Joanna Czerwik-Marcinkowska, Tomasz Zwijacz-Kozica, Wojciech Pusz, and Anna Wojciechowska. Brown bear and diversity of airbone algae and cyanobacteria in the Głowoniowa Nyża Cave. *Journal of Cave and Karst Studies*, v. 81, no. 1, p. 57-67. DOI:10.4311/2018MB0121

THE RELATIONSHIP BETWEEN PRESENCE OF BROWN BEAR (URSUS ARCTOS) AND DIVERSITY OF AIRBONE ALGAE AND CYANOBACTERIA IN THE GLOWONIOWA NYŻA CAVE, TARTRA MOUNTAINS, POLAND

Joanna Czerwik-Marcinkowska^{1,C}, Tomasz Zwijacz-Kozica², Wojciech Pusz³, and Anna Wojciechowska⁴

Abstract

Of the big mammal taxa, the brown bear (*Ursus arctos*) is one of a few surviving species and one of the two largest terrestrial carnivorans that have successfully exploited caves. Greenish and blueish patches were collected in August 2016 from the cave walls and pine twigs in the pseudokarstic Głowoniowa Nyża Cave in the High Tatra Mountains, southern Poland. These materials were cultured and the first appearance of airborne microorganisms (algae and cyanobacteria) during two-three months of cultivation were observed. Overall, 24 species were identified using light microscopy and transmission electron microscopy. The highest number (10) of documented species belonged to Cyanobacteria with the genus *Gloeocapsa* the most diverse. We identified ten Chlorophyta species. Only four taxa of diatoms were found. No correlation between species diversity and physical parameters (temperature and humidity) was found. The materials containing airborne microorganisms growing on the granite walls were most probably brought in by wind, whereas the ones on the twigs were brought in by wind and/or by the bear. The presence of *Ursus arctos* does influence distribution of airborne microorganisms.

Introduction

Caves represent very specific, extreme terrestrial habitats, where growth of airborne microorganisms and vascular plants are limited by unfavorable abiotic factors. They present composite micro-ecosystems that include bacteria, cyanobacteria, algae, fungi, lichens, liverworts, and mosses, in different proportions depending on the environmental conditions. Dayner and Johansen (1991), Pedersen (2000) and Popović et al. (2015) suggested that reduced light intensity, low nutrients, and absence of seasonality are the predominant features that influence distribution and composition of aerial algal and cyanobacterial assemblages in caves, while Mulec et al. (2008) stated that temperature, humidity, and flowing water also play a role in the colonization of aerial habitats. It is possible that animals, and in particular bears, can transport different spores into caves, and an example of such phenomenon is the Głowoniowa Nyża Cave in the High Tatra Mountains, Poland. This cave was discovered in March 2011 by Janusz Łukaszczyk Głowoń while observing a female bear with cubs. The plan and description of cave was prepared by Tomasz Zwijacz-Kozica in collaboration with J. Łukaszczyk Głowoń, M. Strączek Helios, and F. Zięba, during the bears absence. The Głowoniowa Nyża Cave continues to be used for brown bear hibernation.

Ursus arctos is a large brown bear with the widest distribution of any living ursid. The brown bear occurs in the coniferous, mixed, and deciduous forest zones of Europe, however, the bear seasonally visits the tundras and arctic heaths above the timberline. Zedrosser et al. (2001) described the Carpathian population of brown bears in Slovakia, Poland, Ukraine, and Romania which includes about 8,000 bears and is the second largest in Europe. All bear populations are protected by the Habitat Directive in Europe which is compulsory for all EU countries. Almost all the bears in Europe live in large transboundary populations in eastern or northern Europe (Zedrosser et al., 2001). Nielsen et al. (2010) stated that in different regions of the world hibernating bears use various places to build their dens. Linnell et al. (2000) described wintering brown bears in natural caves or in rock cavities, dens dug in snow cover, and also hidden in rotten trees. The European brown bear is omnivorous, but feeds chiefly on vascular plants. Grasslands and shrublands integrated with forests, subalpine meadows, and alpine communities are typical habitat for bears and in particular *Ursus arctos* (Nietfeld et al., 1985). There is the risk of mistaking a briefly exploited summer den for a true bear hibernation site (Mysterud, 1983).

Airborne algae and cyanobacteria are known to colonize non-aquatic habitats including exposed bedrock (Rindi et al., 2010; Ress and Lowe, 2013), soil, terrestrial bryophytes, tree bark, rocks, and anthropogenic structures (Neustupa and Štifterová, 2013). These pioneer species modify the rock surface by producing carbonic acid during respiration

⁴Department of Geobotany and Landscape Planning, Faculty of Biology and Environmental Protection, Nicolas Copernicus University, Lwowska 1, 87-100 Toruń, Poland

¹Department of Botany, Institute of Biology, Jan Kochanowski University, Świętokrzyska 15, 25-406 Kielce, Poland; email: marcinko@kielce.com.pl ²Tatra National Park, Kuźnice 1, 34-500 Zakopane, Poland

³Division of Plant Pathology and Mycology, Department of Plant Protection, Wrocław

University of Environmental and Life Sciences, Grunwaldzki 24a, 53-363 Wrocław, Poland

^cCorresponding author: marcinko@kielce.com.pl

(Smith and Olson, 2007). Airborne microorganisms are generally characterized by small size, high resistance to desiccation, specific preferences for pH, tolerating low nutrient levels, high conductivity, and most of them can be considered as cosmopolitan and distributed worldwide (Falasco et al., 2014). Gorbushina and Broughton (2009) suggested that airborne microorganisms are exposed to harsher and more variable environmental conditions than their aquatic counterparts where the surrounding water usually buffers abrupt changes of radiation and temperature. Many algal species living in aerial habitats are known to produce extracellular mucilage which aides in water retention (Gerrath, 2003). This mucilage contains a considerable amount of moisture and is common in airborne cyanobacterial genera, such as Nostoc, Gloeothece, Gloeocapsa, and Aphanocapsa. Johansen et al. (1983) found that mucilage-producing cyanobacteria and green algae are the first colonizers on moist rock faces, and other algal species begin to colonize the mucilage. Algal cells have been found to be transported by wind, water, and animals, such as birds, bears, and humans (Kristiansen, 1996). Wind is mainly responsible for the atmospheric distribution of airborne microorganisms suppling new inoculum to barren substrata, thus contributing to the cosmopolitan distribution of many microorganisms (Gorbushina 2007). The ecological success of airborne microorganisms in environmentally-harsh habitats such as caves or building facades depends on many factors (Karsten et al., 2007). The nutrients used by organisms to survive, grow, and reproduce, can be supplied by rain, water, snow, aerosols, dust or soil particles, and big animals such as bears. The phototrophs can be transported the same way as the nutrients. In our study we defined the diversity of airborne microorganisms in the Głowoniowa Nyża Cave (High Tatra Mountains, Poland) and we explored morphological adaptations to microhabitat preference. We hypothesize that the presence of Ursus arctos influences distribution of airborne microorganisms.

Materials and Methods

Cave Description

The pseudokarstic Głowoniowa Nyża Cave is located in the High Tatra Mountains of Poland, in the Orla Ściana above the Roztoka Valley in the municipality of Bukowina Tatrzańska (Fig. 1). It was discovered in 29 March 2011 by Janusz Łukaszczyk during observation of a female bear with cubs coming out from the cave after wintering. Due to the necessity to protect the bear wintering place, the exact cave location is not given here. The cave entrance is exposed NE, and its measurements are: width 0.8 m, height 0.9 m, length 4.3 m, and it is situated at 1605 m a.s.l., but the relative height above the Roztoka Valley is 250 m (Fig. 2). The rocky cave bottom is covered by the plant materials brought in by the bear. The cave was formed on a slit in granitoids of the crystalline High Tatras, on a tectonic fracture. The cave is dry and illuminated by the daylight coming through the cave entrance. Its climate depends on outside atmospheric conditions. In the cave light zone close to the entrance, lit by sunlight and well-oxygenated, grow mosses, lichens, grasses, and ferns. Inside the cave the traces of wintering *Ursus arctos* such as the bear's den built with fur, mosses, grasses, and small tree twigs (*Picea abies, Pinus cembra* and *Pinus mugo*), excrement, marks of claws, and fur on the cave walls can be observed. In the whole cave one can find the insects and spiders whose presence confirm good ecological cave conditions (relatively high temperature, humidity, and availability of nutrients).

Sampling

In total, 3 samples were collected in August 2016 from the cave walls (granite A and B) and from twigs of *Pinus cembra* or *Pinus mugo* found inside the cave. The accurate identification of pine twigs was impossible due to the damage inflicted by bear, because the twigs were part of the bear's den. Each sample (greenish and blueish coloured patches) was scraped from cave walls or from pine twigs, placed into labeled sterile plastic bag, and transported to the lab. Material was transferred into Petri dishes with fresh agarized (1%) nutrient Bold's Basal Medium (Bischoff and Bold, 1963), and cultured at 20 °C in a 12-hour light/12-hour dark cycle at $3000 \,\mu \text{Em}^{-2} \text{s}^{-1} \text{lx}$ provided by 40 W cool fluorescent tubes. A microscopic study of cultures began from the first appearance of microorganisms growth during two-three months of cultivation. All the phototrophs were observed in living states and identified using a light microscope Jenamed 2 (Carl Zeiss Jena). The cells for transmission electron microscopy (TEM) were processed according to Massalski et al. (1995) and microphotographs were taken with a TESLA BS 600. Airborne algae and cyanobacteria were identified using the following literature: Anagnostidis and Komárek (1988), Ettl and Gärtner (1995), Komárek and Anagnostidis (2005), and Rindi et al. (2010).

Measurement of Physical Parameters

Temperature *T*, relative humidity *RH*, and dew points *DP* were measured using the Extech Temperature Humidity Meter and Vellman DMV 1300 Luxmeter from September 26, 2017 to October 12, 2017. These parameters were measured 67 times at each sampling site on the same day. For each parameter the mean value with standard error was calculated (Fig. 3).

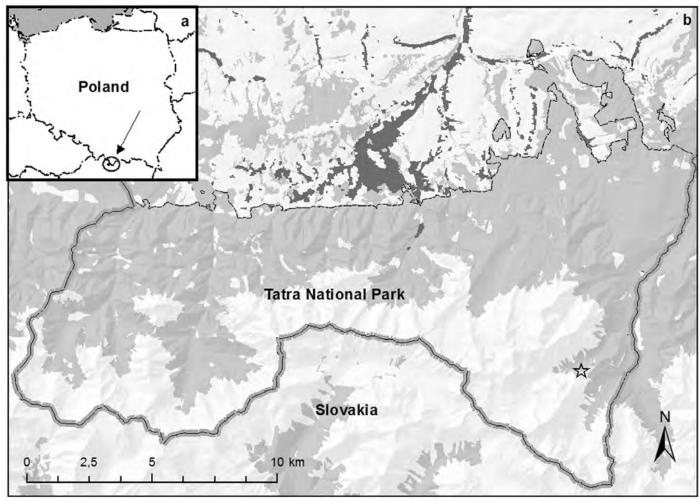


Figure 1. Map of Poland showing the location of the Głowoniowa Nyża Cave. A - A view of the High Tatra Mountains, southern Poland, B - A view of the Tatra National Park with marked location of cave (black star).

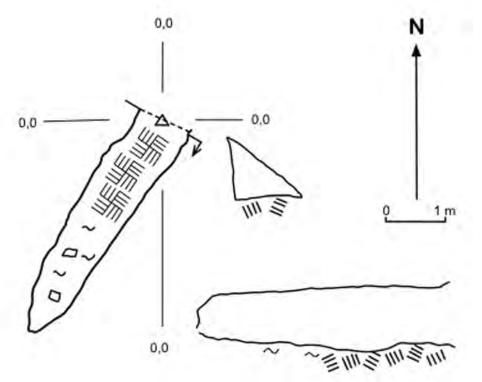


Figure 2. Survey of Głowoniowa Nyża Cave showing a corridor of the cave throughout the length of the cave profile; reproduced from Zwijacz-Kozica (2011). The symbols: four lines all labeled 0.0 are indicating entrance to the cave (origin of the local coordinate system); 3 sketches show: 1 – cave profile, 2 (small triangle) - cross section of the cave entrance (direction indicated by arrow on the cave), 3 - cross section of whole cave; soil - clusters of parallel lines.

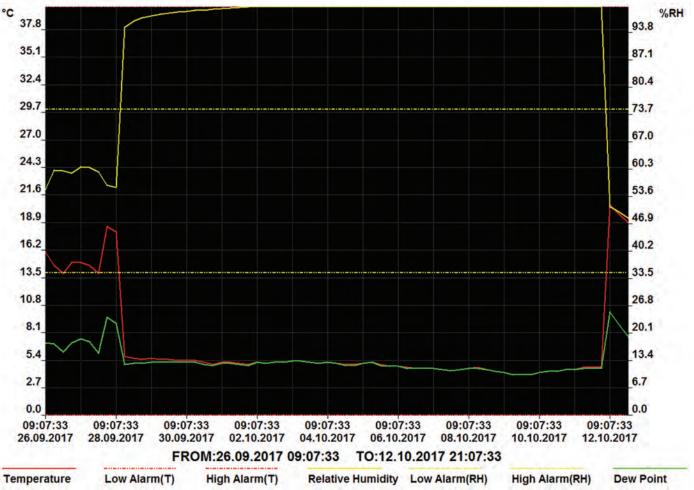


Figure 3. Plot of temperature, relative humidity and dew point values for the cave (from 26 September to 12 October 2017). Two sensors were installed in the bear's den.

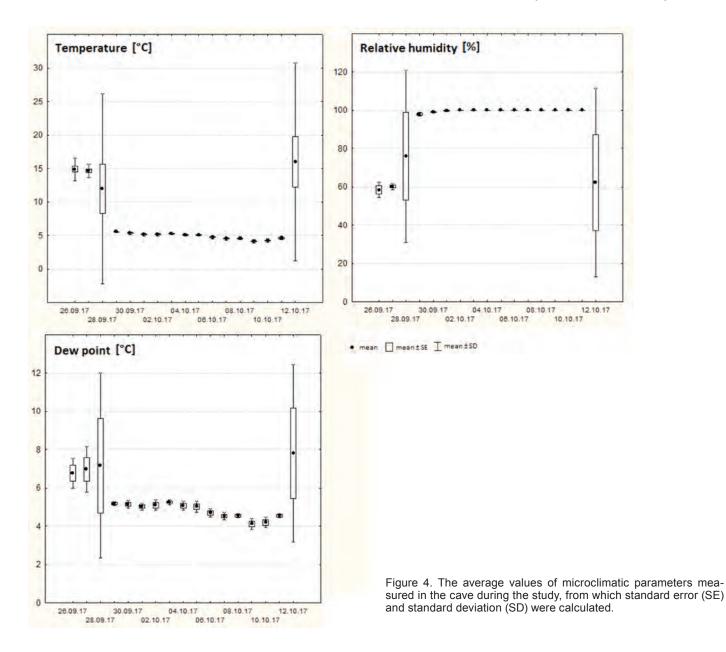
Statistical Analysis

Microhabitat data analysis were presented as diagrams using software Statistica 9.0 (StatSoft Inc., 2009). The diagrams showed basic statistics for temperature, relative humidity, and dew point, i.e. average, standard error (SE) and standard deviation (SD) in the Głowoniowa Nyża Cave. Airborne algae and cyanobacteria species composition and frequency data recorded on three sampling sites (granite A and B, and pine twigs) were subjected to indirect analysis. PCA (Principal Component Analysis) were performed using Canoco 5.0 (ter Braak and Šmilauer, 2012). A graphical representation of this analysis was diagramed, where the vectors indicated sampling sites (particular species were marked by geometric symbols). The species names consisted of three letters of a generic name, dot, and three letters of a species name.

Results and Discussion

Temperature, relative humidity, and dew point were different across sampling sites, with average values 5.0 ± 18.0 , 48.3 ± 99.9 % and 4.0 ± 9.0 respectively (Fig. 4). The lowest *T* value (5 °C) was in September, and highest (18 °C) in October. The highest *RH* value was measured in the brown bear den (99.9 %) because the humidity sensor was placed inside of the den in which were bear excrements and urine, while the lowest *RH* value (48.3 %) was close to the cave entrance. The highest *DP* value was 4.0 and the lowest was 9.0. The presence of brown bear, temperature, and relative humidity were almost constant at all sampling sites, presumably due to their proximity. It is impossible to state whether all the measurements were taken during the presence of bear in the den. The measurements were recorded continuously regardless the bear presence or absence. While the average humidity of the majority of caves in Central Europe is about 85–95 % and the average temperature is in the range 5–8 °C, whereas in the Głowoniowa Nyża Cave, the temperature and humidity at all sampling sites was higher. This dependency was due to the fact that all sampling sites were relatively close to the entrance where *T* and *RH* are influenced by the outside climatic conditions.

In total, 24 species of airborne algae and cyanobacteria were found in the pseudokarstic Głowoniowa Nyża Cave. The dominant group of phototrophs colonizing cave walls and pine twigs were the green algae and cyanobacteria. Ten



Chlorophyta including genera: *Apatococcus*, *Asterochloris, Chroococcidiopsis, Coccomyxa, Klebsormidium, Pseudococomyxa*, and *Stichococcus* were observed both on the cave walls and on the twigs, while *Desmodesmus olivaceus* and *Trentepohlia aurea* were present only on the cave walls (Figs 5-6). These species are typical for different lithophytic substrates in temperate zones. In our laboratory we observed that *Apatococcus minor* grew successfully on solid media (agar) which better mimics its natural growth conditions. It is not able to compete and is rapidly overgrown by other species. *Apatococcus minor* is characterized by different lifestyles and survival strategies, so it is classified as a K-strategist with low growth and mortality rates, long lifespans, and efficient resource utilization capacities (MacArthur and Wilson, 1967). Other airborne algal genera, such as *Coccomyxa* and *Stichococcus* exhibited two or three-fold higher growth rates under the described culture conditions (Gustavs et al., 2010). Consequently, the ecological success of these green algae do not originate from competitive strength based on growth rate but from long-time survival under harsh environmental conditions.

Only four airborne, cosmopolitan and widespread diatoms: *Brachysira* sp., *Hantzschia* sp., *Orthoseira roeseana*, and *Pinnularia borealis*, were identified. Diatoms living in the Głowoniowa Nyża Cave are generally characterized by small size, high resistance to desiccation, specific preferences for pH, and tolerating low nutrient levels. Therefore diatoms were not a significant contribution to the biodiversity of this caves microorganisms. Falasco et al. (2014) and Lauriol et al. (2006) stated that the size of the cave has an important effect on air circulation, and influence the diatom diversity in the deeper zones of caves. The Głowoniowa Nyża Cave is characteristic of small caves with only one main

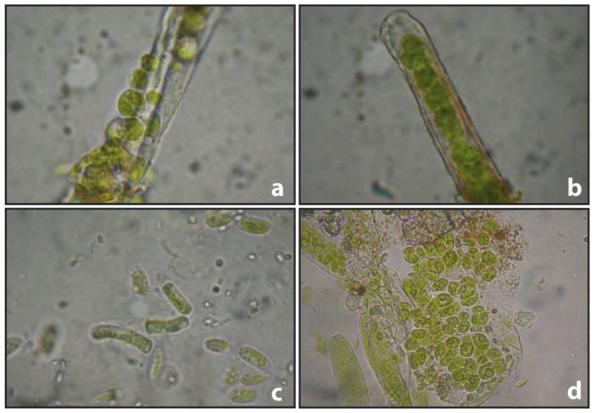


Figure 5. Culturable airborne algae from Głowoniowa Nyża Cave: a-b *Klebsormidium dissectum*, c - *Klebsormidium flaccidum*, d - *Desmococcus olivaceus*. Scale bars 10 µm.

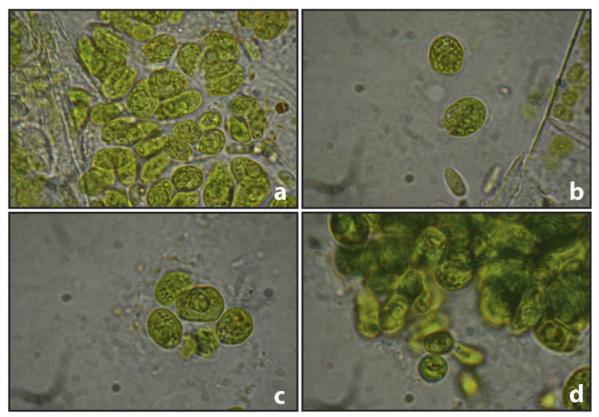


Figure 6. Green algae cells grown aerophytically on a BBM agar slant (Light Microscope view). a-b *Coccomyxa bre-vis*, c-d *Pseudococcomyxa ellipsoidea*. Scale bars 10 µm.

entrance and is typical for brown bear hibernacula. It is possible that colonization of diatom spores being transported by air through the main entrance of cave were hampered because of high temperature and low humidity. Diatoms generally deposit on cave speleothems consequently to air condensation on the walls (Mulec and Kosi, 2009). Water circulation also plays an important role in the cave colonization. Diatom species entering the cave with water are generally adapted to oligotrophic conditions (Falasco et al., 2014). Krammer and Lange-Bertalot (1991) and Germain (1981) described Orthoseira roeseana as aerophilous and xerotic diatom species. It is commonly found on wet walls, moist stones and rocks, mosses, and even on the wet banks of the riparian vegetation (Houk, 2003), and in alkaline areas (Wehr and Sheath, 2003). Falasco et al. (2014) and Garbacki et al. (1999) reported that this species was usually in caves in the liminar zone, exposed to natural light, and Roldán and Hernández-Mariné (2009) found Orthoseira roeseana on artificially illuminated walls. It seems to be adapted to variable environments, on different substrates, both rocks and mosses, and was also found on a woody surface close to the main entrance (Skaloud, 2009). Our study confirmed this species presence only on the pine twigs. Pinnularia borealis is typically an aerophilous and epiphytic species (Taylor et al., 2007), often anemophilous (Krammer, 2000). It is one of the most frequently recorded taxa on submerged bryophytes (Van de Vijver and Beyens, 1997), occurring in wild caves close to the main entrance on very wet walls (Garbacki et al., 1999), but Hoffmann and Darienko (2005) also recorded it in caves opened for tourism. In the Głowoniowa Nyża Cave Pinnularia borealis was observed only sporadically on the pine twigs.

Cyanobacterial species were found mainly on the cave walls but only two taxa. Chroococcus ercegovicii and Gloeocapsa biformis, were present on pine twigs. Ten cyanobacteria taxa in the genera Aphanocapsa, Chroococcus, Gloeocapsa, Gloeothece, Nostoc, and Scytonema were found. The members of Nostocales are typical cyanobacteria among cave microhabitats. The members of the order Oscillatoriales are usually characteristic for caves, however there were not present in the studied cave. Among cyanobacteria, Aphanocapsa muscicola was found on the wall in the Głowoniowa Nyża Cave, but this species occurs in other microhabitats such as soil substrate, bryophytes, and rocks (Matuła et al., 2007). This species according to John et al. (2011) is very abundant on slightly basic substrata, shaded habitats, and is a component of some cyanobacterial mats. During laboratory cultivation, microscopic analyses revealed the presence of atypical cyanobacterial structure, such as single cells embedded in mucilage of dark green color, whereas Aphanothece saxicola grows on wet rock surfaces forming mucilaginous blue-green thallus. This species found in the studied cave is also known from ornamental pools and fountains (Vinogradova, 1999) and as an epilithic cyanobacterium on rocky shores (Nagarkar, 1998). Gloeocapsa atrata among mosses is widespread on wet rocks and Glowoniowa Nyża Cave walls, and less commonly known from wet soil (John et al., 2011). During laboratory cultivation, microscopic analyses revealed the cells of Gloeocapsa atrata occuring in subcolonies with individual envelopes surrounded by colorless mucilaginous envelopes. Gloeocapsa biformis in laboratory cultivation formed irregular colonies, dirty yellow or brownish, and yellow mucilaginous envelopes. The fact that three species Gloeocapsa genus were present at the Głowoniowa Nyża Cave indicates that the airborne microorganisms colonization on the walls is at an intermediate stage, and this agrees with Pentecost (1992), who considered all these species as pioneers in rock colonization. Gloeothece palea grew on wet rocks and other granite surfaces, sometimes among mosses. Nostoc commune is the dominant species responsible for the formation of thick mats containing other airborne algae in the cave. The comparison between the cyanobacteria and algae from Głowoniowa Nyża Cave and three caves in Serbia (Popovíć et al. 2015), shows that in all four caves there is an abundance of Cyanobacteria, with chroococcalean taxa prevailing and species of the genus Gloeocapsa, which occur in various habitats with many different ecological characteristics, indicating its tolerance to a wide range of environmental conditions. Most of the documented cyanobacteria from the investigated caves were typical aerophytic species.

The occurrence frequency of every recorded species based on the observation of 3 samples from each sampling site is shown in Table 1. The ultrastructure of airborne algae and cyanobacteria cells was documented using TEM. The PCA analysis pointed out that micro-environmental factors such as temperature and water availability, and the type of materials used to build the bear's den influenced the distribution of the algae and cyanobacteria. The PCA analysis clearly distinguished the species associated with the brown bear's activity and the spruce twigs in the bear's den (Fig. 7) as presented on one side of the graph, while on the other side of the graph were cyanobacteria collected on the cave granite walls.

Airborne microorganisms can also be observed on hard substrates such as granite. Studies of algal and cyanobacteria on this substrate are very scarce and were carried out previously in Spain (Rifon-Lastra and Noguerol-Seoane, 2001), in Slovakia (Uher, 2010) and in the southern part of Ukraine (Mikhailyuk, 2013). Mikhailyuk and Darienko (2011) studied the epilithic, chasmoendolithic and epiphytic algae from granite outcrops in the south of Ukraine. The results showed that algae never formed macroscopic growth on bare surfaces and occurred only in 40% of the cultivated samples. Cavernicolous airborne microorganisms are rather rich and diverse. Vinogradova and Mikhailyuk (2009) pointed out that algae numbers in speleoecotopes from 340 species (Coûté and Chauveau, 1994) to 542 (Draganov, 1977), among them cyanobacteria account up to 60 % and the rest of the taxonomic groups vary from 1 % to 20 %. Albertano

Таха	Granite A	Pine Twigs	Granite E
Chlorophyta			
Apatococcus minor Brand		1	
Asterochloris pyriformis Tschermak-Woess		3	1
Chroococcidiopsis edaphica Johansen et Flechtner		1	
Coccomyxa brevis (Vischer) Gärtner & Schragi	2	2	
Desmococcus olivaceus Brand			4
Klebsormidium dissectum (Gay) Ettl & Gärtner		3	
Klebsormidium flaccidum var. lubricum (Chodat) Ettl & Gärtner	1	1	
Pseudococcomyxa ellipsoidea Hindák		2	
Stichococcus allas Reisigl	2	2	
Trentepohlia aurea (Linnaeus) Martius	3		
Heterokontophyta			
Bacillariophyceae			
<i>Brachysira</i> sp.	1	1	
Hantzschia sp.		2	2
Orthoseira roeseana (Rabenhorst) Pfitzer		2	
Pinnularia borealis Ehrenberg		1	
Cyanophyta			
Aphanocapsa muscicola (Meneghini) Wille	2		
Aphanothece saxicola Nägeli			2
Chroococcus ercegovicii Komárek & Anagnostidis	1	2	
Gloeocapsa atrata Kützing	1		3
Gloeocapsa biformis Ercegovic	4	4	
Gloeocapsa rupicola Kützing	2		3
Gloeothece palea (Kützing) Rabenhorst	2		5
Nostoc commune Vaucher ex Bornet & Flahault	5		5
Nostoc sp.			2
Scytonema mirabile Bornet	2	2	

Table 1. The list of the documented airborne algal and cyanobacterial species in te Głowoniowa Nyża Cave. Occurence
frequency: 1 = 20 %; 2 = 40 %; 3 = 60 %; 4 = 80 %; 5 = 100 % (after Popović et al., 2015).

et al. (2003) suggested that in well illuminated caves, cyanobacteria, diatoms, and chlorophytes generally colonize lit rock walls causing physical and chemical damage. Pusz et al. (2018) described airborne fungal spores in five bear dens located within Tatra National Park in southern Poland. Thirteen species of fungi were cultured from which seven taxa were present in the Głowoniowa Nyża Cave.

In the Głowoniowa Nyża Cave, airborne algae and cyanobacteria are dominant organisms, and similar species are frequently encountered in European caves (Berberousse et al., 2006). These microorganisms were pioneer species because of their ability to grow diazotrophically (Gallon et al., 1991). Other members of the Oscillatoriales are well-adapted to extremely low irradiance compared to other filamentous cyanobacteria. Albertano et al. (2000) reported the occurrence of cyanobacteria on both marble and granite monuments in different Mediterranean countries. The dominant presence of filamentous cyanobacteria in stable conditions of low-light intensity and high relative humidity has been reported for different caves (Martinez and Asencio, 2010; Roldán and Hernández-Mariné, 2009). *Nostoc* is a cosmopolitan terrestrial genus that can endure desiccation, as well as very low temperatures (Dodds et al., 1995).

Mulec (2012) suggested that heterotrophic microorganisms tend to colonize parts of caves where nutrients have been introduced, such as areas near surface openings, underground rivers, sediments, and surfaces associated with animal excrement. However, Bastian et al. (2009) stated that many caves naturally face increased input of organic matter, while others are subjected to high anthropogenic impact due to drainage of polluted water into the underground or extensive tourist visits of show caves. The airborne algae and cyanobacteria in the Głowoniowa Nyża Cave can be subject to harsh environmental conditions such as an inconsistent availability of moisture, relative increased temperature, and the presence of brown bear. Airborne algae and cyanobacteria can survive frequent and prolonged periods

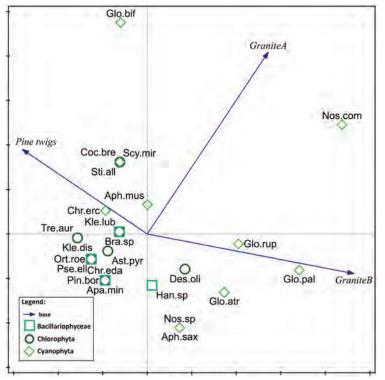


Figure 7. Principal Component Analysis (PCA) of sampling sites based on environmental parameters. The substrates (granite A, granite B and pine twigs) are marked with vectors and microorganisms as geometrical figures: b - square - diatoms (Bacillariophyceae), circle - green algae (Chlorophyta), rhomb - cyanobacteria (Cyanophyta). The 1st ordination axis accounted for 55.6 % of the total variability of data. Abbreviations of species names in Fig. 7 consist of three letters of a generic name and three letters of a species name. Abbreviations of species names: Apatococcus minor (Apa. min.); Aphanocapsa muscicola (Aph. mus.); Aphanothece saxicola (Aph. sax.); Asterochloris pyriformis (Ast. pyr.); Brachysira sp. (Bra. sp.); Chroococcidiopsis edaphica (Chr. eda.); Coccomyxa brevis (Coc. bre.); Chroococcus ercegovicii (Chr. erc); Desmococcus olivaceus (Des. oli.); Hantzschia sp. (Han. sp.); Gloeocapsa atrata (Glo. atr.); Gloeocapsa biformis (Glo. bif.); Gloeocapsa rupicola (Glo. rup.); Gloeothece palea (Glo. pal.); Klebsormidium dissectum (Kle. dis.); Klebsormidium flaccidum var. lubricum (Kle. lub.); Nostoc commune (Nos. com.); Nostoc sp. (Nos. sp.); Orthoseira roeseana (Ort. roe.); Pinnularia borealis (Pin. bor.); Pseudococcomyxa ellipsoidea (Pse. ell.); Scytonema mirabile (Scy. mir.); Stichococcus allas (Sti. all.); Trentepohlia aurea (Tre. aur.).

of desiccation, and moisture availability has been found to be an important factor regulating the algal abundance in these habitats (Lopez-Bautista et al., 2007). Cyanobacteria have been found to dominate communities in aerial habitats (Matthes-Sears et al., 1999) and are also often the first algal colonizer in cave aerial habitats. The tolerance of many cyanobacterial species to a wide range of moisture and light may contribute to their dominance (Whitton and Potts, 2000), and they are able to survive and recover more quickly from desiccation than other microorganism (De Winder et al., 1989).

Species dispersal may play a role in similarities seen between the green algae, cyanobacteria, and diatoms of this cave. Airborne algae have been shown to be easily transported by the wind and animals. Taxa that can withstand desiccation have the potential to survive aerial transport. Many algal species from aerial habitats produce extracellular mucilage which allows them to withstand periods of desiccation (Gerrath, 2003). UV radiation can also influence the survival of cells in aerial transport. Ehling-Schultz et al. (1997) suggested that ability of cyanobacteria to not only withstand desiccation but also to produce photoprotective pigments gives them a competitive advantage for longer distance aerial transport. Johansen et al. (1983) and Ress and Lowe (2013) found that mucilage-producing cyanobacteria and green algae are the first colonizers on moist rock faces, and once established, other species begin to colonize the mucilage. Nutrient availability may play a role in shaping the structure of aerial algae due to possible nutrient limitation in these habitats (Johansen et al., 1983). The ecological phenomena seem to have an influence on the distribution of airborne microalgae in the Cave.

Conclusion

24 species of airborne microorganisms were cultivated from samples collected from various habitats in the pseudokarstic Głowoniowa Nyża Cave in the High Tatra Mountains, southern Poland. These phototrophs using LM and TEM microscopy were identified only from cultivated samples, but not directly from field specimens. In the present work, the diversity of algae and cyanobacteria as the main phototrophic microorganisms colonizing the cave surfaces were studied. There was no correlation between species diversity and physical parameters (temperature and humidity). An investigation of the diversity of airborne microorganisms and presence of brown bear in the Głowoniowa Nyża Cave were conducted for the first time in Poland.

Acknowledgements

We thank Prof. Andrzej Massalski for valuable comments on the manuscript, and PhD Piotr Rafalski for the useful discussion about the statistical methods. Thanks go to anonymous reviewers for improving the manuscript.

References

- Albertano, P., Moscone, D., Palleschi, G., Hermosin, B., Saiz-Jimenez, C., Sanchez-Moral, S., Hernández-Mariné, M., Urzí, C., Groth, I., Schroeckh, V., Saarela, M., Mattila-Sandholm, T., Gallon, J.R., Graziottin, F., Bisconti, F. and Giuliani, R., 2003, Cyanobacteria attack rocks (CATS): Control and preventive strategies to avoid damage caused by cyanobacteria and associated microorganisms in Roman hypogean monuments: *in* Saiz-Jimenez, C., ed, Molecular Biology and Cultural Heritage, Lisse: Swets and Zeitlinger, p. 151–162.
- Albertano, P., Bruno, L., D'Ottavi, D., Moscone, D. and Palleschi, G., 2000, Effect of photosynthesis on pH variation in cyanobacterial biofilms from Roman catacombs: Journal of Applied Phycology, v. 12, p. 279–384. https://doi.org/10.1023/A:1008149529914.
- Anagnostidis, K. and Komárek, J., 1988, Modern approach to the classification system of Cyanophytes. 3. Oscillatoriales: Archiv für Hydrobiologie, Supplement, v. 80, p. 327–472.
- Barberousse, H., Tell, G., Yepremian, C. and Couté, A., 2006, Diversity of algae and cyanobacteria growing on building facades in France: Algological Studies, v. 120, no. 1, p. 81–105. https://doi:10.1127/1864-1318/2006/0120-0081.
- Bastian, F., Alabouvette, C. and Saiz-Jimenez, C., 2009, The impact of arthropods on fungal community structure in Lascaux Cave: Journal of Applied Microbiology, v. 106, p. 1456–1462. https://doi.org/10.1111/j.1365-2672.2008.04121.x.
- Bischoff, H.W. and Bold, H.C., 1963, Phycological Studies IV. Some soil algae from Enchanted Rock and related algal species: University of Texas Publications, Austin, no. 6318, p. 1–95.
- Coûté, A.D. and Chauveau, O., 1994, Algae: *in* Juberthie, C. and Decu, V., eds, Encyclopaedia biospeologica Tome 1. Société de Biospéologie, Moulis, Bucarest, p. 371–380.
- Dayner, D.M. and Johansen, J.R., 1991, Observations on the algal flora of Seneca Cavern, Seneca County, Ohio: Ohio Journal of Science, v. 91, no. 3, p. 118–121.
- De Winder, B., Matthijs, H.C.P. and Mur, L.R., 1989, The role of water retaining substrata on the photosynthetic response of three drought tolerant phototrophic micro-organisms isolated from a terrestrial habitat: Archives of Microbiology, v. 152, p. 458–462. https://doi.org/10.1007/ BF00446929.
- Dodds, W.K., Gudder, D.A. and Mollenhauer, D., 1995, The ecology of Nostoc: Journal of Phycology, v. 31, no. 1, p. 2–18. https://doi.org/10.1111/ j.0022-3646.1995.00002.x.
- Draganov, S., 1977, Taxonomic structure of cave algal flora: in Proc. 7th International Congress of Speleology, Sheffield, England, p. 155-156.
- Ehling-Schultz, M., Bilger, W. and Scherer, S., 1997, UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium Nostoc commune: Journal of Bacteriology, v. 179, no. 6, p. 1940–1945. https://doi: 10.1128/jb.179.6.1940-
- 1945.1997. Ettl, H. and Gärtner, G., 1995, Syllabus der Boden-, Luft- und Flechtenalgen: Gustav Fischer, Stuttgart, Jena, New York, p. 1–721.
- Falasco, E., Ector, L., Isaia, M., Wetzel, C.E., Hoffmann, L. and Bona, F., 2014, Diatom flora in subterranean ecosystems: a review: International
- Journal of Speleology, v. 43, no. 3, p. 231–251. https://doi: 10.5038/1827-806X.43.3.1.
- Gallon, J.R., Hashem, M.A. and Chaplin, A.E., 1991, Nitrogen fixation by *Oscillatoria* sp. under autotrophic and photoheterotrophic conditions: Journal of General Microbiology, v. 137, p. 31–39. https://doi: 10.1099/00221287-137-1-31.
- Garbacki, N., Ector, L., Kostikov, I. and Hoffmann, L., 1999, Contribution to the study of the flora of caves in Belgium: Belgian Journal of Botany, v. 132, no. 1, p. 43–76.
- Germain, H., 1981, Flore des Diatomophycées eaux douces et saumâtres du Massif Armoricain et des contrées voisines d'Europe occidentale: Collection "Faunes et Flores Actuelles", Société Nouvelles des Editions Boublée, Paris, p. 1–444.
- Gerrath, J.F., 2003, Conjugating green algae and desmids: *in* Wehr, J.D. and Sheath, R.G., eds, Freshwater algae of North America. Ecology and classification: Elsevier, New York, p. 353–382. https://doi:10.1016/B978-012741550-5/50010-6.
- Gorbushina, A., 2007, Life on the rocks: Environmental Microbiology, v. 9, p. 1613–1631. https://doi:10.1111/j.1462-2920.2007.01301.x.
- Gorbushina, A.A. and Broughton, W.J., 2009, Microbiology of the atmosphere-rock interface: how biological interactions and physical stresses modulate a sophisticated microbial ecosystem: Annual Review of Microbiology, v. 63, p. 431–450. https://doi:10.1146/annurev.micro.091208.073349.
- Gustavs, L., Eggert, A., Michalik, D. and Karsten, U., 2010, Physiological and biochemical responses of green microalgae from different habitats to osmotic and matric stress: Protoplasma, v. 243, p. 3–14. https://doi.org/10.1007/s00709-009-0060-9.

Hoffmann, L. and Darienko, T., 2005, Algal biodiversity on sandstone in Luxembourg: Ferrantia, v. 44, p. 99-101.

- Houk, V., 2003, Atlas of freshwater centric diatoms with a brief key and descriptions. Part I. Melosiraceae, Orthoseiraceae, Paraliaceae and Aulacoseiraceae: Czech Phycology Supplement, v. 1, p. 1–112.
- Johansen, J.R., Rushfort, S.R., Orbendorfer, R., Fungladda, N. and Grimes, J.A., 1983, The algal flora of selected wet walls in Zion National Park, Utah, USA: Nova Hedwigia, v. 38, p. 765–808.
- John, D.M., Whitton, B.A., and Brook, A.J., 2011, The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae: Cambridge University Press, Cambridge, p. 41–896.
- Karsten, U., Lembcke, S. and Schumann, R., 2007, The effects of ultraviolet radiation on photosynthetic performance, growth and sunscreen compounds in aeroterrestrial biofilm algae isolated from building facades: Planta, v. 225, p. 991–1000. https://doi:10.1007/s00425-006-0406-x-Source.
- Komárek, J. and Anagnostidis, K., 2005, Cyanoprokaryota 2. Oscillatoriales: *in:* Büdel, B., Krienitz, L., Gärdner, G. and Schagerl, M., eds, Süsswasserflora von Mitteleuropa 19/2, Elsevier, Spektrum, Heidelberg, p. 1–759.
- Krammer, K., 2000, The genus *Pinnularia*: *in*: Lange-Bertalot, H. ed, Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats, Ruggel, ARG Gantner Verlag, v.1, p. 703.
- Krammer, K. and Lange-Bertalot, H., 1991, *Bacillariophyceae*. 3. *Centrales, Fragilariace, Eunotiaceae: in:* Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D. eds, Süßwasser von Mitteleuropa, Stuttgart. Band 2/3, Gustav Fischer Verlag, Stuttgart, p. 576.
- Kristiansen, J., 1996, Dispersal of freshwater algae a review: Hydrobiologia, v. 336, p. 151–157. https://doi.org/10.1007/BF00010829.
- Lauriol, B., Prévost, Cl. and Lacelle, D., 2006, The distribution of diatom flora in ice caves of the northern Yukon Territory, Canada: relationship to air circulation and freezing: International Journal of Speleology, v. 35, no. 2, p. 83–92. http://doi:10.5038/1827-806X.35.2.4.
- Linnell, J.D.C., Barnes, B., Swenson, J.E., Andersen, R., 2000, How vulnerable are denning bears to disturbance?: Wildlife Society Bulletin, v. 28, p. 400–413.
- Lopez-Bautista, J.M., Rindi, F. and Casamatta, D., 2007, The systematics of subaerial algae: *in*: Seckbach, J. ed, Algae and Cyanobacteria in Extreme Environments: p. 599–617. https://doi.org/10.1007/978-1-4020-6112-7_33.
- MacArthur, R.H. and Wilson, E.O., 1967, The Theory of Island Biogeography: University Press, Princeton, p. 215.

- Martinez, A. and Asencio, A.D. 2010, Distribution of cyanobacteria at the Gelada Cave (Spain) by physical parameters: Journal of Cave and Karst Studies, v. 72, p. 11–20. https://doi:10.4311/jcks2009lsc0082.
- Massalski, A., Mrozińska, T. and Olech, M., 1995, *Lobococcus irregularis* (Boye-Pet.) Reisigl var. *antarcicus* var.nov. (Chlorellales, Chlorophyta) from King George Island, South Shetland Islands, Antarctica and its ultrastructure: Nova Hedwigia, v. 61, p. 199–206.
- Matthes-Sears, U., Gerrath, J.A., Gerrath, J.F. and Larson, D.W., 1999, Community structure of epilithic and endolithic algae and cyanobacteria on cliffs of the Niagara Escarpment: Journal of Vegetation Science, v. 10, no. 4, p. 587–598. https://doi.org/10.2307/3237193.
- Matuła, J., Pietryka, M., Richter, D. and Wojtuń, B., 2007, Cyanoprokaryota and algae of Arctic terrestrial ecosystems in the Hornsund area, Spitsbergen: Polish Polar Research, v. 28, no. 4, p. 283–316.
- Mikhailyuk, T.I., 2013, Terrestrial algae from the granite outcrops of River Valleys of the Ukraine: International Journal on Algae, v. 15, no. 4, p. 313–332. https://doi.org/10.1615/InterJAlgae.v15.i4.20.
- Mikhailyuk, T.I. and Darienko, T.M., 2011, Algae of terrestrial habitats of NNP Gutsulschina: Phytosociocentre, p. 142–151.
- Mulec, J., 2012, Lampenflora, *in*: White, W.B. and Culver, D.C. eds, Encyclopedia of Caves: Academic Press, Amsterdam, p. 451-456. doi: 10.1016/B978-0-12-383832-2.00064-5.
- Mulec, J. and Kosi, G., 2009, Lampenflora algae and methods of growth control: Journal of Cave and Karst Studies, v. 71, no. 2, p. 109–115.
- Mulec, J., Kosi, G. and Vrhovšek, D., 2008, Characterization of cave aerophytic algal communities and effects of irradiance levels on production of pigment: Journal of Cave and Karst Studies, v. 70, no. 1, p. 3–12.
- Mysterud, I., 1983, Characteristics of summer beds of European brown bears in Norway: International Conference on Bear Research and Management, v. 5, p. 1–208. https://doi.org/10.2307/3872540.
- Nagarkar, S., 1998, New records of marine cyanobacteria from rocky shores of Hong Kong: Botanica Marina, v. 41, no. 6, p. 527–542. http:// doi:10.1515/botm.1998.41.1-6.527.
- Nestupa, J. and Štifterová, A., 2013, Distribution patterns of subaerial corticolous microalgae in two European regions: Plant Ecology and Evolution, v. 146, p. 279–289. https://doi.org/10.5091/plecevo.2013.862.
- Nielsen, S.E., McDermid, G.J., Stenhouse, G.B. and Boyce, M.S., 2010, Dynamic wildlife habitat models: seasonal foods and mortality risk predict occupancy-abundance and habitat selection in grizzly bears: Biological Conservation, v. 143, no. 7, p. 1623–1634. https://doi:10.1016/j. biocon.2010.04.007.
- Nietfeld, M.J., Wilk, K. Woolnough and Hoskin, B., 1985, Wildlife habitat requirement summaries for selected wildlife species in Alberta: Wildlife resource inventory unit, Alberta Energy and Natural Resources. ENR Technical Report Number T/23.
- Pedersen, K., 2000, Exploration of deep intraterrestrial microbial life: current perspective: MiniReview, FEMS Microbiology Letters, v. 185, p. 9–16. https://doi.org/10.1111/j.1574-6968.2000.tb09033.x.

Pentecost, A., 1992, A note on the colonization of limestone rocks by cyanobacteria: Archiv für Hydrobiology, v. 124, p. 167-172.

Popović, S., Subakov Simić, G., Stupar, M., Unković, N., Predojević, D., Jovanović, J. and Ljaljević, G., 2015, Cyanobacteria, algae and microfungi present in biofilm from Božana Cave (Serbia): International Journal of Speleology, v. 44, no. 2, p. 141–140. https://doi:10.5038/1827-806X.44.2.4.

Pusz, W., Baturo-Cieśniewska, A. and Zwijacz-Kozica, T. 2018. Culturable Fungi in Brown Bear Cave Dens: Polish Journal of Environmental Studies, v. 27, no. 1, p. 247-255.

Pusz, W., Baturo-Cieśniewska, A. and Zwijacz-Kozica, T., 2017, Culturable fungi in brown bear cave dens: Polish Journal of Environmental Studies, v. 27, no. 1, p. 1–9.

- Ress, R.J. and Lowe, R.L., 2013, Contrast and comparison of aerial algal communities from two distinct regions in the U.S.A., the Great Smoky Mountains National Park (TN) and the Lake Superior region: Fottea, v. 13, no. 2, p. 165–172. https://doi.org/10.5507/fot.2013.014.
- Rifon-Lastra, A. and Noguerol-Seoane, A., 2001, Green algae associated with the granite walls of monuments in Galicia (NW Spain): Cryprogamie Algologie, v. 22, p. 305–326. https://doi.org/10.1016/S0181-1568(01)01069-8.
- Rindi, F., Allali, H.A., Lam, D.W. and López-Bautista, J.M., 2010, An overview of the biodiversity and biogeography of terrestrial green algae: *in*: Rescigno, V. and Maletta, S. eds, Biodiversity hotspots. Nova Science Publishers, New York, p. 105–122.
- Roldán, M. and Hernández-Mariné, M., 2009, Exploring the secrets of the three-dimensional architecture of phototrophic biofilms in caves: International Journal of Speleology, v. 38, p. 41–53. https://doi:10.5038/1827-806X.38.1.5.

Škaloud, P., 2009, Species composition and diversity of aero-terrestrial algae and cyanobacteria of the Boreč Hill ventaroles: Fottea, v. 9, p. 65–80. https://doi.org/10.5507/fot.2009.006.

Smith, T. and Olson, R., 2007, A taxonomic survey of Lamp flora (Algae and Cyanobacteria) in electrically lit passages within Mammoth Cave National Park, Kentucky: International Journal of Speleology, v. 36, no. 2, p. 105–114. https://doi:10.5038/1827-806X.36.2.6.

Taylor, J.C., Harding, W.R. and Archibald, C.G.M., 2007, An Illustrated Guide to Some Common Diatom Species from South Africa: Water Research Commission, Pretoria, Report TT 282/07, 34 + 12 p. and 178 pls.

- Ter Braak, C.J.F. and Šmilauer, P., 2012, Canoco Reference Manual and User's Guide: Software for Ordination (version 5.0): Microcomputer Power (Ithaca, NY, USA), p. 496.
- Uher, B., 2010, Cyanobacteria and algae as significant actors of biodeterioration: Saarbrücken, LAP Lambert Academic Publishing AG and Co. KG, p. 161.
- Van de Vijver, B. and Beyens, L., 1997, The epiphytic diatom flora of mosses from Strømness Bay area, South Georgia: Polar Biology, v. 17, p. 492–501. https://doi:10.1007/s003000050148.
- Vinogradova, O.N., 1999, Species composition and distribution of Cyanophyta in water bodies of the Mountain Crimea (Ukraine): International Journal on Algae, v. 1, no. 2, p. 76–87. https://doi.org/10.1615/InterJAlgae.v1.i2.70.

Vinogradova, O.N. and Mikhailyuk, T.I., 2009, Algal flora of the caves and grottoes of the National Nature Park "Podilsky Tovtry" (Ukraine): International Journal on Algae, v. 11, p. 289–404. https://doi.org/10.1615/InterJAlgae.v11.i3.80.

- Vinogradova, O.N., Wasser, S.P. and Nevo, E., 2009, Algae of the Sefunim Cave (Israel): species, diversity affected by light, humidity and rock stresses: International Journal on Algae, v. 11, no. 2, p. 99–116. https://doi.org/10.1615/InterJAlgae.v11.i2.10.
- Wehr, J.D. and Sheath, R.G., 2003, Freshwater habitats of algae: *in*: Wehr, J.D. and Sheat, R.G. eds, Freshwater algae of North America: ecology and classification, Academic Press, San Diego.
- Whitton, B.A., 2012, Ecology of Cyanobacteria II. Their Diversity in Space and Time: Springer Science+Business Media B.V., p. 706. https://doi. org/10.1007/978-94-007-3855-3.
- Whitton, B.A. and Potts, M., 2000, The ecology of cyanobacteria. The diversity in time and space: Springer Netherlands, p. 669. https:// doi:10.1007/0-306-4685507.
- Zedrosser, A., Dahle, B., Swenson, J. and Gerstl, N., 2001, Status and management of the brown bear in Europe: Ursus, v. 12, p. 9–20. Zwijacz-Kozica, T., 2011, Protected or not protected, Tatra Mountains: Tatra National Park, p. 56–58.