Description of the cyanobacterial genus *Desmonostoc* gen. nov. including *D. muscorum* comb. nov. as a distinct, phylogenetically coherent taxon related to the genus *Nostoc*

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Abstract: On the basis of data presented here and in earlier studies, *Desmonostoc* gen. nov. is described. The new genus includes the traditional species *Nostoc muscorum* Agardh ex Bornet et Flahault 1888, and several other strains previously assigned to the genus *Nostoc,* which present similar morphology and phylogenetic placement within the *Desmonostoc* lineage. The *Desmonostoc* clade is phylogenetically coherent according to 16S rRNA gene sequence analysis performed with four distinct approaches. In all phylogenetic trees, *Desmonostoc* formed a supported group separated from strains belonging to the related taxa *Nostoc*, *Trichormus,* and *Mojavia.* We also suggest that other clusters hosting strains which for their morphology resemble *Nostoc*, but are more distant from *Nostoc commune* cluster than *Desmonostoc*, should be reclassified into new genera in the future. Strains belonging to *Desmonostoc* form long vegetative filaments embedded in diffluent mucilaginous envelopes, except for primordial stages they never form a firm periderm, and the filaments are never densely coiled with compact trichomes as found in *Nostoc*. Both terminal and intercalary heterocytes occur, and mostly elliptical akinetes were differentiated apoheterocytically in long chains. *Desmonostoc* strains can be usually found in moist or wet meadow, field and forest soils, more rarely in periphyton, but to our knowledge, they are missing or very rare in desert areas. Some of these strains have been found to grow in association with mosses or as symbionts of cycadean plants and of *Gunnera* sp.

Key words: Cyanobacteria, *Desmonostoc*, ecology, *Nostoc*, taxonomy

Introduction

The genus *Nostoc* is one of the earliest described cyanobacterial genera (Bornet & Flahault 1888). It is also the type genus for the whole order Nostocales, presenting the typical morphological characters for the order: isopolar filaments which differentiate akinetes and heterocytes (Komárek & Anagnostidis 1989). Its morphological description is based on the formation of uniseriate isopolar filaments. Chains of akinetes differentiate following an apoheterocytic scheme, while heterocytes develop in both terminal and intercalar positions. The filaments are non–branched and always embedded in mucilage. The production of mucilaginous colonies is one of two key diagnostic characters of the genus. However, the morphology of the envelopes and the level of slime production strongly vary among *Nostoc* strains, ranging from diffluent matter (*N. linckia, N. ellipsosporum*), to firm envelopes around separate filaments (*N. edaphicum, N. punctiforme*), to a thick peridermal envelope present in many benthic *Nostoc* species (e.g. *N. pruniforme*, *N. zedterstedtii*).

The other primary diagnostic character of the genus is its complex life cycle (Lazaroff & Vishniac 1961; Lazaroff 1966; Mollenhauer 1970). In principle, the formation of the vegetative filaments can be achieved via two different cycles. In the hormogenic cycle, differentiation of hormogonia starts with the fragmentation of old vegetative filaments in positions adjacent to heterocytes; later on, new vegetative filaments will develop from hormogonia. In the sporogenic cycle, akinetes germinate into vegetative filaments (Lazaroff 1966). These changes are accompanied by changes in the entire colony morphology and can extend over very different time ranges or even parts of the cycle can be absent in particular strains (Hrouzek et al. 2003, 2005; Mateo et al. 2012). Thus the genus *Nostoc is* heterogeneous when its life cycle is considered.

On the basis of results obtained using a molecular approach, the genus *Nostoc* was again

found to be genetically heterogeneous. Studies taking into account large strain selections revealed its likely polyphyletic origin (ARIMA et al. 2012; TAMAS et al. 2000; HROUZEK et al. 2005; ŘEHÁKOVÁ et al. 2007; PAPAEFTHIMIOU et al. 2008). However, in those analyses, a large and well supported cluster containing strains of the type species of the genus, *Nostoc commune* VAUCHER ex BORNET et FLAHAULT, the widely used strain *Nostoc punctiforme* PCC 73102, and several other well–defined *Nostoc* species with firm sheath material (*N. desertorum, N. lichenoides, N. indistinguendum, N. edaphicum*) was repeatedly found. This clade had the unifying feature of origin in soils, with many isolates well characterized by the production of massive laminar mucilaginous colonies (the diagnostic feature of the generitype) (ŘEHÁKOVÁ et al. 2007). This observation led some authors to indicate this group as "*Nostoc sensu stricto*" or "main *Nostoc* cluster" (HROUZEK et al. 2005; ŘEHÁKOVÁ et al. 2007; PAPAEFTHIMIOU et al. 2008; LUKEŠOVÁ et al. 2009). At least five other groups of cyanobacterial strains exhibiting *Nostoc* morphology, but falling outside the main cluster were preliminarily identified (HROUZEK et al. 2005). However, relationships between them and other related genera of heterocytous cyanobacteria are not clear. The novel genus *Mojavia*, has been described as one of these clusters with similar morphology but lying out of the *Nostoc commune* cluster (ŘEHÁKOVÁ et al. 2008), but its phylogenetic position with respect to the *Nostoc commune* group*, to Trichormus,* and to a clade called by authors *Nostoc* group II including "*Nostoc muscorum* II", was unstable.

We previously reported a well–supported group of *Nostoc muscorum* strains located outside *Nostoc sensu stricto* (HROUZEK et al. 2005; PAPAEFTHIMIOU et al. 2008), that would require description as a new genus if the genus *Nostoc* is to be rendered monophyletic. *Nostoc muscorum* AGARDH is one of the classical *Nostoc* species circumscribed in the seminal work of BORNET et FLAHAULT (1888) on the basis of its colony morphology and ecology. It was found to be an important member of soil communities (ROGERS et al. 1994; DE CAIRE et al. 1997) as well as a physiological model for stress tolerance and heavy metal uptake (BEKASOVA et al. 1999; EL–SHEEKH et al. 2005); and some of its strains have been found to produce non–ribosomal peptides and cytotoxic substances (TOMEK et al. 2010; HROUZEK et al. 2011). However, as often happens with the designation of cyanobacterial taxa, the species name *Nostoc muscorum* was not always properly used. This study brings up the evidence that strains correctly assigned to *Nostoc muscorum* and other strains closely related to them should be placed into a separate genus, which we designate here as *Desmonostoc*. A taxonomic description for the genus *Desmonostoc* is therefore given.

MATERIALS AND METHODS

DNA extraction, PCR, sequence analysis. The 16S rRNA ge-ne of eight *Desmonostoc* strains was sequenced for this study, with an additional 13 strains sequenced by us or others prior to this study (Table 1). The same gene of one cyanobacterial strain belonging to the *Nostoc commune* clade was also sequenced. For DNA sequence analysis, cyanobacterial strains were cultivated in nitrogen–free $BG11_0$ medium (RIPPKA et al. 1979), and their DNA extracted with PowerPlant™ DNA Isolation Kit (MoBio Laboratories Inc.) as detailed in CUZMAN et al. 2010. A DNA fragment including the 16S rRNA gene plus the adjacent ITS was amplified in vitro with universal primer 16S27F and cyanobacterial specific primer 23S30R, purified and sequenced as previously described (CUZMAN et al. 2010). A 16S rRNA gene data set, including the obtained sequences, a large selection of sequences belonging to the cluster object of this study, and several sequences of reference strains of Nostocaceae, was built using high quality sequences longer than 1100 base pairs. The sequences were retrieved from the public domain through SILVA, a comprehensive on–line resource for quality checked and aligned ribosomal RNA sequence data, available online at http://www.arb–silva.de (QUAST et al. 2013). The sequences were aligned using the SINA aligner available on the same website (PRUESSE et al. 2012), and the alignment was imported in ARB, a software environment for sequence data (Ludwig et al. 2004), where it was visually inspected and manually edited.

Phylogenetic relationships among the sequence dataset were calculated with ARB on a 16S rRNA gene fragment 1411 base pairs long in *Desmonostoc muscorum* NIVA–CYA 817, corresponding to positions 28 to 1491 of the reference *Escherichia coli* gene region. The sequence of *Chroococcidiopsis thermalis* PCC 7203 was added to the alignment as a non–heterocytous counterpart, while *Gloeobacter violaceus* VP3–01 was used to root the phylogenetic trees. A first Neighbor Joining (NJ) analysis on the 160 sequences of the complete data set was used to identify a couple of sequences for each cluster of Nostocaceae, only leaving more representatives in the *Nostoc sensu stricto* (*N. commune*) cluster. In the reduced data set, composed by 56 sequences, Neighbor joining (NJ), Maximum Likelihood (ML), and Maximum Parsimony (MP) analyses were run using ARB. NJ, ML, and MP trees have been bootstrapped 1000, 500, or 100 times respectively. For the Bayesian analysis, two runs of four Markov chains were executed using MrBayes v. 3.1.2 (RONQUIST & HUELSENBECK 2003) for 3 000 000 generations with default parameters, sampling every 100 generations (the final average standard deviation of split frequencies was lower than 0.01). First 25% of sampled trees were discarded as burnin, the rest was used to calculate posterior probabilities of branches.

16S rRNA gene similarities between the N and D clusters and between D1 and D2 subclusters (Fig. 1) were calculated with ARB on a gene fragment 1410 base pairs long, corresponding to positions 28 to 1490 of the reference *Escherichia coli* gene region.

Morphological observations. Many of the strains have had their morphology documented in the past (Table 2). In this study, five additional cultured strains were characterized (Table 2). All observations were performed on cultures growing on agar plates. The morphology of the isolates was studied in young, exponentially growing and old cultures of

Table 1. Origin of the strains belonging to *Desmonostoc* sp. cluster. Strains characterized in this study are in bold. The strains characterized in other studies are marked as: (1) Hrouzek et al. 2003, (2) Papaefthimiou et al. 2008, (3) Mateo et al. 2011.

Strain	Habitat/Locality of isolation	Accession number
D. muscorum NIVA–CYA 817(1)	soil, abandoned field /Dlouhá ves – Czech Republic	AJ630451
D. muscorum II(1)	soil, meadow /Jevany – Czech Republic	AJ630452
D. muscorum NIVA–CYA 818 (2)	soil, wet meadow /Dlouhá ves – Czech Republic	AM711523
D. muscorum 2/91(2)	soil, arable field / Nezamyslice – Czech Republic	AM711524
D. muscorum De(2)	Symbiont of *Dioon edule*/botanical garden in Rome – Italy	AM711534
***Desmonostoc* sp. OSNI32s01**	**Periphyton, Sítinný pond / Poodří – Czech Republic**	**HG004587**
***Nostoc* sp. PCC8306**	**waterlogged soil/ West Africa**	**HG004584**
Desmonostoc sp. TO1SO1(#)	soil, abandoned field /Tuscany – Italy	AM711549
Desmonostoc sp. Cc2(2)	symbiont of *Cycas circinalis* /botanical garden in Pisa – Italy	AM711532
***Desmonostoc* sp. Cr3**	**symbiont of *Cycas revoluta* /botanical garden in Bologna – Italy**	**HG004581**
Desmonostoc sp. Cr4(2)	symbiont of *Cycas revoluta*/botanical garden in Florence – Italy	AM711533
***Desmonostoc* sp. Ds**	**symbiont of *Dioon spinulosum* /botanical garden in Rome – Italy**	**HG004579**
Desmonostoc sp. 8964(2)	symbionf of *Gunnera prorepens* / New Zealand	AM711541
Desmonostoc sp. MA4–UAM307(3)	Biofilm of Matarrana stream/Teruel, Spain	HM623782
***Desmonostoc sp.* PCC9230**	*Cycas* sp. symbiont	**HG004585**
***Desmonostoc sp.* PCC8107**	**na**	**HG004579**
***Desmonostoc sp.* PCC7422**	**symbiont of *Cycas sp.*/botanical garden in Stockholm – Sweden**	**HG004586**
***Desmonostoc sp.* PCC6302**	**na**	**HG004582**
Desmonostoc entophytum IAM M–267	na	AB093490
Desmonostoc linckia IAM M–251	na	AB074503
Desmonostoc sp. 8938(#)	symbiont of *Gunnera dentata/* New Zealand	AY742454

3, 10 and 30 days respectively. Cyanobacteria were examined under an Olympus CX40 light microscope equipped with a digital camera. The morphology of colonies, vegetative filaments, hormogonia, mucilaginous sheaths of filaments, and the different cell types (vegetative cells, heterocytes, and akinetes) were studied. Cell parameters were measured using the DP–SOFT software; 50 to 300 measurements were taken for each parameter to describe the trait variability. The morphology of the mucilaginous sheaths was classified into two main categories: diffluent (where no sharp edges of micro–colony could be observed), and enveloping filament (delicate, not lamellated sheath expanding 2–20 μm around the filament). The collected data were analyzed with the software Statistica for Windows v. 9 (StatSoft).

Ecology observations. Information about the ecology of the *Desmonostoc muscorum* group presented in this report were based on the analysis of algal and cyanobacterial communities from about 500 soil samples collected since 1985. Investigated soils originated from different localities in many countries of Europe, the USA, Canada, Mexico, Cuba, Brazil, Africa (Egypt, Kenya), Indonesia, Siberia, Svalbard,

Arctic, Antarctica. They included soils from cultivated fields and meadows, from grasslands, steppe, prairie, different shrub communities, forests, post–mining reclaimed and unreclaimed sites, post–fire, post–volcanic sites, deglaciated areas, cave sediments, hot desert soils, frost heaved soils, peat soils, garden soils, saline soils, polar soils, guts of soil invertebrates collected in the field, and moss epiphytes. Both direct light and epifluorescence microscopy and cultivation methods (dilution plate method on BBM, BG11) were used for the identification and life cycle studies from the natural samples.

Results and Discussion

After preliminary or partial data were previously published (HROUZEK et al. 2005; PAPAEFTHIMIOU et al. 2008; ŘEHÁKOVÁ et al. 2008; MATEO et al. 2011), the existence of a unique, phylogenetically coherent clade for *Desmonostoc muscorum* and allied strains has been confirmed here by the addition of numerous other strains. We performed a comprehensive phylogenetic analysis including strains and species of *Nostoc, Trichormus,* and *Mojavia* that have been demonstrated in past studies to be closely related to the *Desmonostoc* cluster (HROUZEK et al. 2005; PAPAEFTHIMIOU et al. 2008; ŘEHÁKOVÁ et al. 2008; MATEO et al. 2011); we also added a few other genera representing more distantly related Nostocaceae. Moreover, we characterized four new strains (*Nostoc* sp. OSNI32s01, PCC 8306, Cr3 and Ds), demonstrating that they belong to the genus *Desmonostoc* (Figs 1, 2; Table 1). As shown by a phylogenetic analysis performed with four different methods (Figs 1, 2), the 22 strains of *Desmonostoc* were always joined in a single, monophyletic cluster, distinct but related to the *Nostoc commune* cluster, exactly as it was previously shown with smaller taxon sampling (HROUZEK et al. 2005; PAPAEFTHIMIOU et al. 2008; ŘEHÁKOVÁ et al. 2008). *Desmonostoc* was supported as a monophyletic taxon in analyses performed by NJ, MP, ML approaches with bootstraps values of 95, 98 and 77%, respectively (Fig. 1), and also in a Bayesian analysis where it obtained 100% support (Fig. 2). According to the Bayesian analysis, *Desmonostoc* formed a sister group to the *Nostoc commune* cluster (supported 98%), with *Mojavia* as the nearest relative of this larger cluster (Fig. 2). The placement of these three genus–clusters in the phylogenetic tree was unstable in NJ, MP, and ML analyses and never supported by high bootstrap values. Also for the other genus–clusters included with few representatives as references in the phylogenetic analysis, their existence as monophyletic clusters was fully supported, but their distribution along the tree was not stable. On the other hand, at the deepest level the entire heterocytous clade was fully supported in all analyses. This was not unexpected since, in this phylogenetic study, clusters of Nostocaceae were underrepresented by a single or very few strains each, while *N. commune* and *Desmonostoc* were included in the analysis with 11 and 22 sequences respectively, and phylogenetic inference methods are sensitive to unbalanced taxonomic sampling, and even more sensitive to the presence of orphans. The separation of *Desmonostoc* from the *Nostoc commune* cluster is also supported by the position of strains "*Nostoc muscorum"* I and II in a phylogenetic analysis using *rpoB* sequences (RAJANIEMI et al. 2005). On the basis of this evidence, we also recommend that all other groups of cyanobacteria morphologically resembling the genus *Nostoc*, but lying more distant from *Nostoc sensu stricto* than *Desmonostoc* and showing some morphological support, should be described as new genera in the future.

The sequence similarity between strains within the *Desmonostoc* cluster (subsequently referred to as cluster D, as in Fig. 1) and strains within *Nostoc commune* cluster (subsequently referred to as cluster N) ranged between 94 and 97%. Among the 22 *Desmonostoc* sequences (Fig. 1) in cluster D, two stable and well defined subclusters could be easily recognized. Subcluster D1 hosted 14 *Desmonostoc* sequences of various habitat and geographical origins, sharing sequence similarities higher than 99% (Figs 1, 2; Table 1). Six cycad symbionts (Cc2, Cr3, Ds, Cr4, PCC7422 and PCC 9230), two symbionts of *Gunnera* sp. (8964:3 and 9231), three free–living strains (OSNI32s01, TO1SO1 and PCC8306), and three strains of unknown origin were placed within this subcluster. Strain OSNI32s01, characterized in this study, had been isolated from the periphyton of Sitinný pond (Poodří, Czech Republic). The other two strains, TO1SO1 and PCC 8306, were isolated from wet soils in Tuscany and West Africa, respectively. In addition, one Pasteur culture collection strain (PCC 8107) of unknown origin clustered here. Since the morphology and origin of "*Nostoc entophytum*" and "*Nostoc linckia*" are unknown, we cannot at this time confirm the identity of these species. If these strains were correctly identified, a putative species *D. entophytum* would include at least five of the presently characterized strains.

Subcluster D2, consisting of eight strains with sequence similarities higher than 99%, was supported by the NJ, NP and ML methods (96, 99 and 59% for NJ, MP and ML, respectively) and by 100% by Bayesian analysis; subcluster D2 could be further subdivided into two small subgroups with full support by all phylogenetic methods used. One of them is an extremely tight group made up by four free–living strains isolated from meadow soils (*Desmonostoc muscorum* NIVA–CYA 817, NIVA–CYA 818, II and Lukešová 1/87) and by the symbiont *D. muscorum* De (Fig. 1). Because of the nearly complete 16S rRNA gene identity of these five strains and of their high morphological resemblance (see below), we suggest that they should be assigned to the single species *Desmonostoc muscorum*. We also suggest *D. muscorum* to be the type species of the genus with *Desmonostoc muscorum* NIVA–CYA 818

Table 2. Morphometric characteristics of strains belonging to genus *Desmonostoc*. Strains characterized in this study are in bold. The symbols behind the strain name refers to the studies where the strains has been characterized: (1) Hrouzek et al. 2003, (2) Papaefthimiou et al. 2008, (3) Hrouzek et al. 2005, (4) Mateo et al. 2011.

	D. muscorum NIVA–CYA 817 (1)	*D. muscorum* II (1)	*D. muscorum* 1/87 (2)	*D. muscorum* NIVA–CYA 818 (2)	*D. muscorum* De (2)	***Desmonostoc* sp. OSN132s01**	***Desmonostoc* sp. PCC8306**	*Desmonostoc* sp. TO1SO1 (2)	*Desmonostoc* sp. Cc2 (3)	***Desmonostoc* sp. Cr3**	*Desmonostoc* sp. Cr4 (2)	***Desmonostoc* sp. Ds**	*Desmonostoc* sp. 8964 (2)	*Desmonostoc* sp. MA4–UAM307 (4)
subcluster	D1	D1	D1	D1	D1	D2	D2	D2	D2	D2	D2	D2	D2	D2
veg. cell length mean (range)	5.5 (3.6–7.7)	4.7 (2.8–6.8)	4.7 (3.3–7.9)	5.0 (3.2–7.9)	4.0 (2.3–6.3)	4.0 (2.7–7.0)	4.6 (2.8–7.5)	5.0 (2.9–7.6)	4.1 (2.1–6.4)	4.0 (2.2–5.9)	5.5 (3.2–8.2)	4.3 (2.9–6.3)	4.1 (2.0–6.8)	6.4 (4.5–7.0)
veg. cell width mean (range)	5.7 (4.0–6.8)	4.9 (4.0–5.9)	3.4 (2.7–4.5)	4.9 (3.8–6.3)	4.4 (3.4–5.4)	4.1 (2.7–5.1)	4.4 (3.2–5.9)	3.4 (2.7–4.1)	4.0 (2.7–4.9)	4.6 (3.6–6.5)	4.4 (3.3–5.4)	4.4 (3.4–5.5)	4.5 (2.8–7.0)	5.3 (4.0–6.4)
veg.cell length/width mean	1.0	1.0	1.4	1.0	0.9	1.0	1.1	1.5	1.0	0.9	1.3	1.0	0.9	~1.2
heterocytelength mean (range)	8.6 (6.2–11.1)	7.4 (5.3–10.2)	5.6 (4.0–7.0)	8.1 (5.5–11.3)	7.8 (6.8–8.7)	6.2 (4.2–7.9)	5.7 (3.8–9.2)	4.5 (3.4–7.1)	5.3 (3.2–7.3)	6.1 (4.5–8.8)	7.1 (5.3–10.6)	6.1 (4.4–8.6)	5.7 (4.4–6.9)	6.8 (5.0–8.0)
heterocytewidth mean (range)	6.5 (5.0–8.3)	5.8 (4.4–7.2)	4.5 (3.5–5.1)	6.4 (4.5–8.7)	6.7 (5.8–7.4)	5.7 (4.7–6.3)	4.7 3.6–5.9)	3.6 (2.9–4.7)	4.4 (3.1–5.6)	5.3 (4.5–6.6)	5.2 (4.2–7.7)	4.7 (3.9–6.5)	5.1 (3.4–6.9)	6.3 (5.5–7.5)
heterocyte length/width mean	1.3	1.3	1.3	1.3	1.2	1.1	1.2	1.3	1.2	1.2	1.4	1.3	1.1	~1.0
akinete length mean (range)	8.6 (5.4–12.5)	7.0 (4.5–9.4)	6.4 (4.5–8.1)	7.6 (5.5–8.8)	7.0 (5.9–8.0)	4.7 (3.1–8.3)	9.7 (8.5–9.9)	8.2 (7.1–8.5)	5.2 (4.5–6.1)	5.8 (4.3–7.9)	6.4 (3.7–9.5)	4.8 (3.9–5.9)	6.0 (4.38.2)	8.5 (7.0–11–0)
akinete width mean (range)	5.6 (3.8–8.0)	4.9 (3.4–6.3)	5.0 (3.9–6.3)	4.5 (3.7–5.1)	5.3 (3.6–6.9)	4.9 (3.2–6.0)	5.6 (5.0–6.1)	4.6 (4.4–4.7)	4.5 (3.9–5.4)	5.4 (4.4–6.1)	5.1 (3.8–6.2)	4.3 (3.9–4.8)	4.8 (3.8–5.6)	6.2 (5.5–8.0)
akinete length/width mean	1.6	1.4	1.3	1.7	1.6	1.2	1.7	1.8	1.2	1.1	1.5	1.1	1.3	~1.4

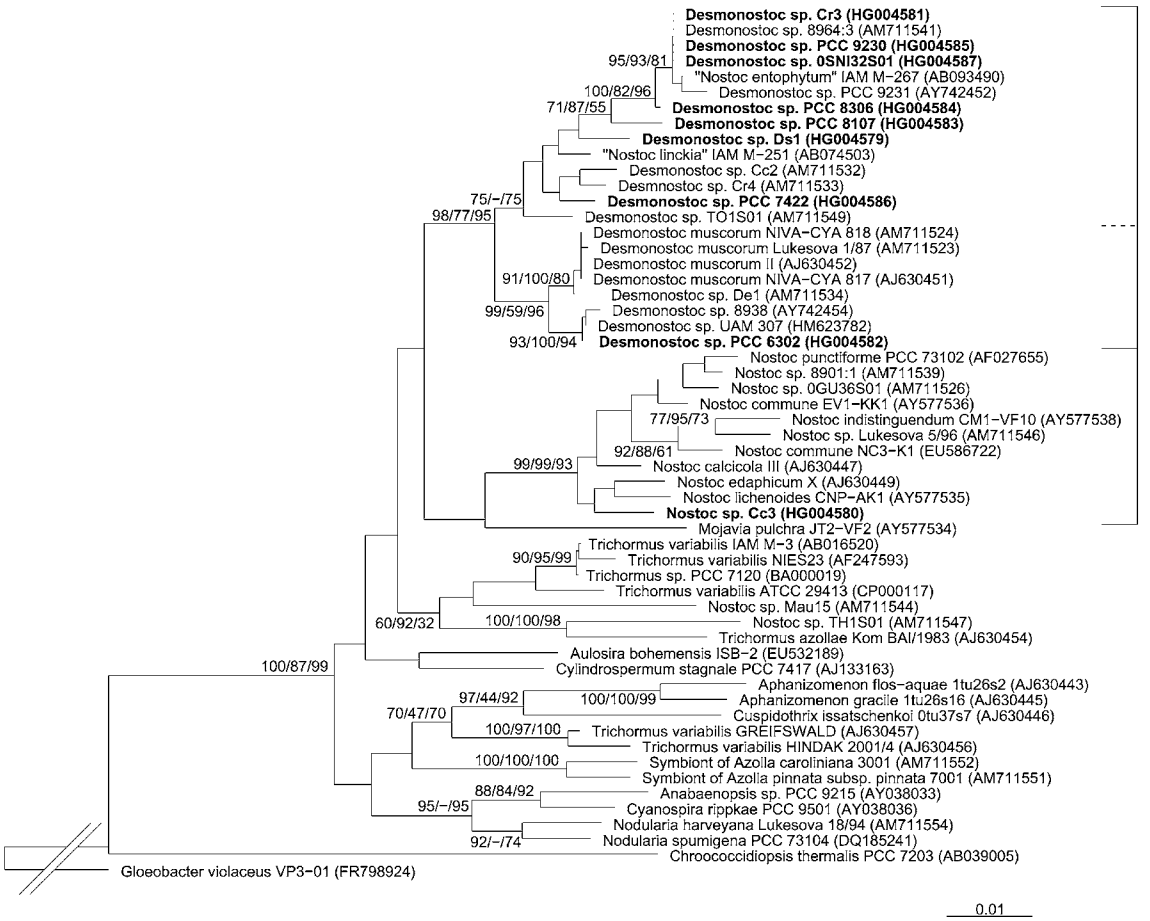

Fig 1. Phylogenetic analysis (NJ tree) based on 16S rRNA gene sequences. Clades of *Nostoc sensu stricto* (marked as N) and *Desmonostoc* cluster (marked as D) can be recognized, as well as the separation of two *Desmonostoc* subclusters (D1 and D2). The numbers above the nodes refer to NJ/ML/MP bootstrap values.

as the reference strain for morphological comparison or the type strain in the future.

A compact and stable group of three sequences (*Nostoc* sp. 8938, UAM307–MA4 and PCC 6302) belonging to strains characterized in other works, is also placed in the D2 subcluster. Strain UAM307–MA4 was isolated from a biofilm in the calcareous Marranta stream in Spain (MATEO et al. 2011), while *Desmonostoc* sp. 8938 is a symbiont of *Gunnera* sp. (SVENING et al. 2005). With the presently available data, it is not clear if this latter subgroup of three strains should be assigned to the same species *D. muscorum* as the other five strains of subcluster D2, or described as a separate taxon.

Morphological characterization did not produce clear separation of taxa nor did it strongly support the phylogenetic differences between the D1 and D2 clades (Table 2). The mean cell/filament width ranged between 3.4 and 5.7 μm. For this character, there is no significant statistical difference between *Desmonostoc* and *Nostoc* strains as tested using t–tests. Cells were isodiametric to barrel–shaped. Also the shape and dimensions of intercalary heterocytes between these two groups did not differ significantly. Previously, we noticed that in the strains of *D. muscorum*, hemispherical terminal heterocytes were lacking, and when terminal heterocytes were present they were oval–shaped and similar to intercalary heterocytes (HROUZEK et al. 2005). However, recently, Mateo and co–authors (MATEO et al. 2011), studying the life cycle of *Desmonostoc* MA4–UAM307 which clustered within the D1 subcluster (Fig.1), reported the occurrence of conical terminal cells on hormogonia from which terminal heterocytes differentiated. Also, we found that strain OSNI32s01 differentiated conical terminal heterocytes at the end of its hormogonia when they were developing into vegetative filaments (Fig. 3j). Since both these strains clustered in the D cluster with good support, the shape of the terminal heterocytes seemed to be variable within this group. We conclude that there are likely multiple species of *Desmonostoc* within our sampled strains, but we lack sufficient morphological and genetic evidence to name these taxa at present. Consequently, we only identify one species, *D. muscorum*, in the present work. Future characterization and study will likely lead workers to identify and characterize more species inside the genus *Desmonostoc*.

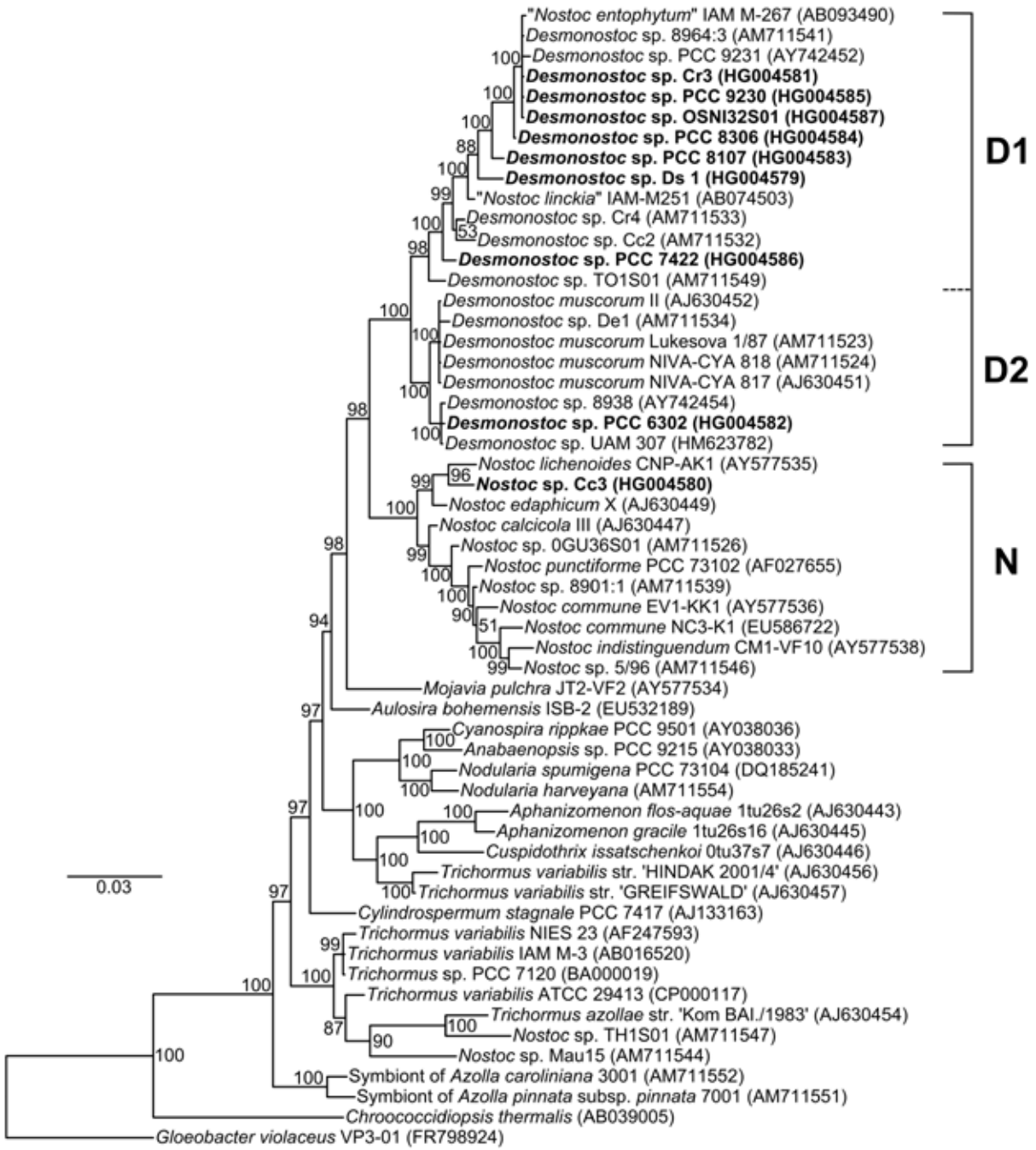

Fig 2. Bayesian inference tree inferred from 16S rRNA sequences. Posterior branch probabilities are given at the nodes. *Nostoc commune* cluster (N) and *Desmonostoc* (D) form sister clades in this tree, with *Mojavia pulchra* branching outside both clusters (cf. Fig. 1.). *Desmonostoc* further divides into two well supported subclusters (D1 and D2).

Despite the morphological similarities between D and N clusters, we found several remarkable differences between these two groups. The morphology of akinetes and vegetative filaments as well as the life cycle were consistent within *Desmonostoc* but different from *Nostoc sensu stricto*. The first similar feature among the strains of the D cluster is the type of life cycle and similar formation of mucilaginous matter. Most of the life cycle the strains are living in long vegetative filaments (Fig. 3) consisting of hundreds of cells (Hrouzek et al. 2005; Mateo et al. 2011). Densely coiled filaments with akinete–like cells (Hrouzek et al. 2003) can be observed only in early stages and not throughout the cell cycle. Instead, this latter feature is frequent in most *Nostoc* strains. The frequent presence of very long vegetative filaments is the reason for the proposed name *Desmonostoc* (from Greek desmus=chain). The filaments are embedded in diffluent mucilaginous matter (e.g. strains Cc2, De, Cr4) or are surrounded by diffluent envelopes as in strains *D. muscorum* NIVA–

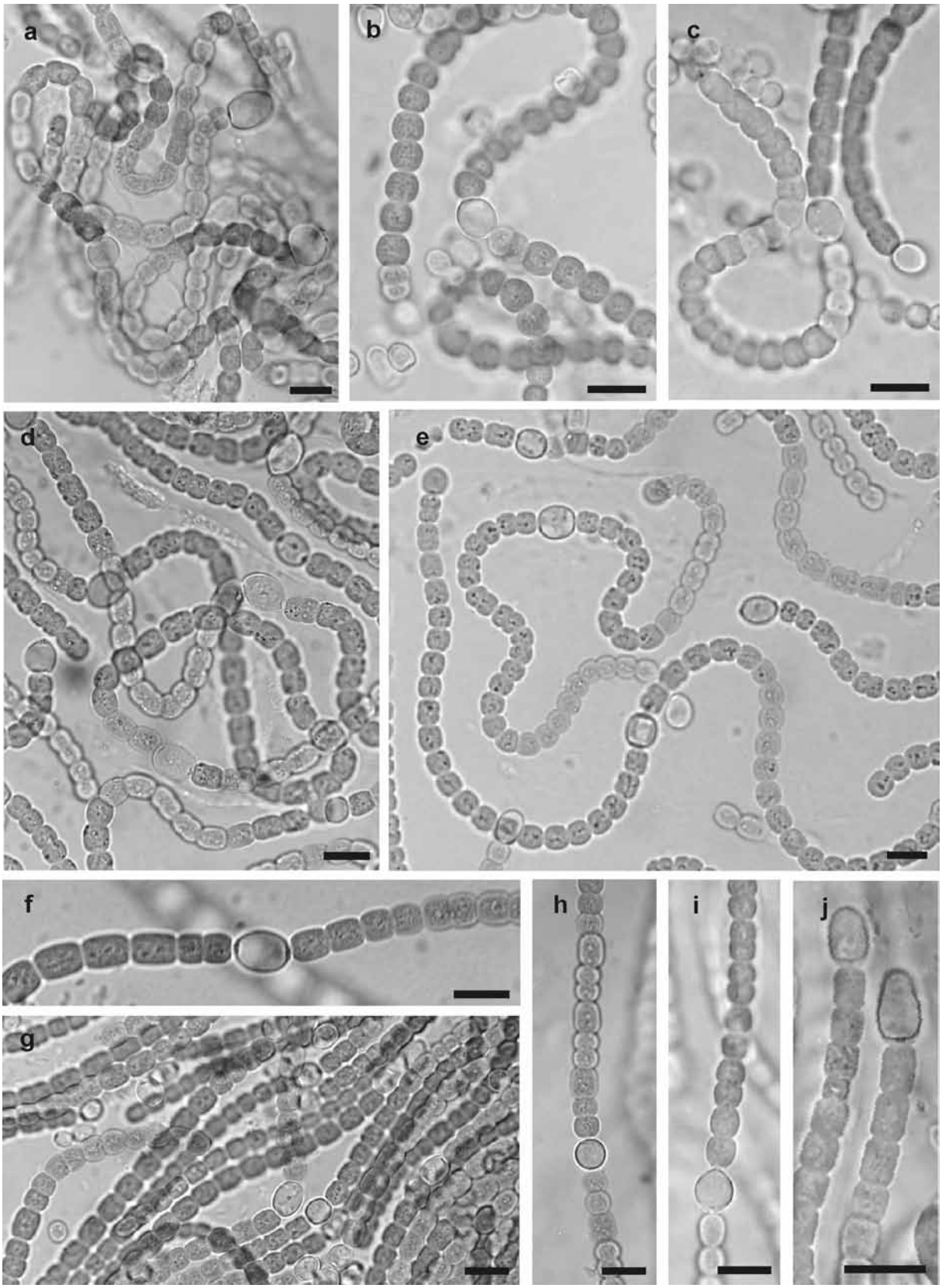

Fig 3. Vegetative filament morphology of *Desmonostoc* strains: (a) *Desmonostoc* sp. Cr4, (b) *Desmonostoc* sp. Cr3, (c) *Desmonostoc* sp. Ds, (d) *Desmonostoc muscorum Desmonostoc muscorum* NIVA–CYA 818, (e) *Desmonostoc muscorum* NIVA–CYA 817, (f) *Desmonostoc muscorum* NIVA–CYA 817, (g) *Desmonostoc* sp. Cc2, (h) *Desmonostoc muscorum* De, (i) *Desmonostoc* sp. OSNI32s01, (j) terminal heterocytes of *Desmonostoc* sp. OSNI32s01.

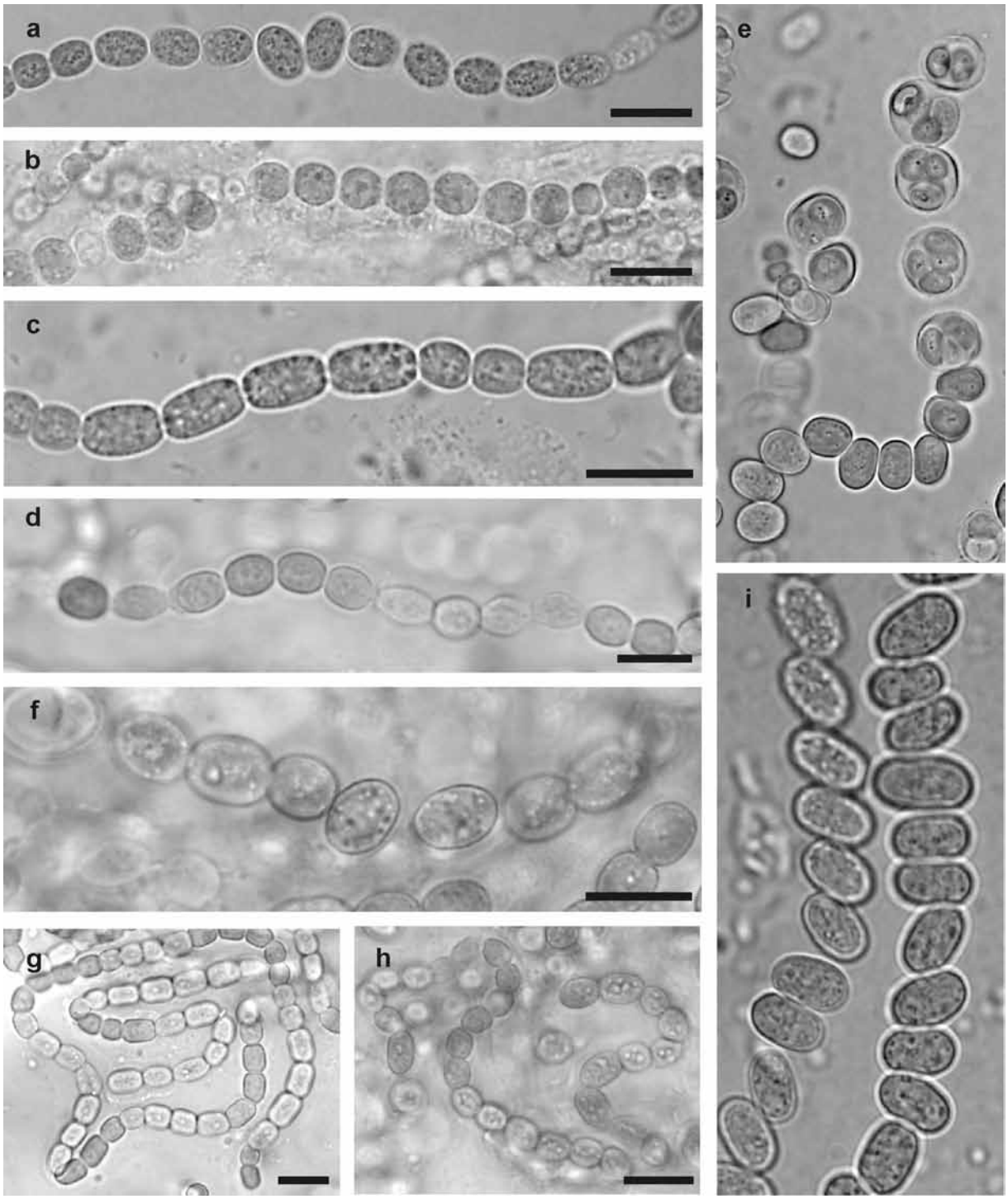

Fig 4. Akinete morphology of *Desmonostoc* strains: (a) *Desmonostoc muscorum* II, (b) *Desmonostoc muscorum* De, (c) *Desmonostoc muscorum* NIVA–CYA 818, (d) *Desmonostoc* sp. Cr4, (e) germinating akinetes of *Desmonostoc muscorum* NIVA–CYA 817, (f) *Desmonostoc muscorum* De, (g) *Desmonostoc* sp. Cr4, (h) *Desmonostoc* sp. Cc2, (i) *Desmonostoc muscorum* NIVA–CYA 817.

Table 3. Localities with significant ocurrence of *Desmonostoc muscorum* with designation of soil type and pH.

country	locality	soil type/habitat	soil type	pH
Czech Republic	Jevany (Prague)	humid cultivated meadow	cambisol	6.2
Czech Republic	České Budějovice, Zavadilka	cultivated garden soil	cambisol	5.7
Czech Republic	České Budějovice, Zavadilka	earthworm gut content	garden soil	
Czech Republic	Třeboň	wet meadow	cambisol	5.2
Czech Republic	Nezamyslice	arable field	cambisol	~ 6.2
Czech Republic	Dlouhá Ves (South Bohemia)	arable field	cambisol	5.7
Czech Republic	Dlouhá Ves (South Bohemia)	abandoned field (fallow)	cambisol	6.0
Czech Republic	Dlouhá Ves (South Bohemia)	wet meadow	cambisol	5.3
Czech Republic	Vintířov (Sokolov)	9 yrs old reclaimed post– brown coal mining site *by Alnus glutinosa* seedlings	tertiary cypris clay	7.1
Czech Republic	Vintířov (Sokolov)	young reclaimed post coal mining site	tertiary cypris clay	7.4
Czech Republic	Pastviny (Sokolov)	young spontaneously succeeded post mining sites, partly vegetated	tertiary cypris clay	7.5
Czech Republic	Dlouhá Ves (South Bohemia)	arable field	cambisol	5.8
Slovakia	Domica cave	cave sediment		7.5
USA, Indiana	Somerville mine	ca 5 yrs old reclaimed post–brown coal mining site (forest area)	silt loam	6.6
USA, Indiana	Indiana Burning Stars	ca 5 yrs old reclaimed post–brown coal mining site (long–leave prairie area)	silt loam	6.7
USA, Tennessee	Laurel grove nr. Oak Ridge	ca 5 yrs old reclaimed and spontaneousely succeded post–brown coal mining sites	loam	6 . 0 – 6.7
USA, Illinois	Carbondale, Sahara–Ashby Kolar Research Plots	different tree plantations on reclaimed or unreclaimed post– brown coal mining sites	shales	~ 7.0
USA, Illinois	Carbondale, Sahara–Ashby Kolar Research	remains of an original prairie	loam	6.8
USA , Illinois	Carbondale, Sahara Coal Company	post coal mining sites reforested by loblolly pine trees	shale	6.9
Africa, Kenya	Tsavo	shoreline of a waterhole	lateritic soil	na
Africa, Kenya	Amboseli	nearby a shallow freshwater lagoon	silt	na

CYA 817, NIVA–CYA 818, Lukešová 2/91 (HROUZEK et al. 2003, 2005) or loosely enveloping the filament as in MA4–UAM 307 (MATEO et al. 2011).

The hormogonial morphology is variable within this group, from long trichomes consisting of more than 50 cells (MA4 UAM 307, NIVA–CYA 817 and NIVA–CYA 818 (HROUZEK et al 2003, 2005, MATEO et al. 2011) to short trichomes as in strain Cr4. As it can be seen in Fig. 4, the members of *Desmonostoc* form robust and clearly recognizable akinetes (Figs 4a,b,c,d,f,i), although we observed also some exceptions in strain Cr4 (Fig. 4d). Long chains of akinetes (usually more then 10) can be observed during sporulation (Figs 4a,c,i). Comparing the akinete

dimensions of *Desmonostoc* with those of the members of *Nostoc sensu stricto*, we found that members of *Desmonostoc* had similar width, but the akinetes tended to be longer (p = 0.031). Their shape was also more elliptic (the statistic difference in length/width ratio was significant at 5% level of significance). Although the akinetes could possess a variable shape even within the same filament, the elliptic shape prevailed. Within *Desmonostoc muscorum* the akinetes were elliptic, strongly granulated and sometimes perpendicular to the filament axis (Fig. 3i). The germination of akinetes inside the filament was also observed in this strain (Fig. 3d).

Desmonostoc Hrouzek et Ventura gen. nov.

Colonies in nature usually pale to dark green to brown, with diffluent mucilage within a firmer surface layer. Filaments long, aggregated into rounded macrocolonies (up to 2 cm in diameter) or in amorphous film, firm mucilage or formation of compact micro–colonies can be observed rarely and only in primordial state or stress conditions. Cells barrel–shaped to oval, 3.5 μm wide or wider on average. Heterocytes terminal and intercalary, solitary. Akinetes frequently occurring, produced apoheterocytically in long chains (10 or more cells), variable in shape but mostly elliptical in the population, easily distinguished from vegetative cells, up to 10 μm long, up to 6 μm wide (average size exceeds 4.8 μm long and 4.2 μm wide). Reproduction both by akinetes and by motile hormogonia which are formed by fragmentation of the vegetative filaments.

Type species: *Desmonostoc muscorum* (Agardh ex Bornet et Flahault) Hrouzek et Ventura comb. nov.

Desmonostoc muscorum (Agardh ex Bornet et Flahault) Hrouzek et Ventura comb. nov.

Basionym: *Nostoc muscorum* Agardh (1812, Dispositio Algarum Sueciae, p. 44) ex Bornet et Flahault (1888, Revision des Nostocacées Hétérocystées, Ann. Sc. Nat. 7th Ser: 7:200).

Holotype specimen: Agardh Herbarium 6154 (LD), collected July 1810, from mosses in Jaders Bruk, Scania, Sweden.

Reference strain: NIVA–CYA 818

Young colonies ± hemispherical, later forming mucilaginous, amorphous mats, up to several cm in diameter, blue–green, later yellow–brown to olive–green, with densely entangled filaments. Long vegetative filaments irregularly flexuous and except for the earliest stages never forming compact microcolonies. Sheaths distinct at the margin of colonies, colourless to yellow–brown. Inside the colony only diffluent mucilage loosely enveloping filaments. Cells shortly barrel–shaped to cylindrical, shorter than wide up to isodiametric, rarely a little longer than wide, 3–5(6.5) μm long, (3.2)4.5–6.8 μm wide. Heterocytes almost spherical or barrel–shaped, (4)6–7.9 μm long, (4)4.5–6.3(7) μm wide. Akinetes occurring in long chains, oval, with smooth, colourless to yellow cell wall, (6.3)8–12 μm long, 4–8 μm wide.

Ecology of *Desmonostoc muscorum*: Strains corresponding morphologically to *D. muscorum* included in this study were found in less than 10% (ca 8%) samples studied by us. No occurrence of *D. muscorum* has been observed in soils below pH = 5.0. However, slightly acid to neutral soil pH 5.3 – 7.1 seemed to be more favourable than alkaline soil (Table 3). From studied localities, *D. muscorum* was isolated most frequently in various meadow soils and arable or abandoned fields (cultivated soils) (e.g. Lukešová 1993), and in young successional partly vegetated sites in post–coal mining sites both reclaimed by different tree species and spontaneously recovered in the Czech Republic (e.g. Lukešová 2001) and in the USA (Indiana, Illinois,Tennessee). We isolated *D. muscorum* also from fecal pellets of the millipede *Glomeris hexasticha* collected from an abandoned field (Lukešová & Tajovský 1999) or gut contents of earthworms from garden soil and found it also growing epiphytically on mosses. We have never observed mass development of *D. muscorum* on the soil surface, and have only rarely found this species as a dominant or subdominant taxon in soil samples.

The species has never been found in completely barren soils, exposed to extreme abiotic conditions, such as in polar or hot sandy deserts, post–mining or saline or other locally arid sites. In soils with pH not limiting cyanobacterial growth, water availability seemed to be the factor determining the distribution of *D. muscorum*, thus local microclimate conditions seem to be very important. According to the literature, *D. muscorum* inhabits a much broader spectrum of biotopes than we recorded in our study. In addition to meadows and cultivated soils, its occurrence was reported also from forests (Aleksachina & Shtina 1984) , paddy fields (de Halperin et al. 1992), wet moss carpets in Antarctica (Broady 1978), cryptogamic soil crusts in subpolar areas (Skuja 1964), peat bogs (Busygina 1976) aerophytic in caves and on rocks (Uzunov et al. 2008), as phycobiont (O`Brien et al. 2006), and even in cryptogamic soil crusts and soils in desert rangelands in biotopes with favourable microclimatic conditions (Johansen et al. 1984; Novičkova–Ivanova 1980). Although it is mentioned also from freshwater habitats (Aboal 1996; Leghari et al. 2005), mainly from periphyton, most reports were published from terrestrial environments.

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