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Biodiversity of phototrophs in illuminated entrance zones of seven caves in Montenegro

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Abstract

The biodiversity of the entrance zones of the Montenegro caves is barely studied, therefore the purpose of this study was to assess the biodiversity of several caves in Montenegro. The samples of phototrophs were taken from various substrates of the entrance zone of 7 caves in July 2017. A total of 87 species of phototrophs were identified, including 64 species of algae and Cyanobacteria, and 21 species of Bryophyta. Comparison of biodiversity was carried out using Jacquard and Shorygin indices. The prevalence of cyanobacteria in the algal flora and the dominance of green algae were revealed. The composition of the phototrophic communities was influenced mainly by the morphology of the entrance zones, not by the spatial proximity of the studied caves.

Key words: karst caves, entrance zone, ecotone, algae, cyanobacteria, bryophyte, Montenegro.

Introduction

The subterranean karst forms represent habitats that considered more climatically stable than the surface. Generally, cave climate is characterized by the low positive temperatures, high relative humidity and lack of lighting (Vanderwolf *et al.* 2013). Seasonal and daily fluctuations of these parameters can be observed at the entrance zones of the caves, yet they remain more stable than on the surface (Prous *et al.* 2015). In the deep zones of the caves there is no natural light and ecosystems are able to function due to the introduction of organic matter from the outside or the activity of chemolithoautotrophic communities (Engel *et al.* 2004; Pentecost & Zhaohui 2001). The entrance areas of caves, include habitats with low levels of photon fluxes, are characterized by a light gradient that allows phototrophs to develop.

Considered close to the communities of the entrance zone of the caves, the lampenflora communities represent photosynthetic species growing in the excursion caves near the lamps. There were not enough studies on the flora in Montenegro prior to 2018, when Mazina & Kozlova (2018) have researched

lampenflora and phototrophic communities of entrance zone of Lipska Cave in the southern part of Montenegro.

The territory of Montenegro is of great interest for the study of hypogean karst formations and has both scientific and cultural significance (see Barović *et al.* 2018). Most of Montenegro is covered by mountain ranges, composed mainly of limestone, with well-developed surface and subterranean karst topographic forms. The largest number of biological studies is focused on the cave fauna of troglophiles and troglobionts (see Pešić *et al.* 2018 for an overview).

In this paper, we studied phototrophic communities of seven caves located in the southern part of Montenegro characterized by the different morphology of entrance zones. The objective of this study was to describe the biodiversity of phototrophic communities at the entrance zones of these caves and to examine the species composition of the selected caves using standard similarity indices.

Material and Methods

Seven caves in Montenegro were the objects of the study (Fig. 1). Obodska Pećina (42.352113° N, 19.005209° E) is located west of the Rijeka Crnojevića, Cetinje. Untitled cave (42. 292351° N, 18.873896° E) is located near the Bečići town, close to the natural monument of Olive tree. Golubinja Pećina (42.2518° N, 18.4757° E) is located on the territory of Lovćen National Park. The remaining caves are located near Lovćen National Park, in the village of Njeguši or near it: Veluštica Pećina (42.432866° N, 18.803783° E), Vrbačka jama (42.433305° N, 18.810832° E), Jama ER-1 (42.4325° N, 18.831949° E), and Njegoš Pećina (42.43301° N, 18.83159° E).

The caves have different types of entrance zones (see Fig. 1 for photographs): large grotto-like entrances (Obodska Pećina, Golubinja Pećina), small horizontal entrances (Njegoš Pećina, Veluštica Pećina, and untitled cave), and vertical entrance areas (Vrbačka jama, Jama ER-1). Obodska Pećina and untitled caves weren't functioned as a spring at the time of sampling due to drought season.

Fieldwork for the study was carried out during July 2017.

We studied the phototrophic epibiotic communities of the cave entrance zones. Samples 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 gametogophytes of bryophytes) were collected into sterile vials. Phototrophs from the epibiotic communities were examined using a Leica DMLS light microscope (Germany). Specimens for examination were prepared by separating small fragments from the communities (biofilms) and placing them in water droplets. The occurrence and abundance of species in the samples were determined, and the data were extrapolated to the entire epibiose area.

Samples of soils and cave communities were incubated on selective culture media. Bristol and Gromov №6 media were used for algae and cyanobacteria. The exposure temperatures were 12 and 24°C and the illumination level was $30\text{-}40 \mu\text{m} \times \text{m}^{-2} \times \text{s}^{-1}$. The methods of growth slides and incubation in a liquid medium were used. For a more complete identification of the phototroph species composition from the illuminated zones, representative samples from communities were incubated in Gromov liquid medium №6. Systematics of cyanobacteria and algae used in the paper correspond to the database available at <http://www.algaebase.org> (Guiry & Guiry 2019). Samples were identified using several field guides: bryophytes with Ignatov & Ignatova (2004), ferns with Mayevsky (2014), and lichens with Andreev (2008). The systematics of bryophytes is given according to Ignatov & Ignatova (2004), ferns according to Cherepanov's report (1995), the lichen according to the database available at mycobank.org. The Jacquard floristic similarity coefficient and the Shorygin community structure similarity index (Rozenderg *et al.* 2000) were used to analyze the similarity of community composition and structure. Cluster analysis was carried out and tree diagrams were constructed using the Euclidean distance in the STATISTICA 7.0 program. Relative abundance was used as a basis for the determination of the dominant species; dominants were also determined in each systematic group: bryophytes, algae, and cyanobacteria.

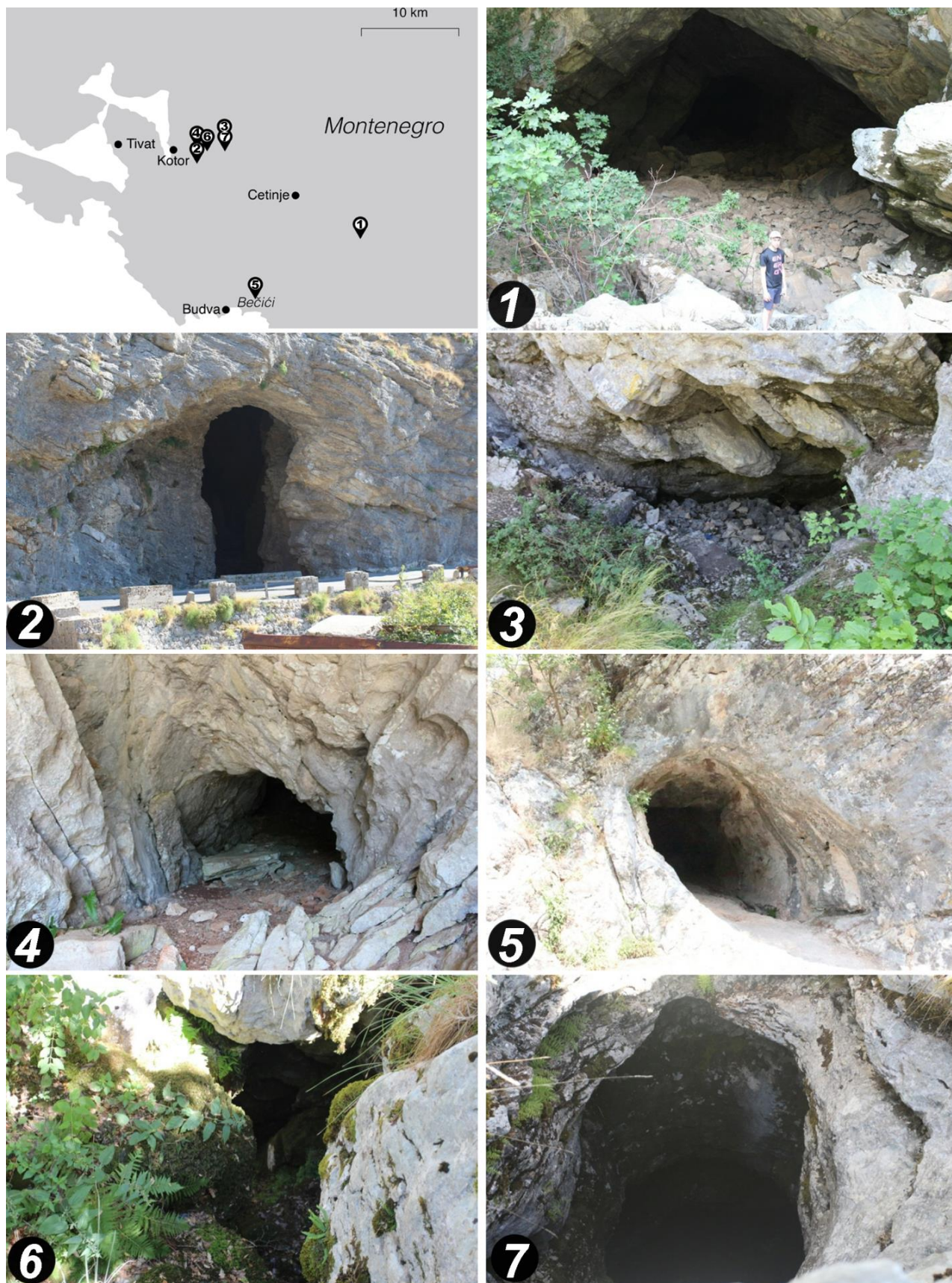


Figure 1. Map of the studied caves in Montenegro and photographs of their entrance zones. Cave numbers correspond to numbers in Map. 1- Obodska Pećina, 2- Golubinja Pećina, 3- Njegoš Pećina, 4- Veluštica Pećina, 5- untitled cave, 6- Vrbačka jama, 7- Jama ER-1.

Results

A total of 87 species (Table 1) of phototrophs were identified in the epibiotic communities of the cave entrance zones. Sixty-four species of cyanobacteria and algae were recorded (Cyanobacteria: 5 orders, 14 families, 18 genera, 42 species; algae: 11 orders, 17 families, 21 genera, 22 species, including: Bacillariophyta: 5 orders, 6 families, 8 genera and 9 species; Chlorophyta: 5 orders, 7 families, 12 genera, 12 species; Ochrophyta: 1 order, 1 family, 1 genera, 1 species). Twenty-one species of Bryophyta were identified (5 orders, 13 families, 17 genera). Protonema of mosses was found in all of the studied caves; one species of Pteridophyta, prothallia of ferns, and one species of lichen were found in the phototroph growth zone of the Veluštica Pećina.

Table 1. The list of the documented phototrophs found in the studied Montenegrin caves.

Phylum	Class	Order	Family	Genus	Species number	
Untitled cave						
Cyanobacteria	Cyanophyceae	Chroococcales	Aphanothecaceae	<i>Aphanothece</i>	1	
			Chroococcaceae	<i>Chroococcus</i>	2	
				<i>Gloeocapsa</i>	3	
			Microcystaceae	<i>Microcystis</i>	3	
		Nostocales	Scytonemataceae	<i>Scytonema</i>	1	
			Stigonemataceae	<i>Stigonema</i>	1	
		Oscillatoriales	Oscillatoriaceae	<i>Oscillatoria</i>	3	
				<i>Phormidium</i>	4	
			Synechococcales	Leptolyngbyaceae	<i>Leptolyngbya</i>	2
				Synechococcaceae	<i>Synechococcus</i>	1
Bacillariophyta	Bacillariophyceae	Naviculales	Diadesmidaceae	<i>Humidophila</i>	1	
		Chlamydomonadales	Palmellopsidaceae	<i>Chlamydocapsa</i>	1	
Chlorophyta	Chlorophyceae	Sphaeropleales	Bracteacoccaceae	<i>Chlorella</i>	1	
			Neochloridaceae	<i>Neochloris</i>	1	
Ochrophyta	Xanthophyceae	Prasiolales	Prasiolaceae	<i>Desmococcus</i>	1	
		Tribonematales	Tribonemataceae	<i>Heterothrix</i>	1	
Bryophyta	Bryopsida	Dicranales	Ditrichaceae	<i>Cynodontium</i>	1	
			Amblystegiaceae	<i>Amblystegium</i>	1	
		Hypnales	Antitrichiaceae	<i>Conardia</i>	1	
Njegoš Pećina						
Cyanobacteria	Cyanophyceae	Chroococcales	Aphanothecaceae	<i>Aphanothece</i>	1	
			Chroococcaceae	<i>Chroococcus</i>	2	
				<i>Gloeocapsa</i>	2	
			Microcystaceae	<i>Microcystis</i>	1	
Bacillariophyta	Bacillariophyceae	Nostocales	Nostocaceae	<i>Anabaeba</i>	1	
		Bacillariales	Bacillariaceae	<i>Nitzschia</i>	1	
		Mastogloiales	Achnantheaceae	<i>Achnanthes</i>	1	
		Naviculales	Diadesmidaceae	<i>Humidophila</i>	1	
		Chaetopeltidales	Chaetopeltidaceae	<i>Floydella</i>	1	
Chlorophyta	Chlorophyceae	Chlamydomonadales	Sphaerocystidaceae	<i>Sphaerocystis</i>	1	
		Sphaeropleales	Bracteacoccaceae	<i>Bracteacoccus</i>	1	
			<i>Chlorella</i>	1		

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TABLE 1

		Chlorellales	Oocystaceae	<i>Oocystis</i>	1		
	Trebouxiophyceae	Prasiolales	Prasiolaceae	<i>Desmococcus</i>	1		
	Hepaticae	Jungermaniales	Jungermanniaceae	<i>Jungermanniaceae</i>	1		
Bryophyta	Bryopsida	Dicranales	Dicranaceae	<i>Amphidium</i>	1		
			Ditrichaceae	<i>Ditrichum</i>	1		
		Hypnales	Plagiotheciaceae	<i>Plagiothecium</i>	1		
		Veluštica Pećina					
			Aphanothecaceae	<i>Aphanothece</i>	1		
		Chroococcales		<i>Gloethece</i>	1		
			Microcystaceae	<i>Gloeocapsa</i>	1		
				<i>Microcystis</i>	1		
			Nostocaceae	<i>Nostoc</i>	2		
			Rivulariaceae	<i>Calothrix</i>	1		
		Nostocales	Scytonemataceae	<i>Scytonema</i>	2		
Cyanobacteria	Cyanophyceae		Stigonemataceae	<i>Stigonema</i>	1		
			Tolypothrichaceae	<i>Borzinema</i>	1		
				<i>Oscillatoria</i>	1		
			Oscillatoriales	Oscillatoriaceae	<i>Phormidium</i>	2	
				Leptolyngbyaceae	<i>Leptolyngbya</i>	2	
				Merismopediaceae	<i>Merismopedia</i>	1	
				Synechococcales			
				familia incertae sedis	<i>Schizothrix</i>	1	
				Bacillariales	Bacillariaceae	<i>Hantzschia</i>	1
		Bacillariophyta	Bacillariophyceae	Naviculales	Diadesmidaceae	<i>Humidophila</i>	1
				<i>Luticola</i>	1		
Surirellales	Surirellaceae			<i>Surirella</i>	1		
Chlorophyta	Chlorophyceae	Sphaeropleales	Bracteacoccaceae	<i>Chlorella</i>	1		
			Mychonastaceae	<i>Mychonastes</i>	1		
	Trebouxiophyceae	Prasiolales	Prasiolaceae	<i>Desmococcus</i>	1		
Pteridophyta	Polypodiopsida	Polypodiales	Aspleniaceae	<i>Asplenium</i>	1		
Bryophyta	Bryopsida	Hypnales	Brachytheciaceae	<i>Sciurohypnum</i>	1		
			Neckeraceae	<i>Homalia</i>	1		
Ascomycota	Lecanoromycetes	Lecanorales	Stereocaulaceae	<i>Lepraria</i>	1		
Golubinja Pećina							
			Aphanothecaceae	<i>Aphanothece</i>	1		
				<i>Gloethece</i>			
Cyanobacteria	Cyanophyceae	Chroococcales	Chroococcaceae	<i>Chroococcus</i>	1		
			Microcystaceae	<i>Gloeocapsa</i>	2		
			Nostocaceae	<i>Nostoc</i>	2		
		Spirulinales	Spirulinaceae	<i>Spirulina</i>	1		
		Synechococcales	Leptolyngbyaceae	<i>Leptolyngbya</i>	1		
Bacillariophyta	Bacillariophyceae	Fragilariales	Fragilariaceae	<i>Fragilaria</i>	1		
		Naviculales	Diadesmidaceae	<i>Humidophila</i>	1		
				<i>Luticola</i>	1		
		Chaetopeltidales	Chaetopeltidaceae	<i>Floydella</i>	1		
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlorococcaceae	<i>Tetracystis</i>	1		
		Sphaeropleales	Bracteacoccaceae	<i>Chlorella</i>	1		

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TABLE 1

	Trebouxiophyceae	Chlorellales	Chlorellaceae	<i>Muriella</i>	1
		Prasiolales	Prasiolaceae	<i>Stichococcus</i>	1
		Bartramiales	Bartramiaceae	<i>Plagiopus</i>	1
Bryophyta	Bryopsida	Dicranales	Dicranaceae	<i>Amphidium</i>	2
		Hypnales	Amblystegiaceae	<i>Campylidium</i>	1
			Brachytheciaceae	<i>Sciurohypnum</i>	1
Vrbačka jama					
		Chroococcales	Microcystaceae	<i>Gloeocapsa</i>	2
				<i>Microcystis</i>	3
Cyanobacteria	Cyanophyceae	Oscillatoriales	Oscillatoriaceae	<i>Phormidium</i>	2
		Synechococcales	Leptolyngbyaceae	<i>Leptolyngbya</i>	1
			Synechococcaceae	<i>Synechococcus</i>	1
Bacillariophyta	Bacillariophyceae	Fragilariales	Fragilariaceae	<i>Fragilaria</i>	1
		Naviculales	Diadesmidaceae	<i>Humidophila</i>	1
Ochrophyta	Trebouxiophyceae	Prasiolales	Prasiolaceae	<i>Stichococcus</i>	1
	Xanthophyceae	Tribonematales	Tribonemataceae	<i>Heterothrix</i>	1
			Amblystegiaceae	<i>Campylidium</i>	1
Bryophyta	Bryopsida	Hypnales	Brachytheciaceae	<i>Homalothecium</i>	1
			Entodontaceae	<i>Entodon</i>	1
		Pottiales	Pottiaceae	<i>Tortella</i>	1
Obodska Pećina					
			Aphanothecaceae	<i>Aphanothece</i>	1
		Chroococcales		<i>Gloeothece</i>	1
Cyanobacteria	Cyanophyceae		Chroococcaceae	<i>Chroococcus</i>	1
			Microcystaceae	<i>Gloeocapsa</i>	2
		Nostocales	Nostocaceae	<i>Nostoc</i>	2
		Oscillatoriales	Oscillatoriaceae	<i>Oscillatoria</i>	1
Bacillariophyta	Bacillariophyceae	Naviculales	Diadesmidaceae	<i>Humidophila</i>	1
			Naviculaceae	<i>Luticola</i>	2
				<i>Navicula</i>	1
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlorococcaceae	<i>Tetracystis</i>	1
		Sphaeropleales	Bracteacoccaceae	<i>Chlorella</i>	1
	Trebouxiophyceae	Chlorellales	Chlorellaceae	<i>Muriella</i>	1
			Fissidentaceae	<i>Fissidens</i>	1
Bryophyta	Bryopsida	Dicranales	Brachytheciaceae	<i>Sciurohypnum</i>	1
			Pseudoleskeellaceae	<i>Pseudoleskeellaceae</i>	1
Jama ER-1					
		Chroococcales	Microcystaceae	<i>Gloeocapsa</i>	2
				<i>Microcystis</i>	4
		Nostocales	Nostocaceae	<i>Nostoc</i>	1
Cyanobacteria	Cyanophyceae	Oscillatoriales	Oscillatoriaceae	<i>Oscillatoria</i>	1
				<i>Phormidium</i>	2
		Synechococcales	Leptolyngbyaceae	<i>Leptolyngbya</i>	1
			Synechococcaceae	<i>Synechococcus</i>	1
Bacillariophyta	Bacillariophyceae	Naviculales	Diadesmidaceae	<i>Humidophila</i>	1
	Chlorophyceae	Chlamydomonadales	Chlorococcaceae	<i>Tetracystis</i>	1
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	<i>Muriella</i>	1
		Prasiolales	Prasiolaceae	<i>Stichococcus</i>	1

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TABLE 1

Bryophyta	Bryopsida	Dicranales	Dicranaceae	<i>Cynodontium</i>	1
			Entodontaceae	<i>Entodon</i>	1
		Hypnales	Plagiotheciaceae	<i>Plagiothecium</i>	1

Relative abundances of identified species were calculated (Tables 2-3). Cyanobacteria *Stigonema* sp. and green algae *Neochloris bilobata* were the dominant species in the untitled cave. Cyanobacteria *Chroococcus minor* and green algae *Bracteacoccus minor* and *Chlorella vulgaris* were the most abundant in the Njegoš Pećina. The cyanobacteria *Scytonema julianum* and the green algae *Chl. vulgaris* were the dominants in the Veluštica Pećina. In the Golubinja Pećina, cyanobacterial species *Chr. limneticus* and *Gloeocapsa compacta*, and the green algae *Chl. vulgaris* were the most abundant. Cyanobacterial species *Gl. compacta*, *Phormidium foveolarum*, *Leptolyngbya voronichiniana*, *Synechococcus elongatus*, diatoms *Humidophila contenta*, green algae *Stichococcus bacillaris* and Ochrophyte *Heterothrix bristoliana* were the dominant species in the Vrbačka jama. Cyanobacterial species *Gloeotheca palea*, *Gl. compacta*, *Nostoc commune* and green algae *Chl. vulgaris* were the dominant species in the Obodska Pećina. In the Jama ER-1, cyanobacterial species *Gl. punctata*, *P. foveolarum* and green algae *S. bacillaris* were the most abundant.

Table 2. Relative abundance of cyanobacteria, algae, fern, and lichen found in investigated caves. Sampling site abbreviations: 1-untitled cave; 2- Njegoš Pećina; 3- Veluštica Pećina; 4- Golubinja Pećina; 5- Vrbačka jama; 6- Obodska Pećina; 7- Jama ER-1.

Taxon	1	2	3	4	5	6	7
Phylum Cyanobacteria							
Class Cyanophyceae							
Order Chroococcales							
Family Aphanothecaceae							
<i>Aphanothece saxicola</i> Nägeli 1849	4,48	4,76	3,75	4,88	-	3,23	-
<i>Gloeotheca caldariorum</i> (Richter) Hollerbach 1938	-	-	2,50	-	-	-	-
<i>Gloeotheca palea</i> (Kützing) Nägeli 1849	-	-	-	4,88	-	9,68	-
Family Chroococcaceae							
<i>Chroococcus limneticus</i> Lemmermann 1898	-	-	-	7,32	-	6,45	-
<i>Chroococcus turgidus</i> (Kützing) Nägeli 1849	2,99	-	-	-	-	-	-
<i>Chroococcus lithophilus</i> Ercegovic 1925	-	4,76	-	-	-	-	-
<i>Chroococcus minor</i> (Kützing) Nägeli 1849	2,99	7,14	-	-	-	-	-
Family Microcystaceae							
<i>Gloeocapsa compacta</i> Kützing 1847	-	-	-	7,32	10,71	9,68	5,00
<i>Gloeocapsa ohaerens</i> (Brébisson) Hollerbach in Elenkin 1937	-	4,76	-	-	-	-	-

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TABLE 2

<i>Gloeocapsa kuetzingiana</i> Nägeli ex Kützing 1849	2,99	-	-	-	-	-	-
<i>Gloeocapsa minuta</i> (Kützing) Hollerbach in Elenkin 1937	2,99	-	-	4,88	-	-	-
<i>Gloeocapsa punctata</i> Nägeli 1849	2,99	7,14	-	-	7,14	-	10,00
<i>Gloeocapsa rupicola</i> Kützing 1849	-	-	5,00	-	-	6,45	-
<i>Microcystis aeruginosa</i> (Kützing) Kützing 1846	2,99	-	-	-	7,14	-	7,50
<i>Microcystis muscicola</i> (Meneghini) Elenkin 1938	5,97	-	2,50	-	7,14	-	5,00
<i>Microcystis pulvereae</i> (H.C.Wood) Forti 1907	4,48	4,76	-	-	-	-	5,00
<i>Microcystis pulvereae f. incerta</i> (Lemmermann) Elenkin 1938	-	-	-	-	7,14	-	-
Order Nostocales							
Family Nostocaceae							
<i>Anabaena sp.</i>	-	4,76	-	-	-	-	-
<i>Nostoc commune</i> Vaucher ex Bornet & Flahault 1888	-	-	3,75	4,88	-	9,68	-
<i>Nostoc microscopicum</i> Carmichael ex Bornet & Flahault 1886	-	-	5,00	4,88	-	6,45	7,50
Family Rivulariaceae							
<i>Calothrix gypsophila</i> (Kützing) Thuret 1875	-	-	5,00	-	-	-	-
Family Scytonemataceae							
<i>Scytonema julianum</i> Meneghini ex B.A.Whitton 2011	4,48	-	6,25	-	-	-	-
<i>Scytonema ocellatum</i> Lyngbye ex Bornet & Flahault 1886	-	-	3,75	-	-	-	-
Family Stigonemataceae							
<i>Stigonema hormoides</i> Bornet & Flahault 1886	-	-	2,50	-	-	-	-
<i>Stigonema sp.</i>	7,46	-	-	-	-	-	-
Family Tolypothrichaceae							
<i>Borzinema rupicola</i> (Borzi) G.De Toni 1936	-	-	5,00	-	-	-	-
Order Oscillatoriales							
Family Oscillatoriaceae							
<i>Oscillatoria amphibia</i> C.Agardh ex Gomont 1892	2,99	-	-	-	-	-	5,00
<i>Oscillatoria granulata</i> N.L.Gardner 1927	2,99	-	-	-	-	-	-
<i>Oscillatoria subtilissima</i> Kützing ex Forti 1892	1,49	-	2,50	-	-	6,45	-

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TABLE 2

<i>Phormidium foveolarum</i> Gomont 1892	4,48	-	-	-	10,71	-	10,00
<i>Phormidium interruptum</i> Kützing ex Forti 1907	2,99	-	-	-	-	-	-
<i>Phormidium irriguum</i> (Kützing ex Gomont) Anagnostidis & Komárek 1988	2,99	-	-	-	-	-	-
<i>Phormidium mucicola</i> Nauman & Huber-Pestalozzi in Huber-Pestalozzi & Nauman 1929	2,99	-	2,50	-	3,57	-	2,50
<i>Phormidium tenue</i> Gomont 1892	-	-	2,50	-	-	-	-
Order Spirulinales							
Family Spirulinaceae							
<i>Spirulina</i> sp.	-	-	-	4,88	-	-	-
Order Synechococcales							
Family Leptolyngbyaceae							
<i>Leptolyngbya leptotrichiformis</i> (Krieger) Anagnostidis & Komárek 2001	2,99	-	-	-	-	-	-
<i>Leptolyngbya voronichiniana</i> Anagnostidis & Komárek 1988	5,97	-	2,50	-	7,14	-	5,00
<i>Leptolyngbya boryana</i> (Gomont) Anagnostidis & Komárek 1988	-	-	3,75	2,44	-	-	-
Family Merismopediaceae							
<i>Merismopedia</i> sp.	-	-	2,50	-	-	-	-
Family Synechococcaceae							
<i>Synechococcus elongatus</i> (Nägeli) Nägeli 1849	2,99	-	-	-	7,14	-	7,50
Family Synechococcales familia incertae sedis							
<i>Schizothrix vaginata</i> Gomont 1892	-	-	3,75	-	-	-	-
Phylum Bacillariophyta							
Class Bacillariophyceae							
Order Bacillariales							
Family Bacillariaceae							
<i>Nitzschia amphibia</i> Grunow 1862	-	2,38	-	-	-	-	-
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow in Cleve & Grunow 1880	-	-	1,25	-	-	-	-
Order Fragilariales							
Family Fragilariaceae							
<i>Fragilaria</i> sp.	-	-	-	2,44	3,57	-	-
Order Mastogloiales							
Family Achnanthaceae							
<i>Achnanthes</i> sp.	-	2,38	-	-	-	-	-

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TABLE 2

Order Naviculales							
Family Diadesmidaceae							
<i>Humidophila contenta</i> (Grunow)							
Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová 2014	4,48	4,76	2,50	4,88	7,14	3,23	7,50
<i>Luticola nivalis</i> (Ehrenberg)							
D.G.Mann in Round, R.M.Crawford & D.G.Mann 1990	-	-	-	4,88	-	6,45	-
<i>Luticola mutica</i> (Kützing)							
D.G.Mann in Round, R.M.Crawford & D.G.Mann 1990	-	-	2,50	-	-	3,23	-
Family Naviculaceae							
<i>Navicula sp.</i>							
	-	-	-	-	-	3,23	-
Order Surirellales							
Family Surirellaceae							
<i>Surirella sp.</i>							
	-	-	1,25	-	-	-	-
Phylum Chlorophyta							
Class Chlorophyceae							
Order Chaetopeltidales							
Family Chaetopeltidaceae							
<i>Floydiella terrestris</i>							
(R.D.Groover & A.M.Hofstetter) Friedl & O'Kelly 2002	-	2,38	-	4,88	-	-	-
Order Chlamydomonadales							
Family Chlorococcaceae							
<i>Tetracystis excentrica</i>							
R.M.Brown & Bold 1964	-	-	-	4,88	-	6,45	5,00
Family Palmellopsidaceae							
<i>Chlamydocapsa lobata</i> Broady							
1977	2,99	-	-	-	-	-	-
Family Sphaerocystidaceae							
<i>Sphaerocystis schroeteri</i> Chodat							
1897	-	7,14	-	-	-	-	-
Order Sphaeropleales							
Family Bracteacoccaceae							
<i>Bracteacoccus minor</i> (Schmidle ex Chodat) Petrová 1931							
	-	11,90	-	-	-	-	-
<i>Chlorella vulgaris</i> Beyerinck							
(Beijerinck) 1890	2,99	11,90	5,00	12,20	-	12,90	-
Family Mychonastaceae							
<i>Mychonastes</i>							
<i>homosphaera</i> (Skuja) Kalina & Puncochárová 1987	-	-	2,50	-	-	-	-
Family Neochloridaceae							
<i>Neochloris bilobata</i> G.Vinatzer							
1975	5,97	-	-	-	-	-	-

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TABLE 2

Class Trebouxiophyceae							
Order Chlorellales							
Family Chlorellaceae							
<i>Muriella terrestris</i> J.B.Petersen 1932	-	-	-	4,88	-	3,23	2,50
Family Oocystaceae							
<i>Oocystis minuta</i> Guillard, Bold & MacEntee 1975	-	7,14	-	-	-	-	-
Order Prasiolales							
Family Prasiolaceae							
<i>Desmococcus olivaceus</i> (Persoon ex Acharius) J.R.Laundon 1985	-	7,14	3,75	-	-	-	-
<i>Stichococcus bacillaris</i> Nägeli 1849	2,99	-	3,75	7,32	7,14	-	10,00
Phylum Ochrophyta							
Class Xanthophyceae							
Order Tribonematales							
Family Tribonemataceae							
<i>Heterothrix bristoliana</i> Pascher 1939	2,99	-	-	-	7,14	-	-
Phylum Ascomycota							
Class Lecanoromycetes							
Order Lecanorales							
Family Stereocaulaceae							
<i>Lepraria sp.</i>	-	-	2,50	-	-	-	-
Phylum Pteridophyta							
Class Polypodiopsida							
Order Polypodiales							
Family Aspleniaceae							
<i>Asplenium trichomanes</i> L., 1753	-	-	2,50	-	-	-	-
Protonemata of mosses	4,48	4,76	5,00	7,32	7,14	3,23	5,00
Prothallia of ferns	-	-	2,50	-	-	-	-

The following species of bryophytes were dominant in the respective caves: untitled cave – *Conardia compacta*; Njegoš Pećina – *Plagiothecium cavifolium*; Veluštica Pećina – *Sciurohypnum starkei* and *Homalia trichomanoides*; Golubinja Pećina – *Amphidium mougeotti*; Vrbačka jama – *Campylidium calcareum*, *Homalothecium philippeanum*, *Entodon schleicheri*; Obodska Pećina – *Sciurohypnum plumosum* and *Pseudoleskeellaceae* sp.

Dominant species of cyanobacteria were the same in the following caves: Golubinja and Vrbačka jama (*Gl. compacta*), Vrbačka jama and Jama ER-1 (*P. foveolarum*). The diatom *S. bacillaris* was dominant in the caves Jama ER-1 and Vrbačka jama. The chlorophyte *Chl. vulgaris* was dominant in the caves Njegoš Pećina, Veluštica Pećina, Golubinja Pećina and Obodska Pećina. Bryophyte dominants were different in each cave.

We assessed the similarity of the species composition and community structure of the studied caves using standard similarity indices. We evaluated biodiversity as a whole and diversity of algal flora (algae and cyanobacteria) separately. Analyses indicated that the similarity of the cave phototrophs is low (Fig. 2). On the other hand, the cave algal flora demonstrated greater similarity. Flora of the caves, which are similar in morphology of the entrance zone (e.g., Golubinja Pećina and Obodska Pećina having a large grotto-like entrances, and Vrbačka jama and Jama ER-1 having entrances in a form of vertical wells) showed the

greatest similarity. We predicted that the caves located close to each other would have high Jaccard and Shorygin indices, but that assumption was not confirmed.

Table 3. Relative abundance of bryophytes found in studied caves. Sampling site abbreviations: 1- untitled cave; 2- Njegoš Pećina; 3- Veluštica Pećina; 4- Golubinja Pećina; 5- Vrbačka jama; 6- Obodska Pećina; 7- Jama ER-1.

Taxon	1	2	3	4	5	6	7
Order Bartramiales							
Family Bartramiaceae							
<i>Plagiopus oederianus</i> (Sw.) Crum et Anderson	-	-	-	7,14	-	-	-
Order Dicranales							
Family Dicranaceae							
<i>Amphidium lapponicum</i> (Hedw.) Schimp., Caroll. Bryol. Eur.	-	-	-	21,43	-	-	-
<i>Amphidium mougeotti</i> (B.S.G.) Schimp	-	30	-	28,57	-	-	-
<i>Cynodontium tenellum</i> B.S.G. (Limpr.)	-	-	-	-	-	-	40
Family Ditrichaceae							
<i>Ditrichum flexicaule</i> (Schwaegr.) Hampe	-	20	-	-	-	-	-
<i>Cynodontium tenellum</i> B.S.G. (Limpr.)	22,22	-	-	-	-	-	-
Family Fissidentaceae							
<i>Fissidens taxifolius</i> Hedw.	-	-	-	-	-	20	-
Order Hypnales							
Family Amblystegiaceae							
<i>Amblystegium serpens</i> (Hedw.) B.S.G.	22,22	-	-	-	-	-	-
<i>Campylidium calcareum</i> Ochyra	-	-	-	21,43	26,67	-	-
Family Antitrichiaceae							
<i>Conardia compacta</i> (Drumm.) Robins	55,56	-	-	-	-	-	-
Family Brachytheciaceae							
<i>Homalothecium philippeanum</i> (Spruce) B. S. G.	-	-	-	-	26,67	-	-
<i>Sciuro-hypnum latifolium</i> (Kindb.) Ignatov et Huttunen	-	-	-	21,43	-	-	-
<i>Sciuro-hypnum plumosum</i> (Hedw.) Ignatov et Huttunen	-	-	-	-	-	40	-
<i>Sciuro-hypnum starkei</i> (Brid.) Ignatov et Huttunen	-	-	50	-	-	-	-
Family Brachytheciaceae							
<i>Entodon schleicheri</i> Demeter	-	-	-	-	26,67	-	40
Family Neckeraceae							
<i>Homalia trichomanoides</i> (Hedw.) B. S. G.	-	-	50	-	-	-	-

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TABLE 3

Family Plagiotheciaceae							
<i>Plagiothecium cavifolium</i> (Brid.)	-	40	-	-	-	-	-
Iwats							
<i>Plagiothecium sp.</i>	-	-	-	-	-	-	20
Family Pseudoleskeellaceae							
<i>Pseudoleskeella sp.</i>	-	-	-	-	-	40	-
Order Pottiales							
Family Pottiaceae							
<i>Tortella tortuosa</i> (Hedw.) Limpr.	-	-	-	-	20	-	-
Class Hepaticae							
Order Jungermanniales							
Family Jungermanniaceae							
<i>Aplozia sphaerocarpa</i> (Hook.)	-	10	-	-	-	-	-
Dum.							

Discussion

In this study, 51 taxa of phototrophs were recorded from seven caves located in the karstic region of southern Montenegro is reported. In terms of taxonomic richness, the communities composition of studied caves was dominated by Cyanobacteria and algae (Bacillariophyta, Chlorophyta and Ochrophyta) and which is consistent with many studies. For example Mazina & Kozlova (2018) found that flora assemblage of Lipska cave include 29 phototrophic species of which 17 species belong to algae and Cyanobacteria, and 12 to Bryophyta.

Diatom *Humidophila contenta* was the only species found in all the caves and had a high score of abundance (more than 7) in the caves-wells. Green algae *Chlorella vulgaris* and *Stichococcus bacillaris* were found in most caves. It is interesting to note that, as a rule, these species dominated in the studied communities (scores of relative abundance were 7-12). However, in the case when both species were simultaneously found in the cave, their relative abundance decreased to 3-5 points.

Aerophytic algae, mainly cyanobacteria, dominate compared with other microorganisms (Czerwik-Marcinkowska 2013; Mulec *et al.* 2008), especially at the cave entrances, where growth conditions are the most suitable (Mulec 2005; Mulec *et al.* 2008).

The cyanobacterium *Aphanothece saxicola* was found in all the caves except the wells; this species is usually found in the form of biofilm on limestone and calcite. The species of the genus *Microcystis* are found in all caves, except the Golubinja Pećina. It should be noted that this cave is rich in organic matters and protonema. A similar abundance of protonema is found in the Vrbačka jama. The genus *Gleocapsa* is represented in all caves.

Aerophilous diatom *H. contenta* (formerly *Diadesmis contenta*) was registered at the entrance areas of all the studied caves. Most members of the *Humidophila* genus are considered cosmopolitan due to their wide distribution (Pouličková & Hašler 2007). *H. contenta* also had the highest occurrence in the caves of Poland (Czerwik-Marcinkowska *et al.* 2015); and was found in the caves of Central Moravia (Czech Republic) (Pouličková & Hašler 2007), Canada (Lauriol *et al.* 2006) and in the Urals (Abdullin 2007).

Species of cyanobacteria and algae that are dominant at the entrance zones are common in caves in various regions: *Gl. compacta* – in Serbia (Popović *et al.* 2017) and UK (Pentecost & Whitton 2012); *Chl. vulgaris* – in the Czech Republic (Pouličková & Hašler 2007), Poland (Czerwik-Marcinkowska & Mrozińska 2009) and Turkey (Selvi & Altuner 2007); *S. bacillaris* – in Slovenia (Mulec *et al.* 2008), Serbia (Popović *et al.* 2017), and the Urals (Gainutdinov *et al.* 2017). *P. foveolarum* is noted as one of the most frequently encountered species of cyanobacteria in European caves (Pentecost & Whitton 2012).

The largest number of bryophyte species were registered at the entrance area of the Golubinja Pećina. This is presumably due to the specific morphological structure of the entrance and the presence of a large amount of organic matter (pigeon droppings).

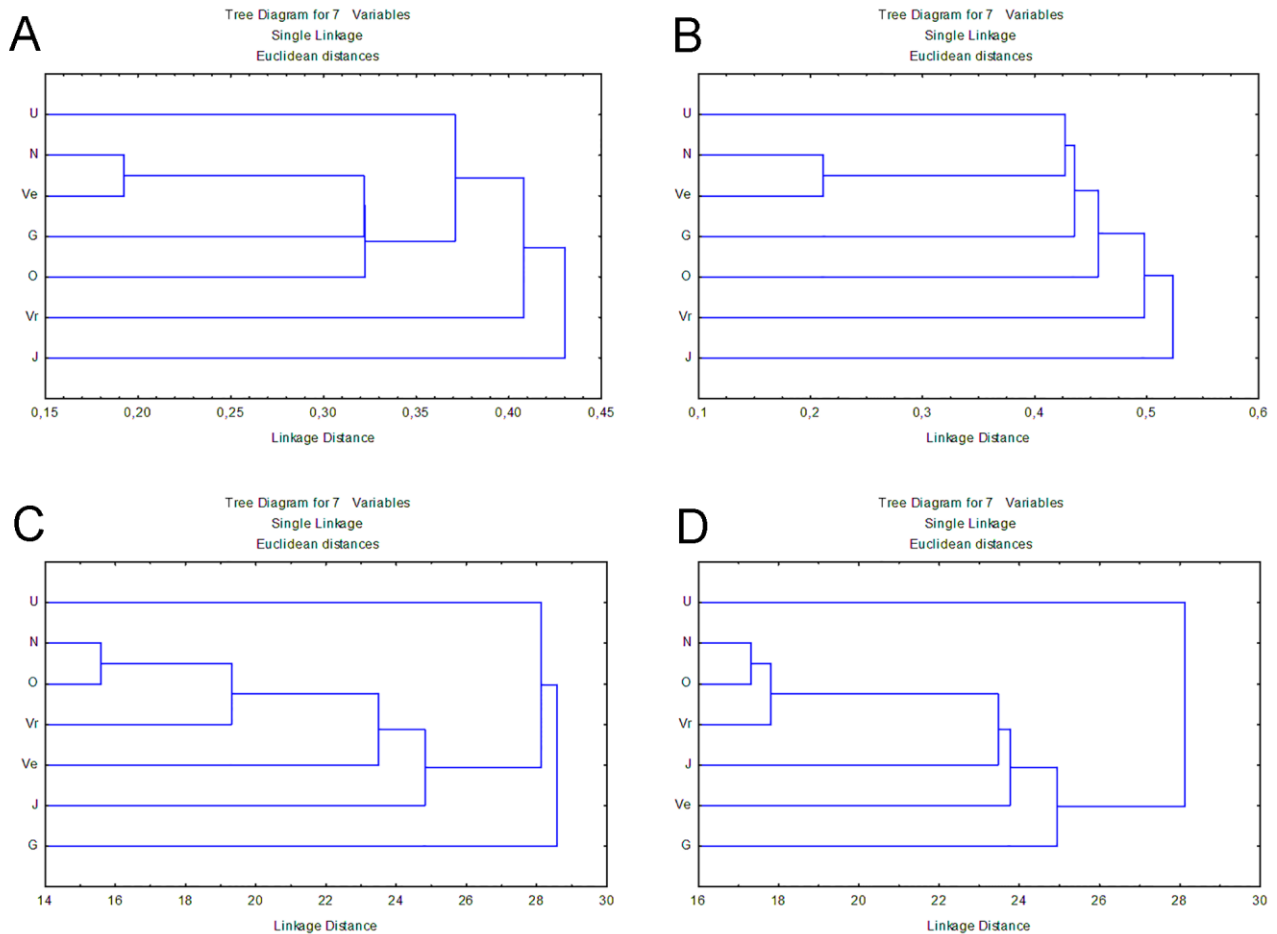


Figure 2. The dendrogram distribution diagram based on a comparison of all flora (A) and algal flora (B) of caves using the Jacquard floristic similarity coefficient. The dendrogram distribution diagram based on the comparison of all flora (C) and algal flora (D) of caves using the Shorygin floristic similarity coefficient. U - Untitled cave; N - Njegoš Pećina; Ve - Veluštica Pećina; G - Golubinja Pećina; Vr - Vrbačka jama; O - Obodska Pećina; J - Jama ER-1.

The species *Amblystegium serpens* and *Campylidium calcareum* have been documented by Mulec & Kubešová (2010) as part of the lampenflora in Slovenian caves. *A. serpens* has been also found in the lampenflora of the caves in Italy (Castello 2014) and the Czech Republic (Kubešová 2001). *Ditrichum flexicaue* and *Fissidens taxifolius* have been also recorded Kubešová (2001) as a part of the lampenflora. *Conardia compacta* and *Plagiopus oederianus* have been registered in the lampenflora of the show caves in Abkhazia and the Krasnodar Territory (Mazina 2016). *A. serpens*, *Tortella tortuosa* and a lichen of the genus *Lepraria* has been registered Pentecost & Zhaohui (2001) in the flora of cave in England (North Yorkshire).

The fern *Asplenium trichomanes*, which we registered in the Veluštica Pećina, is widely distributed and can be found in well-lit entrance areas, as well as in the lampenflora (Castello 2014).

The results of our study showed that the spatial proximity of the investigated caves is not an indicator of assemblage similarity since we did not find any evidence that the species of neighboring caves had coincided more than in the distant ones. Probably, the assemblage similarity caused by the morphology of the entrance zones of the studied caves.

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