



***Cyanocohniella calida* gen. et sp. nov. (Cyanobacteria: Aphanizomenonaceae) a new cyanobacterium from the thermal springs from Karlovy Vary, Czech Republic**

JAN KAŠTOVSKÝ^{1*}, ESTHER BERRENDERO GOMEZ¹, JINDŘICH HLADIL² & JEFFREY R. JOHANSEN^{1,3}

¹ Department of Botany, Faculty of Science, University of South Bohemia, 31 Branišovská, 370 05 České Budějovice, Czech Republic.

² Institute of Geology AS CR, v. v. i., Laboratory of Geological Processes, Rozvojová 269, 165 00 Prague 6, Czech Republic.

³ Department of Biology, John Carroll University, University Heights, OH 44118 USA.

* Corresponding author (hanys@prf.jcu.cz)

Abstract

The thermal springs of Karlovy Vary (Carlsbad) is the *locus classicus* of the well-known thermal cyanobacterium *Mastigocladus laminosus*. In addition to the nominate variety, several other varieties and forms were described based on differences in morphology (true-branching *versus* non-branching) or ecology (thermal *versus* non-thermal). The cyanobacterial strain Kaštovský 1996/2, which was provisionally identified as *M. laminosus* f. *nostocoides*, was also isolated from this locality and discussed in previous work. Based on both morphological and molecular (SSU) analyses, this strain was found to not belong to *Mastigocladus*, but rather to an undescribed genus, presumably within the Nostocaceae. This strain was subsequently lost, and absence of type materials prevented the description of the genus. The species was successfully re-isolated in 2012. The new strain is identical in morphology, life cycle, and 16S rRNA sequence to the lost strain 1996/2. It is herein described as *Cyanocohniella calida* gen. et sp. nov. The genus differs from all other Nostocaceae and Aphanizomenonaceae by the unique combination of these characteristics: 1) thermal-tolerant ecology, 2) life cycle that includes Pseudanabaenaceae-like, *Nostoc*-like and *Chlorogloeopsis*-like stages, 3) absence of aerotopes, and 4) phylogenetic placement in the Aphanizomenonaceae. The sister taxa, based on 16S rRNA gene sequence phylogenetic analysis, are *Cyanospira* and *Anabaenopsis*, both spiral, planktonic, aerotope-bearing, tropical genera and species clearly distinct from *C. calida*.

Key words: Cyanobacteria, new genus, polyphasic approach, thermal springs, Karlovy Vary

Introduction

Cyanobacteria are one of most ecologically important groups of microorganisms in the history of the Earth. Our knowledge about cyanobacterial diversity is still poor, although, with the widespread implementation of the polyphasic approach to taxonomy in the last decade, the scientific community has discovered many new genera and species of cyanobacteria. Many newly described genera are tropical (e.g. *Brasilonema* Fiore *et al.* (2007: 794), *Cyanoaggregatum* Werner *et al.* (2008: 493), *Geminocystis* Korelusová *et al.* (2009: 933), *Cronbergia* Komárek *et al.* (2010: 329), *Ophiothrix* Sant'Anna *et al.* (2010: 218), *Streptostemon*, Sant'Anna *et al.* (2010: 220), *Oxynema* Chatchawan *et al.* (2012: 50), *Calochaete* Hauer *et al.* (2013: 38), *Chakia* Komárková *et al.* (2013: 228), *Limnographis* Komárek *et al.* (2013: 45), but Europe or North America have also been sources of genera new to science (e.g. *Spirirestis* Flechtner *et al.* (2002: 6), *Rexia* Casamatta *et al.* (2006: 23), *Mojavia* Řeháková *et al.* (2007: 488), *Coleofasciculatus* Siegesmund *et al.* (2008: 1575), *Nodosilinea* Perkinson *et al.* (2011: 1404), *Oculatella* Zammit *et al.* (2012: 351), *Anathece* Komárek *et al.* (2011: 321)). However, genera and species are being described from other climates, continents, and habitats and the present is certainly a time of biodiversity discovery in the cyanobacteria that has not been paralleled in the past 60 years.

One of the more complicated questions in cyanobacterial taxonomy is the taxonomy and phylogenetic placement of *Mastigocladus laminosus* (Schwabe 1837: 124) Cohn (1863: 42) ex Kirchner (1898: 81). Ferdinand Cohn described the genus and species from thermal water in the main spring of Karlovy Vary. This is the same locality from which *Fischerella thermalis* Gomont (1895: 52), the type species of that genus, was described, and these two important genera likely share a single population of one species as a common type (Kaštovský & Johansen 2008). Furthermore,

limited phylogenetic evidence available for these taxa (Rajaniemi *et al.* 2005, Kozhevnikov & Kozhevnikova 2011). In contrast, the members of the Nostocaceae that have been sequenced consistently lack aerotopes. Consequently, the presence of aerotopes in a heterocyte-producing genus likely indicates phylogenetic position within the Aphanizomenonaceae, but the absence of aerotopes is uninformative.

Cyanocohniella calida and *Mastigocladus laminosus* appear to be thermal species, but they are actually eurythermal, not stenothermal. Both taxa, collected from Karlovy Vary, occur at elevated (>55 °C) temperatures, but both also grow well at room temperature. It appears that the thermal tolerance gives them a competitive advantage among taxa that apparently overgrow them in less thermal waters. At Karlovy Vary, the springs are frequently flooded by the river, and small springs cool relatively quickly. Truly stenothermal taxa, such as *Thermosynechococcus* Katoh, Itoh, Shen & Ikeuchi (2001: 599, nom. inval.) are absent from this system of hot springs. The aggregation of all true-branching taxa occurring in warm and hot springs into *M. laminosus* suggests that thermal tolerance has taxonomic significance (Kaštovský & Johansen 2008). Only as more populations of cyanobacteria belonging to *Cyanocohniella* are collected and confirmed through molecular sequencing will it be possible to see if thermal tolerance has taxonomic significance in defining this genus.

Stackebrandt & Goebel (1994) suggested that in prokaryotic taxa, those strains with less than 97.5% 16S rRNA sequence similarity should be considered to be separate species, while those with less than 95% similarity should likely be considered to be separate genera. We have shown in this paper that *Cyanocohniella* can certainly not be congeneric with *Mastigocladus*, and report the low similarity in the 16S rRNA gene (<91%) as partial evidence of this. However, this guideline has been misused to group taxa which have highly similar 16S rRNA into the same species or genus (Otsuka *et al.* 1998, 1999). This only works as a recognition guideline, less than the similarity level indicates evidence of genetic separation roughly at these levels. It cannot be used as a grouping criterion to put strains into the same species or genus based on high similarity. The Nostocales in particular are highly similar in the 16S rRNA gene, and this is likely the reason that it is difficult to get bootstrap support along the backbone of phylogenies of heterocyte-producing cyanobacteria. When similarity is high, as it always is in the Nostocales, there are not enough phylogenetically informative sites to resolve relationships when taxon sampling is high. *Cyanocohniella* has 16S rRNA similarity above 95% for 29 different genera in the Nostocales, including taxa from five different families. If the 97.5% similarity cutoff were used to group taxa with *Cyanocohniella*, it would be the same species as *Anabaenopsis elenkini*, *Cyanospira rippkae*, Florenzano, Sili, Pelosi & Vincenzini (1985: 305) *Trichormus variabilis*, *Nodularia baltica* Komárek, Hübel, Hübel & Šmarda (1993: 14) and *Nodularia harveyana*. This is clearly nonsensical, and we strongly discourage the further misuse of this guideline to group multiple morphospecies into a single species, or multiple genera into a single genus. Furthermore, as a separation guideline, in the Nostocales it is far more likely that strains less than 99% similar in 16S sequence are separate species, while the cutoff for closely related genera is possibly as high as 98%. However, even at these levels, similarity should not be used to group same taxa. It is always just part of the evidence.

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