaty
Rzeczyca	1	13.2	left-bank tributary of the Wołosaty
Magura National Park			
Wisłoka	6	164	right-bank tributary of the Vistula
Wisłoka's tributary	1	0.9	nameless, short right-bank tributary of the Wisłoka, drying during summer
Kłopotnica	3	15	left-bank tributary of the Wisłoka
Świerzówka	1	8.3	left-bank tributary of the Wisłoka
Rzeszówka	1	8.2	left-bank tributary of the Wisłoka
Ryjak	3	12	right-bank tributary of the Wisłoka
Ryjak's tributary	1	1.2	nameless, short left-bank tributary of the Ryjak,
Krempna	2	7.3	right-bank tributary of the Wisłoka
Baranie	2	5.8	left-bank tributary of the Wilsznia
Zimna Woda	2	4.8	left-bank tributary of the Hucianka
Zimna Woda's tributary	1	0.8	nameless, short right-bank tributary of the Zimna Woda
Wilsznia	3	11.5	right-bank tributary of the Wisłoka

with chromic mixture (a mixture of sulfuric acid and potassium dichromate), 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 Achromatic objective with differential interference contrast (DIC) for oil immersion (NA 1.4). For scanning electron microscope (SEM) observations, samples were coated with 20 nm of gold using a Quorum Q 150OT ES Turbo-Pumped Sputter Coater and observed under Hitachi SU 8010 and Phenom Pro microscopes.

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

Results and discussion

Luticola frequentissima Levkov, Metzeltin & Pavlov was found only at two watercourses (Wołosaty and Terebowiec) within the Bieszczady National Park, in the Wisłoka River and in two small streams within the Magura National Park. The taxon was also found in the soil of the Wilsznia and Ryjak catchment areas, in the Magura National Park. *Luticola frequentissima* was recorded mostly in small and shallow streams, which dry up in summer.

Taxonomy

Class: Bacillariophyceae Haeckel 1878 emend. Round et al. (1990)

Subclass: Bacillariophycidae D.G. Mann in Round et al. (1990)

Order: Naviculales Bessey 1907

Suborder: Neiidineae D.G. Mann 1990

Family: Diadesmidaceae D.G. Mann 1990

Genus: *Luticola* D.G. Mann 1990

Luticola frequentissima Levkov, Metzeltin & Pavlov (2013).

Morphological description

Valves are lanceolate, rhombic-lanceolate to elliptic (especially small cells) and asymmetrical (Fig. 2a-ad). A detailed morphological description was made by Levkov et al. (2013), which is mostly consistent with our observations (Fig. 3). However, cells in the analyzed samples were much smaller, narrower and contained

a larger number of striae. Valves of the studied material were 6.2-22.5 μm, with a breadth of 4.0-7.6 μm, and the number of striae ranged from 18 to 27 per 10 μm. Whereas Levkov et al. (2013) found that frustules were 12-27 μm in length, 6.5-9.0 μm in breadth and contained 20-24 striae per 10 μm (Table 2). Much smaller and narrower cells were also observed by Ector et al. (2015) in France (7.7-13.8 × 4.8-6.8 μm; 20-24 striae in 10 μm).

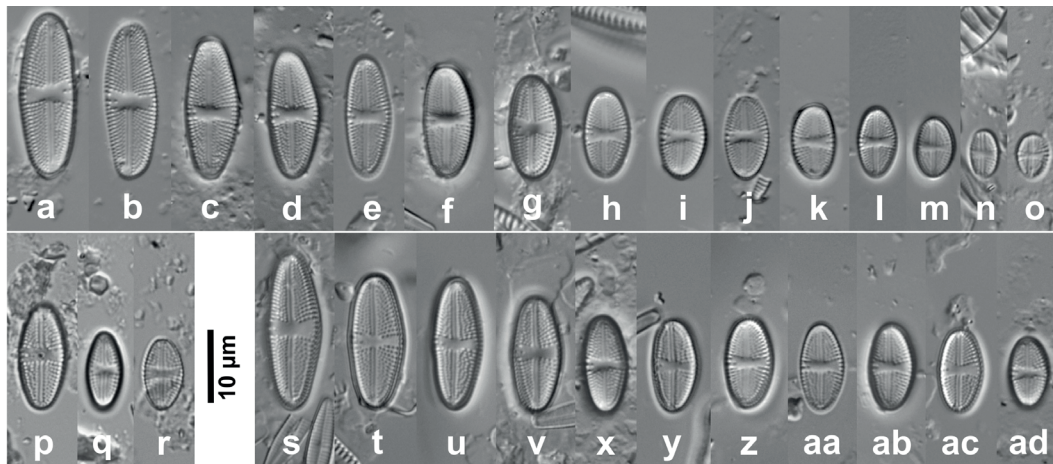
According to Levkov et al. (2013), proximal raphe endings are long and hooked on the same site as proximal endings. In the present study, we found frustules with one proximal raphe end in the opposite direction to the proximal endings (Fig. 3j).

Ecology

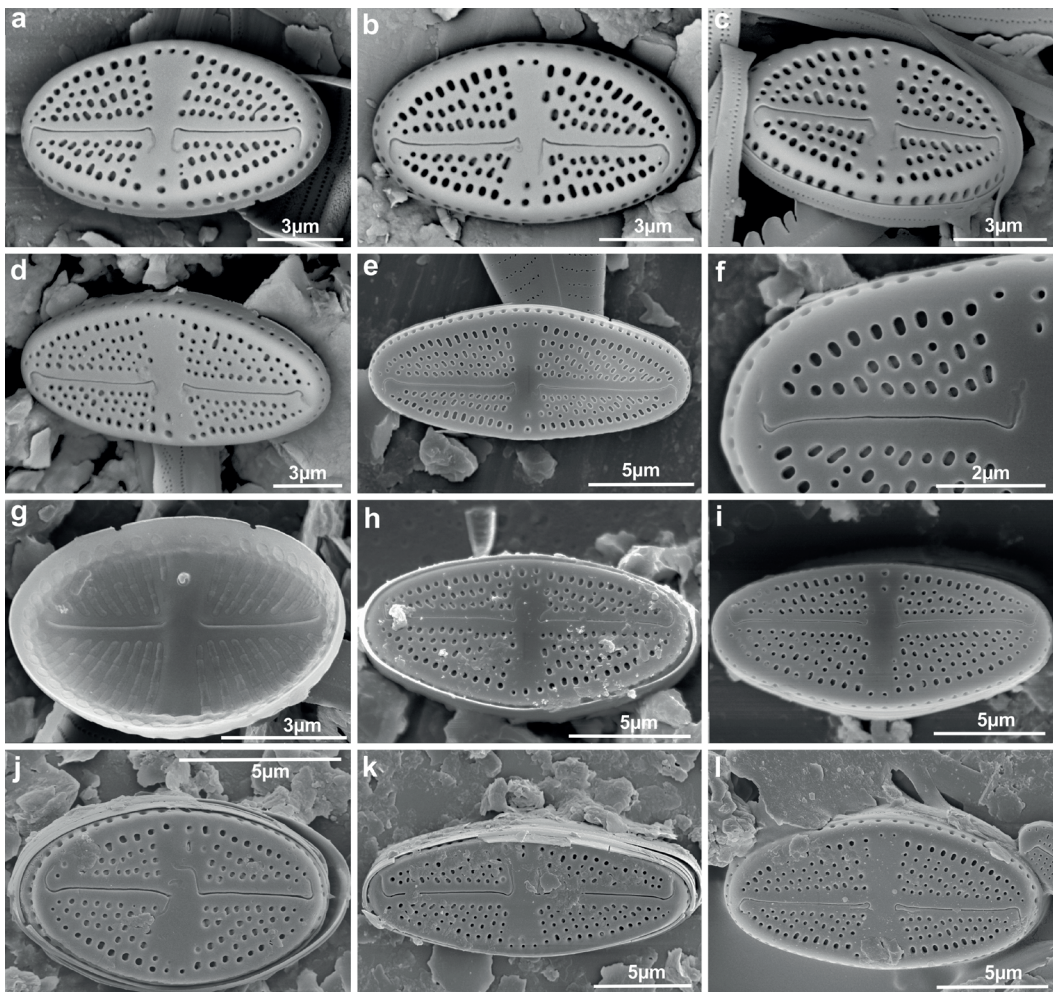
In Bieszczady and Magura National Parks, *Luticola frequentissima* occurred in alkaline and near to neutral waters (pH 6.3-8.5), with conductivity of 138-402 μS cm⁻¹ and water temperature between 5.6 and 13.4°C. Streams with *L. frequentissima* are located in forested and shaded areas (except for well sunlit sites on the Wisłoka River and soils). These streams were mostly shallow, with stony bottoms, mostly overgrown with mosses (Table 2). At the study sites, *L. frequentissima* occurred usually in the form of individual specimens. The species was more abundant only in the Wisłoka's tributary – ca. 1.5% of the assemblage, and in the soil – ca. 1%.

In addition to *L. frequentissima*, the most frequent diatom taxa in the Magura National Park were: *Achnanthes pyrenaicum* (Hust.) Kobayasi, *A. minutissimum* (Kütz.) Czarnecki, *Meridion circulare* (Gréville) A. Agardh and *Gomphonema micropus* Kütz., and *Psammodium grischunum* (Wuthrich) Bukht. & Round in the Wisłoka's tributary. In the Bieszczady National Park, the most frequent were *Achnanthes pyrenaicum* (often more than 70% contribution to the assemblage), *A. minutissimum* and *Diatoma ehrenbergii* Kütz. In the soil, the most frequent were *Hantzschia amphioxys* (Ehrenb.) Grunow and *Luticola acidoclinata* Lange-Bert. Most of these taxa have a wide ecological amplitude and prefer oligo- or mesotrophic waters, rich in calcium (Hofmann et al. 2011; Bąk et al. 2012).

Luticola frequentissima was described for the first time from the Vardar River in Macedonia and is considered a common species in freshwater habitats. It can probably occur in different types of waters, and many records of *Luticola (Navicula) mutica* from freshwater habitats actually represent *L. frequentissima* (Levkov et al. 2013). It has also recently been reported from other parts of Europe, including Belgium (Denys, Oosterlynck 2015) and France (Ector et al. 2015). So far,

**Figure 2**

Luticola frequentissima LM images; population from the Wisłoka's tributary (a-o), from the soil in the Wilsznia catchment (p-r) and from the Wołosaty and Terebowiec streams (z-ad)

**Figure 3**

Luticola frequentissima SEM images: population from the Wisłoka's tributary (a-g), from the Ryjak's tributary (h, i), from the soil in the Wilsznia catchment (j, k) and from the Wołosaty stream (l). Detailed external view of valve apex (f) and internal view of valve (g)

Table 2

Description of the sampling sites and valve dimensions of *L. frequentissima*

Sampling site	Bieszczady National Park		Magura National Park			
	Wolosaty	Terebowiec	Ryjak's tributary	Wisłoka	Wisłoka's tributary	soil (Ryjak, Wilsznia catchments)
Date	03/2014	03/2014	07/2013	10/2013	04/2013 10/2014	04/2014 07/2014 10/2014
Insolation	high	low	low	high	low	high
Type of substrate	stones (large ones covered with moss)	stones	stones (near shore covered with mud)	stones	stones (large ones covered with moss)	soil overgrown with grass in ca. 50%
Temperature (°C)	5.6	6.1	13.4	10.2-11.7	8.1-11.8	-
pH	8.5	8.4	8.1	6.3-6.5	7.3-7.4	5.6-6.5
Conductivity ($\mu\text{S cm}^{-1}$)	166	138	288	378-402	174-341	-
NO_3^- (mg l^{-1})	0.22	0.23	0.84	<0.01	1.91-5.76	-
SO_4^{2-} (mg l^{-1})	16.37	13.74	18.78	20.39-26.53	18.20-21.26	-
<i>L. frequentissima</i> valve dimensions						
Length (μm)	9.4	14.1	8-13.3	11.6-16.5	6.2-22.5	8.6-16.6
Breadth (μm)	5.0	6.6	5.5-6.2	4.0-6.0	4.4-7.6	5.1-6.4
Striae per 10 μm	22	21	23-27	22	18-21	22-27

the species has not been reported from Poland, but it probably occurred in spring waters of southern Poland, where it was incorrectly identified as *Luticola acidoclinata* (Wojtal 2013).

The study conducted in the area of two national parks in SE Poland indicates that *L. frequentissima* can successfully develop in different types of habitats, including soils. Most cells were observed in small streams (Wisłoka's tributaries), which dry up during droughts. Many cells were also observed in soils. In other watercourses, *L. frequentissima* was observed mainly between mosses, but always as individual specimens.

Based on the above observations, this species can be considered aerophilic. It is probably widespread in Poland, but it is often confused with *Luticola mutica* (cells are difficult to distinguish under a light microscope, especially in small specimens). Only SEM observations may allow for proper identification of *L. frequentissima* and exclusion of likely confusion with *L. mutica*. According to Levkov et al. (2013), the central area in *L. mutica* is much smaller, striae are coarser and composed of three areolae covered by ornamented cribrum. The raphe endings are also different: in *L. mutica*, proximal endings are close to each other,

while distal endings extend to the mantle (in *L. frequentissima*, distal fissures extend only onto the valve face).

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