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OBSERVATIONS ON AEROPHYTIC CYANOBACTERIA AND ALGAE FROM TEN CAVES IN THE OJCÓW NATIONAL PARK

Joanna Czerwik-Marcinkowska

Department of Botany, Institute of Biology, Jan Kochanowski University
Świętokrzyska 15, 25-420 Kielce, Poland
e-mail: marcinko@kielce.com.pl

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Abstract

This study, carried out in 2010–11, focuses on species composition and distribution of cyanobacterial and algal communities colonizing ten caves (Biała, Ciemna, Koziarnia, Krakowska, Łokietka, Okopy Wielka Dolna, Sąspowska, Sypialnia, Zbójecka and Złodziejska Caves) in the Ojców National Park (South Poland). A total of 85 taxa were identified, 35 of them belonging to cyanobacteria, 30 chlorophytes, and 20 belonging to other groups of algae. Aerophytic cyanobacteria dominated in these calcareous habitats. Nine species, *Gloeocapsa alpina, Nostoc commune, Chlorella vulgaris, Dilabifilum arthopyreniae, Klebsormidium flaccidum, Muriella decolor, Neocystis subglobosa,* and *Orthoseira roseana*, were the most abundant taxa in all the caves. The investigated microhabitats offer relatively stable microclimatic conditions and are likely to be responsible for the observed vertical distribution of aerophytic cyanobacteria and algae.

Key words: algae, cyanobacteria, caves, Ojców National Park, Poland.

INTRODUCTION

Caves are unique in terms of specific natural characteristics such as: microclimate, temperature, humidity and others. According to M u l e c and K o s i (2009), caves have a special place in human history and many caves are inscribed on the United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage List. Recently, many caves have experienced intensified tourist visits and to make them more attractive for visitors, artificial illumination was installed which changed physico-chemical conditions in the caves. However, most caves, at least in Europe, are damp and the walls at the entrance are covered with green algal gelatinous mass (P o u l i č k o v á and H a š l e r , 2007). At the entrance of limestone caves and on the surfaces around electrical lights, cyanobacteria compete for light with other algae, bryophytes and ferns, but in the deepest recesses of the caves they are usually the only phototrophs (R o u n d , 1981). Most caves represent stable environments characterized by uniform temperatures throughout the year, high humidity and low natural light (H e r n á n d e z - - M a r i n é and C a n a l s , 1994; D u c a r m e et al. 2004; P o u l i č k o v á and H a š l e r , 2007; L a m p r i n o u et al. 2009). According to L a m p r i n o u et al. (2012), a typical cave is described as having three major habitat zones based on light penetration and intensity: the entrance-, transition-, and dim light zone. Moreover, cave characteristics, such as dimensions, morphology, location, orientation and rocky substrate, can play an important role for the biocommunity structure. All caves belong to habitats of extreme conditions characterized by a low content of nutrients (P e d e r s e n , 2000; M u l e c et al. 2008). However, many groups of organisms prefer such conditions for the colonization and growth. The following occur most frequently in caves: liverworts, mosses, some ferns, flowering plants, algae and cyanobacteria (D o b a t , 1970; K u e h n et al. 1992; S a n c h e z et al. 2002; M u l e c , 2005; M u l e c et al. 2008; M u l e c and K o s i , 2009). Algae often play a key role in the food webs and in the colonization processes of rocky habitats (sediments, rocky surfaces, artificial material) as well as they produce colourful effects on the caves walls (G o l u b i č , 1967). These processes are favoured by usually stable environmental conditions prevailing in caves. All these factors make caves attractive for cosmopolitan species, which consequently eliminate the native components (P i p a n , 2005). Although infrequently, but new algal species have also been identified in caves (Jones, 1964; Hernandéz-Mariné and Canals, 1994). Aerophytic algae and cyanobacteria are usually observed in cave entrances illuminated by direct or indirect sunlight and also around the artificial light in caves that are open to tourists (Pentecost, 1992; Mulec, 2005; Mulec et al. 2008; Czerwik-Marcinkowska and Mrozińska, 2011).

In Poland algological studies in caves have been carried out by the following researchers: Starmach (1963); Mrozińska-Broda and Czerwik-Marcinkowska, (2004); Czerwik-Marcinkowska and Mrozińska, (2008, 2009, 2010, 2011).

The aim of this study was to present the spatial distribution of aerophytic algae and cyanobacteria growing in ten caves in the Ojców National Park differing in location, morphology and environmental conditions as part of an extensive study of caves in the Polish Jura.

STUDY AREA

The caves are one of the most characteristic elements of the Ojców National Park and its surroundings. The presence of caves in this area has been noticed for a long time (Chardez and Delhez, 1981; Szelerewicz and Górny, 1986; Bisek et al. 1992; Gradziński et al. 1995a, b, 1996, 1998, 2007; Gradziński and Szelerewicz, 2004; Górny and Szelerewicz, 2008). These caves were created by underground waters dissolving Jurassic marine limestones (Michalik and Partyka, 1992). Some of the caves exceed 300 m in depth. Ten caves: Biała, Ciemna, Koziarnia, Krakowska, Łokietka, Okopy Wielka Dolna, Sąspowska, Sypialnia, Zbójecka and Złodziejska Caves, were studied in the Park (Fig. 1; Table 1). The most attractive caves from the natural and environmental point of view, having corridors longer than 50 m, include the following: Biała, Ciemna, Koziarnia, Krakowska, Łokietka, Okopy Wielka Dolna, Sąspowska and Zbójecka Caves (Partyka, 1997). Only the Łokietka and Ciemna Caves (among the studied ones) are open to tourists.

MATERIALS AND METHODS

Samples for algological analysis were taken from the ten caves of the Ojców National Park in the spring, summer and autumn of 2010 and 2011. Algal crusts were scraped from the walls and ceiling, using a scalpel, into labelled plastic bags and analysed under a light microscope (Jenamed 2) or inoculated for culturing on agar plates (Figs 4–6). Additionally, in the two caves (Łokietka and Ciemna) open to tourists, samples were also collected from around artificial light sources, at different distances from the source, and from sites of most intensive growth. Scraped material was used directly for observation under the light microscope. Two sampling zones were distinguished in each cave: A – light zone, comprising the entrance (usually lit by sunlight and well-oxygenated); and B – dark zone comprising the chambers illuminated only by weak natural daylight or artificial light.

Collected organisms were cultured on standard Bristol agar medium, at 20ºC under a 12/12h light/dark cycle at 3000 $\mu Em^{-2} s^{-1}$ provided by 40W fluorescent tubes. For transmission electron microscopy (TEM), cells were fixed as previously described (Massalski et al. 1995). Ultra-thin sections were cut with glass knives on a Reichert-Jung ultramicrotome. Observations and photographs were made with a TESLA BS 500 electron microscope. Cells for TEM were double fixed with glutaraldehyde in phosphate buffer and postosmicated with osmium tetroxide in the same buffer, then they were dehydrated in series of alcohols, embedded in the synthetic epoxy resin "SPURR" and polymerized at 70ºC for 18 h. For scanning electron microscopy (SEM), the samples were treated with 36% HCl, washed several times with distilled water and boiled in concentrated H_2O_2 with $KClO_3$ in order to remove organic matter. Cyanobacteria and algae were identified according to: Anagnostidis and Komárek (1988); Brown and Bold (1964); Brown and Lean (1969); Etll and Gärtner (1988, 1995); Hoffmann (1986, 2002); Komárek and Anagnostidis (1986, 1989); Krammer and Lange-Bertalot (1986, 1991).

RESULTS

A total of 85 aerophytic algae and cyanobacteria species were identified in the ten caves in the Ojców National Park (Fig. 2; Table 2). Overall, cyanobacteria were dominant and represented by 35 species (40% of the total), green algae 30 species (32.7%), and 12 diatoms species (27.2%). The frequently encountered species among cyanobacteria were as follows: *Aphanocapsa parietina* Näg., *Calothrix fusca* (Kutz.) Born. & Flah., *Chroococcus tenax* (Kirchn.) Hieronymus and *Nostoc commune* Vauch. ex Born. & Flah. They occurred both in the light (A) and dark (B) zones of the studied caves. Microscopic observations revealed that cyanobacteria are arranged in patina assemblages which are blue, brown, green, or gray, and are arranged mosaic-like inside the caves. Among the cyanobacteria present, the following were dominant only in the caves open for tourism, i.e. the Łokietka Cave and the Ciemna Cave: *Gloeocapsa alpina* (Näg.)

F. Brand, *Nodularia harveyana* Thuret ex Born. & Flah. and *Tolypothrix epilithica* Näg. The most rare green algae species were: *Bracteococcus minor* (Chodat) Petrová (Fig. 3), *Desmococcus olivaceum* (Pers. ex Arch.) Laudon, *Muriella decolor* Vischer, *Scotiellopsis terrestris* (Reisigl) Punčochářová & Kalina and *Thelesphaera alpina* Pascher. In the Zbójecka and Ciemna Caves, green algae such as: *Chlorella vulgaris* Beijerinck, *Coleochlamys perforata* (Lee & Bold) Ettl & Gärtn. and *Klebsormidium montanum* (Skuja) Watanabe, were encountered on well-illuminated surfaces. Diatoms were present in the zone of both direct and diffuse sunlight, but they usually preferred the wet habitats (permanently wet or periodically sprinkled with water dripping and sipping from the ceiling and walls). Among the aerophytic diatoms, the most frequently encountered taxa were: *Diadesmis contenta* (Grun. in Van Heurck) D.G. Mann in Round, *Hantzschia amphioxys* (Ehr.) Grunow, *Luticola nivalis* (Ehr.) D.G. Mann, *Orthoseira roseana* (Rabenh.) O'Meara, and *Pinnularia borealis* Ehr. The most species of cyanobacteria and algae (in Biała Cave – 11 species, Ciemna Cave – 16, Koziarnia Cave – 9, Krakowska Cave 20, Łokietka Cave – 12, Okopy Wielka Dolna – 18, Sąspowska Cave – 13, Sypialnia Cave – 23, Zbójecka Cave – 25, Złodziejska Cave – 13) were found on the rocky surfaces and in the indentations close to the entrance (the A bright zone). There were also species found only in the places within direct sunlight: *Chroococcus* cf. *ercegovicii* Kom. & Anagn., *Chlorogloea novacekii* Kom. & Montejano, *Plectonema* cf. *puteale* (Kirchn.) Hansg., *Characium strictum* A. Braun, *Desmococcus olivaceum* (Pers. ex Arch.) Laudon and *Keriochlamys styriaca* Pascher. On the other hand, there were taxa growing only in the close vicinity of electric light: *Chlorobotrys simplex* Pascher, *Klebsormidium montanum* (Skuja) Watanabe, *Stichococcus allas* Reisigl and *S. bacillaris* Näg. Mosses (e.g. the genus *Crotoneuron*) or liverworts (the genus *Polysiphonia*) and ferns (*Asplenium trichomonas* L. and *A. ruta-muraria* L.), forming characteristic abundant clusters, were frequently accompanied by aerophytic diatoms and green algae, e.g.: *Diadesmis contenta* (Grun. in Van Heurck) D.G. Mann in Round, *Gomphonema italicum* Kütz., *Chlorella vulgaris* Beijerinck, *Trentepohlia aurea* (Linné) Martius and *Stichococcus bacillaris* Näg. In the entrances of the caves open to tourists (Łokietka and Ciemna Caves), cyanobacteria, in particular *Calothrix parietina* (Näg.) Thuret, *Gloeocapsopsis* cf. *magma* (Bréb.) Kom. & Anagn., *Nostoc* cf. *microscopicum* Carm., *Phormidium breve* (Kütz. ex Gom.) Anagn. & Kom., and *Pseudocapsa dubia* Erceg., were competing with other algae (e.g. *Chlamydomonas* sp., *Muriella decolor* Vischer, *Leptosira terricola* Bristol Printz), mosses and ferns for the best illuminated surfaces.

Table 1
Location and basic morphometric characteristics of the ten studied caves
(data after Szelerewicz, Górny (1986); Bisek et al. (1992), Gradziński et al. (1995a, b, 1996, 1998, 2007); Górny, Szelerewicz (2008)

Cave	Location	Altitude (m)	Length (m)	Depth (m)	Orientation of cave entrance	Lithology
Biała	Wąwóz Jamki	ca. 398, 407	84	9	NW	Jurassic limestones
Ciemna	Dolina Prądnika	372	209	10	NW, S	Jurassic limestones
Koziarnia	Wąwóz Koziarnia	385	90	11	W	Jurassic limestones
Krakowska	Wąwóz Jamki	410	90	11.5	NW	Jurassic limestones
Łokietka	Dolina Sąspowska	452	320	7	NW	Jurassic limestones
Okopy Wielka Dolna	Dolina Prądnika	380	138	–	W, vertical	Jurassic limestones
Sąspowska	Dolina Sąspowska	370	100	13	E	Jurassic limestones
Sypialnia	Wąwóz Stodoliska	342	44	1.5	S	Jurassic limestones
Zbójecka	Wąwóz Jamki	ca. 370, 372, 376	189	15	NW, W, vertical	Jurassic limestones
Złodziejska	Wąwóz Jamki	380	45	4	E, N	Jurassic limestones

Table 2
List of the identified algae and cyanobacteria species from ten caves of the Ojców National Park

Taxa	Sampling site
Eucaryota	
Chlorophyta	
Chlamydophyceae	
Chlamydomonas sp.	2,4,10
Tetracystis intermedia (Deason & Bold) Brown & Bold	6
Tetracystis cf. *isobilateralis* Brown & Bold	6
Tetracystis cf. *texensis* Brown & Bold	6
Chlorophyceae	
Bracteacoccus minor (Chodat) Petrová	4,6
Characium strictum A. Braun	10
Chlorella vulgaris Beijerinck	1,4,6
Chlorococcum nivale Archibald	1
Chlorosarcina longispinosa Chant. & Bold	3
Choricystis minor (Skuja) Fott	1,9
Coleochlamys perforata (Lee & Bold) Ettl & Gärtner	1,4,6
Desmococcus olivaceum (Persoon ex Archerson) Laudon	2,4
Dilabifilum arthopyreniae (Vischer & Klement)Tschermak-Woess	6,7,8
Gloeocystis polydermatica (Kützing) Hindák	5,6,7
Interfilum paradoxum Chodat & Topali	2
Leptosira terricola (Bristol) Printz	2
Leptosira sp.	8,9
Muriella decolor Vischer	2,4,5,6
Neocystis subglobosa (Pascher) Hindák	2,5,6,8
Podohedra bicaudata Geitler	8
Scotiellopsis terrestris (Reisigl) Punčochářová & Kalina	4,6,8
Thelesphaera alpina Pascher	1
Trebouxiophyceae	
Keriochlamys styriaca Pascher	1
Klebsormidiophyceae	
Klebsormidium flaccidum (Kützing) Silva, Mattox & Blackwell	1,6,7,8
Klebsormidium montanum (Skuja) Watanabe	2,8
Klebsormidium sp.	1
Stichococcus allas Reisigl	8
Stichococcus bacillaris Nägeli	7,8
Stichococcus sp.	6,7,8
Trentepohliophyceae	
Trentepohlia aurea (Linné) Martius	4,6
Heterokontophyta	
Xanthophyceae	

Chlorobotrys simplex Pascher	2
Chlorobotrys terrestris Pascher	2,5,8
Chlorobotrys sp.	2,3,4,5
Gloeobotrys piriformis Reisigl	2
Heterococcus caespitosus Vischer	3,9
Trachychloron sp.	7,10
Trachychloron simplex Pascher	3
Eustigmatophyceae	
Eustigmatos sp.	9
Bacillariophyceae	
Aulacoseira sp.	3
Caloneis silicula (Ehrenberg) Cleve	7
Diadesmis contenta (Grunow in Van Heurck) D.G. Mann in Round	6,7
Gomphonema italicum Kützing	7,10
Grunowia tabellaria (Grunow) Rabenhorst	6
Hantzschia amphioxys (Ehrenberg) Grunow	1,2,8
Luticola sp.	5
Luticola nivalis (Ehrenberg) D.G. Mann	7
Nitzschia sp.	2,7,8
Orthoseira roseana (Rabenhorst) O'Meara	2,7,8
Pinnularia borealis Ehrenberg	3,5
Pinnularia sp.	8,9,10
Prokaryota	
Cyanophyta	
Cyanophyceae	
Anabaena cf. *oscillarioides* Bory de Saint-Vincent	10
Aphanocapsa parietina Nägeli	1
Calothrix fusca (Kützing) Bornet & Flahault	6
Calothrix parietina (Nägeli) Thuret	10
Calothrix sp.	6
Chroococcus cf. *ercegovicii* Komárek & Anagnost.	1
Chroococcus tenax (Kirchner) Hieronymus	8
Chlorogloea novacekii Komárek & Montejano	8
Cyanosarcina sp.	3,4,6,7
Gloeocapsa alpina (Nägeli) F.Brand	3,4,6,7
Gloeocapsa biformis Ercegović	8
Gloeocapsa cf. *decorticans* (A.Braun) Richter	3,4
Gloeocapsa rupicola Kützing	5
Gloeocapsopsis cf. *magma* (Bréb.) Kom. & Anagn.	7
Gloeothece sp.	8,9
Hapalosiphon sp.	4
Leptolyngbya sp.	2,6,7

Microcystis sp.	8,9
Nodularia harveyana Thuret ex Bornet & Flahault	9
Nodularia sp.	3,4,5,10
Nostoc commune Vaucher ex Bornet & Flahault	1,2,3
Nostoc cf. *microscopicum* Carmichael	6
Nostoc punctiforme (Kützing ex Hariot) Hariot	8,9
Nostoc sphaericum Kützing	1,10
Nostoc sp.	5,9
Phormidium amoenum Kütz. ex Gom. & Komárek	7
Phormidium breve (Kütz. ex Gom.) Anagn. & Komárek	6,7,8,9
Phormidium sp.	2,9
Plectonema cf. *puteale* (Kirchner) Hansgirg	2
Pseudocapsa dubia Ercegović	1,2
Schizotrix sp.	2
Synechococcus elongatus Nägeli	3,4
Tolypothrix epilithica Nägeli	5,7,9
Tolypothrix tenuis Kützing ex Bornet & Flahault	6
Tolypothrix sp.	2,8

1 – Sąspowska Cave; 2 – Krakowska Cave; 3 – Łokietka Cave; 4 – Ciemna Cave; 5 – Biała Cave; 6 – Zbójecka Cave; 7 – Okopy Wielka Dolna Cave; 8 – Sypialnia Cave; 9 – Złodziejska Cave; 10 – Koziarnia Cave.

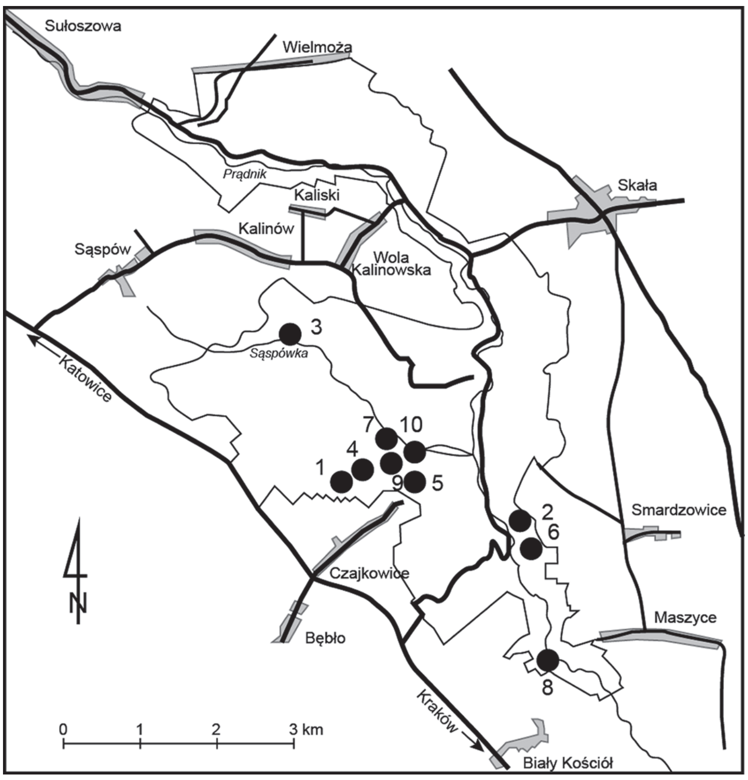

Fig. 1. Location of studied caves in the Ojców National Park (1 – Biała Cave; 2 – Ciemna Cave; 3 – Koziarnia Cave; 4 – Krakowska Cave; 5 – Łokietka Cave; 6 – Okopy Wielka Dolna Cave; 7 – Sąspowska Cave; 8 – Sypialnia Cave; 9 – Zbójecka Cave; 10 – Złodziejska Cave).

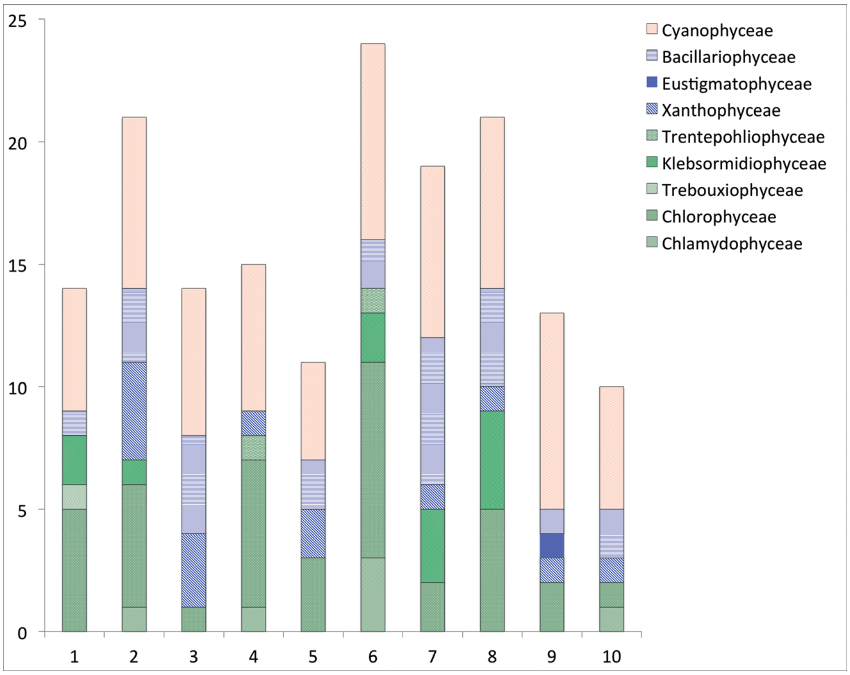

Fig. 2. Participation of particular groups of algae and cyanobacteria in ten studied caves.

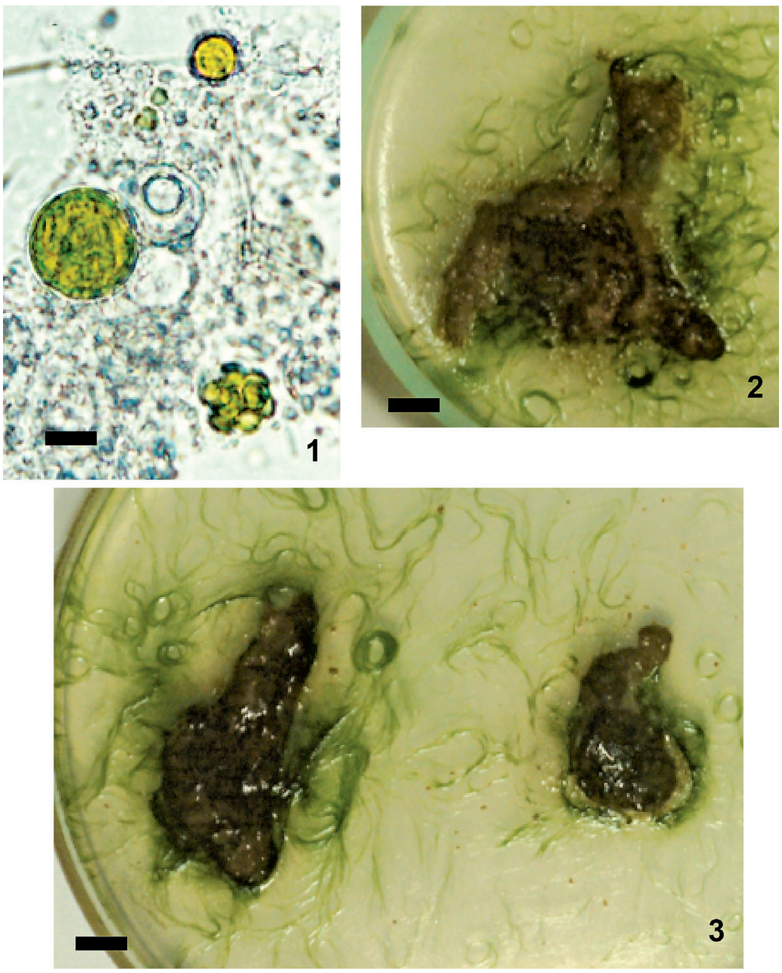

Fig. 3. *Bracteacoccus minor* (Chodat) Petrová. 1– macroscopic view of young cells; 2–3 – algological materials on agar plates in lab. Light micrographs [scale bar: 10 μm].

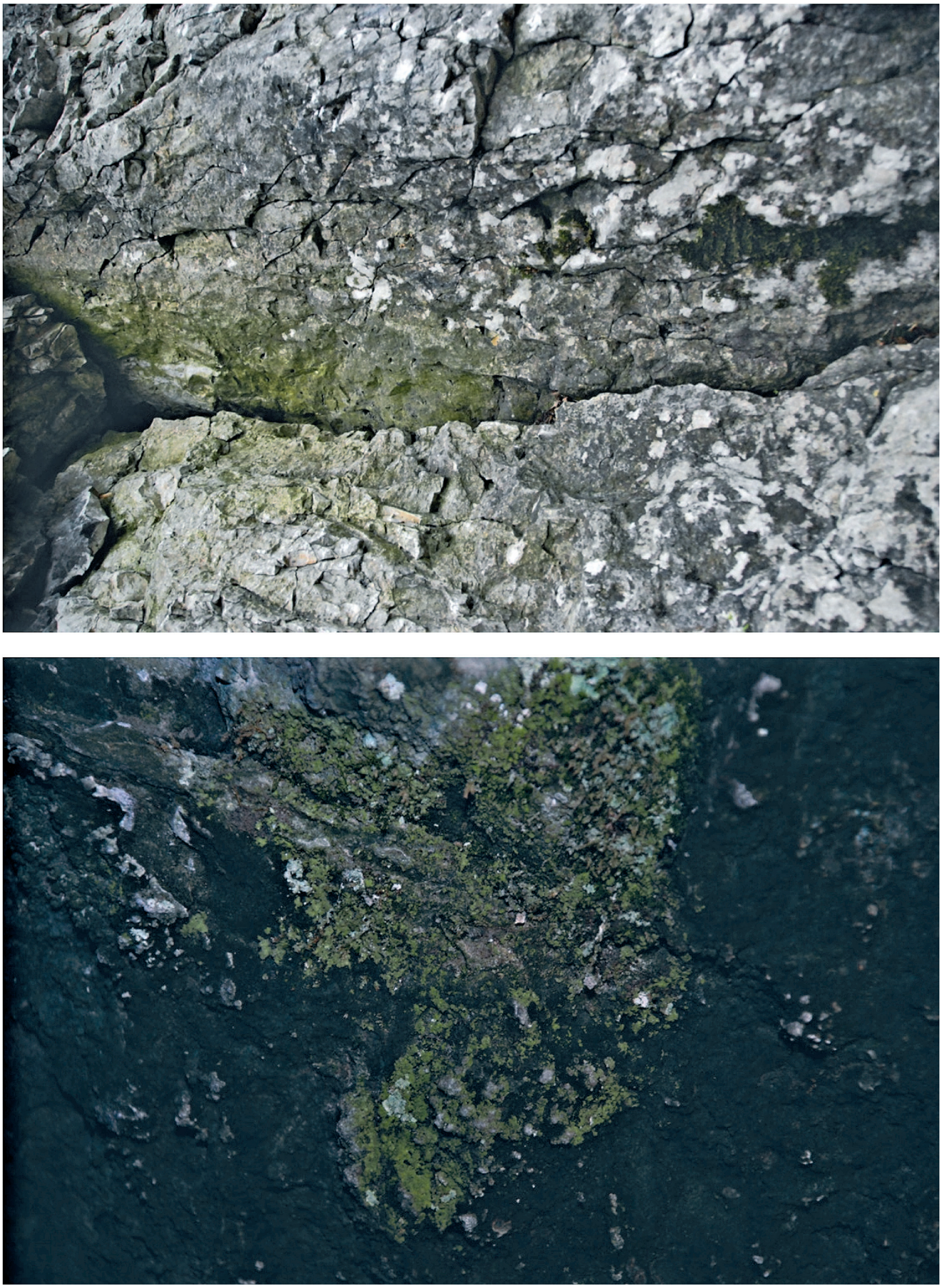

Fig. 4. Epilithic algae and cyanobacteria and their subaeric habitats (Łokietka Cave).

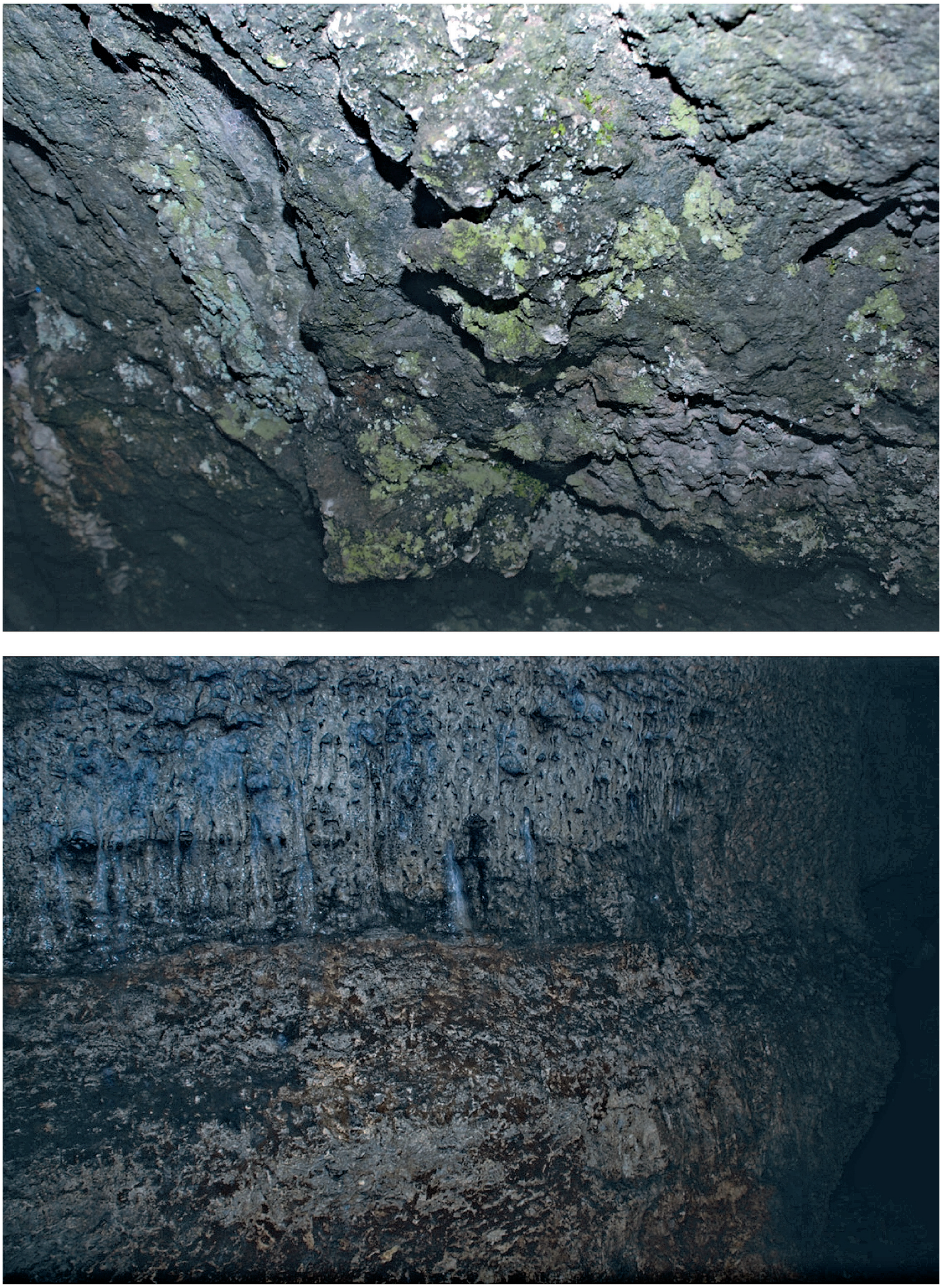

Fig. 5. Aerophytic cyanobacteria and algae in different microhabitat zones in Ciemna Cave.

Fig. 6. The wall of Ciemna Cave cover with growths of aerophytic cyanobacteria and algae, liverworts and mosses.

DISCUSSION

Cyanobacteria prevail in cave entrances compared to other algal groups (Mulec 2005; Mulec and Kosi, 2008). They colonize various parts of cave entrances where biodiversity of organisms is the lowest (Vinogradova et al. 1998). Water relations in caves are as important for cyanobacteria and algae growth and colonization (Pentecost and Whitton, 2000). According to Lamprinou et al. (2009), cave aerophytic assemblages are dominated by cyanobacteria, which represent the first photosynthetic colonizers on the calcareous surfaces usually thriving both as epiliths and endoliths. Epilithic assemblages form extensive dark-green coverings (with *Phormidium breve* as the dominant species) or pale blue-green to whitish coverings (with *Tolypothrix epilithica* as the dominant species). Cyanobacteria, green algae and diatoms constituted an essential component of the flora of the studied caves.

Round (1981) differentiated the distribution of phototrophes depending on the access of either natural or artificial light. The development of algae visible in the form of green cover of cave walls is undoubtedly connected with the availability of light and specific microclimate. The microclimate in caves is influenced by air circulation, hydrological conditions, and isolation of a cave from outside thermal influences (Martinez and Asencio, 2010). Also the presence of tourists usually results in an increase of otherwise constant temperature, with the manner in which algae hibernate. Green algae, which dominate the flora of caves, hibernate in the form of different resting cells (cysts and spores). It was observed that in the studied ten caves of the Ojców National Park green algae and diatoms increased their quantity at a higher level of humidity. According to Asencio and Aboal (2000) as well as Poulíčková and Hašler (2007), the majority of caves in Europe are characterized by average humidity (ca. 70%) and their entrance walls are just covered by green algae and cyanobacteria.

In the Łokietka and Ciemna Caves open for tourism, separate chambers with artificial lighting create habitats in which the species composition of cyanobacteria and algae was very similar. According to Round (1981), Pentecost, (1992) as well as Pentecost and Zhang (2001), the type of substratum is an important factor determining the species composition, distribution and structure of algal communities. It was observed likewise that cyanobacteria such as: *Anabena* cf. *oscillarioides* Bory de Saint-Vin., *Gloeocapsa biformis* Erceg., *Nostoc punctiforme* (Kütz. ex Hariot) Hariot, and some aerophytic diatoms (*Caloneis silicula* (Ehr.) Cl., *Grunowia tabellaria* (Grun.) Rabenh. and *Hantzschia amphioxys* (Ehr.) Grun.) were dependant on temperature, light and humidity. These cyanobacteria and algae prefer humid places during their development, but they also display considerable resistance to drying as well as to low air temperature during winter. The adaptation mechanism of algae living in low temperature is not yet precisely known (Mulec and Kosi, 2008, 2009). It can be therefore concluded that the environmental conditions in the ten caves of the Ojców National Park are stable and conducive to consequent development of cyanobacteria, green algae and aerophytic diatoms.

Protection of aerophytic cyanobacteria and algae in the caves, particularly those open to tourists (i.e. Łokietka Cave, Ciemna Cave), requires full recognition of existing and potential threats regarding the geological structure of the caves. Monitoring of the caves would enable the direction of the most important threats to be documented (e.g. the development of algae on different forms of dripstone formations, including flowstones, stalagmites and small stalactites). Species protection in the caves should be directed towards identification of rare, native, endemic and threatened species (Urban, 2006; Ponikiewicz, 2008).

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Obserwacje aerofitycznych sinic i glonów w dziesięciu jaskiniach Ojcowskiego Parku Narodowego

Streszczenie

Badania algologiczne, przeprowadzone wiosną, latem i jesienią 2010 i 2011 roku wykazały różnorodność występowania sinic i glonów w dziesięciu jaskiniach Ojcowskiego Parku Narodowego (Jaskinia Biała, Ciemna, Koziarnia, Krakowska, Łokietka, Okopy Wielka Dolna, Sąspowska, Sypialnia, Zbójecka and Złodziejska). Zidentyfikowano łącznie 85 gatunków aerofitycznych glonów i sinic, w tym 35 taksonów reprezentujących sinice, 30 gatunków zielenic i 20 przedstawicieli pozostałych grup taksonomicznych badanej algoflory. Niewątpliwie aerofityczne sinice dominują na wapiennych mikrosiedliskach jaskiniowych. Dziewięć gatunków glonów i sinic, spośród występujących w jaskiniach, spotykano najliczniej, były to: *Gloeocapsa alpina, Nostoc commune, Chlorella vulgaris, Dilabifilum arthopyreniae, Klebsormidium flaccidum, Muriella decolor, Neocystis subglobosa, Orthoseira roseana*. Badane mikrosiedliska jaskiniowe wykształcają stosunkowo stabilne warunki mikroklimatyczne, będące odpowiedzią na rozmieszczenie i występowanie aerofitycznych glonów i sinic.