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Cyanophytes on limestone rocks in the Szopczański Gorge (Pieniny Mountains) – their ecomorphology and ultrastructure

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Abstract

This study is devoted to the ecomorphology and ultrastructure of cyanophytes on limestone rocks collected in the Szopczański Gorge (Pieniny Mountains) during the years 2006-2008. There were selected cyanophyte species for examination such as following: *Nostoc microscopicum*, *Phormidium favosum*, *Leptolyngbya foveolarum*, *Tolypothrix distorta* var. *penicillatum*, *Pseudanabaena catenata*. The ultrastructural analysis (TEM) confirmed that the structure and placement of the thylakoids is genus/species specific.

Keywords: Cyanophyta, taxonomy, ultrastructure, limestone rocks, Pieniny Mountains

Introduction

The Szopczański Gorge of the Pieniny Mountains area is an interesting and unique environment. In limestone habitats, some cyanophytes and algae were typically much more abundant than others. Very few detailed studies of limestone epilithic cyanophytes have been carried out in the Gorges of the Pieniny Mountains with the exception of observations made in the last 50 years in many habitats of the Pieniny Mountains that indicate a rich and diversified epilithic phycoflora [1-3]. The mostly published data are from the Dunajec River and other water biotopes in the Pieniny Mountains by Chudyba [4], Kawecka [5], Wasylik [6], Tarnowska [7], Starmach [1], Mrozińska-Webb [8], Mrozińska [9-12], Kawecka and Szczęsny [13], Kawecka and Mrozińska [14], Sanecki [15], Sanecki and Bucka [16], Sanecki et al. [17], Kawecka and Sanecki [18], Mrozińska and Czerwik-Marcinkowska [19], Mrozińska et al. [20,21], Czerwik-Marcinkowska and Mrozińska [22], Wróbel and Zarzycki [23], Wilk-Woźniak et al. [24].

Study area

The Pieniny Mountains consist mainly of limestone and dolomite rock strata with the most picturesque Three Crowns (alt. 982 m) and Sokolica (alt. 747 m) mountains covered by fir, beech, spruce and pine forests. Steep cliffs surrounded by rocky crags rise up to 300 m above the Dunajec River. The most famous gorges of the Pieniny Mountains are the Dunajec River Gorge in Pieniny National Park and Homole Gorge. In addition, there are many swamps, springs and torrents in the Pieniny range which are a very characteristic and important elements of this landscape.

The algological study presented here focused on cyanophytes dwelling on the limestones in the area of Szopczański Gorge (Fig. 1). This Gorge is situated between the western slopes of Three Crowns and the eastern slopes of Podskalnia Mountain in the Pieniny National Park.

Material and methods

The fieldwork was carried out in the years 2006-2008. The sampling procedure was as follows. Generally, for each observation, a method of visual inspection was used by which detectable places hosting cyanophytes in the form of black, brown, grey-green growths/crusts were scraped using a scalpel. This material was collected into small plastic bags and used directly for observation under a light microscope (JENAMED 2). Parts of each sample were used for a cultivation on agar plates in the laboratory. Cultures of cyanophytes were incubated in the standard Bristol agar medium [25] at a temperature of 20° C and in a diurnal regime (12/12 h light/dark cycle, 3000 $\mu Em^{-2}s^{-1}$, 40 W cool fluorescent tubes). The cultivations were examined every 12 days for an isolation of some cyanophyte species to obtain pure cultures [26]. For transmission electron microscopy (TEM) cells were fixed as previously described [27]. Ultra-thin sections were prepared in a way described by Reynolds [28]. TEM was made by a TESLA BS 500 electron microscope. The general taxonomic literature and specific publications, which were used for identification of cyanophyte species, were following: Starmach [29], Anagnostidis and Komárek [30], Komárek and Anagnostidis [31], Whitton [32].

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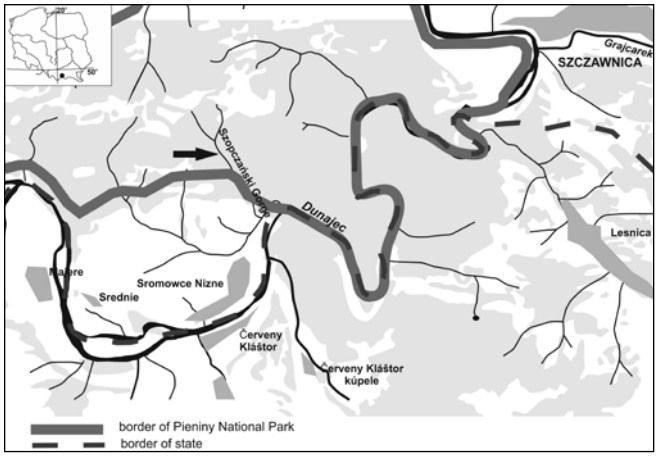

Fig. 1 Situation of Szopczański Gorge within the Pieniny Mountains.

Results and discussion

The limestone rocks in the Szopczański Gorge were usually covered by cyanophytes. These were visually detectable as dark spots on light limestone. Two ecologically different habitats (ecotopes) were recognized, which were clearly distinguished by species composition. The cyanophytes: *Nostoc microscopicum* and *Leptolyngbya foveolarum* grew on the wet rocks, *Tolypothrix distorta* var. *penicillata*, *Phormidium favosum* and *Pseudanabaena catenata* grew on rocky cliffs periodically desiccated. These selected species were analyzed in detail. The resulting description of taxa is given below:

ORDER: NOSTOCALES. (***i***) *Nostoc microscopicum* Carmichael ex Bornet & Flahault (Fig. 2, Fig. 5). LM: colony usually ellipsoidal 50-350 µm, initially blue-green, later olive-green; sheaths clearly yellow or brownish-yellow. Filaments flexuous, cells subspherical 5-7 µm wide. Akinetes ellipsoidal 6-7 × 9-12 µm. Heterocytes subspherical 6.5 µm diameter; wall smooth. TEM: detail of the thylakoids, showing rows of hemidiscoidal phycobilisomes in cross-section. (***ii***) *Tolypothrix distorta* var. *penicillata* (Thuret) Lemmerman (Fig. 3, Fig. 5). LM: cyanophytes (woolly mats) forming tufts, dark blue-green, filaments 8-14 µm wide, trichomes 6.5-10 µm wide, cells 3.5-4.5 µm long. Filaments short, erect and curved, show irregular false branching. Heterocytes basal with a single pore, hemispherical, usually single, located apically, rarely interpolated. False branches forming where heterocytes are rare or absent. Some filaments have cells which are much wider than long, some with external lamellate sheaths. TEM: carboxysomes mainly located in the central zone or near the cell walls, also vacuole-like inclusions of larger dimensions present. Phycocyanin-bodies occur near to the cell membrane.

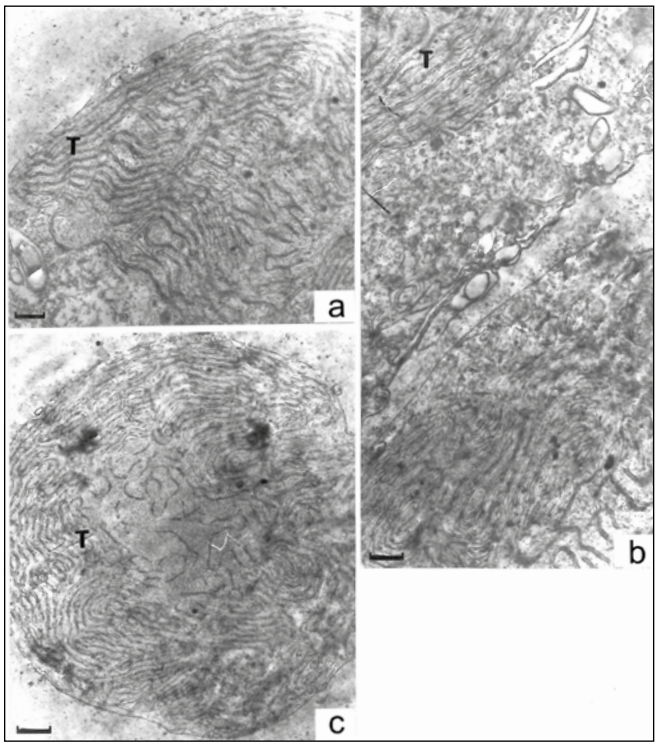

Fig. 2 **a-c** Transmission electron microscopy (TEM) – the transverse sections of *Nostoc microscopicum* Carmichael ex Bornet & Flahault showing an arrangement of thylakoids. T – thylakoids. Scale bar – 1 µm.

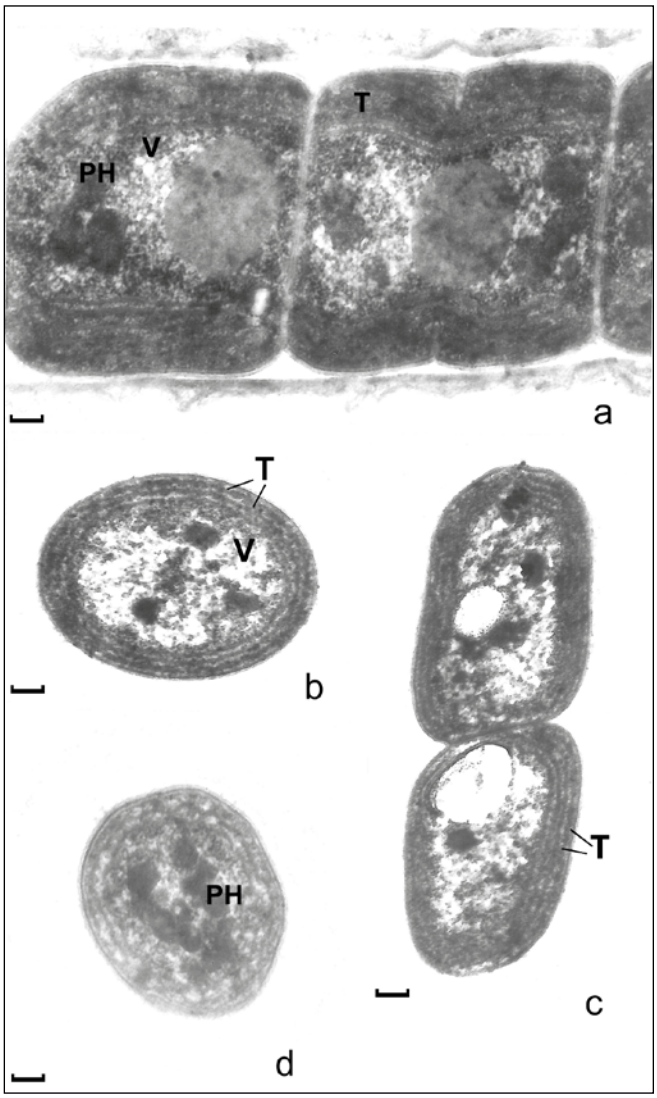

Fig. 3 **a** Longitudinal ultra-thin section of *Tolypothrix distorta* var. *penicilatum* (Thuret) Lemmerman, showing the peripheral arrangement of thylakoids and cell inclusions: polyhedral bodies or carboxysomes and vacuoles. **b-d** *Pseudanabaea catenata* Lauterborn. **b** Longitudinal and transverse section showing a number and an arrangement of thylakoids and cell inclusions: vacuoles, polyhedral bodies or carboxysomes. **c** Two vegetative cells. **d** Transverse section of the vegetative cell with thin wall. PH – polyhedral bodies; T – thylakoids; V – vacuoles. Scale bar – 1 µm.

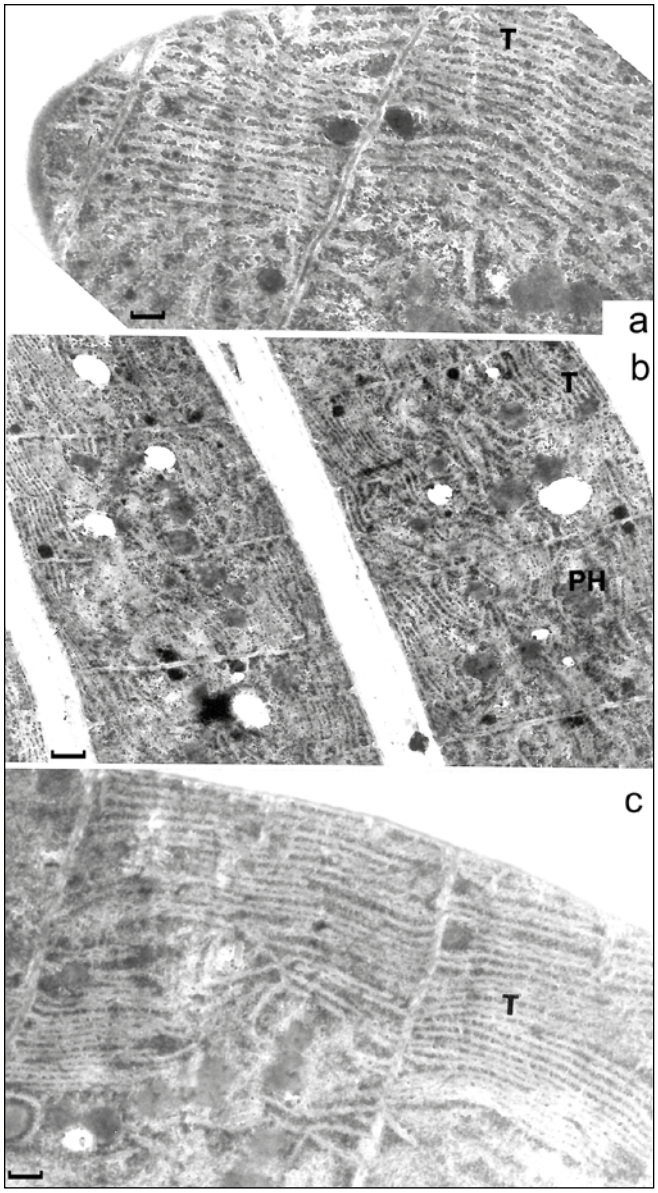

Fig. 4 Longitudinal ultra-thin sections of *Phormidium favosum* (Bory) Gomont. **a** A part of a filament with terminal cell. **b** Cells of two parallel filaments with clearly visible thylakoids and polyhedral bodies. **c** Thylakoid morphology. PH – polyhedral bodies; T – thylakoids. Scale bar – 1 μm.

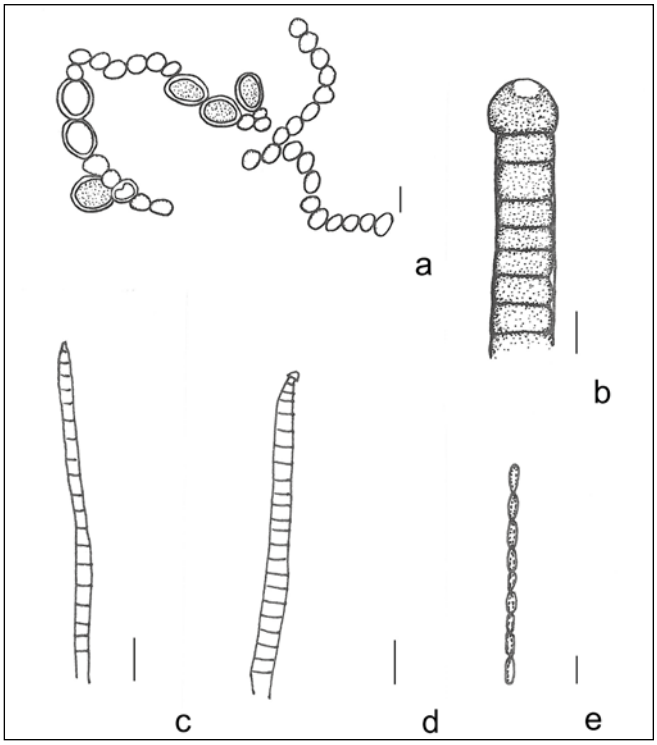

Fig. 5 Macromorphology of studied species. **a** *Nostoc microscopicum* Carmichael ex Bornet & Flahault. **b** *Tolypothrix distorta* var. *Penicillatum* (Thuret) Lemmerman. **c** *Leptolyngbya foveolarum* (Rabenh. ex Gomont) Anagnostidis and Komárek. **d** *Phormidium favosum* (Bory) Gomont. **e** *Pseudanabaea* cf. *catenata* Lauterborn. Scale bar – 1 μm.

ORDER: OSCILLATORIALES. (***i***) *Pseudanabaena catenata* Lauterborn (Fig. 3, Fig. 5). LM: cells cylindrical (always longer than wide) or barrel-shaped, blue-green or pale olive-green, 2.5-5.0 μm long, 1.8-2.6 μm wide. Apical cell cylindrical with rounded end. Trichomes flexuous, isolated. The mean number of cells per trichome relatively constant, between 12 and 18 cells. TEM: the 3 to 5 lamellar thylakoids parallely, peripherally and concentrically arranged, as described for Pseudanabaenaceae by Anagnostidis and Komárek [30]. Carboxisomes situated between the center of the cell and the thylakoids. Vacuole-like inclusions distributed throughout the cell. (***ii***) *Phormidium favosum* (Bory) Gomont (Fig. 4, Fig. 5). LM: filaments unbranched, rarely solitary, usually in dark blue-green mats. Single trichomes within a mucilaginous sheath, 4.2-7 μm wide, crosswalls granulated, apical cell rounded or conical. TEM: thylakoids situated perpendicularly to the cell wall (radially in a cross-section). (***iii***) *Leptolyngbya foveolarum* (Rabenh. ex Gomont) Anagnostidis and Komárek (Fig. 5). LM: dark green mats, thin filaments, trichomes 1-1.8 μm wide, sheaths colourless, crosswalls without granulations. TEM: vacuole-like inclusions, not bounded by a tonoplast and thylakoids distributed peripherally.

Using morphological and ultrastructural analyses, we were able to recognize five taxa of cyanophytes occurring on limestone rocks. The first research carried out by Starmach [1] in the rocky Szopczański Gorge has revealed a particularly interesting phycoflora from the ecological points of view (Tab. 1). We have confirmed the occurrence of five characteristic epilithic taxa of cyanophytes, but many species reported earlier were not detected. Starmach [1] and Mrozińska [11,12] enumerate, in addition to those described in our study, also *Calothrix parietina* (growing on limestone rocks), *Chroococcus kutzingianum*, *Ch. minutus*, *Gloeocapsa alpine*, *Homoeothrix nordstedtii*, *Schizothrix lardacea*, *S. rubra* and *Tholypothrix byssoidea* (found on rocky cliffs covered with slime). It is known that some epilithic species of cyanophytes inhabit also the limestone rocks of the Tatras and Ojców [33]. Rock-surfaces contaminated by mud have recently been observed in Szopczański Gorge. This, together with geological, morphological and climatic changes undoubtedly affected algal and cyanophytic diversity in this area. Only after further studies are carried out on cyanophytes in Szopczański Gorge, it will be possible to compare them with the respective phycoflora of other mountain gorges.

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Tab. 1 Characteristic epilithic cyanophytes found in Szopczański Gorge after [1,11].

Species	Localities	Reference
Aphanothece nidulans P. Richter	dry rocks	[1]
Calothrix gypsophila (Kütz.) Thur.	dry rocks	[1]
Calothrix parietina (Nägeli) Thuret	dry rocks only periodically sprinkled with water	[11]
Chroococcus minutus (Kützing) Nägeli	dry rocks only periodically sprinkled with water	[11]
Gloeocapsa alpina (Nägeli) Brand	dry rocks only periodically sprinkled with water	[1,11]
Gloeocapsa kützingiana Nägeli	dry rocks only periodically sprinkled with water	[1,11]
Homoeothrix nordstedtii (Thuret) Kirchner	rocks continually moistened by waterfalls	[11]
Nostoc microscopicum Carmichael	dry rocks only periodically sprinkled with water	[1,11]
Schizothrix calcarata (Ag.) Gom.	dry rocks	[1]
Schizothrix lardacea (Cesati) Gomont	rocks continually moistened by waterfalls	[11]
Schizothrix rubra P. L. Crouan & H. M. Crouan	rocks continually moistened by waterfalls	[1,11]
Scytonema crustaceum Ag.	dry rocks	[1]
Tolypothrix byssoidea (C. Agardh) Kirchner	rocks continually moistened by waterfalls	[11]
Tolypothrix distorta f. *penicillata* (Ag.) Koss.	dry rocks	[1]
Rivularia calcarata (Woron.) Poljanskij	on rocky cliffs	[1]

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