

UNIVERSITY OF SARAJEVO

VRANICA MOUNTAIN



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- SOURCE OF DIATOMS DIVERSITY -



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**VRANICA MOUNTAIN
"SOURCE OF DIATOMS DIVERSITY"**

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GENERAL PART

ABSTRACT

Freshwater oligotrophic habitats are considered to be threatened due to various anthropogenic influences. The significance of such habitats was recognized by EU authorities and they are listed as important habitats for protection (NATURA 2000). Due to the presence of these habitat types, Vranica mountain was taken for study. Numerous springs, creeks, and small rivers determine the specific hydrological regime of this area. Prokoško lake at the 1.635 meters of altitude gives the subalpine belt a particular ecological value. In addition to numerous springs and mountain creeks, this area is characterized by the presence of peatlands. Water temperature, pH, dissolved oxygen and specific electrical conductivity were measured with a portable multimeter (Orion Star A329). Light microscope observation and micrographs were made using a Best Scope 2020 microscope, equipped with a digital camera (MD-130). Diatoms identification and nomenclature were based on specific diatom literature and identification keys. A total of 174 taxa belonging to 58 genera were determined in the analysed samples. The most species-rich genera were *Eunotia* (22), *Gomphonema* (14) and *Pinnularia* (12). The most frequent and abundant taxa were *Achnantheidium minutissimum*, *Cocconeis placentula*, *Meridion circulare*, *Pinnularia borealis*, *Pinnularia neomajor*, *Staurosirella pinnata* and *Ulnaria ulna*. The largest number of rare and endangered diatom taxa was determined in mountain peatlands (48 taxa), while the smallest number was determined in mountain creeks (16 taxa) and mountain lake (16 taxa). The obtained results from this study could serve, in the future, as a good basis for restoration and conservation of this specific habitat types on a wide area of Vranica mountain, but also Dinaric Alps.

Key words: *Vranica, diatoms, mountain, peatlands, springs, restoration, conservation.*

SAŽETAK

Slatkovodna oligotrofna staništa smatraju se ugroženima zbog različitih antropogenih utjecaja. Značaj ovakvih staništa prepoznati su i od strane EU vlasti i ona su navedena kao važna staništa za zaštitu (NATURA 2000). Zbog prisustva ovih tipova staništa, planina Vranica je uzeta za istraživanje. Brojni izvori, potoci i rječice određuju specifičan hidrološki režim ovog područja. Prokoško jezero na 1.635 metara nadmorske visine daje subalpskom pojasu posebnu ekološku vrijednost. Pored brojnih izvora i planinskih potoka, ovo područje karakteriše prisustvo tresetišta. Temperatura vode, pH, rastvoreni kiseonik i specifična električna provodljivost mjereni su prenosivim multimetrom (Orion Star A329). Istraživanje i fotografisanje dijetomeja izvršeno je svjetlosnim mikroskopom Best Scope 2020 koji je opremljen digitalnom kamerom (MD-130). Identifikacija i nomenklatura dijetomeja zasnovana je na specifičnoj literaturi i ključevima za identifikaciju. U analiziranim uzorcima utvrđeno je ukupno 174 taksona iz 58 rodova. Najbogatiji vrstama su rodovi *Eunotia* (22), *Gomphonema* (14) i *Pinnularia* (12). Najčešći i najzastupljeniji taksoni bili su *Achnantheidium minutissimum*, *Cocconeis placentula*, *Meridion circulare*, *Pinnularia borealis*, *Pinnularia neomajor*, *Staurosirella pinnata* i *Ulnaria ulna*. Najveći broj taksona uvrštenih na Crvenu listu (Hoffman et al. 2018) pronađen je u tresetištima (48 taksona), dok je najmanji broj vrsta prinađen u planinskim potocima (16 taksona) i planinskom jezeru (16 taksona). Dobijeni rezultati ove studije u budućnosti će poslužiti kao dobra osnova za restauraciju i očuvanje ovih specifičnih staništa na širem području planine Vranice, ali i Dinarskih Alpi.

Ključne riječi: *Vranica, dijetomeje, tresetišta, izvori, restauracija, konzervacija.*

INTRODUCTION

Despite the effort to significantly reduce biodiversity loss by 2010, recent research has confirmed the extinction of several plant and animal species listed as endangered in the IUCN Red Lists, while allochthonous taxa are increasing globally (Butchart et al. 2010 in Falasco & Bona, 2011).

Therefore, fragile ecosystems such as Alpine streams should be carefully monitored, as they are exposed to extreme conditions due to their harsh nature (Cantonati & Spitale, 2009 in Falasco & Bona, 2011) and human exploitation (Fráncova et al. 2009 in Falasco & Bona, 2011).

Over the last years, the increasing awareness of the importance of biodiversity has led governmental agencies and the scientific community to improve the knowledge and management of these ecosystems in order to preserve their integrity (Falasco et al. 2011).

In high mountains, freshwater oligotrophic habitats represent usually shelter for high biodiversity due to low variability of environmental factors over the seasons. Because of their low nutrient content, moderate water flow and constant temperature, these types of habitat can be considered suitable for a wide range of species. In that sense, very important habitats are springs, creeks, streams, lakes and peatlands. Indeed, springs offer protection for endangered and rare species taxa in addition to the common oligosaprobous ones (Cantonati 2001; Cantonati & Spitale, 2009 in Falasco et al. 2011).

On the other side mountain creeks and streams are extremely oligotrophic ecosystems where the geology of the substrate represents an important environmental factor in shaping especially algal (diatoms) community. Siliceous substrates shelter the highest biodiversity, with indicator species characteristics of low Ca water content. At local scales, current velocity and pH are the most important variables influencing algal community composition since the geology is the same among habitats (Falasco et al. 2011).

From the literature data, it can be concluded that freshwater oligotrophic habitats which are distributed in the high mountain are very fragile ecosystem types and that from that point of view they must be included in a different monitoring program. At local level, the main threats to this type of habitats include: habitat reduction due to the increase of pastures, the establishment of artificial snow basins, and construction of roads and infrastructure. From a global point of view, an increase in temperature, lengthening of the growing season, habitat fragmentation, and alteration of the snow free-period duration are the most important environmental factors affecting high-mountain ecosystems. As far as climatic warming and global changes are concerned, mountain regions are among the most potentially vulnerable areas, thus being strongly affected by species loss (Körner, 1995 in Falasco et al. 2011).

The ecological status of freshwater oligotrophic habitats in recent years has usually been assessed using bioindicator organisms. Benthic diatoms are regularly used as biological indicators for the environmental assessment of river water quality (included in the Water Framework Directive 2000/06). The analysis of diatom communities is a tool to guarantee an ecological and sustainable use of the water resources and the correct elaboration of the guidelines for their preservation (Falasco & Bona, 2011).

Vranica Mountain is characterized by an extremely high degree of oligotrophic freshwater habitats. Due to global climate change and intense anthropogenic activities, reduction and threatening of these types of habitats in the area of Vranica are increasing each day. In order to protect these habitat types and high diversity of species, it is necessary to assess their condition. In many cases, oligotrophic habitats are used as reference sites due to their high ecological status. A diversity of diatoms were taken as a tool for assessment of the state of oligotrophic freshwater habitats. Our results show that the living world of oligotrophic freshwater habitats, despite their surface, is extremely distinctive and diverse in the wider area of Vranica.

In addition to numerous springs and mountain creeks, this area is also characterized by the presence of peatland ecosystems. These habitat types are extremely valuable elements of biological and ecological diversity. The most prominent plant species with high conservation value are *Alchemilla xanthochlora*, *Eleocharis palustris*, *Eriophorum gracile*, *Parnassia palustris*, *Philonotis fontana*, *Pinguicula leptoceras* and *Saxifraga stellaris*.

Bryophyte species which inhabit oligotrophic freshwater habitats are very rare, and they also have high conservation value and the most prominent are listed as follows: *Bryum schleicheri*, *Calliergon stramineum*, *Climacium dendroides*, *Ctenidium molluscum*, *Plagiomnium affine*, *Sphagnum russowii* and *Sphagnum subsecundum*.

Preliminary research on diversity of diatoms in the peatland ecosystems has revealed a large number, which according to Lange-Bertalot & Steindorf, (1996), have a certain degree of vulnerability, as follows: *Adlafia bryophila*, *Caloneis tenuis*, *Cymbopleura amphicephala*, *Cymbella aspera*, *Diploneis krammeri*, *Diploneis petersenii*, *Encyonema neogracile*, *Eunotia arcubus*, *Eunotia arcus*, *Eunotia glacialis*, *Eunotia tetraodon*, *Frustulia crassinerivia*, *Gomphonema parvulus*, *Neidium affine*, *Neidium bisulcatum*, *Pinnularia subrupestris*, *Pinnularia microstauron*, *Placoneis ignorata* and *Stauroneis phoenicenteron* (Barudanović et al., 2019).

In mountain springs and small creeks, during fieldwork, one crenic species of macroalgae was discovered (*Hydrurus foetidus* (Villars) Trevisan). This species is an indicator of the good ecological state of freshwater oligotrophic habitats, especially mountain springs and creeks. It is indicative that oligotrophic freshwater habitat types on Vranica Mountain are a "hot spot" of biodiversity.

However, due to global climate change and variation in hydrological regime and strong anthropogenic influences, these habitat types and the species contained therein are extremely vulnerable. In order to protect these habitat types, in the future, it is necessary to establish long-term monitoring of biodiversity, as well as their condition.

The aim of this monitoring is to create a plan for the future restoration and conservation activities of these very unique and sensitive habitat types and to protect the high degree of species diversity. According to preliminary data main aim of this study is to establish a database of abiotic and biotic parameters which will enable further action, especially towards their restoration, conservation and long-term monitoring of biodiversity, as well as their conditions. Results of these comprehensive projects might help in establishing reference conditions not only for Bosnia and Herzegovina but also for neighbouring countries.

Study area

Vranica mountain is located in the central part of Bosnia and Herzegovina (Redžić, 2007). Boundaries of the mountain range Vranica are determined by coordinates 43° 30' and 44° 00' N; 17° 30' and 18° 00', E and cover app. 288 km. Vranica has very heterogeneous geology and petrography. Various eruptive rocks and crystal shales, feldspars, muscovite and biotite play the dominant role here.

According to authors (Drešković & Mirić, 2017), the area of Vranica Mountain belongs to the Central Dinarides parts, i.e. lies in the zone of Palaeozoic and Mesozoic limestones. The uniqueness of the geological structure of Vranica Mountain is also reflected in the same geological processes. Palaeozoic deposits are presented by the insufficiently established Silurian to Permian-Triassic. Lower Devon sediments are represented by light grey limestone and dolomites that build the highest part of Vranica Mountain. Middle Devon sediments are represented by massive, rarely layered limestone and dolomite, while deposits upper Devon less represented. The whole system is rich in numerous springs, brooks, and small rivers, which are active throughout the year, and they determine the specific hydrological regime of this area.

Prokoško Lake (46.039 m²) at 1.635 m of altitude gives the subalpine belt a particular ecological value. The most important river which springs on Vranica Mountain is the river Fojnica. This river appears in the area on the northern and eastern slopes of Vranica Mountain from numerous small creeks as follows: Jezernica, Borovnica, Razdobolja, Dobruška Vranica, Pogorelica and Bitovnja. The length of the river basin (river Fojnica) along with spring line is 45.74 km, and the total area is 727,4 km². When flowing through Fojnica town it is called the river Dragača, but when it reaches the river Željeznica which is located near the town, it is called the river Fojnica. Downstream from the river Željeznica, it acquires the characteristics of medium-sized river.

It has numerous tributaries, and the river Čemernica, the Mlava, the Željeznica and the Lepenica are the most important. The river flows through the following towns: Fojnica, Kiseljak and Visoko as a left tributary of the river Bosna an altitude of 430 meters (Drešković & Mirić, 2017; Basler & Benac, 1979).

The vegetation is characterized by the following vegetation belts of climatogenous forests: *Quercion roboris*, *Quercion petraeae*, *Aremonio-Fagion*, *Luzulo-Fagion*, *Piceion excelsae*. Upper timberline makes the presence of *Pinion mugi* and *Alnion viridis*. This area is a habitat for numerous steno-endemic plant species. Vranica Mountain is rich in numerous springs, brooks, and small rivers, which are active throughout the year, and they determine specific hydrological regime of this area. The glacier Lake Prokoško (46 039 m²) at 1635 m of altitude gives the subalpine belt a particular ecological value (Spahić, 2001 in Redžić, 2007).

According to Redžić, (2007) plant coverage of this area could be divided into 28 classes, 44 orders, 73 alliances and 165 communities of the level of association. Very sensitive vegetation from the classes *Scheuzerio-Caricetea fuscae* and *Montio-Cardaminetea* has been developed in this area, including peatland ecosystems.

Despite their relatively small areas, the vegetation from these two classes is very diverse, which has been confirmed by comparative investigations carried out in the other parts of Europe (Figure 1).

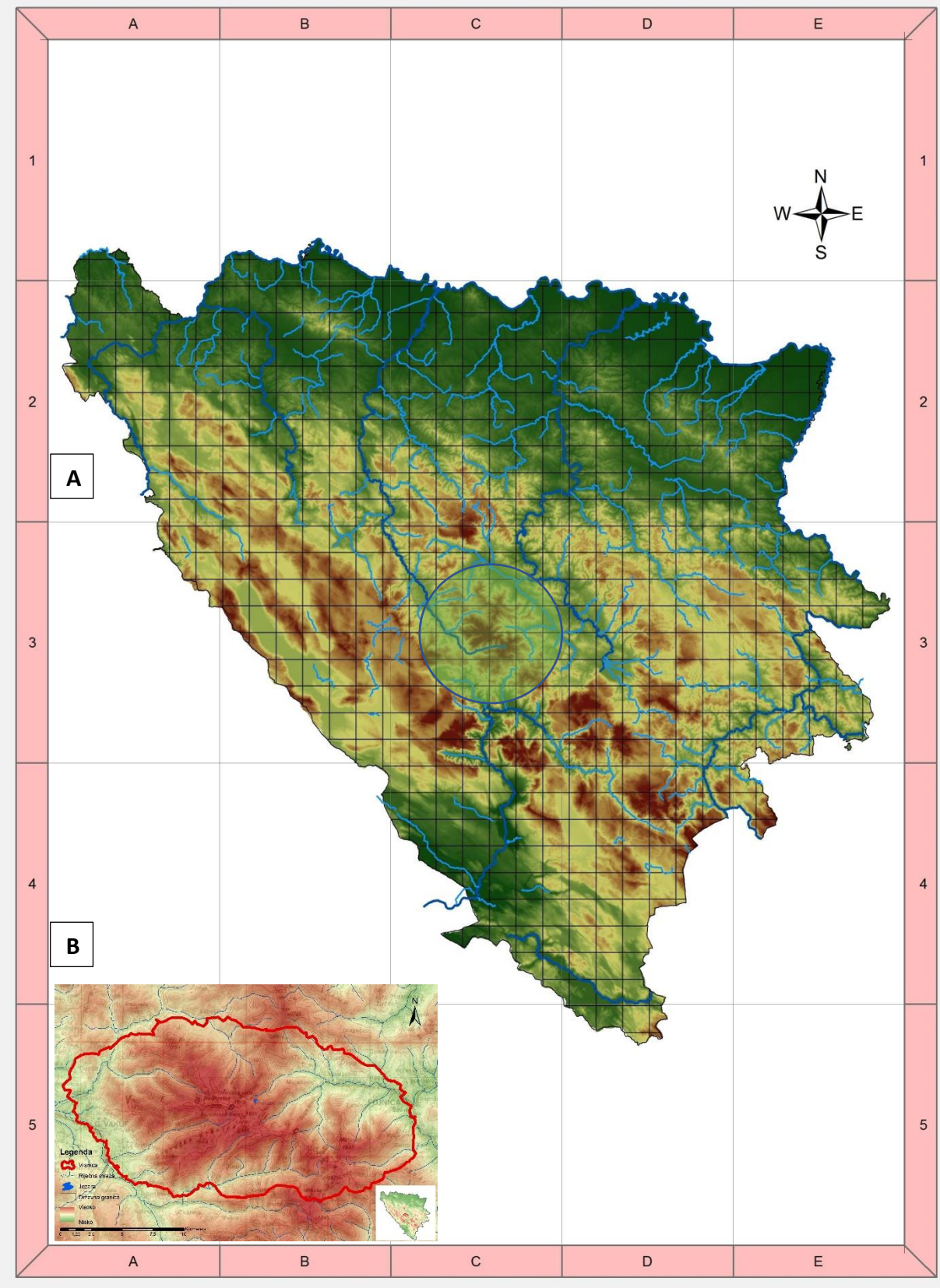


Figure 1. Position of Vranica Mountain in Central Dinarides [A.) Duraković, B. 2019 and B.) Boškailo, A. 2018]

MATERIAL AND METHODS

Physical and chemical analysis

Basic properties of water were measured directly on sampling sites. Water temperature, pH, dissolved oxygen and specific electric conductance were measured with portable multimeter Orion Star A329, while turbidity was measured with Portable turbidimeter AQ3010 and TDS with PCE-CM 41.

Water samples collected on the field were analysed at the Department of Chemistry at Faculty of Science, University of Sarajevo. All samples were collected in plastic bottles. The plastic bottles were soaked in 10% (v/v) HNO₃ and then washed with double distilled water. The bottles were rinsed out three times with the sample water prior to taking the sample.

The analysis of water samples was performed in the following 24 hours after the sampling. All reagents used for the preparation of calibration standard solutions were of analytical grade. Single standard stock solutions (CertPur, Merck, Darmstadt, Germany) containing 1000 mg/L of K, Na, Ca, Mg, Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn were used. Caesium chloride and potassium nitrate (Merck, Darmstadt, Germany) were used as ionization suppressor.

The measurements were performed on an atomic absorption spectrometer model AA240FS, Varian, Australia. The concentration of Na and K in water samples was determined by flame atomic emission spectrometry (FAES). The concentration of Ca, Mg, Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn in water samples was determined by flame atomic absorption spectrometry (FAAS).

Diatom analysis

Samples of phytobenthos were collected from different types of substrates: epilithon, epiphyton, epipsamon and epipelon during summer, autumn and spring seasons in 2018 and 2019. Sample from submerged stones was collected by scraping with a scalpel blade or brushing the upper surface of submerged stones. Stones overgrown by filamentous algae or covered by mud were avoided due to possible contamination by nonepilithic diatoms. Portions of the samples were preserved with 4% formaldehyde solutions.

Laboratory processing of diatoms was carried out applying methods used by Hustedt (1930). In order to obtain pure valves of diatoms, part of the obtained material was digested with sulfuric acid (H₂SO₄), potassium permanganate (KMnO₄) and oxalic acid (C₂H₂O₄). The cleaned valves of diatoms were then mounted in a special mountant (Canada balsam) with a high refractive index in order to make it easier to see surface ornamentation such as striae and other characteristic structures (Mašić et al. 2018).

Five permanent slides have been prepared for each sample and a total of 300 valves were counted to assess relative abundance. Species with content above 5% in a given assemblage were defined as abundant. All slides were scanned for taxa with low relative abundance.

Light microscope observation was conducted using Best Scope 2020 microscope. Species composition and the quantitative relationship of diatoms were estimated from the permanent slides under 1000x magnification.

Species abundance of diatoms were estimated on a five-degree scale as follows: 1-rare (single valve or frustule), 2-sparse (up to 10% of the sample), 3-frequent (11-15% of the sample), 4-very frequent (51-75% of the sample), 5-common (in more than 75% of the sample). The identification of diatom was supported by the following references: Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Lange-Bertalot & Metzeltin (1996), Krammer (1997a,b., 2000, 2001, 2003), Lange-Bertalot, H. (1993, 2001), Reichardt (1999), John et al. (2003), Bey & Ector (2013a-f), Hofman et al. (2013), John (2015), Cantonati et al. (2017) and a number of specialized references listed with respective taxa: Wojtal (2003, 2006), Witkowski et al. (2004), Schmidt et al. (2004), Wojtal & Kwandras (2006), Wojtal (2009), Wojtal et al. (2011), Levkov et al. (2010), Levkov & Ector (2010), Cantonati et al. (2010), Levkov & Williams (2012), Pavlov et al. (2013), Pavlov & Levkov (2013), Bucko et al. (2013), Lange-Bertalot & Wojtal (2014), Pavlov et al. (2016).

The nomenclature of diatoms was adjusted according to the following Internet base: Guiry & Guiry, 2019. In order to further verify the name of the taxon they were used in the addition to the following database: Jahn, R. & Kusber, W.-H. (2019) (AlgaTerra Information System), Spaulding, S.A., Lubinski, D.J. and Potapova, M. 2019 (Diatoms of the United States) and Jüttner, I., Bennion, H., Carter, C., Cox, E.J., Ector, L., Flower, R., Jones, V., Kelly, M.G., Mann, D.G., Sayer, C., Turner, J.A., Williams, D.M. (2019) (Freshwater Diatoms Flora of Britain and Ireland).

Research on diatoms in the wider area of Vranica Mountain has not been carried out so far. First data about the biodiversity of cyanobacteria and algae in some specific habitat types were described by Protić (1926), Kapetanović & Hafner (2007) and Barudanović et al. (2017).

It is very important to highlight that this comprehensive study is first in regards to inventorisation of cyanobacteria and algae in the wider area of Vranica Mountain with special emphasis on diatoms. All collected samples were stored in the Laboratory for Systematics of Algae and Fungi at the Faculty of Science, University of Sarajevo, and also aliquot with diatoms and permanent slides. It is very important to note that from each sample (aliquot), we prepared five permanent slides which are associated with unique field protocol.

Data analysis

Omnidia software (Lecointe et al. 1993) version 6.0.8, was used to calculate diatomaceous indices, including ecological and taxonomic data. The ecological status of freshwater oligotrophic habitats was assessed based on the following diatom indices: IPS – Specific, pollution sensitivity Index (Cemagref, 1982), SLAD - Sládeček's index (Sládeček, 1986), TID - ROTT trophic index (Rott, 1999) and SID - ROTT saprobic index (Rott et al., 1997).

Range of diatom indices varied from 1 to 20 and corresponding to the ecological statuses as follows: bad (1-4), poor (5-8), moderate (9-12), good (13-16) and very good (17-20). In order to determine variables important for a number of species, i.e. determining the correlation between dependent (diversity index) and the independent variables (temperature, pH, dissolved oxygen, specific conductance, turbidity, TDS) Pearson's coefficient of simple linear correlation (r) was used. Pearson's coefficient of correlation between analysed variables was tested using Student t-test at a significance level of <0.05 .

A univariate statistical analysis was performed using the software package PAST v.3.24 (Hammer, 2019). Species diversity in diatom assemblages was determined using Shannon (H') index:

$$H' = - \sum_{i=1}^R p_i \ln p_i$$

where p_i is the proportion of characters belonging to the i_{th} type of letter in the string of interest. p_i is often the proportion of individuals belonging to the i_{th} species in the dataset of interest (Shannon & Weaver, 1949).

Indicator values of diatoms were used (Van Dam et al. 1994) for the purpose of understanding the complexities of environmental conditions in investigated freshwater oligotrophic habitats.

According to the Red List of diatoms (Hoffman et al. 2018), the threatened diatoms were attributed to the categories: Threatened with extinction [1], Highly endangered [2], Endangered [3], At risk [G], Very rare [R] and Declining [V].

In order to analyse the differentiation of individual samples collected at different peatlands ecosystem on Vranica mountain, the Principal Coordinate Analysis (PCoA) was used.

The ordination was conducted on the Bray-Curtis similarity matrix of species data (Legendra & Legendre, 1998; Kamberović et al. 2016; Mašić et al. 2018).

The data were transformed by $\log_{(x+1)}$ after standardising the matrices. Environmental variables (water temperature, pH, dissolved oxygen, specific conductance and turbidity) were presented as vectors after normalisation (Pearson's correlation).

The statistical analysis with graphical interpretation was performed using the software package PRIMER v6 (Anderson et al. 2008).

RESULTS AND DISCUSSION

A. Overview of basic investigated physical, chemical and biological parameters

Results of physical, chemical and biological parameters of freshwater oligotrophic habitat types are shown in Table 1 and Figures 2 - 7. On studied sites the following physical parameters were measured: air temperature, relative humidity and light intensity. From physical and chemical parameters on studied sites the following were measured: water temperature, pH, concentration of dissolved oxygen, specific electroconductivity, turbidity and TDS.

Biological parameters were determined with the presence of diatom taxa, but during the studied period other groups of algae were taken into account. Diatom diversity is presented through two indices as follows: Margalef and Shannon diversity indices, while the ecological state of studied sites was presented through four diatom indices as follows: IPS, SLA, TID and SID.

Average air temperature ranged from 13.26°C (H5) to 18.94°C (H1). The lowest average air temperature was measured in peatlands and the highest air temperature was measured in springs. Some lower temperature was measured in the lake (18.55°C), creeks (18.69°C) and stream (16.79°C). Average air humidity ranged from 60.78% (H4) to 83.51% (H3). The lowest average air humidity was measured in Prokoško Lake and the highest air humidity was measured in streams. Some lower humidity was measured in springs (69,43%), peatlands (68,01%) and creeks (65,65%). The highest average light intensity was measured in springs (9197,00 lux) and the lowest in streams (1596.89 lux). Water temperature ranged from 9.55°C (H1) to 17.43°C (H4). The lowest pH values was measured in peatlands (5.88), while the highest values were measured in streams (8,40).

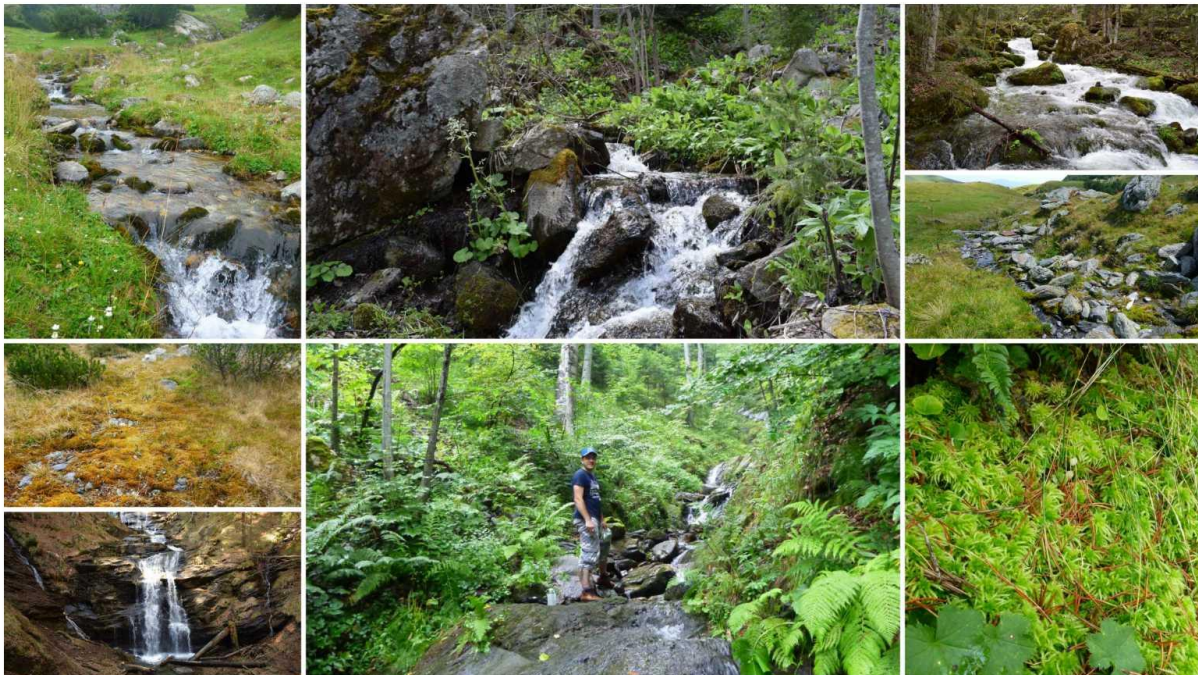


Plate I. Diversity of freshwater oligotrophic habitat types on Vranica Mountain

The values of dissolved oxygen ranged from 7.37 mgL⁻¹ to 9.43 mgL⁻¹. The highest values were measured in streams, and the lowest values were measured in peatlands. The values of electric conductivity ranged from 140.53 µScm⁻¹ (H5) to 223.00 µScm⁻¹ (H3). The highest value of turbidity was recorded in peatlands (314.30 NTU), while the lowest value was recorded in streams (0.80 NTU). TDS ranged from 103.00 ppm (H1) to 204.92 ppm (H4). The value of Margalef diversity index ranged from 4.95 (H4) to 2.68 (H5), while the value of the Shannon diversity index ranged from 2.30 (H4) to 2.68 (H5). The highest number of diatom species was recorded in peatlands (78), while the lowest number of diatom species was recorded in Prokoško Lake (59). Taking into account the other group of algae we can conclude that habitat type (H4) was the richest with the species (121), while the lowest number of species was recorded in streams (61).

Table 1. Overview of physical, chemical and biotic parameters in studied habitats

Habitat types	Springs	Creeks	Streams	Lake	Peatlands
Physical site conditions / ID	H1	H2	H3	H4	H5
Air temperature (°C)	18.94	18.69	16.76	18.55	13.26
Air humidity (%)	69.43	65.65	83.51	60.78	68.01
Light intensity (lux)	9197.00	8344.46	1596.89	8802.00	7337.00
Chemical parameters / ID	H1	H2	H3	H4	H5
Water temperature (°C)	9.55	12.49	11.40	17.43	13.11
pH	7.59	8.15	8.40	8.32	5.88
Dissolved oxygen (mgL ⁻¹)	9.30	9.18	9.43	8.63	7.37
Specific conductance (µScm ⁻¹)	184.35	186.91	223.00	196.59	140.53
Turbidity (NTU)	1.18	1.42	0.80	1.70	314.30
TDS (ppm)	103.00	135.39	163.80	204.92	120.00
Biotic parameters	H1	H2	H3	H4	H5
Diatom taxa	82	61	59	52	78
<i>d</i> – Margalef diversity index	5.44	5.06	5.22	4.05	5.48
<i>H'</i> – Shannon diversity index	2.63	2.65	2.63	2.30	2.68
<i>Bangiophyceae</i>	0	1	0	0	0
<i>Charophceae</i>	0	0	0	1	0
<i>Chlorophyceae</i>	0	0	0	19	0
<i>Chrysophyceae</i>	1	1	0	2	0
<i>Conjugatophyceae</i>	3	9	0	22	14
<i>Cyanophyceae</i>	3	5	1	13	5
<i>Dinophyceae</i>	0	0	0	1	0
<i>Euglenophyceae</i>	0	0	0	7	0
<i>Trebouxiophyceae</i>	0	0	0	4	0
<i>Xanthophyceae</i>	1	1	1	0	1
Total without diatoms	8	17	2	69	20
Total with diatoms	90	78	61	121	98
Rare and endangered diatoms	34	19	16	16	48
Ecological state / ID	H1	H2	H3	H4	H5
IPS/20	18.29	17.11	16.43	17.22	18.79
Sla/20	16.41	16.51	14.85	13.59	16.29
Rott TI/20 (TID)	10.81	9.98	8.41	12.48	16.58
Rott TI/20 (SID)	16.83	16.65	14.54	17.46	19.48

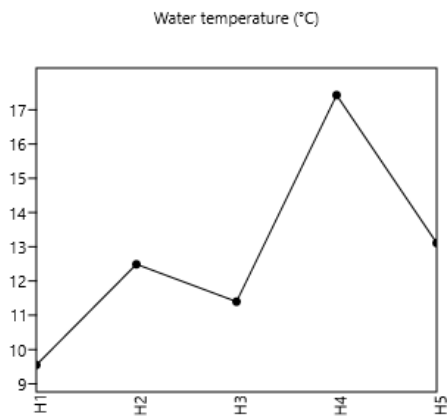


Fig. 2. Variation of water temperature

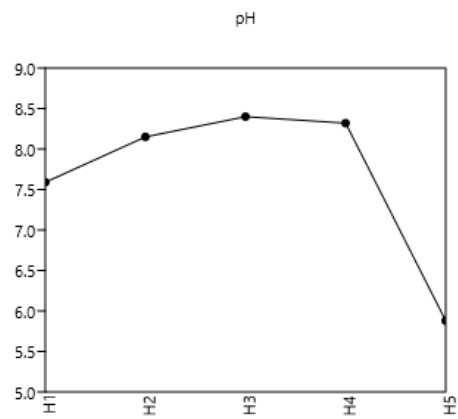


Fig. 3. Variation of pH values

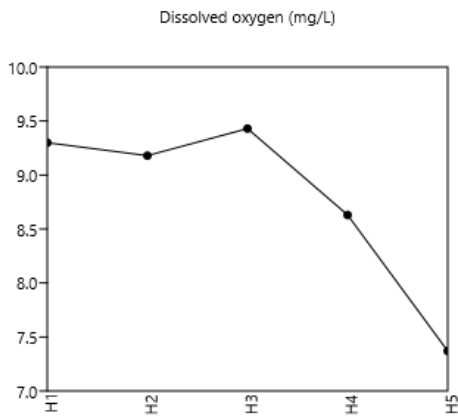


Fig. 4. Variation of dissolved oxygen

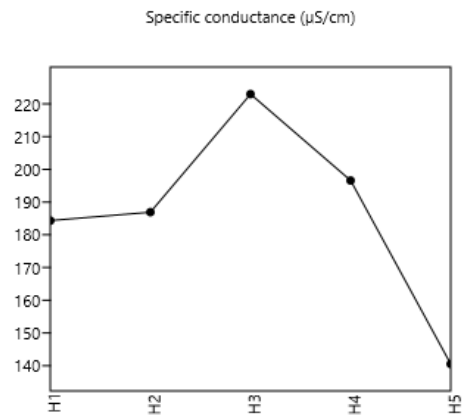


Fig. 5. Variation of specific conductance

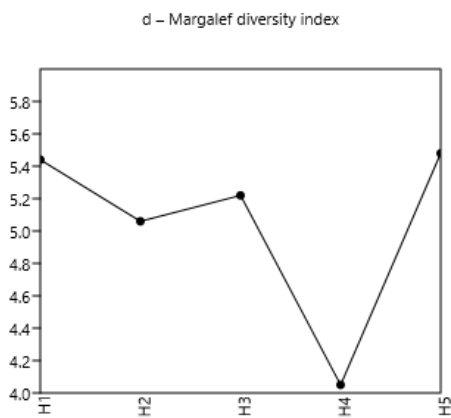


Fig. 6. Variation of Margalef diversity index

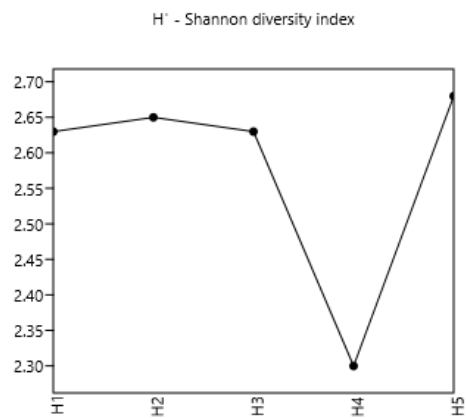


Fig. 7. Variation of Shannon diversity index

B. Heavy metal analysis

In order to analyse the selected heavy metals, water sampling was carried out at six locations as follows: one sample was collected from the lake and peatlands, and two samples were collected from creeks and springs. Sampling from the streams was not performed. A total of 12 chemical parameters of water were analysed. Of the total of six samples collected, cadmium (Cd) was not detected at any site of research. Sodium (Na) and zinc (Zn) were determined at one site, while manganese (Mn) and iron (Fe) were determined at two sites. Chromium (Cr) and nickel (Ni) were determined at five sites, while potassium (K), calcium (Ca), magnesium (Mg), copper (Cu) and lead (Pb) were determined at all investigated sites. The results of the concentration of heavy metals from freshwater oligotrophic habitats collected on the Vranica Mountain are shown in Table 2.

Table 2. Concentration of metals (mgL⁻¹) in water samples collected at Vranica Mountain [CR – creek, LE – lake, SP – spring and PE – Peatland]

Samples	Concentration (mgL ⁻¹)											
	K	Na	Ca	Mg	Cr	Cu	Mn	Fe	Ni	Cd	Pb	Zn
CR26	0.077	n.d.	10.4	0.524	0.026	0.005	n.d.	n.d.	0.006	n.d.	0.039	n.d.
CR27	0.052	n.d.	25.6	1.97	n.d.	0.008	n.d.	n.d.	n.d.	n.d.	0.019	n.d.
17LE	0.185	n.d.	27.1	2.01	0.009	0.011	0.004	0.146	0.007	n.d.	0.003	n.d.
10SP	0.039	n.d.	26.9	1.97	0.008	0.009	n.d.	n.d.	0.017	n.d.	0.005	n.d.
11SP	0.117	n.d.	0.142	0.081	0.004	0.003	n.d.	n.d.	0.002	n.d.	0.029	n.d.
11PE	1.38	0.108	0.691	0.263	0.009	0.004	0.063	0.272	0.013	n.d.	0.022	0.012

n.d. – not detected by using the determination technique

The values of the potassium (K) range from 0.039 to 1.38. The highest values were measured in the peatlands, and the lowest values were measured in the mountain springs. The values of calcium (Ca) range from 0.142 to 10.40. The highest values were measured in the mountain streams, and the lowest values were measured in the mountain springs. The highest values of magnesium (Mg) were measured in the mountain lake (2.01), and the lowest values were measured in the mountain springs (0.081). Within the investigated habitats, there is an extremely low concentration of copper, nickel, lead, and also the remaining measured heavy metals. It is important to note that cadmium (Cd) was not detected by using the determination technique.

C. Ecological state of studied freshwater oligotrophic habitats on Vranica Mountain

In order to assess the ecological status of freshwater oligotrophic habitat types on Vranica Mountain, the results of four diatom indices (IPS, SLA, TID and SID) were taken. The IPS index ranged from 16.43 (H3) to 18.79 (H5). H1, H2, H4 and H5 habitats have very good ecological status, while H3 has good ecological status. The SLA index ranged from 13.59 (H4) to 16.51 (H2). According to the above index, all the analysed habitats have moderately good ecological status. The Rott TID index ranged from 8.41 (H3) to 16.58 (H5). According to the index, H4 and H5 habitats have a good ecological status, while habitats H1, H2 and H3 have a moderate ecological status. The Rott SID index ranged from 14.54 to 19.48. Habitats H1, H2 and H3 have good ecological status, while habitats H4 and H5 have very good ecological status. The results of variation of diatom indices are shown in the figures (Fig 8 – Fig 11).

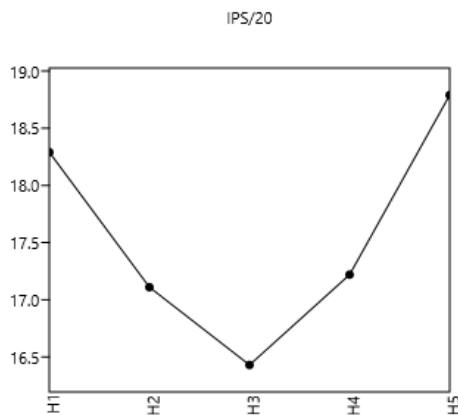


Fig. 8. Variation of IPS index

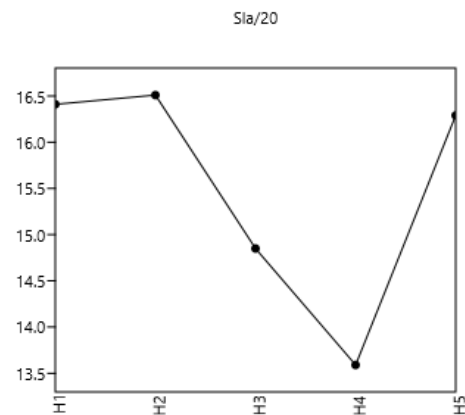


Fig. 9. Variation of Sla index

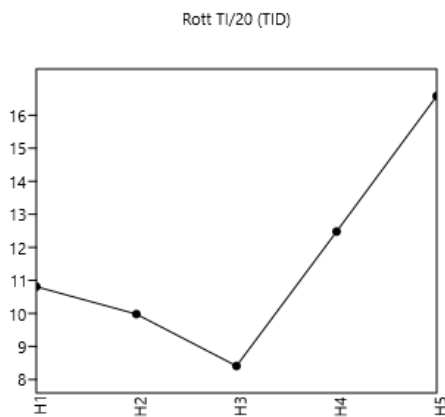


Fig. 10. Variation of TID index

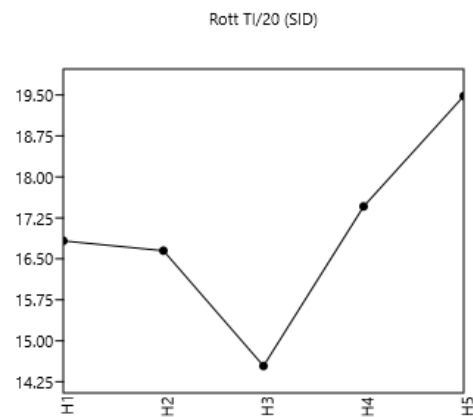


Fig. 11. Variation of SID diatom index

D. Correlation between diversity index and physical and chemical parameters

Diversity indices were in correlation with water temperature and TDS, and slightly with pH. High value of Pearson's correlation coefficient obtained between TDS and water temperature ($r=0.817$), between d and water temperature ($r=-0.879^*$; $p<0.05$), between H' and water temperature ($r=-0.829$), DO and pH ($r=0.860$), SC and pH ($r=0.927^*$; $p<0.05$), SC and DO ($r=0.854$), T2 and pH ($r=-0.953^*$; $p<0.05$), T2 and DO ($r=-0.934^{**}$; $p<0.05$), T2 and SC ($r=-0.859$), d and TDS ($r=-0.896^{**}$; $p<0.05$), H' and TDS ($r=-0.840$) and between H' and d ($r=0.960^*$; $p<0.05$). Statistically significant correlation was marked with an asterisk. Moderate correlation obtained between TDS and pH ($r=0.572$), TDS and SC ($r=0.529$), d and pH ($r=-0.539$). Week values of Pearson's correlation coefficient was obtained between pH and T1 ($r=0.126$), DO and T1 ($r=-0.390$), SC and T1 ($r=-0.034$), T2 and T1 ($r=0.062$), TDS and DO ($r=0.094$), TDS and T2 ($r=-0.354$), d and DO ($r=-0.070$), d and SC ($r=-0.352$), d and T2 ($r=0.410$), H' and DO ($r=-0.024$), H' and SC ($r=-0.304$), H' and T2 ($r=0.362$). Moderate and week values of Pearson's correlation coefficient are not significant at the $p<0.05$ level.

Correlation between diversity indices and measured physical and chemical parameters and level of significance are presented in Table 3 and Figure 12.

Table 3. Correlation between diversity indices and measured physical and chemical parameters and level of significance [H – diversity index, T1 – temperature, DO – dissolved oxygen, SC – specific conductivity, T2 – turbidity, TDS – total dissolved substance, d – Margalef diversity index and H' – Shannon diversity index].

	T1 (°C)	pH	DO (mgL ⁻¹)	SC (μScm ⁻¹)	T2 (NTU)	TDS (ppm)	d	H'
T1 (°C)	1.00
pH	0.126	1.00
DO (mgL ⁻¹)	-0.390	0.860	1.00
SC (μScm ⁻¹)	-0.034	0.927*	0.854	1.00
T2 (NTU)	0.062	-0.953*	-0.934*	-0.859	1.00	.	.	.
TDS (ppm)	0.817	0.572	0.094	0.529	-0.354	1.00	.	.
d	-0.879*	-0.539	-0.070	-0.352	0.410	-0.896*	1.00	.
H'	-0.829	-0.449	-0.024	-0.304	0.362	-0.840	0.960*	1.00

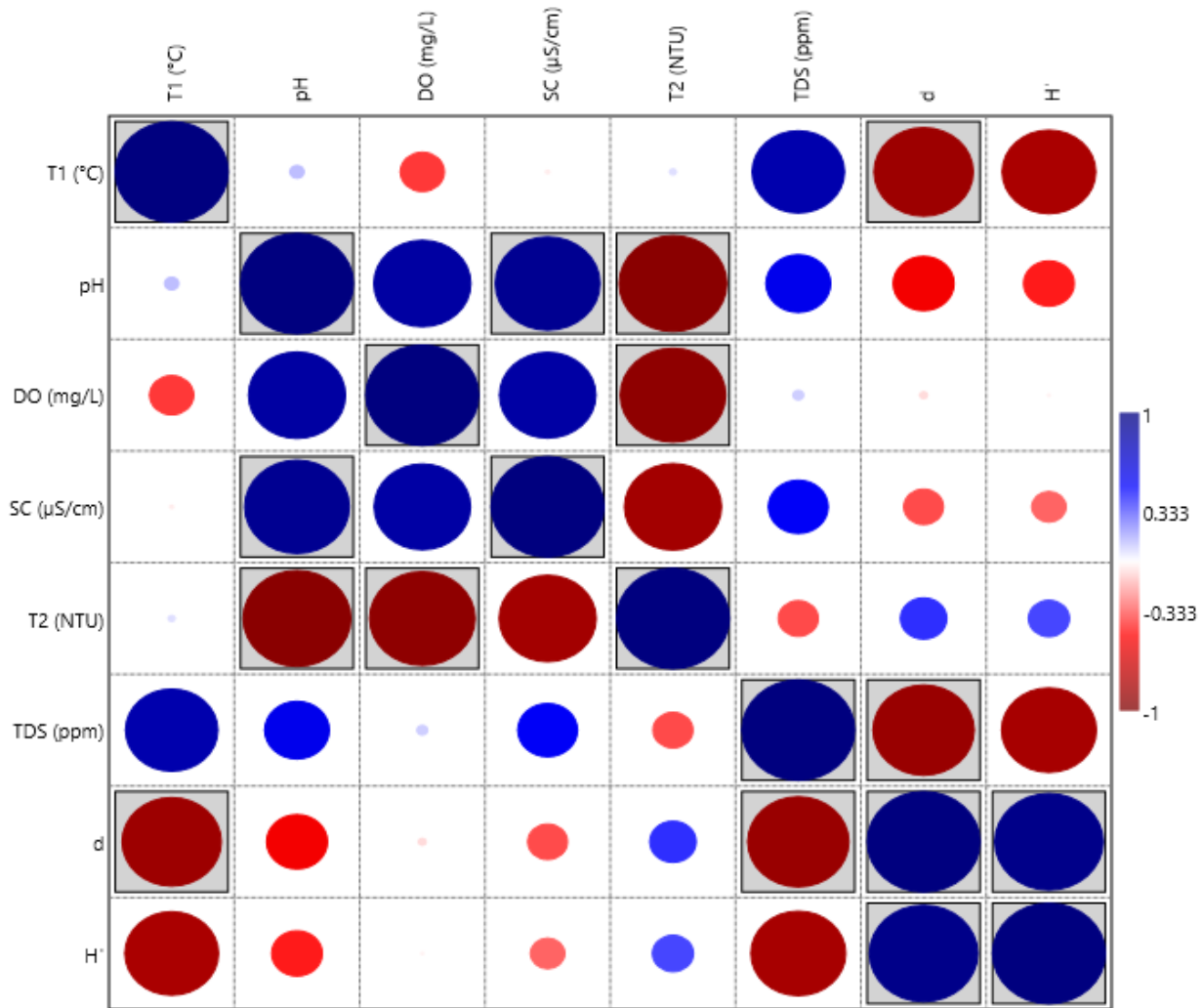


Figure 12. Correlation and level of significance between diversity indices and measured physical and chemical parameters [Statistically significant correlation is marked with a square]

E. Diversity of cyanobacteria and algae in freshwater oligotrophic habitats

Taking into account all groups of algae during the investigated period, a total of 272 taxa were recorded. The highest number of taxa was identified within the class *Bacillariophyceae*, *Chlorophyceae*, *Conjugatophyceae*, while taxa of other classes occurred with a smaller numerical value. The largest number of taxa was found in the lake (121 species or 27.10%), while the smallest number of taxa were recorded in the stream (61 species or 13.62%). In all studied habitat types, algae from class *Bacillariophyceae* were the most dominant. The largest number of species was found in springs (82 species or 24.70%), while the smallest number of species was found in Prokoško Lake (52 species or 15.66%). Summary of the recorded taxa in each investigated habitat type is shown in Table 4. Comparative overview of the recorded number of taxa (*Bacillariophyceae* vs other algal groups) in studied habitat types is shown in Figure 13.

Table 4. Summary of the recorded taxa in each investigated habitat type is shown in tables

Class	Springs	Creeks	Streams	Lake	Peatlands
<i>Bacillariophyceae</i>	82	61	59	52	78
<i>Bangiophyceae</i>	0	1	0	0	0
<i>Charophyceae</i>	0	0	0	1	0
<i>Chlorophyceae</i>	0	0	0	19	0
<i>Chrysophyceae</i>	1	1	0	2	0
<i>Conjugatophyceae</i>	3	9	0	22	14
<i>Cyanophyceae</i>	3	5	1	13	5
<i>Dinophyceae</i>	0	0	0	1	0
<i>Euglenophyceae</i>	0	0	0	7	0
<i>Trebouxiophyceae</i>	0	0	0	4	0
<i>Xanthophyceae</i>	1	1	1	0	1
TOTAL	90	78	61	121	98

F. Diversity of diatoms in freshwater oligotrophic habitats on Vranica Mountain

A total of 174 taxa belonging to 57 genera were determined in the material. Genera with the highest number of species were *Eunotia* (22), *Gomphonema* (14), *Pinnularia* (12), *Encyonema* (7), *Stauroneis* (6), *Cocconeis* (5), *Cymboplectra* (5), *Navicula* (5), *Neidium* (5) and *Nitzschia* (5). Altogether 169 diatom taxa mostly belonging to pennate diatoms were found and five centric diatoms. The most common pennate diatoms were *Cocconeis placentula* Ehrenberg 1838, *Achnantheidium minutissimum* (Kützing) Czarnecki 1994, *Staurosirella pinnata* (Ehrenberg) D.M. Williams et Round 1988, *Meridion circulare* (Gréville) C. Agardh 1831, *Odontidium mesodon* (Ehrenberg) Kützing 1844, *Cocconeis lineata* Ehrenberg 1849, *Gomphonema minusculum* Krasske 1932, *Ulnaria ulna* (Nitzsch) Compère 2001, *Nitzschia fonticola* Grunow in Cleve & Möller 1879, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot 1999, *Navicula radiosa* Kützing 1844, *Diatoma ehrenbergii* Kützing 1844, *Encyonema ventricosum* (C. Agardh) Grunow in A. Schmidt et al. 1885, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot 2004, *Encyonema minutum* (Hilse) D.G. Mann 1990, *Pinnularia borealis* Ehrenberg 1843, *Tabellaria ventricosa* Kützing 1844, *Navicula tripunctata* (O.F. Müller) Bory 1822 and *Pinnularia neomajor* Krammer 1992, while centric diatoms were *Aulacoseira crenulata* (Ehrenberg) Thwaites 1848, *Orthoseira roeseana* (Rabenhorst) O'Meara 1876, *Aulacoseira alpigena* (Grunow) Krammer 1991, *Ellerbeckia arenaria* (Moore ex Ralfs) Crawford 1988 and *Melosira varians* C. Agardh 1827. Within 57 recorded genera (174 taxa), three genus (*Eunotia*, *Gomphonema* and *Pinnularia*) consist of 49 taxa.

Within the genus of *Eunotia* 22 taxa were identified as follows: *E. arcubus*, *E. arcus*, *E. bilunaris*, *E. boreoalpina*, *E. curtagrunowii*, *E. exigua*, *E. glacialifalsa*, *E. glacialis*, *E. implicata*, *E. incisa*, *E. minor*, *E. mucophila*, *E. nymanniana*, *E. paludosa*, *E. praerupta*, *E. rhomboidea*, *E. soleirolii*, *E. subherkiniensis*, *E. tenella*, *E. tetraodon*, *E. triodon* and *E. valida*. Within the genus of *Gomphonema* 14 taxa were identified as follows: *G. acidoclinatum*, *G. acuminatum*, *G. angustum*, *G. exilissimum*, *G. gracile*, *G. hebridense*, *G. italicum*, *G. minusculum*, *G. olivaceum*, *G. parvulum*, *G. productum*, *G. rhombicum*, *G. subclavatum* and *G. truncatum*.

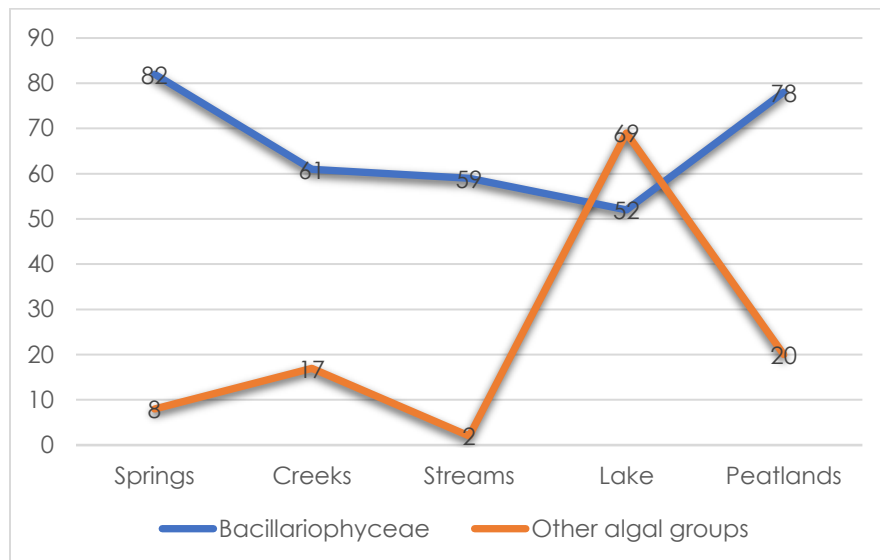


Figure 13. Total number of diatoms vs other algal group found in studied habitat types

Within the genus of *Pinnularia* 12 taxa were identified as follows: *P. appendiculata*, *P. borealis*, *P. microstauron*, *P. neomajor*, *P. obscura*, *P. perirrorata*, *P. rupestris*, *P. schoenfelderii*, *P. stomatophora*, *P. subcapitata* var. *elongata*, *P. subrupestris* and *P. viridis*. It is important to note that these species in the future can serve as significant bioindicators of the state of freshwater oligotrophic habitats in the wider area of Vranica Mountain. Some of these species indicate an oligotrophic state, while some species indicate processes of eutrophication or natural acidification within these freshwater habitats. Also, some of the identified species are extremely rare so that habitats have settled a major conservation significance. During the investigated period in the studied samples of phytobenthos, a certain number of extremely rare diatoms were presented and some of them were presented with only one genus: *Amphipleura pellucida*, *Cavinula cocconeiformis*, *Chamaepinnularia mediocris*, *Craticula cuspidata*, *Cymatopleura solea*, *Decussiphycus hexagonus*, *Denticula tenuis*, *Ellerbeckia arenaria*, *Hanea arcus*, *Hippodonta coxiae*, *Karayevia laterostriata*, *Kobayasiella parasubtilissima*, *Luticola mutica*, *Melosira varians*, *Odontidium mesodon*, *Orthoseira roeseana*, *Paraplaconeis placentula*, *Planothidium lanceolatum*, *Pseudostaurosira parasitica*, *Reimeria sinuata*, *Rhoicosphaenia abbreviata*, *Rhoppalodia gibba*, *Staurosira construens*, *Tabellaria ventricosa* and *Tetracyclus rupestris*. Similar to the previously mentioned diatom taxa, even these rare species can serve as significant bioindicators of the state of freshwater oligotrophic habitat types and in monitoring of certain ecological phenomena. A comprehensive checklist of diatoms species found in freshwater oligotrophic habitat types is presented in Table 5.

G. Diversity of rare and endangered species

According to the Red List of freshwater diatoms (Hoffman et al. 2018) about 79 taxa or 45,40% are cited under various categories: 2 – highly endangered (11 taxa), 3 – endangered (10 taxa), G – at risk (34 taxa), R – very rare (1 taxa), V – declining (23 taxa). The largest number of rare and endangered diatom taxa was determined in mountain peatlands (48 taxa), while the smallest number was determined in mountain creeks (16 taxa) and mountain lake (16 taxa). In total 34 rare and endangered taxa were determined in mountain springs and 19 taxa in mountain streams. Comparative overview of rare and endangered taxa determined in freshwater oligotrophic habitats on Vranica Mountain is shown in Table 6 and 7 and Figure 14.

Table 6. Comparative overview of rare and endangered diatom taxa found in freshwater oligotrophic habitats on Vranica Mountain: H1 – mountain springs, H2 – mountain creeks, H3 – mountain streams, H4 – mountain lake, H5 – mountain peatlands.
[RL – Red List (Hoffman et al. 2018): 2 – highly endangered, 3 – endangered, G – at risk, R – very rare, V – declining]

Habitat code	2	3	G	R	V	Sum
H1	4	2	15	1	12	34
H2	4	0	10	0	5	19
H3	2	2	9	0	3	16
H4	1	2	5	0	8	16
H5	5	7	21	0	15	48

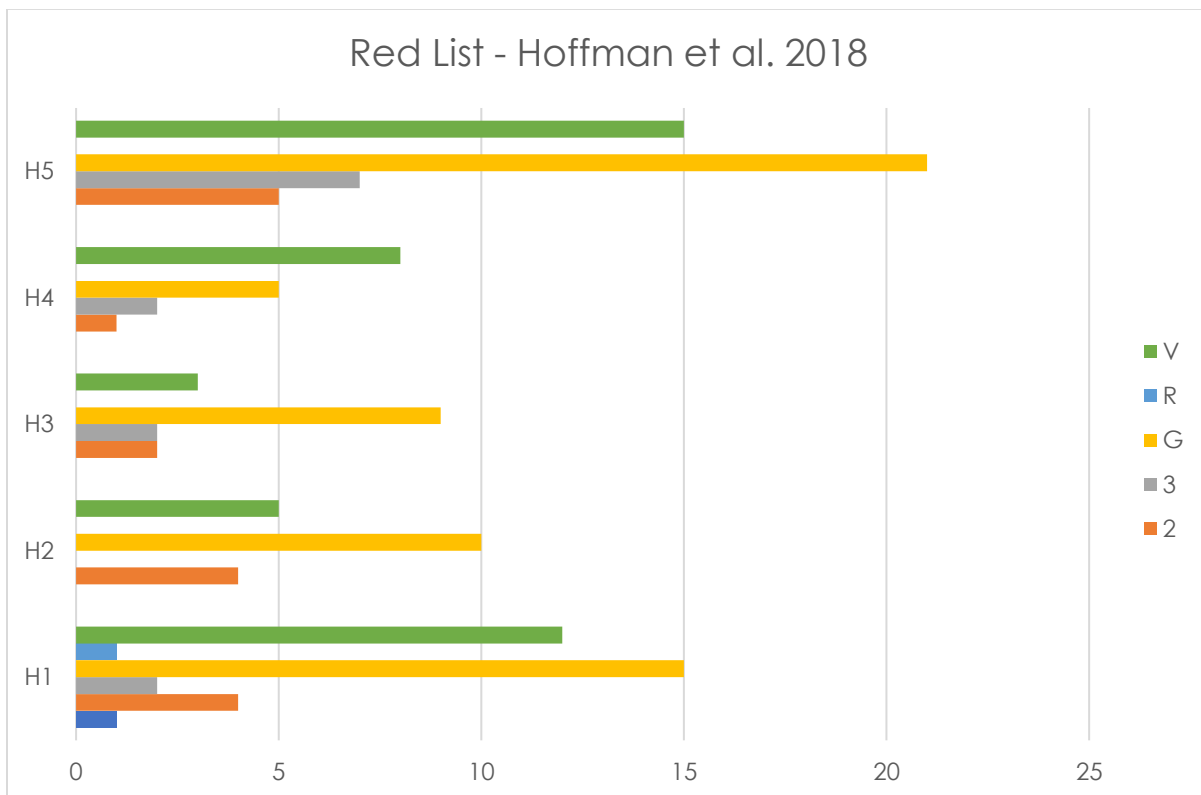


Figure 14. Comparative overview of rare and endangered diatom taxa found in freshwater oligotrophic habitats on Vranica Mountain

Table 7. Detailed overview of rare and endangered diatom taxa found in freshwater oligotrophic habitats on Vranica Mountain

ID	Taxon name	RL	H1	H2	H3	H4	H5
1.	<i>Achnantheidium kranzii</i>	3	0	0	1	0	0
2.	<i>Aulacoseira alpigena</i>	G	0	0	0	0	1
3.	<i>Aulacoseira crenulata</i>	G	1	1	0	0	0
4.	<i>Brachysira brebissonii</i>	3	1	0	0	0	1
5.	<i>Brachysira intermedia</i>	2	0	0	0	1	1
6.	<i>Caloneis aerophila</i>	R	1	0	0	0	0
7.	<i>Caloneis alpestris</i>	G	1	1	0	0	1
8.	<i>Caloneis tenuis</i>	3	0	0	0	0	1
9.	<i>Cavinula cocconeiformis</i>	G	0	0	0	1	0
10.	<i>Chamaepinnularia mediocris</i>	V	0	0	0	0	1
11.	<i>Cymbella aspera</i>	G	1	1	1	0	1
12.	<i>Cymbella proxima</i>	G	0	0	0	1	0
13.	<i>Cymbopleura amphicephala</i>	G	0	1	0	1	1
14.	<i>Cymbopleura austriaca</i>	2	1	1	1	0	1
15.	<i>Cymbopleura cuspidata</i>	G	1	0	0	1	0
16.	<i>Cymbopleura inaequalis</i>	V	0	0	0	1	0
17.	<i>Decussiphycus hexagonus</i>	2	0	1	0	0	0
18.	<i>Diploneis krammeri</i>	V	1	1	0	0	1
19.	<i>Diploneis ovalis</i>	2	0	1	0	0	0
20.	<i>Encyonema perpusillum</i>	3	0	0	0	0	1
21.	<i>Encyonema vulgare</i>	3	0	0	0	0	1
22.	<i>Encyonopsis cesatii</i>	V	0	0	0	1	0
23.	<i>Epithemia goeppertiana</i>	G	0	0	0	0	1
24.	<i>Eunotia arcubus</i>	3	0	0	0	1	0
25.	<i>Eunotia arcus</i>	V	1	0	0	0	0
26.	<i>Eunotia boreoalpina</i>	G	0	0	0	0	1
27.	<i>Eunotia curtagrunowii</i>	G	0	1	0	0	0
28.	<i>Eunotia glacialifalsa</i>	G	0	0	0	0	1
29.	<i>Eunotia glacialis</i>	G	1	0	0	0	0
30.	<i>Eunotia implicata</i>	G	0	0	1	0	1
31.	<i>Eunotia incisa</i>	G	0	0	0	0	1
32.	<i>Eunotia minor</i>	V	0	0	0	1	0
33.	<i>Eunotia mucophila</i>	G	0	0	0	0	1
34.	<i>Eunotia nymanniana</i>	2	0	0	0	0	1
35.	<i>Eunotia paludosa</i>	V	1	0	0	1	1
36.	<i>Eunotia praerupta</i>	2	0	0	1	0	0
37.	<i>Eunotia rhomboidea</i>	G	1	0	1	0	1
38.	<i>Eunotia soleirolii</i>	G	1	0	0	0	0
39.	<i>Eunotia subherkiniensis</i>	2	0	1	0	0	0
40.	<i>Eunotia tenella</i>	G	0	0	1	0	1
41.	<i>Eunotia tetraedon</i>	2	1	0	0	0	1
42.	<i>Eunotia triodon</i>	2	0	0	0	0	1
43.	<i>Eunotia valida</i>	G	1	0	0	0	1
44.	<i>Frustulia crassinervia</i>	V	1	0	0	0	1

45. <i>Frustulia saxonica</i>	V	0	0	0	0	1
46. <i>Gomphonema angustum</i>	G	1	0	1	0	0
47. <i>Gomphonema exilissimum</i>	V	1	0	0	1	1
48. <i>Gomphonema hebridense</i>	V	0	0	0	0	1
49. <i>Gomphonema productum</i>	G	1	1	0	0	0
50. <i>Gomphonema rhombicum</i>	G	0	0	1	0	0
51. <i>Hannea arcus</i>	V	0	1	1	0	1
52. <i>Hippodonta coxiae</i>	G	1	0	0	0	0
53. <i>Karayevia laterostrata</i>	3	1	0	0	0	0
54. <i>Kobayasiella parasubtilissima</i>	V	0	0	0	0	1
55. <i>Navicula concentrica</i>	2	1	0	0	0	0
56. <i>Navicula splendidula</i>	G	0	0	1	0	0
57. <i>Neidium affine</i>	V	1	0	0	0	1
58. <i>Neidium ampliatum</i>	V	1	0	0	0	1
59. <i>Neidium bisulcatum</i>	3	0	0	0	0	1
60. <i>Neidium bisulcatum</i> var. <i>subampliatum</i>	3	0	0	0	0	1
61. <i>Neidium longiceps</i>	G	1	0	0	0	1
62. <i>Nitzschia acidoclinata</i>	V	0	1	0	0	1
63. <i>Pinnularia microstauron</i>	V	1	0	0	1	1
64. <i>Pinnularia neomajor</i>	G	1	1	1	1	1
65. <i>Pinnularia rupestris</i>	G	0	1	0	0	1
66. <i>Pinnularia schoenfelderi</i>	G	0	0	0	0	1
67. <i>Pinnularia stomatophora</i>	G	0	0	0	0	1
68. <i>Pinnularia subrupestris</i>	G	0	1	0	0	1
69. <i>Psammothidium rechtense</i>	2	1	0	0	0	0
70. <i>Psammothidium subatomoides</i>	V	0	1	0	1	1
71. <i>Sellaphora pseudopupula</i>	G	0	0	0	0	1
72. <i>Stauroneis anceps</i>	V	1	0	0	0	0
73. <i>Stauroneis gracilis</i>	V	0	0	1	1	0
74. <i>Stauroneis phoenicenteron</i>	V	1	0	0	0	0
75. <i>Stauroneis separanda</i>	V	1	0	0	0	0
76. <i>Surirella roba</i>	G	1	0	0	0	1
77. <i>Surirella spiralis</i>	V	1	1	1	0	1
78. <i>Tabellaria ventricosa</i>	3	0	0	1	1	1
79. <i>Tetracyclus rupestris</i>	G	1	1	1	0	0
TOTAL NUMBER OF RARE AND ENDANGERED TAXA	-	34	19	16	16	48

Very rare and endangered species identified in freshwater oligotrophic habitat types were: *Achnanthydium kranzii*, *Brachysira brebissonii*, *Brachysira intermedia*, *Caloneis tenuis*, *Cymbopleura austriaca*, *Decussiphycus hexagonus*, *Diploneis ovalis*, *Encyonema perpusillum*, *Encyonema vulgare*, *Eunotia arcubus*, *Eunotia nymanniana*, *Eunotia praerupta*, *Eunotia subherkiniensis*, *Eunotia tetraedon*, *Eunotia triodon*, *Karayevia laterostrata*, *Navicula concentrica*, *Neidium bisulcatum*, *Neidium bisulcatum* var. *subampliatum* *Psammothidium rechtense* and *Tabellaria ventricosa*.

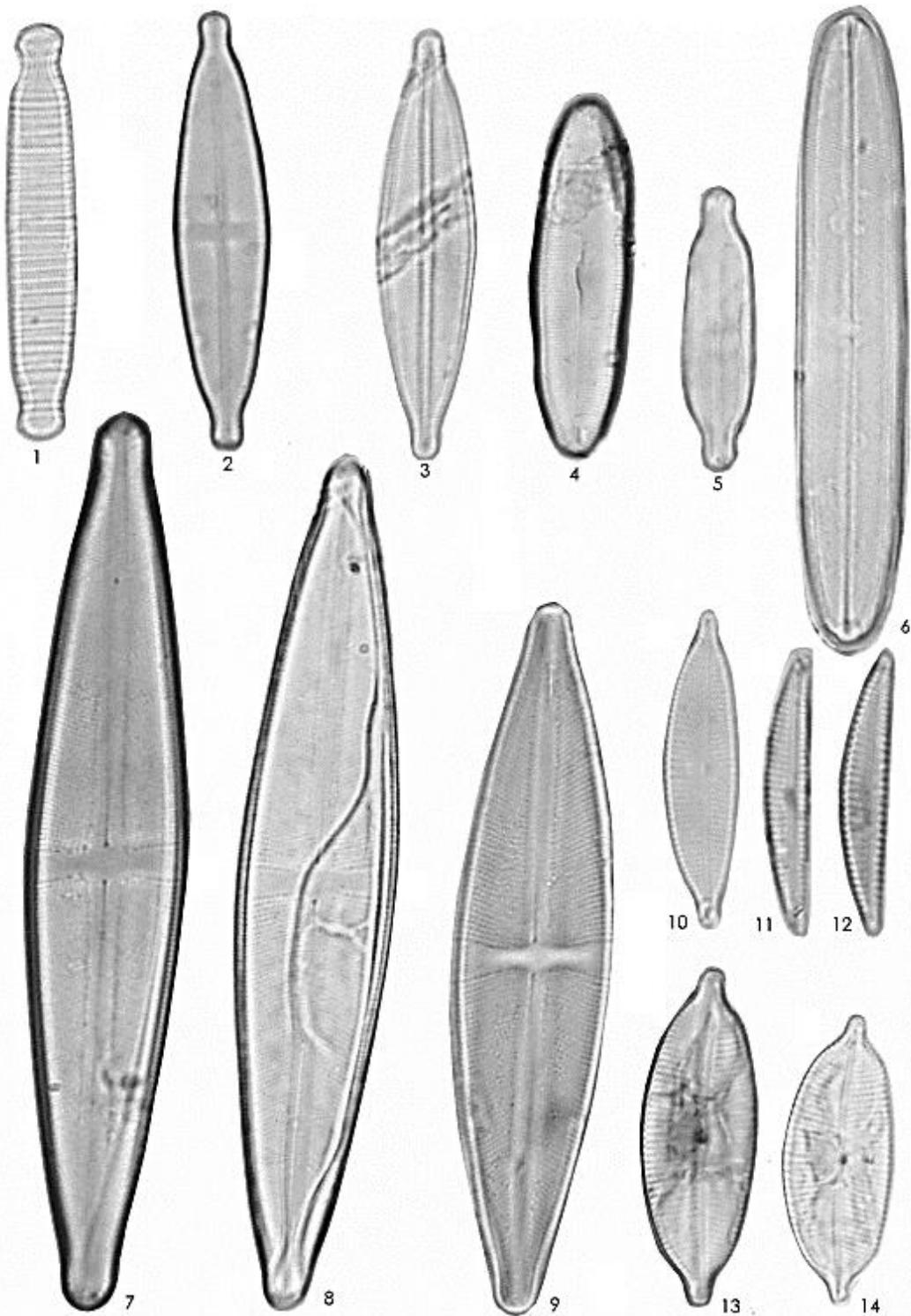


Plate II. Selected diatom taxa (1000x): **1.** *Diatoma ehrenbergii* Kützing, **2.** *Stauroneis anceps* Ehrenberg, **3.** *Frustulia crassinervia* (Bréb. ex W.Smith) Lange-Bertalot & Krammer, **4.** *Caloneis silicula* (Ehrenberg) Cleve, **5.** *Neidium longiceps* (W.Gregory) R. Ross, **6.** *Neidium bisulcatum* (Lagerstedt) Cleve, **7-8.** *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg, **9.** *Stauroneis gracilis* Ehrenberg, **10.** *Cymbopleura naviculiformis* (Auerswald ex Heiberg) Krammer, **11-12.** *Encyonema neogracile* Krammer, **13-14.** *Cymbopleura cuspidata* (Kützing) Krammer.

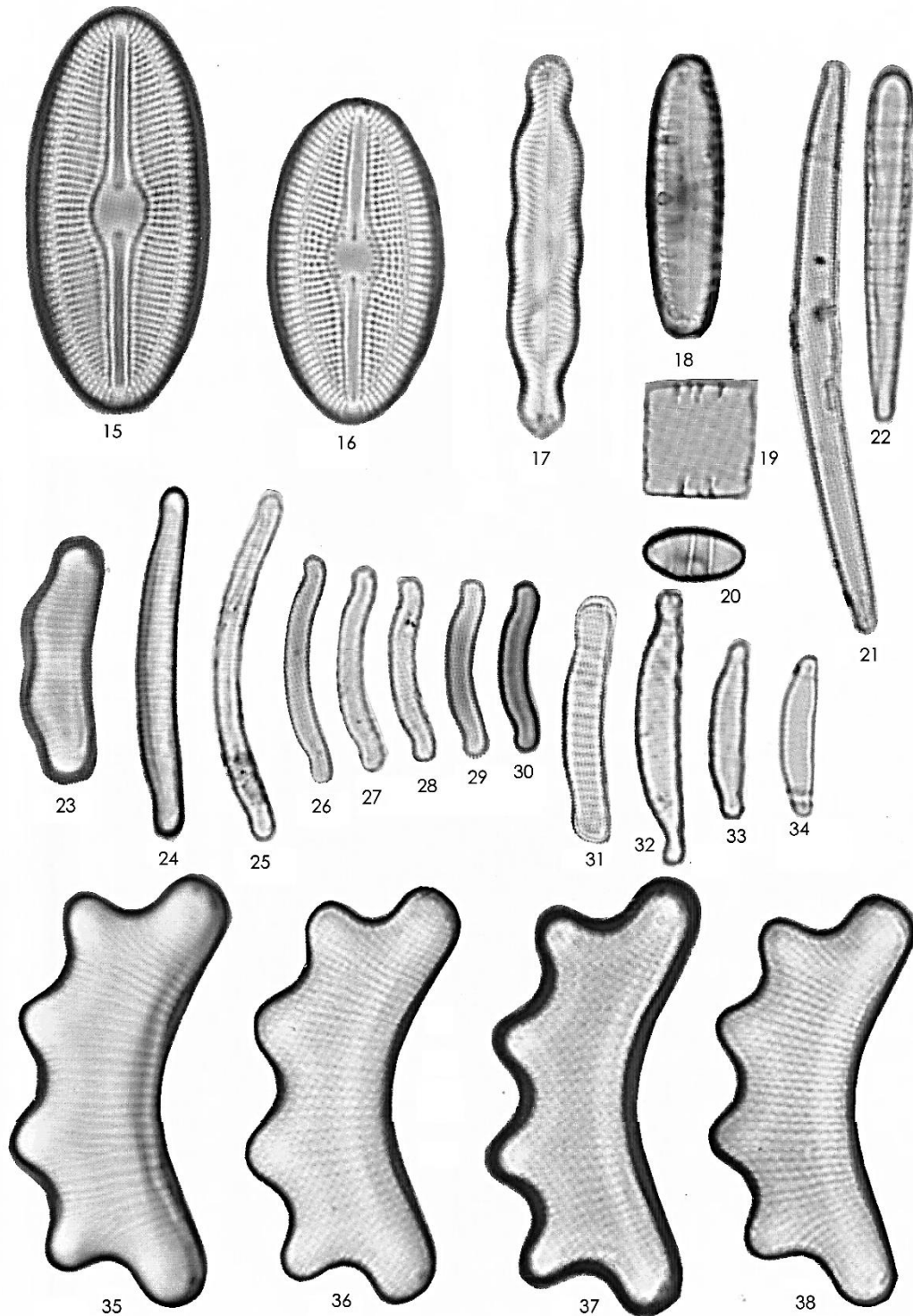


Plate III. Selected diatom taxa (1000x): **15-16.** *Diploneis krammeri* Lange-Bertalot & E. Reichardt, **17.** *Pinnularia grunowii* Krammer, **18.** *Pinnularia borealis* Ehrenberg, **19-20.** *Odontidium mesodon* (Kützing) Kützing, **21.** *Hannaea arcus* (Ehrenberg) R.M.Patrick in R.M.Patrick & C.W.Reimer, **22.** *Meridion circulare* (Greville) C.Agardh, **23.** *Eunotia bigibba* Kützing, **24.** *Eunotia valida* Hustedt, **25.** *Eunotia mucophila* Lange-Bertalot in Metzeltin & Lange-Bertalot, **26-30.** *Eunotia nymanniana* Grunow in Van Heurck, **31.** *Eunotia arcus* Ehrenberg, **32.** *Eunotia boreoalpina* Lange-Bertalot & Nörpel-Schempp **33-34.** *Eunotia incisa* W.Smith ex W.Gregory, **35-38.** *Eunotia tetraodon* Ehrenberg.

H. Results of indicator values of diatoms

Indicator values were used (Van Dam et al., 1994) for the purpose of understanding the complex environmental conditions. Comparative overview of results of the indicator value of diatoms determined in freshwater oligotrophic habitats on Vranica Mountain is shown in table (Table 8).

Table 8. Comparative overview of results of indicator value of diatoms determined in freshwater oligotrophic habitats on Vranica Mountain [M-moisture aerophile, N-nitrogen uptake, P-pH requirements, O-oxygen requirements, S1-salinity, S2-Saprobity and T-trophic state]

Habitat types	M	N	P	O	S1	S2	T
H1	2.66	1.53	3.33	1.66	1.78	1.74	3.69
H2	2.58	1.49	3.63	1.57	1.88	1.82	3.95
H3	2.38	1.63	3.61	1.88	1.87	1.95	4.05
H4	2.42	1.42	3.45	1.77	1.79	1.80	4.29
H5	2.92	1.25	2.88	1.40	1.57	1.42	2.90
Min	2.38	1.25	2.88	1.40	1.57	1.42	2.90
Max	2.92	1.63	3.63	1.88	1.88	1.95	4.29
Average	2.59	1.46	3.38	1.66	1.78	1.75	3.78

A comparison between the spectrum of indicator values of studied freshwater oligotrophic habitat types on Vranica Mountain reveals important differences, such as:

1. Values for humidity ranged from 2.42 (H4) to 2.92 (H5). The obtained results indicate that habitats H2, H3 and H4 inhabit diatom species which have optimum of their development in the water and that partially tolerate short periods of drying. Habitats H1 and H5 inhabit real aquatic taxa, but these habitats also inhabit species that tolerate longer drying periods, i. e. they can live in aerophile conditions.
2. Values for nitrogen uptake ranged from 1.25 (H5) to 1.63 (H3). The obtained results indicate that habitats H2, H4 and H5 inhabit species that are tolerant to the presence of nitrogen, while habitats H1 and H3 inhabit species that require nitrogen.
3. Values for pH requirements ranged from 2.88 (H5) to 3.63 (H2). The obtained results indicate that habitat H5 (peatlands) is inhabited mostly by acidophilic species, while other habitats are inhabited mostly by neutrophilic species.
4. Values for oxygen requirements ranged from 1.4 (H5) to 1.88 (H3). Habitats with lower values are inhabited mostly by species which need more oxygen for their development, while habitats with higher values are inhabited mostly by species which need less oxygen for their development. In studied habitat types species, which need more oxygen for their development are abundant (75% to 100%).
5. Values for salinity ranged from 1.57 (H5) to 1.88 (H3). The obtained results indicate that freshwater oligotrophic habitat types are inhabited mostly by oligohalobous diatom taxa.
6. All studied habitats are inhabited diatom taxa which indicate oligosaprobous condition. Values for saprobity ranged from 1.42 (H5) to 1.95 (H3).
7. Values for trophic state ranged from 2.90 (H5) to 4.29 (H4). The obtained results indicate that the studied habitats are inhabited by diatom taxa which indicate oligo-mesotrophic or meso-eutrophic state. The lowest values for the trophic state are determined in mountain peatlands, while the highest values for the trophic state are determined in Prokoško Lake.

I. Differentiation of diatom assemblages in the studied habitat types

In order to differentiate the composition of diatom assemblages in relation to the gradient of the measured physical-chemical parameters, the method of PCoA analysis was used.

Results of PCoA differentiation of the analysed diatom assemblages are shown in Figure 15.

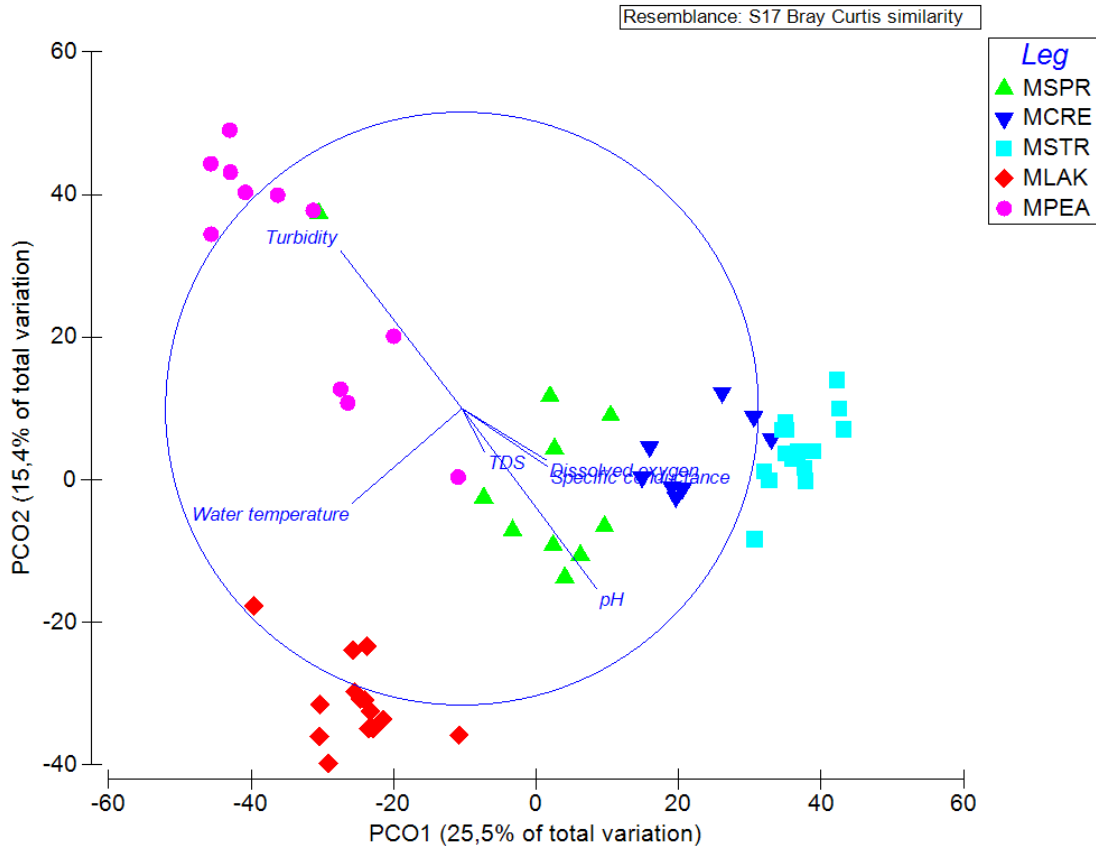


Figure 15. PCo differentiation of the analysed diatom assemblages based on Bray-Curtis similarity matrix with vector overlay showing measured physical-chemical parameters (Pearson's coefficient of correlation with PCoA axes)

The first PCoA axis explained 25.5% total variation, while the second PCoA axis explained 15.4% of the total variation. PCoA analysis clearly shows the differentiation of studied phytobenthos samples on three groups. The first and the largest group consists of samples collected from springs, creeks and streams, the second group consists of samples collected from the lake and the third group consists of samples collected from the peatlands.

It is interesting to note that on the left side of the figure are samples collected from standing water, while on the right side of the figure are samples collected from running water. Within the peatlands, the maximum turbidity of water and the lowest pH value were measured. The highest temperature of the water was measured in Prokoško Lake, while the other physical and chemical parameters had significantly lower values. High values of dissolved oxygen and specific electric conductivity were measured in springs, creeks and streams.

CONCLUSIONS

Based on the conducted study, following remarks and conclusions can be derived:

1. Research for this study was carried out in the wider area of Vranica Mountain. In all studied sites, the physical condition of habitat and physical and chemical parameters of water were measured. Also, algae of phytobenthos were sampled.
2. In addition to the basic physical and chemical parameters, water for selected heavy metals was taken and measured in laboratory condition. Basic physical and chemical parameters in studied sites ranged respectively, and correspond with literature data. The highest value for water temperature was measured in Prokoško lake, and the lowest pH values were measured within mountain peatlands.
3. The highest values of metals were measured in mountain peatlands, while the lowest values were measured within mountain springs. In all studied freshwater oligotrophic habitat types the following metals were identified: potassium (K), Ca (Calcium), Mg (Magnesium), Cu (Copper) and Pb (Lead). From a total of 12 analysed metals, copper, nickel and lead had extremely low values, while cadmium was not detected in the studied habitat types.
4. Diatomaceous indices (IPS, SLA, TID and SID) have confirmed the good ecological state of mounting springs, creeks, streams and peatlands, while the moderate ecological state is confirmed for Prokoško Lake. In order to conclude about the real ecological state of freshwater oligotrophic habitat types on Vranica Mountain, the establishment of long-term monitoring is necessary. This kind of monitoring would be also good for the conservation of biological diversity within these target habitat types. The conducted study represents a good basis for future monitoring of ecological state, but also for monitoring specific ecological phenomena which are in direct connection with water ecosystems (e.g. eutrophication, acidification etc).
5. The highest number of taxa was identified within the class Bacillariophyceae, Chlorophyceae, Conjugatophyceae, while taxa of other classes occurred with a smaller numerical value. The largest number of taxa was found in Prokoško Lake (121 species or 27.10%), while the smallest number of taxa was recorded in the stream Jezernica (61 species or 13.62%). In all studied habitat types, algae from class Bacillariophyceae were the most dominant. The largest number of species was found in springs (82 species or 24.70%), while the smallest number of species was found in Prokoško Lake (52 species or 15.66%).
6. A total of 174 taxa belonging to 57 genera were determined in the material. Genera with the highest number of species were *Eunotia* (22), *Gomphonema* (14), *Pinnularia* (12), *Encyonema* (7), *Stauroneis* (6), *Cocconeis* (5), *Cymbopleura* (5), *Navicula* (5), *Neidium* (5) and *Nitzschia* (5). Altogether 169 diatom taxa mostly belonging to pennate diatoms were found and five centric diatoms. The most common centric diatoms were *Aulacoseira crenulata*, *Orthoseira roeseana*, *Aulacoseira alpigena*, *Ellerbeckia arenaria* and *Melosira varians*.

7. The largest number of rare and endangered diatom taxa was determined in mountain peatlands (48 taxa), while the smallest number was determined in mountain creeks (16 taxa) and mountain lake (16 taxa).
8. Ecological values of diatom taxa have confirmed the oligotrophic state of mountain spring, creeks, streams and peatlands, while the ecological state of Prokoško Lake has been changed. Unfortunately, due to entering of organic matter, but also because of the process of natural eutrophication there was overgrowing of coastal part of the lake with macrophytes vegetation. As a result of this overgrowing, total surface of Prokoško Lake is decreasing. If this trend continues, the faith of Prokoško Lake is similar to neighbouring Suho Lake.
9. With PCoA analysis the most important variables for differentiation between studied freshwater habitats types are identified. Prokoško Lake is characterized by high water temperature, peatlands are characterized by the lowest pH and highest turbidity of water, while mountain springs, creeks and streams are characterized by the highest values of remaining parameters of water.
10. Based on the conducted research it can be concluded that the freshwater oligotrophic habitats on Vranica Mountain are in good ecological state and that they represent a "hot spot of biodiversity". We cannot forget that the studied freshwater habitats are under strong anthropogenic influences. From that point of view, it is important to notice that Prokoško Lake and mountain peatlands which cover only a small part of Vranica Mountain are the most fragile and the most sensitive habitat types in this area. It is very important to notice that other habitat types should not be excluded. Because of the very unique diversity in these habitat types, in the future, it is necessary to pay special attention, especially in regard to their restoration and enhancement of protection measures.
11. Through this study, an extensive work on inventorisation of cyanobacteria and algae was done.
12. Future planned activities which are related directly to this project would be connected with the establishment of long-term monitoring of biodiversity within freshwater oligotrophic habitats on the wide area of Vranica Mountain. In order to conduct this monitoring, 20 representative sites have been selected. During the next period of time, abiotic and biotic parameters will be monitored during vegetation season at least once a month. In addition to cyanobacteria and algae as bioindicator organisms, diversity of aquatic macrophytes will be also taken into account. As a basis for this monitoring, EDGG methodology¹ of phytocoenological relevés will be taken into account as well. Current and future results will serve as a good basis for establishing a long-term monitoring of these very sensitive habitat types and for their better protection.

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¹ Dengler, Jürgen, Steffen Boch, Goffredo Filibeck, Alessandro Chiarucci, Iwona Dembiczyk, Riccardo Guarino, B. Henneberg et al. "Assessing plant diversity and composition in grasslands across spatial scales: the standardized EDGG sampling methodology." *Bulletin of the Eurasian Dry Grassland Group* 32 (2016): 13-33.

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SPECIAL PART

CLASSIFICATION

Empire: Eukaryota

Kingdom: Chromista

Phylum: Bacillariophyta

Subphylum: Coscinodiscophytina (Centric diatoms)

Class: *Coscinodiscophyceae*

Order: Aulacoseirales

Family: *Aulacoseiraceae*

Genus: *Aulacoseira*

Order: Melosirales

Family: *Melosiraceae*

Genus: *Melosira*

Family: *Orthoseiraceae*

Genus: *Orthoseira*

Order: Paraliales

Family: *Radialiplicataceae*

Genus: *Ellerbeckia*

Subphylum: Bacillariophytina (Araphid diatoms)

Class: *Bacillariophyceae*

Subclass: *Eunotiophycidae*

Order: Eunotiales

Family: *Eunotiaceae*

Genus: *Eunotia*

Subclass: *Fragilariophycidae*

Order: Fragilariales

Family: *Staurosiraceae*

Genus: Pseudostaurosira

Genus: Staurosira

Genus: Staurosirella

Family: *Fragilariaceae*

Genus: Fragilaria

Genus: Odontidium

Order: Licmophorales

Family: *Ulnariaceae*

Genus: Ulnaria

Genus: Hannaea

Order: Rhabdonematales

Family: *Tabellariaceae*

Genus: Meridion

Genus: Tabellaria

Genus: Tetracyclus

Genus: Diatoma

Subphylum: Bacillariophytina (Raphide diatoms)

Class: *Bacillariophyceae*

Order: Achnanthes

Family: *Achnanthes*

Genus: Achnanthes

Genus: Karayevia

Genus: Planothidium

Genus: Psammothidium

Family: Cocconeidaceae

Genus: Cocconeis

Order: Bacillariales

Family: Bacillariaceae

Genus: Denticula

Genus: Hantzschia

Genus: Nitzschia

Order: Cymbellales

Family: Anomoeoneidaceae

Genus: Adlafia

Family: Cymbellaceae

Genus: Paraplaconeis

Genus: Cymbella

Genus: Cymbopleura

Genus: Encyonopsis

Family: Gomphonemataceae

Genus: Placoneis

Genus: Encyonema

Genus: Reimeria

Genus: Gomphonema

Order: Naviculales

Family: Cavinulaceae

Genus: Cavinula

Family: Amphipleuraceae

Genus: Amphipleura

Genus: Frustulia

Family: Brachysiraceae

Genus: Brachysira

Family: Naviculales incertae sedis

Genus: Chamaepinnularia

Genus: Kobayasiella

Family: Stauroneidaceae

Genus: Craticula

Family: Diadesmidaceae

Genus: Luticola

Genus: Humidophila

Family: Diploneidaceae

Genus: Diploneis

Family: Naviculaceae

Genus: Caloneis

Genus: Gyrosigma

Genus: Hippodonta

Genus: Navicula

Family: Neidiaceae

Genus: Neidium

Family: Pinnulariaceae

Genus: Pinnularia

Family: *Sellaphoraceae*

Genus: Sellaphora

Family: *Stauroneidaceae*

Genus: Stauroneis

Order: *Rhopalodiales*

Family: *Rhopalodiaceae*

Genus: Epithemia

Genus: Rhopalodia

Order: *Surirellales*

Family: *Surirellaceae*

Genus: Cymatopleura

Genus: Surirella

Order: *Thalassiophysales*

Family: *Catenulaceae*

Genus: Amphora

Order: *Mastogloiales*

Family: *Mastogloiaceae*

Genus: Decussiphycus

CENTRIC DIATOMS

Class: Coscinodiscophycineae

Order: Aulacoseirales

Family: Coscinodiscineae

Aulacoseira Thwaites, 1848

Holotype species: *Melosira crenulata* (Ehrenberg) Kützing

Cells linked tightly to form long straight, curved or even coiled filaments. Plastids discoid. A common freshwater, planktonic genus previously placed in *Melosira*. Valves circular. Valve face plain or with scattered poroids, which are often restricted to its periphery. Valve mantle deep, making a right angle to the planar valve face from which it is sharply differentiated; with vertical or curved rows of areolae. Mantle edge plain. Valve face/mantle junction provided with spines, which are expanded at their apices and fit with the spines of adjacent cells to form a linkage breakable only by damaging the spines. In some species the spines have two 'roots' that straddle a row of pores on the mantle, others have a single 'root' that runs between pore rows. Separation cells occur at intervals in *A. granulata*, the spines on these being lanceolate and of variable length; the areolae of separation valves are often in straight rows, those on other valves spiralling away from the sibling junction in opposite directions. Inside the valve mantle, at the junction between the plain mantle edge and the areolate portion, there is a thickening (ringleiste), which is hollow in *A. ambigua*. Mantle areolae simple, round to rectangular, containing volate occlusions. Small rimoportulae occur inward of the ringleiste and open through it to the outside. The copulae are split rings with ligulae; they are finely areolate, with an advalvar pars media. *Aulacoseira* is one of the most common freshwater diatom taxa, especially abundant in plankton of lakes and large rivers. One of the most successful species of freshwater centric diatoms, it is found in great abundance in fossil diatomites (Spaulding et al. 2022).

During research for this study two species from the genus *Aulacoseira* were identified. Detailed characteristics about the identified species from the genus *Aulacoseira*, distribution and ecological preferences are present further in this study.

1. *Aulacoseira alpigena* (Grunow) Krammer 1991

Dimensions: Diameter: 4-15 µm; Width range: 4-7 µm; Striae: 15-22/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5

Optimal environmental conditions: Oligotraphentic species.

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, acidophilic, polyoxybiontic, halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

2. *Aulacoseira crenulata* (Ehrenberg) Thwaites 1848

Dimensions: Length 5-32 µm; Width 8-20 µm.

Distribution in freshwater habitats on Vranica mountain H1, H2.

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G).

Class: Coscinodiscophyceae

Order: Aulacoseirales

Family: *Melosiraceae*

Melosira C.Agardh, 1824, nom. cons.

Holotype species: *Melosira nummuloides* C.Agardh

Cells cylindrical to subspherical, united in filaments by mucilage pads secreted onto their valve faces; in addition irregular spines may assist the linkage. Cells united distinctively into pairs or triplets by their cingula. Plastids lobed, small plate-like, lying in the peripheral cytoplasm. A common genus in freshwater (*M. varians*) and marine epibenthic habitats (*M. nummuloides*, *M. moniliformis*). Valve face flat or domed, covered with small spines or granules; a more or less well-developed corona consisting of larger irregular spines is sometimes developed. This may be surrounded by a carina (a flat collar-like structure) as in *M. nummuloides*. Valve mantle not readily distinguishable from valve face in most species. Valve mantle edge having a milled appearance. The valve structure is loculate, the loculus being open to the outside via a number of small simple pores and to the inner surface by somewhat larger pores, which may be partially or completely bridged by silica struts (in the latter instance forming rotae). The pattern of these inner pores is independent of the loculi (cf. *Stephanopyxiz*). The loculi may be randomly arranged or lie in rows radiating from the centre of the valve. Rimoportulae occur usually in a ring near the mantle edge and sometimes scattered or grouped on the valve; there is a circular external aperture surrounded by an irregular rim. The internal apertures are in the form of elongate slits also surrounded by an irregular, low rim. Copulae split, ligulate, with regular longitudinal rows of small pores; pars media distinct and near advalvar edge. The two valves are closely associated during the greater part of the cell cycle and the cingula of adjacent daughter cells overlap one another considerably.

In fresh material the cells are frequently connected by their valve surfaces to form filaments. Due to the deep mantle, frustules are usually encountered in girdle view.

During research for this study one species from the genus *Melosira* were identified. Detailed characteristics about the identified species from the genus *Melosira*, distribution and ecological preferences are present further in this study.

1. *Melosira varians* C. Agardh 1827

Dimensions: Diameter 8-35 µm, mantle height 8-17.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Moderately electrolyte-rich running waters with higher trophic levels. Widely distributed in the benthos of smaller and larger running waters, also in springs with high discharge. It is less common in standing freshwaters. Long fine filaments, visible to the naked eye, can develop in shallow, slow-flowing parts of enriched streams, particularly during summer (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is Occasionally aerophilic, facultative N-heterotrophic, alkaliphilic, moderate O₂ (>50% sat.), oligohalobous, α-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Coscinodictophyceae

Order: Aulacoseirales

Family: Orthoseiraceae

Orthoseira Thwaites, 1848

Holotype species: *Melosira americana* Kützing

Cells cylindrical, united to form short filaments with valve faces closely appressed. Interlocking spines can be seen between cells and a characteristic thickening of the girdle bands can be seen in mid-focus. The plastids are numerous, small and discoid. They are peripheral while the nucleus is suspended centrally on cytoplasmic bridges through the vacuole. A small genus of sub-aerial diatoms commonly found among bryophytes especially in alkaline areas. Valve views of the acid-cleaned valves show 2-5 characteristic tube processes at the centre of the face. Rows of areolae radiate from near the centre and pass over the valve rim and down the mantle. At the rim in most species there are well-defined spines. These are simple and triangular in side view or pyramidal with stellate bases and are found between rows of areolae.

In *O. densophila* the ring of spines is interrupted by clusters of distinctive areolae. In intact sibling valves these clusters of pores are precisely aligned with each other. The valve is a simple laminate layer of silica. While the areolae are covered on the inner surface by a velum or some other form of siliceous layer the pores in the clusters are left unoccluded. Though it is improbable that the marginal spines serve to link neighbouring cells, they would prevent torsion or rotation of siblings vis-à-vis one another. Cells are probably bound together by mucilage secretions passed through the tube process. These are unique fluted passages through the valve with simple internal openings and well-defined collars to the outside, described as 'carinoportulae' by Crawford (1979a).

The cingulum is composed of a number of bands whose pars interior is greatly thickened. In *O. dendroteres* the bands are so angled as to give the effect of a spiral arrangement. The bands are sufficiently robust to leave a step on the mantle of one of the sibling valves similar to that observed in *Ellerbeckia*. The frustules of *Orthoseira* are cylindrical and form filamentous colonies linked by marginal spines. The valve surface is flat and possesses one or more unique process, the carinoportula. Internally, the valves

may (or may not) have internal 'caverns', positioned at the valve mantle interface. The marginal spines, if present, are variable in length. *Orthoseira* typically grows in subaerial habitats, often with bryophytes, in soils, and on the bark of tropical trees. The appearance of this genus in lakes and streams is considered incidental. *Orthoseira* is found from tropical to polar regions.

During research for this study one species from the genus *Orthoseira* were identified. Detailed characteristics about the identified species from the genus *Orthoseira*, distribution and ecological preferences are present further in this study.

1. *Orthoseira roeseana* (Rabenhorst) Pfitzer 1871

Dimensions: Diameter 8-70 μm , mantle height 6-13 μm . Rows of areolae: 14-20 in 10 μm .

Distribution in freshwater habitats on Vranica mountain: H1, H 3.

Optimal environmental conditions: Aerial habitat (wet rocks, bryophyte, wet tree bark, riparian zone of stream and rivers). More frequent in mountains regions than in lowlands (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is terrestrial, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous and oligosaprobe.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

Class: Coscinodiphyceae

Order: Aulacoseirales

Family: Radialiplicataceae

Ellerbeckia R.M.Crawford, 1988

Holotype species: *Melosira arenaria* D.Moore ex Ralfs

Cells large, shortly cylindrical, linked to form curved chains of up to 30 or more cells. Plastids numerous, small discoid. Mainly in freshwater, with both fossil and recent forms known. Sometimes visible to the naked eye! Valves robust, circular, with radial markings. Valve face and mantles sharply differentiated. Sibling valves different and complementary, the 'cameo' valve bearing a system of ridges and the 'intaglio' valve an equivalent system of grooves to accommodate them. Intaglio valves have a plain mantle, cameo valves a stepped mantle, as a result of the formation of the cameo valves beneath the edge of the parental hypotheca (Crawford, 1981b).

The valves also have different curvatures and when the concavo-convexity is as pronounced as in *E. arenaria* forma *teres*. this results in cells of unequal perivalvar dimensions; large cells have two (convex) intaglio valves, small cells have two (concave) cameo valves, and intermediate cells have one of each. Valve face without pores except for a peripheral ring in some species. Valve mantle very thick, consisting of a massive inner layer penetrated by long tubes, which connect the cell lumen with a series of shallow chambers; these run the length of the mantle and open to the outside via a finely porous layer of silica. A unique type of tubular process is present on the mantle;

these are easily seen in LM in the forma teres. Each process takes the place of a mantle tube and opens to the inside by a small pore at the top of a dome-like projection. The valvocopula is closed but the other copulae are open. All have rows of small round pores. Valvocopula not easily detached from its valve, bearing a series of crenulations which interdigitate with projections from the edge of the valve mantle.

During research for this study one species from the genus *Ellerbeckia* were identified. Detailed characteristics about the identified species from the genus *Ellerbeckia*, distribution and ecological preferences are present further in this study.

1. *Ellerbeckia arenaria* (D.Moore ex Ralfs) Dorofeyuk & Kulikovskiy 2012

Dimensions: Diameter 37-95 µm, mantle height 10-15 µm. Rows of areolae: 20-22 in 10 µm. Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: Subaerial habitats. Sandy sediments of oligotrophic freshwater habitats. It can develop macroscopic growth in low-altitude, calcium-bicarbonate-rich spring. *E. arenaria* is the only species found in central Europe (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

PENNATE DIATOMS

Class: *Bacillariophyceae* (Araphid diatoms)

Order: Eunotiales

Family: *Eunotiaceae*

Eunotia Ehrenberg, 1837

Lectotype species: *Eunotia arcus* Ehrenberg

Members of the *Eunotiaceae* (*Eunotia*, *Actinella*, *Amphicampa*) are unusual among the raphid diatoms in that frustules have a very short raphe system. The terminal nodules are positioned on the mantle. From the terminal nodule, the raphe slit lies on the valve mantle then slightly, or strongly, curves onto the valve face. As a result of this morphology, raphe branches visible in girdle view. Valves of *Eunotia* are asymmetric to the apical axis. The dorsal margin is convex, smooth, or undulate. The ventral margin is straight or concave. Uniseriate striae extend across the valve face. Areolae of the striae generally lack hymenes and other occlusions. Usually one rimoportula present at an apex of each valve, although occasionally there may be two rimoportulae, or they may be absent. Cells occur singly, free, or attached by mucilaginous stalks, or in long ribbon-like colonies. Species within *Eunotia* are widespread and diverse in acidic and dystrophic habitats (Spaulding et al. 2022).

During research for this study 22 species from the genus *Eunotia* were identified. Comparing results from this study with publications by various authors who have explored algae in Bosnia and Herzegovina, for four species there is not any data. Newly recorded species are: *Eunotia curtagrünowii*, *Eunotia implicata*, *Eunotia mucophila* and *Eunotia subherkiniensis*. The new species were found in reocren spring, stream and peatlands. Detailed characteristics about the identified species from the genus *Eunotia*, distribution and ecological preferences are present further in this study.

1. *Eunotia arcubus* Nörpel et Lange-Bertalot

Dimensions: Length 14-95 µm; Width 4-9 µm. Striae: 8-12/10 µm. Puncta: 30-32/10 µm.

Distribution in freshwater habitats on Vranica mountain: Mountain lake.

Optimal environmental conditions: Occurring in carbonate-rich, oligo- to mesotrophic lakes and springs, very rare in running waters. Studies on the occurrence of *E. arcubus* in springs (Cantonati et al. 2012) and lakes on carbonate substratum suggest that *E. arcubus* prefers low light and very low flow conditions (Cantonati et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, halophobe, oligosaprobe and oligo-mesotrophic species.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

2. *Eunotia arcus* Ehrenberg

Dimensions: Length 19-115 µm; Width 6-11 µm. Striae: 11-14/10 µm. Puncta: 30-36/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Abundant in undisturbed oligo- to dystrophic habitats (Cantonati et al. 2017).

General distribution and ecology: Rather rare, in contrast to northern and eastern Europe and the siliceous Alps (Cantonati et al. 2017). According to Van Dam et al. 1994, this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, halophobe, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

3. *Eunotia bilunaris* (Ehrenberg) Schaarschmidt

Dimensions: Length 14-105 µm; Width 3,5-5,5 µm. Striae: 13-17/10 µm. Puncta: 40-45/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H3, H5.

Optimal environmental conditions: Oligo- to dystrophic, but oligosaprobic freshwater habitats (Cantonati et al. 2017).

General distribution and ecology: Distributed in wide range of conditions, from acidic (due to humic acids) to well-buffered alkaline waters (Cantonati et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, indifferent (euryionic), oxybiontic (75% sat.), oligohalobous, β-mesosaprobe and indifferent.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Eunotia boreoalpina* Lange-Bertalot et Nörpel-Schempp in Lange-Bertalot & Metzeltin

Dimensions: Length 12-15 µm; Width 4-6 µm. Striae: 13-17/10 µm. Puncta: 45-50/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: An indicator of low-alkalinity seepage and pool springs on siliceous substrata (Cantonati et al. 2012 in Cantonati et al. 2017).

General distribution and ecology: Scattered but locally abundant in mountains siliceous substrata (Cantonati et al. 2012). According to Van Dam et al. (1994), this species is acidophilic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

5. *Eunotia curtagrunowii* Nörpel-Schempp & Lange-Bertalot in Lange-Bertalot & Metzeltin

Dimensions: Length 13-45 µm; Width 6-8,5 µm. Striae: 9-14/10 µm. Puncta: 27-32 (35)/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

6. *Eunotia exigua* (Brébisson) Rabenhorst

Dimensions: Length 6-30 (50) µm; Width 3-4 µm. Striae: 19-14/10 µm. Puncta: 45-50/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Wide ecological range from freshwater environments acidified by inorganic acid to minerotrophic fens in humid acids, spring, and mountain streams at pH values from 2 to 7. It can reach important relative abundances in the

presence of high concentration of both naturally-occurring and anthropogenic sulphates (Alles et al. 1991). Desiccation tolerant (Cantonati et al. 2017).

General distribution and ecology: Very frequent and often abundant everywhere (Cantonati et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, acidobiontic, oxybiontic (75% sat.), oligohalophobous, a-mesosaprobe and indifferent.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

7. *Eunotia glacialis* Lange-Bertalot in Krammer & Lange-Bertalot

Dimensions: Length 40-250 µm; Width 4,5-7 µm. Striae: 8,5-10/10 µm. Puncta: 25-28/10 µm. Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Electrolyte-poor acidic, as well bicarbonate-buffered alkaline, oligotrophic usually standing freshwaters (Lange-Bertalot et al. 2017).

General distribution and ecology: Scattered to relatively frequent in central Europe, never really abundant (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

8. *Eunotia glacialis* Meister

Dimensions: Length 60-160 µm; Width 7-12 µm. Striae: 10-12/10 µm. Puncta: 28-30/10 µm. Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Electrolyte-poor, weakly acid, undisturbed habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: Rare in mountains (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

9. *Eunotia implicata* Nörpel-Schempp, Alles et Lange-Bertalot

Dimensions: Length 18-48 µm; Width 3-6 µm. Striae: 14-20/10 µm. Puncta: 35-40/10 µm. Distribution in freshwater habitats on Vranica mountain: H3, H5.

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive, acidophilic and halophobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

10. *Eunotia incisa* Gregory

Dimensions: Length 10-56 µm; Width 3-7 µm. Striae: 16-22/10 µm. Puncta: 40-45/10 µm. Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Electrolyte-poor, oligo- to dystrophic freshwater habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: One of the most frequent *Eunotia* – species, often very abundant in suitable habitats (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic, halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

11. *Eunotia minor* (Kützing) Grunow in Van Heurck

Dimensions: Length 16-62 (73) μm ; Width 4-8 μm . Striae: 9-16/10 μm . Puncta: (35) 40-45/10 μm .

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Wide ecological amplitude, from electrolyte-poor, dystrophic mire habitats to circumneutral springs and streams on siliceous bedrock or on sandy soils, from high mountains to lowlands (Lange-Bertalot et al. 2017).

General distribution and ecology: One of the most frequent *Eunotia* species, often found with high mountains to the lowlands (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aerophilic, acidophilic, halophobe and oligosaprobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

12. *Eunotia mucophila* (Lange-Bertalot et Nörpel) Lange-Bertalot in Metzletin, Lange-Bertalot & García-Rodríguez)

Dimensions: Length 15-70 μm ; Width 2-3 μm . Striae: 20-28/10 μm . Puncta: 40-45/10 μm .

Distribution in freshwater habitats on Vranica mountain: H5.

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, tolerant N-autotrophic, acidophilic, oxybiontic (75% sat.), oligohalobous, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

13. *Eunotia nymanniana* Grunow in Van Heurck

Dimensions: Length 15-55 μm ; Width 2,5-3,4 μm . Striae: 17-21/10 μm . Puncta: 40-45/10 μm .

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Occuring with other small-cell *Eunotia* species in undisturbed and slightly disturbed, electrolyte-poor, weakly acid (not ombrotrophic) habitats on siliceous bedrock (Lange-Bertalot et al. 2017).

General distribution and ecology: Possible under recorded in the past as it was often confused with *E. exigua* and *E. tenella*. Widely distributed and locally abundant (Cantonati et al. 2017). According to Van Dam et al. (1994), According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

14. *Eunotia paludosa* Grunow

Dimensions: Length 6-45 μm ; Width 1,8-3,5 μm . Striae: 18-25/10 μm . Puncta: c. 40/10 μm .

Distribution in freshwater habitats on Vranica mountain: H1, H4, H5.

Optimal environmental conditions: Ombrotrophic raised-bog habitats. Can be very abundant in such habitats. Sporadic in minerotrophic biotopes with *Sphagnum* where it is associated with different dystrophentic *Eunotia* species (e.g. *E. bilunaris*). Strongly associated with the presence of *Sphagnum* spp. (Lange-Bertalot et al. 2017).

General distribution and ecology: Scattered records, due to the loss of suitable habitats (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is neutrophilic, halophilic and oligosaprobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

15. *Eunotia praerupta* Ehrenberg

Dimensions: Length 28-105 µm; Width 10-18 µm. Striae: 5.5-8 (12) /10 µm. Puncta: 27-32/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: Mainly in mountains, never abundant, in undisturbed, oligo-dystrophic, electrolyte-poor habitats on siliceous bedrock (Lange-Bertalot et al. 2017).

General distribution and ecology: Rather rare (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligo-mesotrophic. Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

16. *Eunotia rhomboidea* Husted

Dimensions: Length 10-35 µm; Width 2,5-5 µm. Striae: 13-19/10 µm. Puncta: 40-45/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H3, H4, H5.

Optimal environmental conditions: Acidic minerotrophic fens, electrolyte-poor heathlands-pool, springs and streams and siliceous bedrock (Lange-Bertalot et al. 2017).

General distribution and ecology: Regionally very frequent, and often abundant (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

17. *Eunotia soleirolii* (Kützing) Rabenhorst

Dimensions: Length 15-135 µm; Width 5-8 µm. Striae: 7-12 (16)/10 µm. Puncta: 24-25/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Undisturbed or slightly disturbed, oligo- to weakly eutrophic, not only electrolyte-poor, but usually weakly acidic habitats, for instance minerotrophic fens. Often associated with *E. pectinalis* (Lange-Bertalot et al. 2017).

General distribution and ecology: Frequent and occasionally abundant (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, β-mesosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

18. *Eunotia subherkiniensis* Lange-Bertalot in Lange-Bertalot, Bak & Witkowski

Dimensions: Length 10-30 µm; Width 5-8 µm. Striae: 12-15/10 µm. Puncta: c. 40/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: Undisturbed, electrolyte-poor streams and lakes that are acidic due to the presence of humic acids (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic, halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

19. *Eunotia tenella* (Grunow) Hustedt in A. Schmidt et al.

Dimensions: Length 7-28 µm; Width 3-4,5 µm. Striae: 14-16/10 µm. Puncta: 45-50/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3, H5.

Optimal environmental conditions: Undisturbed, electrolyte-poor, oligo- to dystrophic habitats. Characteristic species of low-alkalinity seepage and pool springs on siliceous substrata (Lange-Bertalot et al. 2017).

General distribution and ecology: Frequent (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

20. *Eunotia tetraodon* Ehrenberg

Dimensions: Length 24-70 µm; Width 9-24 µm. Striae: 6-12/10 µm. Puncta: 24-35/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H5.

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

21. *Eunotia triodon* Ehrenberg

Dimensions: Length 25-100 µm; Width 13-22 µm. Striae: 15-18/10 µm. Puncta: 21-28/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is acidophilic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

22. *Eunotia valida* Hustedt

Dimensions: Length 20-115 µm; Width 4-6 µm. Striae: 11-15/10 µm. Puncta: 30-34/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Electrolyte-poor, oligo- to dystrophic freshwater habitats, rarer also in calcium-bicarbonate-buffered but invariably oligosaprobic inland waters (Lange-Bertalot et al. 2017).

General distribution and ecology: Rather frequent, locally moderately abundant (Lange-Bertalot et al. 2017). According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

Class: Bacillariophyceae

Order: Fragilariales

Family: *Staurosiraceae*

Pseudostaurosira D.M.Williams & Round, 1988

Holotype species: *Fragilaria brevistriata* Grunow

Frustules symmetrical in side view, forming chains in taxa that possess spines. Taxa with incipient or no spines, presumably attached by mucilage stalks. Valves cruciform, bigibbous, lanceolate, rhombic or elliptic. Sternum of variable width and shape. Transition from valve face to mantle varies from abrupt to more gradual, with formation of a transition zone. Striae composed of one to (rarely) a few rows of wide, round, transapically elliptical, or irregularly polygonal areolae running from valve face to mantle. Internally, areolae open into a single depression running from valve face to mantle, which may become chamber-like. Volae highly branched, usually dichotomously, arising from the inner perimeter of the areolae and at different depths. Virgae rectangular or flared at their proximal and distal ends. Viminis short and wide, the one at the valve face-mantle transition usually being wider, giving the impression of a single apical rib running along the valve face margin. Viminules seldom produced and, when formed, occupying only a part of a stria. Flaps or flat siliceous growths originate from several points around the external perimeter of the areolae, close to their external surface. Concentric discs sometimes present, partially occluding the depression into which the areolae open internally. Solid spines can grow from the enlarged vimen that connects the virgae along the valve-face edge or from the virgae themselves, or they can be absent. Spines of varying shape, but usually spatulate, with or without terminal branching, with a cylindrical base, and often bearing stipulae. Stipules produced near the base of spines and projecting downward. Apical pore fields absent, reduced or more fully developed, of the ocellulimbus type; in many cases they are sunken into the apical portion of the valve. They are composed of round poroids, which are sometimes arranged along external troughs parallel to the apical axis of the valve. Mantle plaques present in many species and situated along the abvalvar edge of the valve mantle. Cingulum composed of a larger valvocopula and few to many ligulate copulae, always open, lacking fimbriae and perforations. Emend. E. Morales (2019: 268).

Frustules of *Pseudostaurosira* form chains with frustules linked valve face to valve face. Frustules are rectangular in girdle view. Striae are uniseriate, composed of a few (usually not more than 4) elliptic areolae. Spines are present on the valve margin and may be branched. Note that, for some species, the uniseriate striae and spines are features that are visible in SEM. *Pseudostaurosira* includes the former *Fragilaria brevistriata* and is distinguished by the structure of the striae, rather than the shape of the valve face.

During research for this study one species from the genus *Pseudostaurosira* were identified. Detailed characteristics about the identified species from the genus *Pseudostaurosira*, distribution and ecological preferences are present further in this study.

1. *Pseudostaurosira parasitica* (W.Smith) Morales in Morales & Edlund 2003

Dimensions: Length 10-25 µm; Width 3-5 µm. Striae: 16-20/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Meso- to eutrophic, circumneutral to alkaline freshwater habitats. Saprobity-tolerant up to β -a mesosaprobic level (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, β -mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Staurosira Ehrenberg, 1843

Lectotype species: *Staurosira construens* Ehrenberg

Staurosira valves are elliptic or cruciform in outline. In girdle view, the frustules are rectangular in shape. Cells often form colonies, joined by interlinking spines. The striae are narrow and composed of small, round areolae. Note that, for most species, the areolae are only visible in SEM. Striae do not meet one another in the central sternum. Valves lack rimoportulae. *Staurosira* includes the former *Fragilaria construens* and its relatives. In the light microscope it can be distinguished from *Staurosirella* based on the structure of the striae. The striae of *Staurosira* are composed of round areolae, while the striae of *Staurosirella* are lineolate (Spaulding et al. 2022).

During research for this study one species from the genus *Staurosira* were identified. Detailed characteristics about the identified species from the genus *Staurosira*, distribution and ecological preferences are present further in this study.

1. *Staurosira construens* Ehrenberg 1843

Dimensions: Length 15-27 μm ; Width 5-7 μm . Striae: 13-14/10 μm .

Distribution in freshwater habitats on Vranica mountain: H4, H5.

Optimal environmental conditions: Often epiphytic, it occurs in water with a wide range of trophic states (oligo- to eutrophic lakes and running waters) and electrolyte contents (more or less calcium-rich) (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Staurosirella D.M.Williams & Round, 1988

Holotype species: *Fragilaria lapponica* Grunow

The valves of *Staurosirella* are oval, linear or cruciform. Striae are composed of lineolate areolae. Although it is difficult to resolve the lineolae in the light microscope, they are characteristically broad, and "pearly" in LM. One to two apical porefields are present. Rimoportulae are absent. Neighboring cells may be linked together at valve faces to form colonies. *Staurosirella* includes the former *Fragilaria lapponica*, *F. pinnata*, *F. leptostauron* and their allies. Cells attach to substrates by short stalks secreted by the

apical porefield. Members of the genus are common in both lentic and lotic waters (Spaulding et al. 2022).

During research for this study two species from the genus *Staurosirella* were identified. Detailed characteristics about the identified species from the genus *Staurosirella*, distribution and ecological preferences are present further in this study.

1. *Staurosirella leptostauron* (Ehrenberg) D.M. Williams et Round 1988

Dimensions: Length (6?) 15-36 µm; Width 6,5-13 µm. Striae: 5-9/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H5.

Optimal environmental conditions: Flowing and standing freshwater habitats on carbonate substrata. Ecological amplitude difficult to define: The colonised freshwater habitats are oligo- to eutrophic, but at most slightly saprobiologically impacted (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Staurosirella pinnata* (Ehrenberg) D.M. Williams et Round 1988

Dimensions: Length 3-3,5 µm; Width 2-8 µm. Striae: 5-12/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4, H5.

Optimal environmental conditions: Wide trophic tolerance ranging nearly over the whole spectrum but sensitive to β-mesosaprobic or worse conditions. Also in electrolyte-poor, circumneutral streams and lakes; absent from more or less acid and acidified habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, alkaliphilic, polyoxybiontic (100 % sat.), oligohalobous, beta-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018), this species is not endangered (?).

Class: Bacillariophyceae

Order: Eunotiales

Family: *Fragilariaceae*

Fragilaria Lyngbye, 1819

Lectotype species: *Fragilaria pectinalis* (O.F.Müller) Lyngbye

Fragilaria frustules are rectangular to lanceolate in girdle view. The pattern of ornamentation on the valve face is variable, but a central sternum is generally present. Frustules are joined by small marginal spines to form ribbon like (band-shaped) colonies. A single rimoportula is present, usually positioned at a distal end. Small, apical porefields are also present. Girdle bands are open. Living cells contain plastids composed of 2 plates, positioned against the valve face. *Fragilaria* is often abundant in the plankton of

lakes, including the species *F. crotonensis*, *F. capucina*, and *F. vaucheriae*. Like *Asterionella formosa*, *F. crotonensis* is a species that is considered to have a world wide distribution and is also considered to be introduced by human activities. Note that the concept of the genera *Fragilaria*, *Synedra* and now, *Ulnaria*, have changed substantially. For example, a broad concept of *Fragilaria* is applied in some floras (Krammer and Lange-Bertalot 1991), while a much narrower concept is applied by others (Williams and Round 1987). Later works present a more narrow circumscription of the genus (Lange-Bertalot and Ulrich 2014) (Spaulding et al. 2022).

During research for this study four species from the genus *Fragilaria* were identified. Detailed characteristics about the identified species from the genus *Fragilaria*, distribution and ecological preferences are present further in this study.

1. *Fragilaria recapitellata* L-B & Metzeltin in Metzeltin, Lange-Bertalot & Nergui 2009

Dimensions: Length 11-38 µm; Width 3-5 µm. Striae: 14-18/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: The ecological amplitude is still difficult to appraise. Particularly in large streams and rivers. The species can also live in eutrophic conditions, and tolerates saprobic pollution up to the α-mesosaprobic level (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is neutrophilic, oligohalobous, α-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Fragilaria rumpens* (Kützing) Carlson 1913

Dimensions: Length 20-65 µm; Width 3,5-4 µm. Striae: 18-20/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: Oligo- to mesotrophic, electrolyte poor streams and small rivers on siliceous substrata. Absent from moderately acidified and dystrophic, as well as strongly eutrophic, environments (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is tolerant N-autotrophic, neutrophilic, oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Fragilaria vaucheriae* (Kützing) Petersen 1938

Dimensions: Length <10-50 µm; Width 4-5 µm. Striae: 9-14/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: Distribution poorly known due to identification problems. Krammer & Lange-Bertalot (1991a) state that it is found in freshwater habitats with low to medium electrolyte content. However, it is also frequently recorded from calcium-bicarbonate-rich lakes. Whether these record are *F. tenera* or long-celled morphotypes of *F. gracillis* remains to be seen (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquaric and aerophilic, tolerant N-autotrophic, alkaliphilic, moderate O₂ (>50% sat.), oligohalobous, α-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Fragilaria virescens* (Ralfs) D.M. Williams et Round 1988

Dimensions: Length 10-120 µm; Width 6-10 µm. Striae: 13-19/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, alkaliphilic, moderate O₂ (>50% sat.), oligohalobous, α-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Odontidium Kützing, 1844

Lectotype species: *Odontidium hyemale* (Roth) Kützing

Frustules of *Odontidium* have heavily silicified transapical ribs that extend internally from the valve face. The sternum is distinct, and may be relatively broad, or may be narrow. Spines are simple and located at the valve face/mantle boundary. Spines are considered non-functional. Girdle band structure is complex, with many alternating open copulae and a pleural band. The copulae possess porose ligulae. One rimoportula located sub-apically on each valve, positioned in-line with a single stria and replacing several vimines. Living cells contain multiple elliptic lobed plastids.

Jüttner et al. (2015) propose that *Odontidium* and *Diatoma*, previously thought of as sub-generic sister taxa, should be considered separate genera. Together, *Odontidium* and *Diatoma* comprise one of the two monophyletic araphid groups to evolve heavily silicified transapical ribs.

The other group includes *Meridion*, *Distrionella*, *Tetracyclus* and *Tabellaria* (Spaulding et al. 2022).

During research for this study one species from the genus *Odontidium* were identified. Detailed characteristics about the identified species from the genus *Odontidium*, distribution and ecological preferences are present further in this study.

1. *Odontidium mesodon* (Kützing) Kützing 1849

Dimensions: Length 12-85 µm; Width 4-7 µm. Striae: 18-20/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3.

Optimal environmental conditions: oligo-mesotraphentic species colonizing electrolyte poor, circumneutral to weakly acid running and still waters (Lange-Bertalot et al. 2017).

General distribution and ecology: Oligo-mesotraphentic species colonizing electrolyte-poor, circumneutral to weakly acid running and still waters. According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Eunotiales

Family: *Ulnariaceae*

Ulnaria (Kützing) Compère, 2001

Holotype species: *Ulnaria ulna* (Nitzsch) Compère

Valves are linear and elongate, with some species near 50 µm in length, others reaching greater than 500 µm in length. A narrow central sternum is present, with striae that meet from each side of the valve or are slightly offset. A central area may be present, varying from small and oval to rectangular and extending to the valve margins. The central area may contain "ghost striae", or faint striae that are present over part, or all, of the central area. One to two rimoportulae are present, at one or both apices. In many species, the apices are distinctly rostrate. Striae are punctate and may be uniseriate or biseriate. Girdle bands are closed. Living cells are common in rivers and lakes. They are attached to the substrate by a mucilage pad secreted by an apical porefield. The taxonomic history of *Fragilaria* leading to the establishment of *Ulnaria* is convoluted (Morales 2003, Silva and Hasle 2006, Williams 2011).

The genus *Ulnaria* was proposed to include species within the species complex surrounding *Fragilaria ulna*, that is, *Fragilaria* subgenus *Alterasynedra* Krammer and Lange-Bertalot 1991. It includes *Ulnaria biceps* (Kützing) Compère, *U. capitata* (Ehrenberg) Compère, *U. lanceolata* (Kützing) Compère, *U. ulna* (Nitzsch) Compère, *U. ungeriana* (Grunow) Compère among others.

During research for this study three species from the genus *Ulnaria* were identified. Detailed characteristics about the identified species from the genus *Ulnaria*, distribution and ecological preferences are present further in this study.

1. *Ulnaria acus* (Kützing) Aboal 2003

Dimensions: Length 95-102 µm; Width 4,6-5 µm. Striae: 11,5-13/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3

Optimal environmental conditions: Not known with precision as available data all refer to a group of species. The neotype location is alkaline, with medium to high conductivity, oligosaprobic but eutrophic (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, α-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Ulnaria delicatissima* (W.Smith) Aboal et P.C. Silva 2004

Dimensions: Length 158,2-262 µm; Width 5 µm. Striae: 10-11/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3, H4.

Optimal environmental conditions: *Ulnaria delicatissima* has been reported across a wide geographic range, usually occurring in the plankton, although not reaching high abundance (Patrick and Reimer 1966).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, neutrophilic, oligohalobous and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

3. *Ulnaria ulna* (Nitzsch) Compère 2001

Dimensions: Length 230-320 µm; Width 6,3-7,7 µm. Striae: 9,2-10,4/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4, H5.

Optimal environmental conditions: Poorly known since most existing data refer to a larger group of species. Information available on the epitype locality suggests a slightly alkaline, medium conductivity, oligosaprobic, moderately eutrophic habitat (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, alkaliphilic, moderate O₂ (>50% sat.), oligohalobous, α-meso → polysaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Hannaea R.M.Patrick, 1966

Holotype species: *Hannaea arcus* (Ehrenberg) R.M.Patrick

Valves are linear and arched. One or two rimoportulae occur on each valve, located near the valve ends. The ventral (concave) side of the valve has a central swelling. Faint, or ghost striae, are often present near the center of the valve. *Hannaea* is characteristic of oligotrophic streams and large lakes, where it grows attached in tufted colonies to benthic surfaces (Spaulding et al. 2022).

During research for this study one species from the genus *Hannaea* were identified. Detailed characteristics about the identified species from the genus *Hannaea*, distribution and ecological preferences are present further in this study.

1. *Hannaea arcus* (Ehrenberg) R.M.Patrick

Dimensions: Length 15-150 µm; Width 4-8 µm. Striae: 13-18/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H3, H5.

Optimal environmental conditions: Streams and small rivers. Occurs across a wide range of pH and calcium-bicarbonate content but absent from moderately to strong acid as well as acidified freshwater habitats. In fast-flowing streams of the Alps, this species, which is generally considered to be oligosaprobic, tolerates some organic enrichment. Very rare in standing freshwater habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, β-mesosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

Class: Bacillariophyceae

Order: Eunotiales

Family: *Tabelariaceae*

Meridion C.Agardh, 1824

Holotype species: *Meridion vernale* C.Agardh

Valves of *Meridion* are variable in symmetry to the transverse axis; they are either strongly heteropolar or isopolar. The heteropolar species are clavate (club-shaped) in valve view and wedge-shaped in girdle view. Septae (attached to girdle elements) are present. An apical porefield is present at one end. Valves may have 1-2 rimoportulae. Cells are joined together to form fan-shaped colonies. Chloroplasts are multiple and plate-like in living cells. *Meridion* includes the common species, *M. circulare* and *M. anceps*. *Meridion* and *Diatoma* are closely related, and the features that distinguish them are based on ultrastructure of the copulae, or girdle bands (Williams 1985). Williams states that *Meridion* possesses 1.) an intense poroidal area at each central ligula portion of the valvocopula, 2.) an extended abvalvar process (generally), acting as a functional antiligula, and 3.) absence of a ligula attachment at the valvocopula polar end (Spaulding et al. 2022).

During research for this study two species from the genus *Meridion* were identified. Detailed characteristics about the identified species from the genus *Meridion*, distribution and ecological preferences are present further in this study.

1. *Meridion circulare* (Greville) C.Agardh 1831

Dimensions: Length 10-82 µm; Width 4-8 µm. Striae: 12-16/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4, H5.

Optimal environmental conditions: More frequently recorded from running water with medium electrolyte content than from lakes. More common in clean rather than enriched water (although it can be found in eutrophic condition) (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Meridion constrictum* Ralfs 1843

Dimensions: Length 10-82 µm; Width 4-8 µm. Striae: 12-16/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H 2.

Optimal environmental conditions: Electrolyte-poor, oligo- to mesotrophic streams and small rivers of siliceous regions. Different from *M. Circulare* as concerns sensitivity to (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Tabellaria Ehrenberg ex Kützing, 1844

Holotype species: *Tabellaria flocculosa* (Roth) Kützing

Tabellaria valves are elongate with capitate ends. The valves are generally wider at the center than at the ends. A rimoportula is present in the center of the valve face. Septa are present on the numerous copulae (girdle bands). Pseudosepta may also be present. Cells form zig-zag colonies joined by mucilage pads secreted from apical porefields. *Tabellaria* is considered a close relative of *Oxyneis*. The two genera share the presence of septa, which may be detached in some preparations. Species within the genus are found in a range of water types, from acidic to alkaline waters (Spaulding et al. 2022).

During research for this study one species from the genus *Tabellaria* were identified. Detailed characteristics about the identified species from the genus *Tabellaria*, distribution and ecological preferences are present further in this study.

1. *Tabellaria ventricosa* Kützing 1844

Dimensions: Length 10-16 µm.

Distribution in freshwater habitats on Vranica mountain: H3, H4, H5.

Optimal environmental conditions: Limited to oligo- to dystrophic, acid streams. Rare in lakes. Indicator of very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, beta-mesosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

Tetracyclus Ralfs, 1843

Holotype species: *Tetracyclus lacustris* Ralfs

Frustules rectangular in girdle view, when mature the perivalvar axis exceeding the apical axis. Valves isopolar, their surface flat, the mantle vertical, the margin a sharply stepped-out hyaline lip. Primary internal transapical ribs present, and sometimes secondary and tertiary ribs also. Axial area longitudinal with indefinite margins, sometimes internally thickened to form a sternum. Striae parallel to slightly radiate extending across the mantle as far as the projecting hyaline valve margin. Virgae much coarser than the small vimines and raised above the valve surface close to the margin, become less so towards the axial area. Areolae close to the axial area more widely spaced than elsewhere and somewhat irregularly distributed. Apical pore fields present, consisting of an area where the striae are as closely spaced as the areolae within them. Rimoportulae usually present, varying in number and position. Cingulum consisting of several open bands, their openings at alternate poles. The bands of four kinds: valvocopula bearing a septum; primary copulae each with a ligula and a septum; secondary copulae without septa their ligulae larger than those of the primary copulae; a single pleura. Septa arising from the abvalvar part of the pars exterior of the valvocopula and the primary copulae, extending variable distances into the frustule, with a pore close to the base on the

abvalvar face communicating with a group of pores on the outer face of the band (Spaulding et al. 2022).

During research for this study four species from the genus *Tetracyclus* were identified. Detailed characteristics about the identified species from the genus *Tetracyclus*, distribution and ecological preferences are present further in this study.

1. *Tetracyclus rupestris* (Kützing) Grunow 1881

Dimensions: Length 4-30 µm; Width 3-12 µm. Striae: 20-24/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3.

Optimal environmental conditions: *T. rupestris* is widely distributed in the medium- and high-elevation mountains (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G).

Diatoma Bory de Saint-Vincent 1824

Holotype species: *Diatoma vulgaris* Bory

The genus *Diatoma* possesses a raised central sternum, which may or may not be distinct. Transverse costae are characteristically thickened and septae are absent. Striae are comprised of uniseriate rows of areolae. Each valve has a single rimoportula, positioned near a valve terminus and oriented transapically. A ligula is present on the second girdle band, which attaches to the polar position of the valve, adjacent to the interlocking valvocopula. Spines are scattered near the polar porefields, but are absent elsewhere. Living cells contain numerous discoid, or platelike plastids.

Jüttner et al. (2015) propose that *Diatoma* and *Odontidium*, previously thought of as sub-generic sister taxa, should be considered separate genera. Together, *Diatoma* and *Odontidium* comprise one of the two monophyletic araphid groups to evolve heavily silicified transapical ribs. The other group includes *Meridion*, *Distrionella*, *Tetracyclus* and *Tabellaria* (with the ribs being lost in *Tabellaria*) (Spaulding et al. 2022).

During research for this study four species from the genus *Diatoma* were identified. Detailed characteristics about the identified species from the genus *Diatoma*, distribution and ecological preferences are present further in this study.

1. *Diatoma ehrenbergii* Kützing 1844

Dimensions: Length 30-120 µm; Width 6-9 µm. Striae: >40/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2 i H3.

Optimal environmental conditions: Bicarbonate-rich standing and flowing freshwater habitats. In contrast to *D. vulgaris*, it is a sensitive species that avoids nutrient and organic enrichment (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkalibiontic, oxybiontic (75% sat.), halophilic, a-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is (*).

2. *Diatoma elongatum* (Lyngbye) Agardh 1824

Dimensions: Length 22-120 µm; Width 2-5 µm. Striae: 6-10/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: -

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018), this species is (?).

3. *Diatoma moniliformis* (Kützing) Williams 2012

Dimensions: Length 8-40 µm; Width 2-4,5 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: *D. moniliformis* is usually identified to the species level only due to the uncertainty surrounding the status of the subspecies. Consequently, it is not clear whether or not autecological differences exist. It is however suspected that ssp. *Ovalis* is widely distributed in eutrophic waters. *Diatoma moniliformis* in the northern Batic Sea, and thus evidently tolerates brackish conditions well (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is alkalibiontic, mesohalophobic and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Diatoma vulgare* Bory 1824

Dimensions: Length 8-75 µm; Width 7-18 µm. Striae: >40/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H3.

Optimal environmental conditions: Preference for medium and high trophic levels. Occurring also in standing freshwater habitats but less commonly than in running waters. Widely distributed, and occasionally very abundant. In Germany found in all meso- and eutrophic running-water types (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkalibiontic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Achnanthes

Family: Achnanthidiaceae

Achnanthidium Kützing, 1844

Lectotype species: *Achnanthidium microcephalum* Kützing

Frustules are heterovalvar, that is, one valve possesses a raphe, while the other valve lacks a raphe. Species of *Achnanthidium* are generally small in size, with narrow valves (less than 30 µm in length and 5 µm in breadth). The shape of the valves differs by species, but the ends may be rounded, capitate or rostrate. In girdle view, the frustules appear arched. The raphe valve face is concave, while that of the rapheless valve is convex. The central area of the raphe valve may form a transverse fascia or subfascia. Terminal raphe fissures are usually present and may be highly deflected. The striae are

usually uniseriate. An isolated row of narrow, elongate areolae are present on the mantle, separated from areolae on the valve face by a narrow hyaline area. Cells attach to substrata by a mucilaginous stalk or pad. In some species, sexual reproduction is similar to reproduction in *Planorhynchidium* and *Lemnicola*. *Achnantheidium* was originally described by Kützing, but it was subsumed into *Achnanthes* until the acceptance of more narrow taxonomic boundaries (Round et al. 1980, Kingston 2003).

The genus is distinguished by striae morphology, frustule shape and growth habit. Common species in North America include *A. minutissimum*, *A. rivulare*, and *A. deflexum*. Like many marine *Achnanthes*, *Achnantheidium* typically attaches to benthic substrates by a mucilaginous stalk. *Achnantheidium* species often thrive in flowing waters, often dominating the communities of the high flow zones of rivers and wave zones of lakes. Some taxa are considered to be "oxygen loving" because they are found in turbulent, well-oxygenated water, however, cells may simply be more efficient at obtaining nutrients in such waters. Small cells such as *Achnantheidium minutissimum* (Kütz.) Czarnecki (= *Achnanthes minutissima* Kütz.) are physiologically more active than larger cells, due partly to their large surface to volume ratios.

During research for this study two species from the genus *Achnantheidium* were identified. Detailed characteristics about the identified species from the genus *Achnantheidium*, distribution and ecological preferences are present further in this study.

1. *Achnantheidium kranzii* (Lange-Bertalot) Round et Bukhtiyarova 1996

Dimensions: Length 6-20 µm; Width 3,5-4 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: Oligotrophic, electrolyte-poor, weakly acid to circumneutral streams and springs, very rarely in soft-water lakes. Indicator of very good quality of water (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is (3).

2. *Achnantheidium minutissimum* (Kützing) Czarnecki 1994

Dimensions: Length 5-25 µm; Width 2,5-4 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4.

Optimal environmental conditions: Ecological amplitude apparently very wide: Consistently found in all freshwater habitat types, often in high numbers, and often developing massive growths as first colonizer, for instance on plant substrata in the lake littoral or in electrolyte-poor streams after acidification pulses. Recorded in high proportions over wide range of trophic levels. However, absent from moderately- to strongly-acid and/or very electrolyte-poor environments. Repalced by *A. saprophillum* when organic pollution is strong. Also tolerant to elevated heavy metals, and sometimes developing teratological forms under these conditions (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Karayevia Round & L.Bukhtiyarova ex Round, 1998

Holotype species: *Achnanthes clevei* Grunow

The valves of *Karayevia* are elliptic to lanceolate with rostrate to capitate ends. The rapheless valve possesses striae that are nearly parallel, composed of circular areolae. The raphe valve possesses radial striae, composed of transapically elongate areolae. Externally, the distal raphe fissures curve to the same side. Internally, the areolae are occluded. Cells grow attached to sand grains, with many species preferring alkaline waters. This genus contains species related to *Achnanthes clevei* Grunow in Cleve et Grunow. The original description of the genus, as *Kolbesia*, was invalid because the type was not designated (Fourtanier and Kociolek 1999) Round (1998) later validly published the genus. The type of *Kolbesia* and its members have been transferred to *Karayevia* (Spaulding et al. 2022).

During research for this study one species from the genus *Karayevia* were identified. Detailed characteristics about the identified species from the genus *Karayevia*, distribution and ecological preferences are present further in this study.

1. *Karayevia laterostrata* (Hustedt) Bukhtiyarova 1999

Dimensions: Length 10-36 µm; Width 6-10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Oligotrophic running waters on siliceous substratum; rarer in still waters. Records from alkaline freshwater habitats with medium to higher electrolyte content still need to be verified (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

Planothidium Round & L.Bukhtiyarova, 1996

Holotype species: *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot

Frustules of *Planothidium* are heterovalvar, with slightly concave raphe valve and convex rapheless valve. Valves are elliptic to lanceolate, with rounded, rostrate or capitate apices. The striae are multiseriate. The raphe valve possess radiate striae and a prominent raphe. The terminal raphe fissures are long, curved and deflected unilaterally. Many *Planothidium* species, but not all, possess an asymmetric central area on the rapheless valve. Internally, this central area bears a rimmed depression, and in some cases, a cavum is also present. Note that alternate terms for the cavum are "cave" and "hood". The structure of the rimmed depression and cavum were not recognized until they were examined under the scanning electron microscope. Previously, the structures were termed "hufeisenförmige Fleck" in German, or "hoof-mark" or "horseshoe structure" in English. Cells have an adnate growth habit, in which they are attached by the raphe valve face to the substrata. The genus was split from *Achnantheidium* by both Round and Bukhtiyarova (1996) and Lange-Bertalot (1997). Details of sexual reproduction in *P. lanceolatum* are described in Geitler (1977) (Spaulding et al. 2022).

During research for this study two species from the genus *Planothidium* were identified. Detailed characteristics about the identified species from the genus *Planothidium*, distribution and ecological preferences are present further in this study.

1. *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot 1999

Dimensions: Length 6-40 µm; Width 4,5-10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4.

Optimal environmental conditions: Wide ecological amplitude varied from circumneutral to alkaline, from electrolyte-poor to rich, from oligotrophic to polytrophic freshwater habitats, up to β-mesosaprobic condition. Absent from acid habitats. Also widely distributed in standing freshwater habitats, particularly in electrolyte-poor waters, but less consistently than in running waters (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, alkaliphilic, moderate O₂ (>50% sat.), oligohalobous, α-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot 1999

Dimensions: Length 4-30 µm; Width 3,5-7 µm. Striae: 13-20/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: Mostly similar to that *P. lanceolatum*, with which it frequently co-occures. *P. frequentissimum*, however, is tolerant up to the transition between α-mesosaprobic and polysaprobic conditions (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is tolerant to N-autotrophic, alkaliphilic, moderate O₂ (>50% sat.), oligohalobous, α-meso → polysaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Psammothidium L.Buhtkiyarova & Round, 1996 (Achnanthesiaceae)

Holotype species: *Psammothidium marginulatum* (Grunow) Bukhtiyarova & Round

Frustules are small, elliptic, lanceolate-elliptic, or linear-elliptic in shape. Frustules are bent in girdle view so that the raphe valve is convex and the rapheless is concave, an arrangement that differs from other achnantheid genera. The length to width ratio ranges between 1.5 and 3.0. The valve apices are broadly rounded. The arrangement of areolae is similar on the raphe and rapheless valves. Striae are radiate and closely spaced, with nearly 30 striae in 10 µm. A distinct, rectangular central area is present on the raphe valve. The central area is more expanded on the rapheless valve than the raphe valve, at least in several species. The terminal raphe fissures vary in shape and orientation from very short and almost straight, to long and strongly deflected in opposite directions. In some species, terminal raphe fissures are absent. *Psammothidium* contains a number of species (over 40, according to Index Nominum Algarum) including species formerly treated as *Achnanthes marginulata* Grunow in Cleve and Grunow and *Achnanthes levanderi* Hustedt. The typical habitat of species of *Psammothidium* is on sand grains, where they may grow in abundance.

During research for this study four species from the genus *Psammothidium* were identified. Detailed characteristics about the identified species from the genus *Psammothidium*, distribution and ecological preferences are present further in this study.

1. *Psammothidium bioretii* (H.Germain) Bukhtiyarova & Round 1996

Dimensions: Length 10-30 µm; Width 5-10 µm. Striae: 22-28 (32?)/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Particularly in lakes and smaller running waters on siliceous substrata. However, the ecological amplitude is wide, and the species can be found, independently from the ecoregion, sometimes in moderately high numbers, also in alkaline running and still waters across a wide trophic gradient (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic, oligohalobous, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Psammothidium helveticum* (Hustedt) Bukhtiyarova & Round 1996

Dimensions: Length 7-28 µm; Width 5-7,5 µm. Striae: 23-28/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Characteristic species of electrolyte-poor, strongly acidified streams and still waters of the siliceous central highlands, where it can occur in large number. Also found in small, weakly acid to circumneutral running waters of the lowland on siliceous substrata but less frequently and in lower number (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), halophobe, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Psammothidium rechtense* (Leclercq) Lange-Bertalot 1999

Dimensions: Length 6-25 µm; Width 4-6,5 µm. Striae: 25-30/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Characteristic species of nutrient- and electrolyte-poor, not acidified freshwater habitats. Preference for standing respectively running waters not yet known due to identification problems. Indicator of very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is sensitive N-autotrophic, neutrophilic, polyoxybiontic, halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

4. *Psammothidium subatomoides* (Hustedt) Bukhtiyarova & Round 1996

Dimensions: Length 6-12 µm; Width 3,5-6,5 µm. Striae: 28-40/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H4.

Optimal environmental conditions: Oligotrophic, circumneutral to naturally weakly acid streams and standing freshwater habitats on siliceous substrata. Indicator of very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, acidophilic, polyoxybiontic, halophobe, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

Class: Bacillariophyceae

Order: Cocconeidaceae

Family: Cocconeidaceae

Cocconeis Ehrenberg, 1836

Lectotype species: *Cocconeis scutellum* Ehrenberg

Cocconeis is heterovalvar, that is, the raphe valve ornamentation differs from that of the rapheless valve. Ornamentation of the raphe valve may differ markedly from that of the rapheless valve. The valve mantle is narrow in relation to the valve face, so cells are rarely (if ever) seen in girdle view. Valves may be flexed, or arched, along the apical axis forming a 'saddle' shape. Striae are often uniseriate, but some taxa possess multiseriate striae composed of loculate areolae. In several taxa, the raphe valve is marked by a region near the edge of the valve that is ornamented with a hyaline ring and a more defined mantle than the rapheless valve. Valvocopula may be closed, or complete, with internal projections of silica. The genus contains both marine and freshwater taxa. Living cells have a solitary and adnate habit of growth. Cells possess a single plastid that is flat and C-shaped. Species of *Cocconeis* may be epiphytic on other algae, as well as on hard substrates (e.g. rock, sand). Cells grow with the raphe valve in contact with the substrate (Spaulding et al. 2022).

During research for this study two species from the genus *five* were identified. Detailed characteristics about the identified species from the genus *five* distribution and ecological preferences are present further in this study.

1. *Cocconeis lineata* Ehrenberg 1849

Dimensions: Length 16-80 µm; Width 6-3,5 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4.

Optimal environmental conditions: Still not well known because the taxon has not been identified consistently. Occurs on different substrata (rocks and stones, bryophytes, macroalgae, vascular plants, wood etc) (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Cocconeis pediculus* Ehrenberg 1838

Dimensions: Length 13-54 µm; Width 7-37 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H3.

Optimal environmental conditions: Alkaline, flowing and standing freshwater habitats with medium to high trophic levels. Mostly absent from electrolyte-poor, circumneutral to acid, oligotrophic habitats. Often epiphytic on *Cladophora* (Lange-Bertalot et al. 2017). General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), halophilic, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Cocconeis placentula* Ehrenberg 1838

Dimensions: Length 11-36 µm; Width 7,26 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4, H5.

Optimal environmental conditions: Habitat, distribution, and ecology are not yet known precisely since this variety has only rarely been distinguished using the concept of Jahn et al. (2009). Epitype strain: Epiphytes in slow-flowing alkaline, low temperature, eutrophic running water with medium-high conductivity (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, alkaliphilic, moderate O₂ (>50% sat.), oligohalobous, beta-mesosaprobe and autrophic.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

4. *Cocconeis placentula* var. *klinoraphis* Geitler

Dimensions: Biovolumen: 2828 µm³.

Distribution in freshwater habitats on Vranica mountain: H3.

Distribution in Bosnia and Herzegovina:

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, alkaliphilic, moderate O₂ (50% sat.), oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

5. *Cocconeis pseudolineata* (Geitler) Lange-Bertalot 2004

Dimensions: Length 7,5-38 µm; Width 6-18 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3.

Optimal environmental conditions: Distribution and ecological preferences not precisely known, because this species was often not distinguished from other varieties of the *C. placentula* complex. In lakes and running waters, apparently preferring medium and higher electrolyte contents and trophic levels (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is alkaliphilic and oligohalobous.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Bacillariales

Family: *Bacillaraceae*

Denticula Kützing, 1844

Lectotype species: *Denticula elegans* Kützing

The raphe system of each valve is positioned diagonally opposite to one another, demonstrating nitzschiod symmetry. The raphe system is located within a wide, low keel. The raphe may or may not have proximal raphe ends or be continuous across the valve, a feature difficult to see without SEM. Thick internal bars of silica (fibulae) are present and parallel to the striae. The fibulae widen near the valve margins to form a 'tooth-like' appearance, hence the genus name *Denticula*. *Denticula* species occur in rather diverse habitats. Some species may be locally abundant in carbonate rich waters of moderate. The genus also contains species characteristic of large oligotrophic lakes, as well as warm springs (Spaulding et al. 2022).

During research for this study one species from the genus *Denticula* were identified. Detailed characteristics about the identified species from the genus *Denticula*, distribution and ecological preferences are present further in this study.

1. *Denticula tenuis* Kützing 1844

Dimensions: Length 6-42 µm; Width 3-7 µm. Striae: 25-30/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2.

Optimal environmental conditions: It is frequently found in lake littoral zones where it can occasionally dominate assemblages. However, it is not uncommon also in running waters of different size (from spring-fed stream to river) (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Cymbelales

Family: *Anomoeoneidaceae*

Hantzschia Grunow, 1877, nom. et typ. cons.

Holotype species: *Hantzschia amphioxys* (Ehrenberg) Grunow

Valves are asymmetric to the apical axis. Like *Nitzschia*, the raphe is contained within a canal and eccentrically positioned on the valve margin. In *Hantzschia*, the raphe is always located on the concave, or ventral, margin of the valve. Striae are uniseriate. The genus *Hantzschia* differs from *Nitzschia* in symmetry. Within a *Hantzschia* frustule, the raphes of the two valves are on the margin of the same side. In contrast, within a *Nitzschia* frustule, the raphes of the two valves are on opposite margins. This arrangement of the

raphe is termed "hantzschiod symmetry" and "nitzschiod symmetry", respectively. Hantzschiod or nitzschiod symmetry of a frustule is the result of the position of nucleus at cell division (Pickett-Heaps et al. 1980).

Hantzschia has a greater number of species in marine habitats than in freshwaters. It is characteristic of temporary aquatic habitats and, in particular, the genus is often indicative of soil habitats (Spaulding et al. 2022).

During research for this study two species from the genus *Hantzschia* were identified. Detailed characteristics about the identified species from the genus *Hantzschia*, distribution and ecological preferences are present further in this study.

1. *Hantzschia amphioxys* (Ehrenberg) Grunow 1880

Dimensions: Length 15-50 µm; Width 5-7 µm. Striae: 20-29/10 µm. Puncta: 40-50/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H4.

Optimal environmental conditions: Distributed in the same biotopes as *H. abundans*, and often found together with it. The most frequently encountered species in prolongedly dry and only temporarily wetted (aerial) biotopes, such as soils and rock crevices. It is not, therefore, suitable as an indicator of freshwater quality. If encountered at high relative abundances during ecological assessments, there may be issued with the time, place or manner of sampling (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, tolerant N-autotrophic, neutrophilic, oxybiontic (75% sat.), oligohalobous, a-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Hantzschia calcifuga* Reichardt et Lange-Bertalot 2004

Dimensions: Length 53-93 µm; Width 6,5-8 µm. Striae: 16-20/10 µm. Puncta: 35/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Found in electrolyte-poor, weakly acid to circumneutral habitats (e.g. reed meadows, spring in the siliceous central highlands of Germany), associated with acidophilous diatoms (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic and oligohalobous.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

Nitzschia Hassall, 1845, nom. cons.

Holotype species: *Nitzschia elongata* Hassal

The nitzschiod raphe is positioned eccentrically within a keel which is supported by fibulae. Valves lack a sternum. Within the genus, species have a large range in size, from very small to very large. *Nitzschia* is a very common genus with a large number of species that are often difficult to identify. Some *Nitzschia* species reach great abundance in waters high in organic pollution (Spaulding et al. 2022). Generally two plastids per cell, one towards each ending. Frustules are frequently observed in girdle view (Krammer & Lange-Bertalot, 1988).

During research for this study five species from the genus *Nitzschia* were identified. Detailed characteristics about the identified species from the genus *Nitzschia*, distribution and ecological preferences are present further in this study.

1. *Nitzschia acidoclinata* Lange-Bertalot 1976

Dimensions: Length 8-45 µm; Width 2,5-3 µm. Striae: 10-16/10 µm. Puncta: 27-34/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H5.

Optimal environmental conditions: Oligosaprobic, electrolyte-poor siliceous freshwater environments. However, there are also repeated records from alkaline fens and from springs emerging from Jurassic limestones. It might thus be that the ecological amplitude is wider than supposed, and might depend upon availability of suitable buffering systems (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, beta-mesosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

2. *Nitzschia denticula* Grunow in Cleve & Grunow 1880

Dimensions: Length 16-60 µm; Width 3-8 µm. Striae: 11-20/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H3.

Optimal environmental conditions: Oligo- and mesotrophic lakes on carbonate substrata (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Nitzschia fonticola* Grunow in Cleve & Möller 1879

Dimensions: Length 7-46 µm; Width 2,5-5,5 µm. Fibulae: 9-14/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4.

Optimal environmental conditions: Very frequent in moderately low to high electrolyte content, oligo- to β-mesosaprobic freshwaters. Therefore good indicator species sensitive towards strong saprobic pollution, frequently developing massive occurrence. Apparently avoiding humic and electrolyte- and nutrient-poor, as well as more or less brackish habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Nitzschia recta* Hantzsch in Rabenhost 1862

Dimensions: Length 3,5-100 µm; Width 5-7 µm. Fibulae: 5-8/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Occurrence in the different freshwater habitat types and trophic tolerance not yet definitively to appraise because the species was not

distinguished from similar taxa in the past. Widely distributed and tolerant up to the β -mesosaprobic pollution load (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

5. *Nitzschia sinuata* (W.Smith) Grunow 1880

Dimensions: Length 10-50 μm ; Width 3-8 μm . Striae: 18-25/10 μm .

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: The main occurrence is in artificial still waters (quarry ponds, reservoirs, lakes in decommissioned brown-coal mines) with medium to higher electrolyte contents. Occasionally in high numbers.

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

Class: Bacillariophyceae

Order: Cymbelales

Family: Anomoeoneidaceae

Adlafia Gerd Moser, Lange-Bertalot & Metzeltin, 1998

Holotype species: *Adlafia muscora* (Kociolek & Reviere) Moser, Lange-Bertalot & Metzeltin

Adlafia is a genus characterized by small cells, usually less than 25 μm in length. The valve shape is linear to linear-lanceolate. *Adlafia* is distinguished by terminal raphe fissures that are strongly bent and angular. The shape of the valve ends may be blunt, rostrate, or subcapitate. Striae are radial. Externally, the areolae are covered by hymenes. The striae are uniseriate. Species in this genus are also characterized by the solitary growth of cells. *Adlafia* is comprised of species formerly in *Navicula*, including *A. muscora* (Kociolek and Reviere) Moser, and *A. bryophila* (Petersen) Moser.

Adlafia species are characteristic of aerophilic habitats, especially growing in and around wet mosses. Some species are found in oligotrophic lakes (Spaulding et al. 2022).

During research for this study one species from the genus *Adlafia* were identified. Detailed characteristics about the identified species from the genus *Adlafia*, distribution and ecological preferences are present further in this study.

1. *Adlafia bryophila* (Petersen) Lange-Bertalot in Moser et al. 1998.

Dimensions: Length 10-20(25) μm ; Width (2,5)3-4 μm . Striae: 29-36/10 μm .

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Streams and lakes, rare in larger running waters. Sensitive to organic pollution exceeding weakly β -mesosaprobic but found in a broad electrolyte content range (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is terrestrial, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Cymbelales

Family: *Cymbellaceae*

Paraplaconeis Kulikovskiy, Lange-Bertalot & Metzeltin, 2012

Holotype species: *Paraplaconeis kornevae* Kulikovskiy, Gusev & Lange-Bertalot

Cells possess most of the same characters as those of *Placoneis* species observed with LM, with the exception that striae on the valve face are consistently biseriate with alternating areolae (unfortunately not always well visible with LM). The main distinguishing characteristics with SEM are that areolae open internally (in contrast to *Placoneis*); and are occluded externally by stalked flaps surrounded by reniform slits similar to those in most *Gomphonema* species (Lange-Bertalot et al. 2017).

During research for this study one species from the genus *Paraplaconeis* were identified. Detailed characteristics about the identified species from the genus *Paraplaconies*, distribution and ecological preferences are present further in this study.

1. *Paraplaconeis placentula* (Ehrenberg) Kulikovskiy & Lange-Bertalot 2012

Dimensions: Length 30-50 μm ; Width 12-20 μm . Striae: 8-11/10 μm .

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: Broad spectrum of mesotrophic to weakly brackish, alkaline, flowing and standing habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

Cymbella C.Agardh, 1830, nom. et typ. cons.

Lectotype species: *Cymbella cymbiformis* C.Agardh

The valves of *Cymbella* are slightly to strongly asymmetric to the apical axis. Valves are symmetric to the transapical axis. The terminal raphe fissures are deflected to the dorsal side, an important diagnostic feature. Apical pore fields are present at both poles. Striae are uniseriate. One or more stigmata may be present. If a stigma or stigmata are present, they are located on the ventral side of the central area. Internally, the stigmata are convoluted internal occlusions. Also internally, the proximal raphe may be continuous

across central area. *Cymbella* cells grow predominately in benthic habitats, and often produce mucilaginous stalks that are secreted through the apical porefield. Historically, *Cymbella* has been a large, heterogenous group and is not considered to be a natural, or monophyletic group of species. Recently, a number of genera have been split from *Cymbella*, including *Cymbopleura*, *Delicata* and *Encyonopsis* (Spaulding et al. 2022).

During research for this study four species from the genus *Cymbella* were identified. Detailed characteristics about the identified species from the genus *Cymbella*, distribution and ecological preferences are present further in this study.

1. *Cymbella aspera* (Ehrenberg) H. Peragallo 1889

Dimensions: Length 110-200 µm; Width 26-35 µm. Striae: 6,5-8/10 µm. Puncta: 8-10/10 µm. Stigmata: 7-10.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H5.

Optimal environmental conditions: Ecologically difficult to characterise. Alkaline, meso- to eutrophic running waters and lakes (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

2. *Cymbella neocistula* Krammer 2002

Dimensions: Length 34-110 µm; Width 12-19 µm. Striae: 7-10/10 µm. Puncta: 17-20/10 µm. Stigmata: 3-5.

Distribution in freshwater habitats on Vranica mountain: H1, H3, H4.

Optimal environmental conditions: Meso- to eutrophic, moderately- to more strongly electrolyte-rich, weakly-alkaline freshwater habitats. In Central Europe widely distributed, rather frequent, and locally abundant (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Cymbella neolanceolata* W.Silva 2013

Dimensions: Length 86-225 µm; Width 22-34 µm. Striae: 8-10/10 µm. Puncta: 13-17/10 µm. Stigmata: 5-9.

Distribution in freshwater habitats on Vranica mountain: H2, H4, H5.

Optimal environmental conditions: Oligo- to moderately-eutrophic freshwater habitats on carbonate substrata with moderate electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Cymbella proxima* Reimer in Patrick & Reimer 1975

Dimensions: Length 38-120 µm; Width 18-24 µm. Striae: 7-11/10 µm. Puncta: 12-18/10 µm. Stigmata: 2-5.

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Oligo- to eutrophic but oligosaprobic freshwater habitats with medium electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is halophobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G).

Cymbopleura (Krammer) Krammer, 1999

Holotype species: *Cymbopleura inaequalis* (Ehrenberg) Krammer

Cymbopleura frustules are characteristically large and relatively broad in valve outline. The frustules of *Cymbopleura* are asymmetric to the apical axis, typically with a convex ventral margin and an even broader convex dorsal margin. The striae are composed of rounded areolae. The proximal raphe ends are expanded, while the terminal raphe fissures are hooked and dorsally deflected. Species within *Cymbopleura* lack apical porefields, therefore they do not produce mucilaginous stalks. Stigmata are absent. Cells grow as single individuals. Species within *Cymbopleura* grow in benthic and epipelagic habitats and include *C. amphicephala*, *C. ehrenbergii*, *C. subcapitata*, *C. inaequalis*, *Cymbella lata* and the *Cymbella heteropleura* group (Spaulding et al. 2022).

During research for this study five species from the genus *Cymbopleura* were identified. Detailed characteristics about the identified species from the genus *Cymbopleura*, distribution and ecological preferences are present further in this study.

1. *Cymbopleura amphicephala* (Nägeli) Krammer 2003

Dimensions: Length 22-34 µm; Width 7,2-8,7 µm. Striae: 12-15/10 µm. Puncta: 32-36/10 µm. Distribution in freshwater habitats on Vranica mountain: H2, H4, H5.

Optimal environmental conditions: Oligo- to mesotrophic freshwater habitats with low to medium electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

2. *Cymbopleura austriaca* (Grunow) Krammer 2003

Dimensions: Length 45-90 µm; Width 13,5-19 (23) µm. Striae: 9-14/10 µm. Puncta: 21-25/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4.

Optimal environmental conditions: Often on submerged bryophyte carpets and rock, more rarely in the littoral zone of oligotrophic lakes and in running waters. Indicator of very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

3. *Cymbopleura cuspidata* (Kützing) Krammer 2003

Dimensions: Length 28-61 µm; Width 14-17 µm. Striae: 8-12/10 µm. Puncta: 20-23/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H4.

Optimal environmental conditions: Oligo- to eutrophic, oligosaprobic freshwater habitats with medium electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is neutrophilic, oligohalobous and oligosaprobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

4. *Cymboppleura inaequalis* (Ehrenberg) Krammer 2003

Dimensions: Length 28-61 µm; Width 14-17 µm. Striae: 8-12/10 µm. Puncta: 20-23/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Charactersitics, distinct species of oligo- to weakly-eutrophic, usually calcium-bicarbonate-rich habitats with moderate electrolyte content. In standing and flowing freshwater habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, alkalibiontic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

5. *Cymboppleura naviculiformis* (Auerswald) Krammer 2003

Dimensions: Length 26-50 µm; Width 9-13 µm. Striae: 12-14/10 µm. Puncta: 27-33/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H3, H4, H5.

Optimal environmental conditions: Oligo- to weakly eutrophic freshwater habitats, with preference for siliceous substrata. Single specimens may be found in anthropogenically disturbed sections of running waters (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, neutrophilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Encyonopsis Krammer, 1997 (Cymbellaceae)

Holotype species: *Encyonopsis cesatii* (Rabenhorst) Krammer

Valves are naviculoid and slightly dorsiventral, symmetric to the transapical axis and asymmetric to the apical axis. The raphe is centrally located. Areolae in the striae are round or oblong and oriented with their long axes parallel to the transapical axis. Transapical striae are radiate near the valve center and radiate or parallel near the apices. Proximal raphe ends are deflected toward the dorsal margin. Distal raphe fissures are deflected ventrally. Stigmata and apical pore fields are absent. *Encyonopsis* is distinguished from *Kurtkammeria* by the orientation of the striae at the valve apices, by the shape and orientation of the areolae, and by other features observed in SEM. In *Encyonopsis* species, areolae are round or elongate in a transapical direction. In *Kurtkammeria*, areolae are slit-like or crescent shaped with the long axis oriented apically. In *Encyonopsis*, the terminal striae are radiate or parallel; in *Kurtkammeria*, terminal striae are convergent. Stigmata and apical pore fields may be present in *Kurtkammeria*, but they are absent in *Encyonopsis* (Spaulding et al. 2022).

During research for this study two species from the genus *Encyonopsis* were identified. Detailed characteristics about the identified species from the genus *Encyonopsis*, distribution and ecological preferences are present further in this study.

1. *Encyonopsis cesatii* (Rabenhorst) Krammer 1997

Dimensions: Length 18-60 µm; Width 4-6 (8) µm. Striae: 18-22/10 µm. Puncta: 35-40/10 µm. Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Oligotrophic and mesotrophic, calcium-bicarbonate-rich lakes; more rarely in running waters. The colonised freshwater habitats are mainly calcium-bicarbonate-rich. Indicator for very good ecological quality. *E. cesatii* is abundant in weakly acid and circumneutral mires of the Alps and of central Europe (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

2. *Encyonopsis microcephala* (Grunow) Krammer 1997

Dimensions: Length 10-23 µm; Width 3,5-4,2 µm. Striae: 23-25/10 µm. Puncta: 38-42/10 µm. Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Standing freshwater habitats but also in running waters. Ecological amplitude wide in oligosaprobic environments, from electrolyte-poor, weakly acid to calcium-bicarbonate-rich freshwater but tolerating a narrower range of trophic conditions, from oligo- to moderately-eutrophic. This could be due to the fact that more than only one form occurs but these could not be separated morphologically in the past and were considered together. However, this is not case of the more coarsely striate var. *robusta* (Husted) Krammer, that is known only from the Pyrenees (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Cymbelales

Family: *Gomphonemataceae*

Placoneis Mereschkowsky, 1903

Lectotype species: *Placoneis gastrum* (Ehrenberg) Mereschkowsky

The valve outline of *Placoneis* is linear to lanceolate, sometimes with rostrate or capitate ends. The areolae are loculate and internally occluded. Areolae form uniseriate striae. The axial area is narrow. The proximal raphe ends are straight and slightly expanded externally. The plastid is large, distinct, and divided into two X-shaped plates.

Placoneis contains a number of species that grow in epipelagic habitats of lakes and streams. It includes the former *Placoneis exigua* (Gregory) Mereschkowsky and its allies. The taxonomic position of the genus is unclear, as it has been suggested to hold naviculoid as well as cymbelloid and gomphonemoid affinities (Mann and Stickle 1995, Kociolek and Thomas 2010).

During research for this study two species from the genus *Placoneis* were identified. Detailed characteristics about the identified species from the genus *Placoneis*, distribution and ecological preferences are present further in this study.

1. *Placoneis elginensis* (W.Gregory) E.J.Cox 1988

Dimensions: Length 30-36 µm; Width 9-10 µm. Striae: c. 11/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: Difficult to appraise, as it is often confused with other species (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, alkaliphilic, oxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

2. *Placoneis gastrum* (Ehrenberg) Mereschkowsky 1903

Dimensions: Length 30-60 µm; Width 12-18 µm. Striae: 8-10/10 µm. Puncta: 22-24/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Meso- to eutrophic and oligosaprobic lakes, more rarely in streams and rivers, with medium to high electrolyte contents (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Encyonema Kützing, 1834

Holotype species: *Encyonema paradoxum* Kützing

Encyonema frustules are asymmetric to the apical axis and symmetric to the transapical axis. The dorsal margin is highly arched, while the ventral margin is straight or nearly straight. Distal raphe ends are ventrally deflected. Stigmoids may be present or absent. If a stigmoid is present, it is located on the dorsal side of the central area. Distal raphe ends are ventrally deflected. Apical pore fields are absent. Cells may grow as single cells, produce mucilaginous sheaths, or form colonies within mucilaginous tubes. *Encyonema* is broadly distributed across North America, primarily in benthic habitats (Spaulding et al. 2022).

During research for this study seven species from the genus *Encyonema* were identified. Detailed characteristics about the identified species from the genus *Encyonema*, distribution and ecological preferences are present further in this study.

1. *Encyonema auerswaldii* Rabenhorst 1853

Dimensions: Length 22,6-32,9 µm; Width 9-10,3 µm. Striae: 10-12 in the centre of the valve, 12-15/10 µm near ends.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: *Encyonema auerswaldii* often grows as an epiphyte. It forms long, branching mucilage tubes (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

2. *Encyonema caespitosum* Kützing 1849

Dimensions: Length 22-57 µm; Width 9,5-15 µm. Striae: 9-12/10 µm. Puncta: 18-21/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Eutrophic freshwater habitats with medium electrolyte content. However tolerating conditions from oligo- to eutrophic; in degraded as well as in undisturbed habitats. In flowing habitats rarer, particularly in large stremas and rivers. As regards saprobity, there are records up to over β-a mesosaprobic levels. Besides the nominate variety, there are var. *comensis* Krammer and var. *maxima* Krammer. Possibly the wide ecological amplitude results also from pooling the varieties (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is oligohalobous, α-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Encyonema minutum* (Hilse) D.G. Mann 1990

Dimensions: Length 7-23 µm; Width 4,2-6,9 µm. Striae: 15-18/10 µm. Puncta: 34-38/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4.

Optimal environmental conditions: In antropogenically little disturbed habitats, in the periphyton of oligo- to mesotrophic freshwater habitats with medium electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is neutrophilic and oligohalobous.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Encyonema perpusillum* (A.Cleve) D.G. Mann 1990

Dimensions: Length 12-30 µm; Width 3-5 µm. Striae: 10-14/10 µm. Puncta: 38-45/10 µm.

Distribution in freshwater habitats on Vranica mountain: 5.

Optimal environmental conditions: Characteristic species of oligotrophic, usually acidic freshwater habitats with low electrolyte content on siliceous substrata. Indicator for very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

5. *Encyonema silesiacum* (Bleisch) D.G. Mann 1990

Dimensions: Length 16-42 µm; Width 5,9-9,6 µm. Striae: 11-14/10 µm. Puncta: 28-31/10 µm. Distribution in freshwater habitats on Vranica mountain: H2, H4, H5.

Optimal environmental conditions: Mainly in mountains, in different, anthropogenically little disturbed freshwater habitats (e.g. springs on siliceous bedrock). These freshwater have low to medium electrolyte content, and are usually oligo- to mesotrophic. However, the species can tolerate also eutrophic conditions provided that there is no saprobic pollution, and can occur together with *E. ventricosum* (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, neutrophilic, moderate O₂ (50% sat.), oligohalobous, α-mesosaprobe and indifferent (trophic state).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

6. *Encyonema ventricosum* (C.Agardh) Grunow in A. Schmidt et al. 1885

Dimensions: Length 9-29 µm; Width 4,5-6,9 µm. Striae: (12)14-19/10 µm. Puncta: 33-39/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4.

Optimal environmental conditions: One of the most frequents species of the genus *Encyonema*, mainly in eutrophic, also in more strongly impacted (organic pollution) freshwater habitats with medium electrolyte content, up to the (weakly) α-mesosaprobic level (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is neutrophilic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

7. *Encyonema vulgare* Krammer 1997

Dimensions: Length 28-60 µm; Width 8-13 µm. Striae: 10-13/10 µm. Puncta: 20-26/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Rare to very rare and low numbers in oligotrophic, electrolyte-poor habitats in mountains. Records from freshwater environment on carbonate substrata must be verified (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, alkaliphilic and oligohalobous.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

Reimeria J.P.Kociolek & Stoermer, 1987

Holotype species: *Cymbella sinuata* W.Gregory

Valves asymmetric to the longitudinal axis. Striae of one or two rows of puncta, C-shaped foramina present or absent. Raphe straight, expanded laterally. Central area unilateral, extending to the ventral margin. One dorsal stigma positioned near the proximal raphe ends, internal proximal raphe ends recurved in the same direction toward the apices, central nodule small. Apical pore fields located at the poles, restricted to the ventral margin and mantle or divided into two parts by the curvature of the external terminal end of the raphe, the two parts unequal in size, the smaller on the mantle of the valve apex, the larger on the ventral mantle. Valves of *Reimeria* are symmetric to the

transapical axis and asymmetric to the apical axis. The dorsal margin is slightly arcuate. The ventral margin is markedly expanded, or tumid, at the central area. Apical porefields are present at both poles on the ventral margin and bisected by the distal raphe fissure. The axial area is narrow. A single stigma is positioned on the dorsal side of the central area. Distal raphe fissures are deflected toward the ventral margin. *Reimeria* contains a few species, two of which are broadly distributed across North America in benthic habitats. Older literature contains this group within the *Cymbella sinuata* complex (Spaulding et al. 2022).

During research for this study one species from the genus *Reimeria* were identified. Detailed characteristics about the identified species from the genus *Reimeria*, distribution and ecological preferences are present further in this study.

1. *Reimeria sinuata* (Gregory) Kociolek & Stoermer 1987

Dimensions: Length 9-40 µm; Width 3,5-9 µm. Striae: 8-14/10 µm. Puncta: 45-50/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: Mainly in flowing freshwater habitats. This species includes a group of populations that, taken together, are found over wide ranges of calcium concentrations and trophic levels, although it is more abundant in freshwater habitats with lower levels of nutrient (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, neutrophilic, polyoxybiontic (100% sat), oligohalobous, β-mesosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Gomphonema Ehrenberg, 1832, nom. et typ. cons.

Lectotype species: *Gomphonema acuminatum* Ehrenberg

Valves are symmetric to the apical axis, and asymmetric to the transapical axis. In valve view, valves are club-shaped, or clavate. In girdle view, frustules are wedge-shaped, or cuneate. A stigma may be present on one side of the central area. An apical pore field is present and bisected by the terminal raphe. Striae are uniseriate. Striae are not crossed by longitudinal lines, as in *Gomphoneis*. Many species grow on mucilaginous stalks and can be found in nearly every habitat type within circumneutral lakes and streams of North America (Spaulding et al. 2022).

During research for this study fourteen species from the genus *Gomphonema* were identified. Detailed characteristics about the identified species from the genus *Gomphonema*, distribution and ecological preferences are present further in this study.

1. *Gomphonema acidoclinatum* Lange-Bertalot et Reichardt 2004

Dimensions: Length 20-58 µm; Width 6,6-8,5 µm. Striae: 12-15/10 µm. Puncta: 25-30/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3, H5.

Optimal environmental conditions: Electrolyte-poor, weakly (to stronger?) acid springs and streams (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

2. *Gomphonema acuminatum* Ehrenberg 1836

Dimensions: Length 17,5-57 µm; Width 6,7-10,8 µm. Striae: 9-11/10 µm. Puncta: c. 25/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H4.

Optimal environmental conditions: Widely distributed (cosmopolitan) and frequent, often in high numbers also in oligo- to weakly mesosaprobic, but oligo- to eutrophic freshwater habitats usually with medium electrolyte content. More rarely also in weakly acid, electrolyte-poor milieus.

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Gomphonema angustum* C. Agardh 1831

Dimensions: Length 16-50 µm; Width 5,2-8 µm. Striae: 9-12/10 µm. Puncta: 25-28/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H3.

Optimal environmental conditions: Characteristic species of strongly calcium-rich freshwater habitats, spring on calcareous bedrock and loess, in limestone deposits, incrustated bryophyte carpets; there almost always in high numbers. Where such habitats have disappeared, *G. angustum* is replaced by other, possibly similar *Gomphonema* species (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is neutrophilic, oligohalobous and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

4. *Gomphonema exilissimum* (Grunow) Lange-Bertalot et Reichardt 1996

Dimensions: Length 20-39 µm; Width 4,3-6 µm. Striae: 12-16/10 µm. Puncta: c. 32/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H4, H5.

Optimal environmental conditions: Characteristic species of electrolyte-poor, usually weakly acidic, nutrient-poor freshwater habitats on siliceous substrata (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

5. *Gomphonema gracile* Ehrenberg 1895

Dimensions: Length 20-100 µm; Width 4-11 µm. Striae: 9-17/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

6. *Gomphonema hebridense* Gregory 1854

Dimensions: Length 20-70 µm; Width 4,5-8 µm. Striae: 12-18/10 µm. Puncta: Indistinct.

Distribution in freshwater habitats on Vranica mountain: H5.

Distribution in Bosnia and Herzegovina:

Optimal environmental conditions: The species prefers electrolyte-poor, usually oligotrophic, circumneutral lakes and running waters of the central highlands, more rarely recorded from the lowlands, but also found in the calcareous Alps (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.) and oligohalobous. oligosaprobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

7. *Gomphonema italicum* Kützing 1844

Dimensions: Length 19-53,5 µm; Width 9,3-14 µm. Striae: 10-16/10 µm. Puncta: 23-29/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Distribution in the freshwater habitat types not well known, because this species was formerly not separated from similar taxa and identified as *G. truncatum*. According to Reichardt (2001), it occurs in meso- to eutrophic freshwater habitats but also in lakes that regained an oligotrophic status thanks to restoration project (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

8. *Gomphonema minusculum* Krasske 1932

Dimensions: Length 14-32,7 µm; Width 2,8-4,6 µm. Striae: 12-16/10 µm. Puncta: 13-15/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H5.

Optimal environmental conditions: Widely distributed and frequent, locally in high numbers. Wide ecological amplitude, from oligo- to eutrophic, standing freshwater habitats on carbonate to siliceous substrata (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

9. *Gomphonema olivaceum* (Hornemann) Brébisson 1838

Dimensions: Length 12-42 µm; Width 5,5-9 µm. Striae: 8-12/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3.

Optimal environmental conditions: Mostly eutrophic and moderately electrolyte-rich, carbonate habitats. The saprobic tolerance ranges from oligosaprobic to the threshold between β - to α -mesosaprobic zones (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkalibiontic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

10. *Gomphonema parvulum* (Kützing) Kützing 1849

Dimensions: Length 22-29 µm; Width 5-7,5 µm. Striae: 12-20/10 µm. Puncta: Indistinct.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: Oligosaprobic and mesosaprobic freshwater habitats, often independly from the trophic status (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, facultative N-heterotrophic, neutrophilic, low O₂ (30% sat.), oligohalobous, α-meso - > polysaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

11. *Gomphonema productum* (Grunow) L-B et Reichardt in Lange-Bertalot 1993

Dimensions: Length 20-35 µm; Width 6-7,5 µm. Striae: 8-14/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2.

Optimal environmental conditions: Electrolyte-poor, usually weakly acid freshwater habitats on siliceous substrata. Occurring also in weakly buffered waterholes on other bedrocks types, as long as these are filled by deposition water, together with dying plant materials such as sedges or conifer needles. Indicator of very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

12. *Gomphonema rhombicum* M. Schmidt 1904

Dimensions: Length 36-53 µm; Width 5,4-7,6 µm. Striae: 13-14/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: Oligotrophic, electrolyte-poor streams and lakes on siliceous substrata (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is sensitive N-autotrophic, acidobiontic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

13. *Gomphonema subclavatum* (Grunow) Grunow 1884

Dimensions: Length 25-70 µm; Width 8-10 µm. Striae: 8-10/10 µm. Puncta: 25-30/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H5.

Optimal environmental conditions: Poorly known, because the taxon was included in *G. clavatum* or due to the unclear species concepts of different authors. Oligosaprobic freshwater habitats with a wider amplitude as concerns electrolyte content and pH (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, beta-mesosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

14. *Gomphonema truncatum* Ehrenberg 1832

Dimensions: Length 17-48 µm; Width 8,5-13,5 µm. Striae: 10-15/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Distribution in the different freshwater habitats types not well known, because the species was formerly not distinguished from *G. capitatum* and other similar taxa. The species complex is overall widely distributed in alkaline, oligo- to β-mesosaprobic running- and still waters, is found over a wide range of trophic levels but is rather rarely abundant. According to Reichardt (2001) *G. truncatum* appears to be more widely distributed than *G. capitatum* (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, beta-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Naviculales

Family: Cavinulaceae

Cavinula D.G.Mann & A.J.Stickle, 1990

Holotype species: *Cavinula cocconeiformis* (W.Gregory ex Greville) D.G.Mann & A.J.Stickle

Valves are characterized by uniseriate, radial striae composed of round to elongate areolae. Valves are linear-lanceolate to nearly circular in outline. Externally, the proximal raphe ends are expanded, while the internal proximal raphe ends are straight. Terminal raphe fissures are absent. Internally, the central sternum is thickened. One or two chloroplasts are present in living cells, which are H-shaped in girdle view. This genus is known from oligotrophic lakes and moist subaerial habitats across the United States. It includes the former *Navicula cocconeiformis* Gregory and its allies (Spaulding et al. 2022).

During research for this study one species from the genus *Cavinula* were identified. Detailed characteristics about the identified species from the genus *Cavinula*, distribution and ecological preferences are present further in this study.

1. *Cavinula cocconeiformis* (Gregory ex Grev.) D.G.Mann and Stickle in Round, R.M.Crawford and D.G.Mann 1990

Dimensions: Length 12-40 µm; Width 7-15 µm. Striae: 24-36/10 µm. Puncta: 20-40/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Electrolyte-poor streams and lakes on siliceous substrata with low trophic levels (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

Class: Bacillariophyceae

Order: Naviculales

Family: Amphipleuraceae

Amphipleura Kützing, 1844

Lectotype species: *Amphipleura pellucida* (Kützing) Kützing

Amphipleura is distinguished by the narrow, median rib that extends along the long axis of the valve. This rib is formed on the internal face of the valve, a feature observable in SEM. Near the valve apices, the rib forms two branches, or 'needle eyes', around the raphe system. The raphe of *Amphipleura* is short, limited to the area within the "needle eyes". The valves of *Amphipleura* are linear or spindle-shaped. The striae are composed of extremely fine areolae (0.25 µm in size), which are difficult to resolve, except under conditions of optimal LM resolution. Because of the fine striae, *Amphipleura pellucida* is often used as a test organism for microscope optics. The chloroplast is composed of one H-shaped plastid with a central pyrenoid. *Amphipleura* is common in epipelagic habitats of lentic and slowly moving waters, predominately in alkaline pH ranges. Cells grow singly, or may be enclosed in gelatinous tubes (Spaulding et al. 2022).

During research for this study one species from the genus *Amphipleura* were identified. Detailed characteristics about the identified species from the genus *Amphipleura*, distribution and ecological preferences are present further in this study.

1. *Amphipleura pellucida* (Kützing) Kützing 1844

Dimensions: Length 80-140 µm; Width 7-9 µm. Striae: 37-40/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3

Optimal environmental conditions: More frequent in standing freshwater habitats than in running waters. Calcium-bicarbonate-rich freshwater habitats, occasionally with high electrolyte content, also in weakly-brackish waters. Rare in electrolyte-poor habitats on siliceous substrata (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, α-meso → polysaprobe and oligomesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Frustulia Rabenhorst, 1853, nom. et typ. cons.

Holotype species: *Frustulia saxonica* Rabenhorst

Frustulia valves are rhomboid to linear-lanceolate with straight to undulate margins. The striae are composed of fine areolae, arranged into patterns that appear to form both apical and transapical rows. The genus possesses distinct median, longitudinal ribs that extend most of the length of the valve. The raphe is located between the longitudinal ribs. At the valve terminus, the ribs form a single tip, or porte-crayon. The proximal and distal raphe ends are not clearly observed with light microscopy. Living cells possess one H-shaped plastid. *Frustulia* grows in benthic habitats as single cells or as

colonies in mucilaginous tubes. Species of *Frustulia* are widespread in North America. Cells reach their greatest abundance in waters such as seepage lakes where the water is slightly acidic, dissolved organic carbon (DOC) is high, and specific conductivity is relatively low (Spaulding et al. 2022).

During research for this study three species from the genus *Frustulia* were identified. Detailed characteristics about the identified species from the genus *Frustulia*, distribution and ecological preferences are present further in this study.

1. *Frustulia crassinervia* (Brébisson) Lange-Bertalot et Krammer in Lange-Bertalot & Metzeltin 1996

Dimensions: Length 30-55 µm; Width 8-12,5 µm. Striae: 30-35/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Characteristic species of dystrophic, electrolyte poor freshwater habitats, often forming a large proportion of the assemblage in raised bogs. Indicator of very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidobiontic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

2. *Frustulia saxonica* Rabenhorst 1853

Dimensions: Length 28-105 µm; Width 10-18 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Autecology and occurrence resemble those of *F. crassinervia*, with which it is sometimes associated. Characteristic species of dystrophic, electrolyte-poor freshwater habitats, often forming a large proportion of the assemblages in raised bogs. Indicator of very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidobiontic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

3. *Frustulia vulgaris* (Thwaites) De Toni 1891

Dimensions: Length 40-60 µm (and more); Width 27-32 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2.

Optimal environmental conditions: Wide ecological amplitude. Represent in all freshwater habitat types and sometimes locally abundant. The spectrum ranges from oligotrophic, through slightly dystrophic, to β -a-mesotrophic running waters to tidal, brackish-water habitats of the coastal marshes. It prefers well aerated biotopes, and is also found in pseudoaerial habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous β -mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Naviculales

Family: Brachysiraceae

Brachysira Kützing, 1836

Holotype species: *Brachysira aponina* Kützing

Valves of *Brachysira* are linear to linear-lanceolate in outline. The ends of the valves, or apices, may be rounded to protracted. In general, the valves are symmetric to the apical axis, although some specimens may have a slight, to strong, asymmetry to the transapical axis. The striae are finely punctate, and form longitudinal undulations. The raphe is straight and the axial area is narrow. In the SEM, an elevated siliceous ridge separating the valve face and mantle on the external valve surface is visible. Cells possess a single plastid. *Brachysira* may grow singly and unattached, or it may grow at the ends of mucilaginous stalks. Species within the genus are broadly distributed across North America in a range of trophic states, with individual species characteristic of oligotrophic to dystrophic waters. Cell abundance may be especially high in low conductivity and low pH waters, for example, in bog habitats. The genus is primarily benthic, but may also be found in lake plankton, where cells may become entrained (Spaulding et al. 2022).

Most species of the genus are found exclusively in oligotrophic freshwater habitats and indicate very good ecological quality. However, the generitype is an exception, found in salt-rich waters (Lange-Bertalot et al. 2017).

During research for this study two species from the genus *Brachysira* were identified. Detailed characteristics about the identified species from the genus *Brachysira*, distribution and ecological preferences are present further in this study.

1. *Brachysira brebissonii* Ross in Hartley 1986

Dimensions: Length 12-45 µm; Width 4,5-8,0 µm. Striae: 24-27/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Freshwater habitats with low mineral content, moderately- to strongly-acid due to humic acids but not at risk of acidification. Streams and still waters on siliceous substrata. Good indicator of naturally acid, rather than anthropogenically acidified freshwater habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, oxybiontic (75% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

2. *Brachysira intermedia* (Østrup) Lange-Bertalot in Lange-Bertalot & Moser 1994

Dimensions: Length 25-33 µm; Width 5,0-6,5 µm. Striae: 26-30/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4, H5.

Optimal environmental conditions: Distribution in central Europe is not precisely known, because the species was not separated *B. brebissoni* in the past. It appears to be rare but locally abundant. Characteristic habitats are oligo-to dystrophic, with very low electrolyte content, on siliceous substrata, particularly in mountains (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is acidophilic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

Class: Bacillariophyceae

Order: Naviculales

Family: *Naviculales incertae sedis*

Chamaepinnularia Lange-Bertalot & Krammer, 1996 (Naviculaceae)

Holotype species: *Chamaepinnularia vyvermanii* Lange-Bertalot & Krammer

The valve margins of *Chamaepinnularia* are linear, nearly linear, or undulate. Cells are small, usually less than 25 µm in length and 4 µm in breadth. The striae are composed of simple, chamber-like areolae, with external openings covered by vela. Internally, the areola openings are divided with internal plates of silica. The raphe system may be simple or complex in structure, as in *Pinnularia* or *Navicula*. Externally, the distal raphe fissures are hooked, and terminate internally with a small helictoglossa. The external proximal raphe ends are inconspicuous. Internally, the proximal ends are unilaterally hooked. Cells grow singly, rather than in colonies. Many of the species are aerophilic and grow in splash-zones of streams, moist mosses and lichen habitats. The genus includes a number of smaller species formerly part of *Navicula*, including *N. soehrensii* Krasske (Spaulding et al. 2022).

During research for this study one species from the genus *Chamaepinnularia* were identified. Detailed characteristics about the identified species from the genus *Chamaepinnularia*, distribution and ecological preferences are present further in this study.

1. *Chamaepinnularia mediocris* (Krasske) Lange-Bertalot 1996

Dimensions: Length 9-16 µm; Width 2-3 µm. Striae: 20-23/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Limited to oligo- to dystrophic, acid springs, streams, and standing freshwater habitats. Also frequent in anthropogenically acidified freshwater habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

Kobayasiella Lange-Bertalot, 1999

Holotype species: *Kobayasiella bicuneus* (Lange-Bertalot) Lange-Bertalot

Kobayasiella valves are linear to linear-lanceolate, with ends rostrate or expanded and capitate. Because the striae often number more than 30 in 10 microns, they may not be visible in the light microscope. The striae are radiate in the center of the valve and become convergent at the valve apices. The raphe is filiform, with proximal raphe ends expanded. The raphe has fine, but distinctive 'kinks' or deflections at midvalve. Terminal raphe ends are strongly hooked. *Kobayasiella* is found in low nutrient and acid waters, including peats of the northern hemisphere. As a group, this genus appears to be sensitive to anthropogenic influence, and negatively impacted by human activities. The genus contains the former *Navicula subtilissima* Cleve and its allies. *Kobayasiella* replaces the illegitimate genus *Kobayasia* Lange-Bertalot 1996 (Spaulding et al. 2022).

During research for this study one species from the genus *Kobayasiella* were identified. Detailed characteristics about the identified species from the genus *Kobayasiella*, distribution and ecological preferences are present further in this study.

1. *Kobayasiella parasubtilissima* (Kobayasi et Nagumo) Lange-Bertalot 1999

Dimensions: Length 18-38 µm; Width 3,5-6,0 µm. Striae: 40-42/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Raised bogs and associated habitats where it can form massive growths. In Europe (Germany) rare and exclusively in electrolyte-poor, more or less acid and dystrophic streams and lakes of the siliceous central highlands (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

Class: Bacillariophyceae

Order: Naviculales

Family: Stauroneidaceae

Craticula Grunow, 1868

Holotype species: *Craticula perrotettii* Grunow

The valves of *Craticula* are lanceolate. The striae are distinctly parallel, or nearly parallel. The central area is greatly reduced or absent. The genus is polymorphic, forming different valve morphologies. There are three valve types found for this taxon: vegetative, heribaudii, and craticula forms. The three forms are produced as a cellular strategy for surviving desiccation (Schmid 1979).

Two craticula are formed within the vegetative frustule. Subsequently the heribaudii cell is formed within the craticula. The heribaudii cell usually has different ornamentation compared to the vegetative cell. Note that these stages are not always present in a population, and for many of the smaller species, the craticular and heribaudii stages have not been observed. Living cells possess two elongate, plate-like chloroplasts appressed to the girdle. *Craticula* is a term applied to internal valves that are formed within frustules that are normal in morphology. The craticular valves consist of a raphe-sternum and robust transverse bars. The genus *Craticula* occurs in epipelagic habitats (Spaulding et al. 2022).

During research for this study one species from the genus *Craticula* were identified. Detailed characteristics about the identified species from the genus *Craticula*, distribution and ecological preferences are present further in this study.

1. *Craticula cuspidata* (Kütz.) D.G.Mann 1990

Dimensions: Length 65-170 µm; Width 17-35 µm. Striae: 11-15/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Frequently recorded from inland habitats with high electrolyte content and brackish waters, tolerant of a-mesosaprobic conditions; however rarely recorded in rivers more strongly impacted by industrial waste waters (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is neutrophilic, oligohalobous and oligosaprobe.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Naviculales

Family: Diadesmidaceae

Luticola D.G.Mann, 1990

Holotype species: *Luticola mutica* (Kützing) D.G.Mann

Valves of *Luticola* have an expanded central area with a distinct stigma in the central area. The striae are distinctly punctate. Proximal raphe ends are slightly deflected unilaterally. Distal raphe ends are deflected to the same side as the proximal raphe ends. Living cells possess a single chloroplast. *Luticola* is a widespread aerophilic genus, typical of soil and moss habitats (Spaulding et al. 2022).

During research for this study one species from the genus *Luticola* were identified. Detailed characteristics about the identified species from the genus *Luticola*, distribution and ecological preferences are present further in this study.

1. *Luticola mutica* (Kützing) D.G. Mann 1990

Dimensions: Length 11-28 µm; Width 6,0-9,5 µm. Striae: 16-18/10 µm. Puncta: c.15/10 µm. Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Brackish water and clean freshwater in situation where there are fluctuation in osmotic pressure, for instance in the supralittoral or in lock of larger navigable rivers. This is also one of the most characteristic diatoms in periodically humid sites, e.g. in soil deposits in wall cracks and rock fissures. Saprobic tolerance significantly lower than that of *L. goeppertiana*, only up to the β - α -mesosaprobic level (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, tolerant N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophilic, α -mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Humidophila (Lange-Bertalot & Werum) R.L.Lowe & al., 2014

Holotype species: *Humidophila undulata* R.L.Lowe, Kociolek & J.R.Johansen

Valves are small, usually less than 20 μm . Valves are linear, linear-elliptic or elliptic. The apices are broadly rounded. Striae on the valve face are composed of one usually transapically elongated, elliptic to ovoid areola. The raphe may be secondarily filled with silica. Cells are often joined to form filamentous colonies. There are number of additional morphological features of *Humidophila*, many of which are visible only with SEM. The valve face is flat and may have a narrow rim, or ridge, differentiating the face from the mantle. A single elongated areola lies on the mantle, in line with each stria. Areolae on the mantle may appear to be located on the valve face in species that lack a narrow rim. This arrangement gives the impression of two areolae per stria on the valve face (but there is actually only one). Near the apices, slit-shaped areolae may be present on the mantle. Internally, areolae are internally covered by a porous hymen. The raphe is filiform and straight. The external proximal raphe ends are simple and straight, or they may be expanded pores or anchor-shaped. The proximal ends are not deflected, bent or hooked. Externally, the distal raphe ends are straight and lack terminal fissures. Internally, the distal raphe terminates in a small helictoglossa. The genus *Humidophila* is most abundant in aerial or subaerial habitats, and the genus is particularly diverse in the Antarctic and sub-Antarctic. Cosmopolitan taxa include *H. contenta* (Grunow ex Van Heurck) Lowe et al. and *H. paracontenta* (Lange-Bert. and Werum) Lowe et al. (Spaulding et al. 2022).

During research for this study two species from the genus *Humidophila* were identified. Detailed characteristics about the identified species from the genus *Humidophila*, distribution and ecological preferences are present further in this study.

1. *Humidophila contenta* (Grunow) R.L.Lowe & al. 2014

Dimensions: Length 4-10 μm ; Width 2-3 μm . Striae: c. 40/10 μm .

Distribution in freshwater habitats on Vranica mountain: H1, H2.

Optimal environmental conditions: Pseudoaerial species that often abundant at the air/water interface, for instance rocks overflow by film a water, swamps, bryophyte carpets, spring. Frequently recorded from biotopes with low light intensity, where it is often associated with *D. galica* W. Smith (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, tolerant N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous, β -mesosaprobe and indifferent.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

2. *Humidophila perpusilla* (Grunow) Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová 2014

Dimensions: Length 6-14 μm ; Width 4-5 μm . Striae: 24 to c. 30/10 μm .

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: In aerial biotopes e.g. intermittently wet bryophyte carpets, rocks, earth frequently associated with *H. contenta*. Also found in habitats with low light intensity such as caves and bedrock crevices (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is terrestrial, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Naviculales

Family: Diploneidaceae

Diploneis Ehrenberg ex Cleve, 1894

Lectotype species: *Diploneis didymus* (Ehrenberg) Ehrenberg

Frustules of *Diploneis* are typically elliptic to panduriform, with bluntly rounded apices. Each valve possesses two longitudinal canals, one on each side of the raphe. The canals are positioned within the silica cell wall and open to the exterior through pores, but lack openings to the interior of the cell. The function of these canals is uncertain. The frustules are often heavily silicified, with complex (loculate) areolae. The genus *Diploneis* is large and diverse, with most species primarily from epipelagic habitats. Recent publications clarify a unique set of freshwater species and unappreciated diversity of species in the genus *Diploneis* (Jovanoska 2015, Kulikovskiy et al. 2015, Lange-Bertalot and Fuhrmann 2016, 2017).

These works challenge the long-standing mindset that *Diploneis* is primarily a marine genus with few freshwater representatives. While the freshwater species may be both infrequent and not abundant (Lange-Bertalot and Fuhrmann 2017), there is a much larger species richness in oligotrophic waters. Furthermore, these authors point out that European rivers have been so altered by nutrients and other pollutants that many species of *Diploneis* are simply not encountered in modern surveys. When species of *Diploneis* are encountered in surveys, they are few in number and they are often overlooked (Spaulding et al. 2022).

Diploneis krammeri Lange-Bertalot et Reichardt are often recorded as *D. ovalis* (Hilse) Cleve in the past. True *D. ovalis* was first described from Silesia but rare in central

Europe. It is more frequent in colder European and American areas. It can be distinguished by its large central areas (6-10 µm in diameter).

During research for this study two species from the genus *Diploneis* were identified. Detailed characteristics about the identified species from the genus *Diploneis*, distribution and ecological preferences are present further in this study.

1. *Diploneis krammeri* Lange-Bertalot et Reichardt 2004

Dimensions: Length 20-65 µm; Width 10 to c. 25 µm. Striae: 10-14/10 µm. Puncta: 10-15/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H5.

Optimal environmental conditions: Optimum habitat are well aerated lakes or intermittently wet habitats, often found in wetted bryophyte carpets (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous and oligosaprobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

2. *Diploneis ovalis* (Hilse) Cleve 1891

Dimensions: Length 15-52 µm; Width 9-23 µm. Striae: 10-14/10 µm. Areolae: 16-20/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.), oligohalobous and oligosaprobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

Class: Bacillariophyceae

Order: Naviculales

Family: Naviculaceae

Caloneis Cleve, 1894

Lectotype species: *Caloneis amphisbaena* (Bory) Cleve

Cells solitary, naviculoid. Valves usually linear-lanceolate to elliptical, sometimes with capitate or rostrate poles and sometimes with undulate margins. Valve face flat or curving smoothly into relatively deep mantles. Axial and central areas variable in shape. Terminal fissures of the raphe are usually distinct. Striae basically multiseriate but usually also chambered. The outer wall of the chambers (alveoli) perforated by many rows of small, round poroids occluded by hymenes. The poroids are usually invisible in the light microscope. The inner wall of each alveolus typically opens to the inside of the valve by one fairly large, transapically elongate aperture. The inner apertures usually located

in approximately the same place on each alveolus, thus giving the appearance of one or several longitudinal lines crossing the striae on both sides of the axial area. The girdle consists of a few open bands. *Caloneis* has been reported from fresh, brackish and marine waters. Because the structure of *Caloneis* is so similar to that of *Pinnularia*, some researchers (e. g., Round and others, 1990) have included *Caloneis* within *Pinnularia*. Traditionally, the genera have been separated by the shape of their alveoli. The alveoli of *Caloneis* are usually thinner and denser than those of *Pinnularia*. Species of *Caloneis* are characterized by chambered striae, like those of *Pinnularia*. The chambered striae give the appearance of one to two longitudinal lines. The striae of *Caloneis* are composed of fine alveoli, also like *Pinnularia*. The central area of *Caloneis* may be expanded, and may include silica thickenings that are lunate or irregular in shape. Living cells possess one or two plastids. Several species within *Caloneis* are common in North America in alkaline, brackish and marine habitats. Several taxonomists suggest that *Caloneis* is a synonym of the closely related genus *Pinnularia* and that the two genera should be combined into a single genus (Spaulding et al. 2022).

During research for this study four species from the genus *Caloneis* were identified. Detailed characteristics about the identified species from the genus *Caloneis*, distribution and ecological preferences are present further in this study.

1. *Caloneis aerophila* Bock 1963

Dimensions: Length 14-25 μm ; Width 3-5 μm . Striae: 18-25/10 μm .

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Pseudoaerial, mountain habitats, in particular on damp rocks. Acid springs and headwaters (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is terrestrial and halophobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is very rare (R).

2. *Caloneis alpestris* (Grunow) Cleve 1894

Dimensions: Length 45-92 μm ; Width 10-15 μm . Striae: 19-24/10 μm .

Distribution in freshwater habitats on Vranica mountain: H1, H2, H5.

Optimal environmental conditions: Calcium-bicarbonate-rich, nutrient-poor lakes of the Alpine and peri-Alpine region, rare in running waters (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic, oligohalobous, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

3. *Caloneis silicula* (Ehrenberg) Cleve 1894

Dimensions: Length 27-45 μm ; Width 5-7 μm . Striae: 14-20/10 μm .

Distribution in freshwater habitats on Vranica mountain: H1, H4, H5.

Optimal environmental conditions: Littoral of standing freshwater habitats with medium electrolyte content, usually in low numbers. Population with different outline are commonly found together (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, oligosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Caloneis tenuis* (Gregory) Krammer 1985

Dimensions: Length 20-50 µm; Width 4-7 µm. Striae: 16-24/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Standing, more rarely flowing, oligosaprobic freshwater habitats with low to medium electrolyte content and low trophic level (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

Gyrosigma Hassall, 1845, nom. cons.

Holotype species: *Gyrosigma hippocampus* Hassall

Valves are sigmoid. The axial area is narrow and also sigmoid, containing a sigmoid raphe. The striae are comprised of punctate areolae and form rows that are perpendicular and parallel to the apical and transapical axes. The central area is round to elliptic. External proximal raphe ends are deflected in opposite directions from one another. In living cells, two plastids are present. *Gyrosigma* is characteristic of epipellic and endopellic habitats. The genus is widely distributed in freshwaters, with some species found in brackish waters (Spaulding et al. 2022).

During research for this study two species from the genus *Gyrosigma* were identified. Detailed characteristics about the identified species from the genus *Gyrosigma*, distribution and ecological preferences are present further in this study.

1. *Gyrosigma acuminatum* (Kützing) Rabenhorst 1853

Dimensions: Length 60-180 µm; Width 11-24 µm. Striae: 16-24/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: Alakline freshwater habitats, mainly running waters, with high trophic level. Sensitive to organic pollution above β-a-mesosaprobic level. In Central Europe widely distributed but usually only in small to moderate numbers (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, alkalibiontic, moderate O₂ (>50% sat.), oligohalobous, β-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Gyrosigma attenuatum* (Kützing) Rabenhorst 1853

Dimensions: Length 150-240 µm; Width 23-26 µm. Striae: 10-12/10 µm. Puncta: 14-16/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: In flowing and standing alkaline freshwater habitats with moderately low to high trophic levels, particularly large rivers; sensitive towards organic pollution above the β - α -mesosaprobic levels (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkalibiontic, moderate O₂ (>50% sat.), oligohalobous, β -mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Hippodonta Lange-Bertalot, Witkowski & Metzeltin, 1996

Holotype species: *Hippodonta luneburgensis* (Grunow) L-B, Metzeltin & A.Witkowski

Species in *Hippodonta* possess thick, heavily silicified valves. Valves are generally lanceolate in valve outline with variously shaped poles. The striae are distinct, broad and may be uni-seriate or biseriate. The valve termini are thickened bands of silica, termed polar bars. The raphe is straight and the proximal raphe ends are dilated. Two chloroplasts are present in living cells. *Hippodonta* includes the former *Navicula capitata* Ehrenberg, *N. hungarica* Grunow and allies. Species of *Hippodonta* grow in a wide range of benthic habitats, particularly in mid to higher salinity inland and coastal waters (Spaulding et al. 2022).

During research for this study one species from the genus *Hippodonta* were identified. Detailed characteristics about the identified species from the genus *Hippodonta*, distribution and ecological preferences are present further in this study.

1. *Hippodonta coxiae* Lange-Bertalot 2001

Dimensions: Length 20-30 (47) μ m; Width 5-7 μ m. Striae: 8-10/10 μ m.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Distribution and ecological preferences still not known due to confusion with similar species in the past. Existing records are from the central highlands but a wider distribution is very likely (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is alkaliphilic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G).

Navicula Bory, 1822

Holotype species: *Navicula tripunctata* (O.F.Müller) Bory

Valves of *Navicula* tend to be elliptic to broadly-lanceolate in outline. Valve ends may be capitate, acute, rounded, or not expanded. The central sternum is thickened, and that thickening may be somewhat asymmetric. Pseudosepta may be present, or absent. The raphe is straight and filiform, or lateral in some species. The proximal raphe ends are slightly deflected to one side. The central area may be expanded, but the silica is not thickened into a fascia. A defining character of *Navicula* is the lineate areolae, or lineolae. During the mid-1900's, primarily through the work of Hustedt, *Navicula* became a genus that contained a large number of unrelated lanceolate, biraphid species. Many

of these unrelated species have been separated into new genera or replaced into the older, originally described genera. Note that many species remain within *Navicula* that do not belong to *Navicula* in the strict sense (Spaulding et al. 2022).

During research for this study five species from the genus *Navicula* were identified. Detailed characteristics about the identified species from the genus *Navicula*, distribution and ecological preferences are present further in this study.

1. *Navicula concentrica* Carter 1981

Dimensions: Length 40-75 µm; Width 9-12 µm. Striae: 8-10/10 µm. Lineolae: Distinct, c. 25/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Oligo- to oligo-mesotrophic lakes across a wide range of calcium concentrations, thus also in calcium-bicarbonate-poor, circumneutral lakes. Indicator of very good ecological quality (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is halophobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

2. *Navicula lanceolata* (C.Agardh) Ehrenberg 1893

Dimensions: Length 28-70 µm; Width 8-12 µm. Striae: 10-13/10 µm. Lineolae: c. 32/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3.

Optimal environmental conditions: All running-water types, provided that they are not nutrient-poor and/or more or less acid. Preference for lower temperatures: frequently developing massive occurrence in the winter and spring months. Ecological amplitude wide: from oligosaprobic to α-mesosaprobic conditions but preference for high trophic levels. In lakes distinctly rarer and in lower numbers than in running waters. In central Europe is one of the most frequent diatoms (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, alkaliphilic, moderate O₂ (>50% sat.), halophilic, α-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Navicula radiosa* Kützing 1844

Dimensions: Length 40-120 µm; Width 8-12 µm. Striae: 10-12/10 µm. Lineolae: very fine, 28-32/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H3, H4, H5.

Optimal environmental conditions: Streams, small rivers, and standing freshwater habitats. Rarely also in large rivers. Ecological amplitude wide: from electrolyte-poor to electrolyte-rich, from weakly acid to strongly alkaline, and from oligotrophic to eutrophic conditions. The species is however sensitive towards organic pollution exceeding the β-mesosaprobic level (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, neutrophilic, oxybiontic (75% sat.), oligohalobous, β-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Navicula splendidula* Van Landingham 1875

Dimensions: Length 30-46 µm; Width 7-9 µm. Striae: 12-16/10 µm. Lineolae: Relatively coarse, c. 25/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: Oligo-mesotrophic to eutrophic, calcium-bicarbonate-rich running waters; very rarely (single specimens) also in lakes (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

5. *Navicula tripunctata* (O.F. Müller) Bory 1822

Dimensions: Length 30-70 µm; Width 6-10 µm. Striae: 9-12/10 µm. Lineolae: Often still resolvable, c. 32/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H3.

Optimal environmental conditions: All running waters and lake types with medium to high trophic levels. Absent in oligotrophic as well as in acidic environments. Saprobity tolerant up to the β-a level (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, β-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Naviculales

Family: Neidiaceae

Neidium Pfitzer, 1871

Holotype species: *Neidium affine* (Ehrenberg) Pfitzer

Neidium valves are linear to linear elliptical. Ends are variable in shape across species and may be rounded, capitate, rostrate or protracted. Longitudinal lines are present, formed by internal canals positioned along the valve margins. The proximal raphe ends are bilaterally deflected or straight. In many species, the distal raphe ends appear to bifurcate, forming an apical flap. Interruptions in the striae, or Voigt discontinuities, are frequently present in *Neidium*. Voigt discontinuities are positioned on the secondary side of the axial area along each raphe branch. The striae are uniseriate and composed of rounded, distinct areolae. Two plastids are present in living cells, positioned against the girdle. *Neidium* is rarely abundant in collections, but the genus is broadly distributed. *Neidium* species often grow in slightly acid waters (Spaulding et al. 2022).

During research for this study five species from the genus *Neidium* were identified. Detailed characteristics about the identified species from the genus *Neidium*, distribution and ecological preferences are present further in this study.

1. *Neidium affine* (Ehrenberg) Pfitzer 1871

Dimensions: Length 38-70 µm; Width 8-16 µm. Striae: 22-26/10 µm. Puncta: 21-26/10 µm. Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Oligotrophic to weakly-dystrophic freshwater habitats with low electrolyte content. Records from calcium-bicarbonate-rich freshwater habitats should be considered carefully, because they may represent an independent taxon that is difficult to distinguish from *N. affine* using morphological criteria. Generally, confusion with morphologically similar taxa that do not match the criteria for *N. affine* exactly should not affect ecological-status assessments, because the indicator value of these species is almost identical (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

2. *Neidium ampliatum* (Ehrenberg) Krammer in the sense of K. et Lange-Bertalot 1985

Dimensions: Length 60-105 µm; Width 17-25 µm. Striae: 16-17/10 µm. Puncta: 15-17/10 µm. Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Oligo- to mesotrophic freshwater habitats with low electrolyte-rich habitats, including those located on carbonate substrata should be examined closely to confirm that these do not correspond to other species (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, neutrophilic, oligohalobous and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

3. *Neidium bisulcatum* (Lagerstedt) Cleve 1894

Dimensions: Length 28-82 µm; Width 7-9 µm. Striae: 26-30/10 µm. Puncta: 26-30/10 µm. Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Oligo- to dystrophic, weakly acid or circumneutral freshwater habitats with low electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

4. *Neidium bisulcatum* var. *subampliatum* Krammer 1985

Dimensions: Length 28-82 µm; Width 7-9 µm. Striae: 20-21/10 µm. Puncta: c. 20/10 µm. Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is halophobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is endangered (3).

5. *Neidium longiceps* (Gregory) Ross 1947

Dimensions: Length 20-38 µm; Width 5,5-7,0 µm. Striae: 28-33/10 µm. Puncta: c. 26/10 µm. Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Oligo- to dystrophic, electrolyte-poor, weakly acid freshwater habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G).

Class: Bacillariophyceae

Order: Naviculales

Family: Pinnulariaceae

Pinnularia Ehrenberg, 1843, nom. et typ. cons.

Holotype species: *Pinnularia viridis* (Nitzsch) Ehrenberg

Frustules of *Pinnularia* may be large, up to 300 µm in length. The striae are alveolate. Internally, the striae are positioned within chambers. The openings of the chambers are evident as longitudinal lines that cross the striae. The raphe system may be straight or complex. Externally, the proximal raphe ends are expanded and bent slightly to the same side. Terminal raphe fissures are deflected and may form a distinct, curving bend (a question mark shape). The central area may be expanded to one or both sides. Living cells contain two plastids. *Pinnularia* is closely related to *Caloneis* and some authors suggest they should be considered as the same genus. *Pinnularia* contains a large number of species and is often abundant in low conductance, slightly acid waters (Spaulding et al. 2022).

During research for this study twelve species from the genus *Pinnularia* were identified. Detailed characteristics about the identified species from the genus *Pinnularia*, distribution and ecological preferences are present further in this study.

1. *Pinnularia appendiculata* (C. Agardh) Cleve 1896

Dimensions: Length 16-46 µm; Width 4,7-6,1 µm. Striae: 16-18/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H5.

Optimal environmental conditions: Scattered to rather frequent, occasionally in very high numbers in salt-rich inland waters such as saltworks, alkaline (soda-)lakes with high to medium electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe oligosaprobe and oligo-mesotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Pinnularia borealis* Ehrenberg 1843

Dimensions: Length 24-42 µm; Width 8,5-10 µm. Striae: 5-6/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4, H5.
Optimal environmental conditions: Pseudoaerial. Soils, bryophyte carpets, rock walls; also in different running waters and springs, rarer in lakes (Lange-Bertalot et al. 2017).
General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, β -mesosaprobe and oligo-mesotrophic.
Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Pinnularia microstauron* (Ehrenberg) Cleve 1891

Dimensions: Length 20-100 μm ; Width 7-15 μm . Striae: 2.2-6.6/10 μm . Puncta: 9-14/10 μm .
Distribution in freshwater habitats on Vranica mountain: H1, H4, H5.
Optimal environmental conditions: Scattered in oligotrophic, very electrolyte-poor habitats such as different mires (Lange-Bertalot et al. 2017).
General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic and aerophilic, tolerant N-autotrophic, neutrophilic, moderate (>50% sat.), oligohalobous, β -mesosaprobe and indifferent.
Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

4. *Pinnularia neomajor* Krammer 1992

Dimensions: Length 114-250 μm ; Width 17-30 μm . Striae: 6-8/10 μm .
Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H4, H5.
Optimal environmental conditions: Scattered, mainly in standing oligo- to dystrophic freshwater habitats and mires with low electrolyte content (Lange-Bertalot et al. 2017).
General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, neutrophilic, oxybiontic (75% sat.), oligohalobous, β -mesosaprobe and meso-eutrophic.
Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

5. *Pinnularia obscura* Krasske 1932

Dimensions: Length 12-34 μm ; Width 3-5.4 μm .
Distribution in freshwater habitats on Vranica mountain: H4.
Optimal environmental conditions: Rather frequent, in aerial, intermittently wet habitats, such as submerged bryophyte carpets overflowed by slow currents, damp rocks, and occasionally dampened soils (Lange-Bertalot et al. 2017).
General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous and oligosaprobe.
Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

6. *Pinnularia perirrorata* Krammer 2000

Dimensions: Length 17-30 μm ; Width 4.2-4.4 μm . Striae: 16-18/10 μm .
Distribution in freshwater habitats on Vranica mountain: H5.
Optimal environmental conditions: Electrolyte-poor, acid streams of siliceous regions. Rarer in standing freshwater habitats. Tolerates anthropogenic acidification (Lange-Bertalot et al. 2017).
General distribution and ecology: According to Van Dam et al. (1994), this species is

acidophilic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

7. *Pinnularia rupestris* Hantzsch in Rabenhorst 1861

Dimensions: Length 40-90 µm; Width 9-12,40 µm. Striae: 12-13/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H5.

Optimal environmental conditions: Oligotrophic, oxygen-rich, electrolyte-poor raised bogs (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, neutrophilic, halophobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

8. *Pinnularia schoenfelderi* Krammer 1992

Dimensions: Length 19-37 µm; Width 5-7 µm. Striae: 13-16/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Anthropogenically undisturbed, oligo- to dystrophic, usually electrolyte-poor running waters and peatlands.

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, neutrophilic, moderate O₂ (>50% sat.), oligohalobous, β-mesosaprobe and oligotrophic (Lange-Bertalot et al. 2017).

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

9. *Pinnularia stomatophora* (Grunow) Cleve 1891

Dimensions: Length 55-115 µm; Width 9,5-12,5 µm. Striae: 11-14/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Restricted to cold, freshwater habitats that are acidic due to the presence of humic acids, in particular very electrolyte-poor peatlands with minimal anthropogenic disturbance. Rare but can be abundant in suitable habitats.

General distribution and ecology: According to Van Dam et al. (1994), this species is aerophilic, sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), halophobe, oligosaprobe and oligotrophic (Lange-Bertalot et al. 2017).

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

10. *Pinnularia subcapitata* var. *elongata* Krammer 1992

Dimensions: Length 20-43 µm; Width 4-6 µm. Striae: 11-13/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Oligotrophic, electrolyte-poor and acid habitats.

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, acidophilic, moderate O₂ (>50% sat.), oligohalobous, β-mesosaprobe and oligo-mesotrophic (Lange-Bertalot et al. 2017).

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

11. *Pinnularia subrupestris* Krammer 1992

Dimensions: Length 35-77 µm; Width 8,7-12 µm. Striae: 9-13/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2, H5.

Optimal environmental conditions: Anthropogenically little disturbed, in particular oligo- to dystrophic running waters and springs with low electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

12. *Pinnularia viridis* (Nitzsch) Ehrenberg 1843

Dimensions: Length 100-182 µm; Width 21-30 µm. Striae: 6-7/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H3, H4, H5.

Optimal environmental conditions: Circumneutral, oligo- to weakly eutrophic habitats with low to medium electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, tolerant N-autotrophic, neutrophilic, moderate O₂ (>50% sat.), oligohalobous, β-mesosaprobe and indifferent.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

Class: Bacillariophyceae

Order: Naviculales

Family: Sellaphoraceae

Sellaphora Mereschkowsky, 1902

Lectotype species: *Sellaphora pupula* (Kützing) Mereschkowsky

Valves are linear, lanceolate, or elliptic with bluntly rounded poles. Polar bars, also termed "*transapical thickenings*", are present in some taxa. The axial area is distinct, and may be expanded along the apical axis to form a conopeum. The external proximal raphe ends are dilated. The terminal raphe fissures are deflected. *Sellaphora* is widespread in alkaline to brackish waters of neutral pH. It contains the former *Navicula pupula* Kützing group. The genus also includes a number of small species that have been variously included in *Navicula*, *Eolimna* and *Craticula*. The publication by Wetzel et al. (2015) is an important revision of several small species now within the genus (Spaulding et al. 2022).

During research for this study two species from the genus *Sellaphora* were identified. Detailed characteristics about the identified species from the genus *Sellaphora*, distribution and ecological preferences are present further in this study.

1. *Sellaphora pseudopupula* (Krasske) Lange-Bertalot in Lange-Bertalot et al. 1996

Dimensions: Length c. 15-35 (40) µm; Width 6-7 (8) µm. Striae: 20-22 (24)/10 µm. Puncta: 40-45/10 µm.

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Associated with electrolyte-poor, weakly acidic freshwater habitats on siliceous substrata, in particular springs, spring-fed streams. A very similar phenocodeme, *S. calcicola* nom. Prov., occurs more rarely in moderately calcium-bicarbonate-rich spring or spring-fed mires. It deserves a detailed study (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

2. *Sellaphora pupula* (Kützing) Mereschkowsky 1902 (species group)

Dimensions: Length c. 11-26 µm; Width 6-8 µm. Striae: 18-26/10 µm. Puncta: 50-60/10 µm. Distribution in freshwater habitats on Vranica mountain: H1, H3.

Optimal environmental conditions: The taxonomic uncertainty around *S. pupula* complex means that it is difficult to give precise details of habitat ecology. Appears to be the most common in alkaline, eutrophic to polytrophic, flowing and standing freshwater habitats with medium to increased electrolyte content. This species is characteristic of the epilithon and therefore often under-represented or absent from standard epilithon samples (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, neutrophilic, moderate O₂ (>50% sat.), oligohalobous, α-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

Class: Bacillariophyceae

Order: Naviculales

Family: Stauroneidaceae

Stauroneis Ehrenberg, 1843

Lectotype species: *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg

Stauroneis has naviculoid and mostly solitary cells with two chloroplasts, one on each side of the cell against the cingulum. Valves range from almost linear to lanceolate to elliptic-lanceolate. The central area is a prominent transverse fascia, known as a "stauros". The central fascia typically extends to the valve margins, where one to several short striae may be present. The striae are uniseriate and punctate. The areolae are usually round but are transversely elongate in some species. Pseudosepta are present in some species. Variable and useful diagnostic features for identifying species include: presence or absence of pseudosepta, valve size and shape, width of the axial area, size and shape of the central area, number of striae in 10 µm, shape of areolae and number of areolae in 10 µm, raphe structure, and size and shape of the external proximal raphe ends. *Stauroneis* is a diverse freshwater genus found mostly in the benthos of wetlands and small lakes and ponds. Some species are also found in stream benthos and on moist soils and moss (Spaulding et al. 2022).

During research for this study six species from the genus *Stauroneis* were identified. Detailed characteristics about the identified species from the genus *Stauroneis*, distribution and ecological preferences are present further in this study.

1. *Stauroneis acidoclinata* Lange-Bertalot et Werum 2004

Dimensions: Length 35-60 µm; Width 8,5-10,5 µm. Striae: 21-23/10 µm. Puncta: 25-30/10 µm.

Distribution in freshwater habitats on Vranica mountain: H4, H5.

Optimal environmental conditions: Electrolyte-poor springs and streams, in assemblages of acidophilous diatoms in particular different *Eunotia* species (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

2. *Stauroneis anceps* Ehrenberg 1843

Dimensions: Length 40-70 µm; Width 10-13 µm. Striae: (22 to) 24/10 µm. Puncta: c. 25/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Oligo- to eutrophic, but oligosaprobic freshwater habitats with medium electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, neutrophilic, oxybiontic (75% sat.), oligohalobous, β-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

3. *Stauroneis gracilis* Ehrenberg 1841

Dimensions: Length 72-130 µm; Width 13-21 µm. Striae: 16-21 (usually 18)/10 µm. Puncta: 16-24/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3, H4.

Optimal environmental conditions: Mainly in oligotrophic, circumneutral freshwater habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, neutrophilic, oligohalobous and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

4. *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843

Dimensions: Length 140-220 µm; Width 28-38 µm. Striae: 14-16/10 µm (All radiate). Puncta: 14-17/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Still poor known. Usually oligosaprobic, but oligo- to eutrophic, circumneutral, usually standing freshwater habitats (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, tolerant N-autotrophic, neutrophilic, moderate O₂ (50% sat.), oligohalobous, β-mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

5. *Stauroneis separanda* Lange-Bertalot et Werum 2004

Dimensions: Length 13-17 µm; Width 3,6-5 µm. Striae: 27-29/10 µm. Puncta: Not resolvable with LM.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Scattered, often in high numbers in springs with moderately calcium-rich water. More detailed indications are present still difficult, because this small-celled species was not separated from *S. smithii* in earlier investigations (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

6. *Stauroneis silvahassiaca* Lange-Bertalot et Werum 2004

Dimensions: Length 28-42 μm ; Width 8-9,5 μm . Striae: 30-33/10 μm . Puncta: Very difficult to resolve with LM.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: Electrolyte-poor springs and streams where it co-occurs with acidophilous diatoms (Lange-Bertalot et al. 2017).

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

Class: Bacillariophyceae

Order: Rhopalodiales

Family: Rhopalodiaceae

Epithemia Kützing, 1844

Lectotype species: *Epithemia argus* (Ehrenberg) Kützing

Epithemia has an eccentric raphe system, positioned along the ventral margin. Each branch of the raphe is arched toward the dorsal margin. Externally, the proximal raphe slits terminate in expanded ends, while internally, the raphe slit is continuous through the central nodule. The raphe is also supported internally by large, transapical costae. The valvocopulum, which is the girdle band next to the valve mantle, often possesses septum-like extensions. Areolae are complex. Living cells of *Epithemia* often possess endosymbiotic cyanobacteria capable of fixing atmospheric nitrogen. Species within the genus, therefore, are often tolerant of nitrogen limiting conditions. *Epithemia* is an exclusively freshwater genus. Cells are epiphytic and epipelagic. They are commonly found in carbonate rich (alkaline) waters and can also tolerate relatively high conductivity (Spaulding et al. 2022).

During research for this study four species from the genus *Epithemia* were identified. Detailed characteristics about the identified species from the genus *Epithemia*, distribution and ecological preferences are present further in this study.

1. *Epithemia adnata* (Kützing) Brébisson 1838

Dimensions: Length 15-150 μm ; Width 7-14 μm .

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: Alkaline lakes and running waters with medium to high trophic levels. Mostly epiphytic (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, alkalibiontic, oxybiontic (75% sat.), oligohalobous, β -mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Epithemia goeppertiana* Hilse 1860

Dimensions: Length 40-120 μm ; Width 12-18 μm .

Distribution in freshwater habitats on Vranica mountain: H5.

Optimal environmental conditions: Oligotrophic to weakly-eutrophic and calcium-bicarbonate-rich freshwater habitats. The species appears to be more frequent in northern Europe and in the mountains, where it colonizes freshwater habitats with low to medium electrolyte content as an epiphyte (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aquatic to aerophilic, sensitive N-autotrophic, alkaliphilic, polyoxybiontic (100% sat.) and halophobe.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

3. *Epithemia sores* Kützing 1844

Dimensions: Length 8-70 μm ; Width 6,5-16 μm .

Distribution in freshwater habitats on Vranica mountain: H4.

Optimal environmental conditions: Standing freshwater habitats on carbonate substrata with medium to high trophic levels. Preferentially epiphytic species. Very rare in running waters (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aerophilic, sensitive N-autotrophic, alkalibiontic, oxybiontic (75% sat.), oligohalobous, β -mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

4. *Epithemia turgida* (Ehrenberg) Kützing 1844

Dimensions: Length 45-200 μm ; Width 13-35 μm .

Distribution in freshwater habitats on Vranica mountain: H1, H2, H4.

Optimal environmental conditions: Calcium-bicarbonate-rich standing freshwater habitats and (more rarely) running waters with medium to high trophic levels (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is occasionally aquatic to aerophilic, sensitive N-autotrophic, alkalibiontic, oxybiontic (75% sat.), oligohalobous, β -mesosaprobe and meso-eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Rhopalodia O.Müller, 1895, nom. cons.

Holotype species: *Rhopalodia gibba* (Ehrenberg) O.Müller

Frustules of *Rhopalodia* have a strong dorsiventral symmetry. The raphe is positioned on the dorsal side of each valve in a shallow keel. The valve faces are nearly

planar, that is, each frustule is shaped like 1/2 of an orange. The central endings of the raphe are expanded. The raphe is supported internally by costae. In contrast to *Epithemia*, *Rhopalodia* lacks complex cingula (girdle bands). Cells of *Rhopalodia* grow in nitrogen poor habitats. Like *Epithemia*, cells typically include endosymbiotic cyanobacteria that fix atmospheric nitrogen (Spaulding et al. 2022).

During research for this study one species from the genus *Rhopalodia* were identified. Detailed characteristics about the identified species from the genus *Navicula*, distribution and ecological preferences are present further in this study.

1. *Rhopalodia gibba* (Ehrenberg) O. Müller 1895

Dimensions: Length 22-300 µm; Width 18-30 µm. Striae: Finely punctate, resolvable between the fibular ribs. Puncta: Usually > 27/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H4, H5.

Optimal environmental conditions: Alkaline lakes and running waters. Species usually found in low numbers (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-Autotrophic, alkalibiontic, moderate O₂ (>50% sat.), aoligohalobous, β-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Class: Bacillariophyceae

Order: Surirelales

Family: Surirellaceae

Cymatopleura W.Smith, 1851, nom. et typ. cons. (Surirellaceae)

Holotype species: *Cymatopleura solea* (Brébisson) W.Smith

Note that a number of taxa within *Cymatopleura* have been, or will be, transferred to *Surirella* (Ruck et al. 2016). The genus *Surirella* was revised (Ruck et al. 2016) based on phylogenetic relationships (Ruck and Kociolek 2005, Ruck and Theriot 2011, Ruck et al. 2016a, 2016b). In order for nomenclature to align with evolutionary relationships, a genus-level reclassification was published (Ruck et al. 2016).

Previously, the genus *Cymatopleura* included cells of generally large in size, often over 200 µm in length. The valves were elliptic to panduriform. Species in *Cymatopleura* possessed characteristic undulations on the valve face. In girdle view, *Cymatopleura* was distinct because of the undulating valve face. The raphe was located within a shallow keel, positioned along entire valve margin. In living cells a single plastid, composed of two plates was present. The plastid was highly lobed and extend onto the girdle elements. *Cymatopleura* was epipellic in freshwaters, and found in waters of high conductance and alkaline waters (Spaulding et al. 2022).

The frustules possess canal raphes, that run around the valve at the mantle margin. Very coarse undulations are developed on the valve face; their crests and troughs are clearly visible in both valve and the commonly observed girdle view. The undulations not

interrupted along the median line of the valve. The plastid may be highly lobed and appearing to fill the whole cell in valve view (Lange-Bertalot et al. 2017).

During research for this study one species from the genus *Cymatopleura* were identified. Detailed characteristics about the identified species from the genus *Cymatopleura*, distribution and ecological preferences are present further in this study.

1. *Cymatopleura solea* (Brébisson) W.Smith 1851

Dimensions: Length 30-300 µm; Width 10-45 µm. Fibulae: 6-9/10 µm.

Distribution in freshwater habitats on Vranica mountain: H3.

Optimal environmental conditions: All freshwater habitat types with medium and high trophic levels and electrolyte content (Lange-Bertalot et al. 2017).

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkaliphilic, moderate O₂ (>50% sat.), β-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

Surirella Turpin, 1828

Holotype species: *Surirella striatula* Turpin

Cells of *Surirella* grow as single, isolated cells. The frustules may be either isopolar or heteropolar. Like other genera within the family *Surirellaceae*, the raphe system is positioned along the margin of the valve. The raphe is located within a canal, which may be raised above the valve surface in some species. Cells may be highly silicified, with spines and silica nodules on the valve surface. Species in *Surirella* are common in the benthos, especially epipelagic habitats, across a wide range of water chemistry. Cells may be relatively small, to very large (a few hundred micrometers), depending on the species. Because of the extensive raphe system, species of *Surirella* have high motility as compared to other diatom genera. They are able to live within sand grains and fine sediment, and can move through the sediment by means of the raphe system. The genus *Surirella* was recently revised (Ruck et al. 2016).

The phylogenetic relationships within the lineage were investigated in a number of works (Ruck and Kociolek 2005, Ruck and Theriot 2011, Ruck et al. 2016a, 2016b). In order for nomenclature to align with evolutionary relationships, a genus-level reclassification was published (Ruck et al. 2016).

The genus *Campylodiscus* now includes the "fastuosoid" taxa of *Surirella* and *Campylodiscus*. Many of the marine *Campylodiscus* are now classified within the genus *Coronia*. The revised *Surirella* now includes the *Surirella striatula* clade, the *Surirella Pinnatae* group, and species formerly classified as *Cymatopleura*. Furthermore, the genus *Iconella* was resurrected to accommodate *Stenopterobia* and the "robustoid" taxa *Surirella* and *Campylodiscus* (Spaulding et al. 2022).

During research for this study three species from the genus *Surirella* were identified. Detailed characteristics about the identified species from the genus *Surirella*, distribution and ecological preferences are present further in this study.

1. *Surirella angusta* Kützing 1844

Dimensions: Length 18-70 µm; Width 6-15 µm. Striae: Very delicate, 20-28/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H4.

Optimal environmental conditions: Common in most freshwater habitats, including strongly-eutrophic environments, but usually found in small numbers.

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkaliphilic, oxybiontic (75% sat.), oligohalobous, β-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Surirella roba* Leclercq 1983

Dimensions: Length 22-61 µm; Width 8-11 µm. Striae: 25-30/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H5.

Optimal environmental conditions: Characteristic species of oligotrophic- to mesotrophic, circumneutral to weakly acid streams. Rare in standing freshwater habitats. Indicator of the very good ecological quality.

General distribution and ecology: According to Van Dam et al. (1994), this species is sensitive N-autotrophic, acidophilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is at risk (G)

3. *Surirella spiralis* Kützing 1844

Dimensions: Length 80-124 µm; Width 52-61 µm. Fibulae: 2-3/10 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2, H3, H5.

Optimal environmental conditions: -

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic to aerophilic, sensitive N-autotrophic, neutrophilic, polyoxybiontic (100% sat.), oligohalobous, oligosaprobe and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018), this species is declining (V).

Class: Bacillariophyceae

Order: Thalassiophysales

Family: Catenulaceae

Amphora Ehrenberg ex Kützing, 1844

Lectotype species: *Amphora ovalis* (Kützing) Kützing

Valves are asymmetric to the apical axis and symmetric to transapical axis. On the dorsal margin, the valve mantle is deeper than on the ventral margin. As a result, the frustule is wedge-shaped, similar to a section of an orange. This wedge shape prevents complete focus with a microscope in one focal plane. In most species of *Amphora*, there is an abrupt differentiation between the valve face and mantle by a distinct marginal

ridge. The raphe is moderately to strongly eccentric, and positioned on the ventral side of the valve face. The raphe may be straight, arched or slightly sigmoid. According to Levkov, the proximal raphe ends terminate internally with narrow, elongated rectelevata (SEM feature). Usually, the striae on the dorsal margin are interrupted and a dorsal fascia (hyaline area) is present. The striae on ventral margin are short and may be composed of a single areola. Depending on the orientation of the valve, the striae on the ventral margin may be difficult to discern. Valves lack stigmata. Terminal nodules are indistinct. Species within the genus *Amphora* reach their greatest diversity in marine habitats. Of the freshwater species, *A. ovalis* is one of the widely distributed species (Spaulding et al. 2022).

During research for this study three species from the genus *Amphora* were identified. Detailed characteristics about the identified species from the genus *Amphora*, distribution and ecological preferences are present further in this study.

1. *Amphora copulata* (Kützing) Schoemann et Archibald 1986

Dimensions: Length 19-42 µm; Valve width: 5-7,5 µm; Width of frustules 12-18 µm.

Distribution in freshwater habitats on Vranica mountain: H1, H2.

Optimal environmental conditions: Meso- to polytrophic freshwater habitats. In almost all freshwater habitat types.

General distribution and ecology: According to Van Dam et al. (1994), this species is aquatic, tolerant N-autotrophic, alkalifilic, oxybiontic (75% sat.), oligohalobous, β-mesosaprobe and eutrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

2. *Amphora inariensis* Krammer 1980

Dimensions: Length 15-28 µm; Valve width: 3,5-6 µm; Width of frustules 8-11 µm.

Distribution in freshwater habitats on Vranica mountain: H1.

Optimal environmental conditions: According to Krammer & Lange-Bertalot (1986) *A. inariensis* colonizes oligotrophic, anthropogenically undisturbed standing freshwater habitats with low to medium electrolyte content. However, this species is found in a variety of environments, sometimes moderately impacted.

General distribution and ecology: According to Van Dam et al. (1994), this species is oligohalobous and oligotrophic.

Conservation status: According to Red List (Hoffman et al. 2018) risk not estimated (*).

3. *Amphora minutissima* W. Smith 1853

Dimensions: -

Distribution in freshwater habitats on Vranica mountain: H1, H3.

Optimal environmental conditions: -

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018) for this species not enough data (D).

Class: Bacillariophyceae

Order: Mastogloiales

Family: *Mastogloiaceae*

Decussiphycus Guiry & Gandhi, 2019

Holotype species: *Decussiphycus placenta* (Ehrenberg) Guiry & Gandhi

Cells living solitarily, mostly observed in valve view since in girdle view narrowly rectangular and under light microscopy with or without poor diagnostic significance. Valves broadly elliptic or linear tapering to the cuneate obtusely to broadly rounded or rostrate ends. Valve face flat, valve mantle narrow. Central raphe system generally as in *Navicula* but modified in detail, less complicated. Valve outside: Central raphe endings expanded to deflected drop-like central pores. Terminal fissures at both poles asymmetrically overlapped by a silica fold. Areolae with foramina circular in shape outside, arranged essentially by three systems of striae, which cross each other in angles of 60-80° forming a regular quincunx pattern. Valve inside: Alveoli and areolae pattern similar as outside. Internal raphe fissure running straight in a distinct raphe sternum, ending in larger helictoglossae at the poles and much smaller though somewhat helictoglossa-like silica accumulations at the central nodule. Oblique costae of the quincunx system at least in the central parts with a moderately higher relief than the transapical costae (modified from Lange-Bertalot 2000: 670).

During research for this study one species from the genus *Decussiphycus* were identified. Detailed characteristics about the identified species from the genus *Decussiphycus*, distribution and ecological preferences are present further in this study.

1. *Decussiphycus hexagonus* (Torka) Guiry & Gandhi 2019

Dimensions: Length 25-44 µm; Width 9-13 µm. Striae: 20-25/10 µm.

Distribution in freshwater habitats on Vranica mountain: H2.

Optimal environmental conditions: -

General distribution and ecology: -

Conservation status: According to Red List (Hoffman et al. 2018), this species is highly endangered (2).

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VRANICA MOUNTAIN



Mašić Ermin, PhD

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