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The influence of some factors on cyanobacteria and algae biodiversity in karst speleogenesis

© 2021. Sh. R. Abdullin ORCID: 0000-0002-6946-2321,
Federal Scientific Center of the East Asia Terrestrial Biodiversity,
Far Eastern Branch of the Russian Academy of Sciences,
159, 100-letiya Vladivostoka Prospekt, Vladivostok, Russia, 690022,
e-mail: crplant@mail.ru

The biodiversity of underground habitats is of considerable interest from different standpoints, but information on the biota of the caves is incomplete and fragmentary. In this regard, the identification of different factors that influence the richness of species of various organisms, including cyanobacteria and algae, is important. 166 different samples, which were collected in 2007–2012 from 14 karst caves of natural origin, located in various climatic zones of Russia, were investigated. 121 species and intraspecific taxa of cyanobacteria and algae belonging to five phyla (Cyanobacteria, Bacillariophyta, Ochrophyta, Chlorophyta, Streptophyta) were identified in the studied caves. Based on the obtained material, the influence of some abiotic factors (the stage of karst speleogenesis, the length of the cave, and the area of the entrance, the underlying rocks and the surface climate) on the biodiversity of cyanobacteria and algae was analyzed using statistical methods. The speleogenesis stage (the influence of the factor is 118.4, $p < 0.05$) mostly effect on the species richness of cyanobacteria and algae in the studied caves that directly related to the level of water. Also the underlying rocks (the influence of the factor is 34.7, $p < 0.05$) and the length of the cave (the influence of the factor is 20.3, $p < 0.05$) effect on the biodiversity of cyanobacteria and algae in these caves. It was revealed that the relationship between the total number of species of cyanobacteria and algae and the various stages of speleogenesis is statistically significant ($p < 0.05$), and the species richness of these organisms decreases from the corridor-grotto stage to the corridor-grotto-chamber stage. The number of species of Cyanobacteria and Bacillariophyta also decreased statistically significantly ($p < 0.05$) in the stages of karst speleogenesis: corridor-grotto, corridor-grotto-lake, corridor-grotto-chamber.

Keywords: cyanobacteria and algae, biodiversity, abiotic factors, the stages of karst speleogenesis, caves, Russia.

УДК 581.5

Влияние некоторых факторов на биоразнообразие цианобактерий и водорослей в карстовом спелеогенезе

© 2021. Ш. Р. Абдуллин, д. б. н., в. н. с.,
Федеральный Центр биоразнообразия наземной биоты
Восточной Азии ДВО РАН,
690022, Россия, г. Владивосток, Проспект 100-летия Владивостока, д. 159,
e-mail: crplant@mail.ru

Биоразнообразие подземных местообитаний представляет значительный интерес с различных точек зрения, но информация о биоте пещер является неполной и фрагментарной. В связи с этим важно выявление различных факторов, которые влияют на разнообразие видов различных организмов, включая цианобактерии и водоросли. Исследовано 166 различных проб, отобранных в 2007–2012 гг. из 14 карстовых пещер естественного происхождения, расположенных в различных климатических зонах России. В изученных пещерах идентифицирован 121 вид и внутривидовой таксон цианобактерий и водорослей, относящихся к пяти отделам: Cyanobacteria, Bacillariophyta, Ochrophyta, Chlorophyta и Streptophyta. На основе полученного материала с использованием статистических методов проанализировано влияние некоторых абиотических факторов (стадия карстового спелеогенеза, протяжённость пещеры, площадь входа, вмещающие породы и климат поверхности) на биоразнообразие цианобактерий и водорослей. Установлено, что наибольшее влияние на видовое богатство цианобактерий и водорослей в исследованных пещерах оказывает стадия спелеогенеза (сила влияния фактора – 118,419, $p < 0,05$), непосредственно связанная с уровнем обводнённости полостей, а также залегающие породы (сила влияния фактора – 34,665, $p < 0,05$) и протяжённость пещеры (сила влияния фактора – 20,288, $p < 0,05$). Выявлено, что связь между общим числом видов цианобактерий и водорослей и различными этапами спелеогенеза является статистически значимой ($p < 0,05$), и видовое богатство этих организмов снижается от коридорно-гротовой до коридорно-грото-камерной стадии. Число представителей отделов Cyanobacteria и Bacillariophyta также статистически значимо ($p < 0,05$) и снижается в ряду: коридорно-гротовая, коридорно-грото-озёрная, коридорно-грото-камерная стадии развития пещер.

Ключевые слова: цианобактерии и водоросли, биоразнообразие, абиотические факторы, стадии карстового спелеогенеза, пещеры, Россия.

Many different environmental factors influence the distribution of cyanobacteria and algae in various ecosystems [1, 2]. Caves are the natural subterranean spaces in the underground that are accessible to humans. They are the harbor of unique ecosystems with a peculiar biota including bacteria, fungi, animals, and plants [3]. Some cyanobacteria and algae can be mixotrophs [4] and can grow in dark areas of caves as well. The biodiversity of underground habitats is of considerable interest from taxonomic, evolutionary, ecological, biogeographic and conservation standpoints [3], but information on the biota of the caves is incomplete and fragmentary. In this regard, the identification of different factors (in particular stages of karst speleogenesis) that influence the richness of species of various organisms, including cyanobacteria and algae, is important.

There are various classifications of karst speleogenesis. We suppose, that the classification of [5] is the most convenient to study the differences in cyanobacteria and algae diversity at different stages of the speleogenesis, because it most fully shows changes in abiotic factors that can affect the distribution of these organisms. This classification recognizes following stages: 1. Interstitial stage. The extension of cracks occurs by corrosion and later by erosion. No deposits are formed. 2. Slit stage. In the phreatic conditions, cracks increase in the slits by corrosion, and then erosion. No deposits are formed yet. 3. Canal stage. Due to erosion and corrosion, the slits expand in the elliptical channels in the phreatic conditions. No deposits are formed. 4. Corridor-grotto stage. Underground streams are present in the cave and intensively expand the cave due to erosion and corrosion. Flowstones begin to form. 5. Corridor-grotto-lake stage. Cave consists of grottoes and connecting those corridors with separate flowing and non-flowing lakes. 6. Corridor-grotto-chamber stage. The corridors and grottos are split up on chambers due to landslide and water-chemogenic sediments. There are no permanent watercourses or water bodies [5].

During the evolution of karst caves, degree of their interconnection and exchange of energy and matter with the surface change significantly [6]. That affects the changes in caves ecosystems and biota. It has been known that a change in the stages of the speleogenesis leads to speleofauna transformation [3, 7]. Besides, the richness of animal species could also be influenced by the length of the cave [7] and the surface climate [3, 7]. However, data on the impact of these factors on the biodiversity of cyanobacteria and algae are still absent. That is why, the aim of our study was to analyze using statistical methods the influence of some abiotic factors, including various stages of speleogenesis, on the biodiversity of cyanobacteria and algae in karst caves.

Material and methods

In the Russian Federation, rich in caves [8] cyanobacteria and algae have been studied in over 50 caves [9]. However, for this study, only caves at clearly distinguishable stages of speleogenesis were selected. No caves at the interstitial, slit, and canal stages of speleogenesis were studied due to their inaccessibility. 14 caves of natural origin at the different stages of speleogenesis from various regions situated at varied climatic zones of Russia were studied (Table 1). Most caves overlying in limestone, gypsum, Komarinaya cave – in limestone and sandstone. There are different types of water, accordingly with karst speleogenesis stage in studied caves. The cave parameters are listed in Table 1.

From 2007 to 2012 166 different underground samples were collected. Sampling points were selected randomly along the entire length of each cave. Samples were collected by modified standard methods [11]. Identification of the species composition of cyanobacteria and algae was performed by direct light microscopy and after cultivation of the samples in modified liquid nutrient medium No. 6 [11, 12] in a culture chamber where they were illuminated for 12 hours per day with a light intensity of 2500–3000 lx (17.9–21.4 μmol photons/(m^2 · s)) and temperature +20 to +22 °C. All of the sample material was examined every two weeks during an eight-month cultivation period. All cyanobacteria and algae were identified using a LOMO "Mikmed-1" light microscope. A number of identification keys were consulted [12–20]. The taxonomy of the cyanobacteria and algae is presented according to [21]. The frequency of occurrence (F) of individual species was determined using formula:

$$F = a/A \cdot 100\%,$$

where a – the number of samples in which the species was revealed; A – the total number of samples [22].

To analyse the relationship between cyanobacteria and algae species richness and the potential variables (the stage of karst speleogenesis, the length of the cave, and the area of the entrance, the underlying rocks and the surface

Table 1

The parameters of investigated caves

Caves	I	II	III	IV	V	VI	VII
Golubinskiy Proval	1622	3	PS	I	G	A	1
Pevcheskaya Estrada	305	3	PS	I	G	A	1
Baskunchakskaya	1480	1	PL	II	G	B	2
Rossiyskaya	1800	2	PL	II	L	C	3
Ikskaya	50	1	WHG	III	G	C	4
Kueshta	800	2	PS	I	G	C	5
Hlebodarovskaya	3550	2	WHG	III	L	C	6
Komarinaya	475	2	WHG	III	LS	C	7
Alenushka	108	1	PL	II	L	C	8
Verchniy Ponor	62	1	PS	I	L	C	8
Shumyaschiy Ponor	117	1	PS	I	L	C	8
Essyumskaya	215	1	PS	I	L	C	8
Nizhnyaya Iogachskaya	40	2	WHG	III	L	D	9
Belyy Dvorec	120	3	PL	II	L	E	10

Notes: I – length, m. II – area of the entrance: 1 – < 1 m²; 2 – 1–10 m²; 3 – > 10 m². III – water: PS – permanent stream or streams; PL – permanent lake or lakes; WHG – occasional areas of water, humid ground. IV – speleogenesis stage [5]: I – corridor-grotto; II – corridor-grotto-lake; III – corridor-grotto-chamber. V – overlying rock: G – gypsum, L – limestone, LS – limestone and sandstone. VI – type of surface climate [10]: A – northern part of forest area of temperate climate zone; B – semi-desert area of temperate climate zone; C – temperate continental climate zone; D – sharply continental climate zone; E – temperate monsoon climate zone. VII – location:1 – Arkhangelsk Region, Pinezhsky district; 2 – Astrakhan region, Akhtubinsky district; 3 – Perm Territory, Gubakhinsky district; 4 – Republic of Bashkortostan, Tuymazinsky district; 5 – Republic of Bashkortostan, Iglinsky district; 6 – Republic of Bashkortostan, Meleuzovsky district; 7 – Chelyabinsk region, Ashinsky district; 8 – Chelyabinsk region, Katav-Ivanovsky district; 9 – Republic of Altai, Turochak district; 10 – Primorsky Territory, Partisan district.

Table 2

Biodiversity of cyanobacteria and algae in the studied karst caves at various stages of speleogenesis

Cave	Speleogenesis stage	Cyan	Bacill	Ochr	Chlor	Strept	Total
Essyumskaya	I	14	15	0	9	1	39
Verchniy Ponor		15	17	0	6	0	38
Golubinskiy Proval		14	12	0	10	1	37
Shumyaschiy Ponor		13	16	0	5	1	35
Pevcheskaya Estrada		11	7	0	11	2	31
Kueshta		6	16	0	6	2	30
Total		35	37	0	18	3	93
Baskunchakskaya	II	7	5	0	12	1	25
Belyy Dvorets		7	2	1	9	0	19
Rossiyskaya		6	5	0	6	0	17
Alenushka		5	2	0	8	1	16
Total		13	13	1	22	1	50
Ikskaya	III	3	7	0	5	1	16
Nizhnyaya Iogachskaya		6	3	0	6	0	15
Khlebodarovskaya		5	4	0	2	1	12
Komarinaya		1	1	0	9	0	11
Total		9	11	0	14	1	35

Note: The name of the stages of speleogenesis is the same as in Table 1. Cyan – Cyanobacteria, Bacill – Bacillariophyta, Chlor – Chlorophyta, Strept – Streptophyta.

climate), a dispersion analysis with covariates was applied [23]. To assess the effect of different stages of speleogenesis on the species richness of cyanobacteria and algae, the nonparametric one-way dispersion analysis using Kruskal-Wallis criteria was used, given the low sample size (n = 14 caves) [23]. The analysis was carried out using package Statistica version 10.0.

Results and Discussion

Based upon examination of 166 samples collected in 14 caves, 121 species and intraspecific taxa of cyanobacteria and algae, belonging to five phyla (Cyanobacteria – 40 species and intraspecific taxa, Bacillariophyta – 44 species and intraspecific taxa, Ochrophyta – 1 species, Chlorophyta – 33 species, Streptophyta – 3 species) were identified (Table 2).

The species *Mychonastes homosphaera* (Skuja) Kalina & Punc (F = 93%), *Nostoc punctiforme* Har. (F = 79%), *Leptolyngbya boryana* (Gom.) Anagn. & Kom. (F = 79%), *Leptolyngbya gracillima* (Zopf ex Hansg.) Anagn. & Kom. (F = 79%), *Hantzschia amphioxys* (Ehr.) Grun. in Cleve & Grun. (F = 79%), *Phormidium ambiguum* Gom. (F = 71%), *Muriella terrestris* J.B. Petersen (F = 64%), *Nostoc paludosum* Kütz. ex Bor. & Flah. (F = 57%), *Nitzschia palea* (Kütz.) W.Sm. (F = 57%), *Klebsormidium flaccidum* (Kütz.) P.C. Silva, K.R. Mattox & W.H. Black. (F = 57%), *Chlorococcum infusionum* (Schrank) Menegh. (F = 57%), *Chlorococcum minutum* R.C. Starr (F = 57%), *Stichococcus bacillaris* Näg. (F = 57%), *Sellaphora pupula* (Kütz.) Mereschk. (F = 50%), *Chlorella vulgaris* Beyer. [Beijer.] (F = 50%), *Muriella magna* F.E. Fritsch & R.P. John (F = 50%) were found in most of the caves. The same species appears to be typical for cave algal flora and were often revealed in other studied caves in Russia [9]. In addition, most of these taxa are diagnostic species of syntaxa of various levels belonging to the class of cave cyanobacteria and algae ***Mychonastetea homosphaerae*** [9, 24].

The largest number of cyanobacteria and algae species was found in the caves Essyumskaya, Verkhniy Ponor, Golubinskiy Proval, Shumyashchiy Ponor, Pevcheskaya Estrada and Kueshta (Table 2), which are at the corridor-grotto stage of speleogenesis. The bulck of the flora in caves at corridor-grotto stage was formed by species of Cyanobacteria and Bacillariophyta, and at this stage the largest number of species from the Streptophyta was revealed (Table 2). In the caves of the corridor-grotto-lake and corridor-grotto-chamber stages, species of green algae predominated (Table 2).

Dispersion analysis with covariates showed that the speleogenesis stage has the greatest ef-

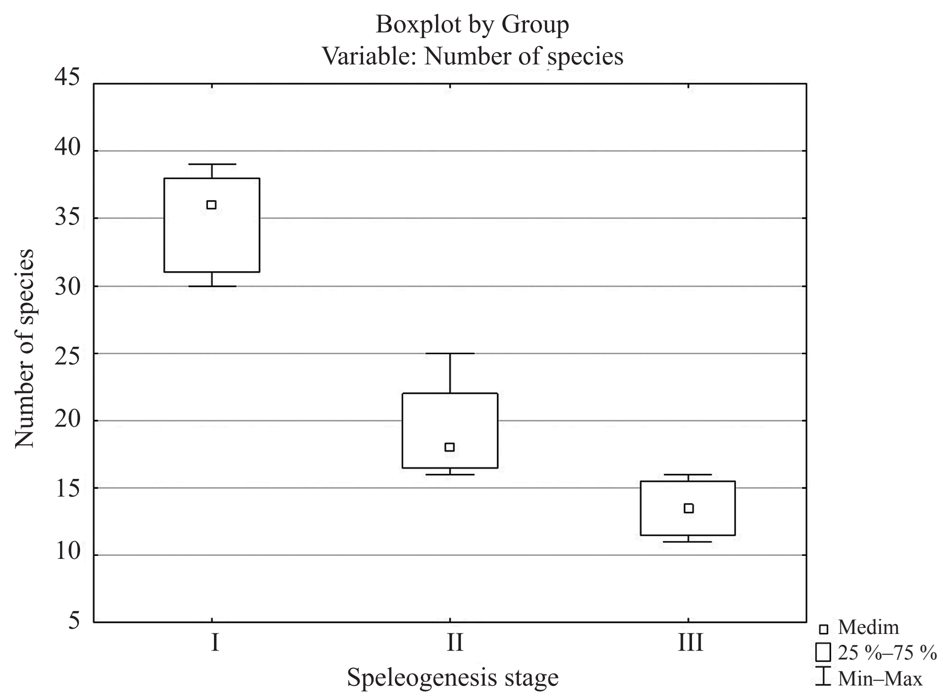

Fig. Influence of different stages of speleogenesis on the number of species of cyanobacteria and algae
Note: The stages of speleogenesis are denoted by numbers:
I – corridor-grotto stage; II – corridor-grotto-lake stage; III – corridor-grotto-chamber stage

fect on the species richness of cyanobacteria and algae in the investigated caves (the influence of the factor is 118.4, $p < 0.05$). Also the overlying rocks (the influence of the factor is 34.7, $p < 0.05$) and the length of the cave (the influence of the factor is 20.3, $p < 0.05$) effect on the biodiversity of cyanobacteria and algae in these caves. The entrance area and surface climate did not have a statistically significant effect on the biodiversity of cyanobacteria and algae ($p > 0.05$). It was also found that the relationship between the total number of species of cyanobacteria and algae and different stages of speleogenesis is valid ($p < 0.05$), and species richness of these organisms decreases from the corridor-grotto stage to the corridor-grotto-chamber stage (Fig.). The number of species of Cyanobacteria and Bacillariophyta also decreased statistically significantly ($p < 0.05$) in the stages of karst speleogenesis: corridor-grotto, corridor-grotto-lake, corridor-grotto-chamber.

It is known that running water plays an important role into the entering of cyanobacteria and algae in caves [3, 11]. That is why, the stage of speleogenesis, which directly relate to the level of watering of the cavities, has the greatest effect on the biodiversity of cyanobacteria and algae in the investigated caves in comparison with other factors. This factor also influenced the biodiversity of the fauna [3, 7]. The decrease in the total number of cyanobacterial and algal species at different stages of speleogenesis is apparently associated with the presence of streams in the corridor-grotto stage, reservoirs – in the corridor-grotto-lake stage, and their absence at the corridor-grotto-chamber stage. The overlying rocks may be responsible for the chemistry of the habitat for cyanobacteria and algae in caves. The length of the cave, as in the case of animals [7], can be directly related to the number and diversity of microhabitats. The absence of a statistically significant effect of the surface climate on the biodiversity of cyanobacteria and algae is apparently due to the fact that all the investigated caves are located in different types of the temperate climatic zone, and their underground microclimate is practically the same. It is not clear why the cave entrance area does not affect the species richness of cyanobacteria and algae, since most of these organisms are phototrophs and should depend on the level of illumination. Probably, this is due to the fact that cyanobacterial-algal cenoses with a relatively constant species composition, belonging to the alliance ***Stichococco minori–Klebsormidion flaccidi*** [24], are formed in the entrance illuminated zone of the caves [9, 24, 25]. While the entering of species into the dark zone and their accumulation there occurs constantly, a “bank of spores” is formed [9], due to which the biodiversity is even higher than in the entrance illuminated zone.

Conclusion

Thus, 121 species and intraspecific taxa of cyanobacteria and algae belonging to five phyla (Cyanobacteria – 40 species and intraspecific taxa, Bacillariophyta – 44 species and intraspecific taxa, Ochrophyta – 1 species, Chlorophyta – 33 species, Streptophyta – 3 species) were identified in 14 karst caves of natural origin, located in different climatic zones of Russia. The species *Mychonastes homosphaera*, *Nostoc punctiforme*, *Leptolyngbya boryana*, *L. gracillima*, *Hantzschia amphioxys*, *Phormidium ambiguum*, *Muriella terrestris*, *Nostoc paludosum*, *Nitzschia palea*, *Klebsormidium flaccidum*, *Chlorococcum infusionum*, *C. minutum*, *Stichococcus bacillaris*, *Sellaphora pupula*, *Chlorella vulgaris* and *Muriella magna* most often found in caves. Most of them are diagnostic species of syntaxa of various levels belonging to the class ***Mychonastetea homosphaerae*** [9]. The largest number of cyanobacteria and algae species was found in the caves, which are at the corridor-grotto stage of speleogenesis. At this stage species of Cyanobacteria and Bacillariophyta dominated, and the largest number of species from the Streptophyta was revealed. In the caves of the corridor-grotto-lake and corridor-grotto-chamber stages, species of green algae predominated. Statistical analyses showed that the speleogenesis stage, directly related to the level of water, has the greatest influence on the species richness of these organisms in the investigated caves. The overlying rocks and the length of the cave also effect on the biodiversity of cyanobacteria and algae in these caves. The surface climate and entrance area did not have a statistically significant influence on the biodiversity of cyanobacteria and algae. It was also revealed that the relationship between the total number of cyanobacteria and algae species and different stages of speleogenesis is valid, and species richness of these organisms, the number of species of Cyanobacteria and Bacillariophyta decreased statistically significantly in the stages of karst speleogenesis: corridor-grotto, corridor-grotto-lake, corridor-grotto-chamber.

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