The micromycetes of fouling communities in the caves of Lovćen National Park, Montenegro

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Abstract

Fouling communities with the dominance of phototrophs such as cyanobacteria, algae, bryophytes, and ferns are formed in the illuminated zone of the caves. These communities include also heterotrophic species—bacteria and micromycetes, often with the high abundance of the latter, indicating significant role of micromycetes for the developing of fouling communities.

In this study we examined the micromycete species composition in the phototrophic communities of five caves, i.e., Golubinja Pećina, Veluštica Pećina, Njegoš Pećina, Vrbačka jama, Jama ER-1 located on the territory of Lovćen National Park in the southwestern Montenegro. Species identification was performed using standard approaches and cultivation methods. In total, 35 species of micromycetes were identified from the phototroph communities of the studied caves. Our study revealed that the species composition of micromycetes was unique for each of the investigated cave. The most frequent species was Rhizopus stolonifer, identified from Golubinja Pećina, Vrbačka jama, and Jama ER-1. Two species, i.e., Aspergillus terreus (found in Veluštica Pećina and Jama ER-1), and Cladosporium oxysporum (found in Golubinja Pećina and Veluštica Pećina) were found each in two caves, whereas all other species were reported from a single cave.

Key words: karst caves, micromycetes, Lovćen National Park, Montenegro.

Introduction

Unlike surface communities, the productivity of caves communities is stable throughout the year. In conditions of limited lighting, high humidity and more stable temperature, cyanobacteria, algae, bryophytes, and ferns are actively developing throughout the year (Burford et al. 2003; Gorbushina 2007; Czerwik-Marcinkowska 2013; Popović et al. 2017). Lichens are typical for rock habitats, as they are most adapted to extreme conditions, but at the same time are very rare in caves, which is due to the increased humidity of underground habitats. Kozlova et al. (2019) studied phototrophic communities of seven caves located in the southern part of Montenegro and found 87 species of phototrophs including 64 species of algae and Cyanobacteria, and 21 species of Bryophyta.
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Microscopic fungi, which are common for non-illuminated parts of caves, due to the high humidity conditions, are also playing an important role in the illuminated zone of the caves (Popović et al. 2015). Their abundance in fouling communities can be high, indicating the significant role of micromycetes for sustainable development of these communities. It is particularly interesting to determine the association of micromycete species with the composition of species of phototrophic communities, including those dominated by bryophytes, ferns, cyanobacteria, or green algae in the community.

The diversity and ecology of the micromycetes of fouling communities of the Montenegrin caves so far remained unstudied. Therefore, the aim of this study is to to provide first data on the micromycetes composition in the phototrophic fouling communities of the entrance zones of Montenegrin caves.

Material and Methods

Five caves in Montenegro located on the territory of Lovćen National Park were the objects of the study: Golubinje Pećina (42.2518° N, 18.4757° E); Veluštica Pećina (42.43286° N, 18.80378° E), Njegoš Pećina (42.43301° N, 18.83159° E), Vrbačka jama (42.433305° N, 18.810832° E) and Jama ER-1 (42.4325° N, 18.831949° E) (Kozlova et al. 2019).

Fieldwork for the study was carried out during July 2017. We studied the micromycetes in the phototrophic fouling communities of the cave entrance zones. Samples of phototrophs were taken from each epibiose area: substrates (limestone, calcite, clay sediments) were sampled, bryophytes and ferns were collected and herbarized, algae and cyanobacteria (including samples from the gametophytes of bryophytes) were collected into sterile vials. Specimens for examination were prepared by separating small fragments from the communities (biofilms) and placing them in water droplets. Czapek-Dox Medium (saccharose concentrations of 0.3% and 3%) and extract from clay deposits (analog of soil extract) were used to detect micromycetes. 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 and isolation of pure cultures was performed weekly in the dark. Systematics of cyanobacteria and algae used in the paper corresponds to the database available at http://www.algaebase.org (Guiry & Guiry 2019). Samples of phototrophs were identified using several field guides: bryophytes with Ignatov & Ignatova (2004), ferns with Mayevsky (2014), and lichens with Andreev (2008). Identifications of micromycetes were performed using the following identification keys: Booth (1971), Domsch (2007), Ellis (1971, 1976), Hoog (2001), Pitt (1991), Ramirez (1982) and Raper (1968). The names of species are given according to the http://www.mycobank.org database (Robert et al. 2005).

Results

In total, 35 species of micromycetes were identified from the phototrophic communities of the studied caves. The relative abundances of identified species were calculated and given in Table 1. The results of the study revealed that the species composition of the micromycete communities was unique for each of the investigated caves.

The most frequent species was Rhizopus stolonifer, reported in our study from Golubinje Pećina, Vrbačka jama, and Jama ER-1. The species Aspergillus terreus was found in Veluštica Pećina and Jama ER-1, whereas Cladosporium oxysporum was found in Golubinje Pećina and Veluštica Pećina.

Kozlova et al. (2019) identified the dominant species of phototrophs in the studied caves. The latter study showed that Chroococcus limneticus, Gloeocapsa compacta, Chlorella vulgaris, and Amphidium mougeottii were the dominant phototrophic species in Golubinje Pećina. In this community, Mucor hiemalis was the most abundant micromycete species (Table 1).

The dominant group of phototrophs in Veluštica Pećina included Scytonema julianum, Chl. vulgaris, Sciurohypnum starkei, and Homalia trichomanoides (Kozlova et al. 2019). Mucor racemosus f. racemosus was the most abundant micromycete species in this community, followed by Cladosporium oxysporum, Fusarium oxysporum, Penicillium aurantiogriseum var. viridicatum and P. simplicissimum (Table 1).
Table 1. The relative abundance of micromycetes in fouling communities of phototrophs, found in studied caves.

|                              | Veluštica Pećina | Golubinja Pećina | Njegoš Pećina | Vrbačka jama | Jama ER-1 |
|------------------------------|-------------------|-------------------|----------------|--------------|-----------|
| **Zygomycota**              |                   |                   |                |              |           |
| *Mucor circinelloides f. circinelloides* Tiegh., Annales des Sciences Naturelles Botanique sér. 6: 1: 94 (1875) | -                  | 0,09             | -             | -           | -         |
| *Mucor hiemalis* Wehmer, Annales Mycologici 1 (1): 37 (1903) | -                  | 0,17             | -             | -           | -         |
| *Mucor luteus* Linnem. ex Wrzosek, Mycotaxon 111: 81 (2010) | -                  | -                | 0,17          | -           | -         |
| *Mucor racemosus f. racemosus* Fresen. (1850) | 0,12              | -                | -             | -           | -         |
| *Rhizopus stolonifer* (Ehrenb.) Vuill., Revue Mycologique Toulouse 24: 54 (1902) | -                  | 0,13             | -             | 0,40        | 0,31      |
| **Ascomycota**              |                   |                   |                |              |           |
| *Alternaria tenuissima* (Nees) Wiltshire, Transactions of the British Mycological Society 18 (2): 157 (1933) | 0,06              | -                | -             | -           | -         |
| *Aspergillus fumigatus* Fresen., Beiträge zur Mykologie 3: 81 (1863) | -                  | -                | -             | 0,10        | -         |
| *Aspergillus ochraceus* K. Wilh., Beiträge zur Kenntnis der Pilzgattung Aspergillus: 66 (1877) | -                  | 0,04             | -             | -           | -         |
| *Aspergillus phoenicis* (Corda) Thom, Journal of Agricultural Research 7: 14 (1916) | 0,06              | -                | -             | -           | -         |
| *Aspergillus terreus* Thom, American Journal of Botany 5 (2): 85 (1918) | 0,06              | -                | -             | -           | 0,15      |
| *Botrytis cinerea* Pers., Neues Magazin für die Botanik 1: 126, t. 3:9 (1794) | -                  | 0,04             | -             | -           | -         |
| *Candida sp.*                |                   |                   |                |              |           |
| *Cladosporium oxysporum* Berk. & M.A. Curtis, Journal of the Linnean Society. Botany 10: 362 (1869) | 0,09              | 0,13             | -             | -           | -         |
| *Cryptococcus albidus* (Saito) C.E. Skinner, The American Midland Naturalist 43: 249 (1950) | -                  | 0,04             | -             | -           | -         |
| *Fusarium oxysporum* Schltldl., Flora Berolinensis, Pars secunda: Cryptogamia: 139 (1824) | 0,09              | -                | -             | -           | -         |

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### TABLE 1

| Species                                      | Source                                                                 | Percentage |
|----------------------------------------------|------------------------------------------------------------------------|------------|
| *Fusarium sporotrichioides* Sherb.           | Memoirs of the Cornell University Agricultural Experimental Station 6: 183 (1915) | 0.23       |
| *Hyphopichia sp.*                           |                                                                        | 0.04       |
| *Neocosmospora solani* (Mart.) L. Lombard & Crous | Studies in Mycology 80: 228 (2015)                                      | 0.20       |
| *Penicillium aurantiogriseum* var. viridicatum Frisvad, Mycologia 81: 850 (1990) |                                            | 0.09       |
| *Penicillium brevicompactum* Dierckx, Annales de la Société Scientifique de Bruxelles 25 (1): 88 (1901) |                                           | 0.06       |
| *Penicillium chrysogenum* Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 58 (1910) |                                           | 0.13       |
| *Penicillium commune* Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 56 (1910) |                                           | 0.03       |
| *Penicillium dierckxii* Biourge, La Cellule 33: 313 (1923) |                                             | 0.06       |
| *Penicillium griseofulvum* Dierckx, Annales de la Société Scientifique de Bruxelles 25 (1): 88 (1901) |                                           | 0.03       |
| *Penicillium lanoso coeruleum* Thom, The Penicillia: 322 (1930) |                                          | 0.03       |
| *Penicillium roqueforti* Thom, U.S.D.A. Bureau of Animal Industry Bulletin 82: 35 (1906) |                                           | 0.17       |
| *Penicillium simplicissimum* (Oudem.) Thom, The Penicillia: 335 (1930) |                                         | 0.09       |
| *Penicillium steckii* K.M. Zalessky, Bulletin International de l'Academie Polonaise des Sciences et des Lettres Série B 1927: 469 (1927) |                                    | 0.09       |
| *Penicillium verrucosum* Dierckx, Annales de la Société Scientifique de Bruxelles 25 (1): 88 (1901) |                                   | 0.06       |
| *Rhodotorula* F.C. Harrison, Transactions of the Royal Society of Canada 21 (2): 349 (1927) |                                        | 0.04       |
| *Scopulariopsis sp.*                         |                                                                        | 0.04       |

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**TABLE 1.**

| Species                  | 0.17 | 0.30 | 0.23 |
|--------------------------|------|------|------|
| *Sporothrix* sp.         | -    | -    | -    |
| *Trichoderma ghanense* Yoshim. Doi, Y. Abe & Sugiy., Bulletin of the National Science Museum Tokyo 13: 3 (1987) | -    | -    | 0.30 |
| *Trichoderma viride* Pers., Neues Magazin für die Botanik 1: 92 (1794) | -    | -    | -    |
| *Trichosporon* sp.       | -    | -    | 0.17 |

*Chroococcus minor, Bracteacoccus minor, Chl. vulgaris and Plagiothecium cavifolium* were the most abundant photrophic species in the Njegoš Pećina (Kozlova et al. 2019). In the latter community, the share of *Mucor luteus, Penicillium roqueforti, Sporothrix* sp., *Trichosporon* sp. in the micromycete community was equal. Phototrophic species *Gl. compacta, Phormidium foveolarum, Leptolyngbya voronichiniaina, Synechococcus elongatus, Humidophila contenta, Stichococcus bacillaris, Heterothrix bristoliana, Campylium calcareum, Homalothecium philippeanum,* and *Entodon schleicheri,* as well as *Rhizopus stolonifer* were dominant in Vrbačka jama, while *Gl. punctata, P. foveolarum, S. bacillaris, Cynodontium tenellum,* and *Entodon schleicheri* dominated in the phototrophic assemblage of Jama ER-1 (Kozlova et al. 2019). *Rhizopus stolonifer* was the most abundant micromycete species in the community of both caves, the Vrbačka jama and the Jama ER-1, respectively.

Cluster analysis indicated the greatest similarity of micromycete composition between Vrbačka jama and Jama ER-1 Caves (Fig. 1), which have entrances in a form of vertical wells.

![Figure 1](image_url)

*Figure 1.* The dendrogram diagram based on the comparison of micromycetes composition in the studied caves.
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Discussion

In this study, 35 species of micromycetes were recorded from fouling communities of phototrophs in the illuminated zone of five caves located on the territory of Lovćen National Park, Montenegro: 14 species were found in Veluštica Pećina, 12 species in Golubinja Pećina, 5 species in Jama ER-1, 4 species in Njegoš Pećina and 4 species in Vrbačka jama.

Species of the genera *Penicillium*, *Mucor*, *Cladosporium*, *Rhizopus* and *Trichoderma*, documented in this study, were the most frequently reported micromycetes in caves of various territories (Gadd 2007; Vanderwolf et al. 2013), whereas the genus *Cladosporium*, documented in Golubinja Pećina and Veluštica Pećina, was commonly found in the air throughout Europe (Sadyś 2017).

*Rhizopus stolonifera* was the most abundant in Vrbačka jama and Jama ER-1. This species has been frequently reported in different parts of the caves in various regions. According to Ogórek et al. (2013) *Rh. solonifer* was most frequently isolated species from the air and the rocks of the Niedźwiedzia Cave in Poland, whereas Nieves-Rivera et al. (2009) found this species in the caves of southwestern Puerto Rico.

Our study revealed that micromycete species composition was unique to each of the studied caves. The greatest similarity was documented between the caves with similar morphology of the entrance zones. This confirms the results of Kozlova et al. (2019) who showed that the composition of phototrophic communities was primarily influenced by the morphology of the entrance zones of the studied caves.

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