

***Pleurocapsa cuprea*, originally described as blue – green alga, is a eukaryotic alga similar to the species *Hildenbrandia rivularis* (Rhodophyta)**

***Pleurocapsa cuprea*, popsaná jako sinice, je eukaryotní řasa z blízkosti druhu *Hildenbrandia rivularis* (Rhodophyta)**

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Abstract

An interesting microorganism was found on stones in the Zbirožský potok stream near the village of Skryje (Rakovník region, Czech Republic). The macroscopic algal mats in the form of flat red patches on stones were macroscopically similar to those of *Hildenbrandia rivularis* (Rhodophyta) growing on stony substrates in freshwater biotopes. However, the identified material in the Zbirožský potok stream differed from *H. rivularis* in its morphology and also in ecology. It corresponded exactly to *Pleurocapsa cuprea* described by HANSGIRG (1892). New classification as red alga was confirmed by investigating samples under a fluorescence microscope after DAPI staining..

Introduction

This article deals with the new classification of a species known as *Pleurocapsa cuprea* HANGS. 1892 (Cyanobacteria) closer to *Hildenbrandia rivularis* (LIEBM.) J. AG. 1852 (Rhodophyta). *P. cuprea* has been recorded in unpolluted mountain streams in the southern Tyrol and in Bohemia (Ždírnice, Ústecký region) (HANSGIRG 1889). Further records come from the surroundings of the village Kladeruby near Mohelno (Vlčí kopec, small stream flowing in the River Oslava) in 1950 (KOMÁREK, pers. comm.) and near Dukovany in 1998 (POULÍČKOVÁ et al. 2004). Both findings were identified as *Pleurocapsa cuprea*.

P. cuprea was ranked among blue-green algae, but its close relation to green algae was considered as well (GEITLER 1932). It was also initially taken for a developmental stage of the genus *Siphononema* GEITL. 1925 or of one from two

species of the genus *Pleurocapsa* THURET IN HAUCK 1885, namely *P. polonica* RACIB. 1910 or *P. aurantiaca* GEITL. 1932. HIERONYMUS speculated about its possible relation to rhodophytes, subclass Bangiophycideae (sec. cit. GEITLER 1932) thanks to the observation of dark round bodies with some kind of a thin cover and wall chloroplasts.

The morphology of macroscopic biofilm and the morphology of cells in particular are to some extent similar to rhodophyte *H. rivularis*, which forms typical red macroscopic matches on stone substrates in running freshwaters. Older records present *H. rivularis* as a species from swift-flowing mountain streams (HINDÁK et al. 1975, 1978, KALINA 2001).. According to the latest records, it is quite abundant in mesotrophic streaming waters. It occurs either in shallow shaded parts or in deeper and more exposed parts of the central stretches of rivers and streams (LEDERER & LHOTSKÝ 2001).

Material and methods

The material was collected at the end of September and the beginning of November 2005 in the inversion valley of the stream Zbirožský potok, about 250 m (blue tourist path, in front of the road across the stream) and 1 km (blue tourist path, behind the road across the stream) upstream from the well-known lakes Skryjská jezírka Rakovnicko, central Bohemia (Fig. 1). The whole area is a part of the protected landscape area Křivoklátsko.

The geological foundation in this inversion valley comprises Palaeozoic Cambrian volcanic rocks, in particular basic andezits and acidic rhyolites and dacits. In some parts can be found outcrops of Cambrian sediments, conglomerates and slates (www.rakovnicko.cz). The Zbirožský potok stream is oligotrophic with an introduced trout population. At the sampling sites, the stream was about 20 – 30 cm deep, swift-running. The bottom was covered with stones. The periphyton consisted solely of diatoms (*Achnanthes* cf. *lanceolata*, *Amphora ovalis*, *Anomoeoneis* sp., *Cocconeis* sp., *Melosira varians*, *Navicula* spp., and *Pleurosigma* sp.).

To prove that the collected material is not a blue-green alga but really a eukaryotic alga, DAPI staining was used (4,6 diamidino 2-phenylindol) with S buffer (KUROIWA & SUZUKI 1980) in concentration 2.5 µg.ml⁻¹. This fluorescent substance bonds to DNA; therefore, stained cell nuclei are then visible under the fluorescent microscope. Thanks to the fluorescent microscope we can also detect the presence or absence of phycobilins. It is also possible to approximately classify the studied algal population into the system of algae, e.g. to exclude or confirm its relation to the group of green algae.

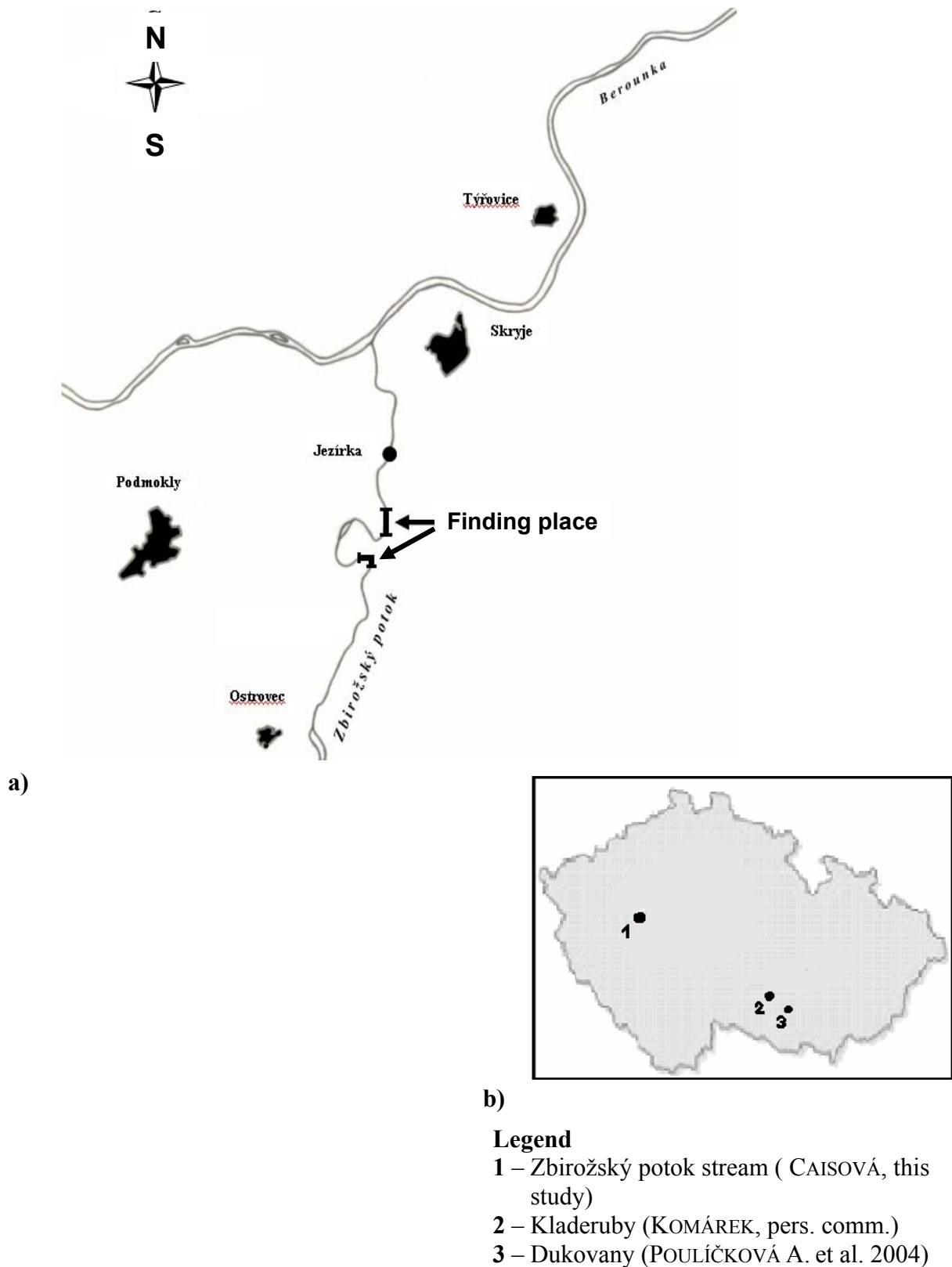


Fig. 1: a) Map of the locality Skryjská jezírka with the finding places of *Pleurocapsa cuprea* in 2005.

b) Map of *Hildenbrandia*-like findings in the Czech Republic.

Results

The macroscopic mats differed at first sight in the production of biomass. September colonies formed approximately 0.5 cm thick deposits on stones, they were of brick colour and could be sampled easily; they formed a stretch almost 7 m long and 4 m wide. November colonies formed much less biomass; they occurred deeper (15 cm below water surface), did not form compact stretches and had to be sampled by scratching with pincers.

The algal cells in a sample (Fig. 2) bunch into irregular, often not precisely bounded spatial macroscopic colonies, which may fall apart. The size of the colonies varies greatly, their width ranges from 20 to 500 μm and their length from 100 to 900 μm ; apparently, one colony often merges into another. Separated parts form smaller flat colonies of various dimensions; some may consist only of a few filamentous forms.

A pseudodichotomic branching occurs within these filamentous forms quite often. The cells have irregular shapes, quadrilateral or hexagonal, seldom approximately oval. The cell dimensions vary from 6 to 10 μm . The cell protoplast is of light green or dark red colour. The cells are surrounded by a relatively thick colourless cell wall. No pyrenoids were observed in parietal chloroplasts. No other kind of reproduction than cell division was recorded.

When observing the DAPI stained material under a fluorescence microscope, cell nuclei were clearly visible (Fig. 3, 4). After using fluorescent filters for the excitation in the green part of the spectral radiation, the presence of phycobilins, which shone red, was proven. On the contrary, after using the filters in the blue part of the spectrum, the affiliation of the biomass to green algae was refuted.

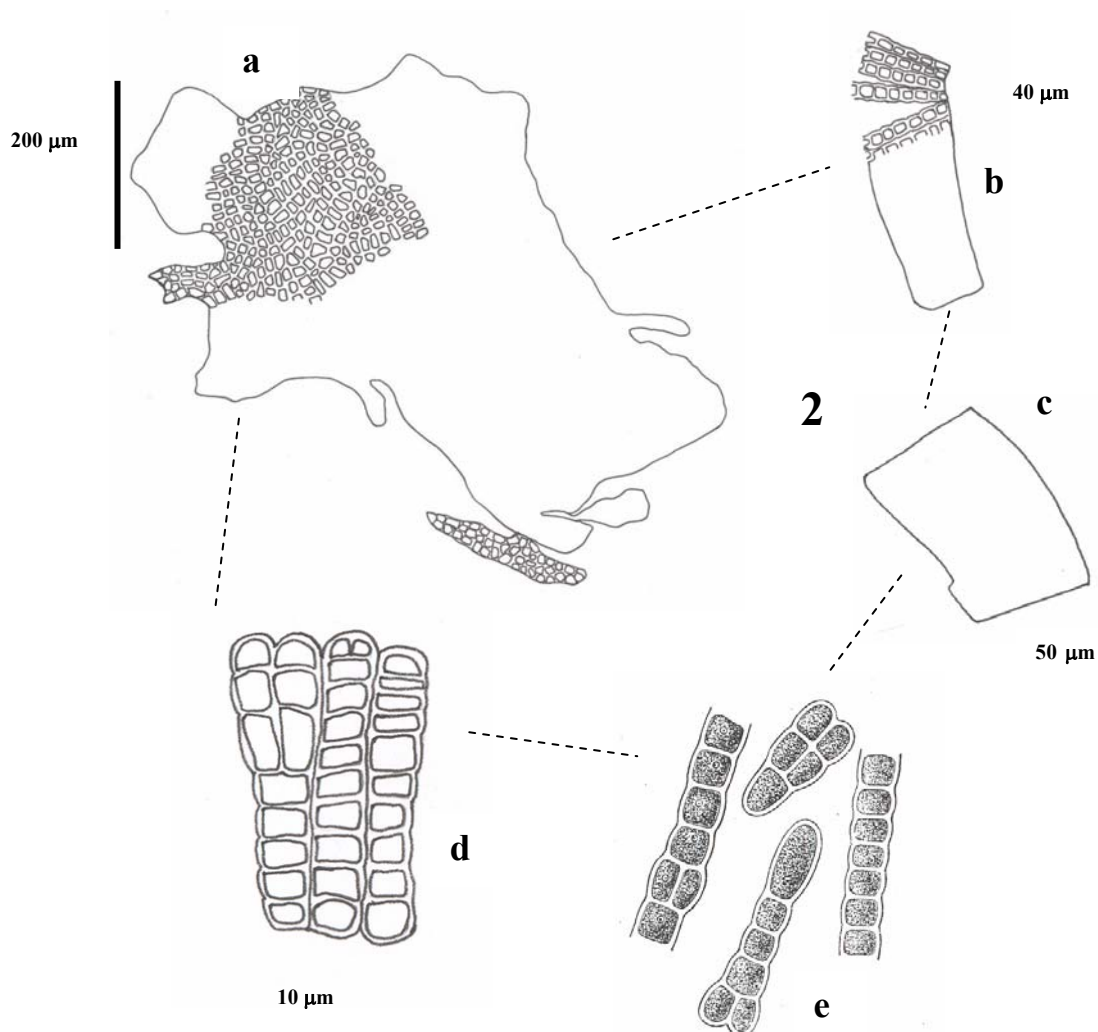


Fig. 2: "The new form of *Hildenbrandia*"; **a** – habitus of the colony, **b-c** – parts of the colony, **d** – detail of pseudodichotomic branching and morphology of cells, **e** – original drawing of "*Pleurocapsa cuprea*" after Hansgirg (1892).

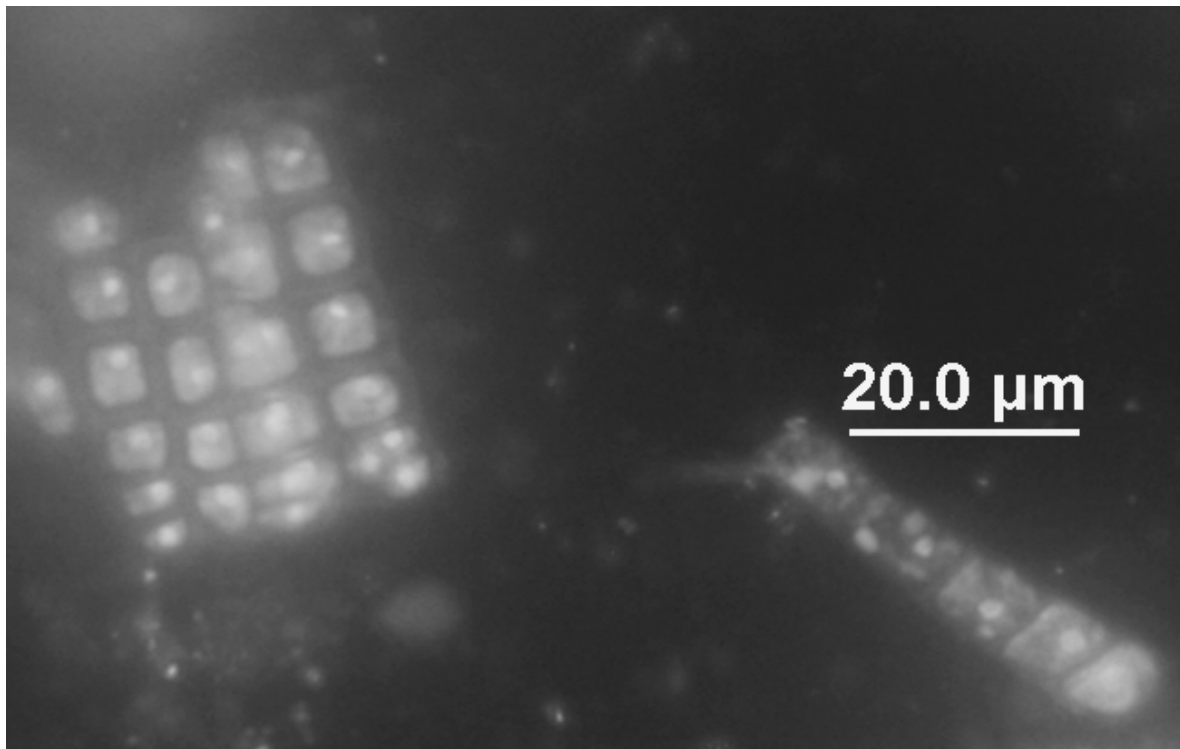


Fig. 3: Stained nuclei observed under fluorescence microscope.

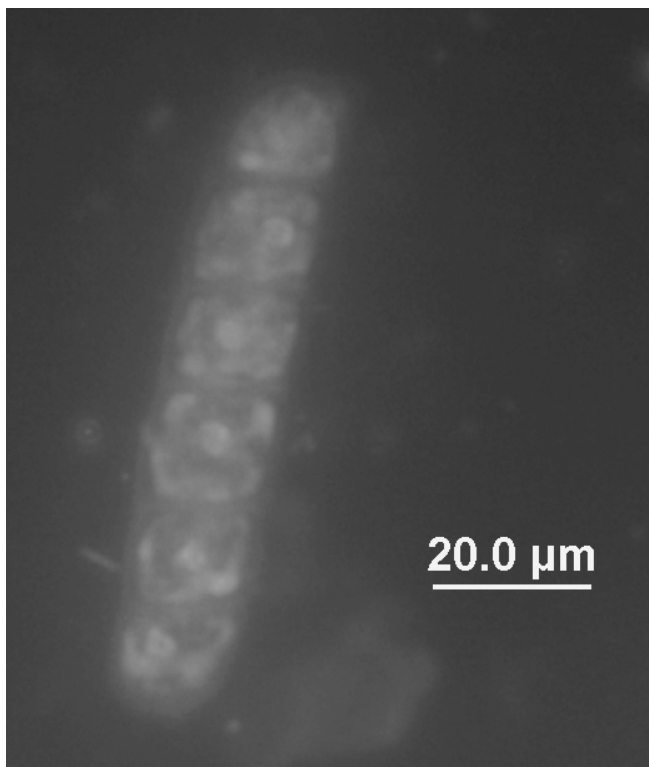


Fig. 4: Stained nuclei under fluorescence microscope in detail.

Discussion and conclusion

The observed microorganism, earlier described and determined as a blue-green alga *Pleurocapsa cuprea*, was detected as eukaryotic, i.e. belonging to algae. The results of fluorescence microscopy suggest that it does not belong to green algae, but it belongs with the group of algae containing phycobilins, i.e. rhodophytes. According to its morphology, it is likely related to genus *Hildenbrandia*.

Unlike *H. rivularis*, the sampled material does not have precisely bounded macroscopic colonies, the occurring biomass forms during its vegetation optimum up to 0.5 cm deposits of orange or red colour. The colonies fall apart into fragments of tabular shape; pseudodichotomic branching of filamentous forms or at least its trace can be recorded quite often.

The studied material, therefore, separates from *Hildenbrandia rivularis* not only morphologically, but it may be ecologically distinct as well. Another difference may be in the manner of attachment to substrate. Unlike *H. rivularis*, it was possible to take the samples of its biomass. No rhizoids that would attach the colonies to the substrate were observed.

The hypothesis that *Hildenbrandia rivularis* and *Pleurocapsa cuprea* are alike is supported also by HUBER – PESTALOZZI, who presents another site of its occurrence – Corsica (cited according to GEITLER 1932). Based on the observation of morphological characteristics, he disagreed with the classification of the species among blue-green algae (GEITLER 1932). The morphological description of his specimens corresponds to our finding, but there is no record of its ecology, or a more detailed description of the site. Nor was the species classified among Chroococcales in the last monograph of KOMÁREK and ANAGNOSTIDIS (1999).

The description of the morphology and ecology of an organism found by Komárek by Kladeruby (right-hand side tributary of the River Oslava) also corresponds with the found rhodophyte. At this site, KOMÁREK (1956) described a new species of blue-green alga, first classified as *Lyngbya rimosa*, later reclassified into genus *Phormidium* as *P. rimosum*, which grew chasmoendolithically in the crevices of stones, on top of which this rhodophyte formed colonies. If *P. rimosum* was found in the stone crevices at this new site, the community of the alga and blue-green alga would be considered highly specific and ecologically distinct.

To discuss the relation of the found rhodophyte to *H. rivularis*, a more detailed comparison of their morphology and ecology would be necessary, as well as a comparison of pigment analysis and electron microscope photography. Last but not least, it is necessary to extract DNA and compare their affinity; only then will it be possible to discuss the differences at the genus or species level.

Acknowledgement

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