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# Lampenflora of Lipska Cave, Montenegro

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**Abstract**: The composition of phototrophic and microfungal communities in the Lipska show cave has been studied. Species were identified from 12 areas containing visible lampenflora, localized throughout the cave, and from 8 sites within the illuminated zone of the cave's natural entrance. Relative occurrence and relative abundance were calculated for the species detected. In total 29 phototrophic species were identified, including 17 species of algae and cyanobacteria, and 12 bryophyte species. Cyanobacteria were the dominant group of phototrophs within the composition of the lampenflora communities. *Leptolyngbya tenuis* and *Gloeocapsa compacta* dominated in the lampenflora communities, whereas *L. tenuis* and *Chroococcus minutus* had the highest abundance in the natural entrance zone. The dominant species of green algae both in the natural entrance zone and within the lampenflora communities were *Stichococcus bacillaris* and *Chlorella vulgaris*. *Fissidens taxifolius* and *Brachythecium tommasinii* dominated in the composition of the lampenflora communities, while *Entodon schleicheri* and *Tortella* sp. had the highest abundance in the natural entrance zone. Additionally, 19 species of microfungi were identified, with *Penicillium chrysogenum* and *P. purpurascens* considered as the dominant species in the composition of the lampenflora, with *P. simplicissimum* and *Fusarium solani* dominating in the natural entrance zone. It is presumed that the further development of lampenflora communities should proceed in line with an increase in the number of mosses.

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## Introduction

Karst caves, which represent unique geological structures, each with its own level of complexity, also provide habitats for a great number of organisms. Currently the study of the microbiota of caves under conditions of artificial illumination is of particular interest and has been investigated widely and in detail in several European countries (e.g. Lamprinou *et al*., 2009, 2012; Martinez and Asencio, 2010; Cennamo *et al*., 2012; Borderie *et al*., 2014; Pusz *et al*., 2014).

Modification of caves to enable safe and interesting tourist excursions requires, among other changes, the installation of artificial lights in the underground environment. This can lead to the growth of photosynthetic organisms, so-called "*lampenflora*". Such organisms cause local destruction of cave substrates and cause deterioration of their aesthetic appearance.

The dominant phototrophic components of cave ecosystems are cyanobacteria and algae (Mulec, 2008). Predominance of cyanobacteria, especially in cave entrance zones, has been detected and described (e.g. Selvi and Altuner, 2007; Mulec and Kosi, 2008; Mazina and Maximov, 2011; Czerwik-Marcinkowska, 2013). Species diversity reduces with decrease of illumination intensity (Falasco *et al*., 2014). Due to their evident resistance to dehydration the most resilient cyanobacteria are encountered under far more-severe conditions than other groups of algae (Potts, 1994). Along with the heterotrophic components of caves represented by bacteria and microfungi, cyanobacteria and algae play a significant role in the genesis of biofilms. Factors that influence the growth and development of aerophilic cyanobacteria and algae are temperature conditions, light intensity, and relative humidity (Popovic *et al*., 2015).


***Figure 1****: Sketch map of part of southwestern Montenegro, showing the approximate location of Lipska Cave.*

Species composition variability within microbial communities depends upon: nutrient availability; type, physical properties and chemical properties of the substrate; morphological details of the cave; and the availability of water (Pantazidou and Roussomoustakaki, 2005; Lamprinou *et al*., 2012; Czerwik-Marcinkowska, 2013).

Development of microfungi communities is determined primarily by nutrition, as is confirmed by the isolation of the greatest number of cultured microfungi from samples with the best-developed biofilms (Popovic *et al*., 2015).

Opened to visitors in 2015, Lipska Cave is Montenegro's first tourist cave, giving access to unique karstic deposits and dripstones. Since the cave was adapted for tourist visits, the first examples of areas where the initial stages of lampenflora overgrowth are clearly visible have appeared. The aim of the research reported here was to study the biodiversity of lampenflora communities within Lipska Cave, and of flora within the cave's naturally lit entrance zone.

## Study area

Lipska Cave (coordinates N 42° 22 0.41057, E 18° 57 11.10848) lies in Dobrosko village, Montenegro, 5km from the city of Cetinje, 33km from Budva (Fig.1) and 35km from the capital city Podgorica. Currently the known length of the cave is about 3.5km; the part of the cave equipped for visitors has a length of 2.5km, with paved paths, railings and stationary lighting. The temperature in the cave is 11–12°C, with 70–80% humidity.

Fieldwork for the study was carried out during July 2017. Samples of phototrophic communities were collected in the illuminated zone of the cave's natural entrance (8 sites), as well as at 12 sites in areas showing visible lampenflora growth along the cave's tourist trail (Fig.2).

## Materials and methods

Examination of phototrophs was carried out using a Leica DMLS (Germany) light microscope. Bristol's Medium (Gaysina *et al*., 2008) and Gromov's Solution No.6 (Gromov, 1965) were used to cultivate cyanobacteria and algae. Growth conditions were: temperatures of 12° and 24°C and light intensity of 30 μmol photons$\times$m$^{-2}\times$s$^{-1}$. The method involved the use of "growth slides" and culturing in a liquid medium. For precise identification of species composition of phototrophs from the illuminated zone, the cultivation of average samples from communities was carried out in Gromov's Solution No.6. In order to detect the propagule of phototrophs introduced into the caves with airflows, samples of soil and water taken from the unlighted zone were cultured in both Gromov's Solution No.6 and Bristol's Medium. Water was filtered through nuclear filters and then the filtrate was placed into the culture medium. All of the sample material was examined routinely during a ten-month cultivation period. Algae were identified using the following classification systems: Komárek and Fott, 1983; Komárek and Anagnostidis, 1998, 2005; Hofmann *et al*., 2013; Komárek, 2013. Taxonomy of cyanobacteria and algae is given according to http://www.algaebase.org.

Czapek-Dox Medium (saccharose concentrations of 0.3% and 3%) and extract from clay deposits (analogue of soil extract) were used to detect microfungi. The first dilutions of soil samples were used for the inoculations, and the "growth slides" method was applied (Netrusov, 2005). Cultivation of fungi was carried out at temperatures of 4, 12 and 24°C, with calculations of grown colonies

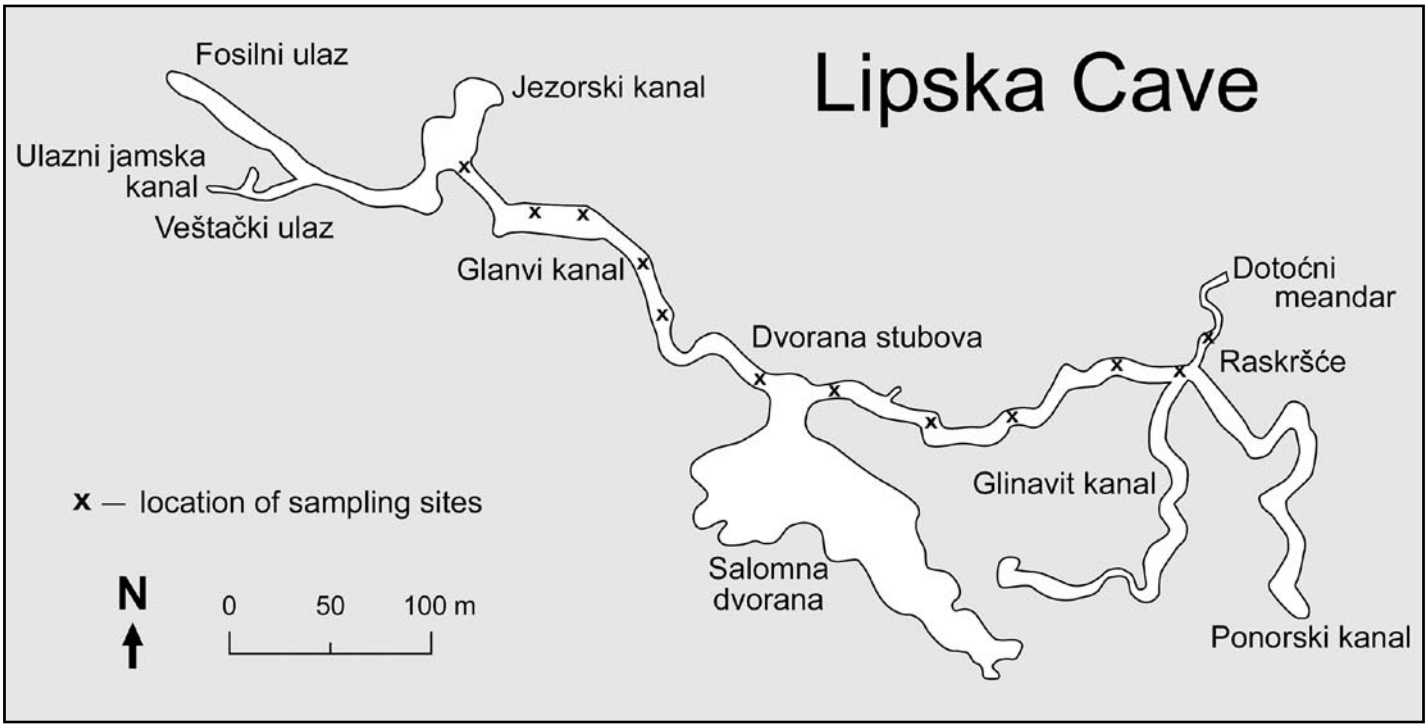


***Figure 2****: Lipska Cave, simplified plan showing locations and spacing of sampling sites along the cave's tourist trail (after Đurović et al., 2002).*

and isolation of pure cultures performed weekly in the dark. Identifications of soil fungi were performed using the following identification keys: Booth, 1971; Ellis, 1976; Domsch *et al*., 2007; Pitt, 1991; Ramirez, 1982; Raper and Fennel, 1965; Raper and Thom, 1968; Simmons, 2007). The names of species are given according to the **http://www.mycobank.org** database Bryophytes were identified according to Ignatov and Ignatova (2003).

Relative abundance and relative occurrence values were determined as the ratio of occurrence (abundance) of the species to the total occurrence (abundance) of all species in percent. The dominant species were considered to have the highest relative abundance (for cyanobacteria, algae and bryophytes) and highest relative occurrence (for microfungi).

| Taxon | Relative occurrence % | Relative abundance % | |
|---|---|---|---|
| | | entrance zone | lampenflora |
| Phylum Cyanobacteria | | | |
| Family Chroococcaceae | | | |
| *Chroococcus minutus* (Kützing) Nägeli | 9.76 | 7.92 | 6.8 |
| Family Microcystaceae | | | |
| *Gloeocapsa compacta* Kützing | 9.76 | 6.93 | 12.2 |
| Family Nostocaceae | | | |
| *Nostoc commune* Vaucher ex Bornet & Flahault | 4.88 | 3.96 | 5.1 |
| *Nostoc microscopicum* Carmichael ex Bornet & Flahault | 4.07 | 4.95 | 1.7 |
| Family Scytonemataceae | | | |
| *Scytonema* sp. | 2.44 | 2.97 | 1.02 |
| *Scytonema hofmannii* C. Agardh ex Bornet & Flahault | 4.88 | 1.98 | 3.06 |
| Family Leptolyngbyaceae | | | |
| *Leptolyngbya tenuis* (Gomont) Anagnostidis & Komárek | 7.32 | 8.91 | 8.5 |
| Phylum Bacillariophyta | | | |
| Family Bacillariaceae | | | |
| *Nitzschia linearis* W.Smith | 1.63 | 2.97 | 0.68 |
| Family Diadesmidaceae | | | |
| *Humidophila contenta* (Grunow) Lowe | 5.69 | 7.92 | 3.74 |
| Family Naviculaceae | | | |
| *Navicula borealis* Ehrenberg | 2.44 | 4.95 | 1.36 |
| Phylum Charophyta | | | |
| Family Klebsormidiaceae | | | |
| *Klebsormidium flaccidum* (Kützing) P.C.Silva | 4.88 | 3.96 | 3.74 |
| Phylum Chlorophyta | | | |
| Family Chlorococcaceae | | | |
| *Chlorococcum minutum* R.C.Starr | 4.07 | 3.96 | 4.42 |
| Family Bracteacoccaceae | | | |
| *Bracteacoccus minor* (Schmidle ex Chodat) Petrová | 3.25 | 3.96 | 3.74 |
| Family Chlorellaceae | | | |
| *Chlorella vulgaris* Beyerinck [Beijerinck] | 9.76 | 9.9 | 14.6 |
| Family Prasiolaceae | | | |
| *Stichococcus bacillaris* Nägeli | 8.13 | 8.91 | 10.5 |
| Family Coccomyxaceae | | | |
| *Choricystis* sp. | 8.94 | 5.94 | 8.5 |
| Family Ulotrichaceae | | | |
| *Ulothrix implexa* (Kützing) Kützing | 2.44 | 5.94 | 1.7 |
| | | | |
| Moss protonemata | 5.69 | 3.96 | 8.5 |

***Table 1**: Cyanobacteriae and algae found in Lipska Cave.*

| Taxon | Relative occurrence % | | Relative abundance % | |
|---|---|---|---|---|
| | Entrance zone | Lampenflora | Entrance zone | Lampenflora |
| Order Dicranales | | | | |
| Family Dicranaceae | | | | |
| *Dicranum* sp. *1* | 9.68 | – | 9.85 | – |
| *Dicranum* sp. *2* | 8.06 | – | 7.58 | – |
| Family Fissidentaceae | | | | |
| *Fissidens taxifolius* Hedw | – | 33.33 | – | 41.38 |
| Family Ephemeraceae | | | | |
| *Trichostomum crispulum* Bruch in F.Muell. | 8.06 | – | 9.09 | – |
| Order Grimmiales | | | | |
| Family Grimmiaceae | | | | |
| *Schistidium agassizii* Sull. Et Lesq. In Sull. | 8.06 | – | 9.85 | – |
| Order Pottiales | | | | |
| Family Pottiaceae | | | | |
| *Tortella* sp. | 8.06 | – | 14.39 | – |
| Order Hypnales | | | | |
| Family Amblystegiaceae | | | | |
| *Campylidium calcareum* Ochyra | 8.06 | – | 6.82 | – |
| Family Brachytheciaceae | | | | |
| *Brachythecium tommasinii* (Sendtn. Ex Boulay) Ignatov et Huttenen | – | 66.67 | – | 58.62 |
| *Homalothecium lutescens* Robins. | 8.06 | – | 6.06 | – |
| Family Entodontaceae | | | | |
| *Entodon schleicheri* Demeter | 8.06 | – | 17.42 | – |
| Family Heterocladiaceae | | | | |
| *Neckera besseri* Jur. | 8.06 | – | 10.61 | – |
| Family Pylaisiaceae | | | | |
| *Stereodon vaucheri* Lindb. Ex Broth. | 8.06 | – | 8.33 | – |

***Table 2**: Bryophyta found in Lipska Cave.*
***Note**: "–" = species absent from samples.*

## Results

Results identified 17 species of cyanobacteria and algae within the compositions both of the natural entrance zone microflora community and the lampenflora communities (Table 1). *Gloeocapsa compacta* and *Chroococcus minutus* were the most widely found cyanobacterial species; *Chlorella vulgaris* and *Choricystis* sp. were the most widespread of the green algae. The highest relative abundances of detected cyanobacterial species were documented for *Leptolyngbya tenuis* and *Chroococcus minutus* within the samples collected from the natural entrance zone, with *Leptolyngbya tenuis* and *Gloeocapsa compacta* the most abundant among the lampenflora communities. Both in the natural entrance zone and the lampenflora communities within the cave the most abundant species of green algae were *Stichococcus bacillaris* and *Chlorella vulgaris*. Prothallia of ferns and protonemata of mosses were also found.

Bryophytes belonging to 12 species were identified: 10 species were present in the cave entrance zone samples and 2 species were identified among the components of the cave's lampenflora (Table 2). The highest bryophyte species occurrences and abundances were documented for *Fissidens taxifolius* and *Brachythecium tommasinii*, which were found only among the components of the cave's sampled lampenflora communities. The most abundant species identified within the cave entrance zone samples were *Entodon schleicheri* and *Tortella* sp.

| Taxon | Relative occurrence, entrance zone % | Relative occurrence, lampenflora % |
|---|---|---|
| Phylum Zygomycota | | |
| *Mucor racemosus f. racemosus* Fresen. | 8.82 | 2.44 |
| *Mucor racemosus f. sphaerosporus* (Hagem) Schipper | – | 1.22 |
| *Rhizopus stolonifer* (Ehrenb.) Vuill. | – | 2.44 |
| Phylum Ascomycota | | |
| *Aspergillus* sp. | 5.88 | 4.88 |
| *Aspergillus ochraceus* K. Wilh. | 11.76 | 2.44 |
| *Aspergillus phoenicis* (Corda) Thom | 14.71 | 2.44 |
| *Botrytis cinerea* Pers. | – | 4.88 |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries | – | 7.32 |
| *Clonostachys rosea f. rosea (Link) Schroers* | – | 2.44 |
| *Fusarium solani (Mart.) Sacc.* | 17.65 | 2.44 |
| *Fusarium sporotrichioides Sherb.* | – | 4.88 |
| *Oidiodendron* sp. | – | 4.88 |
| *Penicillium citrinum* Thom | – | 6.10 |
| *Penicillium chrysogenum* Thom | – | 10.98 |
| *Penicillium rugulosum* Thom | – | 8.54 |
| *Penicillium simplicissimum* (Oudem.) Thom | 20.59 | 8.54 |
| *Penicillium purpurascens* (Sopp) Biourge | 14.71 | 9.76 |
| *Trichoderma* sp. | – | 9.76 |
| *Phoma* sp. | 8.82 | 3.66 |

***Table 3****:Microfungi found in Lipska Cave.*
***Note****: "–" = species absent from samples.*

Additionally, 19 species of microfungi were identified, 3 of which belong to the phylum Zygomicota, with 16 from the phylum Ascomycota (Table 3). Highest occurrences of the microfungi species *Penicillium simplicissimum* and *Fusarium solani* were in the entrance zone; *P. chrysogenum, P. purpurascens* and *Trichoderma* sp. dominated in the composition of the lampenflora communities.

## Discussion

Cyanobacterial species play a significant role under cave environment conditions. Many of them are capable of fixing atmospheric nitrogen – for example the genus *Nostoc* has been noted within the floral composition of various caves (Lamprinou *et al*., 2012; Czerwik-Marcinkowska, 2013).

Cyanobacteria of the order Chroococcales show the greatest abundance both in the entrance zone microfloras and in the lampenflora composition of caves in many areas, because coccoid forms appear best adapted to a low level of illumination (e.g. Mulec *et al*., 2008; Roldán and Hernández-Mariné, 2009; Lamprinou *et al*., 2012; Mazina, 2017). For example, *Chroococcus* spp. are common in caves of various regions (Mulec *et al*., 2008; Lamprinou *et al*., 2009, 2012; Martınez & Asencio, 2010; Cennamo *et al*., 2012; Czerwik-Marcinkowska, 2013). *Chr. minutis* was recorded in caves of Turkey (Selvi and Altuner, 2007), Slovenia (Mulec *et al*., 2008) and Greece (Lamprinou *et al*., 2009). *Gloeocapsa* spp., are the most commonly encountered cyanobacteria in many European caves, including in Poland (Czerwik-Marcinkowska and Mrozińska, 2011), Slovenia (Mulec *et al*., 2008), Spain (Martınez and Asencio, 2010), Italy (Cennamo *et al*., 2012) and Russia (Mazina and Maximov, 2011). Specifically, *G. compacta* was documented in caves of Serbia (Popović *et al*., 2015); Turkey (Selvi and Altuner, 2007), and Slovenia (Krivograd-Klemenčič and Vrhovšek, 2005). *Nostoc* and *Scytonema* are the most common aeroterrestrial cyanobacteria genera (Pattanaik *et al*., 2007). *Nostoc commune* is documented in caves of Serbia (Popović *et al*., 2015) and the Czech Republic (Poulíčková and Hašler, 2007); *N. microscopicum* is recorded in caves of Slovenia (Komárek and Anagnostidis, 2005) and Turkey (Selvi and Altuner, 2007). *Scytonema hofmannii* has been found in caves of the Czech Republic (Poulíčková and Hašler, 2007) and Slovenia (Mulec *et al*., 2012).

Chlorophyta (green algae) and Bacillariophyta (diatoms) are commonly found together with cyanobacteria (Mazina and Maximov, 2011; Cennamo *et al*., 2012; Lamprinou *et al*., 2012; Czerwik-Marcinkowska, 2013). These algae are recorded mainly in places with relatively high humidity (Popović *et al*., 2017). *Chlorella vulgaris* and *Stichococcus bacillaris*, which had the highest occurrence in Lipska Cave, are also documented from caves in the Czech Republic (Poulíčková and Hašler, 2007), Poland (Czerwik-Marcinkowska and Mrozińska, 2009) and Turkey (Selvi and Altuner, 2007).

Moss propagules can be dispersed and transferred into caves by wind, water, vertebrates or invertebrates. Examples from the genus *Fissidens* are commonly noted as a part of the lampenflora (e.g. Mulec and Kubesova, 2010; Mazina, 2016). For example, *F. taxifolius*, was found to be widely distributed close to electric lamps in Crystal Cave (USA) in different years (Thatcher, 1949).

Microfungi from the genera *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Trichoderma* and *Cladosporium* are the most common in cave habitats (Vanderwolf *et al*., 2013). Also, elements of the cave microbiota are sensitive to changes in organic matter: for example, inputs to caves from the external environment are commonly covered rapidly with conidia (spores) of *Aspergillus* spp., *Penicillium* spp. and *Mucor* spp. (Min, 1988).

## Summary

The investigation of the diversity of cyanobacteria, algae, bryophytes and microfungi in caves is the first to have been conducted in Montenegro. Comparison of the species composition within the cave's natural entrance zone and that of lampenflora at its initial stage of growth demonstrated a discrepancy between their dominant species. This can be explained by the distinction between aspects of the entrance zone environmental conditions and the cave microclimate. Cyanobacteria were the dominant group of phototrophs within the composition of the Lipska Cave lampenflora communities. Cyanobacterial species *Leptolyngbya tenuis* and *Chroococcus minutus* were dominant in the natural entrance zone whereas *L. tenuis* and *G. compacta* dominated the lampenflora communities. The most abundant species of green algae both in the natural entrance zone and among the lampenflora communities were *Stichococcus bacillaris* and *Chlorella vulgaris*. Differences between dominant microfungi species in the lampenflora communities and those in the entrance zone are associated with the relatively higher humidity inside the cave and inputs of organic matter suitable for supporting microfungi growth. The low number of bryophyte species within the Lipska Cave lampenflora communities can be characterized as the initial stage of invasive overgrowth. Presumably, an increase in the number of species of bryophytes in the composition of the lampenflora can be expected, because moss protonemata are already common in areas of the cave illuminated by fixed lamps.

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## On-line resources

http://www.algaebase.org
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