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THE DIVERSITY AND MORPHOLOGY OF THE GENUS *LUTICOLA* D.G.MANN (BACILLARIOPHYTA) FROM SUBAERIAL BIOFILMS OF CAVES IN SERBIA

RAZNOLIKOST IN MORFOLOGIJA RODU KREMENASTIH ALG *LUTICOLA* D. G. MANN (BACILLARIOPHYTA) IZ SUBAERSKIH BIOFILMOV JAM V SRBIJI

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Abstract UDC 582.261:551.44(497.11)

Slađana Popović, Danijela Vidaković, Zlatko Levkov, Olga Jakovljević, Nataša Nikolić, Gordana Subakov Simić & Jelena Krizmanić: The diversity and morphology of the genus Luticola D.G.Mann (Bacillariophyta) from subaerial biofilms of caves in Serbia

Research of cave-dwelling diatoms is lacking. In these extreme environments, diatoms are restricted to illuminated zones, and are found at the entrance zone and in the lampenflora community. However, the number of identified species is constantly increasing, and new species are being discovered. The genus *Luticola* is one of the most widely studied genera worldwide and many representatives are characterized as aerophilous and are found in terrestrial habitats. For the first time, *Luticola* species identified from phototrophic subaerial biofilms of 10 caves in Serbia (cave entrances and lampenflora) based on detailed light microscopy (LM) and scanning electron microscopy (SEM) observations were summarized and discussed. In total 11 representatives of the genus were identified at the species level, and morphological features of *Luticola acidoclinata, L. angusta, L. frequentissima, L. dismutica, L. nivalis, L. triundulata,* and *L. quinquenodis* were characterized in detail. For *Luticola angusta,* electron microscopy images are shown for the first time. Additionally, four new taxa (*L. angusta, L. frequentissima, L. kopanjae, and L. poulickovae*) were recorded for the diatom flora of Serbia. The study fills an important gap regarding the occurrence and characteristics of *Luticola* representatives in subaerial habitats.

Keywords: algae, diatoms, *Luticola*, caves, aerophytic habitats.

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Slađana Popović, Danijela Vidaković, Zlatko Levkov, Olga Jakovljević, Nataša Nikolić, Gordana Subakov Simić & Jelena Krizmanić: Raznolikost in morfologija rodu kremenastih alg Luticola D. G. Mann (Bacillariophyta) iz subaerskih biofilmov jam v Srbiji

Raziskav diatomej (kremenastih alg), ki živijo v jamah, je malo. V teh ekstremnih okoljih so diatomeje omejene na osvetljena območja, najdemo jih ob vhodu v jamo in v skupnosti rastlinja, ki uspeva ob stalni osvetljenosti. Število ugotovljenih vrst se nenehno povečuje in odkrivajo se nove vrste. Rod *Luticola* je eden od najbolj raziskanih rodov na svetu, za številne predstavnike rodu pa velja, da so aerofitski in jih najdemo v kopenskih habitatih. Vrste *Luticola*, ki so bile na podlagi natančnih opazovanj s svetlobno mikroskopijo (LM) in vrstično elektronsko mikroskopijo (SEM) opredeljene v fototrofnih subaerskih biofilmih v 10 jamah v Srbiji (ob vhodu v jamo in v skupnosti rastlinja, ki uspeva ob stalni osvetljenosti), so zdaj prvič povzete in obravnavane. Skupaj je bilo na ravni vrste opredeljenih 11 predstavnikov rodu, poleg tega so podrobno opisane morfološke značilnosti vrst *Luticola acidoclinata, L. angusta, L. frequentissima, L. dismutica, L. nivalis, L. triundulata* in *L. quinquenodis*. Za vrsto *Luticola angusta* so prvič prikazane slike elektronske mikroskopije. Poleg tega so bili za kremenaste alge (diatomeje) v Srbiji evidentirani štirje novi taksoni (*L. angusta, L. frequentissima, L. kopanjae in L. poulickovae*). Študija zapolnjuje pomembno vrzel glede pojavljanja in značilnosti predstavnikov vrste *Luticola* v subaerskih habitatih.

Ključne besede*:* alge, diatomeje, *Luticola*, jame, aerofitski habitati.

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1. INTRODUCTION

Diatoms are photosynthetic, eukaryotic microalgae that can be found in many water and wet habitats, but their presence is also recorded in caves (Kociolek et al., 2017). Many studies (of which most originate from Europe, Asia and North America) have discussed their presence in caves (Falasco et al., 2014 and references therein; Lauriol et al., 2006 and references therein; Van Landingham, 1964 and references therein) and, accordingly, diatoms in these habitats are limited to the illuminated zones and prefer humid places and stable environmental conditions (Falasco et al., 2014). Many representatives, however, can tolerate temporary desiccation as resting spores or in protective mucilage (Sterrenburg et al., 2007), especially if they are embedded in well-developed extracellular polymeric substances in subaerial biofilms (Albertano, 2012).

One of the most widely studied diatom genera in caves and subaerial habitats is the genus *Luticola* D.G.Mann. Species from the genus *Luticola* previously belonged to *Punctulata* (Grunow) Patrick which was a subgenus of *Navicula* Bory. After a detailed examination, the mentioned subgenus was established as a new genus *Luticola* in 1990 by Mann in Round et al. (1990). The newly separated genus included all species of *Navicula* with the following features: a longitudinal canal positioned near the valve margin, simple filiform raphe with variable raphe proximal and distal endings (hook, bent, or deflected), presence of a single isolated pore, and uniseriate striae with more-or-less round to transapically elongated areolae covered internally by perforated hymenes. Not only valve morphology and frustule ultrastructure are different from *Navicula*, but also in almost every feature that involves cell organization (i.e. cytoplasmic differences, chloroplast number, and arrangement), as well as reproductive strategies (*Luticola* representatives are predominantly allogamous) (Round et al., 1990; Poulíčková & Hašler, 2007; Poulíčková, 2008; Pavlov et al., 2009; Levkov et al., 2013; Chattová et al., 2017; Wetzel et al., 2021).

Regarding ecology, the genus *Luticola* is widespread in freshwater, brackish, and terrestrial environments. Still, it can be ecologically characterized as an aerophilous genus and it is often recorded on permanently moist rocks, wet walls, cave walls, among mosses, wet soil, splashing zones of waterfalls, deserts, in lichens (Round et al., 1990; Poulíčková & Hašler, 2007; Pavlov et al., 2009; Kociolek et al., 2017; Da Silva-Lehmkuhl et al., 2019; Peszek et al., 2021; Rybak et al., 2021; Wetzel et al., 2021). It is worth mentioning that this is a genus with both cosmopolitan and endemic species (Kociolek et al., 2017; Da Silva-Lehmkuhl et al., 2019; Peszek et al., 2021; Rybak et al., 2021).

The study of this genus expanded from freshwater habitats that were extensively studied earlier to more diverse and extreme habitats in the whole world, which resulted in an increasing number of described species (Hindáková & Noga, 2021 and references therein). A detailed and extensive revision of the genus has been carried out by Levkov et al. (2013), where 200 species are described, of which 93 are marked as new. Currently, there are 261 listed species (year 2022) according to Algaebase (Guiry & Guiry, 2020).

Despite extensive study of diatoms in the last twenty years in Serbia, the knowledge about subaerial diatom assemblages is limited, since most studies focused on freshwater diatoms (e.g., Andrejić et al., 2012, Vidaković et al., 2014; Krizmanić et al., 2015a; Vidaković et al. 2020a, 2020b, Jakovljević et al., 2021a, 2021b). For this reason, the number of recorded *Luticola* species in Serbia is very low. According to published data, only 16 *Luticola* species have been recorded, mostly from high mountain rivers and streams (*L. acidoclinata*, *L. cohnii*, *L. dismutica*, *L. mutica*, *L. lanceolata*, *L. muticopsis*, *L. nivalis*, *L. paramutica*, *L. triundulata*, *L. ventriconfusa, L. goeppertiana* and *L. ventricosa* (e.g., Krizmanić et al., 2015b; Jakovljević et al., 2016; Vasiljević et al., 2017; Vidaković et al., 2018), than from soda pans ((*L. pulchra* (Ćirić et al., 2021)) and artificial sandpit lake ((*L. imbricata*, *L. pseudoimbricata*, *L. pulchra* i *L. rotunda* (Vidaković et al., 2022)).

Therefore, the goal of this study is to document and discuss *Luticola* species identified from phototrophic subaerial biofilms of 10 caves in Serbia (cave entrances and lampenflora (complex community of phototrophic organisms that develop in the vicinity of artificial light) based on detailed light microscopy (LM) and scanning electron microscopy (SEM) observations. These are the detailed data from subterranean environments in Serbia considering this genus. In addition, four new taxa were recorded for the diatom flora of Serbia, and the first electron microscopy illustrations will be shown for *Luticola angustata*.

2. MATERIALS AND METHODS

2.1. STUDY SITES AND CAVE DESCRIPTIONS

During the exploration of phototrophic microorganisms in caves in Serbia, the genus *Luticola* was documented in 10 caves of which three are located in west Serbia (Hadži Prodanova – HP, Rćanska – RC and Stopić Cave – ST), 6 in east/southeast Serbia (Bogovinska – BG, Cerjanska (Kravlje spring – C), Jezava – J, Lazar – L, Samar – S, Vernjikica – V) and one in central Serbia (Risovača Cave – RIS) (Table 1).

Caves of Western Serbia. Stopić Cave is an outflow of a complex stream-sink system and a hydrologically active cave with a large entrance about 18 m high and 35 m wide, which allows external factors to influence the conditions inside. It is one of the most important tourist attractions in Western Serbia with the most admired part of the cave, called Hall with Tubs (Đurović, 1998; Kličković, 2014; Lazarević, 2012). The Rćanska Caves also represent a very complex system consisting of the Velika, Suva and Slepa Caves and the Bezdan Pit. Sampling was carried out at the entrance (approx. 13 m wide and 17 m high) of the lower part of the Velika Cave (Đurović, 1998; Popović et al., 2017). Hadži Prodanova Cave consists of an entrance channel, the central hall and radiating side channels with a total length of around 420 meters. The cave entrance is narrow and high, and the cave passages are dry, with no cave flow (Đurović, 1998, Popović et al., 2017).

Caves of Eastern Serbia. The Bogovinska Cave is a complex of three levels of galleries, upper, main and lower and its total length is 5842 meters. The main gallery is characterized by the presence of a stream flowing through it sinking several times, and the lower gallery is constantly hydrologically active. It is characterized by a not very large entrance (Đurović, 1998). Lazar Cave is a spring cave consisting of dry fossils and active channels. During the first phase of reconstruction in 1953, a 15.2 m wide and 5.42 m high entrance was opened. However, due to the extreme influence of external factors on the cave environment, the entrance channel was closed with a massive wall of stones and concrete in 1978. The cave is an active show cave and very popular in this part of Serbia. The tourist trail is short compared to the more than 16 km of channels explored so far (Lazarević, 1998; Popović et al. 2023). Vernjikica Cave is located not far from Lazar Cave and is a typical dry cave in dry limestone with a length of 1015 m of explored channels. It is characterized by a very small entrance (Đurović, 1998; Milanović, 2012).

Caves of South-Eastern Serbia. Samar Cave has so far explored 3829 m of subterranean channels and is characterized by two sink entrances and one spring entrance called Veliki pešter (Đurović, 1998, Nešić et al., 2007), where sampling was carried out. Very close to Samar is also the small entrance to Jezava Cave, a speleological object with a complex hydrogeological function (sink–spring) with a total length of 903 m (Milanovic, 2012; Nešić et al., 2007; Popović et al., 2020). The Cerjanska Cave system includes several caves, including the place where the sampling was carried out, a spring in Kravlje, from which the Kravljanski stream flows (Nešić & Jović, 2016).

Caves of Central Serbia. Risovača Cave is 187 m long and is characterized by a small entrance. It is one of the most visited tourist caves and one of the most important archaeological sites of the Palaeolithic in Serbia (Đurović 1998).

2.2. SUBAERIAL BIOFILM SAMPLING

A sampling of phototrophic subaerial biofilm was performed at the entrance zone of caves and in the case of active show caves – Lazar, Risovača, and Stopić caves,

*Tab. 1***:** *List of visited caves and their locations.*

Caves	Coordinates	Location
Hadži Prodanova Cave	43° 37' 38,78'' N; 20° 14' 25,30'' E	Western Serbia
Rćanska Cave	43° 44' 2,70'' N; 20° 14' 29,37'' E	Western Serbia
Stopić Cave	43° 42' 12,00'' N; 19° 51' 12,40'' E	Western Serbia
Bogovinska Cave	43° 53' 49,41'' N; 21° 55' 30,64'' E	Eastern Serbia
Cerjanska Cave	43° 27' 07,74'' N; 21° 55' 22,81'' E	South-Eastern Serbia
Jezava Cave	43° 26' 47,77'' N; 21° 58' 33,26'' E	South-Eastern Serbia
Lazar Cave	44° 01' 44,07'' N; 21° 57' 44,54'' E	Eastern Serbia
Samar Cave	43° 26' 45,65'' N; 21° 58' 34,41'' E	South-Eastern Serbia
Vernjikica Cave	44° 01' 34,85'' N; 21° 56' 59,90'' E	Eastern Serbia
Risovača Cave	44° 18' 10,20" N; 20° 34' 54,50" E	Central Serbia

also at places around artificial light (lampenflora). Samar and Jezava caves were sampled seasonally (four times a year), Lazar, Risovača and Stopić caves three times a year during the touristic season and others once a year. The number of sampling sites depended on the developed subaerial biofilm and three to eight (mainly located on a cave wall, but also on ceiling or sediment) were chosen per cave. Subaerial biofilms were sampled with a flame-sterilized scalpel and transported into the laboratory in sterile plastic bags. The sampling was performed in the period from 2014 to 2017.

2.3. SAMPLE PROCESSING AND IDENTIFICATION

For removing organic matter, samples have been processed according to Taylor et al. (2005): they were treated with $KMnO_4$ solution and concentrated HCl and then rinsed with distilled water of pH 6-7. Such prepared diatom sample was used for the preparation of permanent microscopic slides mounted with Naphrax (Brunel Microscopes Ltd). Slides were observed with Zeiss AxioImager-M.1 light microscope and Axio-Vision Release 4.9 software. *Luticola* morphological features were observed with a scanning electron microscope. Part of the material was observed with a Tescan Mira 3 XMU field emission scanning electron microscope (FESEM) (Tescan, Brno, Czech Republic) at the Faculty of Technology and Metallurgy, University of Belgrade, Serbia where prior to analysis, the samples were coated with gold for 45 s using a Polaron SC502 Sputter Coater (Fisons, VG Microtech, East Sussex, England). Additionally, the material was observed with a Cambridge S4 Stereoscan (Cambridge Instruments Ltd, Cambridge, UK) at the Friedrich Hustedt Study Centre for Diatoms (BRM) in Bremerhaven, Germany and SEM stubs were prepared using cleaned diatom material coated with gold-palladium (Polaron SC7640 sputter coater, Quorum Technologies, Ashford, UK). The identification of *Luticola* species was performed using the identification key book Levkov et al. (2013).

2.4. DATA ANALYSIS

Canonical correspondence analysis (CCA) was performed to show *Luticola* species in relation to variables referring to the zone of the cave from which samples are taken (entrance or lampenflora zone). Identified *Luticola* species (presence/absence) were included as response data, while the cave zone from which samples were taken represented a nominal explanatory variable. The unimodal method was used due to data represented as presence/absence (Ter Braak & Šmilauer, 2012). The results are shown in the form of a pie ordination diagram (pictorial form of data representation used to better understand the proportionate parts of a data set).

3. RESULTS

3.1. IDENTIFIED LUTICOLA TAXA

Representatives of the genus *Luticola* were documented in samples from 10 caves in Serbia (Table 2). In total, 11 representatives of the genus were identified at the species level. The characteristic features of one representative were not specific for only one taxon and it was rare in the sample to observe it with SEM, so we left it as *Luticola* sp. 1. *Luticola* spp. refers only to the remains of the genus *Luticola* frustule, never the entire individual. Many of the identified representatives were documented at only one locality: *L. acidoclinata, L. angusta, L. dismutica, L. imbricata, L. poulickovae*. The most frequently encountered were *L. frequentissima* (4), *L. triundulata* (6), *L. nivalis* (7) and *L. quinquenodis* (9 localities). The highest diversity was observed in Samar (7 representatives identified to the species level) and the lowest in Jezava Cave where only residues of this genus (parts of a few *Luticola* representatives that could not be identified) were found. New species for Serbia (marked with an asterisk in Table 2) are *L. angusta, L. frequentissima, L. kopanjae, L. poulickovae.*

Canonical correspondence analysis (CCA) showing *Luticola* species in relation to variable referring to the zone of the cave from which samples are taken (entrance zone or lampenflora) is represented in Figure 1. Taxa that are found both in subaerial biofilms from the entrance zone and in lampenflora samples are placed in the middle of the ordination diagram and those are *L. frequentissima, L. nivalis, L. triundulata and L. quinquenodis*.

3.2. DESCRIPTION OF SELECTED *LUTICOLA* SPECIES

Luticola species that were frequently documented (abundant) in samples, even though found in only one locality, were described below. Dimensions of valves are given acording to our data for each recorded taxa.

Tab. 2: List of identified Luticola taxa from subaerial biofilm samples of caves in Serbia (BG – Bogovinska Cave, C – Cerjanska Cave (Kravlje spring), HP – Hadži Prodanova Cave, J – Jezava, L – Lazar Cave, RC – Rćanska Cave, RIS – Risovača, S – Samar, ST – Stopić Cave, V – Vernjikica). Luticola spp. refers to different parts of Luticola representatives that could not be identified; # New records for Serbia.

Identified taxa	**BG**	**C**	**HP**	**J**	**L**	**RC**	**RIS**	**S**	**ST**	**V**
Luticola acidoclinata Lange-Bertalot			*							
#Luticola angusta Solak & Levkov								*		
Luticola dismutica (Hustedt) D.G.Mann										*
#Luticola frequentissima Levkov, Metzeltin, A.Pavlov	*				*			*	*	
Luticola imbricata (Bock) Levkov, Metzeltin, A.Pavlov						*				
#Luticola kopanjae Levkov, A.Pavlov, Cvetkoska						*		*		*
Luticola nivalis (Ehrenberg) D.G.Mann	*	*	*		*	*		*		*
#Luticola poulickovae Levkov, Metzeltin, A.Pavlov	*									
Luticola quinquenodis Levkov, Metzeltin, A.Pavlov	*	*	*		*	*	*	*	*	*
Luticola triundulata Levkov, Metzeltin, A.Pavlov	*		*			*		*	*	*
Luticola ventricosa (Kützing) D.G.Mann								*		*
Luticola sp. 1 D.G.Mann								*		
Luticola spp. D.G.Mann	*	*	*	*	*	*	*	*	*	*

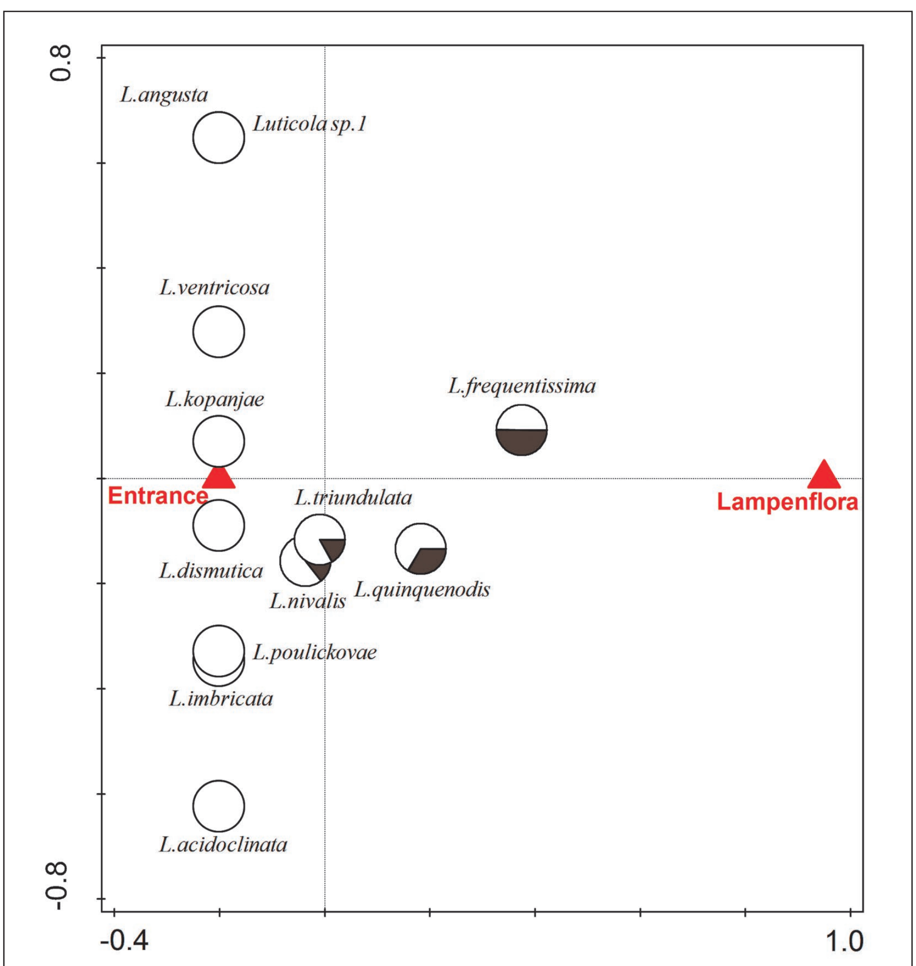

Figure 1: CCA showing Luticola species in relation to variable referring to the zone of the cave from which samples are taken (entrance or lampenflora zone), more precisely, the relative proportion of a certain taxon presence at the entrance cave zone or in lampenflora. White color - entrance, gray - lampenflora.

Luticola acidoclinata Lange-Bertalot (Figure 2: 3-6, 8-10)

LM (Figure 2: 3-6): Valves lanceolate to rhombic-lanceolate, tumid in middle, 15.9-20.7 µm long, 7-7.8 µm wide with slightly protracted and broadly rounded apices. Axial area narrow, linear, slightly expanded near central area. Raphe almost straight. Central area quite wide, slightly asymmetrical, bow tie shaped, bordered by 3-4 isolated round areolae on both valve margins. Single, round stigma, always present in central area, closer to valve margin. Striae distinctly punctate, moderately radiate near mid-valve becoming strongly radiate towards apices, 20-22 in 10 µm.

SEM (Figure 2: 8-10): Externally, proximal raphe endings deflected to side opposite of stigma (Figure 2: 8, 10). Distal raphe fissures strongly hooked, first weak deflected towards same side as proximal endings and then abruptly hooked towards opposite side, continuing to valve mantle, terminating shortly before valve edge (Figure 2: 10). Striae usually composed of 3-4 round to slightly elongated areolae that are larger close to valve margin (Figure 2: 8, 10). Internally, proximal raphe endings slightly deflected opposite to stigma. Distal raphe endings terminating with small helictoglossae (Figure: 2: 9).

Distribution in Serbia (this study and published data): Hadži Prodanova Cave (Table 1), Mlava River, Vrla River, Radovanska River, Cvetića zaliv (Vidaković et al., 2018).

Luticola angusta Solak & Levkov (Figure 2: 7, 12, 13)

LM (Figure 2: 7): Valve linear-lanceolate, 13.1 µm long, 5.7 µm wide, with protracted, rostrate and rounded apices. Axial area narrow, linear. Proximal raphe endings slightly deflected opposite to stigma. Central area wide, asymemetrical, bow tie shaped, bordered on each margin by row of areolae. Single, isolated stigma clearly visible. Striae distinctly punctate, moderately radiate, 22 in 10 µm.

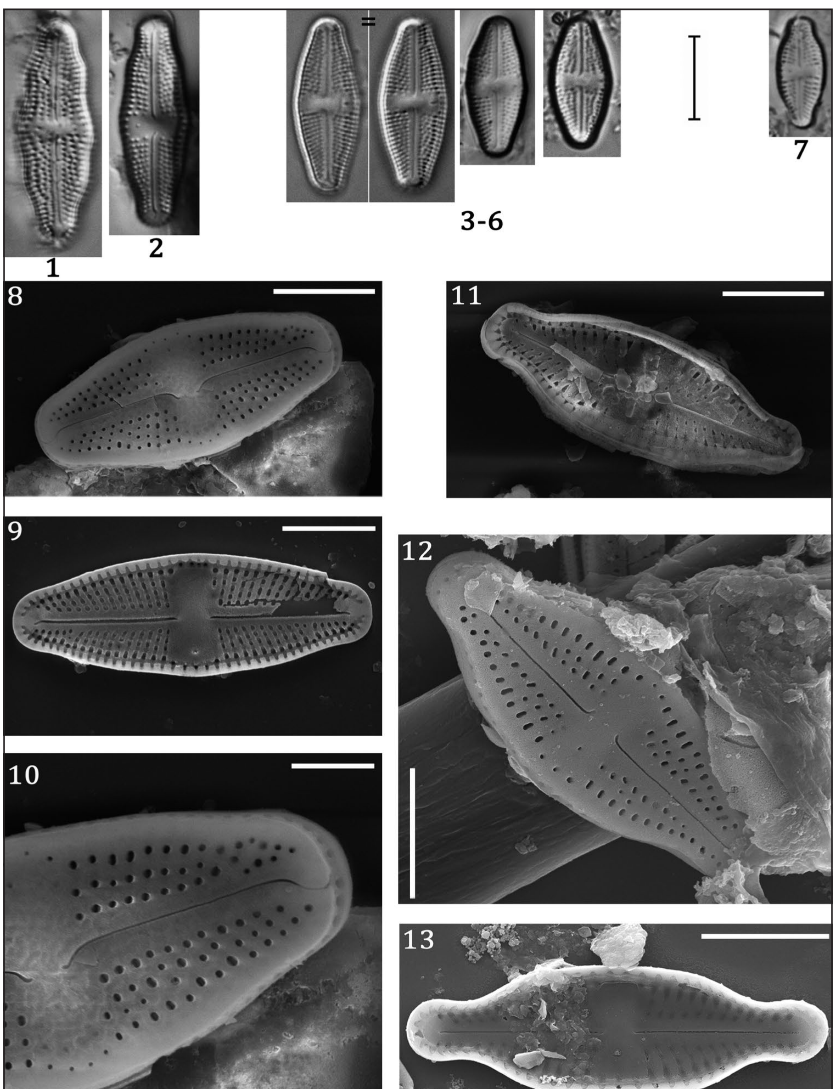

Figure 2: SEM and LM micrographs. 1, 2, 11 Luticola dismutica; 3-6, 8-10 L. acidoclinata; 7, 12, 13 L. angusta. Scale bars: Picture 10 = 2 µm; Pictures 8, 9, 11-13 = 5 µm; Pictures 1-7 = 10 µm.

SEM (Figure 2: 12, 13): Externally, proximal raphe fissures slightly deflected to side opposite stigma (Figure 2: 12). Striae composed of 2-3 round to slightly elongated areolae that are larger close to valve margin (Figure 2: 12-13). Internally, areolae are occluded by hymens, forming a continuous strip across valve. Proximal raphe branches slightly deflected opposite to stigma. Distal raphe branches straight (Figure 2: 13).

Distribution in Serbia: Samar Cave (Table 1).

Luticola dismutica (Hustedt) D.G.Mann (Figure 2: 1, 2, 11)

Basionym: *Navicula dismutica* Hustedt

LM (Figure 2: 1, 2): Valves lanceolate, in the middle weakly tumid with undulate margins, 25.4-27.1 μm long, 7.7-8.8 μm wide, with rostrate apices. Axial area narrow, linear throughout. Proximal raphe endings slightly curved. Central area wide, transversally elongated, bordered by 2-4 isolated round areolae on both valve margins. Single isolated, transapically elongated stigma present in central area. Striae coarsely punctate, irregularly arranged, radiate, 16-17 in 10 μm.

SEM (Figure 2: 11): Internally, areolae are occluded by hymens, forming a continuous strip across valve. Raphe slit simple and straight.

Distribution in Serbia (this study and published data): Vernjikica Cave (Table 1), Veliko Jažinačko and Malo Jažinačko lakes, Šar Mountains (Urošević, 1994, 1998, reported as *Navicula dismutica*), Nišava River (Andrejić et al., 2012), Sava River (Simić et al., 2015).

Luticola frequentissima Levkov, Metzeltin & Pavlov (Figure 3: 1-11)

LM (Figure 3: 1-7): Valves asymmetrical, rhombic-lanceolate to elliptic, 8.7-22.7 μm long, 5.7-7.7 μm wide, with rounded apices. Axial area narrow. Raphe straight to

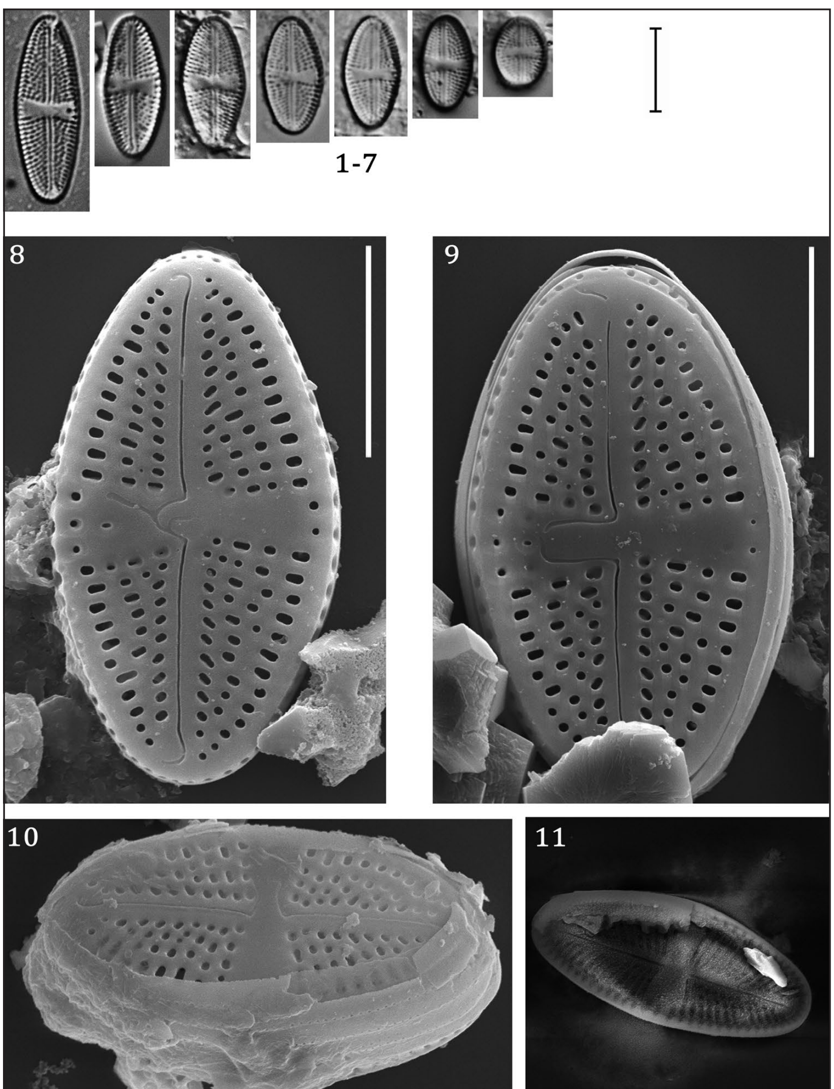

Figure 3: SEM and LM micrographs of Luticola frequntissima. Scale bars: Pictures 1-7 = 10 μm; Pictures 8-11 = 5 μm.

slightly curved. Central area bow tie shaped and slightly asymmetrical, bordered by 3-4 isolated round areolae on both valve margins. Close to valve margin, in the central area present a single, round stigma. Striae punctate and strongly radiate throughout, 18-24 in 10 µm.

SEM (Figure 3: 8-11): Externally, proximal raphe ending hook shaped and towards stigma, continuing with irregular, shallow groove (Figure 3: 8-10). Distal raphe ending hooked on the same side as proximal endings, and also continuing into a shallow groove (Figure 3: 8, 9). Striae composed of 3-4 round areolae (Figure 3: 8-10). Single row of round areolae on valve mantle (Figure 3: 10). Internally, areolae are occluded by hymens. Raphe slit simple and straight (Figure 3: 11).

Distribution in Serbia: Bogovinska, Lazar, Samar, and Stopić caves (Table 1).

Luticola quinquenodis (Grunow) Levkov, Metzeltin & A.Pavlov (Figure 4: 1-18, Figure 5: 1-8)
Basionym *Navicula quinquenodis* Grunow

LM (Figure 4: 1-18): Valves linear with undulate margins, central undulation wider, 12.4-29.1 µm long, 7.1-10.1 µm wide, with apices weakly protracted in smaller specimens to distinctly rostrate to subcapitate in larger specimens. Axial area narrow. Central area wide, bow tie shaped to transversally elongated, bordered by 2-4 isolated round to elongated areolae on both valve margins. Close to valve margin, a single, round stigma present in the central area. Striae punctate, radiate near mid-valve and strongly radiate toward apices, 17-18 in 10 µm.

SEM (Figure 5: 1-8): Externally, single row of round areolae on valve mantle (Figure 5: 1, 5, 7, 8). Proximal raphe ending slightly deflected opposite to stigma (Fig-

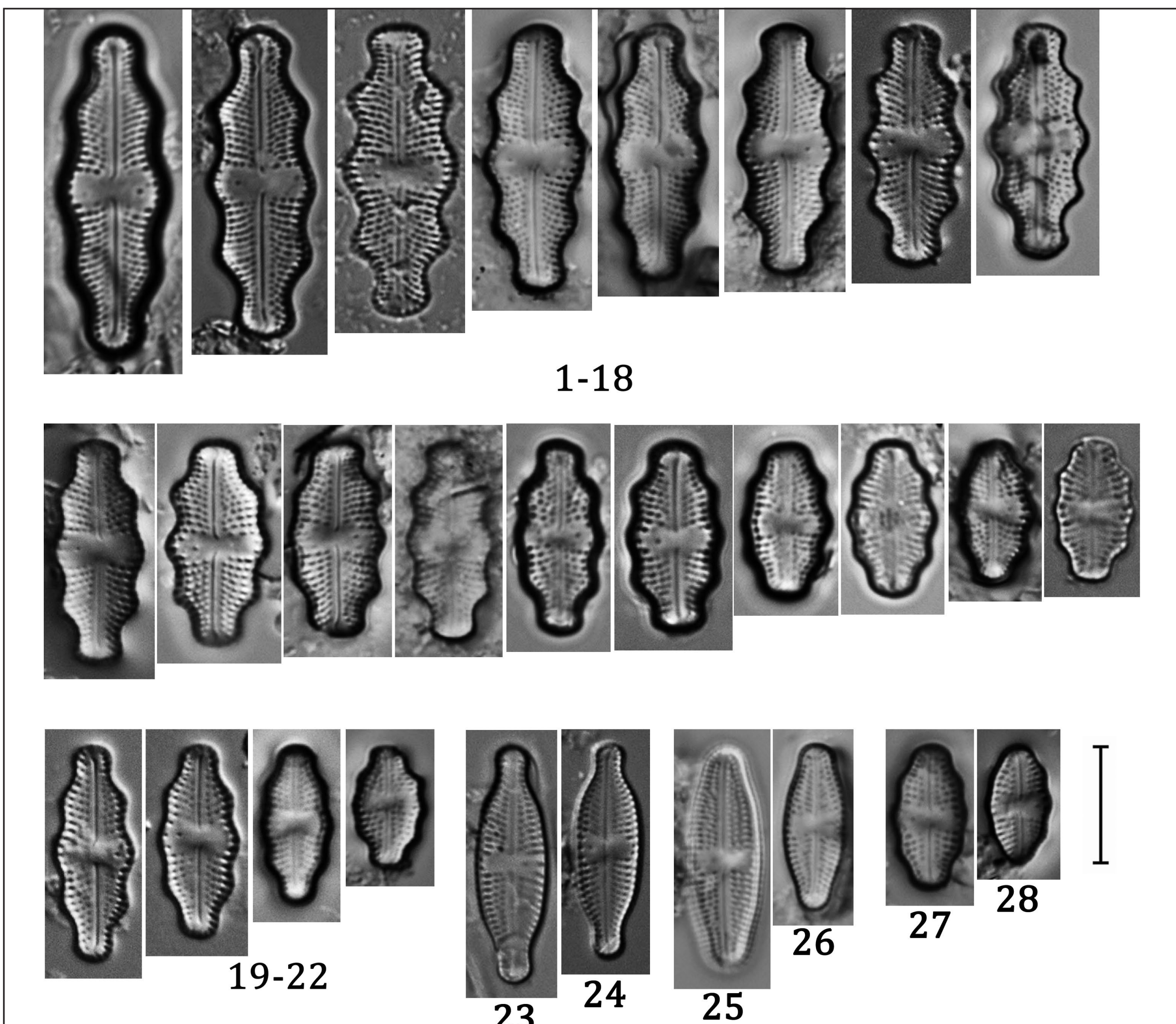

Figure 4: LM micrographs. 1-18 L. quinquenodis; 19-22 L. nivalis; 23, 24 L. ventricosa; 25, 26 L. poulickovae; 27, 28 L. kopanjae. Scale bar 10 µm.

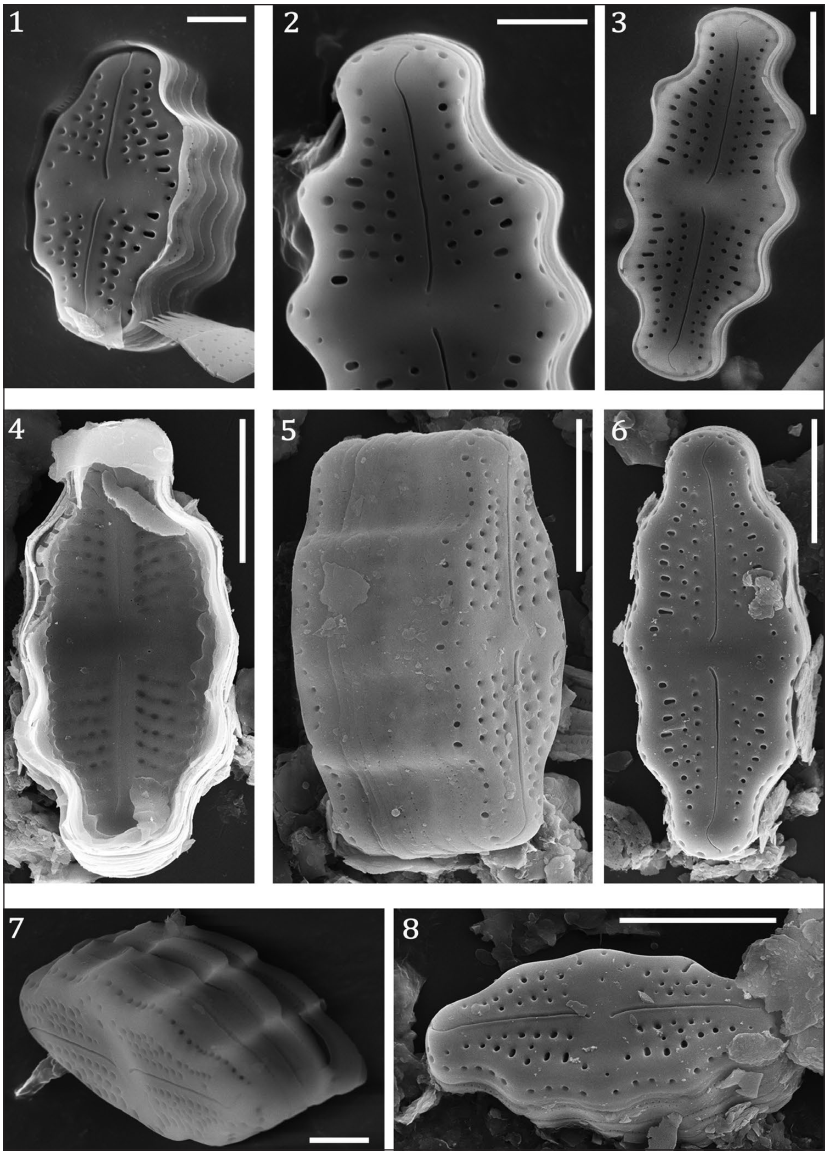

Figure 5: SEM micrographs of Luticola quinquenodis. Scale bars: Pictures 1, 2, 7 = 2 µm; Pictures 3-6, 8 = 5 µm.

ure 5: 1-3, 6-8). Distal raphe fissures hooked, first toward the same side as proximal, than on the other side (Figure 5: 2, 3, 6) extending onto the valve mantle (Figure 5: 5, 6). Striae composed of 2-3 round to elongated areolae. Areolae close to valve margin larger (Figure 5: 1-3, 5, 6, 8). Internally, areolae are occluded by hymens. Raphe slit simple, straight to slightly curved (Figure 5: 4).

Distribution in Serbia: Bogovinska Cave, Cerjanska Cave (Kravlje spring), Hadži Prodanova Cave, Lazar Cave, Rćanska Cave, Risovača, Samar, Stopić Cave, Vernjikica (Table 1).

■ ***Luticola triundulata*** Levkov, Metzeltin & A.Pavlov (Figure 6: 1-13)

LM (Figure 6: 1-11): Valves linear with tri-undulate margins, 12.9-19.6 µm long, 6.6-7.8 µm wide; with capitate, broadly rounded apices. Axial area narrow. Central area wide, elliptic, transversally elongated, bordered by 3-5 round areolae. Single stigma present in central area close to valve margin. Striae punctate and weakly radiate throughout, 18-20 in 10 µm.

SEM (Figure 6: 12, 13): Single row of round areolae on valve mantle (Figure 6: 13). Proximal raphe endings weakly deflected opposite of the stigma (Figure 6: 12). Distal raphe endings simple linear not extending onto the valve mantle and terminating on the valve face (Figure 6: 12). Striae composed of 2-3 round to transapically elongated areolae (Figure 6: 12).

Distribution in Serbia (this study and published data): Bogovinska Cave, Hadži Prodanova Cave, Rćanska Cave, Samar, Stopić Cave, Vernjikica (Table 1), Mlava River (Vidaković et al., 2018).

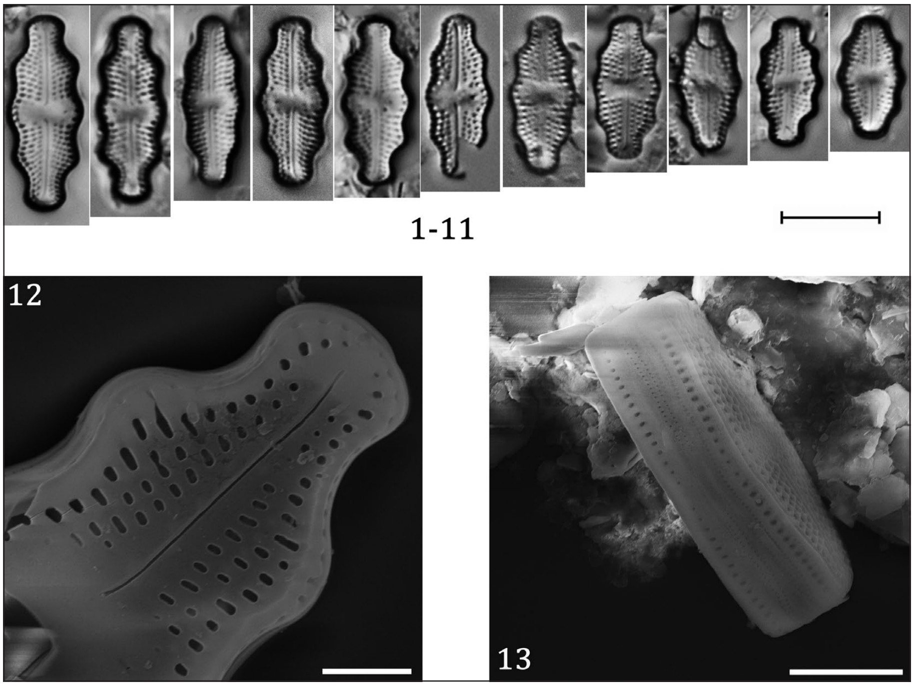

Figure 6: LM and SEM micrographs. 1 - 13 Luticola triundulata. Scale bars: Picture 12 = 2 µm; Picture 13 = 5 µm; Pictures 1-11 = 10 µm.

- ***Luticola nivalis*** (Ehrenberg) D.G.Mann (Figure 4: 19-22)
  Basionym: *Navicula nivalis* Ehrenberg

LM (Figure 4: 19-22): Valves linear with equally formed undulate margins, 10.6-24.4 µm long, 6-8.4 µm wide. Apices subcapitate in larger specimens to rostrate in smaller specimens, broadly rounded to truncate. Axial area narrow. Central area wide, transversally elongated to bow tie shaped, bordered by single row of areolae on both valve margins. Single, isolated stigma present in central area. Striae punctate and strongly radiate throughout, 18-22 in 10 µm.

Distribution in Serbia (this study and published data): Bogovinska Cave, Cerjanska Cave (Kravlje spring), Hadži Prodanova Cave, Lazar Cave, Rćanska Cave, Samar, Vernjikica (Table 1), Niška Spa (Cvijan, 1992-1993, as *N. nivalis*), Vlasina Lake (Cvijan & Laušević, 1997, as *N. nivalis*), Kopaonik (Laušević & Cvijan, 1996, as *N. nivalis*), Novo Ilje I, Melenci (Fužinato et al., 2007), Rćanska Cave (Popović et al., 2017), Sava River (Vasiljević et al., 2017), Slano Kopovo (Ćirić et al., 2021).

For some *Luticula* species (rarely encountered in samples) only LM micrographs are obtained and presented on Figure 4, while their distribution in caves in Serbia is shown in Table 2. Except in caves, *L. ventricosa* is observed in Zapadna Morava River (Jurišić, 2004, as *N. mutica* var. *ventricosa*), Kamenica River, *Čemernica* River (Jurišić, 2004, as *N. mutica* var. *ventricosa*), Sava River (Vasiljević et al., 2017), Crnica River (Jakovljević et al., 2021b, 2021c). *Luticola nivalis*, as one of the most frequent *Luticola* species in the caves (confirmed by references mentioned later in the Discussion section), is described above in detail even presented only with LM micrographs.

4. DISCUSSION

4.1. CAVE ENVIRONMENT

A deep cave zone (which is considered a true cave environment) is characterized by the constant temperature, high relative air humidity, the absence of light, and the existence of a balance (equilibrium) of microclimatic parameters (Czerwik-Marcinkowska & Mrozińska, 2011). On the contrary, cave entrance zones are more susceptible to the influence of the external climate, where ecological factors are prone to alterations. However, the mentioned existing equilibrium of ecological parameters deep inside the cave can be disturbed during the transformation of a natural cave environment into a show cave and as a consequence of the introduction of infrastructure (paths, stairs, electricity) and especially, the installation of artificial light (Albertano, 2012; Baquedano Estévez et al., 2019). Any subterranean environment connected with the outside becomes subject to the input of organic matter and various microorganisms. Non-residential microorganisms that occasionally enter the cave via air, water, sediments, and spores, or are introduced by animals or humans are called accidental organisms, and according to Falasco et al. (2014), diatoms are included in this category. Depending on the conditions, they can shortly stay in the cave (transitory microorganisms), or they can have a bigger impact on the cave environment (Falasco et al., 2014).

4.2. FACTORS INFLUENCING DIATOM DISTRIBUTION

The most significant environmental factors that influence the diatom distribution in caves can not be easily assumed (Pouličková & Hašler, 2007) and most of the ecological preferences of the species are still unknown (Falasco et al., 2014). The driving factor that is necessary for phototrophs to exist is light (day-light at the cave entrance zone or artificial light in show caves). Artificial light in show caves can cause changes in relative air humidity, air temperature or microclimatic parameters, creating more favorable conditions for the survival and/or proliferation of algae. According to Falasco et al. (2014) light can be related to phototrophic cave community richness. However, even in the presence of light, the speleoenvironment is still harsh and limiting in a number of ways (Pouličková & Hašler, 2007). On the other hand, it is considered that moisture level is the most important limiting factor; however, exposure to long periods of desiccation may be more crucial (Pouličková & Hašler, 2007 and references therein). Many diatoms indeed can tolerate temporary desiccation by forming resting spores or in protective mucilage envelopes. In that manner, *L. nivalis* can easily withstand long periods of drought (Lauriol et al., 2006), while *L. mutica sensu lato*, even though can be found in various water habitats, also inhabits dry rocks and stone walls (Sterrenburg, 2007). According to Czerwik-Marcinkowska and Mrozińska (2011), *L. nivalis* and *L. mutica sensu lato* are particularly sensitive to environmental conditions and have a high resistance to drying out and too low ambient temperature (the adaptation to low temperatures is not well understood). Humidity fluctuations are also related to the species richness since illuminated and wet habitats are more convenient for diatoms than illuminated and dry ones (Falasco et al., 2014). The higher richness in the case of diatoms is also connected with the presence of mosses. According to Poulíčková and Hašler (2007) *L. mutica sensu lato* is rare on lithic substrates, but more common on mosses and is very common as an epiphytic diatom. Diatoms are also sensitive to rock surface structure (Czerwik-Marcinkowska & Mrozińska, 2011). The nature and properties of the rock substratum, its texture, and porosity, water content, as well as potential bioreceptivity, influence settlement and development of phototrophs (Albertano, 2012). The chemical composition of the rock as well as pH can also influence species composition (Falasco et al., 2014) and for example, low diatom diversity in some caves could be related to the lack of silica (Selvi & Altuner, 2007). Limestone and sandstone surfaces can have similar moist regimes, temperature extremes, and low nutrient availability, but flora is notably different due to differences in pH (Falasco et al., 2014). However, if diatom taxa are found in well-developed subaerial biofilms, they can be growing in a completely different environment from those at the very surface of a rock; they are usually incorporated and well protected by other organisms and highly hydrated matrix. Caves are also oligotrophic habitats, characterized by little organic matter present unless it is imported from the outside. However, according to Van Dam et al. (1994) most aerial diatom species are indicative of low nutrient availability.

4.3. RECORDS OF GENUS LUTICOLA IN CAVES AND ITS FEATURES

Representatives of the genus *Luticola* are recorded in subterranean habitats by many researchers and some examples are given below. *Luticola dismutica* sensu Pouličková (2008) – later confirmed to be *Luticola poulickovae* sensu Levkov 2013, was collected from the Podkova natural cave in Czech Republic and its morphology, cytology, and sexual reproduction were explored by Pouličková (2008). Pouličková and Hašler (2007) represented the first study of the diatoms in three caves (entrance and lampenflora) in the Czech Republic. *Luticola mutica sensu lato*, *L. nivalis*, *L. nivaloides* and *L. paramutica* were found, of which *L. mutica sensu lato* was closer investigated. *L. mutica sensu lato* was also found by Škaloud (2009) among phototrophs of the Boreč Hill ventaroles (diatom flora of the ventaroles (karst fissures) in general resembles well the species composition found in the caves according to Garbacki et al. (1999) cited in Škaloud (2009)). Very frequently encountered in different studies was *L. nivalis* and was recorded by Selvi and Altuner (2007) in Ballıca Cave (Tokat-Turkey), Czerwik-Marcinkowska (2013) in 10 caves in the Ojców National Park in Poland, in lampenflora samples from Mammoth Cave National Park, Kentucky (Smith & Olson, 2007). Czerwik-Marcinkowska and Mrozińska (2011) in caves in Poland found both *L. mutica sensu lato* and *L. nivalis*. According to Falasco et al. (2014) among the most frequent and abundant species recorded in subterranean ecosystems are *L. nivalis* and *L. mutica sensu lato* which are aerophilous and often recorded together, colonizing the same portion of the walls. They conclude however that these taxa can proliferate in a variety of environmental conditions. Van Vuuren et al. (2019) recorded the genus *Luticola* without referring to the specific taxon in caves in South Africa. Lauriol et al. (2006) examined diatom flora in ice caves of the northern Yukon Territory, Canada, and recorded *L. goeppertiana*, *L. mutica sensu lato*, *L. muticopsis*, *L. nivalis* of which *L. nivalis* was numerous in the sample of grus. Peszek et al. (2021) examined samples of soil and organic detritus from the ground of the cave entrance at Rapa Nui (Easter Island = Isla de Pasqua). Four species of the genus *Luticola* were recorded, *Luticola ectorii* Levkov, Metzeltin & Pavlov and three that are described as new to science: *Luticola georgzizkae* Witkowski, Lange-Bertalot, M. Rybak & Peszek, *Luticola rapanuiensis* M.Rybak, Peszek, Witkowski & Lange-Bertalot and *Luticola moaiorum* Peszek, M. Rybak, Witkowski & Lange-Bertalot. This is of interest to mention due to the potential of finding more and more new *Luticola* species in cave habitats.

It should be noted that many above-studies are done before the revision of the genus *Luticola* published by Levkov et al. (2013), so the mentioned *Luticola* species, which especially refers to *L. mutica sensu lato* and *L. nivalis* may now bear a different name. Earlier findings of *L. mutica sensu lato* in freshwaters may be related to similar species such as *L. frequentissima*, *L. imbricata*, *L. pseudoimbricata* etc. Many observed specimens of *L. nivalis* in the past are related to *L. undulata* (Levkov et al., 2013). There are many findings of the mentioned two taxa in Serbia from different habitats. Details of findings for *L. nivalis* are given in the results section in the description of the species, and considering *L. mutica sensu lato,* it was recorded in the following studies in Serbia: ponds near Kostolac (Cvijan, 1985, as *N. mutica*); lakes of Sirinićka side of *Šar* mountain (Veliko Jažinačko, Malo Jažinačko, Donje Blateštičko) (Urošević, 1994, 1998, as *N. mutica*); Tisa, Bečej (Ržaničanin, 2004a; Ržaničanin et al., 2005, as *N. mutica*); Rasina (Mudrakovac, Malo Golovode, Bivolje) (Ržaničanin, 2004b, as *N. mutica*); Nišava (Andrejić et al., 2012); Dojkinačka river (Krizmanić et al., 2015b); Sava Lake (Trbojević et al., 2021); Sava River (Sremska Mitrovica, Beograd) (Vasiljević et al., 2017), Crnica River, Radovanska River (Jakovljević et al., 2021b).

Many *Luticola* taxa can be wrong identified since they are similar and can not be well distinguished using only a light microscope. For example, according to Noga et al. (2017) *L. frequentissima* was usually confused with *L. mutica sensu lato,* especially when observing small specimens.

Undulate representatives (in studies usually mentioned *L. nivalis*) are very common in cave environments or abundant in samples (as mentioned by Lauriol et al. (2006)). By analyzing our samples we also found that undulate representatives *L. nivalis*, *L. triundulata* and *L. quinquenodis* are very widespread and way more frequently found and abundant in samples than others that are mainly sporadically encountered. Also, they were the only found *Luticola* representatives in lampenflora. This may indicate that this community, considering the phototrophic organisms, could be unique and different from the phototrophic communities in subaerial biofilms at the cave entrances. That coincides with the conclusion of Burgoyne et al. (2021) according to which members of lampenflora communities may originate from different sources and are distinct from microorganism communities in nearby unlit caves.

Accordingly, even though sporadically found *Luticola* representatives indeed can be considered as accidental organisms in caves, we can not be sure about undulate ones. Considering ecology, these species are easily found in subaerial habitats (Levkov et al., 2013).

According to Falasco et al. (2014), some diatom taxa can exhibit smaller sizes when they are not in their optimal habitat and when they are subjected to extreme living conditions. The dimensions of the described species in this study correspond with those in the identification

Tab. 3: Comparison of dimensions of described taxa from identification key and this study. L – length, W – width.

Described taxa	Levkov et al. (2013) L/W (µm)	This study L/W (µm)
Luticola acidoclinata	10-30/5.0-8.5	15.9-20.7/7.0-7.8
Luticola angusta	12.19/4.5-6.5	13.1/5.7
Luticola dismutica	18-40/6.0-9.5	25.4-27.1/7.7-8.8
Luticola frequentissima	12-27/6.4-9.0	8.7-22.7/5.7-7.7
Luticola nivalis	11-21/6.0-8.0	10.6-24.4/6.0-8.4
Luticola quinquenodis	13-32/7.0-10.5	12.4-29.1/7.1-10.1
Luticola triundulata	14-24/6.0-7.5	12.9-19.6/6.6-7.8

key, but at a closer look, we can distinguish two groups (Table 3). *L. acidoclinata*, *L. angusta*, *L. dismutica*, and partially *L. frequentissima*, do not reach upper L/W limit and their dimensions were in the middle of the dimensions range. On the contrary, *L. nivalis*, *L. quinquenodis* and *L. triundulata* almost reached the upper L/W limit or were larger.

Phototrophic microorganisms, among which are diatoms, are still not explored enough in cave habitats. Even when explored, diatoms are not always studied in detail, since sample preparation and identification that requires SEM are demanding. However, they deserve more attention. Speleoenvironments offer extreme but relatively stable conditions to diatoms and are a good model system for future studies (Poulíčková & Hašler, 2007) and records of rare or new species.

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