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SUBAQUEOUS STALACTOIDS IN THE DAL'NEE VERKHNEE LAKE OF THE SHULGAN-TASH CAVE (SOUTHERN URALS)

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The relevance of the work is conditioned by the need for a comprehensive study of the mineralogical and microbiological parameters of the Shulgan-Tash (Kapova) cave, which is an archeological monument of universal importance.
The purpose of the work: to describe findings of subaquatic stalactites in the Shulgan-Tash cave and to solve mineralogical and genetic issues.
Research methodology: hydrochemical, electron microscopic and microbiological techniques of research were used in the present work.
Results. This article describes subaqueous stalactoids for the first time in Russia. In the foreign literature they are called "Pool Fingers". They are the carbonate forming with the origin, which is not yet definitely installed. They are emerging in the coastal zones of the cave waters. Subaqueous stalactoids were found in the Dal'nee Verkhnee lake on the second floor of the Shulgan-Tash cave 700 meters from the entrance. Subaqueous stalactoids are widespread along the Eastern and Western shores of the lake. They are thin and elongated aggregates of cream-colored up to a length of 30 cm, composed of calcite. They were formed in the lake of calcium bicarbonate type water with total mineralization of 390–510 mg/l, their hydrogen index is close to neutral (pH 6.8–7.3) and minor (not more than 5 mg/l) magnesium, sulfates and chlorides. Stalactitical surfaces are covered with the biofilm of the extracellular polymeric substances (EPS) and microorganisms. In their environment, the crystallization of calcite takes place. The "filaments", whose taxonomic affiliation is not clear are found inside the units in the flattened strands of biological origin. Calcite is a block crystal subindividuals, which possess the elements of splitting. Their size is about 200–300 μm. The manifestation of skeletal crystal growth happens rather often. Researches prove the participation of microorganisms in the origin of subaqueous stalactoids. Apparently, filamentous bacterial mats play the role of the primers and a "framework", which provides a gravity-oriented growth of the aggregates. Extracellular polymeric substance (EPS) has the ability to form complex compounds with ions of Ca^{2+}. It may indirectly lead to the precipitation of calcite.
Conclusions. The microbial mediated genesis, similar to that described in the literature of the North American counterparts, was substantiated for the subaqueous stalactoids (Pool Fingers).

Keywords: subaqueous stalactoids, Pool Fingers, secondary mineral formation, the carbonates, the calcite skeletal crystals, bacteria, Shulgan-Tash cave.

Introduction

The relevance and purpose of the work

The subject of our research is the microclimate and gas composition of the atmosphere [1, 2]. The subject consequently includes modern mineral formation, the study of rare mineral species [3], and the isotopic Geochemistry of elements in the minerals of caves. One of the objects of our research is the cave Shulgan-Tash in Bashkortostan. It is famous for its Paleolithic paintings. To preserve them, the monitoring and study of the microclimatic features of modern mineral formation and activities of microorganisms and microflora (bacteria, fungi, and algae) was carried out [4].

The present work describes rare mineral formations, the so-called *subaqueous stalactoid*s, for the first time in Russia. In the foreign terminology they are called "Pool Fingers" or stalactoids [1, 2, 5–8]. Similar formations belong to subaqual mineral deposits of caves [8–10]. The urgency of the work is conditioned by the need for a comprehensive study of mineralogical and microbiological parameters of the cave Shulgan-Tash (Kapova), which is an archeological site of universal importance.

The purpose of this work is the description of the findings of subaqueous stalactoids of the cave Shulgan-Tash and the solution of mineralogical and genetic issues.

Research methodology

Hydrochemical, electron-microscopic and microbiological methods of research were used in this work.

Hydrochemical monitoring of lake had been performed since 2010. The ionic composition of samples was determined by titrimetric method (estimated accuracy of ±5–10 %); the pH solution was determined using pH meter Sartorius PB (±0.03 pH). Calculations of saturation indices for calcite were performed in the program Aqion 4.15.5. The study of the morphology and the elemental composition was performed using electron scanning microscopy on the devices TESCAN Vega 3 SBH with an energy dispersive spectrometer X-ACT Oxford Instruments (Institute of problems of superplasticity of metals of RAS, Ufa, analysts I. I. Musabirov and S. N. Sergeyev) and JEOL JSM 6390LV with EMF-prefix INCA Energy 450 X-max 80 at the Institute of Geology and Geochemistry of the Russian Academy of Sciences, Ekaterinburg (analyst L. V. Leonova).

In the study of the number of microorganisms in the "Pool Fingers" for comparison the takyr clay was taken from the Diamond hall of the same cave. Selection and accounting of the number of microorganisms in the samples was carried out by means of of sowing suspension on solid nutrient media [4]. The number of heterotrophic bacteria on agar mesopatamia, oligotrophs on starvation agar, micromycetes in the environment of Chapek was studied. Incubation of crops was produced at a temperature of +28 °C and +10 °C for 7–14 days. For the convenience of microbiota analysis we used the coefficient of psychrotolerant, which is the ratio of the number of colonies of microorganisms grown at +6 °C to the number of colonies grown at +28 °C. This figure was determined at a significant difference in the number of colonies in samples incubated at specified temperatures [11].

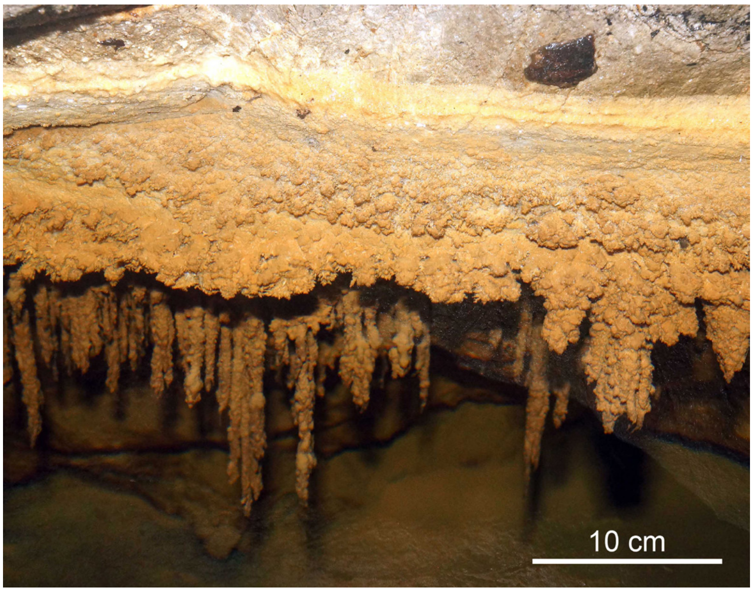

Figure 1. The subaqueous stalactoids "Pool Fingers" in the Dal'nee Verkhnee lake of the Shulgan-Tash cave.
Рисунок 1. Субаквальные сталактоиды «Pool Fngers» в Дальнем Верхнем озере пещеры Шульган-Таш.

Research results

Subaqueous stalactoids is a carbonate formation with definitely not of the installed origin. They are emerging in the coastal zones of the cave water bodies [12]. Their origin is associated with the calcification of the filamentous colonial forms of bacteria [6]. However, it was not possible to identify these organisms due to the fact that the researchers usually got inactive forms. These formations are considered as microbialite or "biothemes" which are the mineral deposits formed with the help of leading microorganisms.

Subaqueous stalactoids are typical for caves in the Guadalupe Mountains in the southern United States. They were first described in 1990 in a Lechuguilla cave [13]. Currently they occur in several caves in Germany, Austria, Switzerland, and Spain [8]. No literary data on the "Pool Fingers" in the caves of Russia are known to us. Perhaps subaqueous stalactite or "Pool Fingers", was discovered in 2006 for the first time in Russia. It was found in the Dal'nee Verkhnee lake cave called Shulgan-Tash in the southern Urals by the Director of the "Bashkortostan" movie company R. M. Isakov. It appeared in the underwater filming of the movie called "In search of Akbuzat". We study the finding of these formations in this work.

Dal'nee Verkhnee lake is located on the second floor of the cave at ≈ 700 m from the entrance and represents the slow groundwater reservoir area of about 90 m^2 with a maximum depth of 2 meters. Subaqueous stalactoids are widespread along the Eastern and Western shores of the lake. It is a thin elongated aggregate of cream-colored, length 5–15 cm; it can rarely reach the length of 30 cm (Fig. 1).

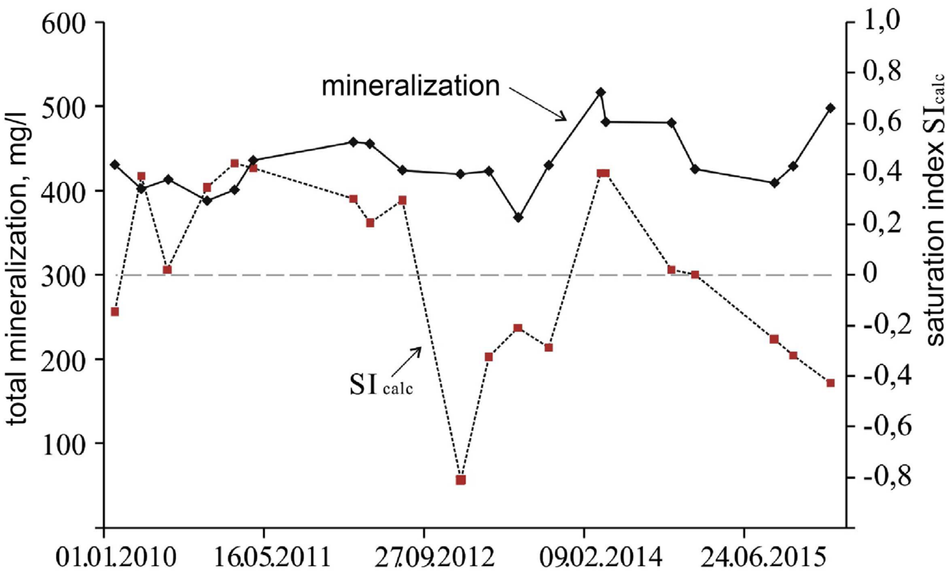

Figure 2. Changes in the total mineralization and the index of saturation for calcite according to the monitoring during the years 2010–2016.
Рисунок 2. Динамика общей минерализации и индекса насыщения к кальциту по данным мониторинга 2010–2016 гг.

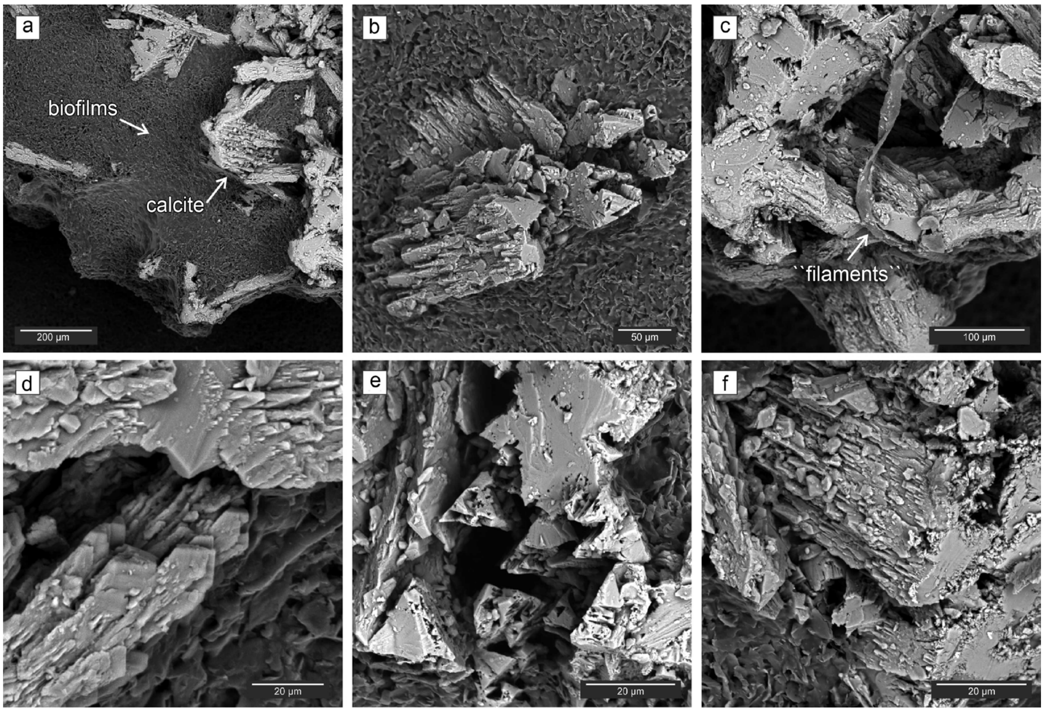

Figure 3. Electron micrographs of samples of subaquatic stalactoids.
Рисунок 3. Электронные микрофотографии образцов субаквальных сталактоидов.

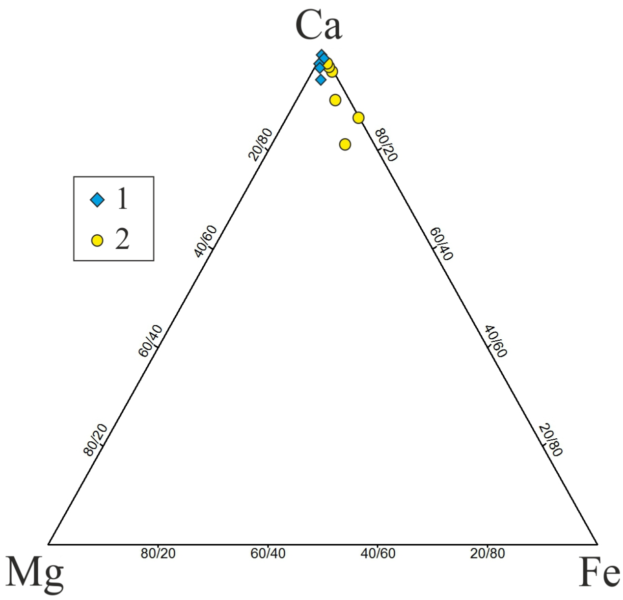

Figure 4. The ratio of Ca–Mg–Fe mineral formations in the Shulgan-Tash cave. 1 – the subaerial speleothems (sinter aggregates, coralloid, fibrous-acicular calcite; 2 – subaqueous stalactoids of the Dal'nee Verkhnee lake.
Рисунок 4. Соотношения Ca–Mg–Fe в минеральных образованиях пещеры Шульган-Таш: 1 – субаэральные спелеотемы (натечные агрегаты, кораллоиды, волокнисто-игольчатый кальцит; 2 – субаквальные сталактоиды Дальнего Верхнего озера.

The hydrochemical monitoring of the lake waters has been carried out since 2010. The Ionic composition of samples was determined by titrimetric method (estimated accuracy of ±5–10 %); the solution pH was determined by means of using pH meter Sartorius PB (±0.03 pH). Calculations of saturation indices for calcite were performed in the program Aqion 4.15.5. The study of the morphology and of the elemental composition was performed using electron scanning microscopy on the devices TESCAN Vega 3 SBH with an energy dispersive spectrometer X-ACT Oxford Instruments (Institute of problems of superplasticity of metals of RAS, Ufa, analysts I. I. Musabirov and S. N. Sergeyev) and a JEOL JSM 6390LV with EDS-attachment INCA Energy 450 X-max 80 at the Institute of Geology and Geochemistry of the Ural Department of the Russian Academy of Sciences, Ekaterinburg (analyst V. L. Leonova).

The clay takyr from the Diamond hall of the same cave was taken for comparison when studying the number of microorganisms in the “Pool Fingers”. Allocation and accounting of the number of microorganisms in the samples was carried out by cultur-

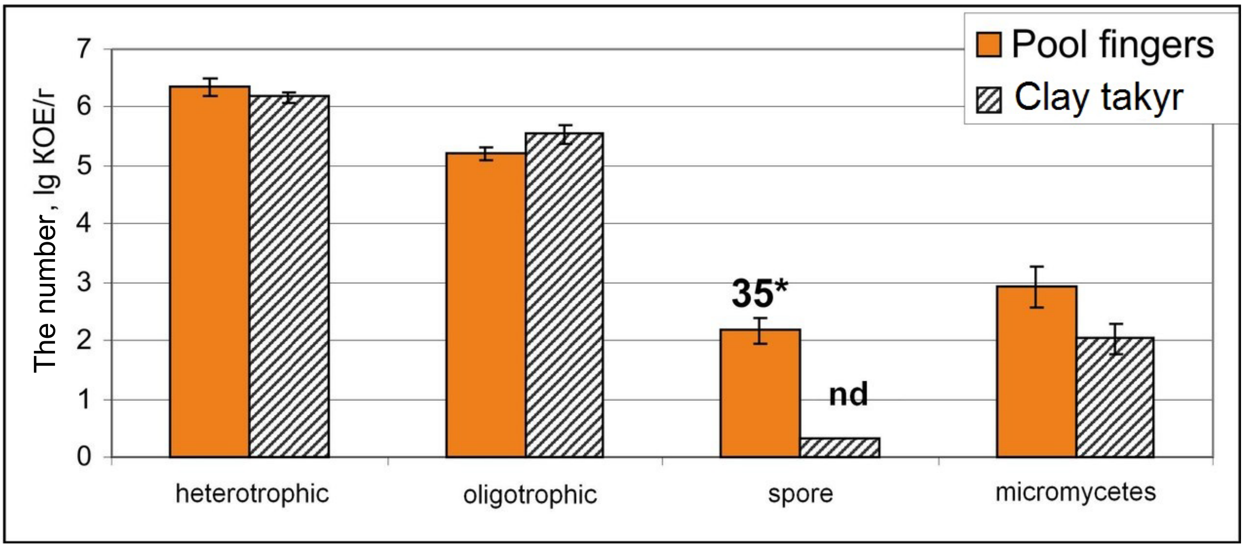

Figure 5. The number of bacteria and micromycetes in the subaqueous stalactoids called “Pool Fingers” and clay takyr from the audience Brilliantovaya (nd – no data; *ratio of psychrophiles, the data indicated are statistically significant at $P < 0.05$).
Рисунок 5. Численность бактерий и микромицетов в субаквальных сталактоидах «Pool Fingers» и глине такыра из зала Блиллиантовый (нд – нет данных, * – коэффициент психрофильности, статистически значимые данные обозначены при $P < 0.05$).

ing suspensions on solid nutrient medium [14]. The number of heterotrophic bacteria on the meat infusion agar, oligotrophs on starvation agar, and micromycetes in the environment of Čapek were studied as well. The incubation of crops was produced at a temperature of +28 °C and +10 °C for 7–14 days. To analyze the microbiota a coefficient of psychrotolerance, which is a ratio of the number of colonies of microorganisms grown at +6 °C by the number of colonies grown at 28 °C, was used. This figure was determined by the reliable difference in the number of colonies from samples incubated at the indicated temperatures [4].

The hydrochemical type of water in the Dal'nee Verkhnee lake is bicarbonate calcium, total mineralization of 390–510 mg/l, pH is close to neutral (pH 6.8–7.3), the content of magnesium, sulfates and chlorides is insignificant (< 5 mg/l). Graph of the changes in the total mineralization and saturation index for calcite is shown in Fig. 2. The saturation index for calcite is subject to considerable fluctuations and rarely reaches a critical value (SI = 0.3), when the crystallization of calcite is possible. When a new portions of water enriched in aggressive carbon dioxide (for example, in autumn floods in 2012), come to the lake the index of saturation decreases to negative values (see Fig. 2), and then with some delay an increase in salinity is seen due to the dissolution of calcite.

Previously, we found that the stalactoids of the Dal'nee Verkhnee lake are composed of calcite. Electron micrographs of stalactoids are shown in Fig. 3. Stalactoids surface is covered by the biofilm of extracellular polymeric substances (extracellular polymeric substances EPS) and microorganisms. The crystallization of calcite takes place in their environment (see Fig. 3, *a*, *b*). Inside the units, there occur the flattened strands of biological origin called the “filaments”, their taxonomic affiliation is not clear (see Fig. 3, *b*). Calcite is a block crystal subindividuals of the size 200–300 μm. They are characterized by some elements of splitting. It often happens that the manifestation of the skeletal structure of crystals takes place (see Fig. 3, *d–f*).

According to the energy dispersive spectroscopy (Inca Energy 450) calcite occurs in the admixture of iron, potassium, magnesium; sometimes there are titanium and manganese. The ratio of calcium and its typical impurities (calculated for atomic %) of magnesium and iron in the samples of stalactoids ($n = 6$) and normal subaerial speleothem the cave Shulgan-Tash ($n = 16$) is shown in Fig. 4. It is clear that stalactoids are significantly enriched in iron in comparison with conventional speleothem, which are characterized by significantly more pure chemical composition. It is close to stoichiometric composition. The split and skeletal growth is apparently linked with the presence of the structural impurities in the calcite.

The Fig. 5 shows the data of microbiological studies of mineral formation “Pool Fingers” and clay takyr. The analysis showed a high number of microorganisms in both samples, where the number of bacteria was as follows: heterotrophic 1.5–2.2 × 10^6 CFU/g, oligotrophic 1.6–3.5 × 10^5 CFU/g, spore 135 CFU/g and micromycetes 100–800 CFU/g. Meanwhile high abundance of psychrotolerant spore bacteria was observed in the mineral formation of stalactoids “Pool Fingers” (index 35). It should be noted that in the samples of the “Pool Fingers” the number of micromycetes was 8 times more than in the soil of the cave (in the takyr clay). Thus, microbiological studies have shown that “Pool Fingers” biofilms contained a high number of bacteria, such as takyr clay, and the number of micromycetes even surpassed it.

We believe that the accumulation of metals in calcite (which is not typical for the investigated conventional calcite deposits in caves) is connected, with the microbial activity, since microorganisms are able to locally change the redox potential of the medium and the conditions of migration of metals. The accumulation of metals may also be due to the adsorption of colloids on a bacterial slime. Thus, the participation of the microorganisms in the origin of subaqueous stalactoids is confirmed by the presence of biogenic components in the structure of aggregates. I. e., in the EPS biofilm where the crystallization of calcite and microbial filaments take place. Apparently, filamentous bacterial mats play the role of primers and a “framework”, which provides a gravity-oriented growth of the aggregates. Extracellular polymeric substance (EPS), have the ability to form complex compounds with ions of Ca^{2+}. This substance may indirectly lead to the precipitation of calcite [3]. We can assume that it is due to the buffering role of EPS that the calcite crystals do not dissolve during the periods of the appearance of aggressive carbonic acid in the water of the lake. This issue is open to discussion.

Conclusion

As follows from the above, of subaquatic stalactoids (Pool Fingers) of the Shulgan-Tash cave justified the microbial-mediated genesis, similar to that described in the literature of the North American counterparts.

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Субаквальные сталактоиды в Дальнем Верхнем озере пещеры Шульган-Таш (Южный Урал)

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Актуальность работы обусловлена необходимостью комплексного изучения минералогических и микробиологических параметров пещеры Шульган-Таш (Каповой), представляющей собой археологический памятник мирового значения.
Цель работы: описание находки субаквальных сталактоидов из пещеры Шульган-Таш и решение минералого-генетических вопросов.
Методология исследования: в работе применялись гидрохимические, электронно-микроскопические и микробиологические методы исследований.
Результаты. В статье впервые для России описаны субаквальные сталактоиды (в зарубежной литературе называемые *«Pool Fingers» – бассейновые пальцы*) – карбонатные образования пещер с однозначно не установленным происхождением, формирующиеся в прибрежных зонах пещерных водоемов. Субаквальные сталактоиды найдены в Дальнем Верхнем озере, на втором этаже пещеры Шульган-Таш, в 700 м от входа. Субаквальные сталактоиды распространены вдоль восточного и западного берегов озера. Это тонкие вытянутые агрегаты кремового цвета длиной до 30 см, сложенные кальцитом. Образовались они в озере с гидрокарбонатно-кальциевым типом воды, общей минерализацией 390–510 мг/л, водородным показателем, близким к нейтральному (pH 6,8–7,3) и с незначительным (не более 5 мг/л) содержанием магния, сульфатов и хлоридов. Поверхность сталактоидов покрывает биопленка внеклеточных полимерных веществ (*extracellular polymeric substances – EPS*) и микроорганизмов, в среде которой происходит кристаллизация кальцита. Внутри агрегатов встречаются уплощенные нити биологического происхождения «филаменты», таксономическая принадлежность которых неясна. Кальцит представляет собой блочные, с элементами расщепления кристаллические субиндивиды размером 200–300 μm. Нередко наблюдается проявление скелетного роста кристаллов. Исследованиями подтверждается участие микроорганизмов в генезисе субаквальных сталактитоидов. По всей видимости, нитчатые бактериальные маты играют роль затравок и «каркаса», обеспечивающего гравитационно-ориентированный рост агрегатов. Внеклеточное полимерное вещество (EPS), обладающее способностью образовывать комплексные соединения с ионами Ca2+, может опосредованно приводить к осаждению кальцита.
Выводы: для субаквальных сталактоидов (Pool Fingers) обоснован микробно-опосредованный генезис, схожий с описанными в литературе североамериканскими аналогами.

Ключевые слова: субаквальные сталактоиды; Pool Fingers; вторичные минеральные образования; карбонаты; кальцит; скелетные кристаллы; бактерии; пещера Шульган-Таш.

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