Algae in the aerophytic habitat of Račiške ponikve cave (Slovenia)

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Abstract. In the cave entrance of Račiške ponikve, four selected sites with confluent algal growth and different sun irradiance levels were sampled for algological analysis. Altogether, 42 algal taxa were determined, with the highest proportion of cyanobacteria, 30 taxa (71%), followed by 8 taxa (19%) of Chlorophyta and 4 taxa (10%) of Chrysophyta. The favourable ratio of Oscillatoriales vs. Nostocales indicates that flora in Račiške ponikve cave is generally exposed to low photo flux. In the twilight zone of the cave, coccoid cyanobacteria prevailed. Moreover, at all sampling points algae characteristic of water and/or soil habitats were also identified besides typical aerophytic algae.

Keywords: algae, cave, Račiške ponikve, Slovenia

Izvleček. ALGE V AEROFITSKEM HABITATU JAME RAČIŠKE PONIKVE (SLOVENIJA) - Za algološko vrstno sestavo smo v jamskem vhodu Račiških ponikev opravili vzorčenje na štirih izbranih mestih s konfluentno obrastjo alg in različno izpostavljenostjo sončni osvetlitvi. Skupaj smo identificirali 42 taksonov alg, z največjim deležem cianobakterij, 30 taksonov (71%), ki so jim sledili predstavniki Chlorophyta z 8 taksoni (19%) in s 4 taksoni (10%) Chrysophyta. Višji delež Oscillatoriales v primerjavi s predstavniki Nostocales nakazuje, da je flora v jamskem vhodu Račiških ponikev izpostavljena pretežno šibkim intenzitetam svetlobe. V jami v pasu somraka prevladujejo kokoidne cianobakterije. Na vseh vzorčnih mestih smo poleg alg, ki so značilne aerofitske, identificirali tudi alge, ki navadno uspevajo v vodnih in terestičnih habitatih.

Ključne besede: alge, jama, Račiške ponikve, Slovenija

Introduction

Karst caves are considered to be an extreme environment (Culver et al. 2004). In caves, cyanobacteria and algae can be identified in different types of water bodies (Kuehn et al. 1992, Sanchez et al. 2002) and in aerophytic habitats (Golubić 1967, Dobat 1970). Phototrophs are easily found in cave entrances illuminated by direct or indirect sunlight and in show caves equipped with artificial illumination as part of a lampenflora community around lamps (Dobat 1998, Mulec 2005). In the cave entrance illuminated by sunlight, it is worth mentioning a peculiar phototrophic community of stromatolitic stalagmites and stalactites, which enhances precipitation of $CaCO_3$ and consequently the growth of speleothems. In Slovenia, the best examples of such biogenic speleothems can be found in Škocjanske jame (Mulec et al. 2007). To date, 197 cyanobacterial and algal taxa have been identified in Slovenian subaerophytic karst habitats (Golubić 1967, Dobat 1973, Martinčič 1978, Martinčič et al. 1981, Krivograd-Klemenčič & Vrhovšek 2005, Mulec 2005, Krivograd-Klemenčič 2007, Mulec et al. in press). In such habitats, mainly cyanobacteria can be found that can prosper at low photon flux, even lower than the light compensation point (Mulec et al. in press). A typical cave cyanobacterium *Geitleria calcarea* was identified on poorly illuminated walls in Škocjanske jame and Huda luknja (Mulec 2005, Krivograd-Klemenčič 2007).

In the last decade, there has been considerable progress in understanding different ecological interactions in various habitats. However, there are still only few ecophysiological studies on subaerial algae and their adaptations (Golubić 1967, Cox 1977, Cox et al. 1981, Couté 1982, Aboal et al. 1994, Couté & Chauveau 1994, Hernandez-Marine et al. 1999, Giordano et al. 2000, Whitton & Potts 2000, Hoffmann 2002, Mulec et al. in press). Environmental factors in aerophytic habitats are not always friendly to algae and do not always support successful establishment of a phototrophic community (Mulec et al. in press). The purpose of the study was to identify the species composition of a phototroph community in a weakly illuminated ponor cave, and its response to low illumination rates.

Description of study site

Račiške ponikve cave is located in the SW part of Slovenia and it is developed in Paleogene limestone (Fig. 1). An intermittently flowing stream sinks at the entrance. Sampling of aerophytic algae was conducted in the cave entrance of the ponor cave Račiške ponikve (Fig. 1). To obtain illumination gradient samples were taken where confluent phototrophic growth was observed, at distances of 1.5, 2.0, 4.5 and 12.0 metres from the entrance toward the cave interior (Fig. 2).

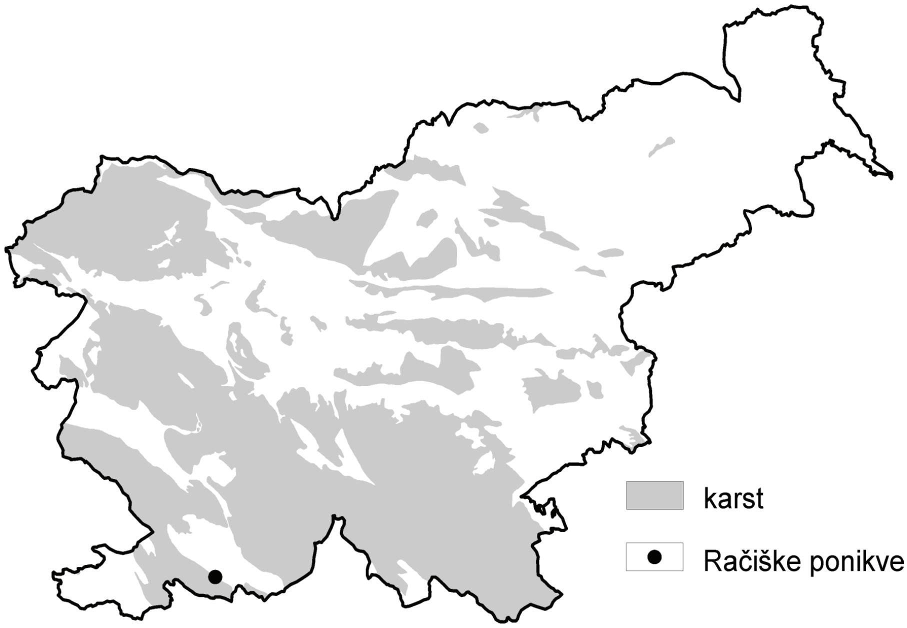

Figure 1. Geographical location of Račiške ponikve cave in Slovenia.
Slika 1. Geografski položaj jame Račiške ponikve v Sloveniji.

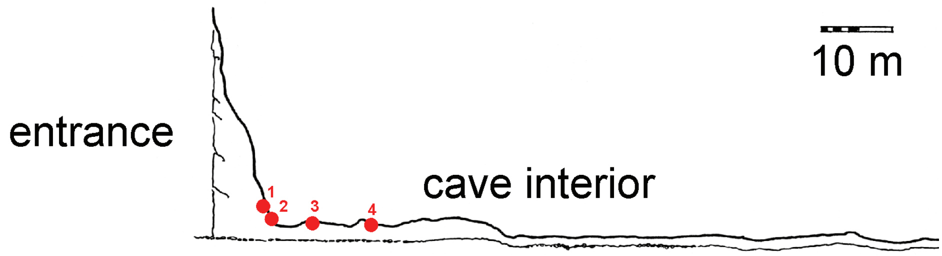

Figure 2. Sampling sites (1-4) of the aerophytic algae in Račiške ponikve cave (Source: Cave register IZRK ZRC SAZU and JZS).
Slika 2. Mesta vzorčenja (1-4) aerofitskih alg v jami Račiške ponikve (Vir: Kataster jam IZRK ZRC SAZU in JZS).

Matherial and methods

Prior to collecting, photon flux density (PAR, photosynthetically active radiation) at the sites was measured using a LICOR LI-1000 DataLogger, USA (10 June 2003, 13:30, 9°C; weather outside the cave was sunny and clear). At each sampling sites Jaworski medium (Warren et al. 1997) was aseptically inoculated with scrapes of the algal mat. Cultures of algae were isolated in liquid Jaworski and on 1% solid Jaworski media. Cyanobacteria were selectively isolated in the medium supplemented with 100 μg ml^{-1} of DCMU (N-3, 4-dichlorophenyl-N'-dimetil urea). Cultivation laboratory conditions were the following: 20°C, 8:16 light/dark period with a photon flux density of 100 μmol m^{-2} s^{-1} for several weeks. Cultures were regularly screened each second day for several weeks under a magnifying glass and light microscope (Nikon Eclipse TE300). Floristic data obtained from culture material were supplemented with microscopic data of the same field material previously fixed with 4% formalin solution.

Diatom samples were processed and identified as described by Clesceri et al. 1998. Sputtered gold specimens were screened using an SEM microscope (JSM-840, JEOL, USA). Several literature sources were used to identify subaerial algal species (Abdelahad 1989, Asencio & Aboal 2000, Couté 1982, Ettl & Gärtner 1995, Garbacki et al. 1999, Geitler 1932, Golubić 1967, Hindak 1996, Hoffmann 1986, Komárek & Anagnostidis 2000, 2005, Krammer & Lange-Bertalot 1986, 1988, 1991, Lemmermann et al. 1915, Sulek 1969).

The Pearson (r) correlation coefficient was applied to compare two independent random variables. r ranges from -1 (negative correlation) to +1 (perfect positive correlation), the t test was also used (≤0.05). For cluster analysis the Bray-Curtis similarity measure was applied.

Results

From four macroscopically distinctive samples of algal mats 42 taxa were identified, 30 taxa (71%) of cyanobacteria, 8 taxa (19%) of Chlorophyta and 4 taxa (10%) of Chrysophyta. From all sampling sites cyanobacterium *Gloeocapsa punctata* and green alga *Chlorella* sp. were determined (Tab. 1)

The negative correlation between proportion of coccoid type of cyanobacteria and PAR in the cyanobacterial community was statistically significant (-0.93, p=0.03). Coccoid cyanobacteria became more abundant in the community as distance into the cave increased. The abundance of algae at the sampling site with the lowest photon flux density was the lowest (sampling site 4). Algal communities differed among sampling sites (Fig. 3).

Table 1. Species composition of the aerophytic algal community in Račiške ponikve cave at each sampling site (in bold are designated species typical for aerophytic habitats after Lazar 1960, Ettl & Gärtner 1995, Komárek & Anagnostidis 2000, 2005)
Tabela 1. Sestava vrst v združbi aerofitskih alg jame Račiške ponikve na vsakem vzorčnem mestu (s krepko so označene vrste, značilne za aerofitske habitate po Lazar 1960, Ettl & Gärtner 1995, Komárek & Anagnostidis 2000, 2005)

	Sampling site			
	1	**2**	**3**	**4**
Species \ PAR (µmol m^{-2} s^{-1})	1.94	1.02	0.65	0.19
PROKARYOTA				
CYANOPHYTA				
Aphanocapsa sp. Nägeli				+
***Aphanocapsa fusco-lutea* Hansgirg**	+			
***Aphanocapsa muscicola* (Meneghini) Wille**		+	+	+
***Aphanothece saxicola* Nägeli**	+		+	
Borzia sp. Cohn		+		
Borzia trilocularis Cohn	+		+	
***Chondrocystis dermochroa* (Nägeli) Komárek et Anagnostidis**			+	
Chroococcidipsis sp. Geitler		+	+	
Chroococcus sp. Nägeli	+			
***Chroococcus lithophilus* Ercegović**	+	+	+	
Chroococcus minutus (Kützing) Nägeli	+	+	+	
***Chroococcus varius* A. Braun in Rabehorst**			+	
***Gloeocapsa* sp. Kützing**	+	+		
***Gloeocapsa atrata* Kützing**	+	+		
***Gloeocapsa punctata* Nägeli**	+	+	+	+
***Gloeocapsa rupestris* Kützing**	+			
Gloeocapsopsis sp. Geitler ex Komárek			+	+
***Gloeothece palea* (Kützing) Rabenhorst**			+	
Leptolyngbya foveolarum (Rabenhorst ex Gomont) Anagnostidis et Komárek	+	+		
Leptolyngbya fragilis (Gomont) Anagnostidis et Komárek	+		+	
Leptolyngbya perelegans (Lemmermann) Anagnostidis et Komárek	+			
Lyngbya sp. Agardh				+
Nostoc. sp. Vaucher	+			
***Nostoc microscopicum* Carmichael**			+	
Oscillatoria sp. Vaucher			+	+
Plectonema sp. Thuret	+		+	

	Sampling site			
	1	**2**	**3**	**4**
Plectonema cf. *puteale* (Kirchner) Hansgirg	+	+		
Pleurocapsa minor Hansgirg			+	
Pseudocapsa sp. Ercegović		+		
Synechocystis sp. Sauvageau				+
EUKARYOTA				
CHRYSOPHYTA				
Chlorocloster sp. Pascher	+			+
Navicula sp. Bory	+			
***Navicula contenta* var. *biceps* (Arnott, Grunow in Van Heurck) Cleve**				+
***Navicula gallica* var. *perpusilla* (Grun) Lange-Bertalot**			+	
CHLOROPHYTA				
Chlorella sp. Beijerinck	+	+	+	+
Cosmarium tinctum Ralfs		+		
Kirchneriella sp. Schmidle	+			
Pediastrum boryanum (Turpin) Meneghini	+	+		
Pseudoclonium cf. *basiliense* Vischer			+	
***Stichococcus bacillaris* Nägeli**		+		
***Trentepohlia aurea* Martius**			+	
Ulothrix variabilis Kützing		+		

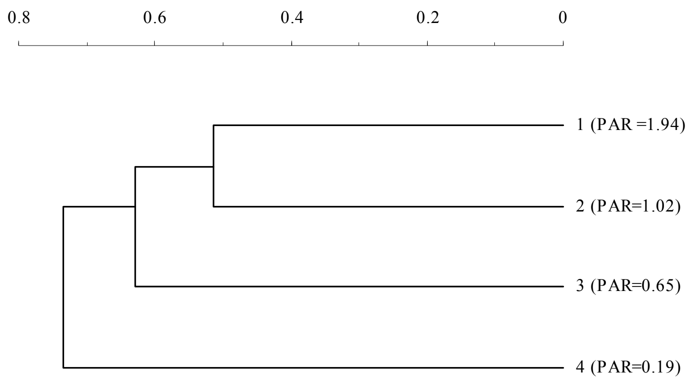

Figure 3. Comparison of the sampling sites of aerophytic algae in Račiške ponikve cave based on the Bray–Curtis measure of similarity (PAR values at the sampling sites are designated).
Slika 3. Primerjava vzorčnih mest aerofitskih alg v jami Račiške ponikve na osnovi Bray-Curtisove mere podobnosti (označene so PAR vrednosti vsakega vzorčnega mesta).

Discussion

In the cave entrances cyanobacteria prevail compared to other algal groups (Golubić 1967, Martinčič 1978, Mulec 2005, Mulec et al. in press). They can colonize the deepest parts of the cave entrance where biodiversity of organisms is the lowest (Vinogradova et al. 1998). Interestingly, in the study of algae from the cave entrance of Huda luknja Krivograd-Klemenčič (2007) found that the most abundant group were diatoms.

In the aerophytic algal community of Račiške ponikve cave, cyanobacteria dominated (71%) as was the case at the cave entrance of Škocjanske jame in the part named Schmidlova dvorana where cyanobacteria comprised 74% (59 taxa) of the community (Mulec 2005, Mulec et al. in press). With regard to cyanobacteria in the cave entrance of Račiške ponikve the ratio of Oscillatoriales vs. Nostocales was 9 : 2. Albertano et al. (2000) explained the favourable ratio of Oscillatoriales regarding Nostocales with better adaptation of Oscillatoriales to extremely low irradiance levels. Our results indicate that flora in Račiške ponikve cave is generally exposed to low illumination rates (Tab. 1). In the twilight zone (sampling site 4) of the cave, coccoid cyanobacteria prevailed. The community composition varied notably with decreasing of irradiance levels when approaching deeper in the cave interior (Fig. 3).

The presence of aerophytic community on a certain spot in a cave indicates that there are favourable conditions for its development, e.g. illumination, higher humidity. Despite the fact that cave walls of Račiške ponikve are a typical aerophytic habitat at all sampling points also algae typical for water or soil habitats were identified (Tab. 1). Golubić (1967) pointed out that even in typical aerophytic habitats especially small sized cyanobacteria and eukaryotic algae can live submerged under tiny droplets of water. The 3D biofilm structure of an aerophytic algal mat in Račiške ponikve cave can act as a »trap« for planktonic and phytobenthic algal species brought in by the intermittent stream. Beside the intermittent stream into the aerophytic community from the upper layers, seeping and dripping water bring through many crevices and cracks constantly new organisms and viable propagules, not only soil algae, which interact with the aerophytic community. This zone named epikarst has been recently established as a very rich and diverse habitat (Pipan 2005, Pipan & Culver 2007). Organisms originating above the cave and from intermittent stream enrich the phototrophic community and can considerably contribute to aquatic food chain for stygobiotic fauna.

Povzetek

Kraške jame predstavljajo ekstremno okolje. V jamah cianobakterije in alge identificiramo v različnih vodnih telesih in v aerofitskih habitatih. V nekaterih primerih so v vhodnih delih jam cianobakterije odgovorne za rast kapnikov. Čeprav je bil v zadnjem času narejen precejšen napredek v smislu identifikacije alg in habitatov kraških jam v Sloveniji in po svetu, pa še vedno manjkajo podrobne fiziološke in ekološke raziskave teh organizmov. Namen pričujoče raziskave je bil ugotoviti vpliv šibke intenzitete svetlobe v vhodu jame Račiške ponikve na sestavo združbe alg. V aerofitski združbi alg ponorne jame Račiške ponikve smo identificirali celokupno 42 taksonov z največjim deležem cianobakterij (71%). Rezultati kažejo, da je delež kokalnih oblik cianobakterij višji globlje v jamskem vhodu. Da je proučevani habitat v jami Račiške ponikve podvržen le šibkim intenzitetam svetlobe, nakazuje tudi višje število pripadnikov reda Oscillatoriales v primerjavi z Nostocales. Sestava združbe alg se med vzorčnimi mesti z zmanjševanjem intenzitete svetlobe proti notranjosti jame razlikuje. Čeprav so stene Račiških ponikev značilen aerofitski habitat, so bile na vseh vzočnih mestih identificirane tudi alge, ki so sicer značilne za vodne habitate. Poleg teh alg, ki jih prinese v aerofitsko združbo presihajoči potok, se v tej združbi znajdejo tudi terestrične alge in drugi organizmi, ki jih iz zgornjih plasti, vključno iz epikraške cone, prinese mezeča oziroma kapljajoča voda.

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