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Microbiota of Hypogean Habitats in Otap Head Cave

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The investigation presents the assessment of species composition and structure of microbiota communities in the Otap Head Cave. Species were identified using standard approaches and cultivation methods. The abundance of algae and cyanobacteria was estimated applying the 5-point Brown-Blank scale. Biodiversity of biofouling communities was revealed. Cyanobacteria were the dominant group of phototrophs colonizing the cave wall and water streams. The most frequently documented cyanobacteria were species from genera *Chroococcus*, *Gloeocapsa*, *Oscillatoria*, and *Phormidium*. Among micromycetes prevailed Ascomycetes (genera *Aspergillus*, *Penicillum*, *Trichoderma*). The development of so-called lampenflora around artificial lights was not observed. The presence of sulphate-reducing and sulphur-oxidizing bacteria was detected, which possibly indicates that a small circulation of sulphur occurs in the cave at present time.

Keywords: outflow caves, cyanobacteria, algae, micromycetes, hypogean habitats.

Introduction

Hypogean environments represent unique habitats with stable climatic conditions and absence of seasonality. Based on light intensity, the cave can be divided into three zones: the entrance, which is strongly impacted by surface conditions; the twilight zone, where partially direct or indirect light penetrates; and the deep or dark zone, characterized by an absence of light (Northup & Lavoie, 2001). Depending on the conditions and resources in these zones, different organisms prevail. In the entrance, the development of the representatives of orders Magnoliophyta and Pteridophyta (Martinčič et al., 1981; Kubešová, 2001), as well as Bryophyta (Mulec & Kubešová, 2010; Kubešová, 2001), is noted. Algae and cyanobacteria dominate in the twilight and dark zones. In addition to the phototrophic component, fungi can be detected in cave communities (Mazina & Popkova,

2017). Therefore, the entrance and adjacent zones show a gradient of physical and biological features; in other words, the cave entrance can be considered as an ecotone (Prous et al., 2015).

In the conditions of cave habitats, relatively isolated communities are formed, the structure and functioning of which can differ from the surface ones. In the dark zone, heterotrophic species predominate. Often they are represented by psychrotolerants and oligotrophs. This fact can be explained by relatively stable, low temperatures, as well as the limited nutrient input (Vanderwolf et al., 2013). The discreteness of the micromycetes distribution in the caves is caused by uneven distribution of organic matter. In the presence of constant fluxes from the surface and a large number of species of troglobionts or troglophiles, micromycetes introduced into the trophic chains of native cave subterranean species predominate (Vanderwolf et al., 2013).

Cave water streams are oligotrophic and, as a consequence, communities functioning is dependent on allohtonic substances and availability of light (Culver, 1982). In the presence of hydrogen sulphide in subterranean water streams, microbial mats are formed. The dominant group of bacteria in above-mentioned mats is filamentous chemolithoautotrophic Epsilonproteobacteria (Engel et al., 2004; Engel et al., 2009). Specific microbiota of sulphur circulation can be identified using molecular methods, for example, representatives of genus *Thiobacillus, which receive energy by the oxidation of sulfur compounds (Hose et al., 2000).*

Generally, cave communities are epilithic and develop on the surface of walls, ceiling and speleothems (Roldán & Hernández-Mariné, 2009). However, endolithic communities can be detected in caves, too. In this case, microorganisms penetrate deep into the substrate up to several millimetres (Golubic et al., 1981). Microorganisms can be found in the aerothytic and water habitats of caves, for example, in the outflow cave, where communities can proliferate in water streams and irrigated areas.

Many caves are used as tourist objects, which is accompanied by their equipment and installation of artificial light. As a consequence, the biotopes of the caves change, and the so-called lamp flora develops.

The aim of present study is to assess the species composition and structure of microbiota communities in Otap Head Cave.

Methods

Otap Head Cave (the name in Abkhazian language is Uatap ahy) is located in the Otap village, Republic of Abkhazia. The absolute height of the entrance is 222 m above the sea level (42 ° 55 '17.8 "N, 41 ° 32' 18.8" E).

The length of the cave is more than 1,000 m. Otap Head Cave is laid in the layered Upper Cretaceous limestone along the strata of the bedding. The cave opens with a large arch located in the gorge of the river Uatapahy, flowing from Otap Head Cave and flowing into the river Otap. The flow rate of the outflowing river is estimated at about 30 L/sec. Approximately 70 m from the entrance, there is a lake. Somewhat further, there is a hall connected to the surface by a vertical well. Since 2013, the exploitation of the cave as a touristic object has begun.

The investigation was carried out in 2011–2012 and in May 2017. Temperature and relative humidity were measured using electronic devices Elin Complex iB-DLR-L-U (thermochron) with an error of 0.1°C and 1%. The pH was measured with pH meters PH200 (error ± 0.02 pH) and PH-013 (error ± 0.01 pH). The initial estimation of CO_2 emission and atmospheric gas content in the cave was carried out using the gas analyzer Testo.

A stable air temperature (12–13°C) was reported in the cave. Fluctuations are caused by changes in water temperature, which can vary during periods of floods. Otap Head is the outflow cave and, consequently, most of it is occupied by an underground river. In the entrance zone, a gradual increase of air temperature was detected, but it was always somewhat lower (in summer) or higher (in winter) than the air temperature on the surface. The relative humidity was 72–86%.

The water temperature in cave streams was 13°C, and water pH differed from 8.2 to 8.3 at the same time while soil pH was 7.2–8.6.

The concentration of carbon dioxide in the air was 400–1,500 ppm, depended insufficiently on the season and increased in the deep zones of the cave. Periodically,

an increase of the hydrogen sulphide concentration (12 ppm) was recorded in the entrance grotto zone. In subterranean waters closer to the cave entrance, the concentration of hydrogen sulphide was periodically increased to 250–300 mg/L.

The sampling sites were located in the cave stream, the irrigated zone between the stream and cave walls, as well as on the walls. Material for algological and microbiological investigation was collected from visually visible fouling communities by scraping the cave substratum with a sterile scalpel. Samples were deposited into sterile vials at a temperature of the cave until laboratory processing. Scraped material was directly observed using the light microscope Leica DMLS (Germany) and Biolam MBS-9 (Russia), as well as SEM JSM-25 S. The abundance of algae was estimated by applying the 5-point Brown- Blank scale. To clarify a systematic position of species, algae and cyanobacteria were transferred to culture media with the help of a microcapillary. Gromov medium No. 6 and extract from substrates (analogue of soil extract) were used. Cultures were incubated at 11°C and 25°C and light intensity 30–40 μmol m^{-2} s^{-1}. The method of fouling glasses and cultivation in a liquid medium was used. In addition, to identify the propagules of phototrophs introduced into the caves by streams, samples of soil and water from the dark zone were cultured in Gromov No. 6 medium. The water was pre-filtered through nuclear filters, and then the filtrate was inoculated in a culture medium (Netrusov, 2005). The cultivation period was 11 months.

Algae and cyanobacteria were identified using the following literature: Andreeva (1998), Gollerbach et al. (1953), Zabelina et al. (1951), Komarek and Anagnostidis (2005), and Whitton (2011). Taxonomy was presented by database http://www.algaebase.org.

To identify micromycetes, soil samples in the first dilution were inoculated. In addition, the fouling method was used (Netrusov, 2005). Standard agar mediums Czapek and Czapek-Dox (sucrose concentration 0.3%), potato-glucose agar, soil extract and hungry agar were used (Netrusov, 2005). Cultivation of micromycetes was carried out in the dark at a temperature of 4, 12 and 24°C. The growing colonies were accounted and pure cultures were isolated every week. The cultivation time at low temperatures was not less than 4 weeks (maximum 12 weeks). After an incubation period, fungi were identified based on colony macromorphology and microscopic features of fungal reproductive structures using a light microscope (Leica DMLS (Germany) and Biolam MBS-9 (Russia)). Fungi were identified using the following literature: Raper and Fennel (1965); Raper and Thom (1968); Booth (1971); Ramirez (1982); Ellis & Ellis (1997); Domsch et al. (2007). The names of species were given according to the database http://www.mycobank.org. The number of micromycetes in the cave air was estimated using the sedimentation method on the Chapek-Doks medium (Netrusov, 2005).

An analysis of the sanitary-indicative microflora was carried out, and the total microbial number (TMN) in water and soil was determined using a standard approach. The number of microorganisms was accounted using an MPA medium. Bacteria of the *Escherichia coli* group were identified by membrane filters using the Endo medium at 37°C. Swabs from lactose-positive colonies were investigated using the Gram stain, and an oxidase test was performed. Bacteria *Clostridium perfingens* were identified on a Wilson-Blair medium at 43°C. The number of bacteria was estimated in colony-forming units (CFU) per the weight of dry soil.

The number of microorganisms in the air was determined by sedimentation with an exposure of 30 minutes on the MPA medium. The calculation of the CFU amount per unit volume of air was carried out according to the Omelyansky formula.

Identification of various groups of bacteria was carried out using selective media. The study was conducted using standard microbiological methods. The method of inoculation from serial dilutions to agar nutrient media was used. Scrapings were made from dense specimens. Incubation of the samples was carried out at a temperature of +28°C, +12°C and +4°C for 14–30 days.

To identify sulphate-reducing bacteria, the Postgate medium was used in time while the Beyerinck medium was used for sulphur-oxidizing bacteria and the Letten medium for iron-oxidizing (ferro-bacteria) bacteria. The Saburo medium was used for yeast isolation. The presence of sulphur-oxidizing bacteria was determined by the appearance of a white film of molecular sulphur on the surface of the medium. The turbidity of the medium and the appearance of black colour indicated the presence of sulphate-reducing bacteria. The presence of iron bacteria was estimated by the appearance of ocherous sediments.

Results and Discussion

Photothropic microbiota. Fifty species of photosynthetic organisms were identified. Cyanobacteria were the dominant group, represented by 36 species (72% of the reported species, 1 Class, 5 Orders, 14 Families, 20 Genera). Phylum Bacillariophyta included 8 species (16%, 1 Class, 3 Orders, 6 Families, 6 Genera), phylum Chlorophyta was represented by 3 species (6%, 2 Classes, 3 Orders, 3 Families, 3 Genera), phylum Ochrophyta by 2 species (4%, 1 Class, 1 Order, 1 Family, 2 Genera) and phylum Rhodophyta by 1 species (2%, 1 Class, 1 Order, 1 Family, 1 Genus). A total number of orders, classes, families and genus are presented in Table 1.

Phototrophic communities developed in zones where direct or indirect light penetrated. However, at the time

Table 1. *Taxonomic structure of the Otap Head Cave flora*

Phylum	Class	Order	Family	Genus	Species number
Cyanobacteria	Cyanophyceae	Chroococcales	Microcystaceae	Gloeocapsa	4
				Microcystis	1
			Chroococcaceae	Chroococcus	4
				Gloeocapsopsis	1
			Aphanothecaceae	Aphanothece	1
				Gloeothece	2
		Nostocales	Nostocaceae	Nostoc	2
			Scytonemataceae	Scytonema	1
			Stigonemataceae	Stigonema	1
			Tolypothrichaceae	Tolypothrix	1
		Oscillatoriales	Oscillatoriaceae	Oscillatoria	3
				Phormidium	4
				Potamolinea	1
			Cyanothecaceae	Cyanothece	1
			Microcoleaceae	Symploca	1
		Synechococcales	Merismopediaceae	Aphanocapsa	1
				Synechocystis	2
			Leptolyngbyaceae	Leptolyngbya	3
			Pseudanabaenaceae	Jaaginema	1
		Pseudanabaenales	Schizotrichaceae	Schizothrix	1
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	Chlamydomonas	1
	Trebouxiophyceae	Chlorellales	Chlorellaceae	Chlorella	1
		Prasiolales	Prasiolaceae	Stichococcus	1
Bacillariophyta	Bacillariophyceae	Thalassiophysales	Catenulaceae	Amphora	1
		Tabellariales	Tabellariaceae	Diatoma	1
		Naviculales	Diadesmidaceae	Humidophila	1
			Naviculaceae	Navicula	2
			Neidiaceae	Neidium	1
			Pinnulariaceae	Pinnularia	2
Ochrophyta	Xanthophyceae	Tribonematales	Tribonemataceae	Heterothrix	1
				Tribonema	1
Rhodophyta	Florideophyceae	Hildenbrandiales	Hildenbrandiaceae	Hildenbrandia	1

of the study, biofouling of phototrophs around stationary lights was not observed. In other words, the absence of a lampenflora was revealed, which indicates a moderate exploitation regime of the cave.

Due to the presence of a powerful water stream in the cave, there was a difference in the species composition of the biofouling communities, depending on their location relative to the stream (cave walls far from the water stream, the water stream and the zone between the stream and the cave wall). The variations in occurrence and relative abundance of identified taxa among the various zones are shown in Table 2.

It should be noted that communities were mostly located discretely, forming visually distinct spots. By the maximum abundance, the dominants of communities in different cave habitats were identified.

Table 2. *Cyanobacteria and algae of Otap Head Cave*

	Occurrence			Relative abundance (%) in cave
	Zone 1	Zone 2	Zone 3	
Phylum Cyanobacteria				
Gloeocapsa atrata Kützing	1		1	2.30
Gloeocapsa sp.	1		1	1.72
Gloeocapsa magma (Brébisson) Kützing	1		1	2.30
Gloeocapsa punctata Nägeli	1		1	2.87
Microcystis pulverea (H.C.Wood) Forti	1		1	2.87
Chroococcus minutus (Kützing) Nägeli	1		1	4.60
Chroococcus dispersus (Keissler) Lemmermann	1		1	2.30
Chroococcus lithophilus Ercegovic	1		1	1.72
Chroococcus turgidus (Kützing) Nägeli	1		1	2.30
Gloeocapsopsis magma (Brébisson) Komárek & Anagnostidis ex Komárek	1		1	1.72
Aphanothece microscopica Nägeli	1		1	2.87
Gloeothece rupestris (Lyngbye) Bornet	1		1	1.72
Gloeothece palea (Kützing) Nägeli	1		1	1.72
Nostoc microscopicum Carmichael ex Bornet & Flahault	1			1.72
Nostoc punctiforme Hariot	1			1.15
Scytonema drilosiphon Elenkin & V.I.Polyansky [Polyanski]	1			2.87
Stigonema sp.	1			1.15
Tolypothrix calcarata Schmidle	1			2.87
Oscillatoria limosa var. tenuis Seckt		1		1.15
Oscillatoria rupicola (Hansgirg) Hansgirg ex Forti	1		1	1.72
Oscillatoria tenuis C.Agardh ex Gomont	1		1	1.72
Phormidium aerugineo-coeruleum (Gomont) Anagnostidis & Komárek	1	1	1	4.02
Phormidium irriguum (Kützing ex Gomont) Anagnostidis & Komárek		1	1	1.72
Phormidium lividum (Hansgirg) Forti		1		1.15

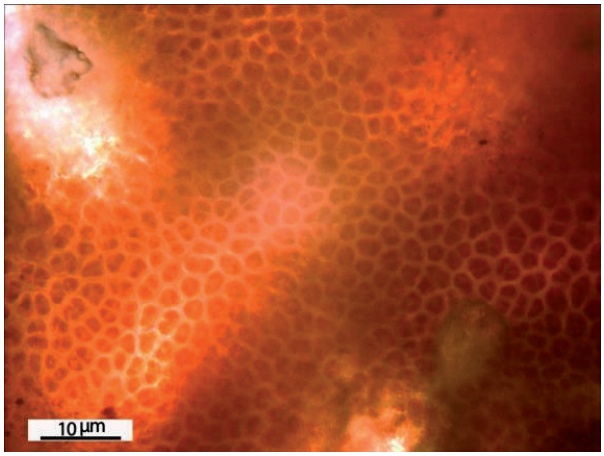
	Occurrence			Relative abundance (%) in cave
	Zone 1	Zone 2	Zone 3	
Phormidium sp.		1	1	1.15
Potamolinea aerugineo-caerulea f. calcarea (Gomont) M.D.Martins & L.H.Z.Branco	1			1.15
Cyanothece aeruginosa (Nägeli) Komárek	1		1	2.30
Symploca muscorum Gomont ex Gomont	1			2.87
Aphanocapsa grevillei (Berkeley) Rabenhorst	1		1	2.30
Synechocystis crassa Woronichin	1		1	1.72
Synechocystis minuscula Woronichin	1		1	1.15
Leptolyngbya angustissima (West & G.S.West) Anagnostidis & Komárek	1			1.15
Leptolyngbya tenuis (Gomont) Anagnostidis & Komárek	1			1.72
Leptolyngbya voronichiniana Anagnostidis & Komárek	1			1.15
Jaaginema subtilissimum (Kützing ex Forti) Anagnostidis & Komárek	1			1.15
Schizothrix sp.	1		1	1.72
Phylum Chlorophyta				
Chlamydomonas intermedia Chodat	1		1	1.15
Chlorella vulgaris Beyerinck [Beijerinck]	1		1	4.60
Stichococcus minor Nägeli	1		1	1.15
Phylum Bacillariophyta				
Amphora sp.		1		1.72
Diatoma vulgaris Bory de Saint-Vincent	1	1	1	2.30
Humidophila contenta (Grunow) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová	1	1	1	3.45
Navicula cryptocephala Kützing			1	0.57
Navicula sp.		1	1	1.15
Neidium affine (Ehrenberg) Pfizer		1	1	1.72
Pinnularia borealis Ehrenberg	1		1	2.30
Pinnularia elegans (W.Smith) K.Krammer	1		1	1.72
Phylum Ochrophyta				
Heterothrix bristoliana Pascher	1		1	2.30
Tribonema minus (Wille) Hazen	1		1	2.30
Phylum Rhodophyta				
Hildenbrandia rivularis (Liebm.) J. Ag.		1	1	1.72

** Zone 1 – cave walls far from the water stream; zone 2 – the water stream; zone 3 – the zone between the stream and the cave wall.*

In the cave stream, three types of communities were detected.

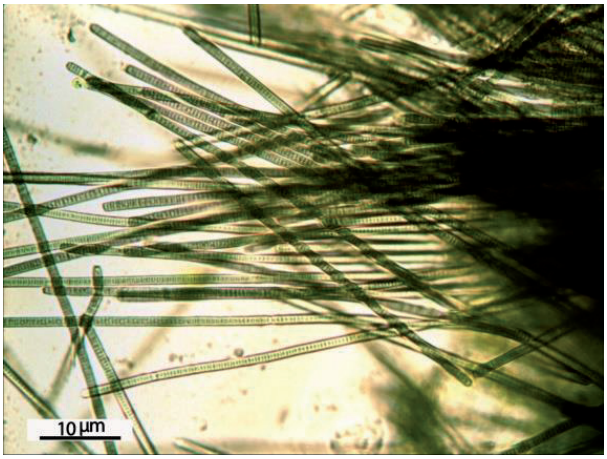
Type 1. Mono-species communities of red alga *Hildenbrandia rivularis* (*Fig. 1*). Biofoulings were closely adhered to the substrate. They were located on limestone or pebbles and were submerged in a stream or were in an irrigated zone.

Fig. 1. *Hildenbrandia rivularis*



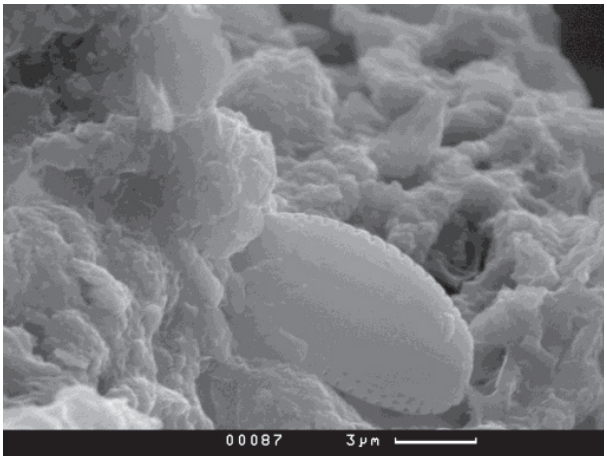
Type 2. Communities forming mucilaginous biofilms with the dominance of filamentous forms of cyanobacteria (genus *Oscillatoria* and *Phormidium*) (Fig. 2). Crystals of calcium carbonate were detected in the structure of biofilms. Probably they are the limestone particles on which biofilms are formed, as the disintegration of the rock under the communities was observed.

Fig. 2. *Phormidium irriguum*



Type 3. Communities of diatom algae, which had a different coloration from red-brown to greenish-brown. *Humidophila contenta*, *Amphora sp.* (Fig. 3), species of the genus *Navicula* were dominant. These communities were found either in the form of dense biofoulings on limestone or they were in association with cyanobacteria (diatoms were in the upper layer of biofilm).

Fig. 3. *Amphora sp. in cyanobacterial biofilm Phormidium irriguum*



In the irrigated zone between the stream and cave walls, the following types of communities were revealed:

Type 1. Communities with the dominance of green algae *Chlorella vulgaris*;

Type 2. Communities with a predominance of coccal forms of cyanobacteria, dominated by *Gloeocapsa punctata* or *Microcystis pulverea*;

Type 3. Communities with a predominance of diatom algae *Humidophila contenta*.

On the cave walls far from the water stream, other types of communities were identified:

Type 1. Communities of filamentous cyanobacteria, dominated by *Tolypotrix calcarata*, *Scytonema drilosiphon*, and *Potamolinea aerugineo-caerulea f. calcarea*;

Type 2. Communities with a predominance of green alga *Chlorella vulgaris*;

Type 3. Communities with the predominance of species of the genus *Chroococcus*, dominant *Chroococcus minutus* (Kützing) Nägeli.

The cause of discreteness of communities and the change of dominants in communities with similar life forms are unclear and require further research.

Table 3. *Species composition and occurrence of micromycetes in Otap Head Cave*

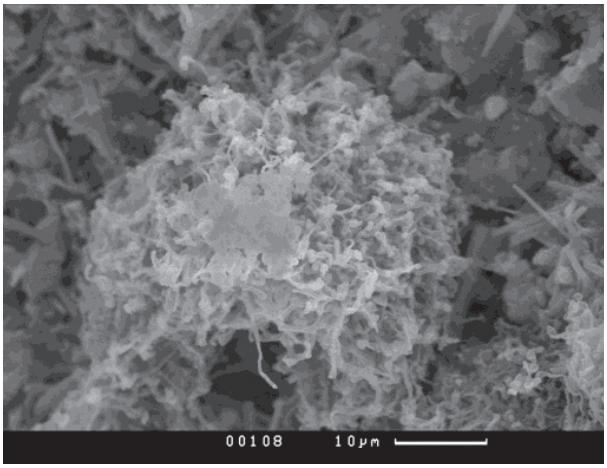
ZYGOMYCOTA	Zone 1	Zone 2	Zone 3
Absidia sp.	1		
Absidia cylindrospora Hagem	1		1
Mortierella alpina Peyronel	1	1	1
Mucor hiemalis Wehmer	1	1	
Mucor rouxii (Calmette) Wehmer	1		1
Rhizopus arrhizus A. Fisch.	1		1
Rhizopus stolonifer (Ehrenb.) Vuill.	1	1	1
Thamnidium sp.	1		1

ASCOMYCOTA	Zone 1	Zone 2	Zone 3
Acremonium sp.	1		
Acremonium charticola (Lindau) W. Gams	1	1	
Alternaria alternata (Fr.) Keissl	1	1	1
Aspergillus flavipes (Bainier et R. Sartory) Thom et Church	1	1	1
Aspergillus flavus Link	1		1
Aspergillus flavus var. oryzae (Ahlb.) Kurtzman	1		1
Aspergillus fumigatus Fresen	1	1	1
Aspergillus niger Tiegh.	1	1	1
Aspergillus ochraceus G. Wilh.	1		1
Aspergillus sp.	1	1	
Aspergillus restrictus G. Sm.	1		1
Aspergillus terreus Thom	1		1
Aspergillus ustus (Bainier) Thom & Church	1		1
Aspergillus versicolor (Vuill.) Tirab.	1		1
Aureobasidium pullulans (de Bary) G. Arnaud	1	1	1
Botrytis cinerea Pers.	1		1
Chaetomium globosum Kunze ex Fr.	1		1
Chaetomium sp1.	1		
Cladosporium cladosporioides (Fresenius) G.A. de Vries	1	1	1
Cladosporium herbarum (Persoon) Link	1		1
Cladosporium sphaerospermum Penz.	1		
Fusarium sp.	1		
Fusarium oxysporum Schltdl.	1		1
Fusarium solani (Mart.) Sacc.	1		1
Fusarium sporotrichioides Sherb.	1		1
Geomyces pannorum (Link) Sigler et J.W. Carmichael	1	1	1
Humicola sp.	1		1
Penicillium aurantiogriseum Dierckx	1	1	1
Penicillium chrysogenum Thom	1	1	1
Penicillium simplicissimum (Oudem.) Thom	1		
Penicillium sp.	1		
Talaromyces flavus (Klöcker) Stolk & Samson	1		
Talaromyces purpurogenus (Stoll) Samson	1		1
Trichoderma hamatum (Bonord.) Bainier	1		
Trichoderma harzianum Rifai	1		1
Trichoderma polysporum (Link) Rifai	1		
Trichoderma viride Pers.	1		1

Fungal microbiota. A total of 45 species of micromycetes (Table 3) were identified in the communities of the cave entrance. Zygomycota included 8 species, and the remaining species belonged to the Ascomycota. The dominant were species of the genus *Aspergillus* (11 species), *Trichoderma* (4 species), and *Penicillium* (4 species), including the telomorphs *Talaromyces* (2 species).

After analyzing the soil of the cave walls not irrigated by water streams, it was revealed that the surface of clay and mineral deposits was covered with a mycelium, in time when biofoulings were not visible to the unaided eye (Fig. 4).

Fig. 4. *Micromycetes on the surface of clay deposits*



The number of micromycetes in the air in the entrance zone was 150 CFU/m^3, in the deeper part of the cave it was 3–6 CFU/m^3, and in the hall under the well it was 8–12 CFU/m^3. Most of the identified species belong to typical representatives of the soil mycobiota. The number of propagules of micromycetes in the cave air is insufficient. On the cave walls, species of the genus *Trichoderma* and *Cladosporium* predominated. Species of the genus *Aspergillus* were most often isolated from the phototrophic communities. Species *Aureobasidium pullulans*, *Botrytis cinerea*, *Chaetomium globosum* and representatives of Zygomycota were found only in the dark part of the cave, in the hall, i.e., at the end of the excursion route. All identified species were able to grow at the temperature of the cave, which indicates their adaptation to the conditions of the cave environment. The species composition of fungi identified in 2011 and 2017 was similar.

Bacterial microbiota. In the cave grounds, the total microbial number (TMN) was 106 CFU/g in the entrance area, and 104–105 CFU/g in the deeper parts of the cave. In the cave waters, the TMN was 100–150 CFU/L at the beginning of the water stream. In the entrance zone, TMN increased to 1,800–2,300 CFU/L. Bacteria of *Escherichia coli* group were not found in cave streams. Only in the entrance zone, in the water, they appear in the amount of 2–12 CFU/L.

In the air of the cave, the TMN was 200–600 CFU/m^3 in the deeper parts of the cave, and 1,800 CFU/m^3 in the hall under the well. In the entrance zone, the estimation of the TMN in the air was not carried out because of strong air currents.

Bacteria *Clostridium perfingens* were found in the soils of the cave entrance (4–18 CFU/g) and in the hall under the well (13–25 CFU/g). *Clostridium perfingens* were absent in the soil of the deeper part of the cave.

As a result of microbiological studies, sulphuric or thiobacillic bacteria were isolated both on the rock and in the deposits of the cave, which can indicate the oxidation of sulphur compounds. Moreover, the development of sulphate-reducing bacteria was detected. Acidophilic iron bacteria were found in rock and sediment samples. The presence of sulphuric and sulphate-reducing bacteria in the analyzed samples indicates that at the present time in the cave there is a simultaneous activity of sulphate-reducing and sulphur-oxidizing bacteria, that is, a small circulation of sulphur occurs. Changes in biodiversity or abundance of micromycetes and phototrophs in the areas of hydrogen sulphide outputs were not detected.

Species belonging to the phylum Cyanobacteria were the most abundant in all investigated zones of Otap Head Cave. Cyanobacteria have been frequently encountered in European caves as a dominant phylum in cave communities (Selvi & Altuner, 2007; Czerwik-Marcinkowska & Mrozińska, 2009, 2011; Cennamo et al., 2012; Czerwik-Marcinkowska, 2013; Popović et al., 2015). They are pioneer organisms in the genesis of cave biofilms being able to grow diazotrophically (Gallon et al., 1991) and produce exopolymeric substances that allow the formation of the microbial community and its adhesion to substratum (Stal, 2000). *Chroococcus minutes*, one of the dominant cyanobacteria in Otap Head Cave, was also recorded from Greece (Lamprinou et al., 2009), Slovenia (Mulec, 2008, Mulec & Kosi, 2008), Slovakia (Uher

& Kováčik, 2003), Turkey (Selvi & Altuner, 2007), and Hungary (Buczko & Rajczy, 1989). *Gloeocapsa* spp. are also common in caves (Vinogradova et al., 1998; Czerwik-Marcinkowska & Mrozińska, 2011). The occurrence of *Chroococcus* and *Gloeocapsa* species has been reported not only in dim cave environments, but also on monuments exposed to daylight (Scheerer et al., 2009), confirming the tolerance of these algae to a wide range of environmental conditions. Coccoid forms of cyanobacteria represent a major part of the biofilm communities in caves. There is a hypothesis that coccoid forms are adapted to lower light intensity, as they tolerate low irradiance easily than other taxa of cyanobacteria (Mulec et al., 2008). Oscillatoriales are also very abundant in cave zones with lower light intensity (Roldán & Hernández-Mariné, 2009; Lamprinou et al., 2012), because they are well adapted to extremely low irradiance in comparison with other filamentous Cyanobacteria (Albertano et al., 2000). Representatives of genus Scytonema are considered to be one of the most dominant aeroterrestrial cyanobacteria (Pattanaik et al., 2007). *Scytonema drilosiphon* has the ability to deposit crystals of calcium carbonate (CaCO3) in their sheaths that allow the higher survivor capacity (Whitton, 2012). Chlorophyta genera, such as *Chlorella* and *Stichococcus*, and Bacillariophyta genera, such as *Navicula* and *Pinnularia*, are ubiquitous, cosmopolitan and capable to colonize a wide range of substrata, regardless of microclimatic and environmental conditions (Macedo et al., 2009). Red algae *Hildenbrandia rivularis* was not recorded in cave communities.

The cave entrance is usually characterized by the richness of the species composition of fungi (Ogórek et al., 2013); as a consequence, the high biodiversity of micromycetes noted in the Otap Head Cave is consistent with the literature data.

In a review of Vanderwolf et al. (2013), it was shown that the most abundant fungi in cave habitats are *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Trichoderma,* and *Cladosporium*. Most of these fungal species are cosmopolitan saprotrophs associated with soil, plant material, or invertebrates (Vanderwolf et al., 2013). In addition, they are able to grow on nutrient-poor substrata (Sterflinger et al., 2012).

Usually, researchers note differences in the species composition of micromycetes in the air, substrates, in water and on rock, which is associated with imperfection of methods, features of isolated species and insufficient inspection of the object (Ogórek et al., 2013). However, in the present study, such a difference was noted by using the standard method for different zones.

There are several ways of propagules input in caves: with air streams and soil particles and plant residues that penetrate the ground with water streams that are most powerful during the high water, when even large accumulations of organic matter can be transported over long distances (Hsu & Agoramoorthy, 2001).

According to the results of the study, it can be concluded that the sanitary-microbiological situation in the cavity is satisfactory. The outputs of hydrogen sulphide are periodic and can induce the development of thionic bacteria. In neutral and weakly alkaline cavern conditions, bacterial sulphur deposition and sulphate formation can be expected, which leads to the development of sulphate reducers. Probably, sulphuric acid speleogenesis (SAS) occurs in the subaqueous environment of the cave at or close to the water table but also in subaerial conditions in moisture films and droplets in the cave environment. This phenomenon is widespread in caves, but it was described mainly in caves with higher temperatures (De Waele et al., 2015).

Otap Head Cave can be considered as an example of successful limitation of anthropogenic load on a touristic cave, which is more associated with the primary eutrophication of the cave and its poor attendance, than with the optimal mode of exploitation.

Stable temperature conditions are documented in caves; moreover, they are weakly dependent on surface temperature, so this object can be convenient for considering the effects of global climate change, in particular changes in biodiversity. At the moment, the stability of the composition of the cave microbiota indicates the absence of the influence of both global planetary changes and anthropogenic impact.

Conclusions

As a result of the research, the biodiversity of phototrophs and micromycetes of the Otap Head Cave was revealed at the first time. The species composition of phototrophs included 50 species, in comparison with mycromycetes 45. Among the micromycetes, the species of the genus *Aspergillus* predominated. The main types of phototrophic biofouling communities were determined. The periodic local increase in hydrogen sulphide in the cave air and waters was revealed. Given into account that there are thermal springs near the cave, it can be assumed that in time of changes in the hydrological regime of the cave, for example, in floods or low water, thermal waters with

hydrogen sulphide can flow or the gas can escape into the cave due to the elimination of the water backstop. Microbiological studies using culture methods demonstrated the presence in the cave of sulphur bacteria and sulphate-reducers, as well as iron bacteria; as a consequence it is necessary to continue research in this direction using molecular methods.

Despite the fact that Otap Head Cave was equipped for sightseeing purposes in 2013, the composition of the species has not changed. This may be explained by that, firstly, the flow of visitors is small, secondly, the lampenflora did not have time to form, and thirdly, the entrance zone of the cave is rich in organic matter due to the presence of guano from bats, as well as periodic falling into the cave debris branches and tree trunks.

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