Cyanoprokaryotes of genus *Petalonema* – taxonomically and molecularly interesting taxa

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Introduction

The genus *Petalonema* contains six subaerial habitats associated endo/epilithic cyanobacteria requiring special substrate conditions for growth in the nature. The cyanoprokaryotes can be described as filamentous, heterocytous, falsely branching with yellowish-brown lamellated mucilage sheaths. Their cells are widely rounded, heterocytes are intercalar or basal. On the rocks and stones, they form soft gelatinous colonies. The areal of this genus is azonal, they can be find in Antarctica, Australia, India, North America and several places in Europe. At least one species, *P. crustaceum*, can be found also in the Czech Republic (Komárek & Hauer 2007).

Apart from the type species *Petalonema alatum*, other known *Petalonema* species are *P. crustaceum*, *P. densum*, *P. involvens*, *P. pulchrum* and *P. velutinum*.

This study focuses mainly on *Petalonema alatum*, which was first described from Scotland by Berkeley in 1833. Since that time, the taxonomical position of the species (and genus) changed several times. Borzi in 1879 considered *Petalonema* to be synonymous to *Scytonema*. Other opinion was published by Itzigsohn in 1855, who didn't accept the genus Petalonema at all and considered *P. alatum* to be just morphotypes inside *Scytonema myochrous*. During the 133 years since *Petalonema alatum* description, authors chose one of the three taxonomical possibilities.
Although Petalonema exhibits high morphological similarity with *Scytonema*, Komárek & Anagnostidis (1989) didn't place it to the family *Scytonemataceae*, but to the family *Microchaetaceae*. The only molecular study done on the genus *Petalonema* (species *P.* cf. *involvens* in particular) was published by Taton et al. (2006) who sequenced 16S and 23S rRNA gene and placed Petalonema to the *Nostoc* clade.

The aim of the study was and is to confirm the validity and clarify the taxonomical position of genus *Petalonema*, based both in morphological variations and molecular taxonomical data.

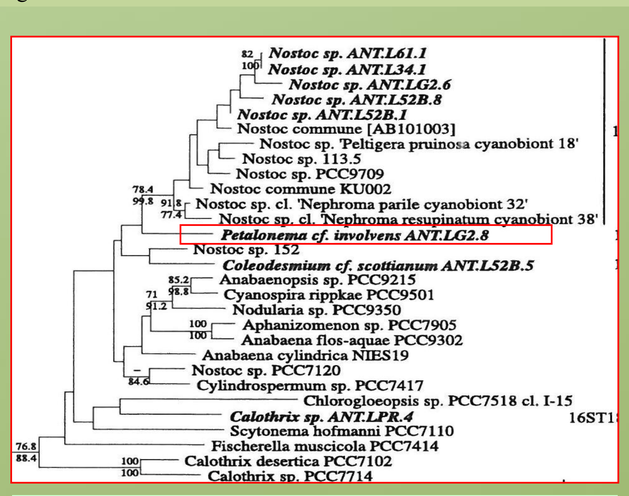


Fig. 1: Position of *Petalonema* cf. *Involvens* in the phylogeny tree. Taken from Taton et al. (2006)

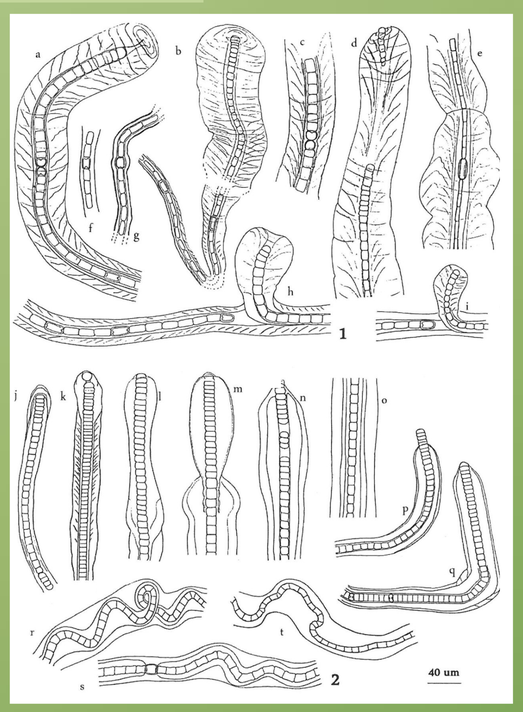


Fig. 2: Iconographs of *Petalonema alatum*: (a-i) natural samples; (j-s) culture; (a-c, e-g, j-m, o-q) heteropolarity of filaments and sheath stratification; (d, n) hormogonia formation;, (r-s) trichome spiral-curved in culture. Orig. B. Uher.

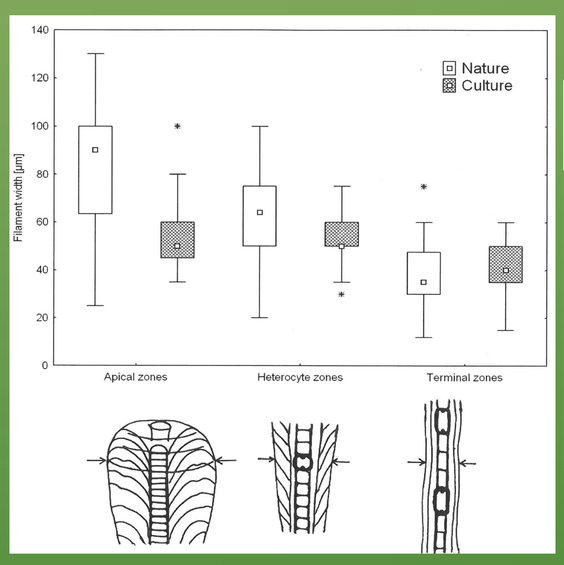
Materials and methods

Samples used for the study were taken from wet calcareous wall located in Piecky Gorge in the National Park Slovenský raj (Slovakia) by random scraping of the rock surface into sterile tubes, irregularly during summer seasons 1998 - 2007. At the same time, relative humidity and temperature were measured using a thermo-hygrometer and irradiance values using a radiometer with quantum sensor.

Prokaryotes taken from the nature were cultured in Petri dishes and tubes with BBM medium under controlled conditions (21 C, humidity 60%, irradiance 10.3 $\mu m.s^{-1}.m^{-2}$, light 660 lx, PhAR 2.14 $W.m^{-2}$ and in a photoperiod 12:12 light:dark).

Both natural isolates and isolates from the culture were observed and documented using a stereomicroscope Olympus SZH and Olympus BX 50 light microscope equipped wit Lucia Image Analysis.

After the initial cultivation, the clones were isolated. At both isolates from nature and culture (60 each) the morphometric data were measured: width of filament apex, width of the first heterocyte in the heterocyte zone and width of the terminal zone. These data were then statistically visualised using STATISTICA (Statsoft®) program. The dimensions of cells of both types of isolates were compared using Wilcoxon Signed Ranks Test.



Wilcoxon Signed Ranks Test

significant value 0.05;

Apical zones:

Z = -5,62,

$p_{apex} < 0.0001$;

Heterocyte zones:

Z = -3,16,

$p_{heterocyte} = 0.0016$;

Terminal zones:

Z = -2,09,

$p_{terminal} = 0.0370$

Fig. 3: Comparison of filament measurements of *Petalonema alatum* – in the nature vs. culture, * outliers, median, ┬ non outlier, box border quantil 25% and 75%

Fig. 5: *Petalonema alatum* – the queen of cyanobacteria. Orig. B. Uher.

Results

Petalonema alatum was the most prevalent organism in the natural samples, accompanied by bacteria, fungi, coccal algae or diatoms. Colonies formed on limestone were mucilaginous, yellowish green or greyish green, sometimes blackish brown, the major parts of filaments were observed inside the rock, just the apical parts were out of the rock.

Petalonema grown in the wild formed flexuous filaments, loosely entangled, with obvious heteropolarity. False branching was present at heterocytes. Sheaths were thick, diverged into many layers, inner yellowish, outer mostly colourless (Fig. 4).When maintained in the culture on agar plate, *Petalonema* formed mucilaginous, compact, dark-green colonies; filaments were horizontal or erect, mostly unbranched, rarely with single false branching. Heteropolarity was reduced . The sheaths were lamellated, but most of the mucilage layers were lost. Only old filaments were yellowish, other were colourless (Fig. 4: g, h). Cells were the same shape as in the wild, but smaller. Heterocytes were rare, basal or intercalary. The comparison of filaments formed in the wild and in the culture is in Fig. 2.

A significant difference between morphological characteristics of organisms from the wild and culture has been found. Petalonema grown on agar plate was narrower then in the wild in all of three monitored characteristics -width of apical, heterocyte and terminal zone (Fig. 3). Other features, such as heteropolarity, false branching and presence of meristematic zones were found relatively stable.

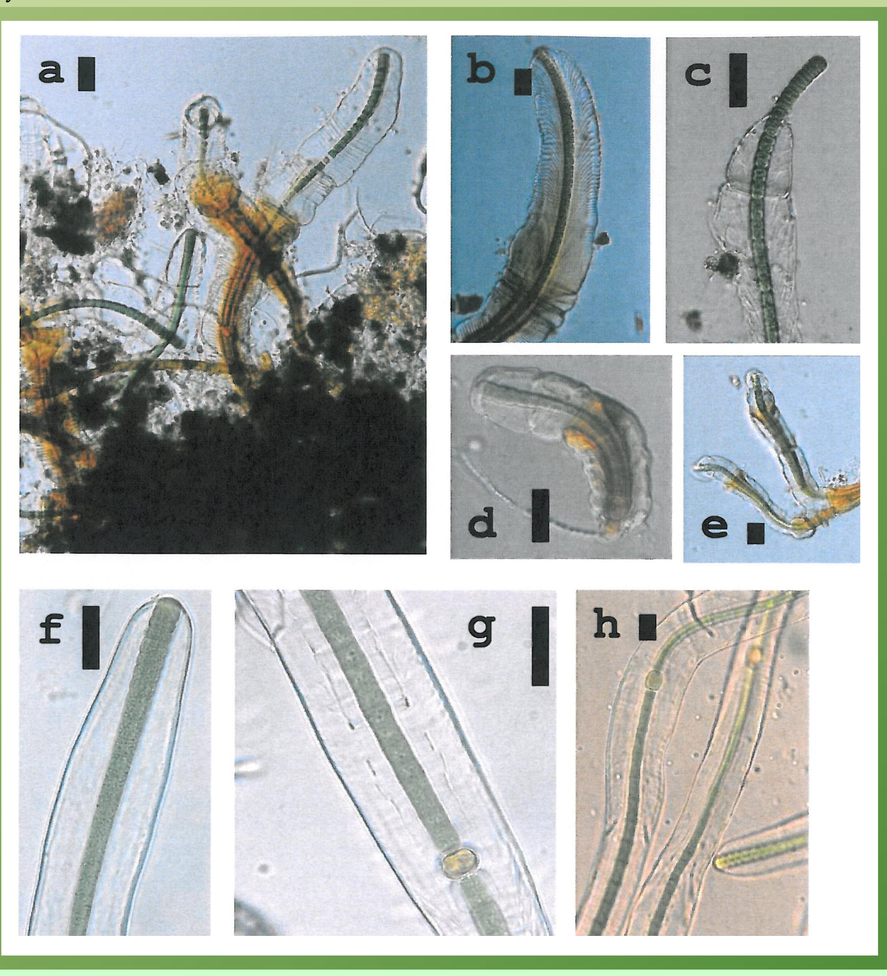


Fig. 4: *Petalonema alatum*: (a-e) natural sample; (f-h) culture; (a) filament arising from the substrate, (b, c, f) apices with apical rounded cell, (d) tolypotrichoid fragment of filament; (e) false branching; (g, h) well established sheath stratification. Scale bars 30 µm. Orig. B. Uher

Discussion

The previous and recent taxonomical position of *Petalonema* is based only in morphological features. The oldest descriptions focused basically only on the structure types of trichome sheaths and filaments. Other features, such as presence of heterocytes or meristematic zones, were found crucial later.

Phycologist of 20th century represented different approaches to the taxonomy and also considered different features to be diagnostic for Petalonema; three main streams can be recognized. The most important is the first group, based on Berkeley's description of *Petalonema alatum* as separate taxon, but also among these people there were differences. For example Berkeley stressed the branching, filament lined by lamella, and envelope structure, on the other side, Komárek & Anagnostidis (1989) stressed the type of heterocytes and branching, apex of filament and structured sheath. This moved *Petalonema* to the family *Microchaetaceae*.

This taxonomical position seems to be correct and better than other approaches. Also presented results of this study confirms that the morphological variability inside the species is very high and there is no reason why to put *Petalonema* inside other species.

In the only molecular study, Taton *et al.* (2006) didn t include many *Microchaetaceae* species in the tree, and so *Petalonema* appeared in the Nostoc clade, which it probably doesn't belong to.

Conclusion

The morphological part of the study confirmed the validity of genus *Petalonema* and the differentiated it from other genera it used to belong to, such as *Scytonema* or *Tolypothrix*. Now the molecular approvement of these results is needed. New samples will be collected in Slovakia and other places in Europe, rRNA and *rbcL* genes will be sequenced, so that we will be able to place *P. alatum* more accurately in the phylogeny tree of cyanobacteria.

Acknowledgement

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