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Morphological and ecological characteristics of diatom species *Stauroneis neofossilis* Lange-Bertalot & Metzeltin 1996 (*Bacillariophyceae*) from Bosnia and Herzegovina

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Abstract

In mine pit lake Bistrik, which is located near Kakanj Municipality, a diatom species from the genus *Stauroneis* has been recorded and identified. In addition to the analysis of the composition of cyanobacteria and algae, the basic physical and chemical parameters of water were also measured. By comparing the morphometric characteristics of the valve of *Stauroneis neofossilis*, a similarity with the literature data was determined. The paper also presents LM and SEM micrographs of *S. neofossilis* and a list of identified species of the genus *Stauroneis* in Bosnia and Herzegovina. To obtain a more complete picture of morphometric characteristics, distribution and ecology, a detailed investigation of this genus from freshwater habitats in Bosnia and Herzegovina is necessary.

Key words: Biodiversity, Diatoms, Rare Species, Balkan Penninsula, Dinarides.

1. Introduction

Diatoms are eukaryotic, unicellular microbes (Round et al., 1990). The majority of species exhibit very specific preferences for various environmental elements like pH, nutrients, and salinity, making them one of the most often used bioindicators for determining the quality of water and doing paleolimnological research (Stevenson et al., 1999). Ehrenberg (1843) created the genus *Stauroneis* without specifying a generic type, although Boyer later characterized it (1927).

The genus is distinguished by uniseriate striae, navicular, single, long, narrow, generally linear-lanceolate to lanceolate valves, and a thickened center region

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known as a "stauros" (Casa et al., 2017). There are currently more than 800 taxa in the genus, which live in a variety of habitats such as freshwater, brackish water, marine, and coastal environments (Levkov et al., 2016).

Species of the genus *Stauroneis* are generally widely distributed in various freshwater habitats. Although most of them are characteristic of oligotrophic waters in the temperate zone (Lange-Bertalot & Metzeltin, 1996). They are also frequently found in Arctic and sub-Antarctic regions (Van de Vijver et al., 2005; Zidarova et al., 2014).

This species also can be found in subaerial habitats (Van de Vijver et al., 2004; John, 2014) or even in caves (Falasco et al., 2015). Additionally, it is one of the most frequently recorded genus in high mountain peat-bogs (Levkov et al., 2005). According to Guiry & Guiry (2021), *S. neofossilis* is very rare in Europe and its presence was reported only in a few publications (Metzeltin, 1996; Denys, 2009; Buczkó, 2016; Karlason et al., 2018).

In Bosnia and Herzegovina, 21 taxa from the genus *Stauroneis* have been recorded until now from various freshwater habitats. Main morphometric data and the list of identified species from the genus *Stauroneis* in Bosnia and Herzegovina was given in the table (Table 1).

Table 1. *Main morphometric features of diatoms from the genus Stauroneis identified in Bosnia and Herzegovina (Lecointe et al. 1993).*

Taxa	Length range	Width range	Striae in 10 µm	Areolae count per 10 µm
1. Stauroneis neofossilis Lange-Bertalot & Metzeltin*	94-100	17-19	22-23	18-20
2. *Stauroneis acidoclinata* Lange-Bertalot & Werum in Werum & Lange-Bertalot	35-70	8-12	19-23	20-30
3. *Stauroneis acuta* W.Smith	80-180	12-18	11-15	15-18
4. *Stauroneis anceps* Ehrenberg	40-70	10-13	22-24	25-25
5. *Stauroneis anceps* f. *linearis* Rabenhorst	40-70	20-25	-	-
6. *Stauroneis anceps* var. *amphicephala* Kützing	51-58	11-11	18-21	-
7. *Stauroneis dilatata* Ehrenberg	-	-	-	-
8. *Stauroneis gracilis* Ehrenberg	68-130	13-24	15-21	16-24
9. *Stauroneis kriegeri* Patrick	17-24	4-6	26-30	16-19
10. *Stauroneis lanceolata* Kützing	-	-	-	-
11. *Stauroneis legumen* (Ehrenberg) Kützing	16-45	4-10	24-29	25-25
12. *Stauroneis meniscus* J.Schumann	-	-	-	-
13. *Stauroneis microbtusa* Reichardt	-	-	-	-
14. *Stauroneis neohyalina* Lange-Bertalot & Krammer	38-49	6-9	32-40	-

15. *Stauroneis parathermicola* Lange-Bertalot	8-17	3-14	24-24	-
16. *Stauroneis parvula* (Grunow) Cleve	-	-	-	-
17. *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg	129-220	26-38	14-18	13-17
18. *Stauroneis platystoma* Ehrenberg	-	-	-	-
19. *Stauroneis producta* Grunow in Van Heurck	30-50	8-11	22-28	28-30
20. *Stauroneis smithii* Grunow	20-40	6-10	24-26	26-30
21. *Stauroneis thermicola* (J.B.Petersen) J.W.G.Lund	8-17	3-5	20-24	30-36

* First record for Bosnia and Herzegovina

The main aim of this study was to present a new locality where *S. neofossilis* was identified, with a description of the ecological characteristics of the habitat. This paper presents the first LM and SEM documentation of the external and internal view of a valve of the species *S. neofossilis* from Bosnia and Herzegovina.

2. Material and Methods

2.1. Study area

Mine pit lake Bistrik is located in the Municipality of Kakanj (Figure 1). The lake is located at an altitude of 454 meters, 44°06’06,42’’ N and 18°09’45,73’’ E, near the village Haljinići. The direction of the lake is northwest (NW)-southeast (SE). The mine pit lake was formed by the subsidence of the terrain, which occurred under the influence of underground coal exploitation. The investigated lake is fed by surface and rainwater. The hydrological regime of mine pit lake is unpredictable throughout the year. It was possible to see the water level fluctuate both during dry and rainy spells. There are obvious water oscillations throughout the year of one to two meters. During the study period, the lake's water had a greenish-blue to bluish-green hue. Well-established stands of floodplain forests can be found in the lake Bistrik complex in addition to the developed hygrophilous and hydrophilic vegetation.

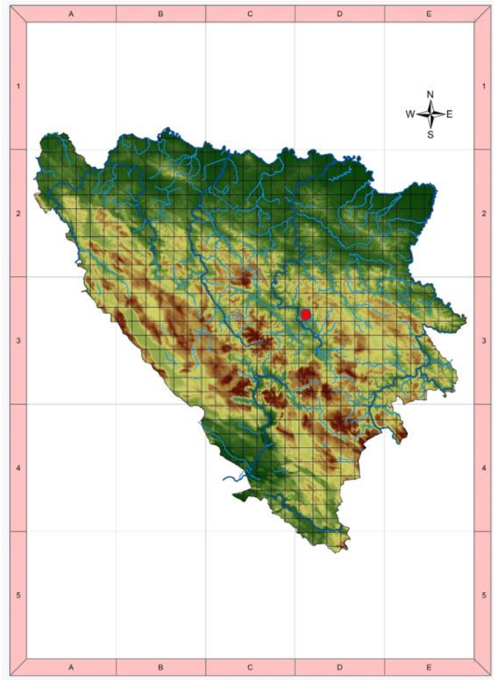

Figure 1. Position of mine pit lake Bistrik

2.2. Sampling and species analysis

Algological material was collected from three micro-localities during summer seasons (2013-2014) and transported to the Laboratory for the study of the systematics of algae and fungi, Department of Biology, Faculty of Science, University of Sarajevo (Bosnia and Herzegovina). Epipelon samples were collected from the uppermost layer of mud with a spoon or pipette aspirator. The collected material was fixed with 4% formalin. Laboratory processing of diatoms was carried out applying methods used by Hustedt (1930). Light microscope observation was conducted using Best Scope 2020 microscope. Scanning electron microscope (SEM) observations were made using a Hitachi, S 4500 at the Goethe Universität Frankfurt am Main. Species composition and the quantitative relationship of diatoms are estimated from the permanent slides under 1000x magnification. At least 400 valves in each slide were counted for all samples. The identification of diatoms was supported by the following references: Lange-Bertalot & Metzeltin (1996); Cantonati et al. (2017). The nomenclature of identified algae was adjusted according to the following Guiry&Guiry (2021). Omnidia software version 6.0.8, was used for ecological data (Lecointe et al. 1993).

3. Results

S. neofossilis Lange-Bertalot & Metzeltin was identified as the diatom after micro-phytobenthos samples analysis. Recently, *S. neofossilis* was identified as a new species for Bosnia and Herzegovina's algal flora (Tomović et al., 2021). The remainder of this report provided information on the valves characteristics as well as notes on their distribution and ecology.

Description: Valves are lanceolate to rhombic-lanceolate with pronounced capitate protruding ends. Valve length 94-100 µm and width 16,5-18,5 µm. The rapha is strongly lateral and completely straight in small but distinct confluent marked central pores. The axial area is linear, neither narrow nor wide. Central area/stauros is approximately linear (i.e., bounded parallel by the transapical strips and 3 µm wide). Striae proximally moderate, distally more radially, 22-23/10 µm. Areolae count per 10 µm varied from 18-20. Biovolume is 11,666 µm 3 (Lecointe et al., 1993; Lange-Bertalot & Metzeltin, 1996). LM microphotographs were shown in Figure 2, while external and internal valve view (SEM) of species *S. neofossils* were shown in Figure 3-8.

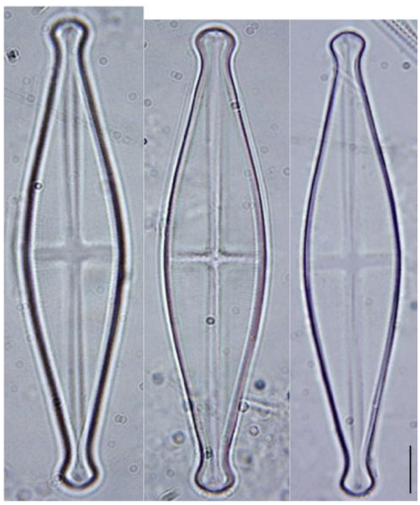

Figure 2. LP micrographs of S. neofossilis; scale bar = 10 µm

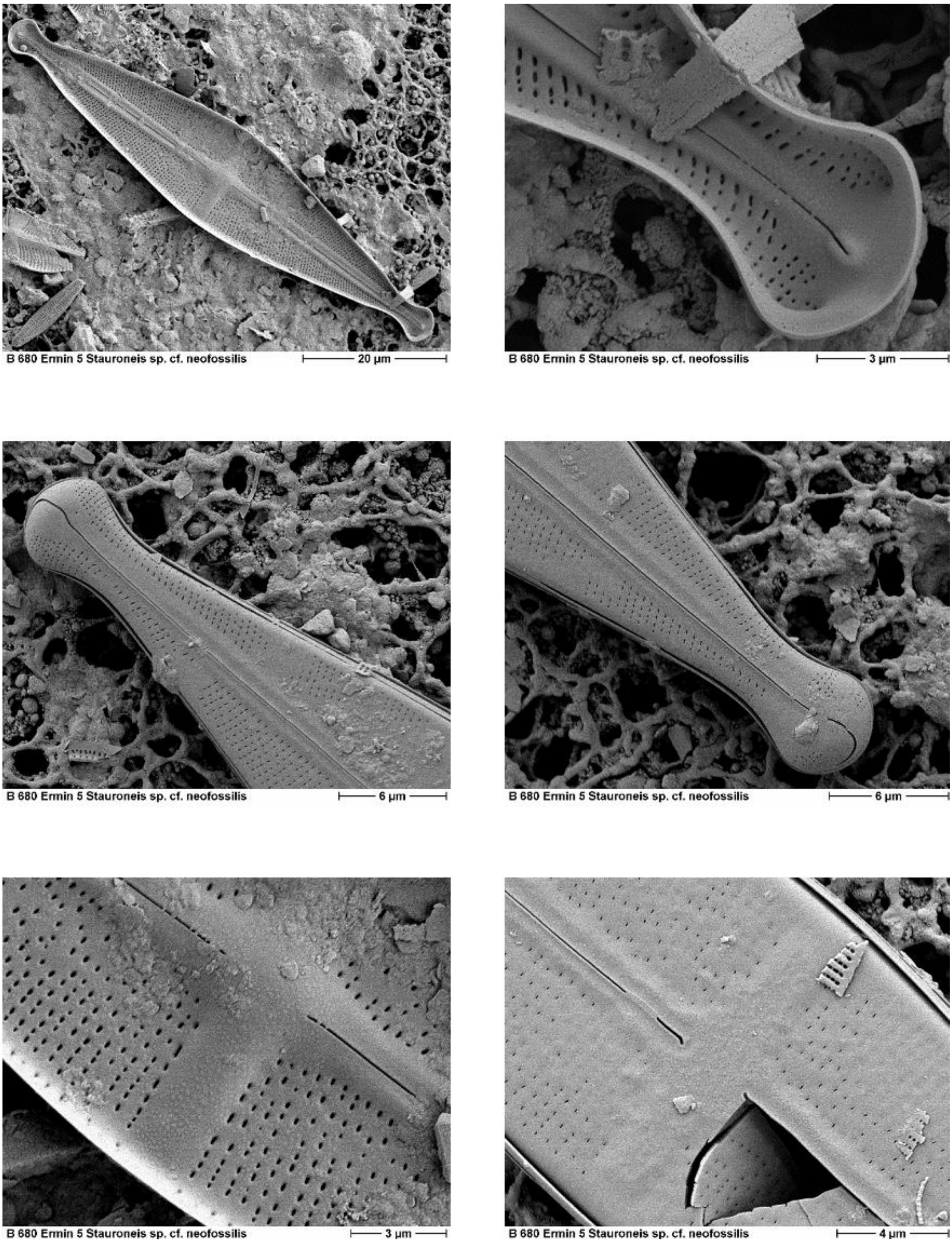

Figure 3-8. SEM images of Stauroneis neofossilis Lange-Bertalot & Metzeltin. 3. Internal view of the entire valve. 4. Internal view of valve apex. 5-6. External view of valve apex. 7. Internal view of the central area. 8. External view of the central area. Scale bars: Fig 1: 20 µm, Figs 3-4: 6 µm, Fig 6: 4 µm, Figs 2-5: 3 µm

Ecology: Species was found in epipelic assemblages in the mine pit lake Bistrik. During the investigated period, the pH value ranged from 7.27-7.63. Electrical conductivity varied from 258-285 µS/cm. In mine pit lake Bistrik selected heavy metals were measured as follows: Al (41 mg/l), Cr (0.33 mg/l), Zn (2.51 mg/l), Ni (3.79 mg/l), Mn (370 mg/l), Pb (37.50 mg/l). The concentration of silicate (Si) was 1.48 mg/l (Tomović et al. 2021; Mašić 2018).

Associated algal flora: In the qualitative composition of microphytobenthos of the mine pit lake Bistrik (Kakanj), a total of 97 species of cyanobacteria and algae were found. The largest number of species was found within the classes: *Bacillariophyceae* (75 taxa or 77.32%), *Chlorophyceae* (7 taxa or 7.22%), *Conjugatophyceae* (5 taxa or 5.15%) and *Cyanophyceae* (5 taxa or 5.15%). The dominant species in the phytobenthos of the investigated mine pit lake are *Achnanthidium minutissimum* (Kützing) Czarnecki, *Rhopalodia gibba* (Ehrenberg) O. Müller, *Hantzschia amphioxys* (Ehrenberg) Grunow etc. Rare and endangered species of algae in investigated mine pit lake was: *Caloneis schumanniana* (Grunow) Cleve, *Cymbella aspera* (Ehrenberg) Cleve, *Gomphonema dichotomum* Kützing, *Gomphonema sarcophagus* W.Gregory, *Gomphonema acuminatum* Ehrenberg, *Navicula oblonga* (Kützing) Kützing, *Neidium affine* (Ehrenberg) Pfitzer, *Neidium ampliatum* (Ehrenberg) Krammer in Krammer & Lange-Bertalot, *S. anceps* Ehrenberg and *S. phoenicenteron* (Nitzsch) Ehrenberg.

4. Discussion

A rare diatom taxon from the genus *Stauroneis* known as *S. neofossilis* was discovered through the examination of microphytobenthos samples obtained in the mine pit lake Bistrik (Tomović et al., 2021). Basic morphometric characteristics were compared, and it was discovered that they matched the data from the literature (Lange-Bertalot & Metzeltin, 1996; Buczkó, 2016; Karlason et al., 2018). 21 taxa of the genus *Stauroneis*, including rare and endangered species (Kapetanović et al., 2011; Mašić & Barudanović, 2018), as well as *S. neofossilis* (Tomović et al., 2021) were identified after an analysis of the literature data available for phycological research in Bosnia and Herzegovina. A monograph by Lange- Bertalot & Metzeltin contains the taxon's initial discovery and a thorough description (1996). The species was found in the oligotrophic lake Julma Ölkky in Finland.

In the monograph Buczkó, another finding of this taxon was published where on Retzat mountain, the taxon was discovered in the glacier lake Brazi (Buczkó, 2016). While Denys (2009) discovered the species in Belgium's shallow lowland lakes, Carlson et al. (2018) list it in the electronic database for the region of Sweden. The following *Stauroneis* species have recently been characterized as new to science: *S. kingstonii* (Burge et al. 2015), *S. fuegiana* (Casa et al. 2017), *S. cavalcantei*, and *S. karstica* (Tusset et al., 2018).

Numerous writers do global study on the flora of the genus *Stauroneis*. In this regard, data are discovered for North Macedonia (Zidarova et al., 2014), Antarctica (Van de Vijver et al., 2005), and Korea (Joh, 2014). (Levkov et al., 2016). The discovery of the endangered *S. balatonis* Pantocsek species in Serbia's Sava Lake (Trbojevi et al., 2019) is noteworthy. A revision of the *S. smithii* Grunow complex for Macedonia is provided by Levkov et al. (2016).

The authors looked into 20 infraspecies taxa for this paper. The *S. blazenciciae* species, which differs from *S. smithii* in having a valve-like form, was described by the authors as a brand-new species for science. *Stauroneis* species are primarily found in freshwater habitats, although they also live in the Arctic and Antarctica, a variety of wetland habitats, and even caverns (Levkov et al., 2016; Tusset et al., 2018). Van de Vijver et al. (2004) present data for 63 species of Stauroneis and report 40 new species of this genus from the Arctic and (Sub)-Antarctic areas.

5. Conclusions

The distribution and morphometric characteristics of *S. neofossilis* are discussed in this publication. This taxon has only been found in a few places in Europe, mostly in natural habitats, whereas in Bosnia and Herzegovina it has been found in an artificial habitat—a mine pit lake. Furthermore, LM and SEM micrographs were used to display the properties of valve structure.

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