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First record of *Bacillicladium lobatum* (Chaetothyriales) in Caucasus (second finding in the world)

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Keywords: *Bacillicladium*, cryoconits, Chaetothyriales, microfungi, mountains of Caucasus.

Summary. *Bacillicladium lobatum* was isolated from cryoconits at the mountains of the Caucasus, and it is represented by strain CZ-25. Macro- and micromorphology of the isolate were examined along with partial sequences of internal transcribed spacer of rDNA (ITS1-5.8S-ITS2). The isolate *Bacillicladium lobatum* CZ-25 had a number of features that distinguished it from the strain *Bacillicladium lobatum* from speleothem biofilm covering bare granite walls in the KungstraÈdgården metro station in Stockholm. Strain from Caucasus had conidia larger and hyphae wide, and the maximum growth temperature was higher. As a result of our study, we first discovered the microfungi *Bacillicladium lobatum* at the Caucasus. Our study shows that *Bacillicladium lobatum* is a species found at towns of Northern Europe and at the mountains of Caucasus.

Первая находка *Bacillicladium lobatum* (Chaetothyriales) на Кавказе (вторая находка в мире)

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Ключевые слова: горы Кавказа, криоконiты, микроскопические грибы, *Bacillicladium*, Chaetothyriales.

Аннотация. *Bacillicladium lobatum* выделен из криоконитов гор Кавказа и представлен штаммом CZ-25. Исследовали макро- и микроморфологию изолята, а также частичные последовательности внутреннего транскрибируемого спейсерного участка рДНК (ITS1-5.8S-ITS2). Изолят *Bacillicladium lobatum* CZ-25 имел ряд особенностей, которые отличали его от штамма *Bacillicladium lobatum* из биопленки образований, покрывающих голые гранитные стены на станции метро KungstraÈdgården в Стокгольме. Штамм с Кавказа имел более крупные конидии и широкие гифы, его максимальная температура роста была выше. В результате наших исследований мы впервые обнаружили микрогрибы *Bacillicladium lobatum* в горах Кавказа. Наше исследование показывает, что *Bacillicladium lobatum* является видом, встречающимся в городах Северной Европы и в горах Кавказа.

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Introduction

Currently, there is melting and retreat of glaciers in all mountain systems and high latitudes of the Arctic and Antarctic and alpine system (Belozerov et al., 2020; Scher et al., 2020). Formation of biological soil crusts began of organisms from cryoconits (Takeuchi et al., 2018). However, there is very little evidence addressing whether these organisms are functioning on debris-covered glaciers, or whether they are just inactive (dormant) atmospheric transients that are easily detectable in a low biomass environment (Darcy, Schmidt, 2016).

Cryoconits are granular/spherical mineral particles connected with archaea, cyanobacteria, heterotrophic bacteria, algae, fungi, and micro animals (Zawierucha et al., 2017). Microorganisms and in particular fungi are the first life forms to colonize freshly exposed substrates and play a tremendous role in soil formation (Sigler et al., 2002; Sigler, Zeyer, 2002, 2004; Nicol et al., 2005; Bardgett et al., 2007; Nemergut et al., 2007; Schmidt et al., 2008, 2012; Ciccazzo et al., 2016).

Cryoconite is a matrix of mineral particles and biological material deposited on glaciers by wind and meltwater, most likely of local origin (Porazinska et al., 2004). Large granules and thick accumulations of cryoconite material enable formation of anoxic zones (Poniecka et al., 2018). They also provide protection from extreme fluctuations in air temperature and partial UV screening, either by ice lidding or by the formation of granules (Hodson et al., 2008; Bagshaw et al., 2016).

The formation of communities of microorganisms (including fungi) in the cryoconites of glaciers can occur due to the environment (primary mountain soils and stone substrates).

In the cryoconites of the Caucasus the species *Bacillicladium lobatum* Hubka, Reblova et Thureborn was isolated. The genus *Bacillicladium* was described in 2016 by Hubka, Reblova and Thureborn with the type species *Bacillicladium lobatum*. Cultures of this species was isolated from speleothem biofilm covering bare granite walls in the KungstraÈdgården metro station in Stockholm. *Bacillicladium* is distantly related to the known five chaetothyrialean families and is unique in the Chaetothyriales by variable morphology showing hyphal, meristematic and yeast-like growth *in vitro* (Reblova et al., 2016). Chaetothyrialean fungi, one of the black yeast orders, inhabiting plant or stone surfaces under stressful and extreme conditions are usually characterized by compact colonies, slow growth, heavily melanised mycelium and predominantly asexual reproduction (Sterflinger et al., 1995; Selbmann, et al., 2005; Gueidan et al., 2008; Ruibal et al., 2008).

In 2019, the new species *Bacillicladium clematidis* Crous et R. K. Schumach. was described (CBS:H-23828 culture from holotype of *Bacillicladium clematidis*: CPC:33882, CBS:145035 MycoBank MB829299). Name reflects the host genus *Clematis* from which it was isolated. Typus: "Austria, Gaaden, branch of *Clematis* vitalbae (Ranunculaceae), 21 IV 2017, M. Mann et R. K. Schumacher, HPC 2101, RKS 102" (holotype CBS H-23828, culture ex-type CPC 33882 = CBS 145035) (Crous et al., 2019). To the date, according to the database Index Fungorum, the genus *Bacillicladium* had 2 species (Index Fungorum. URL: http://www.indexfungorum.org).

Materials and methods

Samples were taken in August 2019 from Glacier Shkheld, Gorge Adyl-Su (N43°11', E042°38'), the Kabardino-Balkarian Republic. It is part of the Russian Federation, North Caucasian Federal District and the North Caucasian Economic Region. The method of serial dilutions with spread on agar plates was used to isolate fungi cultures (Goldman, Green, 2015). Single spore isolation was used to obtain pure culture. Inoculations were prepared from spore suspensions made in a 0.2 % agar and 0.05 % Tween 80 solution and agar plates were inoculated as described by Samson et al. (2014). The strain was designated as CZ-25.

The pure culture was grown on Czapek agar medium without antibiotics at 20 °C for 15 days for molecular analysis. DNA was extracted by using a Diamond DNA Plant kit (ABT, Russia, Barnaul) according to the manufacturer's instructions. Internal transcribed spacer of rDNA (ITS1-5.8S-ITS2) (White et al., 1990) were used as a phylogenetic marker. Internal transcribed spacer of rDNA (ITS1-5.8S-ITS2) was amplified using the PCR-primers ITS1 (5′-TCC-GTA-GGT-GAA-CCT-TGC-GG-3′) and ITS4 (5′-TCC-TCC-GCT-TAT-TGA-TAT-GC-3′). At the end of amplification, the samples were detected by agarose gel electrophoretic method; sequencing of the obtained DNA fragments was carried out in the commercial organization BioBeagle (St. Petersburg) using the Sanger method. Sequences were proofread and edited using BioEdit version 7.1.9. Newly generated sequences were

compared to the available sequences in the GenBank database (NCBI) by using BLAST instrument (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The obtained sequences were entered into the genebank under the number ON239759. Label number LE F-349784 in HERBARIUM MYCOLOGICUM Instituti botanici nomine V. L. Komarovii Academia Scientiarum Rossicae.

The isolate was cultivated on Czapek agar (Raper, Thom, 1949), malt extract agar (MEA) (Samson et al., 2010), Sabouraud Agar (SDA) (Acharya, Hare, 2022), potato dextrose agar (PCA) for follow-up morphological observations. The isolate was inoculated on 90 mm Petri dishes and incubated for 30 days at 2, 7, 14, 20, 25, 30 and 35 °C. Color determination was performed according to the ISCC-NBS Centroid Color Charts (Kelly, 1964), according to the recommendations of Nováková et al. (2012). For micro-morphological examination, microscopy by Carl Zeiss AxioImager A1 was used. Statistical processing (medium size) was performed using the statistical software package MS Excel 2007.

Results and discussion

As a result of studies of mycobiota cryoconites of the Caucasus mountains, we isolated strain CZ-25. BLAST analysis of the ITS region showed 100 % similarity of the isolate CZ-25 (ON239759) and *Bacillicladium lobatum* CBS:141179, CCF5199. The species *Bacillicladium lobatum* is the type species. The observed micro- and macromorphology of isolate CZ-25 also corresponded to the micro- and macromorphology of *Bacillicladium lobatum* (Reblova et al., 2016). Therefore, molecular and morphological data led to the conclusion that the obtained isolate CZ-25 belongs to the species *Bacillicladium lobatum*.

Macromorphology. Colonies on Czapek agar at 25 °C in 30 d attaining 20–38 mm diam Dark Grayish Olive (No. 111), velvety to crusty, raised, cerebriform, margin lobed, flat with a broad zone of submerged growth, reverse black.

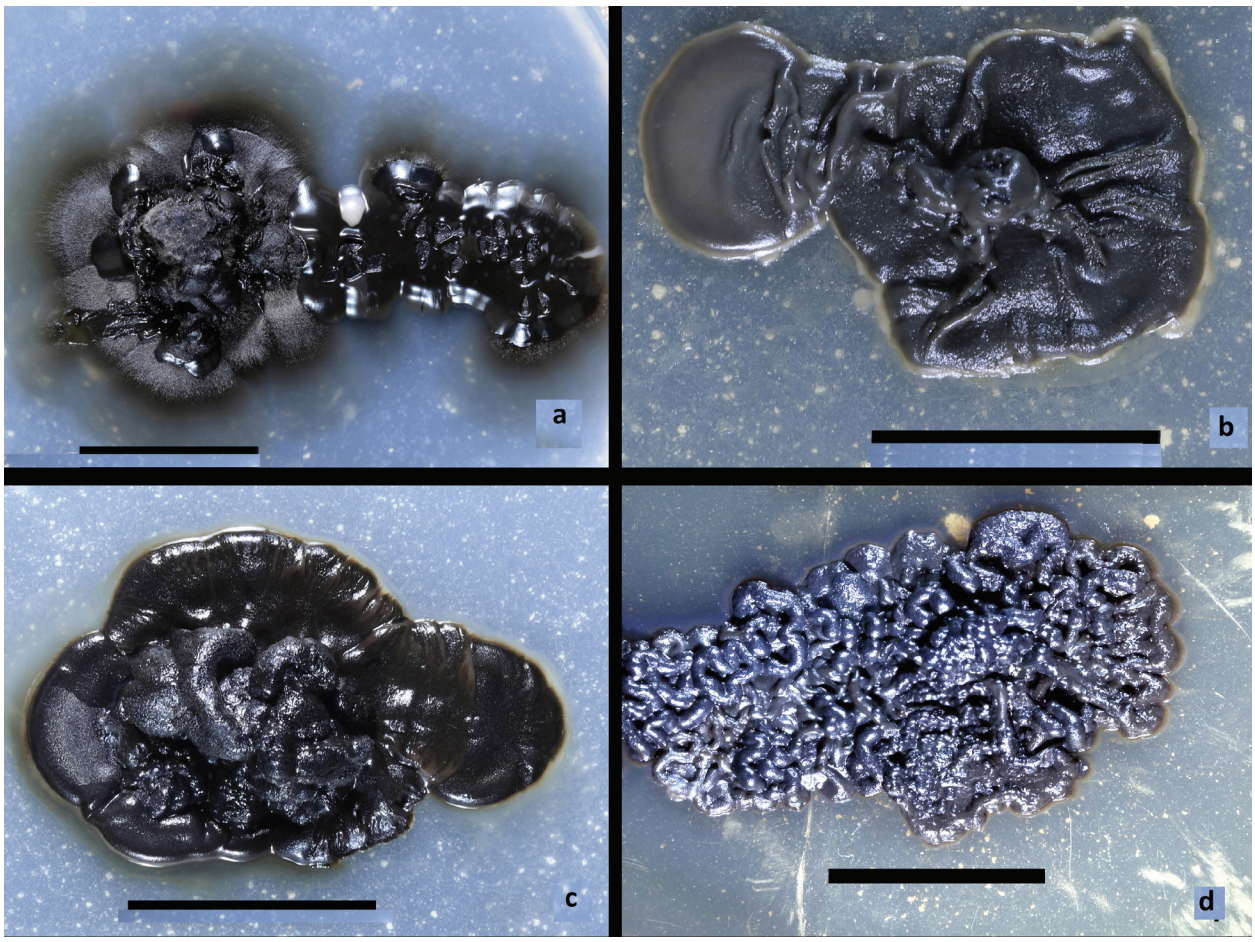

Fig. 1. Macromorphology of *Bacillicladium lobatum*. Phenotypic variability of 40-day-old colonies. Morphogenesis of colony growing on Czapek agar (a); MEA (b) PCA(c) and SDA (d) at 25 °C. Bar = 1 cm (a–d).

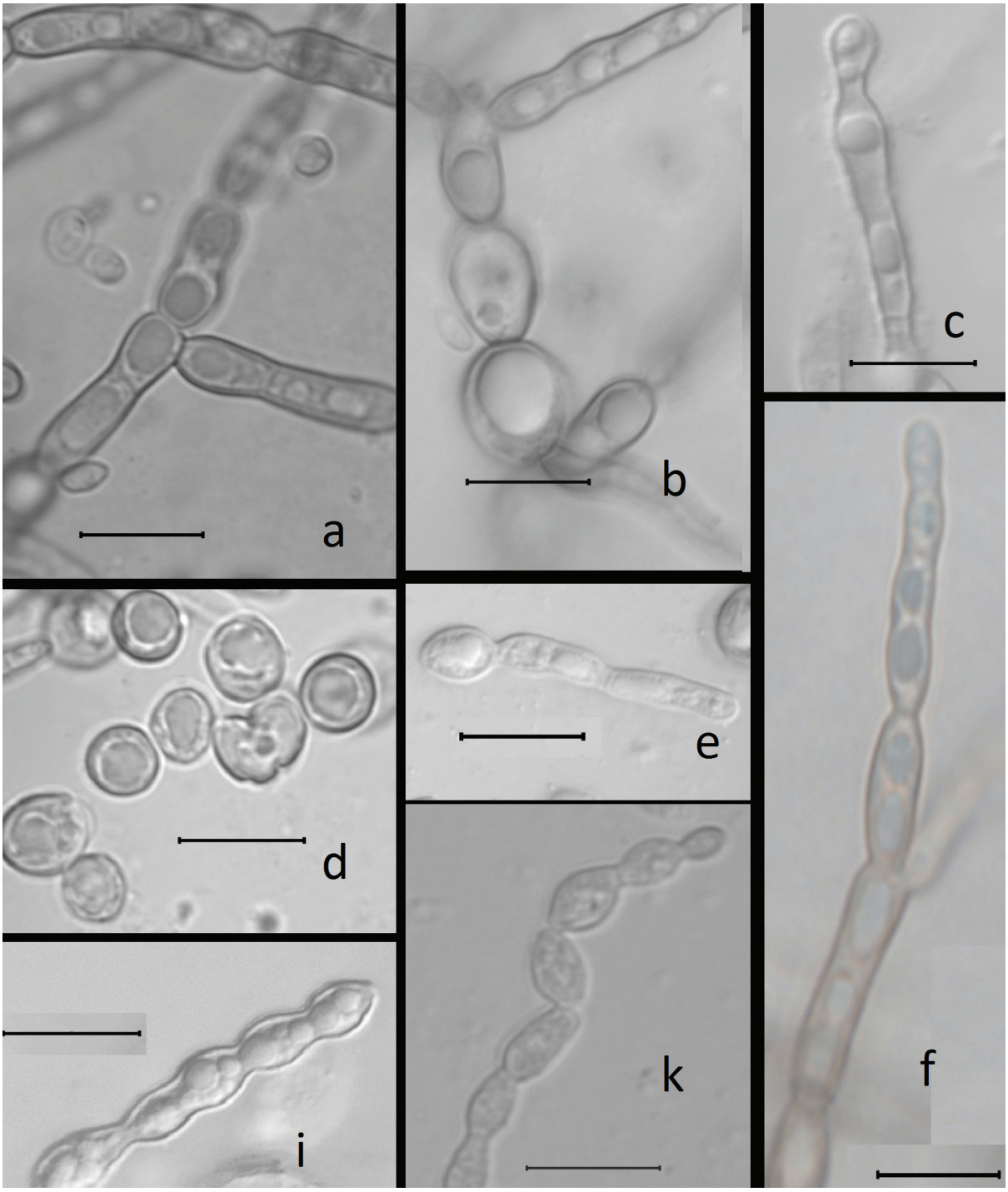

Fig. 2. Micromorphology of *Bacillicladium lobatum*. Yeast-like state (d), budding (c), germinating by hyphae (e) or forming short chains (I, k); Uni- or multicellular bodies, single or in chains (a, b, f). Bar = 10 µm (a–k).

Colonies slow-growing, on MEA at 25 °C in 30 d attaining 18–32 mm diam Grayish Olive (ISCC-NBS Colour No. 109) to Dark Grayish Olive (No. 111), velvety, raised, cerebriform, margin lobate, reverse black.

Colonies slow-growing, on PCA at 25 °C in 30 d attaining 25–40 mm diam Grayish Olive (ISCC-NBS Colour No. 109) to Dark Grayish Olive (No. 111), velvety, cerebriform, margin lobate, reverse black.

Colonies on SDA at 25 °C in 30 d attaining 30–43 mm diam (7–10 mm in 14 d), Olive Black (No. 114) in the center, Moderate Olive (No. 107) in the marginal part, moist, covered by sparse aerial mycelium, flat with a broad zone of submerged growth, margin entire, reverse black.

Colonies at 20 °C showed similar growth parameters and slight morphological differences. Colonies at 30 °C showed the highest growth rate and slight

morphological differences. Colonies at 2 and 35 °C in 30 d no growth were observed on all media. Colonies at 7 and 14 °C in 30 d attained 5–6 and 16–19 mm diam. Colonies on MEA and PCA were less folded, colonies on PCA had a moister appearance with less abundant aerial mycelium on the surface. Colonies on SDA were more raised, cerebriform.

Thus, the colonies correspond to the description (Reblova et al., 2016), however, the optimum growth temperature is slightly increased (growth was noted at 30 °C). The presence of melanin in cells improves their resistance to UV irradiation and survival in extreme, nutritionally poor environments.

The temperature range of growth of this isolate (lack of growth at low temperatures) apparently indicates its development on rocky surfaces in the mountains, and not in glacier cryoconites. Macromorphology is shown in Fig. 1.

Micromorphology. Mycelium from hyaline to olive-brown, predominantly smooth. Hyphae are 2.0–6.0 μm wide. Conidiophores are mainly reduced to a short chain of ramoconidia on a scar, simple, poorly developed, up to 200 μm long, up to 4 μm broad. Conidia are brown to dark brown, disposed in long, simple or branchy chains, smooth- and thick-walled, mostly broadly lens-shaped, ovoid-, cylindrical-, lemon-shaped 4–6 × 4–10 μm. Micromorphology is shown in Fig. 2.

The dimensional characteristics of the isolated strain CZ-25, in general, correspond to the description of Reblova et al. (2016). The exceptions are sizes of conidia and wide of hyphae.

Thus, *Bacillicladium lobatum* has a wider range, this species found in both the northern parts of Europe (Sweden) and the mountains of Caucasus.

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