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New insights in the morphology and phylogeny of heterocytous cyanobacteria from Peru, including the description of new taxa

Master's thesis

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Annotation:
Morphology and phylogeny for 36 heterocytous cyanobacterial strains are studied. Discussion with morphological, ecological, and phylogenetically related taxa is given for each strain. Potentially new genera and species are found, one of them being proposed as a novel genus.

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CONTENTS

I. INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes of crucial importance for life development on Earth. Their presence date since the Precrambian, and are the responsible for the Great Oxidation Event (GOE) (Schirrmeister et al. 2015, 2016). Cyanobacteria are able to colonize a wide spectrum of biotopes due to their ability to tolerate extreme conditions (Chrismas et al. 2015, Oren 2015). They play key roles in aquatic and terrestrial trophic chains, being the primary producers, providing organic nitrogen to other organisms, among others roles (Whitton 2012). In addition, the endosymbiosis process between a cyanobacterium and a heterotrophic eukaryote originated several life lineages on Earth (De Vries & Archibald 2017, Rodríguez-Ezpeleta et al. 2005). Due to these facts, Cyanobacteria are one of the most important organisms on Earth.

In last centuries, several approaches to study cyanobacteria taxonomy have been used. Historically, cyanobacteria were studied based only on morphological traits (Gomont, 1892, Bornet & Flahault, 1886-1888), which in several cases were found to be plastic and dependent of environmental factors (Berrendero et al. 2011). Culture techniques applied to cyanobacteria demonstrated to be a useful tool to study their taxonomy, although with some limitations (Komárek 2006). The use of molecular techniques improved our knowledge in cyanobacterial evolution (Boyer et al. 2001, Johansen & Casamatta 2005), especially the use of the 16S rRNA gene in phylogenetic analysis, which has demonstrated accurate results at genus level (Mareš 2017). In last decades, the redefinition of genus and species concept in cyanobacteria (Dvořák et al. 2015, Johansen & Casamatta 2005), and the use of the "polyphasic approach" (method that combine mainly morphological, ecological, and molecular criteria) (Komárek 2006, 2017), have led the revision and recognition of multiple taxa (Komárek et al. 2014).

Heterocytous cyanobacteria are the most recently evolved group within the cyanobacteria, and the most morphological diverse of all prokaryotes (Komárek 2013). Due their richness in morphotypes, heterocytous cyanobacteria were classified in different taxonomical levels: the Stigonematales, which show true-branching (Anagnostidis & Komárek 1990), and the Nostocales, with false-branching or no branching at all (Komárek & Anagnostidis 1989). Nowadays, we know that all heterocytous cyanobacteria derive from a common single ancestor (Tomitani et al. 2006), and that the true-branching feature arise several times in their

evolution (Gugger & Hoffmann 2004, Mareš et al. 2015), which derive in their unification into one order: the Nostocales. Families and genera within the Nostocales were classically separated based on phenotypic traits, as well with other cyanobacteria. Nonetheless, several studies using the "polyphasic approach" show that traditional taxa within the Nostocales are not monophyletic (Gugger & Hoffmann 2004, Rajaniemi et al. 2005, Sihvonen et al. 2007). For example, traditional families like Microchaetaceae or Fischerellaceae were strongly modified in their original sense, being reduced (Hauer et al. 2014), or included in other families (Komárek et al. 2014) respectively. In the same sense, traditional and widely-used genera like *Calothrix* or *Nostoc* were found to be polyphyletic (Berrendero-Gomez et al. 2016, Hrouzek et al. 2013), and are in urgent need of revision. Furthermore, other issues present in taxonomy of heterocytous cyanobacteria are: (1) having complex life cycles with different morphological stages resembling other taxa (e.g. *Cyanocohniella*, Kaštovský et al. 2014), (2) changes in morphology due to effect of different nutrient concentrations in media of cultured strains (Berrendero et al. 2008, Livingstone & Whitton 1983), and (3) having distinct genotypes with highly similar morphologies ("cryptotaxa", Řeháková et al. 2007, Shalygin et al. 2017). For all of these reasons, the heterocytous cyanobacteria are one of the most taxonomical challenging groups within the prokaryotes.

Diversity of cyanobacteria, including the heterocytous types, is not enough study, being estimated that more than half of species are still undescribed (Nabout et al. 2013). Most works on cyanobacterial diversity had occurred on temperate zones, and focused mainly in inland-aquatic biotopes (Hauer et al. 2015). Notwithstanding, recent studies on tropical and subtropical areas, using the polyphasic approach, had led to the discovery of novel heterocytous cyanobacteria (Fiore et al. 2007, González-Resendiz et al. 2018, Hentschke et al. 2016, Komarkova et al. 2013, Miscoe et al. 2016). In this sense, tropical and subtropical countries possibly harbor a huge diversity of cyanobacteria, mainly due to their variety on geography, topography and climate.

Peru is a tropical country that shows different types of climates and varied geography on its territory. The Andean cordillera, the Humboldt Current, and the trade winds are the main factors that influence climate in Peru (Tovar Narváez et al. 2010, Rundel et al. 1991, Young et al. 2002), creating different and unique ecosystems across the country (Rodríguez & Young 2000). Comprehensible, such diverse of ecosystems harbors a huge biological diversity, which makes Peru being considered one of the most biodiverse countries in the

world (MINAM 2013). In addition, Peru has two important biodiversity hotspots, including the richest and most biodiverse one on Earth: the Tropical Andes (Myers et al. 2000, Rodriguez-Mahecha et al. 2004).

Even though Peru is an important tropical country, the knowledge of its cyanobacterial diversity is outdated and incomplete. The last compilation of cyanobacteria diversity from Peru correspond to Acleto et al. (1978), which report 180 infra-generic taxa and 44 genera. Up to date, no published work using the "polyphasic approach" on any Peruvian cyanobacteria exist, with most studies based on limnological or floristic approaches. In addition, the Tropical Andes hotspot show few studies focused on cyanobacterial diversity, although some of its areas are threatened by deforestation, mining, and other human activities.

Studies on diversity heterocytous cyanobacteria from Peru using the "polyphasic approach" are urgently needed. The use of morphological evaluation, ecological characterization, and molecular techniques applied to heterocytous cyanobacteria from Peru, would possibly give new and interesting cyanobacteria not yet described, helping to solve some taxonomic problems within families and genera of the Nostocales, and increasing the knowledge of the cyanobacterial diversity in Peru.

II. MATERIAL AND METHODS

A. Site description

a. Coastal region

1. La Encantada lagoon (Fig. 1A)

La Encantada lagoon locates at 120km north of Lima (11°08'07"S 77°33'12"W), distant 5.3 km from the seashore. It has an elevation of 135 m a.s.l. and a surface area around 35 ha. Small arid hills, agricultural lands, and urbanistic territories recently formed surround La Encantada. The water has pH 8.4 – 9.9, conductivity between 4.6 – 5.8 ms/cm and salinity rises up to 4.4 ppm. White salty crusts were observed near the edge of the lagoon.

2. Lomas de Lachay (Fig. 1B)

Lomas de Lachay („Lachay hills“) is a national reserve in the desert of central Peru, 105 km north of Lima (11°22'S - 77°22'W), with an area of 5070 ha and altitudes ranging between 50 to 750 m a.s.l. (SERNANP 2013). *Lomas de Lachay* is under two climatic periods: a dry season (November to May) and a wet season (June to October). The presence of fog in the wet season is one of the most important factors for the formation of the seasonal vegetation (Arana et al. 2016, Rundel et al. 1991).

b. Andean region

1. Tuctuca lagoon (Fig. 1F)

Tuctuca lagoon locates 225 km east from Lima (11°34'26"S - 74°57'28"W), and locates in the Tropical Andes hotspot. It has an area of 6 ha, and altitude of 4309 m a.s.l. The climatic conditions are typical of the Andean valleys, dry in autumn and winter, and rainy season from December to march (SENAMHI 2008).

c. Amazon region

1. Villa Rica (Fig. 1D)

Villa Rica is a small city placing at 230 km northeast of Lima (10°44'S 75°16'W), showing a complex topography, with the highest altitude up to 2500 m a.s.l. The annual temperature rate is 21° C, annual precipitation rate of 1500 mm, and rainy season between January to May. Villa Rica belongs to "Peruvian Yungas" biogeographical region located in the western slope of the Andes, within the Tropical Andes hotspot, which has a high biological diversity (Young & Leon 1999, Tovar Narváez et al. 2010). In addition, Villa Rica has an important wetland: El Oconal lagoon. This aquatic ecosystem is characterized by the abundance of macrophytes with high diversity of associated algae and cyanobacteria (Mendoza 2015).

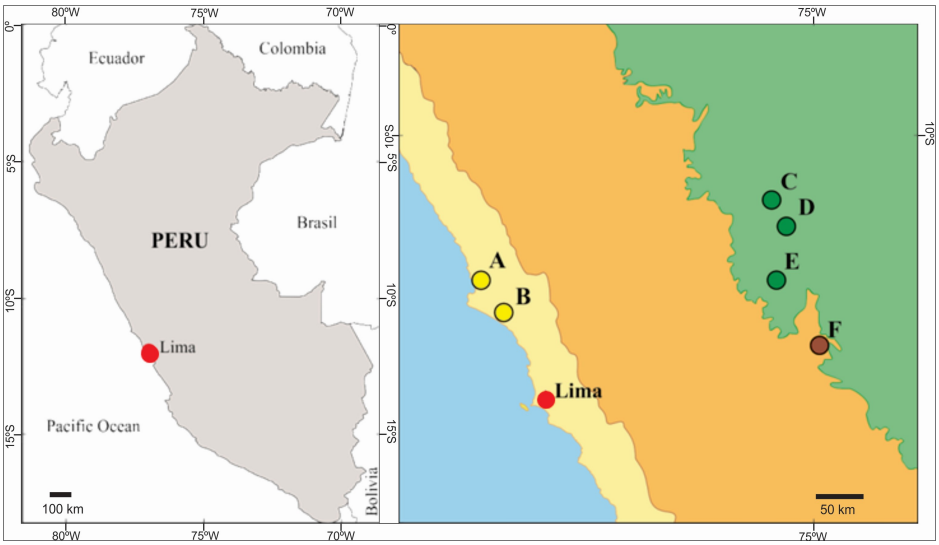


Figure 1. Left: Map of Peru and its borders. Right: Map showing localities for sampling. Colored areas show the Pacific Ocean (light blue), Coastal region (yellow), Andean region (brownish-orange) and Amazon region (green). Localities: (A) La Encantada lagoon, (B) *Lomas de Lachay*, (C) Oxapampa, (D) Villa Rica, (E) San Ramón, (F) Tuctuca lagoon.

2. Oxapampa (Fig. 1C)

Oxapampa is a city located at 230 km east from Lima (10°34'37"S 75°24'10"W). Oxapampa belongs to the "Peruvian Yungas" biogeographical region (Tovar Narváez et al. 2010), with similar characteristics to the previously mentioned for Villa Rica. Elevation is 1700 - 2500 m a.s.l., annual temperature rate of 23° C, and annual precipitation rate of 1500 mm.

3. San Ramón (Fig. 1E)

San Ramón is a small city located at 200 km east of Lima (11°07'S 75°21'W), with altitudes between 800 - 1000 m a.s.l. San Ramón belongs to the "Peruvian Yungas" biogeographical region (Tovar Narváez et al. 2010), and is surrounded by hills with montane humid forests (Young & Leon 1999). Climatic conditions are similar to Oxapampa and Villa Rica.

B. Sampling and methods of collection

Sixteen samples from terrestrial and aquatic biotopes were collected (Table 1), from which four belong to the Coastal region, one to the Andes, and eleven to the Amazon. Soil samples were collected using sterile petri dishes (6 x 1.5 cm) and a spatula, being dried and storaged. Mosses were scrapped off from the bark of the trees with the help of a spatula. Epiphyton from macrophytes were collected by scraping or cutting with a blade the submerged parts of plants (roots, stems, leaves). Crusts attached to *T. dominguensis* were obtained by cutting part of the roots, and then scraping the crusts with a sharpened blade. Samples were kept in a refrigerator box for their transportation to the laboratory.

Tables 1. List of collected samples, their habitat, origin, and collection information. Codes: LA = Lomas de Lachay, LE = La Encantada lagoon, TU = Tuctuca lagoon, SR = San Ramón, VR = Villa Rica, OX = Oxapampa.

Code	Habitat	Geographical coordinates	Locality	Region	Date of collection	Collector
LA001	Biological Soil Crust	11°23'04"S 77°22'44"W	Lomas de Lachay	Coast	Jun 2014	Cesar Arana
LA004	Biological Soil Crust	11°24'13"S 77°23'18"W	Lomas de Lachay	Coast	Jun 2014	Cesar Arana
LA007	Biological Soil Crust	11°22'27"S 77°22'30"W	Lomas de Lachay	Coast	Jun 2014	Cesar Arana
LE006	Mineralized crusts attached to roots of *Typha dominguensis*	11°08'04.1"S 77°33'02.9"W	La Encantada lagoon	Coast	9 May 2015	Leonardo Mendoza
TU003	mud associated to aquatic plants	11°34'44.6"S 74°57'05.9"W	Tuctuca lagoon	Andes	15 May 2015	Jose Roque
SR004	soil with mosses	11°08'19.2"S 75°20'21.1"W	near El Tirol waterfall, San Ramón	Amazon	22 May 2016	Cesar Quin
SR005	moss	11°08'19.9"S 75°20'12.7"W	near El Tirol waterfall, San Ramón	Amazon	22 May 2016	Cesar Quin
VR002	muddy soil	10°44'11.6"S 75°16'06.8"W	Villa Rica	Amazon	10 September 2015	Leonardo Mendoza
VR003	muddy soil	10°44'10.7"S 75°16'07.4"W	Villa Rica	Amazon	10 September 2015	Leonardo Mendoza
VR004	muddy soil	10°44'09.7"S 75°16'08.1"W	Villa Rica	Amazon	10 September 2015	Leonardo Mendoza
VR006	moss	10°45'06.7"S 75°16'10.3"W	El Oconal lagoon	Amazon	10 September 2015	Leonardo Mendoza
VR007	soil with green biofilm	10°45'05.3"S 75°16'09.8"W	El Oconal lagoon	Amazon	10 September 2015	Leonardo Mendoza
VR009	epiphytic in *Hydrocotyle bonarienses*	10°45'12.1"S 75°16'15.6"W	El Oconal lagoon	Amazon	10 September 2015	Leonardo Mendoza
VR010	epiphytic in *Thelypteris interrupta*	10°45'07.6"S 75°16'18.1"W	El Oconal lagoon	Amazon	10 September 2015	Leonardo Mendoza
VR011	epiphytic in *Miriophyllum aquaticum*	10°45'10.0"S 75°16'22.7"W	El Oconal lagoon	Amazon	10 September 2015	Leonardo Mendoza
OX001	mosses 6-8 m high in bark of tree	10°32'43"S 75°22'16"W	near Yanachaga-Chemillén National Park, Oxapampa	Amazon	11 August 2014	Bryan Espinoza

C. Isolation and cultivation techniques

For cyanobacterial isolation, aliquots from samples were inoculated to petri dishes (10x12cm) with solidified nutrient media (1.5% agar). For soil samples, small subsamples were hydrated with sterile distilled water, later transferred to the petri dishes, and were spread using a glass spatula. For mosses, short pieces of thallus and single leaves were cut and placed in equidistant positions on the agar plate. For mineralized crust sample, a subsample was crushed with a sterilized glass stick, and then scattered on the agar plate.

After 1 to 3 weeks, mixed colonies and filaments of cyanobacteria were detected. Single filaments per morphotype observed were picked up with a sterile Pasteur pipette for verification on the presence of any contaminant under the optical microscope. Healthy filaments were transferred each one to a single well of millipore microplates (8 x 12.5cm, 24 wells, well area 2.5cm^2) containing solidified nutrient media (1.5% agar). After, 2 to 4 weeks, mass growing on the microplates were transferred to sterilized agar slant tubes with growth medium. The same media were used for each isolation in each transferring step. All the process of growing of cyanobacteria were performed with 16:9 light:dark cycle at 22°C.

Three culture media were used to evaluate possible morphological variations of studied cyanobacteria. CHU10 medium (Chu 1942) was modified to give a final phosphorus concentration of 0.2 mg/l (Berrendero et al. 2008), being a low nutrient medium. BG-11$_0$ (Rippka et al. 1979) is the most used medium for cultivation of cyanobacteria, and has higher nutrient concentrations than CHU10. Z8 (Kotai 1972) has a higher nutrient concentrations compared to CHU10 and BG-11$_0$, being suitable for soil cyanobacteria. All media were prepared without nitrogen source to promote growing of heterocytous types, and 0.1 µg/ml cycloheximide (Biochemica, Applichem GmbH, Germany) was added to avoid eukaryote contamination.

D. Morphological characterization

Morphological observations were performed using an Olympus SZ51 Stereo Microscope and Olympus BX 51 microscope equipped with Nomarski DIC optics and Olympus DP71 digital camera for micrographs. Morphological measurements were performed using ImageJ (https://imagej.nih.gov/ij/) from micrographs obtained, measuring at least 20 trichomes and filaments per strain. To investigate strain morphology throughout the cyanobacterial life cycle, observations of cultures were made every three to six months. Strains were identified

using Geitler (1932), Komárek & Anagnostidis (1989), Komárek (2013), and papers of heterocytous cyanobacteria from topical areas. Features considered for descriptions were the shape of colony; morphology and measures of filaments, trichomes, cells, and hormogonia; presence/absence of terminal hairs, sheath, branching; and position of heterocytes, akinetes and arthrospores along the trichome. Figures were prepared using ®CorelDraw2017 v.19.1.

E. Molecular characterization

a. DNA extraction and PCR amplification

A total of 60-100 mg of cyanobacterial strain biomass was dried for 48 to 72 hours in silica gel and then pulverized in a Retsch MM200 mixer mill (Retsch GmbH, Haan, Germany) for 8 min at 24 s^{-1}, using wolfram carbide beads and lysis buffer of the genome isolation kit. Isolation of total genomic DNA was performed using Invisorb™ Spin Plant Mini Kit (STRATEC Molecular GmbH, Berlin, Germany), following the manufacturer's instructions, and stored at -20°C until their use.

Amplification of almost complete 16S rRNA gene, adjacent 16S-23S internal transcribed spacer (ITS) region, and a small fragment of 23S rRNA gene (total of 2,300 – 2,700 bp) was performed with the primers pA (Edwards et al. 1989) and KP.591R (Haugen et al. 2007). Each PCR reaction contained 10 ng of DNA, 10 µL Master Mix (Plain PP Master Mix, Top Bio, Czech Republic, Prague) with Taq polymerase, and 6 pmol of each primer, with a final volume of 20 µL. The conditions described by Gkelis et al. (2005) were used. Amplified DNA fragments were checked by agarose gel electrophoresis.

b. Ligation, transformation, and clone selection

PCR products were purified by electrophoresis for 45 min at 60 V using 1.5% low melting point agarose gel and TAE buffer, being cloned using the pGEM-T Easy vector system (Promega Corp., Madison, WI, USA) and chemically competent cells according to manufacturer's specifications. Eight clones were randomly selected and checked for the correct insert using the cyanobacteria-specific primers and PCR conditions described by Nübel et al. (1997). All positive clones were checked again for distinguishing different 16S-23S ITS regions using the primers CYA 781Fa (forward complement of CYA 781Ra, Nübel et al. 1997) and KP.591R (Haugen et al. 2007), and PCR conditions described by Gkelis et al. (2005). At least three clones of each ITS type observed were selected to grow in liquid LB medium with ampicillin.

c. Isolation of plasmidic DNA and sequencing

The plasmids were purified using UltraClean® Standard Mini Plasmid Prep Kit according to manufacturer's instructions. Sequencing was performed by the company SEQme (Dobříš, Czech Republic), using the original primers for amplification, primer 14 (Wilmotte et al. 1993), and 16S1494R (Wilmotte et al. 2002).

Table 2. List of primers used in this study

Primer	Type	Sequence	Author
pA	Forward	AGA GTT TGA TCC TGG CTC AG	Edwards et al. 1989
KP.591R	Reverse	TCG CCG GCT CAT TCT TCA	Haugen et al. 2007
14	Forward	TGT ACA CAC CGC CCC GTC	Wilmotte et al. 1993
16S1494r	Reverse	GTA CGG CTA CCT TGT TAC GAC	Wilmotte et al. 2002
CYA 359F	Forward	GGG GAA TYT TCC GCA ATG GG	Nübel et al. 1997
CYA 781Ra	Reverse	GAC TAC TGG GGT ATC TAA TCC CAT T	Nübel et al. 1997
Cyano 7f*	Forward	AAT GGG ATT AGA TAC CCC AGT AGT C	Nübel et al. 1997

* reverse complement to primer CYA781Ra (Nübel et al. 1997)

F. Phylogenetic analysis

a. Selection of sequences and alignment

16S rRNA gene sequences obtained were compared with available sequences in the National Center for Biotechnology Information (NCBI) database using BLAST (http://www.ncbi.hlm.nih.gov/BLAST), and with internal dataset of sequences from PhD. Esther Berrendero. Closely related sequences from NCBI and the internal database, three outgroups (*Gloeobacter violaceus* PCC 7421, *Chroococcidiopsis thermalis* PCC 7203, *C. cubana* SAG 39.79), and sequences obtained in this study were aligned using MAFFT v.7.380 (Katoh et al. 2017), with G-INS-I algorithm and default parameters, checked using BioEdit 7.0.1 (Hall 1999). Final alignment had sequences of 1,000 - 1,514 bp lenght.

b. Construction of phylogenetic trees and calculation of sequence identities

Four trees were built: "Rivulariaceae Tree" (RT), "Scytonemataceae Tree" (ST), "Fortieaceae – Tolypothrichaceae Tree" (FTT), and "Aphanizomenonaceae – Nostocaceae – Hapalosiphonaceae Tree" (ANHT), all comprising 16S rRNA gene sequences from almost all heterocytous cyanobacteria, and each tree with more sequences from specific taxa according

to their families. The total number of sequences used for tree construction is 189, 185, 196, and 227 for RT, ST, FTT, and ANHT respectively.

For all trees, GTR+I+G evolutionary model was selected using the Akaike Information Criterion in jModelTest2 v.2.1.6 (Darriba et al. 2012), and used for the Bayesian Analysis (BA) and Maximum Likelihood (ML). BA was performed in MrBayes v.3.2.6 (Ronquist et al. 2012), with two runs of eight Markov chains and 22 million generations, sampling every 100 generations, temperature of 0.08 (except for ANHT, temp=0.06), and 25% burn-in. The exact parameters for the models were taken from jModelTest2 (table 3). ML was run using RAxML v.8.2.10 (Stamatakis et al. 2008) with 1,000 bootstrap replicated, and default parameters. jModelTest2, BA, and ML analysis were run using the CIPRES Portal (Miller et al. 2012). In BA, the trees had final average standard deviation of split frequencies < 0.01, and estimated sampled size (ESS) of 2289.34 – 9201.79 for all parameters, above the average of 200 accepted as sufficient by phylogeneticists (Drummond et al. 2006). The potential scale reduction factor (PSRF) was 1.000 – 1.002 for all the parameters in BA, indicating the statistical convergence of the MCMC chains (Gelman & Rubin 1992). The topology from BA is showed; each node contains the support values from BA and ML respectively. Colored circles were drawn next to each taxa on the trees, with colors according to type of climates following Peel et al. (2006), while triangles and squares indicate two broad habitats: terrestrial or aquatic, respectively, with colors showing specific habitats. Trees were viewed using FigTree v.1.4.2 (Rambaut 2012), and re-drawn using Inkscape v.0.91. The identities of 16S rRNA gene sequences were obtained with the formula: 100*[1-(p-distance)]. P-distances were calculated in MEGA v.7 (Kumar et al. 2016).

Table 3. List of parameters obtained from jModelTest2 and used in BA. f = base/codon frequency, R=substitution rate, α=alpha parameter of the gamma distribution, i=proportion of invariant sites.

Parameter	RT	ST	FTT	ANHT
f(A)	0.26	0.27	0.25	0.26
f(C)	0.22	0.22	0.23	0.22
f(G)	0.31	0.32	0.31	0.30
f(T)	0.21	0.19	0.21	0.22
R(A↔C)	0.853	0.807	0.907	0.957
R(A↔G)	2.015	1.734	2.016	2.139
R(A↔T)	1.345	1.313	1.200	1.190
R(C↔G)	0.524	0.476	0.480	0.565
R(C↔T)	2.832	3.062	2.586	2.897
R(G↔C)	1.000	1.000	1.000	1.000
α	0.58	0.58	0.60	0.58
i	0.53	0.53	0.59	0.55

III. RESULTS

A total of 36 strains were obtained in the present study, from which five belong to the coastal region in Peru, one to the Peruvian Andean region, thirty to the Peruvian Amazon, one from Czech Republic, one from an unknown locality in the United States of America, and one from Kenya (table 4). Strains T014, T064, T067, T030, T035, T019, T029, and T044 correspond to *Calothrix*, with strain T014 identified as *Calothrix* cf. *elenkinii* and strains T064 and T067 as *Calothrix* cf. *marchica*. Strains T027 and P3C2e correspond to *Skacelovkia*, a novel genus similar to *Calothrix*, with strain T027 designated as *S. peruviana* sp. nov., type species of the genus. Strains T008, T017 and T048 correspond to a cyanobacteria showing features from different genera, provisionally identified as "*Dichothrix*". Strains T001 and T076 correspond to *Brasilonema*, while T001 identified as *B. octagenarum* Aguiar et al. Strains T051, T056 and T063 belong to *Scytonema*. Strain T037 resembles *Calochaete* in morphology, confirmed after phylogenetic evaluation. Strain T054 resembles *Camptylonemopsis* in morphology, identified as *Camptylonemopsis* cf. *pulneyensis*. Strains T042, T047, T060, JOH06, and JOH42 correspond to *Microchaete* in morphology, with strains T042 and T047 being designated as *Microchaete* sp. type 1, and T060 as *Microchaete* sp. type 2. Strains T002 and T079 belong to *Tolypothrix*, with only T002 idenfitied as *Tolypothrix* cf. *helicophila*. Strains T061, T075 and T081 fit the description of *Hassallia*, and strains T061 and T081 were identified as *H. californica* Johansen et Flechtnet. Strains T033 and T065 correspond to *Anabaena*. Strains T020 and T082 belong to *Trichormus*, being identified as *T. variabilis* (Kützing ex Bornet et Flahault) Komárek & Anagnostidis, and *Trichormus* cf. *indicus* respectively. Strain T049 corresponds to *Nodularia*, while strain T028 to *Westiellopsis*.

The genera *Brasilonema, Calochaete*, *Camptylonemopsis*, *Hassallia*, *Trichormus*, and *Westiellopsis* and the species *Calothrix* cf. *marchica*, *Calothrix* cf. *elenkinii*, *B. octagenarum*, *H. californica*, *Tolypothrix* cf. *helicophila*, *Trichormus* cf. *indicus*, and *T. variabilis* are new records for Peru. The present study is the second, after Acleto (1969), which reports aerophytic cyanobacteria from mosses in Peru. Furthermore, this work is the fourth that study cyanobacteria from the Peruvian Yungas biogeographic zone, within the Tropical Andes hotspot, after Acleto (1969), Young & León (1990), and Huapalla (2000).

Table 4. List of isolated strains used in this study, including information their origin, media used for isolation, and type of sequences obtained.(*)=sequences obtained by PhD. Esther Berrendero.

Taxa	Strain code	Information of the strain		Sequences information		
		Original Sample	Media	# 16S rRNA sequences	ITS with no tRNA	ITS with $tRNA^{Ile}$ and $tRNA^{Ala}$
Calothrix cf. *elenkinii*	T014	VR003	CHU10-N	2	X	X
Calothrix cf. *marchica*	T064	JU005	Z8-N	1	X	X
	T067	JU004	Z8-N			X
Calothrix sp. type 1	T030	VR002	CHU10-N	1	X	
Calothrix sp. type 2	T035	VR006	CHU10-N	2	X	X
Calothrix sp. type 3	T019	VR006	CHU10-N	2	X	X
Calothrix sp. type 4	T029	VR011	CHU10-N	1	X	X
Calothrix sp. type 5	T044	LE006	BG11-N	1		X
Skacelovkia peruviana sp. nov.	T027	VR009	CHU10-N	1	X	X
Skacelovkia sp.	P3C2e*	-	CHU10-N	1	-	-
"*Dichothrix*" sp.	T008	VR010	BG11-N	2	X	X
	T017	VR011	CHU10-N			X
	T048	VR009	BG11-N		X	X
Brasilonema octagenarum	T001	VR006	BG11-N	1	X	X
Brasilonema sp.	T076	JU004	Z8-N	1	X	X
Scytonema sp. type 2	T063	LA001	Z8-N	1		X
Scytonema sp. type 1	T051	VR003	Z8-N	1	X	
	T056	VR004	Z8-N	1	X	X
Calochaete sp.	T037	VR006	CHU10-N	1	X	X
Camptylonemopsis cf. *pulneyensis*	T054	VR007	Z8-N	1	X	X
Hassallia californica	T061	LA001	Z8-N	2		X
	T081	LA004	Z8-N			X
Hassallia sp.	T075	LA007	Z8-N	1		X
Microchaete sp. type 1	T047	VR002	BG11-N	2	X	X
	T042	VR002	BG11-N	1	X	X
Microchaete sp. type 2	T060	OX001	Z8-N	1 1	X	X
-	JOH6*	-	Z8-N			
-	JOH42*	-	Z8-N			
Tolypothrix sp.	T079	JU004	Z8-N	1		X
Tolypothrix cf. *helicophila*[2]	T002	VR006	BG11-N	1		X
Anabaena sp. type 1	T065	VR007	Z8-N	1	X	X
Anabaena sp. type 2	T033	VR006	CHU10-N	1	X	
Nodularia sp.	T049	VR002	BG11-N	1	X	X
Trichormus variabilis	T020	VR002	CHU10-N	1	X	X
Trichormus cf. *indicus*	T082	JU003	Z8-N	1	X	X
Westiellopsis sp.	T028	VR003	CHU10-N	1		X

A. Family Rivulariaceae

Thirteen strains belonging to ten taxa are described in this section. Nine *Calothrix* morphotypes were isolated, from which two belong to a new genus *Skacelovkia* gen. nov. Three strains show features from *Dichothrix, Scytonematopsis* and *Calothrix*, thus, they were provisionally named "*Dichothrix*". Measurements of main morphological traits are in table 5, while comparison of main features of "*Dichothrix*" with similar genera are listed in table 6. The Rivulariaceae Tree is represented in Fig. 3.

a. Morphological and ecological descriptions

Group I. *Calothrix* Freshwater & Soil

1. *Calothrix* cf. *elenkinii*, strain T014 (Fig. 6 A-C): Heteropolar filament up to ± 800 µm, with one basal heterocyte (Fig. 6B), strongly and densely coiled trichomes (Fig. 6 A,C) within the filament sheath, no hair-like ending trichome in low nutrient media (fig. 6B). *C.* cf. *elenkinii* was isolated from muddy soil with some Poaceae plants near, in Villa Rica.
2. *Calothrix* cf. *marchica*, strains T064, T067 (Fig. 6 D-J): Long heteropolar filaments (up to ± 1,200 µm) without hair (fig.6E), one basal heterocyte (fig. 6D), simple (Fig. 6F) or double (Fig. 6G) false branching rarely observed. Akinetes ellipsoidal to slightly oval, 6.6 – 12.5 x 3.4 – 5.4 µm (Fig. 6 H, I). Hormogonia from 2 cells up to more (Fig. 6J). Both strains were isolated from moss samples near El Tirol waterfall in San Ramon.
3. *Calothrix* sp. type 1, strain T030 (Fig. 6 K, L): Heteropolar filaments without hair-like ending trichome, bearing one basal heterocyte (Fig. 6K), simple false branching (Fig. 6L), and with distinctive circular mucilaginous thickening at the end of the trichome (Fig. 6K, with arrow). Strain T030 was isolated form muddy soil sample in Villa Rica.
4. *Calothrix* sp. type 2, strain T035 (Fig. 6 M-O): Heteropolar filaments with simple false branching (Fig. 6N). Trichomes ending in conically rounded cells, and coiling within the sheath (Fig. 6M). One or two basal heterocytes (Fig. 6O), but intercalary heterocytes also present. Strain T035 was isolated from moss sample located on the entrance of El Oconal lagoon, with young herbaceous vegetation.
5. *Calothrix* sp. type 3, strain T019 (Fig. 6 P-R): Long heteropolar filaments (up to ± 700 µm), no hair-like ending trichome (Fig. 6P), but sometimes the last two to three apical cells where longer than usual and colorless (Fig. 6Q). One or rarely two basal heterocytes (Fig. 6R). Strain T019 was obtained from moss sample on the entrance of El Oconal lagoon, same as in *Calothrix* sp. type 2.

Table 5. Measurements of main morphological features from *Calothrix*-like strains isolated in the present study.

Taxa	Vegetative cells (lenght)			Trichome (width)			Filament (width)			Basal Heterocyte	
	Basal	Middle	Apical	Basal	Middle	Apical	Basal	Middle	Apical	Lenght	Width
Calothrix cf. *elenkinii*	2.9 - 6.2	2.0 - 6.6	1.9 - 7.1	5.5 - 14.6	3.8 - 8.2	3.3 - 6.4	6.0 - 15.1	4.2 - 10.8	3.3 - 12.6	5.0 - 12.0	6.4 - 12.8
Calothrix cf. *marchica*	2.6 - 7.4	3.0 - 5.8	2.4 - 8.8	4.4 - 8.2	2.4 - 5.0	2.3 - 4.3	4.8 - 8.6	3.1 - 5.4	2.7 - 4.5	5.5 - 11.5	4.4 - 7.7
Calothrix sp. type 1	2.8 - 9.9	3.0 - 11.4	3.5 - 10.5	4.4 - 12.5	3.2 - 6.6	2.3 - 5.0	5.5 - 12.9	4.1 - 7.9	4.1 - 7.7	4.5 - 13.7	4.5 - 13.4
Calothrix sp. type 2	2.0 - 4.3	1.6 - 3.3	1.8 - 3.5	6.3 - 9.1	2.7 - 3.7	3.2 - 4.2	6.7 - 10.4	3.7 - 4.7	3.7 - 5.2	5.4 - 13.4	5.4 - 11.0
Calothrix sp. type 3	2.8 - 7.5	2.1 - 6.1	2.0 - 6.1	6.9 - 13.3	4.8 - 7.6	3.9 - 6.4	8.2 - 16.5	5.7 - 9.0	5.6 - 8.8	4.3 - 8.9	6.9 - 13.8
Calothrix sp. type 4	2.0 - 7.8	1.6 - 5.5	3.6 - 10.6	6.7 - 17.1	5.0 - 10.6	2.8 - 4.1	7.5 - 21.3	5.9 - 13.8	-	3.5 - 12.8	6.0 - 14.7
Calothrix sp. type 5	1.6 - 6.2	1.4 - 5.1	1.5 - 10.4	2.4 - 11.2	2.0 - 6.8	1.1 - 3.4	2.4 - 13.9	2.1 - 7.4	1.8 - 3.9	2.5 - 8.8	1.5 - 10.7
Skacelovkia peruviana sp. nov.	2.7 - 5.1	1.5 - 4.1	1.9 - 3.9	4.1 - 6.9	1.8 - 4.4	1.5 - 3.8	4.2 - 7.1	2.0 - 4.5	1.6 - 3.8	4.8 - 8.7	4.2 - 7.6
Skacelovkia sp.	3.2 - 6.2	3.2 - 5.1	2.4 - 3.7	3.8 - 6.7	3.8 - 5.3	2.4 - 3.3	5.4 - 8.4	4.6 - 6.7	-	2.0 - 6.0	2.1 - 7.3

Group II. *Calothrix* Brackish - I

6. *Calothrix* sp. type 4, strain T029 (Fig. 7 A-H): Young filaments show U or C-like curvature (Fig. 7E), but old filaments show this feature only at basal parts (Fig. 7F). Young trichomes straight, with no polarity (Fig. 7B), later becoming heteropolar and curved (Fig. 7C). Trichomes ending in shortly and roundly conical cells. False branching observed in old stages (Fig. 7G). Hormogonia isopolar, joined together and parallel disposed, forming green spots when observed at low magnification (Fig. 7H). Strain T029

was isolated from epiphyton attached to the roots of *Miriophyllum aquaticum* in El Oconal lagoon, with brownish detritus particles.

Group III. Rivulariaceae sensu stricto

7. *Calothrix* sp. type 5, strain T044 (Fig. 7 I-F): Thallus irregularly distributed in the surface, penetrating the agar, with creeping and some erect filaments, not forming fascicles. Filaments solitary, rarely irregularly grouped (Fig. 7I), ± irregularly coiled, flexuous, becoming narrowed toward ends, ending in hyaline hairs (Fig. 7J). Single false branching common (Fig. 7L), double false branching rarely observed. Sheath colorless (Fig. 7 R-S) to yellowish-brown (Fig. 7T), firm to sometimes funnel-like opened at the ends or along the filaments (Fig. 7 R-S). Trichomes cylindrical, sometimes coiling, constricted at cross-walls (Fig. 7 M, R). Cells are ± barrel-shaped, shorter than wide to ± isodiametric; apical cells conically rounded without hairs (Fig. 7N), or hyaline and longer than wide when forming hairs (Fig. 7J). Heterocytes at basal and intercalar positions, single or up to four in a row (Fig. 7M). Basal heterocytes hemispherical, rounded, sometimes conically rounded, rarely ± ellipsoidal. Sub-basal heterocytes shortly cylindrical to barrel-shaped, sometimes hemispherical with flat ends to roundly quadratic. Intercalar heterocytes cylindrical, rarely discoid, flattened rounded (Fig. 7M). Hormogonia isopolar (Fig. 7N) or slightly polarized (Fig. 7O), later becoming clearly heteropolar (Fig. 7Q), with shortly cylindrical cells. In fresh material *Scytonematopsis*-like stages frequent, with up to four intercalar heterocytes joined (Fig. 7M). The filaments are longer, usually incrusted in mineralized substrates (Fig. 7S), with only basal or main part of trichomes being outside the substrate. The mucilaginous sheath is thicker (Fig. 7R), usually lamellate and brownish or yellowish (Fig. 7T), especially in parts of the trichome within the substrate. *Calothrix* sp. type 5 was isolated from a mineralized crust attached to submerged roots of *Typha dominguensis* in La Encantada lagoon. The crusts were thick and hard in consistence, with some *Leptolyngbya* and *Phormidium* filaments, and a high number of small pennate diatoms.

Group IV. *Skacelovkia*

8. *Skacelovkia* Mendoza-Carbajal & Berrendero-Gómez *gen. nov.*

Description: Heterocytous cyanobacterium with heteropolar thallus. Filaments creeping, solitary or in irregular clusters, not forming colonies. Simple or double false branching rarely observed, especially in old stages. Adult filaments up to 1 mm long. Sheath colorless, hyaline, firm, attached to trichomes, thin, sometimes layered and thickened in old cultures.

Trichomes always constricted at cross walls, isopolar in very young stages, becoming tapered with age. Cells ± isodiametric, barrel shaped to cylindrical. End cells conically rounded. No hair-like ending trichomes. One or rarely two basal heterocytes. Intercalar heterocytes only before false branching formation. Reproduction by production of hormogonia, disintegration of trichomes by necridic cells, liberation of secondary branches, or breakage of trichome in the apical part.

Etymology: Named in honor of Olga Lepšova-Skacelová.

Type species: *Skacelovkia peruviana* Mendoza-Carbajal & Berrendero-Gómez *spec. nov*.

Diagnosis: Morphologically indistinguishable from *Calothrix*, and similar to *Macrochaete*, but different from the latter because *Skacelovkia* does not produce long hyaline hairs in low phosphorous media, and have different cell shape. Phylogenetically distant from *Calothrix, Macrochaete*, and from other heteropolar genera (*Rivularia*, *Cyanomargarita*, *Gloeotrichia*).

9. *Skacelovkia peruviana* Mendoza-Carbajal & Berrendero-Gómez *spec. nov.*, strain T027 (Fig. 8 A-C)

Description: Filaments solitary, heteropolar, up to ± 300 µm long, straight to irregular curved (Fig. 8A), single false branching only in old cultures (Fig. 8B). Sheaths thin, colourless, not lamellated. Trichomes constricted at cross walls, narrowing towards ends without hair-like formation (Fig. 8A), wider in lower parts only in developed trichomes (Fig. 8B). Cells barrel-shaped, cylindrical or quadratic, ± isodiametric, apical cell conically rounded. Heterocytes single or rarely in pairs, hemispherical, rounded, or widely conically rounded (Fig. 8 A, B); intercalar heterocyst rarely present (Fig. 8C). Hormogonia isopolar, becoming heteropolar before heterocyst formation, sometimes resembling *Phormidium* trichomes.

Type locality: Peru, Oxapampa, El Oconal lagoon, epiphyton attached to *Hydrocotyle* 10°45'12.1"S 75°16'15.6"W, 190 m a.s.l., 10 September 2015. Collector: Leonardo Mendoza.

Etymology: from the Latin *peruviana* (= from Peru), referring to the country where the taxon was obtained.

Ecology and distribution: Epiphytic, attached to *Hydrocotyle* stems with detritus in a tropical lagoon, "Peruvian Yungas" biogeographical region, Tropical Andes hotspot.

Observations: When isolated, the trichome resembled *Phormidium* because the lack of heterocytes and being notoriously long. The branches are frequently observed in old cultures, but are almost absent at young phases. Hair endings not formed even when strains are cultured in low nutrient media. Morphological description is based on cultures.

10. *Skacelovkia* sp. strain P3C2e (Fig. 8 D-G)

Description: Filaments heteropolar, solitary, straight to irregular curved (Fig. 8E), pale blue green color. Sheaths thin, colourless, rarely widely lamellated (Fig. 8E). Single false branching only in old cultures, double false branching less frequent (Fig. 8D). Trichomes constricted at cross walls, narrowing towards ends with no hair-like formation, wider in lower parts only in developed trichomes, with necridic cells. Cells cylindrical, rounded to barrel-shaped, usually shorter than wide up to isodiametric, ending in conically rounded apical cells (Fig. 8G). Heterocytes single or rarely in pairs, hemispherical to rounded. Hormogonia isopolar when released, rapidly becoming heterpolar (Fig. 8F).

Type locality: Czech Republic, Kutnař. Collector: Olga Lepšova-Skacelová. Isolator: Esther Berrendero Gómez

Ecology and distribution: wet stone on the pond.

Observations: The branches are frequent in old stages, and mucilaginous sheath becoming wider with age. Morphological description bases exclusively on cultures.

Group V "*Scytonematopsis contorta*" group

11. "*Dichothrix*" sp., strains T008, T017 and T048 (Fig. 9): Colonies on the agar surface, at first formed as small aggregations of filaments attached by their basal heterocytes next to each other, later filaments become radially oriented to the center of the flattened hemispherical colonies but without common mucilage enveloping the colony (Fig. 9 A, B). Mature colonies macroscopically observed as dark brown circles on the agar, in old cultures with some fascicles perpendicularly arising from the colony. Bush-like fascicles (*Dichothrix*-like stage) part of mature colonies, or separated (Fig. 9C). *Calothrix*- (Fig. 9I) or *Dichothrix*-like stages (Fig. 9C) more frequent, sometimes *Scytonematopsis*-like stages present, which arise from hormogonia formed at basal parts of *Calothrix*-like filaments (Fig. 9 D-H). Sheaths colorless to slightly yellowish, delimited, attached, usually extending the trichomes end, more evident when trichomes are coiling, thin in young cultures, lamellated in old stages, sometimes at the end of filaments funnel-like widened (Fig. 9N). False branching simple (Fig. 9M) or double (Fig. 9J). Trichomes blue-green, dark green or slightly brownish green in color, constricted at cross walls (Fig. 9F), continuously narrowed towards ends (Fig. 9I), sometimes coiling inside the sheath (Fig. 9M), forming long hair-like endings in low nutrient media (Fig. 9 O, P). Cells quadratic, cylindrical, barrel-shaped, flattened rounded, shorter than wide up to ± isodiametric. Heterocytes yellowish, green, or light brownish color, heteromorphic. Basal heterocytes hemispherical to rounded, conical to conically rounded

(Fig. 9K), sometimes flattened or elongated, up to four functional heterocytes joined. Intercalar heterocyts cylindrical, sometimes barrel-shaped (Fig. 9L), up to two joined. Hormogonia of two types: Heteropolar hormogonia with basal cell conically rounded, inner cells barrel-shaped and constricted, with high motility, resembling hormogonia from *Rivularia* (Fig. 9Q). Isopolar hormogonia are strongly to slightly constricted, with barrel-shaped cells, later becoming heteropolar (Fig. 9S), longer, with cylindrical, narrowed, notoriously constricted cells. Arthrospores rarely observed, rounded to shortly spherical (Fig. 9R). In fresh material only few filaments observed, not differing in morphology from the description based on cultures. "*Dichothrix*" was isolated from samples of epiphyton on *Thelypteris interrupta*, *Hydrocotyle* sp.., and *Miriophyllum aquaticum* from El Oconal lagoon. The epiphyton samples were rich in detritus and green algae, as described in Mendoza (2015). This cyanobacterium was densely aggregated in hemispherical to spherical colonies on the young pinnate leaves, and especially on the sori of submerged leafs of *T. interrupta*, joining together with an *Hormotila*-like green algae at the base of colonies (Mendoza 2015).

Table 6. Generic features of "*Dichothrix*" compared with other morphologically related genera. Code **I**: *Dichothrix* Zanardini ex Bornet et Flahault; **II**, *Scytonematopsis* Kiseleva; **III**, *Calothrix* Agardh ex Bornet et Flahault; **IV**, *Macrochaete* Berrendero, Johansen et Kaštovský; **V**, *Rivularia* Agardh ex Bornet ex Flahault; **VI**, *Cyanomargarita* Shalygin, Shalygina et Johansen; **VII**, *Gloeotrichia* Agardh ex Bornet et Flahault. P: present, A: absent, S: in some species only.

Morphological characteristics	**"*Dichothrix*"**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
Polarity	Heteropolar	P	A	P	P	P	P	P
	Radiating from center	A	A	A	A	P	P	P
Colony	Bush-like fascicles	P	A	A	A	A	A	A
	Prostate, grass-like	A	P	A	A	A	A	A
	Single or geminate	P	P	S	S	P	P	P
Branching	Repeated false branching	P	A	A	A	P	P	P
	Divaricated branching	P	A	A	A	A	A	A
Trichome ending	Hair-like	P	P	S	P	P	P	P
Arthrospores	Yes	A	P	A	A	A	S	A

b. Analysis based on 16S rRNA gene

Strains morphologically belonging to the Rivulariaceae family are distributed in three clades: Clade A ("Calothrichaceae"), Clade B ("*Scytonematopsis contorta*" group), and Clade C (Rivulariaceae sensu stricto). Clade A is composed mainly by *Calothrix* and *Macrochaete* strains. Clade B contains mostly two *S. contorta* strains described from Hawaii (Vaccarino & Johansen, 2011), and two sequences of "*Dichothrix*" obtained in the present work. Clade C principally *Nunduva, Rivularia,* and strains identified as *Calothrix*, one of them from the present study. Identities of 16S rRNA gene sequences of rivulariacean strains are show in tables 7, 8 and 9.

Clade A. "Calothrichaceae"

Seven taxa studied in the present work fall in this clade, five corresponding to *Calothrix*, and two to *Skacelovkia*. Most of *Calothrix* strains fall in three different groups separated by their ecological preferences and genetic distances: *Calothrix* Freshwater & Soil, *Calothrix* Brackish – I, and *Calothrix* Brackish – II. One novel group located separately from the previous *Calothrix* groups is composed of two strains, and due to its phylogenetic position and lowest identity compared to other cyanobacteria is proposed as *Skacelovkia* gen nov.

Five strains fall in *Calothrix* Freshwater & Soil, which comprises mostly *Calothrix* strains from freshwater and terrestrial habitats. *Calothrix* cf. *elenkinii* strain T014 forms a subcluster with *Calothrix* sp. PCC 7715 (= *C. thermalis* SAG 37.79) and "*Rivularia*" sp. IAM M-261, being identical in 16S rRNA gene sequence. *Calothrix* sp. PCC 7715 was isolated from a thermal pool in France (Rippka et al. 2001), and *Rivularia* sp. IAM M-261 from an unknown locality in Japan. *Calothrix* sp. type 1 strain T030 places as a sister taxa of a subcluster containing strains UAM 373, UAM 372, and UAM 315, isolated from different brackish rivers in Spain (Berrendero et al. 2008), and identity of 16S rRNA gene sequence comparing strain T030 with the Spanish strains is 98.5 – 99.1%. *Calothrix* cf. *marchica* strains T064 and T067 form a subcluster with *C. marchica* PCC 7114, isolated from a pool in India (Rippka et al. 2001), being 99.3 – 99.7% similar in genetic identity. *Calothrix* sp. type 3 strain T019 and *Calothrix* sp. type 2 strain T035 have two 16S rRNA sequences each one. *Calothrix* sp. type 3 strain T019 appears as a sister of the subcluster containing strains T064, T067 and PCC 7714, sharing 97.1 – 97.2% of identity. *Calothrix* sp. type 2 strain T035 and *Calothrix* sp. HA4395-MV3 from Hawaii form a well-supported subcluster, being 99.0% identical in 16S rRNA gene sequence.

Table 7. Comparison of the 16S rRNA gene sequence identity of *Calothrix* strains from Clade A isolated in the present study (in bold), and phylogenetically related taxa.

	Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Calothrix* cf. *elenkinii* T014**																
2	*Calothrix* sp. PCC 7715	100.0															
3	*Rivularia* sp. IAM M-261	100.0	100.0														
4	*Calothrix* sp. PCC 7103	98.2	98.2	98.2													
5	***Calothrix* sp. type 1 T030**	97.8	97.8	97.8	98.8												
6	*Calothrix* sp. UAM 373	97.3	97.3	97.3	98.0	98.5											
7	*Calothrix* sp. MU27 UAM 315	97.7	97.7	97.7	98.2	99.2	98.5										
8	*Calothrix* sp. TJ12 UAM 372	97.7	97.7	97.7	98.2	99.1	98.9	99.6									
9	***Calothrix* sp. type 3 T019**	96.7	96.7	96.7	96.7	97.0	96.6	96.8	96.9								
10	***Calothrix* cf. *marchica* T064**	96.6	96.7	96.6	96.3	96.1	95.6	95.4	95.4	97.2							
11	***Calothrix* cf. *marchica* T067**	96.5	96.6	96.5	96.2	95.9	95.6	95.2	95.2	97.1	99.3						
12	*Calothrix marchica* PCC 7714	96.5	96.6	96.5	96.2	95.8	95.5	95.2	95.2	97.2	99.7	99.3					
13	***Calothrix* sp. type 2 T035**	97.3	97.3	97.3	97.1	97.3	97.3	97.3	97.4	98.1	96.7	96.5	96.6				
14	*Calothrix* sp. HA4395-MV3	97.1	97.1	97.1	97.5	97.6	97.6	97.9	97.9	97.9	96.9	96.6	96.7	99.0			
15	***Calothrix* sp. type 4 T029**	91.0	90.9	90.9	91.1	90.9	91.3	90.8	90.7	91.5	91.1	91.0	91.2	91.8	92.7		
16	Cf. *Calothrix* sp. Musc. cyanobiont 5	90.9	90.9	90.9	91.0	90.8	91.3	90.6	90.7	91.2	90.9	90.9	91.1	90.8	91.4	97.7	
17	*Calothrix parietina* CCAP 1410/11	90.7	90.7	90.7	91.5	91.2	91.7	91.1	91.1	91.2	91.1	91.1	91.2	91.5	92.7	98.3	97.9

The strain *Calothrix* sp. type 4 T029 falls as a sister branch of *Calothrix* Brackish – I subcluster, *Calothrix* sp. CAL3361 from a lake in Finland (Sihvonen et al. 2007), and *Calothrix parietina* CCAP 1410/11 from an unknown locality in Denmark. The closest strains in identity of 16S rRNA to *Calothrix* sp. type 4 T029 are *Calothrix parietina* CCAP 1410/11 (98.3%), and cf. *Calothrix* sp. Muscicolous cyanobiont 5 (97.7%). cf. *Calothrix* sp. Muscicolous cyanobiont 5 was isolated as a photobiont in *Blasia pusilla* L. from Finland (Rikkinen et al. 2002). Two strains form the cluster of the novel genus *Skacelovkia*, being located as sister of *C. elsteri* from Antarctica (Komárek et al. 2012). *Skacelovkia* strains are 99.7% identical in 16S rRNA gene sequence, while the closest taxa in identity with *Skacelovkia* is *C. elsteri* (95.1%). The identities of *Skacelovkia* strains with other representatives of *Calothrix* and *Rivularia* stains are 90.9 – 93.8%.

Table 8. Comparison of the 16S rRNA gene sequence identity of *Skacelovkia peruviana, Skacelovkia* sp. and *Calothrix* sp. type 5 (in bold) compared with related rivulariacean strains

	Strains	1	2	3	4	5	6	7	8	9	10	11	12
1	***Skacelovkia peruviana* T027**												
2	***Skacelovkia* sp. P3C2e**	99.6											
3	*Calothrix elsteri* CCALA 953	95.2	95.3										
4	*Calothrix* sp. PCC 7714	93.3	93.2	94.6									
5	*Calothrix* sp. BECID1	92.6	92.6	93.7	91.7								
6	*Calothrix* sp. XP4B	92.1	92.2	93.4	91.1	93.2							
7	*Macrochaete psychrophila* CCALA 1092	94.0	94.0	94.3	94.0	93.3	94.7						
8	***Calothrix* sp. type 5 T044**	91.5	91.6	92.4	90.3	92.4	90.6	93.0					
9	*Calothrix* sp. CCMEE-5093	90.9	91.0	92.8	89.9	92.1	90.4	92.8	99.8				
10	*Rivularia* sp. PUNA-NP3-PCI185B	91.9	91.8	92.6	90.7	92.7	91.8	92.9	99.2	99.9			
11	*Rivularia atra* BIR MGR1	91.5	91.5	92.5	90.3	92.3	90.6	93.2	98.6	99.0	98.9		
12	*Nunduva fasciculata*	92.6	92.5	92.8	90.7	93.2	91.9	92.3	96.9	97.4	96.8	97.1	
13	*Gloeotrichia pisum* SL6-1-1	91.8	91.7	92.8	91.6	91.0	91.6	93.4	93.3	92.9	93.2	92.8	91.7

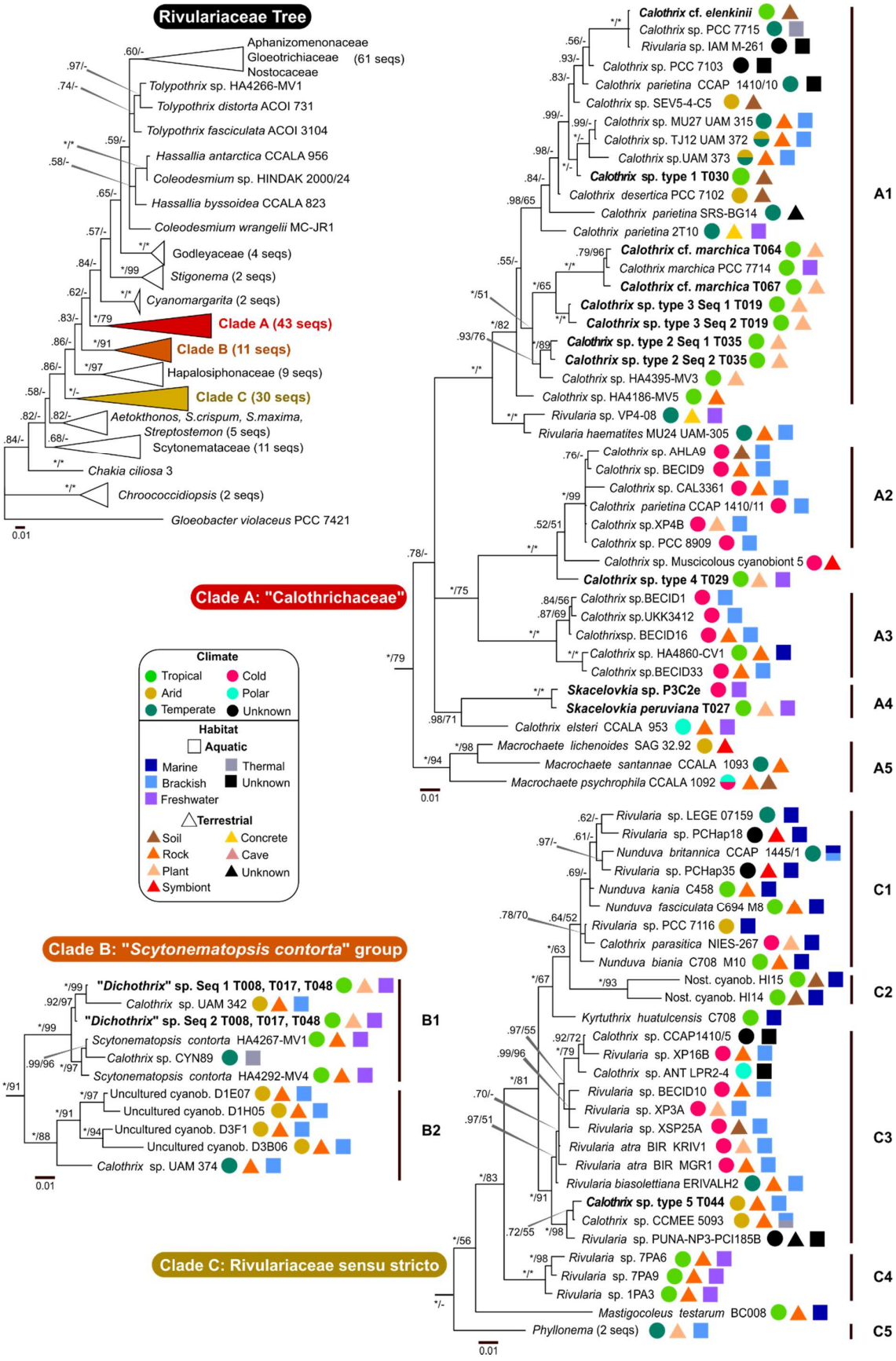
Clade B. "*Scytonematopsis contorta*" group

This clade contains nine sequences assigned to *Calothrix, S. contorta,* "*Dichothrix*" (from the present study), and from uncultured cyanobacteria. Two well-supported subclusters, B1 and B2, are within Clade B. Subcluster B1 is composed of two sequences of "*Dichothrix*" strains T008, T017, and T048, *Calothrix* sp. UAM 342 from epilithon in calcareous river in Spain (Berrendero et al. 2011), *S. contorta* strains HA267-MV1 and HA2492-MV4 from from wet rock walls and waterfall splash zones in Hawaii (Vaccarino & Johansen 2011), and *Calothrix* sp. CYN89 from a thermal spring in New Zealand (Martineau et al. 2013). Identity of 16S rRNA gene between members of subcluster B1 is 97.3 – 99.6%. Identity of the two sequences of "*Dichothrix*" is 99.5%. Subcluster B2 is composed of four sequences of uncultured cyanobacteria from stromatolites in Ruidera pools (Santos et al. 2010), and *Calothrix* sp. UAM 374 from Tejada stream (Berrendero et al. 2011), all from Spain.

Table 9. Comparison of the 16S rRNA gene sequence identity of "*Dichothrix*" sequences with phylogenetically and morphologically related taxa.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
1	***"Dichothrix"* sp. Seq 1 strains T008, T017, T048**													
2	***"Dichothrix"* sp. Seq 2 strains T008, T017, T048**	99.5												
3	*Calothrix* sp. UAM 342	98.1	98.6											
4	*Scytonematopsis contorta* HA4292-MV4	99.2	98.9	97.5										
5	*Scytonematopsis contorta* HA4267-MV1	99.3	98.8	97.4	99.6									
6	*Calothrix* sp. CYN89	98.5	98.2	97.3	98.8	99.3								
7	Uncultured cyanob. clone D1E07	95.6	95.2	94.7	95.3	95.3	94.5							
8	*Calothrix* sp. UAM 374	95.7	95.3	94.3	95.5	95.5	94.5	97.0						
9	*Calothrix desertica* PCC 7102	92.0	91.9	91.4	91.8	92.0	92.0	92.7	92.6					
10	*Rivularia atra* BIR MGR1	92.3	92.1	92.2	92.3	92.3	92.3	91.3	91.9	90.1				
11	*Macrochaete psychrophila* CCALA 1092	92.8	92.7	91.9	92.6	92.6	92.0	92.0	93.8	94.4	93.1			
12	*Cyanomargarita melechinii* APA-RS9	92.7	92.7	92.2	92.6	92.6	93.1	92.0	93.6	91.8	92.3	94.3		
13	*Gloeotrichia echinulata* URA3	92.7	92.8	92.9	92.3	92.5	92.8	91.5	93.9	91.5	92.8	93.5	95.2	
14	*Scytonematopsis maxima* LCR-FBC	91.4	91.2	91.0	91.3	91.3	91.6	90.3	91.4	91.1	91.6	91.9	93.0	92.3

Fig. 3. Phylogenetic tree based on 16S rRNA gene sequences of heterocytous cyanobacteria, focused on the rivulariacean taxa. Clades A, B and C are shown in detail. The strains studied in the present work are in bold. Nodes report bootstrap support for Bayesian analysis and maximum likelihood. The climate classification follows Peel et al. (2007).


Rivulariaceae Tree
Aphanizomenonaceae
Gloeotrichiaceae (61 seqs)
Nostocaceae
Tolypothrix sp. HA4266-MV1
Tolypothrix distorta ACOI 731
Tolypothrix fasciculata ACOI 3104
Hassallia antarctica CCALA 956
Coleodesmium sp. HINDAK 2000/24
Hassallia byssoidea CCALA 823
Coleodesmium wrangelii MC-JR1
Godleyaceae (4 seqs)
Stigonema (2 seqs)
Cyanomargarita (2 seqs)
Clade A (43 seqs)
Clade B (11 seqs)
Hapalosiphonaceae (9 seqs)
Clade C (30 seqs)
Aetokthonos, S.crispum, S.maxima,
Streptostemon (5 seqs)
Scytonemataceae (11 seqs)
Chakia ciliosa 3
Chroococcidiopsis (2 seqs)
Gloeobacter violaceus PCC 7421
0.01
Clade A: "Calothrichaceae"
Calothrix cf. elenkinii
Calothrix sp. PCC 7715
Rivularia sp. IAM M-261
Calothrix sp. PCC 7103
Calothrix parietina CCAP 1410/10
Calothrix sp. SEV5-4-C5
Calothrix sp. MU27 UAM 315
Calothrix sp. TJ12 UAM 372
Calothrix sp.UAM 373
Calothrix sp. type 1 T030
Calothrix desertica PCC 7102
Calothrix parietina SRS-BG14
Calothrix parietina 2T10
Calothrix cf. marchica T064
Calothrix marchica PCC 7714
Calothrix cf. marchica T067
Calothrix sp. type 3 Seq 1 T019
Calothrix sp. type 3 Seq 2 T019
Calothrix sp. type 2 Seq 1 T035
Calothrix sp. type 2 Seq 2 T035
Calothrix sp. HA4395-MV3
Calothrix sp. HA4186-MV5
Rivularia sp. VP4-08
Rivularia haematites MU24 UAM-305
A1
Calothrix sp. AHLA9
Calothrix sp. BECID9
Calothrix sp. CAL3361
Calothrix parietina CCAP 1410/11
Calothrix sp.XP4B
Calothrix sp. PCC 8909
Calothrix sp. Muscicolous cyanobiont 5
Calothrix sp. type 4 T029
A2
Calothrix sp.BECID1
Calothrix sp.UKK3412
Calothrixsp. BECID16
Calothrix sp. HA4860-CV1
Calothrix sp.BECID33
A3
Skacelovkia sp. P3C2e
Skacelovkia peruviana T027
A4
Calothrix elsteri CCALA 953
Macrochaete lichenoides SAG 32.92
Macrochaete santannae CCALA 1093
Macrochaete psychrophila CCALA 1092
A5
0.01
Climate
Tropical
Cold
Arid
Polar
Temperate
Unknown
Habitat
Aquatic
Marine
Thermal
Brackish
Unknown
Freshwater
Terrestrial
Soil
Concrete
Rock
Cave
Plant
Unknown
Symbiont
Clade B: "Scytonematopsis contorta" group
"Dichothrix" sp. Seq 1 T008, T017, T048
Calothrix sp. UAM 342
"Dichothrix" sp. Seq 2 T008, T017, T048
Scytonematopsis contorta HA4267-MV1
Calothrix sp. CYN89
Scytonematopsis contorta HA4292-MV4
B1
Uncultured cyanob. D1E07
Uncultured cyanob. D1H05
Uncultured cyanob. D3F1
Uncultured cyanob. D3B06
Calothrix sp. UAM 374
B2
0.01
Rivularia sp. LEGE 07159
Rivularia sp. PCHap18
Nunduva britannica CCAP 1445/1
Rivularia sp. PCHap35
Nunduva kania C458
Nunduva fasciculata C694 M8
C1
Rivularia sp. PCC 7116
Calothrix parasitica NIES-267
Nunduva biania C708 M10
Nost. cyanob. HI15
Nost. cyanob. HI14
C2
Kyrtuthrix huatulcensis C708
Calothrix sp. CCAP1410/5
Rivularia sp. XP16B
Calothrix sp. ANT LPR2-4
Rivularia sp. BECID10
Rivularia sp. XP3A
Rivularia sp. XSP25A
Rivularia atra BIR KRIV1
Rivularia atra BIR MGR1
Rivularia biasolettiana ERIVALH2
Calothrix sp. type 5 T044
Calothrix sp. CCMEE 5093
Rivularia sp. PUNA-NP3-PCI185B
C3
Clade C: Rivulariaceae sensu stricto
Rivularia sp. 7PA6
Rivularia sp. 7PA9
Rivularia sp. 1PA3
C4
Mastigocoleus testarum BC008
Phyllonema (2 seqs)
C5
0.01

Clade C. Rivulariaceae sensu stricto

This clade harbors *Calothrix, Kyrtuthrix, Mastigocoleus, Nunduva, Phyllonema, Rivularia*, and "Nostocales cyanobacterium" strains, all of them from marine or brackish waters, except the three *Rivularia* sequences from Pozas Azules, Mexico (Dominguez-Escobar et al. 2011). Five distinct subclusters are recognized: C1, composed of *Nunduva* and *Rivularia* sequences; C2, with two "Nostocales" sequences; C3, including *R. atra*; C4, containing three *Rivularia* sequences from freshwater microbial mats; and C5, composed by two *Phyllonema* strains.

Calothrix sp. type 5 strain T044 falls within the high-supported subcluster C3 (*Rivularia* sensu stricto), and is located as a sister of *Rivularia* sp. PUNA-NP3-PCI185B from rocks in Argentina (Shalygin et al. 2017), and *Calothrix* sp. CCMEE 5093 from a brackish hot spring in Wyoming, United States (Dillon & Castenholz 2003). Identity of *Calothrix* sp. type 5 T044 compared to strains CCMEE-5093 and PUNA-NP3-PCI185B is 99.8 and 99.3% respectively, and when compared to *R. atra* BIR MGR1 is 98.6%.

B. Family Scytonemataceae

Five strains belonging to four taxa fall within this family. Two taxa correspond to *Brasilonema*, while the other two correspond to *Scytonema,* but only one of them belongs to *Scytonema* sensu stricto in the phylogenetic tree (Fig. 8).

a. Morphological and ecological descriptions

Group I. *Brasilonema*

1. *Brasilonema octagenarum*, strain T001 (Fig. 10 A-C): Thallus macroscopic, with erect parallel filaments. Filaments uniseriate (Fig. 10A), with simple and double false branching (Fig. 10B), 9.6-24.4 µm wide. Trichomes cylindrical, constricted at cross-walls, not attenuated toward ends, 7.8-22.0 µm wide. Cells 2.2-24.0 µm long. Heterocytes single or in pairs (Fig. 10B), mostly intercalary, 3.8-16.8 x 10.9-19.4 µm. Hormogonia isopolar after fragmentation, then heteropolar (Fig. 10C). *Brasilonema* sp. type 1 was isolated from moss, near the entrance of El Oconal lagoon, Villa Rica, tropical humid forest in Peru.
2. *Brasilonema* sp., strain T076 (Fig. 10 D-F): Filaments uniseriate (Fig. 10D), with single and double false branching (Fig. 10E), 9.6-14.9 µm wide. Sheaths colorless, not lamellated. Trichomes cylindrical, constricted at cross-walls (Fig. 10D), attenuated toward the apices in well-developed trichomes (Fig. 10E), 9.3-13.2 µm wide. Cells 2.6-6.3 µm long. Heterocytes intercalar, rarely basal, 3.7-14.3 x 6.9-12.8 µm. Hormogonia isopolar

when released, later heteropolar (Fig. 10F). This cyanobacterium was isolated from moss sample mixed with soil, near El Tirol waterfall in San Ramon, tropical humid forest in Peru.

Group II. *Scytonema* – I (sensu stricto)

3. *Scytonema* sp. type 1, strains T051 and T056 (fig. 10 G-I): Thallus above the agar surface. Filaments creeping, with simple and double false branching (Fig. 10G), 3.8-8.2 µm wide. Sheaths thin, colorless. Trichomes constricted to slightly constricted at cross walls (Fig. 10 G, H), ending in rounded cells (Fig. 10H), 2.7-6.7 µm wide. Cells 1.6-7.6 µm long. Intercalar heterocytes 3.3-14.1 x 4.1-8.1 µm. Hormogonia isopolar (Fig. 10I). Strain T051 was collected from a muddy soil sample with sparced herbaceous vegetation surrounding, while strain T056 was obtained from bottom mud in an almost empty human-made water channel. Both were collected in Villa Rica, Amazon humid forest in Peru.

Group III. *Scytonema* – II

4. *Scytonema* sp. type 2*,* strain T063 (fig. 10 J-L): Thallus caespitose, Filaments long, creeping and erected, above the agar. False branching simple and double (Fig. 10K), 9.0-19.2 µm wide. Sheaths colorless to yellow brownish (Fig. 10J). Trichomes cylindrical, not attenuated toward ends, slightly to notoriously constricted at cross walls (Fig. 10J), 6.7-16.6 µm wide. Cells cylindrical, quadratic, ± rectangular (Fig. 10K), barrel-shaped (Fig. 10J), ± discoid in meristematic zones (Fig. 10L), 3.9-36.2 µm long. Apical cell broadly conically rounded or hemispherical (Fig. 10L). Heterocytes intercalar, barrel shaped, cylindrical, rectangular, single or in pairs (Fig. 10J), 6.7-29.5 x 7.9-16.2 µm. This taxa was isolated from a biological soil crust in *Lomas de Lachay* National Reserve, arid coastal dessert in Peru.

b. Analysis based on 16S rRNA gene

Strains identified as genera from the Scytonemataceae locate in seven main clades (A – G, Fig. 4). Clade A includes "*Tolypothrix*" PCC, *A. laxa*, *Calochaete*, *Roholtiella*, and *S. mirabile* SAG 83.79. Clade B comprises *Anabaenopsis*, *Cyanocohniella*, *Cyanospira*, *Chrysosporum*, *Nodularia,* and *S. bohneri* SAG 255.80. Clade C possess five *Scytonema* strains from freshwater habitats in New Zealand (Smith 2012, Smith et al. 2012, Smith et al. 2011). Clade D is composed by *Iningainema, Petalonema, Scytonema*, and *Scytonematopsis*. Clade E correspond to *S. hyalinum* group Type 1 Operon sensu Johansen et al. (2017), and is

Table 10. Comparison of 16S rRNA gene sequence identities of *Brasilonema* strains and related taxa. Studied strains from the present work are in bold.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Brasilonema octagenarum* T001**																
2	*Brasilonema* sp. CENA382	99.6															
3	*Brasilonema* sp CENA360	99.5	99.9														
4	*Brasilonema* sp. RKST-322	99.4	99.8	99.7													
5	*Brasilonema octagenarum* UFV-E1	99.4	99.9	99.8	99.9												
6	*Scytonema* sp. SAG 67.81	99.1	99.4	99.4	99.5	99.6											
7	*Brasilonema* sp. CDV2	98.2	98.6	98.5	98.6	98.8	98.9										
8	*B. angustatum* HA4187-MV1	98.5	98.9	98.8	98.9	99.0	99.1	98.5									
9	*Brasilonema burkei* HA4348-LM4	97.1	97.3	97.2	97.4	97.4	97.5	97.3	97.8								
10	*Brasilonema bromeliae* SPC 951	96.5	96.7	96.6	96.6	96.7	97.1	96.4	97.2	97.6							
11	*B. tolantongensis*Tolantongo	98.1	98.4	98.3	98.5	98.6	98.6	98.0	98.1	97.1	97.5						
12	*Scytonema* sp. 00557 00001	97.7	97.9	97.8	98.1	98.1	98.3	97.9	98.2	97.3	97.9	98.5					
13	***Brasilonema* sp. T076**	97.4	97.6	97.6	97.6	97.7	98.0	97.2	98.0	97.5	97.6	98.4	99.7				
14	*Brasilonema terrestre* CENA116	97.5	97.8	97.7	97.7	97.8	97.9	96.9	98.0	98.3	97.5	98.2	98.3	98.4			
15	*Symphyonemopsis* sp. VAPOR1	95.7	96.1	96.0	96.2	96.1	96.2	95.5	96.2	96.7	96.2	96.5	96.5	96.2	96.6		
16	*Iphinoe spelaeobios* LO2-B1	95.4	95.7	95.6	95.9	95.7	95.6	95.3	95.8	96.6	95.7	95.8	96.1	95.7	96.2	99.2	
17	*Scytonema hofmanni* PCC 7110	92.8	93.1	93.0	93.1	93.1	93.1	92.3	93.1	93.7	93.1	93.3	94.0	93.3	93.3	93.6	93.4

divided in subclusters E1 and E2. Subcluster E1 comprises *Scytonema* strains mostly from soil biotopes in arid localities, including *Scytonema* sp. type 2 strain T063 isolated in the present work. Subcluster E2 habors two *Symphyonema* strains. Clade F comprise four subclades (F1, F2, F3, and F4). Subclade F1 possess *Brasilonema* strains, including two obtained in the present work. Subclade F2 shows *Iphinoe* and *Symphyonemopsis* strains. Subclade F3 corresponds to *Scytonema* sensu stricto, including *S. hofmannii*, type species of the genus, and strains *Scytonema* sp. T051 and T056 from the present work. Subclade F4 comprises *Scytonema* strains from localities with temperate climate. Clade G is analogous to *S. hyalinum* group Type 2 Operon sensu Johansen et al. (2017), and comprises mostly strains from soil arid habitats. *Chakia* locates as a basal group of all heterocytous taxa. *Ewamiamia* locates as a sister branch of subclades F3 and F4. *Petalonema alatum*, type species of *Petalonema*, places as a sister taxa of the subclade F4. Identities of 16S rRNA gene sequences from *Brasilonema* and *Scytonema* are in tables 10 and 11, the Scytonemataceae

Table 11. Comparison of 16S rRNA identity of *Scytonema* strains. Sequences from the present study are in bold.

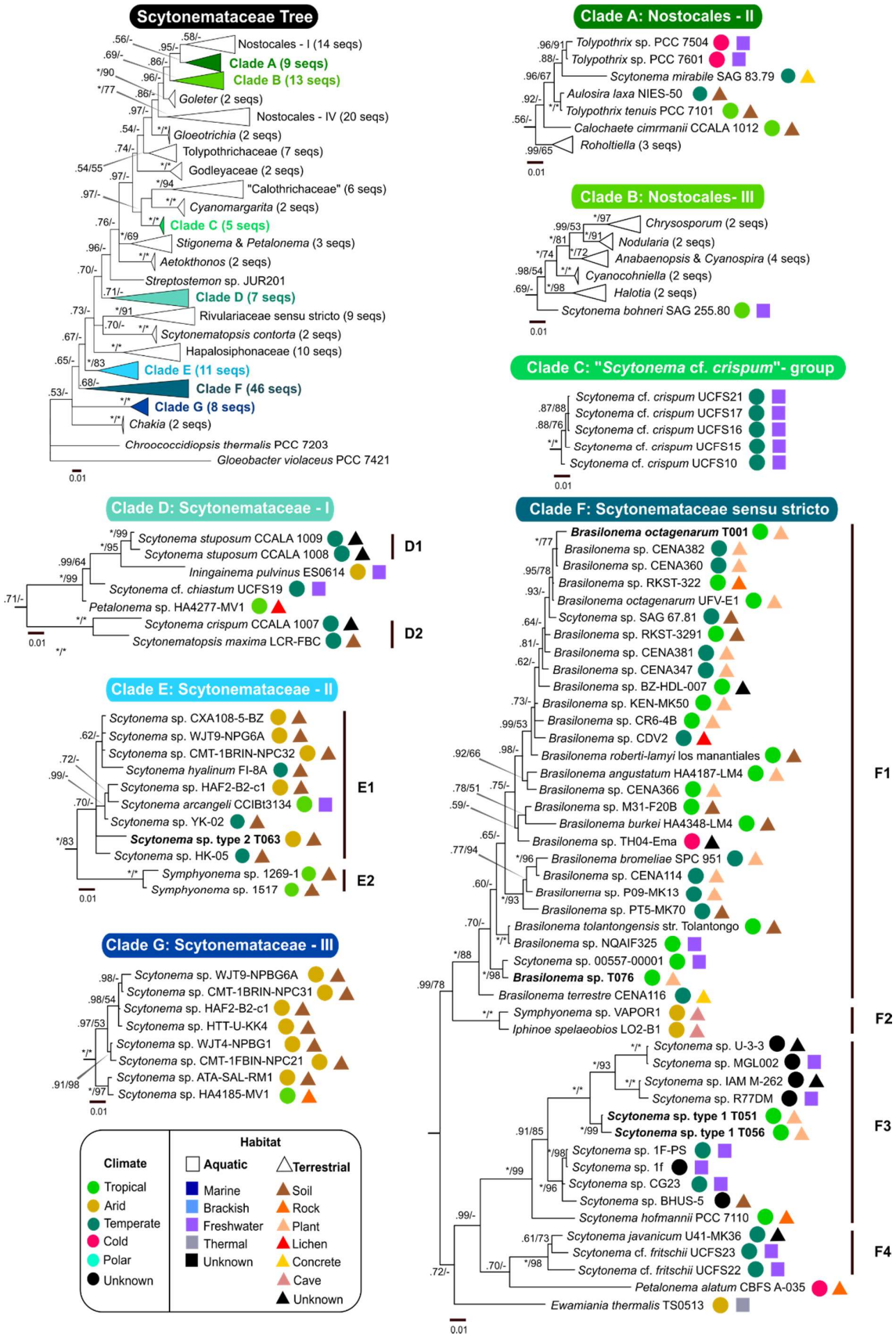
	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Scytonema* sp. type 1 strain T051**																
2	***Scytonema* sp. type 1 strain T056**	99.6															
3	*Scytonema* sp. MGL002	97.1	96.8														
4	*Scytonema* sp. IAM M-262	97.2	96.8	97.4													
5	*Scytonema* sp. 1F-PS	96.7	96.4	96.1	96.2												
6	*Scytonema hofmanni* PCC 7110	95.7	95.2	95.1	95.4	96.7											
7	*Scytonema javanicum* U41-MK36	92.6	92.2	92.6	93.1	94.3	94.2										
8	***Scytonema* sp. type 2 strain T063**	92.4	92.0	92.0	91.8	93.2	93.1	94.8									
9	*Scytonema hyalinum* FI-8A	91.8	91.4	92.0	91.6	92.9	93.2	93.8	96.7								
10	*Scytonema arcangeli* CCIBt3134	92.8	92.4	92.5	91.7	93.4	93.6	94.5	97.9	97.7							
11	*Scytonema* sp. WJT9-NPBG6A PS3C	90.6	90.3	90.4	90.5	91.7	89.8	90.1	91.5	91.0	91.4						
12	*Scytonema stuposum* P15-MK34	92.8	92.5	92.9	92.2	92.7	93.5	91.9	93.2	92.5	93.6	88.8					
13	*Scytonema* cf. *chiastum* UCFS19	92.5	92.2	92.8	91.8	92.5	93.3	92.2	93.7	93.5	94.2	90.5	97.4				
14	*Scytonema crispum* U55-MK38	91.4	91.1	91.6	90.8	91.4	91.2	92.0	92.1	91.7	92.6	89.9	91.6	93.2			
15	*Scytonema* cf. *crispum* UCFS15	91.0	90.7	90.1	89.9	91.6	91.4	91.1	92.0	92.3	92.4	90.3	93.0	93.9	92.0		
16	*Scytonema bohneri* SAG 255.80	90.7	90.3	91.3	89.9	91.6	91.3	91.3	92.6	92.7	93.1	89.5	91.9	93.5	91.7	94.9	
17	*Scytonema mirabile* SAG 83.79	91.8	91.4	91.4	91.1	92.5	91.9	91.3	93.0	93.5	93.5	89.8	92.5	93.9	91.5	93.7	96.9

Tree is show in Fig. 4.

Subclade F1 (Clade F) contains *Brasilonema octagenarum* strain T001 and *Brasilonema* sp. T076. Both strains sharing identites of 96.2% and 97.4% when compared to *B. bromeliae*, type species of the genus. *B. octagenarum* T001 places together with strains *Brasilonema* sp. CENA382, *Brasilonema* sp. CENA 360, *B. octagenarum* UFV-E1, and *Brasilonema* sp. RKST-322, the first four of them sharing identities between 99.4 to 99.9%. *Brasilonema* sp. T076 places together with "*Scytonema*" sp. 00557_00001 from Hawaii, both with identity of 99.6%.

Scytonema sp. type 1 strains T051 and T056 fall within subcluster F3 (Clade F). Both strains locate related to other *Scytonema* strains mostly from India. Identities of 16S rRNA gene sequence between strains T051 and T056 is 99.6%, and both compared to *S. hofmanni* is 95.2 – 95.7%.

Scytonema sp. type 2 strain T063 fall in subcluster E1 (Clade E) with other *Scytonema* strains mostly from soil biotopes in arid localities. Identities between strains T063 compared to CIBt3134 is 97.9%, while compared to *S. hofmanni* is 93.1%.


Scytonemataceae Tree
Nostocales - I (14 seqs)
Clade A (9 seqs)
Clade B (13 seqs)
Goleter (2 seqs)
Nostocales - IV (20 seqs)
Gloeotrichia (2 seqs)
Tolypothrichaceae (7 seqs)
Godleyaceae (2 seqs)
"Calothrichaceae" (6 seqs)
Cyanomargarita (2 seqs)
Clade C (5 seqs)
Stigonema & Petalonema (3 seqs)
Aetokthonos (2 seqs)
Streptostemon sp. JUR201
Clade D (7 seqs)
Rivulariaceae sensu stricto (9 seqs)
Scytonematopsis contorta (2 seqs)
Hapalosiphonaceae (10 seqs)
Clade E (11 seqs)
Clade F (46 seqs)
Clade G (8 seqs)
Chakia (2 seqs)
Chroococcidiopsis thermalis PCC 7203
Gloeobacter violaceus PCC 7421
0.01
Clade A: Nostocales - II
Tolypothrix sp. PCC 7504
Tolypothrix sp. PCC 7601
Scytonema mirabile SAG 83.79
Aulosira laxa NIES-50
Tolypothrix tenuis PCC 7101
Calochaete cimrmanii CCALA 1012
Roholtiella (3 seqs)
0.01
Clade B: Nostocales- III
Chrysosporum (2 seqs)
Nodularia (2 seqs)
Anabaenopsis & Cyanospira (4 seqs)
Cyanocohniella (2 seqs)
Halotia (2 seqs)
Scytonema bohneri SAG 255.80
0.01
Clade C: "Scytonema cf. crispum"- group
Scytonema cf. crispum UCFS21
Scytonema cf. crispum UCFS17
Scytonema cf. crispum UCFS16
Scytonema cf. crispum UCFS15
Scytonema cf. crispum UCFS10
0.01
Clade D: Scytonemataceae - I
Scytonema stuposum CCALA 1009
Scytonema stuposum CCALA 1008
Iningainema pulvinus ES0614
Scytonema cf. chiastum UCFS19
Petalonema sp. HA4277-MV1
Scytonema crispum CCALA 1007
Scytonematopsis maxima LCR-FBC
D1
D2
0.01
Clade E: Scytonemataceae - II
Scytonema sp. CXA108-5-BZ
Scytonema sp. WJT9-NPG6A
Scytonema sp. CMT-1BRIN-NPC32
Scytonema hyalinum FI-8A
Scytonema sp. HAF2-B2-c1
Scytonema arcangeli CCIBt3134
Scytonema sp. YK-02
Scytonema sp. type 2 T063
Scytonema sp. HK-05
Symphyonema sp. 1269-1
Symphyonema sp. 1517
E1
E2
0.01
Clade G: Scytonemataceae - III
Scytonema sp. WJT9-NPBG6A
Scytonema sp. CMT-1BRIN-NPC31
Scytonema sp. HAF2-B2-c1
Scytonema sp. HTT-U-KK4
Scytonema sp. WJT4-NPBG1
Scytonema sp. CMT-1FBIN-NPC21
Scytonema sp. ATA-SAL-RM1
Scytonema sp. HA4185-MV1
0.01
Climate
Tropical
Arid
Temperate
Cold
Polar
Unknown
Habitat
Aquatic
Marine
Brackish
Freshwater
Thermal
Unknown
Terrestrial
Soil
Rock
Plant
Lichen
Concrete
Cave
Unknown
Clade F: Scytonemataceae sensu stricto
Brasilonema octagenarum T001
Brasilonema sp. CENA382
Brasilonema sp. CENA360
Brasilonema sp. RKST-322
Brasilonema octagenarum UFV-E1
Scytonema sp. SAG 67.81
Brasilonema sp. RKST-3291
Brasilonema sp. CENA381
Brasilonema sp. CENA347
Brasilonema sp. BZ-HDL-007
Brasilonema sp. KEN-MK50
Brasilonema sp. CR6-4B
Brasilonema sp. CDV2
Brasilonema roberti-lamyi los manantiales
Brasilonema angustatum HA4187-LM4
Brasilonema sp. CENA366
Brasilonema sp. M31-F20B
Brasilonema burkei HA4348-LM4
Brasilonema sp. TH04-Ema
Brasilonema bromeliae SPC 951
Brasilonema sp. CENA114
Brasilonema sp. P09-MK13
Brasilonema sp. PT5-MK70
Brasilonema tolantongensis str. Tolantongo
Brasilonema sp. NQAIF325
Scytonema sp. 00557-00001
Brasilonema sp. T076
Brasilonema terrestre CENA116
Symphyonema sp. VAPOR1
Iphinoe spelaeobios LO2-B1
Scytonema sp. U-3-3
Scytonema sp. MGL002
Scytonema sp. IAM M-262
Scytonema sp. R77DM
Scytonema sp. type 1 T051
Scytonema sp. type 1 T056
Scytonema sp. 1F-PS
Scytonema sp. 1f
Scytonema sp. CG23
Scytonema sp. BHUS-5
Scytonema hofmannii PCC 7110
Scytonema javanicum U41-MK36
Scytonema cf. fritschii UCFS23
Scytonema cf. fritschii UCFS22
Petalonema alatum CBFS A-035
Ewamiania thermalis TS0513
F1
F2
F3
F4
0.01

C. "Fortieaceae", Tolypothrichaceae, and *Camptylonema*

Twelve strains belong to this section, five correspond to the Tolypothrichaceae, five resemble *Microchaete*, one corresponds to *Camptylonemopsis*, and one to *Calochaete*. Table 12 shows measurements of *Microchaete* and *Calochaete* strains from the present study. Tables 13, 14 and 15 show identities of strains from this section.

a. Morphological and ecological descriptions

Group I. *Calochaete*

- *Calochaete* sp. strain T037 (Fig. 11 H-O):

Colony amorphous, purple to brownish in color (Fig. 11 H). Filaments heteropolar, slightly to notoriously swollen, without hairs. False branching rarely observed, simple and double. Sheath thin, colorless. Trichomes constricted at cross-walls, tapered towards ends (Fig. 11 I, J). Vegetative cells cylindrical, barrel shaped, to shortly rounded. Terminal cells roundly conical. Basal heterocytes up to 5 in one row (Fig. 11K), sometimes present at both ends in short trichomes (Fig. 11M). Hormogonia isopolar to rarely heteropolar, with cylindrical, barrel-shaped, shorter to isodiametric cells (Fig. 11 N, O). Yellow cells with slightly thicker walls resembling arthrospores observed (Fig. 11 L). *Calochaete* sp. was isolated from moss sample, near the entrance of El Oconal lagoon, Villa Rica, tropical humid forest in Peru.

Group II. *Camptylonemopsis*

- *Camptylonemopsis* cf. *pulneyensis*, strain T054 (Fig. 11 P-AA)

Filaments irregularly widespread in the agar (Fig. 11P), uniseriate, isopolar (Fig. 11 Q-S), seeming heteropolar after filament breakage (Fig. 11 T), crescent to U-shaped (Fig. 11 S, U), rarely double or single branched (Fig. 11 W, X). Sheaths thin, colorless, firm, sometimes extending beyond the end of trichome (Fig. 11V). Trichomes cylindrical, constricted at cross walls, irregularly flexuous, slightly attenuated toward ends in young stages (Fig. 11 Q, R), later with widened ends in well-developed trichomes (Fig. 11 T, V). Initially young isopolar trichomes formed after hormogonia development (Fig. 11 Q, R), later both trichome ends start to erect (Fig. 11S), and internal cells show different morphologies across the trichome:

Fig. 4. Phylogenetic tree based on 16S rRNA gene sequences of heterocytous cyanobacteria, focused on the scytonematacean taxa. Clades A – G are shown in detail. The strains studied in the present work are in bold. Nodes report bootstrap support for Bayesian analysis and maximum likelihood. The climate classification follows Peel et al. (2006).

shorter than wide and roundly barrel-shaped in basal parts vs. longer than wide and cylindrical in erected parts (Fig. 11 U, V). Breakage of trichomes at intercalar heterocyte position produce filaments resembling *Fortiea* (Fig. 11T), or *Microchaete* (fig. 11Y). In old cultures filaments are creeping, with only isodiametric cells, starting the formation of akinetes, similar to *Aulosira* (Fig. 11Z). Heterocytes intercalar, barrel-shaped, ± cylindrical, ± quadratic, sometimes discoid, usually solitary, rarely in pairs (Fig. 11 Q-S, U, W-Y); basal heterocytes present after filament breakage (Fig. 11Y). Akinetes apoheterocytical, shortly cylindrical to barrel-shaped, in rows, developing all the way up to the closest heterocyte, with brownish-orange exospore (Fig. 11Z). Hormogonia with barrel-shaped, isodiametric cells (Fig. 11 AA). *Camptylonemopsis* cf. *pulneyensis* was isolated from a soil sample with green biofilm, outside the entrance to Oconal lagoon in Villa Rica, tropical humid forest in Peru.

Group III. *Microchaete*

- *Microchaete* sp. type 2, strain T060 (Fig. 11 A-G)

Colony blue-green, diffuse, spreading irregularly on the agar surface (Fig. 11A). Sheaths sometimes extending beyond the trichome. Trichomes cylindrical, slightly widened at the base (Fig. 11B), slightly narrowed toward ends. Vegetative cells cylindrical to barrel shaped (Fig. 11 B, C). Single and double false branching rarely observed (Fig. 11E), filaments with numerous branches in old cultures (Fig. 11D). Basal heterocytes rounded, hemispherical, single, rarely in pairs (but only one functional) (Fig. 11 B, E). Intercalar hemispherical heterocytes arranged in pairs before filament breakage (Fig. 11C), more frequent in old cultures. Hormogonia isopolar, constricted (Fig. 11F), becoming later heteropolar, and forming a young trichome by formation of basal heterocyte (Fig. 11G). *Microchaete* sp. type 2 was isolated from a moss sample collected from a bark of tree at 6 - 8 meters above the surface, in the surroundings of Yanachaga-Chemillén National Park, Oxapampa, tropical Amazon forest in Peru. Several *Stigonema* filaments were observed during the process of isolation, but they died after some days of culturing.

Group IV. "*Microchaete*"-like group

- *Microchaete* sp. type 1, strains T042 and T047 (Fig. 13 A-I)

Filaments solitary, heteropolar (Fig. 13 A, B), up to ± 500 μm long. Sheaths thin, colorless, not lamellated (Fig. 13C), sometimes extending the beyond of trichomes (Fig 13D). Trichomes, attenuated towards ends (Fig. 13H), notoriously constricted at cross-walls,

sometimes coiled within the sheath (Fig. 13I). Cells cylindrical, barrel-shaped, longer than wide up to isodiametric (Fig. 13 A, D), shorter than wide only in old stages, apical cells roundly conical (Fig. 13H). False branching frequent in adult and old cultures, simple and double. Basal heterocytes from 1 in young cultures, up to 3 in one row (Fig. 13F), in mature old cultures, hemispherical, oval, cylindrical, rarely elliptic. Intercalar heterocytes cylindrical, subspherical, shortly spherical, rarely trapezoidal or rectangular, up to 5 consecutively in one row only in mature filaments (Fig. 13E), becoming terminal when trichome breaks. Arthrospores pale green, oval, shortly rounded, walls slightly thick, giving quadratic appearance, in rows, developed from basal (Fig. 13H) up to trichome end (Fig. 13G). Hormogonia with cylindrical cells, isopolar at first, then heteropolar, formed by breakage of trichomes, or necridic cells, not directly observed by germination from arthrospore. Strains T042 and T047 were obtained from a muddy soil sample in Villa Rica, tropical high rainforest in Peruvian Amazon.

- JOH06 (Fig. 13J)

Filaments heteropolar, irregularly distributed in the agar surface. Sheath colorless, hyaline, not lamellated. Trichomes constricted, with cylindrical, rhomboid to barrel-shaped vegetative cells. Apical cells conically rounded. Heterocytes basal, conically rounded, and intercalar. False branching not common, simple. This strain was isolated by PhD. Marketa Bohunická from a soil sample collected in an unknown locality from USA.

- JOH42 (Fig. 13 K-N)

Filaments heteropolar, irregularly distributed in the agar surface. Sheath colorless, hyaline, not lamellated. Trichomes constricted (Fig. 13K). Vegetative cells barrel-shaped to cylindrical, longer than wide or ± isodiametric. Apical cells usually roundly conical (Fig. 13M). Heterocytes basal (Fig. 13 K, L) and intercalar, up to three in a row. Simple false branching observed, frequent in long filaments (Fig. 13K). Arthrospores pale green to pale yellowish, cylindrical to roundly barrel-shaped (Fig. 13N), in rows, developed from basal up to apex of trichome. This strain was isolated by PhD. Marketa Bohunická from a soil sample collected in Kenya.

Table 12. Measurements of main morphological features of "Fortieaceae", Tolypothrichaceae, and *Microchaete* strains isolated in the present study.

Taxa	Strain code	Filament	Vegetative cells (length)			Trichomes (width)			Basal Heterocyte		Intercalar Heterocyte	
		Length	Basal	Middle	Apex	Basal	Middle	Apex	Length	Width	Length	Width
Calochaete sp.	T037	up to ± 320	3.4 - 8.9	1.9 - 5.5	2.0 - 5.8	4.1 - 7.9	3.1 - 4.9	2.2 - 3.8	3.2 - 7.0	3.8 - 6.8	5.5 - 8.1	4.1 - 5.8
Microchaete sp. type 1	T042, T047	up to ± 500	3.4 - 11.9	3.2 - 10.0	3.9 - 10.0	2.0 - 7.4	2.3 - 4.0	2.5 - 3.4	4.2 - 9.7	3.8 - 9.1	3.5 - 11.6	3.9 - 9.0
Microchaete sp. type 2	T060	up to ± 1000	3.4 - 8.9	2.9 - 9.4	3.6 - 6.5	4.4 - 7.1	3.0 - 7.4	3.6 - 4.6	3.9 - 7.6	4.4 - 7.9	3.5 - 14.7	4.2 - 8.6
-	JOH6	up to ± 220	4.1 - 7.9	3.9 - 7.2	3.5 - 7.1	3.0 - 7.1	2.8 - 7.0	2.8 - 6.5	3.5 - 6.9	3.4 - 7.0	-	-
-	JOH42	up to ± 350	3.5 - 8.2	3.2 - 7.6	3.0 - 7.2	2.8 - 6.8	2.7 - 5.9	2.2 - 5.5	4.1 - 8.2	3.8 - 8.8	4.0 - 9.1	4.2 - 8.9
Hassallia californica	T061, T081	up to ± 350	2.9 - 8.3	2.2 - 6.0	2.2 - 5.2	9.2 - 14.4	10.2 - 13.9	8.3 - 12.9	7.0 -11.4	10.0 - 13.7	-	-
Hassallia sp.	T075	-	3.6 - 7.2	3.4 - 6.9	3.3 - 6.8	9.9 - 14.0	9.5 - 13.1	9.4 - 10.8	6.8 - 10.2	8.3 - 14.1	-	-
Tolypothrix cf. *helicophila*	T002	-		3.8 - 11.3			4.0 - 10.3		5.4 - 13.2	5.7 - 9.8	-	-
Tolypothrix sp.	T079	-	5.4 - 13.1	3.5 - 7.6	3.3 - 8.5	5.6 - 8.4	5.5 - 8.7	5.5 - 7.8	7.6 - 34.5	6.5 - 10.7	5.7 - 12.7	7.7 - 12.8

Group V. Tolypothrichaceae

- *Tolypothrix* cf. *helicophila* T002 (Fig. 12 A-C)

Filaments were spread all over the surface, with typical tolypothricoid branching, heteropolar (Fig. 12A), with up to four false branches arising from a single point (Fig. 12B). Trichomes cylindrical, constricted at cross-walls with rounded ends (Fig. 12C). Heterocytes solitaries (Fig. 12 B, C) or up to two in rows. Strain T002 was isolated from a moss sample collected in Villa Rica, tropical high forest in the Peruvian Amazon.

- *Tolypothrix* sp. T079 (Fig. 12 D-F)

Colonies dispersed, with filaments spreading and penetrating the agar. Filaments with numerous false branches (Fig. 12D), up to three branches arise from one single point (Fig. 12E). Sheath colorless, thin. Cells cylindrical, ± quadratic to barrel-shaped; basal cells

usually longer than wide, rarely isodiametric; middle cells shorter than wide up to isodiametric, apical cells conically rounded, usually shorter than wide (Fig. 12F). Heterocytes basal or intercalar, single or up to 3 in a row (Fig. 12E), heteromorphic: ± ellipsoid, ± rounded, ± ovate, rarely trapezoidal with rounded borders, isodiametric up to longer than wide. Sub-basal heterocyte mostly cylindrical, much longer than wide (Fig. 12E). Intercalar heterocytes rounded, rarely cylindrical, shorter than wide or isodiametric. Strain T079 was obtained from moss sample in San Ramon, Amazonian humid rainforest of Peru.

- *Hassallia californica* strains T061 and T081 (Fig. 12 G-J)

Colonies bushy, rough (Fig. 12G). Filaments with false branches that arise laterally after formation of intercalar heterocytes, mostly perpendicular to main axis (Fig. 12H). Sheaths thin, narrow, rarely funnel-like at the base, colorless to brown-yellowish, slightly lamellated in old trichomes. Trichomes slightly attenuated toward ends. Cells blue-green, discoid to shortly barrel-shaped, shorter than wide. Apical cell short, widely rounded. Heterocytes basal and intercalar, rounded, oval, hemispherical, rarely more or less barrel-shaped (Fig. 12I). Hormogonia formed after breakage of trichomes (Fig. 12J), or necridia. This taxa was isolated from biological soil crust (BSC) in *Lomas de Lachay*, arid coastal dessert in Peru.

- *Hassallia* sp. strain T075 (Fig. 12 K-N)

Colonies bushy. Filaments false branched (Fig. 12K), uniseriate, erect, C- or J-shaped curved (Fig. 12L). Sheath not lamellated, sometimes funnel-like. Trichomes slightly attenuated toward ends. Cells discoid, shorter than wide, apical cell slight rounded. Heterocytes shortly hemispherical, rounded, basal and intercalary (Fig. 12M). Hormogonia with discoidal cells (Fig. 12N). This taxa was isolated from BSC in *Lomas de Lachay*, coastal dessert in Peru.

b. Analysis based on 16S rRNA gene

Twelve strains corresponding to fortieacean, tolypothrichacean, *Camptylonema* or *Microchaete* taxa are found in four clades (A – D, Fig. 10). Clade A is composed mostly by heteropolar ("*Calothrix*", *Calochaete, Microchaete, Tolypothrix*) and isopolar (e.g. *Mojavia, Nostoc*) genera, including *Calochaete* sp. T037 from this study. Clade B also comprises heteropolar (*Fortea, Microchaete*) and isopolar (*Cylindrospermum, Desmonostoc*) taxa, including *Microchaete* sp. type 2 T060 and *Camptylonemopsis* cf. *pulneyensis* T054, both isolated in this work. Clade C includes mainly strains identified as *Calothrix* or *Microchaete*,

Table 13. Identities of 16S rRNA gene from *Camptylonemopsis* cf. *pulneyensis* T054, *Calochaete* sp. T037, and *Microchaete* sp. type 2 T060 (all in bold) compared with related taxa.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Camptylonemopsis* cf. *pulneyensis* T054**																
2	*Fortiea contorta* PCC 7126	97.1															
3	*Fortiea laiensis* HA4221-MV2	97.8	97.1														
4	*Fortiea coimbrae* ACOI 1451	96.2	97.6	96.6													
5	*Microchaete* sp. SAG 47.93	96.1	97.5	96.2	99.6												
6	***Microchaete* sp. type 2 T060**	96.1	96.3	95.9	97.5	97.2											
7	*Microchaete tenera* ACOI 630	96.3	96.9	97.1	97.7	98.1	99.2										
8	*Aulosira bohemensis* ISB-2	96.4	96.9	97.2	97.6	98.0	99.0	99.6									
9	***Calochaete* sp. T037**	95.8	96.6	95.9	96.9	96.9	96.6	97.5	97.7								
10	*Calochaete cimrmanii* CCALA 1012	97.1	96.2	97.0	96.6	97.0	96.7	96.9	97.2	99.1							
11	*Roholtiella edaphica* CCALA 1063	96.7	96.7	96.5	96.9	97.3	97.9	98.0	97.9	98.5	97.9						
12	*Desmonostoc muscorum* Lukesova 1/87	96.0	96.4	95.3	96.3	96.6	96.8	96.5	96.3	95.9	95.9	97.1					
13	*Microchaete diplosiphon* CCALA 811	95.5	95.9	95.2	96.2	97.0	96.6	96.5	96.6	97.2	97.5	97.7	96.3				
14	*Aulosira laxa* NIES-50	96.6	96.0	95.8	96.5	96.9	96.7	96.8	97.1	96.9	98.0	98.0	96.3	98.3			
15	*Camptylonemopsis* sp. HA4241-MV5	95.6	95.5	94.8	95.4	95.7	95.8	96.1	96.3	95.5	96.6	97.4	95.5	96.6	97.8		
16	*Camptylonemopsis* sp. MGCY355I	95.1	95.2	94.6	95.6	95.4	95.3	96.3	96.6	95.5	96.6	97.3	94.8	96.3	97.3	98.8	
17	*Calothrix anomala* SAG 1410.4	95.4	95.5	94.8	95.4	95.1	95.5	96.3	96.6	95.7	96.6	97.3	95.1	95.9	97.1	99.2	98.2

Clade A. Nostocales – I

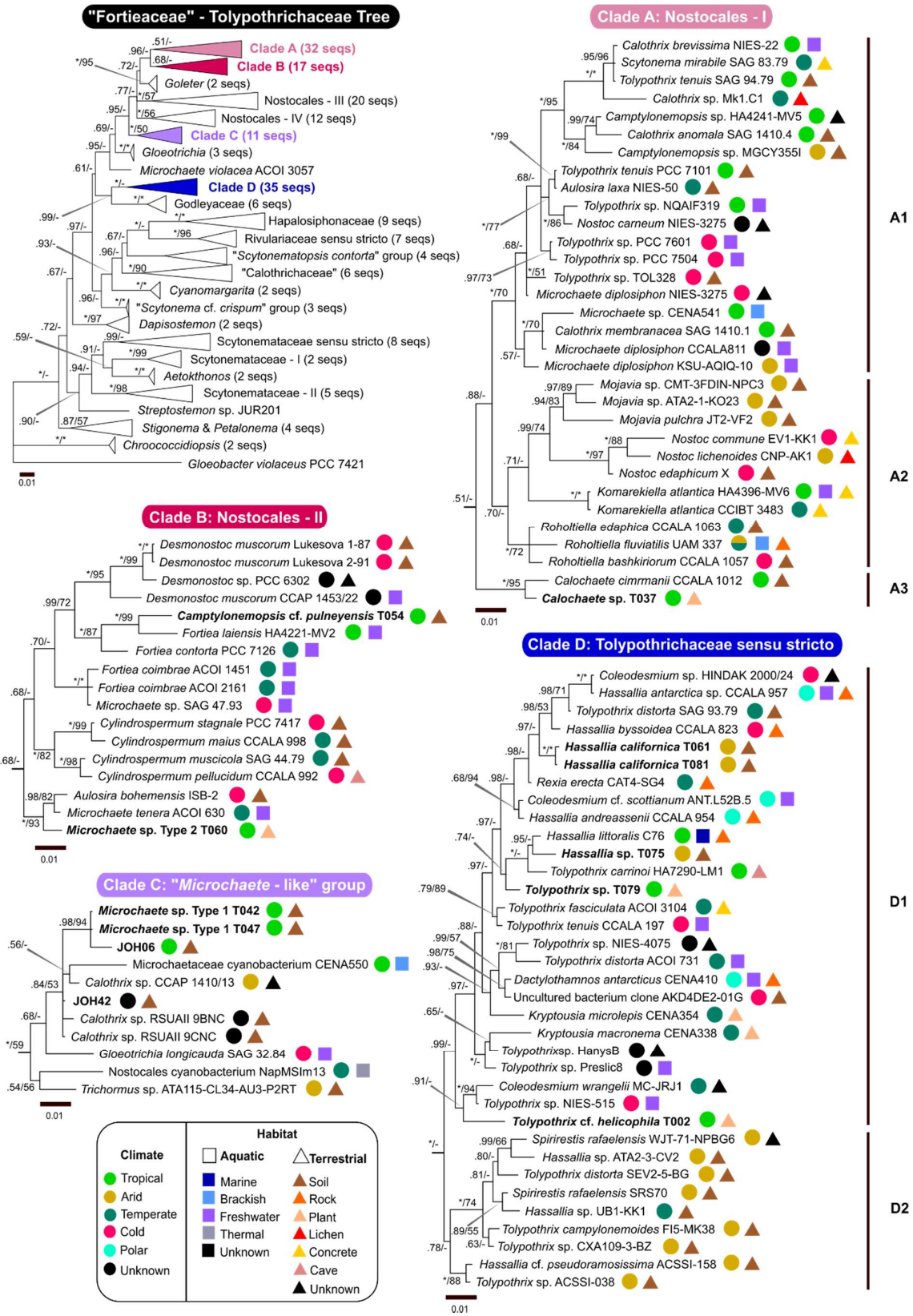
This clade mostly includes strains identified as *Microchaete*, *Tolypothrix*, *Calothrix*, and *Nostoc*, including the type species of the genera *Aulosira*, *Calochaete*, *Komarekiella*, *Mojavia*, *Nostoc*, and including *Microchaete* sp. type 1 strains T042 and T047, isolated and characterized by the author, and strains JOH06 and JOH42, isolated by PhD. Marketa Bohunická and sequenced by PhD. Esther Berrendero Gómez. Clade D corresponds to the Tolypothrichaceae sensu Hauer et al. (2014), and possess four strains isolated in the present work: *H. californica* strains T061 and T081, *Hassallia* sp. T075, *Tolypothrix* cf. *helocophila* T002, and *Tolypothrix* sp. T079. Identities of 16S rRNA gene between selected taxa of clades A and B are listed table 13, and from clusters C and D are in tables 14 and 15 respectively.

Table 14. Comparison of 16S rRNA identity of members of Clade C, including strains from the present study (in bold) and related taxa.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Microchaete* sp. type 1 T042**																
2	***Microchaete* sp. type 1 T047**	100.0															
3	**JOH06**	99.3	99.3														
4	**JOH42**	99.2	99.2	98.4													
5	Microchaetaceae cyanob. CENA550	97.4	97.4	97.2	98.3												
6	*Calothrix* sp. CCAP 1410/13	98.8	98.8	98.2	99.7	98.1											
7	*Calothrix* sp. RSUAII 9BNC	99.0	99.0	98.1	99.7	98.0	99.4										
8	*Calothrix* sp. RSUAII 9CNC	99.2	99.2	98.4	100.0	98.3	99.7	99.7									
9	*Gloeotrichia longicauda* SAG 32.84	97.3	97.3	97.2	98.0	96.9	97.4	97.8	98.0								
10	Nostocales cyanob. NapMSlm13	97.1	97.1	96.9	97.8	96.0	96.5	97.6	97.8	96.1							
11	*Trichormus* sp. ATA115	97.7	97.7	97.2	98.5	97.2	98.5	98.2	98.5	97.2	97.3						
12	*Microchaete tenera* ACOI 630	96.8	96.8	96.3	97.0	96.2	96.7	96.8	97.0	96.2	96.2	96.0					
13	*Roholtiella edaphica* CCALA 1063	97.1	97.1	96.9	97.5	96.5	97.1	97.2	97.5	97.2	96.4	96.6	98.0				
14	*Microchaete diplosiphon* NIES-3275	95.9	95.9	95.7	97.8	96.0	96.6	97.6	97.8	95.7	95.7	97.3	96.8	98.0			
15	*Microchaete diplosiphon* CCALA 811	95.5	95.5	95.4	97.4	95.8	95.9	97.1	97.4	95.9	95.5	96.9	96.5	97.7	98.9		
16	*Microchaete violacea* ACOI 3057	96.7	96.7	97.0	97.0	95.8	96.9	96.8	97.0	96.8	96.2	96.1	95.4	96.8	96.2	96.0	
17	*Calothrix* sp. PCC 7714	92.4	92.4	92.1	92.6	91.3	92.0	92.4	92.6	91.9	91.3	91.8	91.6	92.0	90.9	90.2	92.3

Roholtiella. Clade A is divided in three subclusters: A1, A2, and A3. Subcluster A1 is well-supported, and possess mostly strains assigned to *Microchaete* and *Tolypothrix,* including *A. laxa*, type species of *Aulosira*. Subcluster A2 harbors *Komarekiella*, *Nostoc sensu stricto*, *Mojavia*, and *Roholtiella*. Subcluster A3 is highly supported, and corresponds to *Calochaete*, including *C. cimrmanii*, type species of the genus isolated from surface soil in "páramo" zone, Costa Rica (Hauer et al. 2013), and *Calochaete* sp. T037. Identity of 16S rRNA gene between both *Calochaete* strains is 99.1%.

Fig. 5. Phylogenetic tree based on 16S rRNA gene sequences of heterocytous cyanobacteria, focused on taxa from the "Fortieaceae", Tolypothrichaceae, *Camptylonemopsis*, and *Microchaete*. Clades A – D are shown in detail. Strains studied in the present work are in bold. Nodes report bootstrap support for Bayesian analysis and maximum likelihood. The climate classification follows Peel et al. (2007).


"Fortieaceae" - Tolypothrichaceae Tree
Clade A (32 seqs)
Clade B (17 seqs)
Goleter (2 seqs)
Nostocales - III (20 seqs)
Nostocales - IV (12 seqs)
Clade C (11 seqs)
Gloeotrichia (3 seqs)
Microchaete violacea ACOI 3057
Clade D (35 seqs)
Godleyaceae (6 seqs)
Hapalosiphonaceae (9 seqs)
Rivulariaceae sensu stricto (7 seqs)
"Scytonematopsis contorta" group (4 seqs)
"Calothrichaceae" (6 seqs)
Cyanomargarita (2 seqs)
"Scytonema cf. crispum" group (3 seqs)
Dapisostemon (2 seqs)
Scytonemataceae sensu stricto (8 seqs)
Scytonemataceae - I (2 seqs)
Aetokthonos (2 seqs)
Scytonemataceae - II (5 seqs)
Streptostemon sp. JUR201
Stigonema & Petalonema (4 seqs)
Chroococcidiopsis (2 seqs)
Gloeobacter violaceus PCC 7421
0.01
Clade A: Nostocales - I
Calothrix brevissima NIES-22
Scytonema mirabile SAG 83.79
Tolypothrix tenuis SAG 94.79
Calothrix sp. Mk1.C1
Camptylonemopsis sp. HA4241-MV5
Calothrix anomala SAG 1410.4
Camptylonemopsis sp. MGCY355I
Tolypothrix tenuis PCC 7101
Aulosira laxa NIES-50
Tolypothrix sp. NQAIF319
Nostoc carneum NIES-3275
Tolypothrix sp. PCC 7601
Tolypothrix sp. PCC 7504
Tolypothrix sp. TOL328
Microchaete diplosiphon NIES-3275
Microchaete sp. CENA541
Calothrix membranacea SAG 1410.1
Microchaete diplosiphon CCALA811
Microchaete diplosiphon KSU-AQIQ-10
Mojavia sp. CMT-3FDIN-NPC3
Mojavia sp. ATA2-1-KO23
Mojavia pulchra JT2-VF2
Nostoc commune EV1-KK1
Nostoc lichenoides CNP-AK1
Nostoc edaphicum X
Komarekiella atlantica HA4396-MV6
Komarekiella atlantica CCIBT 3483
Roholtiella edaphica CCALA 1063
Roholtiella fluviatilis UAM 337
Roholtiella bashkiriorum CCALA 1057
Calochaete cimrmanii CCALA 1012
Calochaete sp. T037
A1
A2
A3
0.01
Clade B: Nostocales - II
Desmonostoc muscorum Lukesova 1-87
Desmonostoc muscorum Lukesova 2-91
Desmonostoc sp. PCC 6302
Desmonostoc muscorum CCAP 1453/22
Camptylonemopsis cf. pulneyensis T054
Fortiea laiensis HA4221-MV2
Fortiea contorta PCC 7126
Fortiea coimbrae ACOI 1451
Fortiea coimbrae ACOI 2161
Microchaete sp. SAG 47.93
Cylindrospermum stagnale PCC 7417
Cylindrospermum maius CCALA 998
Cylindrospermum muscicola SAG 44.79
Cylindrospermum pellucidum CCALA 992
Aulosira bohemensis ISB-2
Microchaete tenera ACOI 630
Microchaete sp. Type 2 T060
0.01
Clade C: "Microchaete - like" group
Microchaete sp. Type 1 T042
Microchaete sp. Type 1 T047
JOH06
Microchaetaceae cyanobacterium CENA550
Calothrix sp. CCAP 1410/13
JOH42
Calothrix sp. RSUAII 9BNC
Calothrix sp. RSUAII 9CNC
Gloeotrichia longicauda SAG 32.84
Nostocales cyanobacterium NapMSIm13
Trichormus sp. ATA115-CL34-AU3-P2RT
0.01
Clade D: Tolypothrichaceae sensu stricto
Coleodesmium sp. HINDAK 2000/24
Hassallia antarctica sp. CCALA 957
Tolypothrix distorta SAG 93.79
Hassallia byssoidea CCALA 823
Hassallia californica T061
Hassallia californica T081
Rexia erecta CAT4-SG4
Coleodesmium cf. scottianum ANT.L52B.5
Hassallia andreassenii CCALA 954
Hassallia littoralis C76
Hassallia sp. T075
Tolypothrix carrinoi HA7290-LM1
Tolypothrix sp. T079
Tolypothrix fasciculata ACOI 3104
Tolypothrix tenuis CCALA 197
Tolypothrix sp. NIES-4075
Tolypothrix distorta ACOI 731
Dactylothamnos antarcticus CENA410
Uncultured bacterium clone AKD4DE2-01G
Kryptousia microlepis CENA354
Kryptousia macronema CENA338
Tolypothrixsp. HanysB
Tolypothrix sp. Preslic8
Coleodesmium wrangelii MC-JRJ1
Tolypothrix sp. NIES-515
Tolypothrix cf. helicophila T002
Spirirestis rafaelensis WJT-71-NPBG6
Hassallia sp. ATA2-3-CV2
Tolypothrix distorta SEV2-5-BG
Spirirestis rafaelensis SRS70
Hassallia sp. UB1-KK1
Tolypothrix campylonemoides FI5-MK38
Tolypothrix sp. CXA109-3-BZ
Hassallia cf. pseudoramosissima ACSSI-158
Tolypothrix sp. ACSSI-038
D1
D2
0.01
Climate
Tropical
Arid
Temperate
Cold
Polar
Unknown
Habitat
Aquatic
Marine
Brackish
Freshwater
Thermal
Unknown
Terrestrial
Soil
Rock
Plant
Lichen
Concrete
Cave
Unknown

Clade B. Nostocales – II

This clade shows strains identified as *Cylindrospermum*, *Desmonostoc, Fortiea*, *Microchaete*, and *Aulosira*, including *Camptylonemopsis* cf. *pulneyensis* T054 and *Microchaete* sp. type 2 T060 from the present study. *Camptylonemopsis* cf. *pulneyensis* T054 clusters with *F. laiensis* HA4221-MV2 from Hawaii, and *F. contorta* PCC 7126 from California (Hauer et al. 2014, Herdman et al. 2011b). Identities of 16S rRNA gene sequence of strain T054 compared to *F. laiensis* and *F. contorta* are 97.8 and 97.1% respectively, while compared to *Camptylonemopsis* sp. HA4241-MV5 from Hawaii and *Camptylonemopsis* sp. MGCY355I from Iran, are 95.6 and 95.1% respectively. *Microchaete* sp. type 2 T060 forms a compact sub-cluster at the base of Clade B, together with *A. bohemensis* ISB-2, from soils in Czech Republic (Lukešová et al. 2009), and *M. tenera* ACOI 630, from freshwaternin Portugal (Hauer et al. 2014). Identities between these three strains are 99.0 – 99.6%.

Clade C. "*Microchaete*"-like group

This clade comprises 11 sequences, including heteropolar strains: *Microchaete* sp. type 1 strains T042 and T047, strain JOH06, strain JOH42, Microchaetaceae cyanobacterium CENA 550, three *Calothrix* strains (CCAP 1410/13, RSUAII 9BNC, RSUAII 9CNC), *G. longicauda* SAG 32.84; and isopolar strains: Nostocales cyanobacterium NapMSIm13 and *Trichormus* sp. ATA115. Strains T042 and T047 are identical in 16S rRNA gene sequence, while both compared to JOH06 and JOH42 show 99.3 and 99.2% of identity respectively. All members of Clade C show identities of 96.0 – 100%. Identities of *Microchaete* sp. type 1 strains T042 and T047, strain JOH06, and strain JOH42 with other *Microchaete* strains are 95.4 – 97.8%

Clade D. Tolypothrichaceae sensu stricto

Clade D shows high support, and contains five strains isolated in the present study, three of them morphologically correspond to *Hassallia*, and two to *Tolypothrix*. Clade D is divided in two subclusters, D1 and D2. Subcluster D1 includes strains of one uncultured cyanobacteria, *Coleodesmiusm, Dactylothamnos, Hassallia, Kryptousia, Rexia*, and *Tolypotrix*, including strains T002, T061, T075, T079 and T081 from the present study. Two strains of *T. distorta* (ACOI 731 and SAG 93.79), type species of *Tolypothrix*, fall within subcluster D1. Subcluster D2 is composed exclusively by strains isolated from soil bitotopes, mostly from arid localities, including *Hassallia, Tolypothrix*, and *Spirirestis*, including the type species of *Spirirestis*, *S. rafaelensis*, and one strain (SEV2-5-BG) identified as *T. distorta*.

Table 15. Comparison of 16S rRNA identity of tolypothrichacean strain from the present study (bold) with related strains.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Tolypothrix* cf. *helicophila* T002**																
2	***Tolypothrix* sp. T079**	97.8															
3	*Tolypothrix tenuis* CCALA 197	97.8	99.0														
4	*Tolypothrix carrinoi* HA7290-LM1	97.3	98.5	98.4													
5	*Tolypothrix* sp. NIES-515	98.3	98.0	98.0	97.6												
6	*Tolypothrix distorta* ACOI 731	97.4	97.9	98.3	97.5	98.2											
7	*Tolypothrix distorta* SAG 93.79	98.3	98.3	98.5	97.5	97.9	98.1										
8	*Tolypothrix distorta* SEV2-5-2Ca	98.0	97.6	97.1	96.8	97.1	96.2	97.5									
9	***Hassalia californica* T061**	97.6	98.4	98.4	97.6	98.2	97.8	98.6	97.3								
10	***Hassalia californica* T081**	97.6	98.5	98.5	97.7	98.3	97.9	98.7	97.4	99.9							
11	***Hassalia* sp. T075**	98.2	98.0	98.3	98.5	97.8	97.7	98.6	97.0	97.7	97.8						
12	*Hassallia antarctica* CCALA 957	97.9	98.5	98.5	97.5	97.5	97.8	99.3	97.5	98.3	98.4	98.0					
13	*Hassalia littoralis* C76	97.7	98.5	98.2	99.0	97.7	97.4	98.6	97.4	98.5	98.5	98.9	98.2				
14	*Hassallia byssoidea* CCALA 823	97.6	98.1	98.1	97.1	97.7	97.7	99.1	96.5	98.8	98.8	98.2	98.4	98.0			
15	*Coleodesmium wrangelii* MC-JRJ1	97.6	97.6	97.6	97.2	99.5	97.8	97.5	96.7	97.9	98.0	97.2	97.1	97.1	97.3		
16	*Coleodesmium* sp. Hindak 2000/24	97.9	98.5	98.5	97.5	97.5	97.8	99.3	97.5	98.3	98.4	98.0	100.0	98.2	98.4	97.1	
17	*Rexia erecta* CAT4-SG4	97.4	98.9	98.7	97.9	97.9	97.9	98.6	96.8	99.1	99.2	97.8	98.5	98.9	98.9	97.6	98.5

Hassallia californica strains T061 and T081 forms a high supported subcluster with *H. byssoidea* CCALA 823, type species of *Hassallia*, *T. distorta* SAG 93.79, *H. antarctica* CCALA 957, and *Coleodesmium* sp. HINDAK 200/24. Identity of 16S rRNA gene sequence between strains T061 and T081 is 99.9%, while when both strains are compared to *H. bysoidea* CCALA 957, *T. distorta* SAG 93.79, *H. antarctica* CCALA 957, and *Coleodesmium* sp. HINDAK 200/24, the identities are 98.8, 97.8 – 97.9, 98.3 – 98.4, and 98.3 – 98.4% respectively. *Hassallia* sp. T075 is part of a subcluster containing *H. littoralis* from rocky marine littoral in Mexico (González-Resendiz et al. 2013), and *T. carrinoi* from a cave wall in Hawaii (Miscoe et al. 2016), showing identities of 98.9 and 98.5% respectively, and when compared to *H. byssoidea* CCALA 823, the identity is 98.2%.

Tolypothrix sp. T079 places as a sister taxa of the subcluster containing *Hassallia* sp. T075, *H. littoralis,* and *T. carrinoi*, being 98.0 – 98.5% similar in identity with these strains. *Tolypothrix* cf. *helicophila* T002 forms a branch sister of a subcluster comprising *C. wrangelii* from Meigs

Creek, Great Smoky Mountains National Park, USA (Flechtner et al. 2002), and *Tolypothrix* sp. NIES-545 from Japan (NIES webpage). Identities of strain T002 compared to *C. wrangelii* MC-JR1 and *Tolypothrix* sp. NIES-515 are 97.6 and 98.3% respectively.

D. Aphanizomenonaceae, Nostocaceae, and Hapalosiphonaceae

Six strains belong to this section, one of *Nodularia* (Aphanizomenonaceae), two of *Anabaena* (Nostocaceae), two of *Trichormus* (Nostocaceae), and one to *Westiellopsis* (Hapalosiphonaceae). Measurements of main morphological features are in Table 16, while comparison of the identities of *Anabaena*/*Hydrocoryne*, *Trichormus, Nodularia*, and hapalosiphonacean strains are listed in tables 17, 18, 19, and 20 respectively. The phylogenetic tree from this section is represented in Fig. 6.

a. Morphological and ecological descriptions

Group I. *Anabaena*

- *Anabaena* sp. type 1, strain T065 (Fig. 14 A-D)

Filaments entangled (Fig. 14A), irregularly curved, with 1 up to 4 parallel trichomes. Sheaths colorless, diffuse, hyaline, rarely well-delimited (Fig. 14B). Trichomes cylindrical, constricted at cross-walls, clearly attenuated toward ends (Fig. 14C). Cells barrel-shaped or cylindrical with rounded edges (Fig. 14 B, C). Heterocytes only intercalar, shortly barrel-shaped to ± rounded. Akinetes at both side of the heterocytes, one up to three per side, ellipsoidal, cylindrical (Fig. 14D). This taxa was isolated from a soil sample covered by a green biofilm, outside the entrance of El Oconal lagoon, Villa Rica, tropical humid forest in the Peruvian Amazon.

- *Anabaena* sp. type 2, strain T033 (Fig. 14 E-I)

Mats blue-green, flat, gelatinous. Filaments straight to ± irregularly entangled (Fig. 14E), with 1 or more parallel trichomes per filament (Fig. 14F). Sheaths colorless, diffuse, hyaline, not well-delimited (Fig. 14F). Trichomes cylindrical, constricted at cross-walls, slightly attenuated towards both ends. Cells barrel-shaped, subglobose. Basal heterocytes conically rounded, shortly drop-like (Fig. 14G), always present in young trichomes, sometimes at both ends (Fig. 14I); intercalary heterocytes isodiametric to slightly longer than wide (Fig. 14 G, H). Akinetes ellipsoidal to cylindrical, solitary or up to 5 in a row, at both sides of heterocytes (Fig. 14G) or distant from them (Fig. 14H). *Anabaena* sp. type 2 was isolated from moss sample, near the entrance of El Oconal lagoon, Villa Rica, tropical humid forest in Peru.

Table 16. Measurements (µm) of the main morphological features of isopolar heterocytous strains isolated in the present study.

Taxa	Strain code	Vegetative cells		Terminal Heterocytes		Intercalar Heterocytes		Akinetes	
		Length	Width	Length	Width	Length	Width	Length	Width
Anabaena sp. type 1	T065	1.9 - 5.0	3.2 - 6.1	-	-	2.9 - 5.1	3.9 - 5.6	6.3 - 19.0	5.4 - 8.1
Anabaena sp. type 2	T033	2.8 - 7.6	3.7 - 9.6	4.0 - 7.6	3.2 - 5.7	5.2 - 9.6	5.2 - 7.0	9.3 - 17.8	5.4 - 7.0
Trichormus variabilis	T020	2.2 - 6.3	3.9 - 5.7	4.0 - 4.2	4.9 - 5.4	5.4 - 8.7	4.9 - 7.0	5.5 - 12.8	5.2- 6.9
Trichormus cf. *indicus*	T082	2.4 - 5.1	3.8 - 5.0	3.7 - 7.7	3.4 - 5.8	5.3 - 8.2	3.8 - 7.2	10.8 - 13.5	5.8 - 6.8
Nodularia sp.	T049	1.5 - 4.3	5.9 - 8.8	-	-	2.7 - 5.6	6.4 - 9.4	3.6 - 8.4	7.4 - 11.6

Group II. *Trichormus*

- *Trichormus variabilis*, strain T020 (Fig. 14 J-N)

Mats flat, mucilaginous. Filaments comprising from 1 to several straight, coiled or curved trichomes (Fig. 14 J, K, N). Sheaths colorless, delimited (Fig. 14K). Trichomes cylindrical, straight, irregularly flexuous (Fig. 14 L, M). Cells barrel-shaped, subspherical, ± quadratic, isodiametric or shorter than wide (Fig. 14L). Heterocytes mostly intercalar (Fig. 14M), rarely basal, solitary, subspherical, ± ellipsoidal, conically rounded only in basal heterocytes. Akinetes ellipsoidal, with smooth colorless exospore, ± oval to ellipsoidal, arising apoheterocitically in rows (Fig. 14N). *T. variabilis* was isolated from a soil sample in Villa Rica, tropical humid forest in Peru.

- *Trichormus* cf. *indicus* T082 (Fig. 14 O-S)

Mats mucilaginous, initially grouped into tube-like filaments (Fig. 14O), then irregular. Filaments with delimited mucilaginous envelope, with one to several trichomes (Fig. 14P). Sheaths gelatinous, not adjacent to trichomes, sometimes not evident (Fig. 14P). Trichomes isopolar, cylindrical, straight (Fig. 14Q), irregularly curved or spirally coiled in tube-like filaments (Fig. 14 O, P), slightly narrowed toward both ends in well-developed trichomes (more evident in young trichomes, Fig. 14 R, S). Cells barrel-shaped, cylindrical; terminal cells conically rounded, usually isodiametric or longer than wide (Fig. 14R). Heterocytes intercalar, solitary, ± spherical to barrel-shaped (Fig. 14 P, R); basal heterocytes conically rounded to slightly ovate (Fig. 14S). Akinetes developed apoheterocytically, in rows (Fig. 14Q), ellipsoidal with flattened ends (Fig. 14P), rarely obliquely positioned in the trichome. *Trichormus* cf. *indicus* was isolated from sediments associated with aquatic plants, near Tuctuca lagoon, located at 4450 meters a.s.l, in the Peruvian Andes.

Group III. ***Nodularia***

- *Nodularia* sp. T049 (Fig. 15 A-C)

Colonies filamentous, dendroid, tube-like (Fig. 15A), penetrating the agar, with trichomes longitudinally spirally coiled (Fig. 15A), surrounded by a hyaline, colorless, not firm, mucilaginous sheath. Trichomes uniseriate, in colonies (Fig. 15A), or solitaries (Fig. 15B), constricted at cross-walls. Cells shorter than wide, discoid or shortly barrel-shaped (Fig. 15C). Heterocytes intercalar, rarely basal, shortly conically rounded only in basal heterocytes (Fig. 15B). Akinetes compressed, subspherical, wider than long, in rows, with brown cell wall and yellowish granular content (Fig. 15C). This taxa was isolated from a soil sample in Villa Rica, tropical humid forest in Peru.

Group IV. ***Westiellopsis***

- *Westiellopsis* cf. *prolifica* T028 (Fig. 15 D-H)

Thallus ± radially disposed (Fig. 15D), forming circular or rounded patches on the agar surface. Primary filaments true-branched, torulous, flexuous (Fig. 15 E, H). Secondary filaments swollen at the base (Fig. 15E). Sheaths colourless. Primary trichomes constricted, usually slightly narrowed at terminal parts, usually monoseriate (Fig. 15 E, F), rarely biseriate to multiseriate (Fig. 15 G, H), with cells barrel-shaped, cylindrical, ± rounded, usually isodiametric or longer than wide, 3.8-15.1 x 5.4-11.0 μm. Secondary trichomes constricted, swollen at the base and slightly attenuated toward apex, sometimes *Pseudanabaena*-like form, mostly monoseriate, with cells mostly cylindrical and barrel-shaped, isodiametric to shorter than wide, rarely longer than wide (Fig. 15E), 1.8-7.3 x 3.4-6.5 μm. Terminal (basal) heterocytes present only in young trichomes of after germination from the monocytes, conically rounded to hemispherical. Intercalary heterocytes heteromorphic in primary filaments, usually longer than wide, 4.1-16.2 x 5.6-10.5 μm; in secondary filaments cylindrical, subspherical, shortly rounded, 4.2-9.3 x 4.8-8.2 μm. Lateral heterocytes rarely present, oval to subspherical, slightly smaller than intercalary heterocytes (Fig. 15F). Pseudohormocytes as a result of transversal and longitudinal division of cells, formed first at the end of lateral branches, then along all trichomes (Fig. 15H), mono- up to 4-seriate in secondary filaments, and multiseriate in primary filaments. Monocytes pale yellowish, rounded, ± spherical, shortly conical, grouped in rows in the pseudohormocytes, 3.0-6.4 x 3.6-8.1 μm. *Westiellopsis* sp. was isolated from a soil sample surrounded by small herbs in Villa Rica, tropical humid forest in Peru.

Table 17. Comparison of 16S rRNA identity of *Anabaena* and *Hydrocoryne* strains, including taxa from the present study (in bold).

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Anabaena* sp. type 1 T065**																
2	***Anabaena* sp. type 2 T033**	97.9															
3	*Anabaena* sp. XP35A	97.0	97.4														
4	*Anabaena* sp. 5-Kutnar09	98.2	98.0	97.2													
5	Cyanob. culture CAWBG85	98.2	98.1	97.5	99.5												
6	*Anabaena cylindrica* PCC 7122	98.5	98.4	97.9	99.1	99.4											
7	*Anabaena cylindrica* DC-3	99.2	98.8	98.3	99.1	99.4	100.0										
8	*Anabaena vaginicola* ISC90	98.1	98.2	97.5	99.2	99.3	99.4	99.5									
9	*A.augstumalis* SCMIDKE-JAHNKE/4a	98.4	98.4	97.7	99.1	99.1	99.6	99.8	99.6								
10	*Anabaena* sp. 15-Soos11-BA	98.3	98.3	97.5	99.0	99.1	99.6	99.7	99.5	99.9							
11	*Hydrocoryne* sp. CENA398	98.1	98.0	97.6	99.0	98.9	99.2	99.3	99.1	99.1	99.0						
12	*Hydrocoryne* sp. UFV-ANT31	98.1	98.0	97.6	98.9	98.9	99.2	99.2	99.1	99.1	99.0	99.7					
13	*Anabaena* sp. 4-Vresova10-L1	98.3	98.3	97.5	98.1	98.0	98.2	98.4	98.1	98.3	98.2	98.5	98.4				
14	Cyanobacterium BECID34	97.8	97.6	97.3	98.3	98.3	98.3	98.6	98.4	98.3	98.3	98.5	98.5	99.3			
15	*Hydrocoryne spongiosa* HA4387-MV2	98.0	98.3	97.0	98.3	98.2	98.2	98.4	98.4	98.4	98.2	98.6	98.6	99.2	98.8		
16	*A. oscillarioides* BO-HINDAK 1984/43	95.7	96.2	95.9	96.1	96.2	96.6	96.3	96.4	96.4	96.3	96.6	96.6	96.1	96.3	95.9	
17	*Anabaena oscillarioides* BECID22	95.0	96.1	95.4	95.1	95.4	95.7	95.5	95.5	95.5	95.3	95.4	95.4	95.1	95.3	95.5	95.6

b. Analysis based on 16S rRNA gene

The six strains described previously fall in Clade A (Nostocales – I), Clade B (Nostocales – II), Clade C (Nostocales – III), and Clade F (Hapalosiphonaceae). Clade A contains strains identified as *Anabaena, Hydrocoryne, Raphidiopsis, Sphaeropsermopsis, Wollea,* and one strain incorrectly named "*Nostoc azollae*", which does not exist in the literature. Clade B is composed by strains named *Anabaena, Aphanizomenon, Cuspidothrix, Dolichospermum* and *Trichormus*. Cluster C harbors strains of *Anabaenopsis, Chrysosporum, Cyanocohniella, Cyanospira*, and *Nodularia*. Clade F possess only the true-branching cyanobacteria *Fischerella*, *Hapalosiphon*, *Mastigocladus*, *Nostochopsis*, *Pelatocladus*, *Westiella*, and *Westiellopsis*. Clades D (*Halotia*) and E (Nostocales – IV), containing *Trichormus* sequences are shown for discussion.

Fig. 6. Phylogenetic tree based on 16S rRNA gene sequences of heterocytous cyanobacteria, focused on the Aphanizomenonaceae, Nostocaceae, and Hapalosiphonaceae families. Clades A – F are shown in detail. Strains studied in the present work are in bold. Nodes report bootstrap support for Bayesian analysis and maximum likelihood. Climate classification follows Peel et al. (2007).

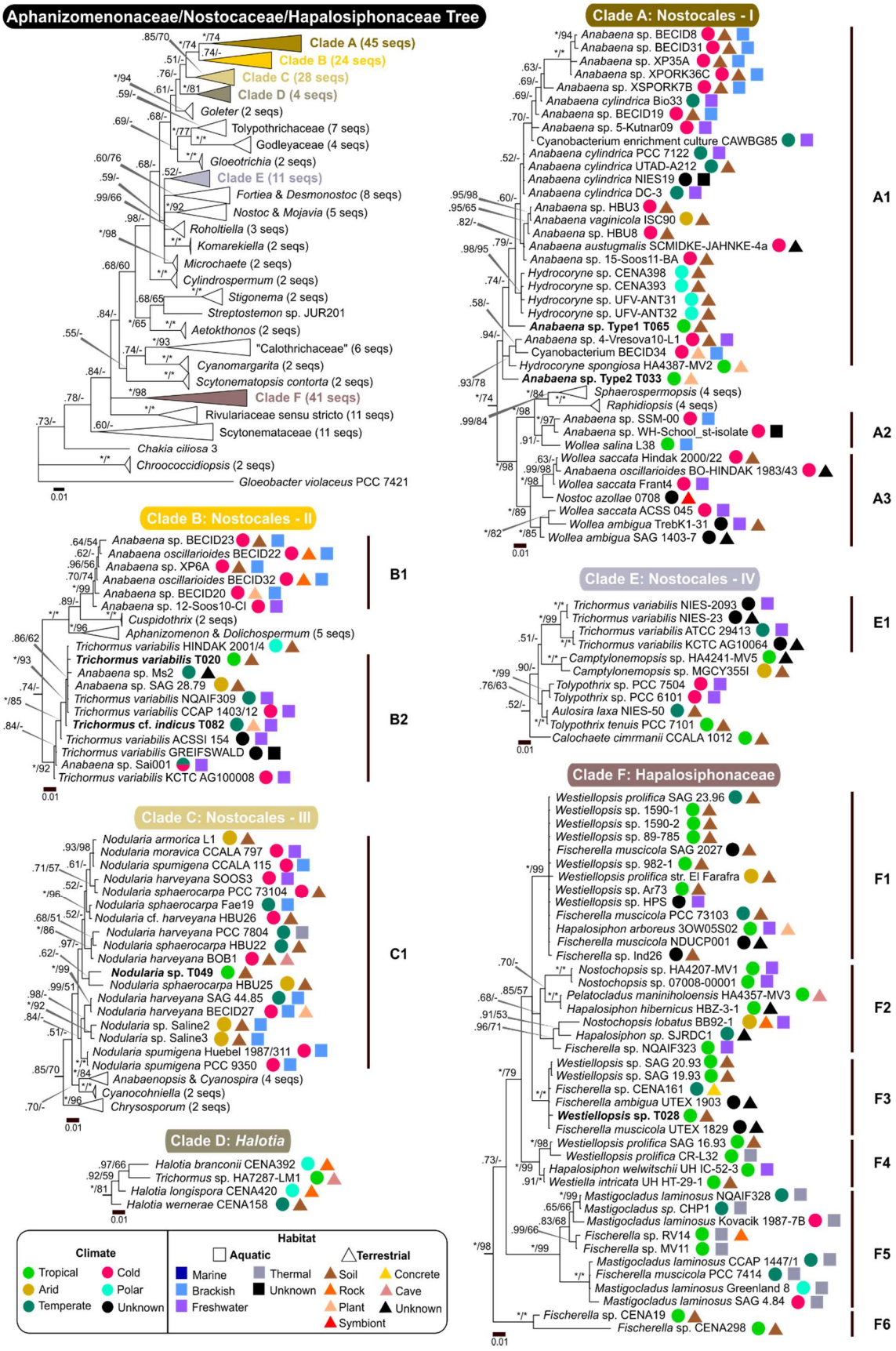

Aphanizomenonaceae/Nostocaceae/Hapalosiphonaceae Tree
Clade A (45 seqs)
Clade B (24 seqs)
Clade C (28 seqs)
Clade D (4 seqs)
Goleter (2 seqs)
Tolypothrichaceae (7 seqs)
Godleyaceae (4 seqs)
Gloeotrichia (2 seqs)
Clade E (11 seqs)
Fortiea & Desmonostoc (8 seqs)
Nostoc & Mojavia (5 seqs)
Roholtiella (3 seqs)
Komarekiella (2 seqs)
Microchaete (2 seqs)
Cylindrospermum (2 seqs)
Stigonema (2 seqs)
Streptostemon sp. JUR201
Aetokthonos (2 seqs)
"Calothrichaceae" (6 seqs)
Cyanomargarita (2 seqs)
Scytonematopsis contorta (2 seqs)
Clade F (41 seqs)
Rivulariaceae sensu stricto (11 seqs)
Scytonemataceae (11 seqs)
Chakia ciliosa 3
Chroococcidiopsis (2 seqs)
Gloeobacter violaceus PCC 7421
0.01
Clade A: Nostocales - I
Anabaena sp. BECID8
Anabaena sp. BECID31
Anabaena sp. XP35A
Anabaena sp. XPORK36C
Anabaena sp. XSPORK7B
Anabaena cylindrica Bio33
Anabaena sp. BECID19
Anabaena sp. 5-Kutnar09
Cyanobacterium enrichment culture CAWBG85
Anabaena cylindrica PCC 7122
Anabaena cylindrica UTAD-A212
Anabaena cylindrica NIES19
Anabaena cylindrica DC-3
Anabaena sp. HBU3
Anabaena vaginicola ISC90
Anabaena sp. HBU8
Anabaena austugmalis SCMIDKE-JAHNKE-4a
Anabaena sp. 15-Soos11-BA
Hydrocoryne sp. CENA398
Hydrocoryne sp. CENA393
Hydrocoryne sp. UFV-ANT31
Hydrocoryne sp. UFV-ANT32
Anabaena sp. Type1 T065
Anabaena sp. 4-Vresova10-L1
Cyanobacterium BECID34
Hydrocoryne spongiosa HA4387-MV2
Anabaena sp. Type2 T033
Sphaerospermopsis (4 seqs)
Raphidiopsis (4 seqs)
Anabaena sp. SSM-00
Anabaena sp. WH-School_st-isolate
Wollea salina L38
Wollea saccata Hindak 2000/22
Anabaena oscillarioides BO-HINDAK 1983/43
Wollea saccata Frant4
Nostoc azollae 0708
Wollea saccata ACSS 045
Wollea ambigua TrebK1-31
Wollea ambigua SAG 1403-7
A1
A2
A3
Clade B: Nostocales - II
Anabaena sp. BECID23
Anabaena oscillarioides BECID22
Anabaena sp. XP6A
Anabaena oscillarioides BECID32
Anabaena sp. BECID20
Anabaena sp. 12-Soos10-CI
Cuspidothrix (2 seqs)
Aphanizomenon & Dolichospermum (5 seqs)
Trichormus variabilis HINDAK 2001/4
Trichormus variabilis T020
Anabaena sp. Ms2
Anabaena sp. SAG 28.79
Trichormus variabilis NQAIF309
Trichormus variabilis CCAP 1403/12
Trichormus cf. indicus T082
Trichormus variabilis ACSSI 154
Trichormus variabilis GREIFSWALD
Anabaena sp. Sai001
Trichormus variabilis KCTC AG100008
B1
B2
Clade E: Nostocales - IV
Trichormus variabilis NIES-2093
Trichormus variabilis NIES-23
Trichormus variabilis ATCC 29413
Trichormus variabilis KCTC AG10064
Camptylonemopsis sp. HA4241-MV5
Camptylonemopsis sp. MGCY355I
Tolypothrix sp. PCC 7504
Tolypothrix sp. PCC 6101
Aulosira laxa NIES-50
Tolypothrix tenuis PCC 7101
Calochaete cimrmanii CCALA 1012
E1
Clade C: Nostocales - III
Nodularia armorica L1
Nodularia moravica CCALA 797
Nodularia spumigena CCALA 115
Nodularia harveyana SOOS3
Nodularia sphaerocarpa PCC 73104
Nodularia sphaerocarpa Fae19
Nodularia cf. harveyana HBU26
Nodularia harveyana PCC 7804
Nodularia sphaerocarpa HBU22
Nodularia harveyana BOB1
Nodularia sp. T049
Nodularia sphaerocarpa HBU25
Nodularia harveyana SAG 44.85
Nodularia harveyana BECID27
Nodularia sp. Saline2
Nodularia sp. Saline3
Nodularia spumigena Huebel 1987/311
Nodularia spumigena PCC 9350
Anabaenopsis & Cyanospira (4 seqs)
Cyanocohniella (2 seqs)
Chrysosporum (2 seqs)
C1
Clade F: Hapalosiphonaceae
Westiellopsis prolifica SAG 23.96
Westiellopsis sp. 1590-1
Westiellopsis sp. 1590-2
Westiellopsis sp. 89-785
Fischerella muscicola SAG 2027
Westiellopsis sp. 982-1
Westiellopsis prolifica str. El Farafra
Westiellopsis sp. Ar73
Westiellopsis sp. HPS
Fischerella muscicola PCC 73103
Hapalosiphon arboreus 3OW05S02
Fischerella muscicola NDUCP001
Fischerella sp. Ind26
Nostochopsis sp. HA4207-MV1
Nostochopsis sp. 07008-00001
Pelatocladus maniniholoensis HA4357-MV3
Hapalosiphon hibernicus HBZ-3-1
Nostochopsis lobatus BB92-1
Hapalosiphon sp. SJRDC1
Fischerella sp. NQAIF323
Westiellopsis sp. SAG 20.93
Westiellopsis sp. SAG 19.93
Fischerella sp. CENA161
Fischerella ambigua UTEX 1903
Westiellopsis sp. T028
Fischerella muscicola UTEX 1829
Westiellopsis prolifica SAG 16.93
Westiellopsis prolifica CR-L32
Hapalosiphon welwitschii UH IC-52-3
Westiella intricata UH HT-29-1
Mastigocladus laminosus NQAIF328
Mastigocladus sp. CHP1
Mastigocladus laminosus Kovacik 1987-7B
Fischerella sp. RV14
Fischerella sp. MV11
Mastigocladus laminosus CCAP 1447/1
Fischerella muscicola PCC 7414
Mastigocladus laminosus Greenland 8
Mastigocladus laminosus SAG 4.84
Fischerella sp. CENA19
Fischerella sp. CENA298
F1
F2
F3
F4
F5
F6
Clade D: Halotia
Halotia branconii CENA392
Trichormus sp. HA7287-LM1
Halotia longispora CENA420
Halotia wernerae CENA158
Climate
Tropical
Arid
Temperate
Cold
Polar
Unknown
Habitat
Aquatic
Marine
Brackish
Freshwater
Thermal
Unknown
Terrestrial
Soil
Rock
Plant
Symbiont
Concrete
Cave
Unknown

Table 18. Comparison of 16S rRNA identity of *Trichormus* strains, including taxa from the present study (in bold), and related taxa.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Trichormus variabilis* T020**																
2	***Trichormus* cf. *indicus* T082**	99.8															
3	*Trichormus variabilis* HINDAK 2001/4	99.9	99.9														
4	*Anabaena* sp. Ms2	99.7	99.8	99.9													
5	*Anabaena* sp. SAG 28.79	99.9	99.9	100.0	99.9												
6	*Trichormus variabilis* NQAIF309	99.9	99.9	100.0	99.9	100.0											
7	*Trichormus variabilis* CCAP 1403/12	99.9	99.9	100.0	99.8	100.0	100.0										
8	*Trichormus variabilis* ACSSI 154	99.7	99.8	99.8	99.6	99.8	99.8	99.7									
9	*Trichormus variabilis* GREIFSWALD	99.1	99.3	99.2	99.1	99.2	99.2	99.1	99.8								
10	*Anabaena* sp. Sai001	99.1	99.3	99.2	99.1	99.2	99.2	99.0	99.8	100.0							
11	*Trichormus variabilis* KCTC AG10008	99.1	99.2	99.1	99.0	99.1	99.1	99.1	99.8	99.9	99.9						
12	*Wollea saccata* Hindak 2000/22	96.0	95.9	95.9	95.7	95.9	96.1	95.7	96.4	96.6	96.5	96.6					
13	*Anabaena oscillarioides* BECID22	95.9	95.9	95.9	95.9	95.9	96.2	95.8	96.4	96.5	96.5	96.6	95.4				
14	*Hydrocoryne spongiosa* HA4387-MV2	97.3	97.2	97.3	97.1	97.2	97.2	97.2	97.4	97.1	97.1	97.1	95.7	95.4			
15	*Trichormus* sp. HA7287-LM1	96.0	96.2	96.1	95.9	96.1	96.3	96.2	96.3	96.3	96.2	96.3	94.9	94.4	95.7		
16	*Halotia branconii* CENA392	94.4	94.4	94.4	94.3	94.3	94.6	94.9	95.5	94.9	94.9	95.0	94.1	93.8	94.4	97.5	
17	*Trichormus variabilis* ATCC 29413	94.7	94.6	94.6	94.4	94.5	94.9	94.8	95.1	95.2	95.2	95.3	95.7	93.8	95.1	95.4	94.7

Clade A. Nostocales – I

This clade divides in subclusters A1 – A3, and *Sphaerospermopsis* and *Raphidiopsis* strains. Subcluster A1 contains *Anabaena* and *Hydrocoryne* strains, including two not identified (strains CAWBBG85 and BECID34), two *Anabaena* taxa isolated in the present work, and strain HA4387-MV2, identified as *H. spongiosa*, type species of *Hydrocoryne.* Subcluster A2 shows two *Anabaena* strains from cold localities at USA (Stevenson & Waterbury 2006, Tomitani et al. 2006), and *Wollea salina* from brackish biotope in Thailand (Kozliková-Zapomělová et al. 2016). Subcluster A3 is composed of *Anabaena, Wollea*, "*Nostoc azollae*" 0708, and includes strain BO-HINDAK 1983/43, identified as *A. oscillarioides*, type species of *Anabaena*, and three strains (Hindak 2000/22, Frant4, and ACSS 045) identified as *W. saccata*, type species of *Wollea*.

Anabaena sp. type 1 T065 and *Anabaena* sp. type 2 T033 cluster within Subclade A2. Both strains place separated and as

Table 19. Comparison of 16S rRNA identities of selected *Nodularia* and related strains, including *Nodularia* sp. T049 from the present study (in bold).

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Nodularia* sp. T049**																
2	*Nodularia sphaerocarpa* HBU25	98.6															
3	*Nodularia harveyana* BOB1	98.6	98.4														
4	*Nodularia harveyana* PCC7804	98.1	98.5	99.5													
5	*Nodularia sphaerocarpa* Fae19	98.8	98.4	99.2	98.9												
6	*Nodularia sphaerocarpa* PCC73104	98.7	99.1	98.9	98.8	99.1											
7	*Nodularia spumigena* CCALA 115	98.3	98.7	99.0	99.1	99.2	99.4										
8	*Nodularia moravica* CCALA 797	98.4	98.7	99.1	99.2	99.3	99.4	100.0									
9	*Nodularia armorica* L1	98.2	98.5	98.9	98.9	99.1	99.2	99.8	99.8								
10	*Nodularia harveyana* SAG 44.85	98.2	98.4	98.2	98.1	98.4	98.5	98.5	98.4	98.2							
11	*Nodularia* sp. Saline2	98.6	98.6	98.3	98.0	98.3	98.8	98.5	98.6	98.4	98.8						
12	*Nodularia* sp. Saline3	98.7	98.9	98.4	98.4	98.5	98.6	98.8	98.9	98.7	99.0	99.6					
13	*Nodularia spumigena* PCC9350	98.6	98.9	98.9	99.0	99.1	99.1	99.4	99.4	99.1	98.8	98.7	99.1				
14	*Anabaenopsis elenkinii* SAG 252.80	97.7	97.0	97.1	97.2	97.7	97.3	97.4	97.4	97.1	97.2	97.7	97.7	97.7			
15	*Cyanospira rippkae*	97.5	97.0	97.5	97.5	97.5	97.2	96.7	97.0	96.7	97.3	97.4	97.5	97.7	98.2		
16	*Cyanocohniella calida* CCALA 1049	97.4	97.0	97.4	97.2	97.5	96.7	97.3	97.0	96.9	97.5	97.2	97.5	97.2	97.2	97.8	
17	*Chrysosporum bergii* 09-02	97.0	96.6	96.4	96.2	96.7	96.6	96.4	96.5	96.3	97.2	96.7	96.8	96.9	96.7	96.5	96.0

independent branches within Subclade A1. Identities of 16S rRNA gene sequence between *Anabaena* sp. type 1 compared to strains BO HINDAK 1984-43 and BECID22, both identified as *A. oscillarioides*, are 95.7 and 95.0% respectively, while when *Anabaena* sp. type 2 is compared to both *A. oscillarioides* strains, the identities are 96.2 and 96.1% respectevly.

Clade B. Nostocales – II

This clade contains subclusters B1, B2, and strains of *Cuspidothrix, Aphanizomenon* and *Dolichospermum*. Subcluster B1 shows six *Anabaena* sequences, two of them identified as *A. oscillarioides*, all of them from cold localities in Finland (Halinen et al. 2008, Rajaniemi et al. 2005), and Czech Republic (Kozlikova-Zapomelova et al. 2016, Kust et al. 2015). Subcluster B2 contains eleven strains, three of *Anabaena,* and eight of *Trichormus*, six identified as *T. variabilis*, type species of the *Trichormus*. Two strains isolated from the present work fall within Subcluster B2: *T. variabilis* T020, and *Trichormus* cf. *indicus* T082. Identities of 16S rRNA gene

Table 18. Comparison of 16S rRNA identity of selected hapalosiphonacean strains, including *Westiellopsis* sp. T028 from the present study (in bold).

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	***Westiellopsis* sp. T028**																
2	*Westiellopsis* sp. SAG 20.93	99.6															
3	*Westiellopsis* sp. SAG 19.93	99.6	100.0														
4	*Fischerella* sp. CENA161	99.6	99.6	99.6													
5	*Fischerella ambigua* UTEX 1903	99.8	99.8	99.8	99.9												
6	*Fischerella muscicola* UTEX 1829	99.9	100.0	100.0	99.8	100.0											
7	*P. maniniholoensis* HA4357-MV3	97.2	97.2	97.2	97.3	97.4	98.0										
8	*Hapalosiphon hibernicus* BZ-3-1	97.2	97.2	97.2	97.2	97.4	97.9	100.0									
9	*Nostochopsis lobatus* BB92.1	96.9	96.9	96.9	96.9	97.1	97.7	97.0	97.0								
10	*Westiellopsis prolifica* SAG 23.96	97.9	98.1	98.1	97.9	98.1	98.5	97.2	97.2	96.8							
11	*Fischerella muscicola* SAG 2027	97.9	98.1	98.1	98.0	98.1	98.5	97.3	97.2	96.9	100.0						
12	*Hapalosiphon arboreus* 3OW05S02	97.6	97.8	97.8	97.7	97.8	98.5	97.3	97.2	96.9	100.0	100.0					
13	*Westiellopsis prolifica* SAG 16.93	97.6	97.6	97.6	97.8	97.8	97.9	96.8	96.8	95.9	97.2	97.2	97.2				
14	*Hapalosiphon welwitschii* UH IC-52-3	97.1	97.1	97.1	97.2	97.3	97.8	97.5	97.4	96.6	97.4	97.5	97.4	99.2			
15	*Westiella intricata* UH HT-29-1	97.1	97.1	97.1	97.2	97.3	97.8	97.5	97.4	96.6	97.4	97.5	97.4	99.2	100.0		
16	*M. laminosus* Kovacik 1987/7B	94.2	94.5	94.5	94.4	94.5	94.1	95.1	95.0	95.0	95.1	95.2	95.2	95.0	95.3	95.3	
17	*Fischerella* sp. CENA19	95.3	95.3	95.3	95.3	95.5	95.6	95.0	95.0	94.6	94.7	94.8	94.5	95.4	95.3	95.3	93.4

sequences within members of subcluster B2 are 99.0 – 100%.

Clade C. Nostocales – III

Clade C contains subcluster C1, *Anabaenopsis*, *Cyanospira*, *Cyanocohniella*, and *Chrysosporum*. Subcluster C1 includes eighteen *Nodularia* strains, including three different strains identified as *N. spumigena* Mertens ex Bornet & Flahault, type species of the genus. *Nodularia* sp. strain T049, isolated in the present study, fall within subcluster C1. Identities of 16S rRNA gene sequence between *Nodularia* sp. T049 and *N. spumigena* strains CCALA 115, Huebel 1987/311, and PCC 9350 are 98.3, 98.6, and 98.6% respectively. Members of subcluster C1 are 98.0 - 100% similar in genetic identity.

Clade D. Hapalosiphonaceae

This clade divides in subclusters F1 – F4. Subcluster F1 is analogous to "W-clade" sensu Saber et al. (2017), and comprises eight strains of *Westiellopsis*, including two of *W. prolifica* Janet, type species of the genus, four identified as *Fischerella*, and one of *Hapalosiphon arboreus* West & G.S.West. Most of these

strains come from soil samples in tropical localities (Finsinger et al. 2008, Gugger & Hoffmann 2004, Komárek et al. 2017, Saber et al. 2017). Subcluster F2 includes three *Nostochopsis* strains, two *Hapalosiphon*, one *Fischerella*, and one *Pelatocladus*. Subcluster F3 has three *Fischerella*, and three *Westiellopsis* strains, including *Westiellopsis* sp. strain T028 isolated in the present study. Subcluster F4 includes two *W. prolifica* strains, one *Hapalosiphon*, and one strain identified as *Westiella intricata*, type species of *Westiella*. Subcluster F5 comprises only thermal strains of the genera *Mastigocladus,* including its type species *M. laminosus*, and *Fischerella*. Subcluster F6 shows two *Fischerella* strains from soil biotopes in Brazil.

Westiellopsis sp. T028 falls in Sucbcluster F3 (Clade F), related to *Westiellopsis* sp. SAG 20.93 and 19.93 from soil habitat in Thailand (SAG webpage), *Fischerella* sp. CENA 162 from water sample in Piracicaba at São Paulo State, Brazil (Fiore et al. 2009), *F. muscicola* UTEX 1829, and *F. ambigua* UTEX 1903. Identities of 16S rRNA gene sequence between *Westiellopsis* sp. strain T028 and other strains from subcluster F3 ranges from 99.6 to 99.9%.

IV. DISCUSSION

A. The Family Rivulariaceae

The Rivulariaceae is morphologically defined as having tapered filaments usually ending in hairs, obligatory basal heterocytes, and having facultative false branching and intercalary heterocytes (Komárek 2013). In last years, *Phyllonema, Nunduva, Cyanomargarita* and *Macrochaete*, were erected as novel genera within the Rivulariaceae, the last two considered cryptotaxa. (Berrendero-Gómez et al. 2016, Shalygin et al. 2017). Sequences of *Gardnerula, Isactis,* and *Sacconema* are not available, and their position within the Rivulariaceae has not been demostrated. In the phylogenetic tree (Fig. 2), rivulariacean taxa locate in clades A – C, which confirms the polyphyletic status of the Rivulariaceae (Berrendero-Gomez et al. 2016, Berrendero et al. 2008, González-Resendiz et al. 2018, León-Tejera et al. 2016, Shalygin et al. 2017, Sihvonen et al. 2007). Clade A comprises most of the strains identified as *Calothrix,* and divides in subcluster A1 – A5*,* 3 non-grouped *Calothrix* strains, and 2 *Rivularia* strains. Subcluster A4 belongs to a novel cluster, containing the new genus *Skacelovkia*. Clade B comprises two sequences of *S. contorta*, three of *Calothrix,* four of uncultured cyanobacteria, and two of "*Dichothrix*" from the present study. Clade C includes *K. huatulcensis, Nunduva, Mastigocoleus, Phyllonema,* most of the strains identified as *Rivularia*, and some strains identified as *Calothrix,* including strain T044 from the present study. All these results are in agreement with previous papers (Alvarenga et al. 2016, González-Resendiz et al. 2018, León-Tejera et al. 2016, Ramírez-Reinat & Garcia-Pichel 2012).

a. The genus *Calothrix*

Strains identified as *Calothrix* are distributed in the clades A – C, confirming its polyphyletic status (Berrendero-Gomez et al. 2016, Sihvonen et al. 2007). Most of the strains within Clade A are separated in three groups, each one with ecologically similar taxa: subcluster A1 (*Calothrix* Freshwater & Soil), subcluster A2 (*Calothrix* Brackish – I), subcluster A3 (*Calothrix* Brackish – II). In addition, subcluster A4 corresponds to the novel genus *Skacelovkia,* and subcluster A5 to *Macrochaete.* These findings, except for the *Skacelovkia* group, are supported by different works (Berrendero-Gomez et al. 2016, Berrendero et al. 2008, Shalygin et al. 2017, Sihvonen et al. 2007). Five *Calothrix* strains from the present work fall within subcluster A1, all of them from soil or moss samples. *Calothrix* sp. type 4 T029 falls as a sister branch of subcluster A2. Two *Skacelovkia* strains isolated in this study form a well-supported subcluster, separated from other *Calothrix* groups, showing low identities compared with other cyanobacteria. Three *Calothrix* strains from aquatic biotopes

fall within Clade B, which will be discussed in the "*Dichothrix*" section. Four *Calothrix* strains are present in Clade C (Rivulariaceae sensu stricto). These strains are mostly from marine or brackish biotopes, and one of them, *Calothrix* sp. type 5 T044, was isolated in the present study.

Calothrix cf. *elenkinii* T014 is similar to *C. elenkinii* Kosinskaja in having long clusters of filaments entangled together, with rarely false branching, and trichomes without hairs (Komárek 2013). However, *C. elenkinii* does not have isodiametric cells like in strain T014, and it was described based on a liquid culture from a river in Russia, while *C.* cf. *elenkinii* was isolated from muddy soil in Villa Rica, Amazon region in Peru. *Calothrix* cf. *elenkinii* forms a subcluster together with *Calothrix* sp. PCC 7715, and *Rivularia* sp. IAM M-261, within *Calothrix* Freshwater & Soil (subcluster A1), the three being identical in 16S rRNA gene sequence (table 7). Strain PCC 7715 was identified as *C. thermalis* Hansgirg ex Bornet et Flahault in the Durham Culture Collection (DCC), and following Komárek (2013), *C. thermalis* possess trichomes ending in hairs, isodiametric to longer than wide cells, and akinetes, features never observed in *Calothrix* cf. *elenkinii* T014. In addition, strains PCC 7715 and T014 have different ecology: soils in Peruvian Amazon vs. thermal waters in France, respectively. There is no information of *Rivularia* IAM M-261. Based on identities and phylogenetic position of *Calothrix* cf. *elenkinii* T014, *Calothrix* sp. PCC 7715, and *Rivularia* sp. IAM M-261, it is likely that they correspond to the same species. Therefore, this species would have broad ecological and morphological spectrums. However, further studies using the polyphasic approach on *Calothrix* sp. PCC 7715 and *Rivularia* sp. IAM M-261 are desirable.

Calothrix cf. *marchica* strains T064 and T067 resembles *C. marchica* Lemmermann in showing constricted trichomes, conically rounded apical cell, colorless sheath, and similar cell dimensions (Komárek 2013). However, it differs from it in having longer filaments (up to ± 1.2mm), false branching, akinetes, slightly different ecology (epiphytic in mosses in strains T064 and T067, while epiphytic in *Nostoc* and green algae in *C. marchica*), and different distribution (Europe for *C. marchica,* Peru for *Calothrix* cf *marchica*). Nevertheless, comparison on morphology of *Calothrix* cf. *marchica* strains T064 and T067 and *C. marchica* must consider that the latter is described based on natural populations, while the Peruvian strains rely on cultures. In the phylogenetic tree (Fig. 2), *Calothrix* cf. *marchica* strains T064 and T067 cluster with *Calothrix* sp. PCC 7714, forming a high-supported group.

Identity of 16S rRNA gene sequence between strains T064 and T067 is 99.3%, and both compared to PCC 7714 is 99.3 – 99.7% (table 7), suggesting that the three strains belong to one single species according to proposed limit for species separation (98.7 – 99%) by Stackebrandt & Ebers (2006). *Calothrix* sp. PCC 7714 is the same strain as *C. marchica* DCC D0202 from the Durhma Culture Collection (DCC) (Rippka et al. 2001). Morphology of PCC 7714 is not documented, but it was isolated from a pool in India, a different habitat from that described for the species (Komárek 2013), but resembling more to the habitat of var. *intermedia*, described by Rao (1937). These evidences will suggest that strains T064, T067, and PCC 7714 could belong to the same species, possibly corresponding to *C. marchica*. Morphological evaluation (especially verification of akinetes) and studies on the 16S-23S ITS structure of PCC 7714 must confirm this theory

Calothrix sp. type 2 strain T035 and *Calothrix* sp. type 3 strain T019 correspond morphologically to *Calothrix* possessing solitary heteropolar filaments, basal heterocytes, and facultative false branching (Komárek 2013). Both taxa differ in morphology, but share similar habitats: mosses in tropical forest. Hairs were not produced in both strains, even though they were cultured in low nutrient media. In the phylogenetic tree *Calothrix* sp. type 2 clusters with *Calothrix* sp. HA4395-MV3 from Hawaii, both with similar habitat (aerophytic in moss), and being 99% identical in 16S rRNA gene sequence (table 7). Following Stackebrandt & Ebers (2006), identities above 98.7 – 99.0% between strains would suggest that they belong to the same species. Considering that there is no published morphological information of strain HA4395-MV3, and based on the discussed information, we cannot assure that *Calothrix* sp. type 2 is conspecific with the Hawaiian strain. On the other hand, *Calothrix* sp. type 3 strain T019 clusters as a sister taxa of the "*C. marchica*" group (strains T064, T067, and PCC 7714) with high support. Strain T019 shows identities of 97.1 – 97.2% with *Calothrix* cf. *marchica* strains T064 and T067, and *Calothrix* sp. PCC 7714, below the cut-off for species separation (Stackebrandt & Ebers 2006), and well above 95%, the limit for genus recognition (Stackebrandt & Goebel 1994). Strains T019, T064, T067 were isolated from similar habitat (mosses), while PCC 7714 was isolated from a pool. Morphologically, *Calothrix* sp. type 3 differs from *Calothrix* cf. *marchica* in showing shorter filaments, and not producing akinetes and hairs. However, these differences must consider that strain T019 and strains T064 and T067 were cultured using different culture media. Based on all these evidences, *Calothrix* sp. type 3 T019 possibly correspond to a novel species, however, further studies using the 16S-23S ITS sequence must be performed.

Calothrix sp. type 1 strain T030 shows the typical features of *Calothrix*, but identification at species level was not possible. Strain T030 shows constricted, gradually narrowed trichomes with shortly barrel-shaped cells ending in rounded or conically rounded, resembling *C. desertica* Schwabe. However, *C. desertica* has creeping and erect filaments and sheaths yellow near the base, features not observed for *Calothrix* sp. type 1. *Calothrix* sp. type 1 was isolated from muddy soils, common habitat from several *Calothrix* taxa (Komárek 2013). In the phylogenetic tree, *Calothrix* sp. type 1 locates as a sister branch of a group of *Calothrix* strains from brackish rivers in Spain (Fig. 2). Identity of 16S rRNA gene sequence between *Calothrix* sp. type 1 compared to *Calothrix* sp. UAM 373, UAM 315, and UAM 372 is 98.5, 99.2, and 99.1% respectively. Following Stackebrandt & Ebers (2006), strains T030, UAM 315, and UAM 372 could belong to the same species because they are above the cut-off for species separation, while strains T030 and UAM 373 would be different species. *Calothrix* sp. UAM 315 form colonies (Fig. 2H, Berrendero et al. 2008) and "akinetes" (Fig. 28N, Berrendero 2008), while *Calothrix* sp. UAM 372 shows brownish to yellowish sheaths and multicellular hairs (Berrendero et al. 2011), any of these features being present in strain T030. Furthermore, strains UAM 315 and UAM 372 were collected from temperate brackish rivers (Berrendero et al. 2008), different from the habitat of *Calothrix* sp. type 1. Consequently, based on morphological and ecological information, *Calothrix* sp. type 1 is likely not conspecific with strains UAM 315 and UAM 372. However, further analysis using the 16S-23S ITS sequence must confirm this hypothesis.

Calothrix sp. type 4 strain T029 fits the description of *Calothrix* in terms of morphology, although it could not be identified at species level. Strain T029 does not show hairs, even though it was cultured using the modified Chu10 medium. Two morphological features are notorious in *Calothrix* sp. type 4: (1) The U or C-like curvature in its filaments (Fig. 7 A, E, F), and, (2) the agglomeration of parallel hormogonia and young trichomes, appearing like green dots when observed macroscopically (Fig. 7H). *Calothrix* sp. type 4 was isolated from epiphyton on submersed aquatic plant in a tropical lagoon (El Oconal), similar habitat and locality to that of *C. clavata* G.S. West and *C. columbiana* G.S. West (Komárek 2013). However, *C. clavata* and *C. columbiana* differ notoriously from *Calothrix* sp. type 4 in cell dimensions. Strikingly, *Calothrix* sp. type 4 clusters as a sister taxa of cf. *Calothrix* sp. Muscicolous cyanobiont 5, an hepatic cyanobiont, and subcluster A2, including strains from cold and brackish habitats (fig. 2). Identities of 16S rRNA gene sequence between strain

T029 compared to cf. *Calothrix* sp. Muscicolous cyanobiont 5, and *C. parietina* CCAP 1410/11 (representative strain from subcluster A2) are 97.7 and 98.3% respectively, below the cut-off for species separation (Stackebrandt & Ebers 2006). Based on these evidences, *Calothrix* sp. type 4 would probably correspond to a novel species, but further studies including the 16S-23S ITS sequence of phylogenetically related strains are necessary.

b. The genus *Rivularia*

Our results show clades A and B containing *Rivularia* sequences, which confirms its polyphyly (Berrendero et al. 2008). Clade A shows two *Rivularia* strains from a brackish river in Spain (UAM 305), and concrete fountain in Italy (VP4-08) locate as a sister group of subcluster A1. Clade C includes *Rivularia* strains that fall within subclusters C1 and C3 (marine or brackish habitats), and C4 (freshwater). Subcluster C1 is weakly supported, and comprises *Rivularia* and *Nunduva* strains. Subclusters C2 and C4 contain sequences of microbial crusts in marine and freshwater biotopes from Australia and Mexico respectively. Subcluster C3 comprises strains of *Rivularia* and *Calothrix*, all from brackish waters, including *Calothrix* sp. type 5 strain T044 from the present study. All these results are in agreement with previous works (León-Tejera et al. 2016, González-Resendiz et al. 2017).

Calothrix sp. type 5 T044 corresponds morphologically to *Calothrix*, and differs from *Rivularia* in the absence of colonies. However, some confusion existed on the identification of strain T044 because some filaments resembled *Scytonematopsis*, mostly observed in fresh material, rarely on cultures. Hyaline hairs was frequently observed when first isolated, but rarely after some months, possibly because strain T044 is cultured using BG11-N, which could influence hair development in *Calothrix* and *Rivularia* (Berrendero et al. 2008). The locality from which strain T044 was isolated (La Encantada lagoon) probably contain high levels of NaCl, SO_4Na_2, SO_4Ca, and $CaCO_3$, main salts from similar localities in the coast of Peru (Maldonado 1943). Therefore, it is probably that BG11-N would not be the most suitable medium for culturing *Calothrix* sp. type 5. On the other hand, *Calothrix* sp. type 5 is morphologically similar to *C. scopulorum* (Weber et Mohr) Agardh ex Bornet et Flahault, but they differ in branching pattern, and constriction of trichomes (Komárek 2013). In addition, *C. scopulorum* occurs mostly as epilithic in marine biotopes, while strain T044 was isolated from mineralized crusts attached to roots of *Typha dominguensis* in a coastal brackish lagoon, both habitats not markedly different. Phylogenetically, *Calothrix* sp. type 5 forms a well-supported group with *Calothrix* sp. CCMEE 5093 from Yellowstone National Park, USA

(Dillon et al. 2003), and *Rivularia* sp. PUNA-NP3-PCI185B from Argentina (Shalygin et al. 2017). Identity of 16S rRNA gene sequence between strains T044 compared to CCMEE 5093 and PUNA-NP3-PCI185B is 99.8 and 99.3% respectively, well above the cut-off for species separation (Stackebrandt & Ebers 2006), suggesting they belong to a single species. There is no published data on the morphology of *Calothrix* sp. CCMEE 5093 and *Rivularia* sp. PUNA-NP3-PCI185B, however, both show similar ecological conditions compared to *Calothrix* sp. type 5. *Calothrix* sp. CCMEE 5093 and strain T044 were isolated from habitats with high content in mineral compounds, the first one from siliceous substrate of shallow, tepid (<35°C) hot spring effluents, and the second one from mineralized rocks attached to *T. dominguensis* in brackish coastal lagoon. Dillon et al. (2003) mention that *Calothrix* sp. CCMEE 5093 shows brown-blackish filaments due to scytonemin in its sheath, working as protection for high UV radiation. In the same sense, the central coast of Peru, region from where *Calothrix* sp. type 5 was isolated, shows high rates of UV radiation and temperatures that rise up to 35°C in summer (Rundel et al. 1991). This could explain the brownish, lamellated sheath (Fig. 7T) most frequently observed in fresh material of *Calothrix* sp. type 5. On the other hand, *Rivularia* sp. PUNA-NP3-PCI185B was isolated from an unknown locality within the Argentinian Andes, thus, it is probably that this taxa is also exposed to high UV radiation, as typical for the highlands across the Andean Cordillera. Based on these data, it is probably that *Calothrix* sp. type 5, *Calothrix* sp. CCMEE 5093 and *Rivularia* sp. PUNA-NP3-PCI185B belong to a single species, but morphological evaluation and characterization of the 16S-23S ITS sequence of the last two strains is necessary. On the other hand, genetic identities of strains T044, CCMEE 5093, and PUNA-NP3-PCI185B compared to *R. atra* BIR MGR1, representative of subcluster C3, are 98.6 – 99.0%, overlapping the cut-off values for species separation (98.7 – 99.0%, Stackebrandt & Ebers 2006). *Calothrix* sp. type 5 does not form typical hemispherical gelatinous colonies and "akinetes" like in *R. atra* BIR MGR1 (Sihvonen et al. 2007), but both share similar habitats (epilithic in brackish waters). Characterization and comparison of the 16S-23S ITS sequences of *Calothrix* sp. type 5 and *R. atra* BIR MGR1 would ellucidate their taxonomic relationships.

c. The genus *Skacelovkia*

The genus *Skacelovkia* is morphologically indistinguishable from *Calothrix*. However, *Skacelovkia* clearly differs from other heteropolar cyanobacteria like *Macrochaete, Dichothrix, Rivularia,* and *Gloeotrichia* in not having terminal hairs, fasciculated and

divaricated filaments, rounded to hemispherical colonies, and akinetes, respectively (Komárek 2013). The two strains of *Skacelovkia*, *S. peruviana* strain T027 and *Skacelovkia* sp. strain P3C2e are morphologically similar, differing only in the shape of vegetative cells. At young stages, *S. peruviana* resembled *C. galpinii* Cholnoký-Pfannkuche, but later observations on the Peruvian strain showed false branching and intercalar heterocytes, features not described for *C. galpinii* (Komárek 2013). In addition, *S. peruviana* T027 and *Skacelovkia* sp. P3C2e were isolated from freshwaters, but at localities with different climatic conditions (cold in P3C2e, tropical in T027). In the phylogenetic tree, *Skacelovkia* is represented by the high-supported subcluster A4, within Clade A (Fig. 2), well separated from the recognized *Calothrix* groups in subclusters A1 (Freshwater & Soil), A2 (Brackish – I), A3 (Brackish – II), and A5 (*Macrochaete*). Most heteropolar cyanobacteria are ≤ 94% similar to *Skacelovkia* in identities of 16S rRNA gene sequence, except for *C. elsteri* CCALA 953 which is 95.2 – 95.3% similar in identity with *Skacelovkia* (table 8). If the cut-off for genus separation is follow (95%, Stackebrant & Goebel 1994), then *Skacelovkia* is undoubtfully a distinct genus from other heteropolar cyanobacteria, but could belong to the same genus as *C. elsteri*. Considering that *C. elsteri* clusters as a sister branch of *Skacelovkia* (subcluster A4), and does not differ greatly in morphology, further analysis using the 16S-23S ITS sequence are necessary to evaluate if they correspond to the same genus. On the other hand, *S. peruviana* T027 and *Skacelovkia* sp. P3C2e are 99.7% identical in 16S rRNA gene sequence, well above 98.7 – 99.0%, the limit for species separation (Stackebrandt & Ebers 2006). Based on similarities on morphology, habitat, and high identity of 16S rRNA, it seems probably that the Peruvian and Czech strains correspond to the same species. However, this require confirmation by further studies using 16S-23S ITS sequence.

d. The genus "*Dichothrix*"

"*Dichothrix*" sp. show features corresponding to different genera (table 6): heteropolar *Calothrix*-like and *Dichothrix*-like stages, isopolar *Scytonematopsis*-like stage, simple and double false branching, and arthrospores. Therefore, a confident genus designation was not possible. In "*Dichothrix*", the *Scytonematopsis*-like stage is similar to the one observed in *S. contorta*, but differing in coiling intensity (Vaccarino & Johansen 2011). The fasciculated *Dichothrix*-like stage are similar to the described for *D. baueriana* (Grunow) Bornet et Flahault, but the latter shows that feature almost permanently. The colonial morphology of "*Dichothrix*" is more or less similar as in *Rivularia* (Komárek 2013), except that the first does not show a colonial mucilaginous envelope. "*Dichothrix*" sp. strain T017 shows trichomes

ending in long hairs during most of its life cycle (Fig. 9P), while in strains T008 and T048 the hairs started to detach after some months of cultivation. This is probably because T017 was cultured using modified CHU10, while T008 and T048 were cultured using BG11. The effect of culture media in hair lost is well documented in rivulariacean taxa (Berrendero et al. 2008, Livingstone & Whitton 1983). The three strains of "*Dichothrix*" sp. were isolated from epiphyton on roots of aquatic plants in El Oconal lagoon, Amazon region in Peru.

Phylogenetically, the two "*Dichothrix*" sequences form a high supported subcluster (B1) within Clade B, together with *Calothrix* sp. strains UAM 342 and CYN89, and *S. contorta* strains HA4292-MV4 and HA4267-MV1, all of them sharing 16S rRNA gene sequence identities ≤ 97.3%, well above the cut-off for genus separation (95%, Stackebrandt & Goebel 1994). Identities between strains of subcluster B1 compared to *Calothrix* sp. UAM 374 and Uncultured cyanobacterium clone D1E07, representatives of subcluster B2, are 94.3 – 95.7%, overlapping the cut-off for genus recognition (95%, Stackebrandt & Goebel 1994). Identities of strains from Clade B (including subclusters B1 and B2) compared with representatives of other heteropolar cyanobacteria are ≤ 93.9% (table 9), below the limit for genus separation (Stackebrandt & Goebel 1994). Therefore, phylogenetic position and genetic identities support the separation of Clade B at generic level, but it is not clear if it contains one single genus, or two genera represented by subclusters B1 and B2 respectively. On the other hand, "*Dichothrix*" sequences (Seq 1 and Seq 2) are 99.5% identical in 16S rRNA gene sequence (table 9). Both "*Dichothrix*" sequences are 98.8 – 99.3% similar in genetic identity to both *S. contorta* strains, which overlaps the cut-off for species separation (98.7 – 99.0%, Stackebrandt & Ebers 2006). Considering the clear differences between "*Dichothrix*" and *S. contorta* in morphology (trichome structure, colony formation, shape and position of heterocytes, and arrangement of filaments), and ecology (epiphyton for "*Dichothrix*", epilithon for *S. contorta*), it seems unlikely that they belong to the same species. In addition, *Calothrix* sp. UAM 342 clusters within the two "*Dichothrix*" sequences in the phylogenetic tree (Fig. 2), and they are 98.1 -98.6% similar in genetic identity, below the limit for species separation. Despite their phylogenetic position, and considering the differences in morphology (colony structure, branching pattern, different arrangement of filaments), ecology (epilithic in arid brackish river for UAM 342, epiphytic in tropical freshwater lagoon for "*Dichothrix*"), and identity, it is clear that *Calothrix* sp. UAM 342 and "*Dichothrix*" correspond to different species. All these conclusions must be confirmed after the evaluation on the secondary structure of the 16S-23S ITS sequence from discussed strains.

B. The family Scytonemataceae

The Scytonemataceae has been modified in its original concept by recent studies (e.g. hormogonia development, Vaccarino & Johansen 2012). In addition, several works suggest the polyphyletic status of the Scytonemataceae authors (Komárek et al. 2013, Vaccarino & Johansen 2012), which is confirmed by the results from the present study. In the phylogenetic tree, genera morphologically corresponding to the Scytonemataceae are distributed in clades A – F (Fig. 3). Clade F represents Scytonemataceae sensu stricto, and divides in subclusters F1 (*Brasilonema*), F2 (*Iphinoe* and *Symphyonemopsis*), F3 (*Scytonema* sensu stricto), F4 (*Scytonema javanicum*-group), and solitary branches of *Ewamiana* and *Petalonema alatum* (Borzì ex Bornet & Flahault) Correns. *Chakia, Iningainema,* and other strains identified as *Scytonema*, *Scytonematopsis* (*S. contorta* and *S. maxima)*, and *Petalonema* (strains HA277-MV1 and ANT-LG2-8), do not belong to the Scytonemataceae sensu stricto, in agreement with previous works (Komárek et al. 2013, Mareš et al. 2015, Smith et al. 2012, Vaccarino & Johansen 2011). The exception is *Iningainema*, which its afiliation in the Scytonemataceae was stated as "provisional" (McGregor & Sendall 2017).

a. The genus *Scytonema*

Strains identified as *Scytonema* fall within clades A – F in the phylogenetic tree (Fig. 3). *Scytonema hofmannii* Agardh ex Bornet et Flahault, type species of *Scytonema*, falls within subcluster F3 (Clade F), and therefore represents *Scytonema* sensu stricto. Other four *Scytonema* groups are located in clades C ("*Scytonema* cf. *crispum*" group), G (Scytonemataceae – III), subclusters D1 and D2 within Clade D (Scytonemataceae – I), subcluster E1 within Clade E (Scytonemataceae – II), and subcluster F4 within Clade F (Scytonenatamceae sensu stricto). Most of these groups have been found and characterized by others authors, although differently named (Hentschke et al. 2016, Komárek et al. 2013, Shalygin et al. 2017). The five *Scytonema* groups found in this study are in agreement with the four clades described by Komárek et al. (2013), except that these authors included *S. javanicum*-group within *Scytonema* sensu stricto. Single sequences of *S. mirabile* SAG 83.79 and *S. bohneri* SAG 255.80 fall within clades A and B, respectively.

Scytonema sp. type 1 strains T051 and T056 morphologically correspond to *Scytonema*, but it does not fit any species listed in Komárek (2013). *Scytonema* sp. type 1 differs from *S. hofmannii* in dimensions of filaments, structure of the sheath, and characteristics of trichomes. Ecologically, *Scytonema* sp. type 1 is similar to *S. guyanense* (Montagne) Bornet

et Flahault, both from soil and tropical localities, but they have different morphology (filaments characteristics, sheath color, and dimensions). *Scytonema* sp. type 1 strains T051 and T056 fall within subcluster F3 (Cluster F), corresponding to *Scytonema* sensu stricto. Strains T051 and T056 share 99.6% of 16S rRNA gene sequence identity, and are ≤ 97.2% similar in identity with other *Scytonema* strains from subcluster F3, well below the cut-off for species separation (98.7 - 99%, Stackebrandt & Ebers 2006). Based on phylogeny and genetic identity, *Scytonema* sp. type 1 probably belongs to a new undescribed species. Nevertheless, this should be confirmed with further studies on the morphology and 16S-23S ITS sequence of related *Scytonema* strains. On the other hand, strains from *Scytonema* sensu stricto (subcluster F3) are ≥ 95.1% similar in identity, but compared to other phylogenetically distant *Scytonema,* the identities are ≤ 92.8% (table 11). Following the limit for genus recognition (95%, Stackebrandt & Goebel 1994) and phylogenetic position, there are strong evidences that *Scytonema* taxa outside *Scytonema* sensu stricto belong to different genera, which is suggested by Komárek et al. (2013). More studies are needed to split *Scytonema* from related taxa, even though they share similar morphologies.

Scytonema sp. type 2 strain T063 falls within subcluster E1 (Clade E, Fig. 3), analogous to Cluster B (*S. hyalinum* group) sensu Komárek et al. (2013) and *Scytonema hyalinum* group Type 1 Operon sensu Johansen et al. (2017). *Scytonema* sp type 2 morphologically differs from members of Cluster B sensu Komárek et al. (2013) in sheath color and constriction at cross-walls. *S. crispum* sensu Montoya et al. (1998) resembles *Scytonema* sp. type 2 in ecology (both from *Lomas* ecosystems in Peru), and slightly in morphology, differing in the length of vegetative cells. Interestingly, *Scytonema* sp. type 2 and most of strains from subcluster E1 were obtained from soils in arid localities. Identities of 16S rRNA gene sequence of *Scytonema* sp. type 2 compared to *S. hyalinum* FI-8A and *S. arcangeli* CCIBt3134 are 96.7 and 97.9% respectively, below 98.7 – 99%, the cut-off for species separation (Stackebrandt & Ebers 2006), and above 95%, the limit for genus recognition (Stackebrandt & Goebel 1994). *S. hyalinum* and *S. arcangeli* mainly differ from *Scytonema* sp. type 2 in filament structure, measurements of cells and trichomes, and thallus formation. In addition, *Scytonema* sp. type 2 and *S. hofmannii* are 93.1% similar in identity, below the cut-off for genus separation. Therefore, based on morphology and genetic identity, and following conclusions from the previous paragraph, *Scytonema* sp. type 2 probably correspond to a novel species within a new genus (subcluster E1) separated from *Scytonema* sensu stricto. Despite this, careful must be taken considering the presence of an highly

divergent rrn Operon within *S. hyalinum* group (Johansen et al. 2017), which may complicate taxonomic conclusions of studies using only ribosomal sequences.

b. The genus *Brasilonema*

Brasilonema is a monophyletic, well-defined genus, with predominantly tropical or subtropical distribution (Komárek 2013, Sant'Anna et al. 2011). According to our results, *Brasilonema* forms a well-supported subcluster (F1) within Clade F (Scytonemataceae sensu stricto), consistent with several studies (Becerra-Absalón et al. 2013, Sant'Anna et al. 2011, Vaccarino & Johansen 2012, Villanueva et al. 2018). Subcluster F1 contains *B. octagenarum* strain T001, and *Brasilonema* sp. strain T076, both from the present study, and two *Scytonema* strains (SAG 67.81 and 00557-00001), which must be transferred to *Brasilonema*. Subcluster F2, sister of *Brasilonema*, includes *Iphinoe* and *Symphyonemopsis,* in agreement with different works (Becerra-Absalón et al. 2013, Fiore et al. 2007, Miscoe et al. 2016, Vaccarino & Johansen 2012).

B. octagenarum strain T001 fits the species diagnosis given by Aguiar et al. (2008). Indeed, strain T001 only differs from the original description in the plant substrate, which is a moss in the Peruvian strain, and *Eucalyptus grandis* Hill ex Maiden in the Brazilian strain. *B. octagenarum* is also known to colonize orchid surface leaves (Aguiar et al. 2008, Sant'Anna et al. 2011), which could justify its presence to mosses and probably to other epiphytic plants common for tropical forests (e.g. bromeliads, hepatics). In the phylogenetic tree (Fig. 3), *B. octagenarum* strain T001 forms a high supported sub-cluster with *Brasilonema* sp. strains CENA 382, CENA360, RKST-322, and *B. octagenarum* UFV-E1. All these strains share 16S rRNA gene sequence identities of 99.4 – 99.9%, well above 98.7 – 99%, the proposed limit for species separation (Stackebrandt & Ebers 2006). While the morphological and ecological data from *Brasilonema* sp. CENA382 and CENA360 correspond to *B. octagenarum* Andreote (2013), the habitat of *Brasilonema* sp. RKST-322 is different (rock susbstrate, NCBI webpage). Confirmation that *B. octagenarum* T001, *Brasilonema* sp. CENA382, *Brasilonema* sp. CENA360, and *Brasilonema* sp. RKST-322, are conspecific with *B. octagenarum* UFV-ER1 (reference strain), studies using the 16S-23S ITS must be performed. If this theory is validated, *B. octagenarum* will broad its habitat and ecological preferences, being able to colonize different plant substrates (palms, orchids, mosses), and rock substrates in tropical and subtropical regions.

Brasilonema sp. strain T076 does not differing substantially from other *Brasilonema* species (table 9). Notoriously, *Brasilonema* sp. is the second species, after *B. angustatum* Vaccarino et Johansen that shows tapered filaments and do not form fasciculated thallus (Vaccarino & Johansen 2012). Both species are morphologically and ecologically similar, differing only in their dimensions. In contrast, *Brasilonema* sp. and *B. angustatum* place distantly in the phylogenetic tree (fig. 3), and are 98.0% identical in 16S rRNA gene sequence, below 98.7 – 99%, the proposed limit for species separation (Stackebrandt & Ebers 2006). In addition, *Brasilonema* sp. T076 forms a well-supported sub-cluster with *Scytonema* sp. 00557_0001, isolated from a fishtank in Hawaii (NCBI webpage). Both strains are 99.6% identical in 16S rRNA gene sequence, well above the cut-off for species separation (Stackebrandt & Ebers 2006). Even though there is no available morphological data of *Scytonema* sp. 00557_0001, phylogenetic position and high identities support that it is conspecific with *Brasilonema* sp. T076. Furthermore, *Brasilonema* sp. T076 does not correspond to *B. angustatum* based on phylogeny and genetic identity, although they have similar morphology and ecology. Further studies using the 16S-23S ITS sequence are required to validate both conclusions.

C. "Fortieaceae", Tolypothrichaceae, *Camptylonemopsis,* and *Microchaete*

The families "Fortieaceae" and Tolypothrichaceae, and the genera *Camptylonemopsis* and *Microchaete*, partly correspond to the family Microchaetaceae sensu Komárek (2013). However, the Microchaetaceae was reduced to contain only the type species *M. grisea* Thuret ex Bornet et Flahault (Hauer et al. 2014), and probably other marine *Microchaete* (Komárek 2013). The "Fortieaceae" was listed as a family in Komárek et al. (2014), although was not validly published, and contains *Aulosira* Kirchner ex Bornet et Flahault, *Calochaete* Hauer et al., *Coleospermum* Kirchner in Cohn (still not validated, includes the freshwater *Microchaete*, Komárek 2013), *Fortiea* De-Toni, and *Roholtiella* Bohunická et al. These genera are found in clades A and B in the phylogenetic tree (Fig. 4), suggesting that the "Fortieaeae" is not monophyletic. *Aulosira*, *Calochaete* and *Roholtiella* fall within subclusters A1, A2 and A3 (Clade A), while *Fortiea* strains belong to Clade B. On the other hand, Hauer et al. (2014) erected the Tolypothrichaceae containing *Coelodesmium* Borzi ex Geitler, *Hassallia* Berkeley ex Bornet et Flahault, *Spirirestis* Flechtner et Johansen, *Rexia* Casamatta, Gomez et Johansen, and *Tolypothrix* Kützing ex Bornet et Flahault. In the phylogenetic tree, strains identified as tolypothrichacean genera fall in clades A and D (Fig. 4). The foundational genera *Coelodesmium*, *Hassallia*, *Spirirestis*, *Rexia*, *Tolypothrix* (including three strains identified as *T. distorta* Kützing ex Bornet et Flahault, type species of

Tolypothrix), and the recently described *Kryptousia* and *Dactylothamnos,* are part of Clade D, which represents Tolypothrichaceae sensu stricto. This results are similar to previous studies (Alvarenga et al. 2017, Hauer et al. 2014, Komárek et al. 2014, 2015). Other "*Tolypothrix*" strains fall within Clade A, placing together with other strains mostly assigned to heteropolar heterocytous cyanobacteria, consistent with results from Hauer et al. (2014) and Bohunická et al. (2015). In addition, *Streptostemon* Sant'Anna et al. was also included in the Tolypothrichaceae (Komárek et al. 2014), but according to Hentschke et al. (2016) it forms an independent branch, separated from the Tolypothrichaceae, which is confirmed in the present work (Fig. 4). The two *Kryptousia* sequences used in the phylogenetic tree (*K. microlepis* CENA354 and *K. macronema* CENA338) do not form a monophyletic group, contradicting results of Alvarenga et al. (2017).

a. The genus *Tolypothrix*

Strains assigned to *Tolypothrix* are placed in two distant clusters in the phylogenetic tree (fig. 4): Clade A (Nostocales – I), and D (Tolypothrichaceae sensu stricto). Concerning the genus *Tolypothrix* we find four main taxonomical problems:

i) The bacteriological reference strains for *Tolypothrix* (Herdman et al. 2011b) place far from the Tolypothrichaceae sensu stricto. This agrees with Hauer et al. (2014), which demonstrate that morphology and phylogenetic position of "*Tolypothrix*" PCC 7504, and PCC 7415 (reclassified as *Roholtiella* by Bohunická et al. (2015)) are different from the true *Tolypothrix*. In addition, our results show that strains "*Tolypothrix*" PCC 7101, PCC 7601, and PCC 7708 (=*Calothrix membranacea* SAG 1410-1) do not belong into the Tolypothrichaceae sensu stricto, and probably do not share typical *Tolypothrix* features.

ii) Even if we do not consider the bacteriological reference strains for *Tolypothrix*, the genus is still polyphyletic. *T. tenuis* SAG 94.79 places within Clade A, far from Tolypothrichaceae sensu stricto (Clade D). Furthermore, within Clade D, several *Tolypothrix* strains are splittered in different clusters, intermixed with species of *Hassallia*, *Coleodesmium*, *Kryptousia*, *Dactylothamnos*, and *Spirirestis*. This is confirmed by several works (Berrendero et al. 2011, Bohunická et al. 2015, Hauer et al. 2013, Miscoe et al. 2016, Shalygin et al. 2017).

iii) Strains SAG 93.79, SEV2-5-2Ca, and ACOI 3104 identified as *T. distorta,* type species of the genus, place distantly from each other within Clade D, making difficult the selection of *Tolypothrix* sensu stricto. Morphological description is available for strain SEV2-5-2Ca (Flechtner et al. 2002), while photo-documentation exist for strain ACOI

3104 (Hauer et al. 2014). However, information for strain SAG 93.79 (Hess 1962) is limited to physiological analysis only. Based on these incomplete data, designation of the strain which truly correspond to *T. distorta* seems precipitate at this time.

In this work, two *Tolypothrix* strains were isolated and studied in detail. Both taxa fall inside Tolypothrichaceae sensu stricto, one was identified as *T.* cf. *helicophila*, while the other could not be assigned to any existing *Tolypothrix* species.

Tolypothrix cf. *helicophila* strain T002 does not differ greatly in morphology with other *Tolypothrix* species described so far. Indeed, strain T002 is similar to *T. helicophila* Lemmermann, except that does not show wide sheaths (Komárek 2013). In addition, both taxa show different ecology: *T. helicophila* attaches to water plants and shells of mollusks in stagnant water bodies from northern hemispheres (Komárek 2013), while *Tolypothrix* cf. *helocophila* T002 was isolated from mosses, in tropical humid forest from Peru. Phylogenetically, *Tolypothrix* cf. *helicophila* T002 locates in a small subcluster together with *Tolypothrix* sp. NIES-515 and *Coleodesmum wrangelii* MC-JRJ1, from freshwater and terrestrial habitats respectively (NIES webpage, Flechtner et al. 2002). Identities of 16S rRNA gene sequence between strains T002 compared to NIES-515 and MC-JRJ1 are 98.3% and 97.6% respectively, below 98.7 – 99.0%, the cut-off for species recognition in prokaryotes (Stackebrandt & Ebers 2006). No information on morphology is available for strain NIES-515, while description of strain MC-JR1 is given in Flechtner et al. (2002), but the latter clearly differs from *Tolypothrix* cf. *helicophila* in width of filaments, number of trichomes per sheath, and constriction at cross walls. Identities of *Tolypothrix* cf. *helicophila* T002 compared to *T. distorta* strains ACOI 731, SAG 93.79, and SEV2-5-2Ca are 97.4, 98.3, and 98.0% respectively, all below the cut-off for species separation, but above the limit for genus recognition (95%, Stackbrandt & Goebel 1994). Clearly, *Tolypothrix* cf. *helicophila* T002 is a different species from other *Tolypothrix* species sequenced so far, but its affiliation with the true *T. helicophila* must be further investigated.

Tolypothrix sp. strain T079 is morphologically different from other *Tolypothrix* species by its distinctives size and shape of heterocytes. Three *Tolypothrix* species show similar heterocytes: *T. calcalrata* Schmidle, *T. delicatula* Philson, and *T. lanata* Wartmann ex Bornet & Flahault, but they clearly differ from *Tolypothrix* sp. T079 in cell and trichome morphologies, and ecology. In the phylogenetic tree, *Tolypothrix* sp. T079 places as a sister

branch of a well-supported subcluster containing *Hassallia littoralis* González-Resendiz et León-Tejera, *Hassallia* sp. T075, and *T. carrinoi* Miscoe, Pietrasiak et Johansen. These three strains are 98.0 – 98.5% identical in 16S rRNA gene sequence compared to strain T079, below the cut-off for species separation (98.7 – 99%, Stackebrandt & Ebers 2006). Furthermore, *H. littoralis*, *Hassallia* sp. T075, and *T. carrinoi* show clear morphological (shape of heterocytes, sheath morphology) and ecological (habitat) differences with *Tolypothrix* sp. T079. On the other hand, strain T079 show identities of 97.6 – 98.3% compared to *T. distorta* strains ACOI 731, SAG 93.79, and SEV2-5-2Ca, below the cut-off for species recognition (Stackebrandt & Ebers 2006). Comparing the identities of *Tolypothrix* sp. T079 with *T. tenuis* CCALA 197 and *R. erecta* CAT4-SG4, these overlap with the limit for species separation. However, *T. tenuis* CCALA 197 and *R. erecta* CAT4-SG4 differ in phylogenetic position, morphology, and ecology with strain T079. Based on, morphology, ecology, phylogenetic position, and genetic identities suggest that *Tolypothrix* sp. T079 would correspond to a new species, which will require confirmation using the 16S-23S ITS sequence.

b. The genus *Hassallia*

Hassallia strains are present within Clade D, and appears to be polyphyletic. Noticeable, *Hassallia* includes the only tolypothricoid species from marine biotopes sequenced so far: *H. littoralis* (González-Resendiz et al. 2013). This would suggest broad ecological preferences of *Hassallia*, and also that probably new tolypotrichoid taxa are present in marine littoral biotopes (González-Resendiz et al. 2013). In the present study, three strains were isolated and identified as *H. californica* (strains T061 and T081) and *Hassallia* sp. (strain T075).

Hassallia californica strains T061 and T081, matches the species diagnosis given by Flechtner et al. (2008), except that the Peruvian strains show more variability in cell length, and yellow-brownish sheath color. *H. californica* was described from soil samples at San Nicholas Island, California, USA (Flechtner et al. 2008), while in the present study it was collected from samples of Biological Soil Crust at *Lomas de Lachay*, central coast of Peru, both localities sharing similar climatic conditions (arid in *Lomas de Lachay*, and semi-arid to temperate in St. Nicholas Island). Bird migration could explain the presence of *H. californica* in both localities, with some birds like the Franklin's gull (*Larus pipixcan*) migrating from North America to the Peruvian coast in Austral summer (Burger et al. 2010). In the phylogenetic tree (Fig. 4), *H. californica* forms a subcluster containing *H. byssoidea* CCALA

823, *H. antarctica* CCALA 957, *Coleodesmium* sp. Hindak 2000/24, and *T. distorta* SAG 93.79. Identities of 16S rRNA gene sequence between these four strains with *H. californica* strains T061 and T081 are slightly below or overlapping the cut-off for species recognition (98.7 – 99.0%, Stackebrandt & Ebers 2006). Surprisingly, identity between *H. californica* strains T061 and T081 compared to *Rexia erecta* CAT4-SG4 is above the limit for species separation (Stackebrandt & Ebers 2006). However, *Rexia* is evidently different in morphology compared to *Hassallia,* justifying the separation of *R. erecta* and *H. californica*. Certainly, *H. californica* strains T061 and T081 is a clearly separate species within the Tolypothrichaceae, extending its geographic range to the coast of Peru.

Hassallia sp. strain T075 shows features corresponding to *Hassalia*; however, it does not correspond to any species. *Hassallia* sp. T075 is similar in morphology and ecology to *H. californica* and *H. pseudoramossisima* Johansen et Flechtner, but it differs from them in not possessing the perpendicular branching, and not being abundantly pseudobranched, respectively. In the phylogenetic tree (Fig. 4), *Hassallia* sp. T075 falls within a subcluster that includes *H. littoralis* and *T. carrinoi*. Identities of 16S rRNA gene sequence between these three taxa are between 98.5 – 99.0%, partially overlapping the cut-off for species separation (98.7 – 99.0%, Stackebrandt & Goebel 2006). Despite this, the three taxa show great differences in morphology (filaments arrangement, branching pattern, sheath morphology) and habitat preferences. *T. carrinoi* was described showing rare false branching, cells shorter than wide, and colonies having upright filaments (Miscoe et al. 2016). Although the rare false branching is not typical for *Hassallia* and *Tolypothrix*, short cells and upright filaments correspond to the main features described for *Hassallia* (Komárek 2013). Certainly, *Hassallia* sp. T075 separates from other tolypothricoid taxa based on its morphology and phylogenetic position, probably belonging to a new undescribed species. Studies using the 16S-23S ITS sequence are necessary to confirm this theory.

c. The genus *Calochaete*

Calochaete was described by Hauer et al. (2013) showing tapered filaments swollen at the base, basal or intercalary heterocytes, frequent false branching, constricted trichomes, and cylindrical or barrel-shaped cells. All these features are also present in *Calochaete* sp. strain T037, isolated in the present study. However the Peruvian strain has two more unique features: the presence of up to five basal heterocytes (Fig. 11K), and a yellowish cell with slightly thick walls locating above the basal heterocyte, resembling an akinete (Fig. 11L). In

addition, arthrospores and division in two planes were not observed in *Calochete* sp. T037, important features in this genus (Hauer et al. 2013). The described differences in morphology between *Calochaete* sp. T037 and *C. cimrmanii* Hauer et al., type species of *Calochaete,* must be taken with care, considering that both were cultured using different media. Ecologically, both *Calochaete* sp. T037 and *C. cimrmannii* were isolated from aerophytic habitats in tropical localities: mosses at tropical Amazon forest in Peru, and soils at "paramo" zone in Costa Rica, respectively. Considering *Calochaete* sp. described by Mühlsteinová & Hauer (2013) from a bromeliad leaf pool, the genus *Calochaete* could have broader habitat preferences than the already mentioned in this study. On the other hand, Hauer et al. (2013) place *Calochaete* as a basal group of a clade containing *Tolypothrix* strains UAM 332, 334, 337, PCC 7504 (all transferred to *Roholtiella* by Bohunická et al. 2015), *Tolypothrix* PCC 7504, 7601, 7101, TOL328, and *M. diplosiphon* Gomont ex Bornet et Flahault CCALA 811. These findings are in agreement with results from the present work, which locates *Calochaete* sp. strain T037 and *C. cimrmanii* within subcluster A3, related to subclusters A2 (which includes *Roholtiella, Nostoc, Mojavia, Komarekiella*) and A1 (that mainly contains *M. diplosiphon*, "*Tolypothrix*" PCC strains, and *Camptylonemopsis*). Other papers also show similar results on the phylogenetic position of *Calochaete* (Bohunická et al. 2015, Shalygin et al. 2017). Identity of 16S rRNA gene sequence between *Calochaete* sp. T037 and *C. cimrmanii* is 99.1% (table 17), slightly above 98.7 – 99%, the limit for species separation (Stackebrandt & Ebers 2006). This finding would suggest that both *Calochaete* sp. T037 and *C. cimrmanii* could belong to the same species. However, differences in morphology and comparison of the 16S-23S ITS sequence (data not show) of both taxa show that they vary enough to support their separation as different species.

d. The genus *Camptylonemopsis*

Camptylonemopsis is distinguished from other genera in showing crescent-shaped filaments, basal parts attached to the substrate, ends of filaments growing upwards, rare false branching, cells longer and narrower in central part of trichomes, and shorter and wider at the ends (Desikachary 1948, Komárek 2013). In the present study, strain T054 shows all these features, clearly corresponding to *Camptylonemopsis* in terms of morphology. However, depending on the age of cultures, sometimes strain T054 resembles *Aulosira*, *Fortiea*, or *Microchaete*, which is also reported by Desikachary (1948). On the other hand, strain T054 partially resembles *C. pulneyensis* Desikachary, differing in having slightly different dimensions, not lamellated sheaths, and showing rows of akinetes. Considering that

Camptylonemopsis cf. *pulneyensis* strain T054 was described based on culture material and that the original description of *C. pulneyensis* relies on natural populations (Desikachary 1948), small differences between both descriptions could be reasonable. In addition, *C. pulneyensis* and strain T054 come from different habitats within the tropics (algal sample in *C. pulneyensis*, soil in strain T054).

The phylogeny of *Camptylonemopsis* is poorly known because the type species, *C. lahorensis* (Ghose) Desikachary has not been studied using the modern methods, and only eight *Camptylonemopsis* 16S rRNA gene sequences are available in the NCBI webpage (March 2018). Four of these sequences are longer than 1000 bp: *Camptylonemopsis* sp. HA4241-MV5 clone B2-3 + p4 (JN385292), *Camptylonemopsis* sp. HA4241-MV5 clone B2-3 + p3 (HQ847564), *Camptylonemopsis* sp. MGCY355I (KY056812), and *Camptylonemopsis* sp. MGCY385II (KY056808), the first two from Hawaii (Sherwood et al. 2015), and the latter belonging to unpublished sequences from Iran (NCBI webpage). We include *Camptylonemopsis* sp. HA4241-MV5 clone B2-3 + p4 and *Camptylonemopsis* sp. MGCY355I in the present study, both forming a strong supported cluster with *Calothrix anomala* SAG 1410.4, and locating as sister of a group of strains referred as "*C. brevissima*", "*S. mirabile*", "*T. tenuis*", and *"Calothrix* sp.", within subcluster A1 (Clade A). Similar results are found in different papers (Bohunická et al. 2015, Bravakos et al. 2016, Hentschke et al. 2017, Silva et al. 2014). On the other hand, *Camptylonemopsis* cf. *pulneyensis* T054 clusters with *Fortiea laiensis* Vaccarino et Johansen and *F. contorta* Hauer, Bohunická et Mareš, forming a strong subcluster sister of *Desmonostoc,* within Clade B (Fig. 4). Identities of 16S rRNA gene sequence between *Camptylonemopsis* cf. *pulneyensis* with *F. laiensis* and *F. contorta* is 97.8 and 97.1% respectively, below the cut-off for species separation (Stackebrandt & Ebers 2006), but above the limit for genus recognition (Stackebrandt & Goebel 1994). However, *Camptylonemopsis* cf. *pulneyensis* strain T054 clearly differs from *F. laiensis* and *F. contorta* in the polarity of filaments and position of heterocytes along the trichome. On the other hand, identities of *Camptylonemopsis* cf. *pulneyensis* strain T054 with *Camptylonemopsis* sp. HA4241-MV5 clone B2-3 + p4 and *Camptylonemopsis* sp. MGCY355I are 95.6 and 95.1% respectively, slightly above the cut-off for genus separation (Stackebrandt & Goebel 1994), suggesting that the three *Camptylonemopsis* strains would belong to the same genus. Nevertheless, based on their different phylogenetic positions (Fig. 4) it is clear that the Peruvian *Camptylonemopsis* belongs to a different genus than *Camptylonemopsis* sp. HA4241-MV5 clone B2-3 + p4 and *Camptylonemopsis* sp.

MGCY355I. In the absence on morphological data of the latter two strains, further discussion is not possible. The polyphasic characterization of other *Camptylonemopsis* taxa, including the type species, are necessary to delimitate the phylogenetic position of the true *Camptylonemopsis*.

e. The genus *Microchaete*

Within *Microchaete*, several morpho- and ecotypes are described, which probably belong to different genera (Komárek 2013), but the freshwater *Microchaete* would correspond to *Coleospermum* according to Komárek (2013, 2017). In recent studies, freshwater *Microchaete* species are placed in different positions within the Nostocaceae (Bohunická et al. 2015, Genuário et al. 2017, Shalygin et al. 2017). The present study support these results, locating strains identified as *Microchaete* in clades A, B, and C, and as an independent branch (Fig. 4). In Clade A, *M. diplosiphon* strains NIES-3275, CCALA811, and KSU-AQIQ-10, and *Microchaete* sp. CENA541 are located within subcluster A1, while in Clade B, a subcluster containing *A. bohemensis* ISB-2 and two *Microchate* strains (including *Microchaete* sp. type 2 strain T060 isolated in the present study) locates as a basal group. Clade C is composed of four strains from the present study showing *Microchaete* features, related to other strains identified as *Calothrix*, *Gloeotrichia*, *Trichormus*, and two unidentified strains (Microchaetaceae cyanobacterium CENA 550, and Nostocales cyanobacterium NapMSIm13). *M. violacea* ACOI 3057 places as a sister taxa of the cluster comprising *Gloeotrichia*, *Goleter*, Clade C, and all the Nostocaceae and Aphanozimenonaceae (clades A, C, Nostocales – III , and Nostocales – IV; Fig. 4).

The *Microchaete* strains studied in this work cluster in clades B and C (Fig. 4). *Microchaete* sp. type 1 (strains T042 and T047), and strains JOH06 and JOH42 are located within Clade C, being discussed in the following section. *Microchaete* sp. type 2 strain T060 locates within Clade B, and fits the description of *Microchaete*, however, its identification at species level was not possible. The only taxa morphological and ecological similar to *Microchaete* sp. type 2 is *Microchaete* cf. *aequalis* sensu Watanabe & Komárek. Both taxa differ in the width of the trichome, and habiting localities with different climatic conditions (tropical in *Microchaete* sp. type 2, temperate in *Microchaete* cf. *aequalis* sensu Watanabe & Komárek) (Komárek 2013). On the other hand, *Microchaete* sp. type 2 T060 forms a high-supported cluster with *A. bohemensis* ISB-2 and *M. tenera* ACOI 630. Identities of 16S rRNA gene sequence between *Microchaete* sp. type 2 with *A. bohemensis* and *M. tenera* are slightly

above the cut-off (98.7 – 99.0%) for species separation (Stackebrandt & Ebers 2006). *Microchaete* sp. type 2 T060, *A. bohemensis*, and *M. tenera* show few morphological differences (nature of the filament, and akinete formation), but are markedly different in ecology: aerophytic in moss at 8 m. high on a bark of tree for strain T060, soil in temperate locality for strain ISB-2, and freshwater in temperate locality for strain ACOI 630. Likely, *Microchaete* sp. type 2 T060, *A. bohemensis* ISB-2 and *M. tenera* ACOI 630 belong to a single genus (*Coleospermum*?), as mentioned by Hauer et al. (2014) for the latter two strains. Further studies using the 16S-23S ITS sequences are necessary to evaluate if the three strains correspond to one single species or not.

f. The Novel *Microchaete*-like group

The remaining *Microchaete*-like strains studied in the present work (T042, T047, JOH06, and JOH42) fall within Clade C (Fig. 4), together with heteropolar (*Calothrix* sp. CCAP 1410/13, *Calothrix* sp. RSUAII 9BNC, *Calothrix* sp. RSUAII 9CNC, *Gloeotrichia longicauda* SAG 32.84, Microchaetaceae cyanobacterium CENA 550), and isopolar (Nostocales cyanobacterium NapMSIm13, and *Trichormus* sp. ATA115) strains. *Microchaete* sp. type 1 strains T042 and T047 show phenotypic traits similar to *Calothrix*, *Microchaete*, and *Roholtiella*, and the unique ability to form up to 5 basal or intercalar functional heterocytes in a row within a trichome. Strains JOH42 and JOH06 share similar characteristics from *Microchaete* sp. type 1, but in JOH06 the production of arthrospores was not observed, and presence of up to 5 heterocytes in both JOH06 and JOH42 was undetected. Further studies of the life cycle in strains JOH06 and JOH42 are necessary to evaluate if they are able to show all *Microchaete* sp. type 1 features. Most of the strains conforming Clade C have not been published, and their morphology remain unknown. The exceptions are Microchaetaceae cyanobacterium CENA 550 and Nostocales cyanobacterium NapMSIm13, the first one showing heteropolarity, intercalar and basal heterocytes, and cylindrical cells (Fig. 1, Panel 3 in Genuário et al., 2017), and the second being isopolar and resembling *Nostoc* (Bravakos et al. 2016). On the other hand, *Microchaete* sp. type 1, JOH42, and JOH06 were isolated from soil samples in different localities in Peru, USA, and Kenya, respectively, while other members of Clade C are edaphic (strains CCAP 1410/13, RSUAII 9BNC, RSUAII 9CNC), or aquatic (strains SAG 32.84, CENA 550). The two nostocacean members of Clade C, strains NapMSIm13 and ATA115, were isolated from thermal waters in Greece (Bravakos et al. 2016), and soil at Atacama desert in Chile (NCBI webpage) respectively.

Phylogenetic positions of *Microchaete* sp. type 1 strains T042 and T047, strain JOH06, and strain JOH42 are highly supported within Clade C. Strains T042 and T047 are identical in 16S rRNA gene sequence, while both compared to JOH06 and JOH042 show identities of 99.3 and 99.2% respectively, above the cut-off for species separation (89.7 – 90%, Stackebrandt & Ebers 2006). Genetic identities between all strains from Clade C are ≤ 96.0% well above the cut-off for genus recognition (95%, Stackebrandt & Goebel 1994). Clade C forms a separate, well-supported cluster from other heterocytous genera, including other *Microchaete* strains. Komárek (2013, 2016) states that the freshwater *Microchaete* would correspond to the genus *Coleospermum*. In addition, Komárek (2016) suggest that within *Coleospermum* there are three distant groups of species separated by morphology and life strategies: (a) with cylindrical trichomes (e.g. *M. tenera,* typical *Coleospermum*), (b) with narrowed trichomes toward the ends (e.g. *M. diplosiphon*), and (c) with obligatory formation of akinetes in long rows (e.g. *M. articulata* Komárek et al.). Following this classification, and considering that *Microchaete* sensu stricto is restricted to contain the type *M. grisea* and other marine species (Hauer et al. 2014, Komárek 2013), *Microchaete* sp. type 1 strains T042 and T047, strains JOH06, and strain JOH42 do not correspond to any *Coleospermum* group sensu Komárek (2016), neither to *Microchaete*. Consequently, these strains would belong to a novel genus based on their separated phylogenetic position, and morphological features. Likely, this novel genus would comprise also the heteropolar strains from Clade C based on similar ecology (almost all from soil biotopes) and close phylogenetic position to studied strains T042, T047, JOH06, and JOH42, while phylogenetic status of the isopolar strains (NapMSIm13 and ATA115) will require further studies using more sequences. Characterization using the 16S-23S ITS sequence and further morphological evaluation are necessary for the heteropolar strains from Clade C to confirm that they belong to the novel genus.

D. The family Nostoaceae

The Nostocaceae comprises mostly isopolar, unbranched heterocytous cyanobacteria producing akinetes (Komárek et al. 2014, Komárek 2013). Almost all genera from the Nostocaceae have been studied using polyphasic approach, except for *Isocystis* Borzì ex Bornet et Flahault, and *Macrospermum* Komárek (Komárek et al. 2014). The phylogenetic tree (Fig. 5) also shows the polyphyly of the Nostocaceae, which is intermixed with genera from different families (“Fortieaceae”, Aphanizomenonaceae). Phylogenetic trees in different

works also show the polyphyletic status of the Nostocaceae, although not directly mentioned (Kozlíková-Zapomělová et al. 2016, Kust et al. 2015, Lukešová et al. 2009).

a. The genus *Anabaena*

Anabaena is an isopolar heterocytous genus comprising non-planktic taxa (Komárek 2013), however several studies suggest its polyphyly (Halinen et al. 2008, Komárek 2013, Kozlíková-Zapomělová et al. 2016, Kust et al. 2015). In the present work, strains identified as *Anabaena* are splitted in clades A and B in the phylogenetic tree (Fig. 5), which confirms its polyphyletic status. Kozlíková-Zapomělová et al. (2016) states that the correct concept of *Anabaena* correspond to cluster "F" (Fig. 3, Kozlíková-Zapomělová et al. 2016), locating as sister of a cluster containing *Cuspidothrix*, *Dolichospermum*, and one "*A. oscillarioides*" group. Cluster "F" sensu Kozlíková-Zapomělová et al. (2016) is not represented in our phylogenetic tree, however, it is clearly separated from other clusters containing *Anabaena* strains (Kozlíková-Zapomělová et al. 2016), which are analogous to subclusters A1, A2, and A3 (Clade A), and subclusters B1 and B2 (Clade B) from the present study. Two *Anabaena* strains (type 1 and type 2) isolated in the present study fall in subcluster A1 (Clade A), which also contains one strain identified as *H. spongiosa* Schwabe ex Bornet et Flahault, type species of the genus *Hydrocoryne*.

Anabaena sp. type 1 strain T065 corresponds morphologically to *Anabaena*, but does not fit any species. It differs from *A. oscillarioides* Bory ex Bornet et Flahault, type species of *Anabaena*, in showing smaller heterocytes and akinetes, and akinetes with oval to cylindrical shape (Kozlikova-Zapomelova et al. 2016). In the isolation process, a hyaline, not-well delimited mucilaginous enveloping 1 – 3 trichomes was noticed. This envelope was evident only because attachment of detritus particles along the sheath. This feature was no longer observed after transferring to test tubes with agar medium. Ecology of *Anabaena* sp. type 1 is similar to that of other *Anabaena* species, but different for *A. oscillarioides* (temperate aquatic biotopes, Komárek 2013). On the other hand, *Anabaena* sp. type 1 falls within subcluster A1 (Clade A), forming an independent branch. Based on identity of the 16S rRNA gene sequence, *A. cylindrica* DC-3 shows the highest identity (99.2%) with *Anabaena* sp. type 1, above the cut-off for species recognition (Stackebrandt & Ebers 2006). However, *A. cylindrica* DC-3 and *Anabaena* sp. type 1 differ in phylogenetic position, morphology (position of akinetes along the trichome), and ecology (Pan et al. 2008), demonstrating that they do not belong into a single species. Genetic identities of *Anabaena* sp. type 1 with *A.*

oscillarioides BO-HINDAK 1984/43 and *A. oscillarioides* BECID 22 are 95.7 and 95.0% respectively, slightly above or in the limit of cut-off for genus separation (95%, Stackebrandt & Goebel 1994). Despite this, strains BO-HINDAK 1984/43 and BECID 22 fall distantly from *Anabaena* sp. type 1 (Fig. 5), not corresponding to the same genus. Further analysis using the 16S-23S ITS are recommended clarifying the taxonomic status of *Anabaena* sp. T065, which probably belong to a new undescribed species.

Taxonomic identification of *Anabaena* sp. type 2 strain T033 was doubtful because it shows parallel trichomes, and sometimes enclosed within a not-well delimited sheath (Fig. 14F). Parallel trichomes may suggest presence of filaments or fascicles, however these were not observed. It is possible that in nature, the sheath is clearly delimited, similar to *Hydrocoryne*. Despite this, *Anabaena* sp. type 2 shows basal and intercalary heterocytes, and akinetes joined or distant from the heterocytes, features not corresponding to *Hydrocoryne* (Komárek 2013), confirming its affiliation to *Anabaena*. Only two species have heterocytes in basal and intercalary positions, and akinetes joined and distant from heterocytes: *A. echinospora* Skuja, and *A. pirinica* Petkoff. *Anabaena* sp. type 2 differs from the latter two species in having colourless akinete exospore and cells shorter than wide, respectively. *A. sedovii* Kosinskaja is the only species that share similar habitat that of *Anabaena* sp. type 2, but the first locates in Nordic countries, while the latter in Peru. Phylogenetically, *Anabaena* sp. type 2 places in a solitary basal branch within subcluster A1, related to other *Anabaena* and *Hydrocoryne* strains. Identities of the 16S rRNA gene sequence of *Anabaena* sp. type 2 compared to *A. oscillarioides* BO-HINDAK 1984/43 and *A. oscillarioides* BECID 22 are 96.2 and 96.1% respectively, above the limit for genus separation (95%, Stackebrandt & Goebel 1994). Despite this, *Anabaena* sp. type 2 places in different positions compared to strains BO-HINDAK 1984/43 and BECID 22, therefore, do not belong into the same genus. Other strains from subcluster A1 are ≤ 98.8% similar in identity with *Anabaena* sp. type 2 (table 17) below or in the limit for species recognition (Stackebrandt & Ebers 2006). Consequently, based on phylogenetic position, ecology, and genetic identity, it seems that *Anabaena* sp. type 2 belongs to a new undescribed species, which requires confirmation using the 16S-23S ITS.

Anabaena sp. type 1 and type 2 fall in subcluster A1, within Clade A, which does not correspond to the original concept of *Anabaena* following Kozlíková-Zapomělová et al. (2016). Therefore, Subcluster A1 must be reclassified to a new or existing genus. Interestingly, one strain identified as *H. spongiosa,* type species of *Hydrocoryne,* fall in

subcluster A1, however information on its morphology and ecology is limited. If this strain truly correspond with *H. spongiosa*, all other strains within A1 should be transferred to *Hydrocoryne*. Despite this, *Anabaena* sp. type 1 and type 2 do not fit the description of *Hydrocoryne*, therefore the diagnostic characters of the genus must be reevaluated to accommodate the Peruvian *Anabaena* taxa (and probably others strains in the group). Indeed, the "firm, delimited" sheath typical of *Hydrocoryne* could not be considered as diacritical for the whole genus but to some species only, similar to the case of *Microcoleus* (Strunecký et al. 2013).

b. The genus *Trichormus*

Strains identified as *Trichormus* are present within subclade B2 in Clade B (Nostocales – II), Clade D (*Halotia* group), and Clade E (Nostocales – IV), similar results are found in Choi et al. (2012), Gladkikh et al. (2008), and Miscoe et al. (2016). Subclade B2 forms a compact cluster composed of several strains of *T. variabilis*, type species of the genus, and three strains identified as *Anabaena*, in agreement with previous works (Choi et al. 2012, Kozliková-Zapomělová et al. 2016, Kust et al. 2015, Papaefthimiou et al. 2008). *Trichormus* sp. HA7287-LM1 clusters within the *Halotia* group (Genuário et al. 2015), which suggest affiliation within this genus. The cluster "*T. variabilis*", affiliated with *Camptylonemopsis,* "*Tolypothrix*" PCC strains, and *A. laxa*, forms a highly supported cluster in the phylogenetic tree. However, some strains from this cluster are named differently in the literature ("*Anabaena variabilis*", "*Nostoc*", "*Cylindrospermum*", "*Anabaena flos-aquae*"; Rippka et al. 1979, 2001, UTEX webpage), and their habitat is referred as plankton in freshwater (Choi et al. 2012) or are unknown (Rippka et al. 1979, Herdman et al. 2001a).

Trichormus variabilis strain T020 fits the description of the species; however, it shows slightly shorter akinetes, and rarely basal heterocytes. This last feature is not mentioned by Komárek (2013) neither Fjerdingstad (1969) in their description for *T. variabilis*. *T. variabilis* T020 was isolated from soils in tropical Amazon forest, which is also in agreement with the occurrence of the species (Komárek 2013). In the phylogenetic tree, *T. variabilis* T020 clusters within subclade B2 (Clade B), forming a highly compact cluster with other *T. variabilis* strains and two *Anabaena* strains from soil samples at different localities. Identities of 16S rRNA gene sequence between all members of subclade B2 are 99.0 – 100% identical, above the limit for species separation (98.7 - 99%, Stackebrandt & Ebers 2006). Further studies using the 16S-23S ITS sequence are necessary to confirm this theory.

Trichormus cf. *indicus* stain T082 is similar to *T. indicus* Komárek, differing in dimensions of akinetes, in having basal heterocytes in young trichomes (present in T082, not described for *T. indicus*), sheath structure, and showing slightly shorter cells. *Trichormus.* cf. *indicus* T082 was isolated from mud sample associated to aquatic plants in a highland lake at Peruvian Andes, while *T. indicus* was described from rivers (Komárek 2013). In the phylogenetic tree, *Trichosmus* cf. *indicus* appears in subclade B2, and identities of 16S rRNA gene sequence compared with the other strains from this cluster are 99.2 – 99.9%, well above the cut-off for species recognition (98.7 – 99.0%, Stackebrandt & Ebers 2006). This suggests that all strains from subclade B2 belong to a single species. Further studies using 16S-23S ITS sequences would confirm this theory.

E. The family Aphanizomenonaceae

The family Aphanizomenonaceae is defined as having mostly planktic, aerotoped, isopolar heterocytous genera, some of them producing toxins (e.g. *Aphanizomenon*), and all of them being studied using molecular methods (Komárek et al. 2014). In the phylogenetic tree (Fig. 5), the Aphanizomenonaceae shows to be polyphyletic, locating typical planktic aerotoped genera intermixed with benthic aquatic genera never forming aerotopes (e.g. *Sphaerospermopsis* and *Raphidiopsis* clustering with benthic *Anabaena* and *Wollea* in Clade A). These results are in agreement with phylogenetic trees found in several works, although the status of the Aphanizomenonaceae is not directly mentioned (Genuário et al. 2013, Kozlíková-Zapomělová et al. 2016). In the present study, strain T049 identified as *Nodularia* sp. was isolated, and discussion on this taxa is presented below.

a. The genus *Nodularia*

Nodularia is a well-defined genus distinguished by having cells shorter than wide and apoheterocytic formation of akinetes (Komárek 2013). *Nodularia* species form a high supported subcluster (C1) within Clade C (Fig. 5), but their internal phylogenetic relationships are not clear. This is in agreement with results of Řeháková et al. (2014), even though they use more *Nodularia* sequences in their study.

Nodularia sp. strain T049 correspond to the benthic, metaphytic, and soil *Nodularia* morphotypes (Komárek 2013). *N. willei* Gardner is the most similar species to *Nodularia* sp. T049, both in morphology, with ± same dimensions of cells, as well as ecology (mats with

vegetation; Komárek 2013). *Nodularia* sp. T049 presents a unique feature not described for any *Nodularia* species so far: the spirally coiled trichomes inside the mucilaginous envelope (Fig. 15A). Identity of 16S rRNA gene sequence between *Nodularia* sp. compared with other *Nodularia* species used in the tree is 98.0 – 98.8%, below or overlapping the cut-off for species separation (Stackebrandt & Ebers 2006). Based on morphology and genetic identity, it is probably that *Nodularia* sp. is a new species. Further evaluation using 16S-23S ITS is necessary to confirm this theory.

F. The family Hapalosiphonaceae

The Hapalosiphonaceae is a true-branching, monophyletic family represented by Clade F in the phylogenetic tree (fig. 5), containing strains assigned to *Fischerella* (Bornet et Flahault) Gomont, *Hapalosiphon* Nägeli in Kützing ex Bornet et Flahault*, Mastigocladus* Cohn ex Kirchner, *Nostochopsis* Wood ex Bornet et Flahault*, Pelatocladus* Johansen et Vaccarino*, Westiella* Borzì*,* and *Westiellopsis* Janet. Despite this, several hapalosiphonacean genera have not been studied using modern methods, for example *Albrightia* Copeland, *Geitleria* Friedmann, and *Loriella* Borzi. Recently, Komárek et al. (2017) described *Dictyophoron* as a new genu within the Hapalosiphonaceae; however, its affiliation within this family is doubtful because *Dictyophoron* clusters next to *Brasilonema*, within the Scytonemataceae.

a. The genus *Westiellopsis*

Strains identified as *Westiellopsis* fall in subclusters F1, F3, and F4 within Clade F, which suggest the polyphyletic status of the genus. Subcluster F1 has several strains of *Fischerella Hapalosiphon,* and *Westiellopsis,* and is analogous to "W clade" sensu Saber et al. (2017). These authors mentioned that "W clade" belongs to *Westiellopsis*, and strains within this clade that are designated as different genera require further evaluation. However, the recently well-studied *H. arboreus* 3OW05S02 from Belize (Komárek et al. 2017) falls within subcluster F1, and is clearly morphological different to *Westiellopsis*. Subcluster F3 shows three *Westiellopsis* and three *Fischerella* strains with high support. Saber et al. (2017) show an unsupported cluster that is analogous to subcluster F3 from the present study, but they include more sequences. Subcluster F4 contains strains labelled as *Hapalosiphon*, *Westiella,* and *Westiellopsis,* and following recommendations by Kaštovský & Johansen (2008) and Saber et al. (2017), these will require further analysis to evaluate their final generic status.

Westiellopsis sp. strain T028 isolated in the present study shows the diagnostic features for the genus *Westiellopsis*: chroococcoid stages, pseudohormocytes and monocytes (Janet 1941, Komárek 2013). Despite this, it differs from other species of the genus in having a typical radially disposed thallus forming circular patches on the agar surface, and showing lateral branches constricted with short to isodiametric cells (Fig. 15E), and lateral heterocytes in main trichomes without being located in short branches (Fig. 15F). Even though descriptions of some *Westiellopsis* species rely on culture material, these do not mention any specific feature of the thallus (Janet 1941, Komárek 2013, Saber et al. 2017). In addition, the lateral heterocytes in *Westiellopsis* sp. T028 is a feature described for *Pelatocladus*, being the only morphological feature to separate the Hawaiian genus from other hapalosiphonacean genera (Miscoe et al. 2016). Despite this, it is important to note that descriptions of *W. prolifica* (Janet 1941, Saber et al. 2017), *Westiellopsis* sp. T028, and *Pelatocladus* (Miscoe et al. 2016) rely on strains cultured under different media (Moore's solution, BG11, modified Chu N°10, Z8). Therefore, to reduce the possibility of induced morphological variations, studies on their morphology should rely on strains cultured under same conditions. Phylogenetically, *Westiellopsis* sp. T028 belongs to subcluster F3 within Clade F with high support. Identities of 16S rRNA gene sequence between members of this subcluster are 99.6 – 100% (table 19), well above the cut-off for species recognition (98.7 – 99%, Stackebrandt & Ebers 2006). Furthermore, identities between subclusters F1 – F4 are ≤ 95.9% , which could justify their affiliation to one single genus following the limit for genus separation (95%) proposed by Stackebrandt & Goebel (1994). In this line, Gugger & Hoffmann (2004) suggest that strains of cluster 1 (analogous to the cluster containing subclusters F1 – F4) identified as *Fischerella*, *Hapalosiphon*, *Nostochopsis*, and *Westiellopsis* may belong to a single genus based on their high identity. Kaštovský & Johansen (2008) refute this idea based on morphological and ecological diversity within these genera. In addition, evidence shows that in some hapalosiphonean taxa, the acquisition of novel phenotypic characteristics precedes the fixation of genotypes (Koch et al. 2017), which could explain the similar identity between morphological different taxa. Therefore, the high identity of 16S rRNA gene sequence between strains from subclusters F1 – F4, including *Westiellopsis* sp. T028, would not indicate that they correspond to the same species or genus, as mentioned by other heterocytous cyanobacteria (Flechtner et al. 2002, Kaštovský et al. 2014). Further analysis using other genetic markers, and considering the ecology of taxa, must be performed to solve this taxonomic problem.

V. CONCLUSIONS

In the present thesis, the phylogenetic studies using the 16S rRNA gene sequences show to be a good tool for taxonomical studies above the species level, and are a suitable tool for defining cyanobacterial genera together with morphological and ecological characterization. In this study, *Skacelovkia* gen. nov. is proposed based on this criteria, and other two potentially new genera await further erection as novel taxa ("*Dichothrix*", "*Microchaete*"-like group). However, limitations exist when only the 16S rRNA gene is used for phylogenies, and species separation is problematic. Therefore, further studies using other other DNA sequences are necessary. According to several studies, the use of 16S-23S ITS sequences improve the correctly recognition between distinct species, and it is suggested that it mus be applied to those taxa that could not be assigned to a novel species, or which species designation was doubtful in the present work. On the other hand, the several unindentified cyanobacteria and new records demonstrate that the Tropical Andes hotspot habors potentially novel taxa, and that the cyanobacterial knowledge from Peru is still far to be completely known. Furthermore, several taxa within the heterocytous cyanobacteria were found to be polyphyletic, thus, further taxonomic work is needed to obtain monophyletic genera and families.

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VII. APPENDIX

A. Figures 6 – 15: photomicrographs of studied strains.

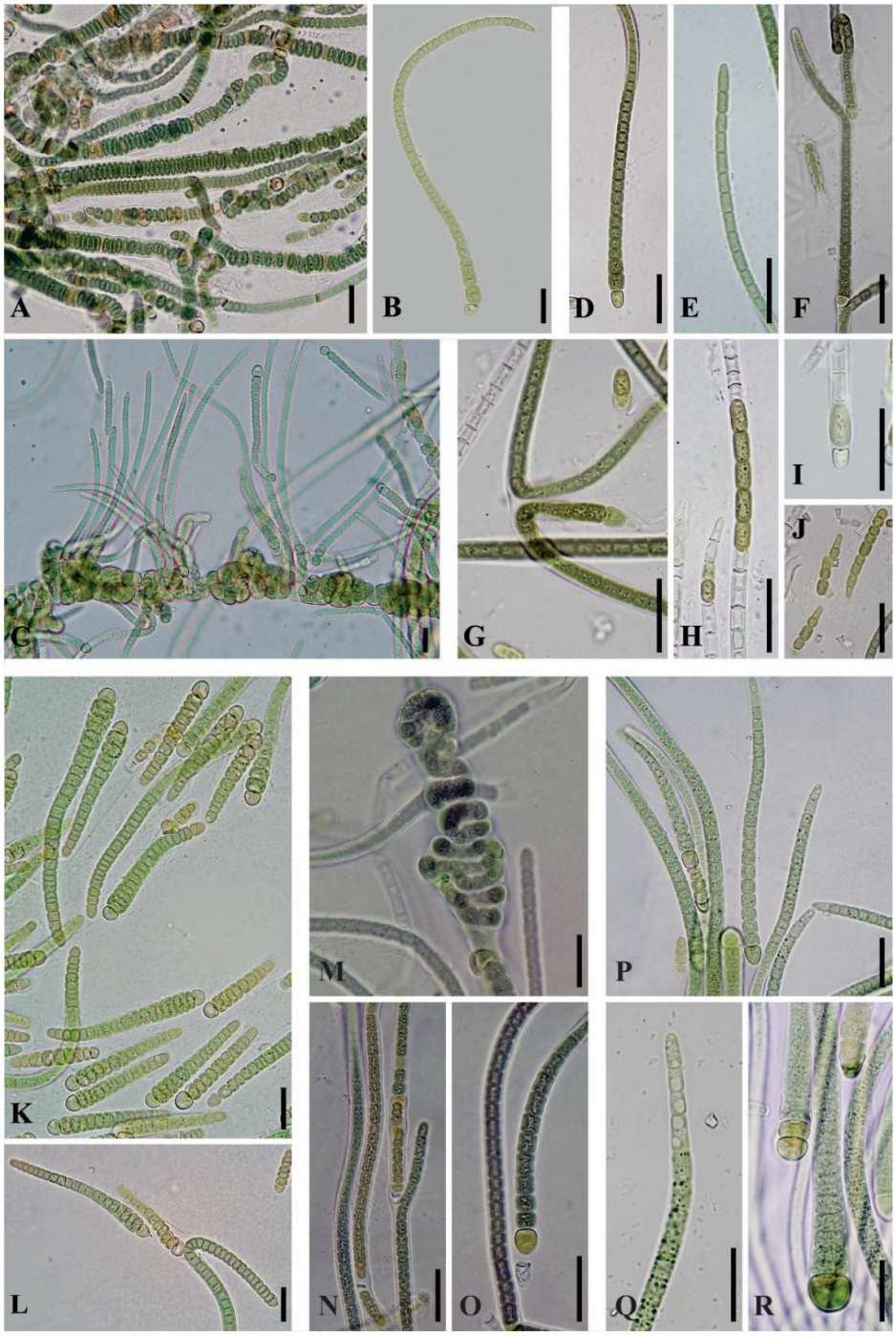

Fig. 6. Light photomicrographs of *Calothrix* strains. A-C: *Calothrix* cf. *elenkinii*, strain T014; D-J: *Calothrix* cf. *marchica*, strains T064 and T067; K-L: *Calothrix* sp. type 1, strain T030; M-O: *Calothrix* sp. type 2, strain T035; P-R: *Calothrix* sp. type 3, strain T019. Scale bar = 100 µm for A and C, 20 µm for C-R.

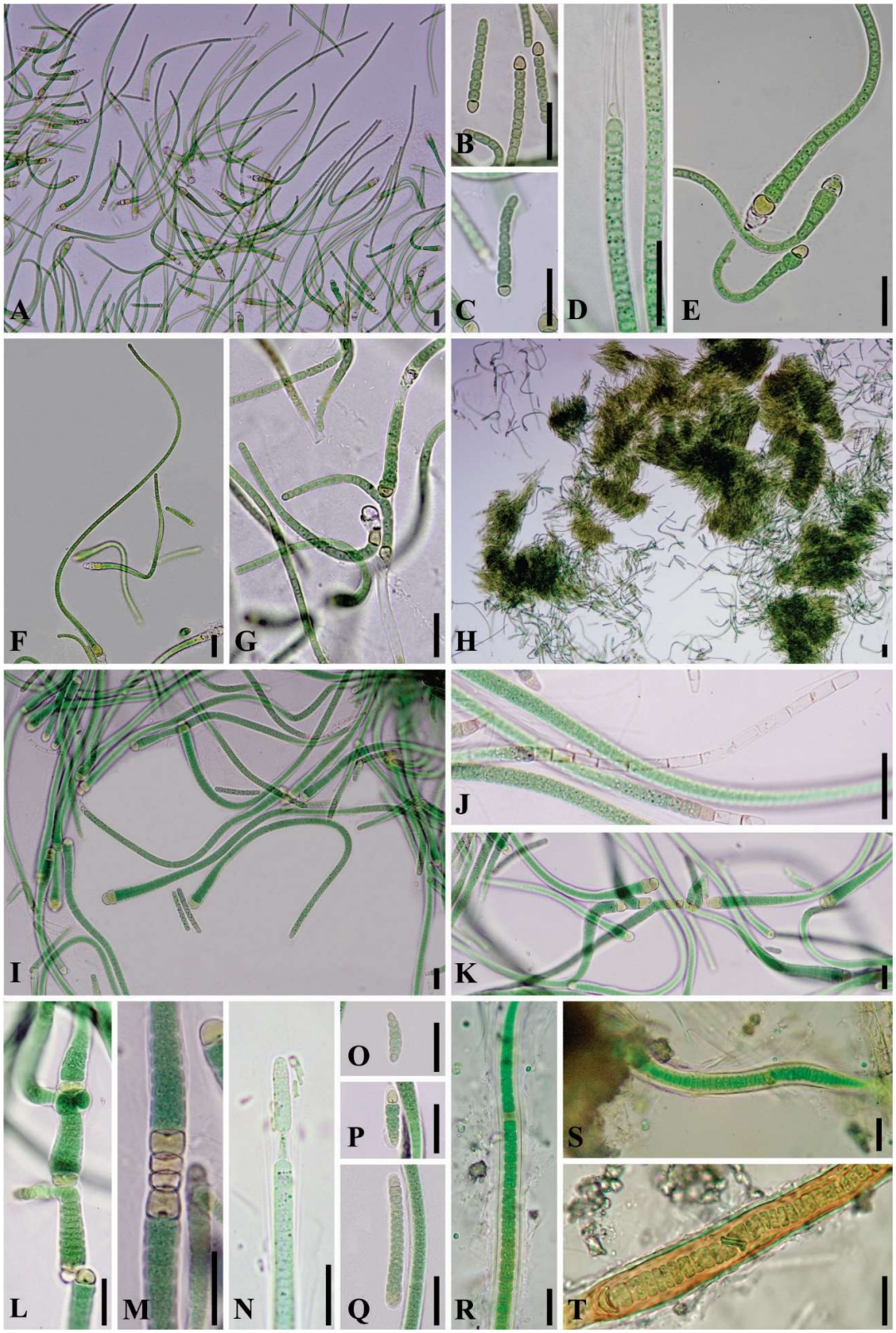

Fig. 7. Light photomicrographs of *Calothrix* strains. A-H: *Calothrix* sp. type 4, strain T029; I-F: *Calothrix* sp. type 5, strain T044. Scale bar = 20 µm.

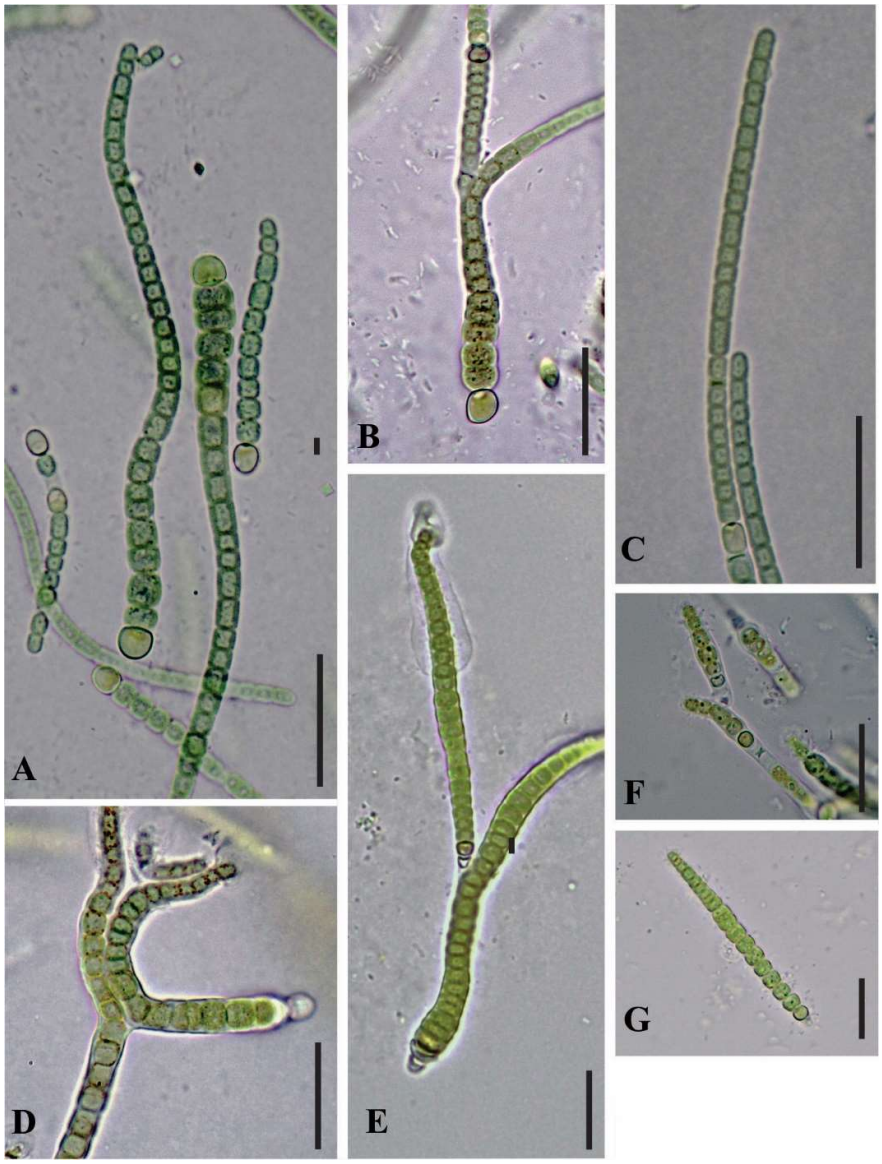

Fig. 8. Light photomicrographs of *Skacelovkia* strains. A-C: *Skacelovkia peruviana*, strain T027, D-G: *Skacelovkia* sp. strain P3C2e. Scale bar = 20 µm.

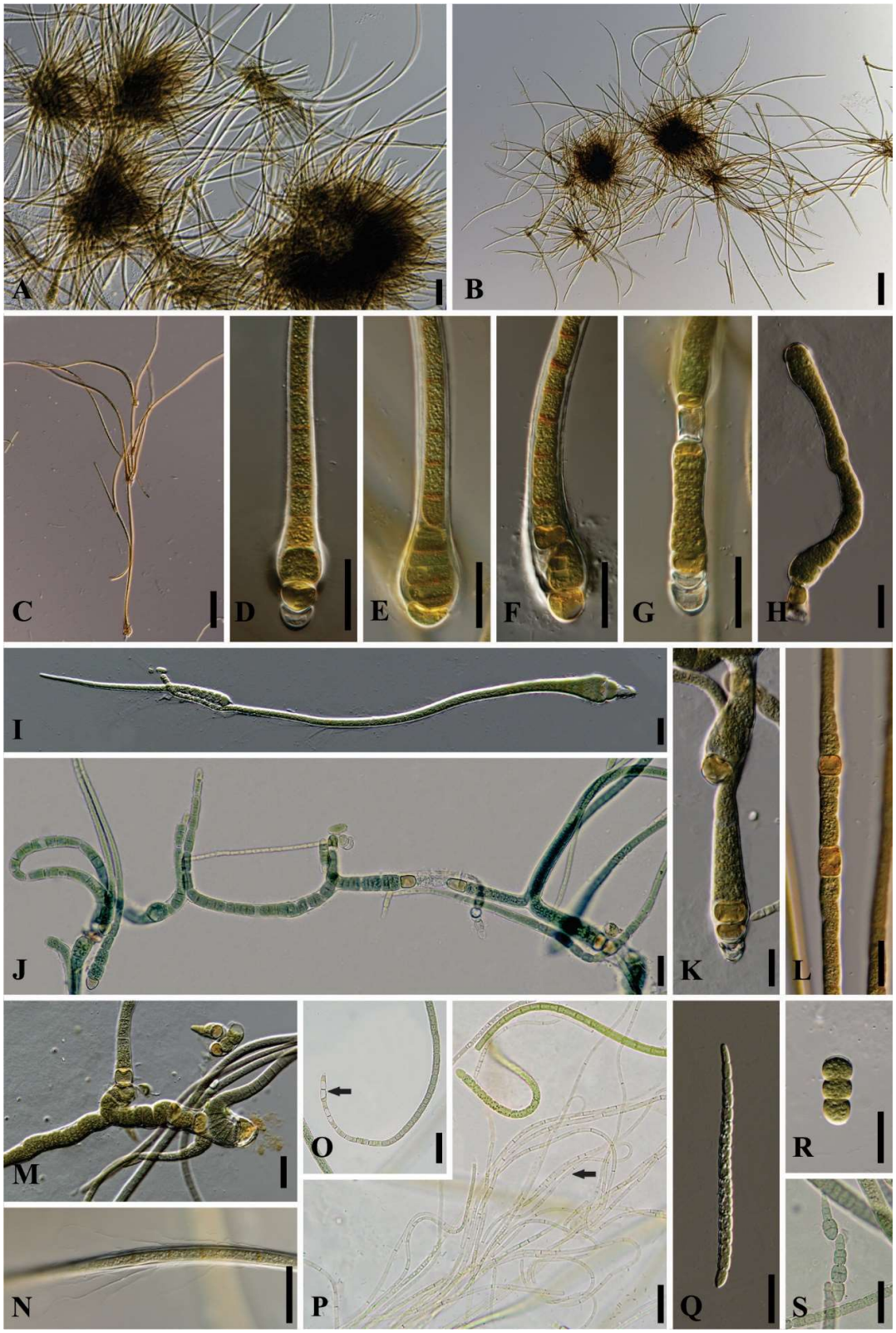

Fig. 9. Light photomicrographs of "*Dichothrix*" sp., strains T008, T017, and T048. Scale bar = 100 µm for A – C; 20 µm for D – S.

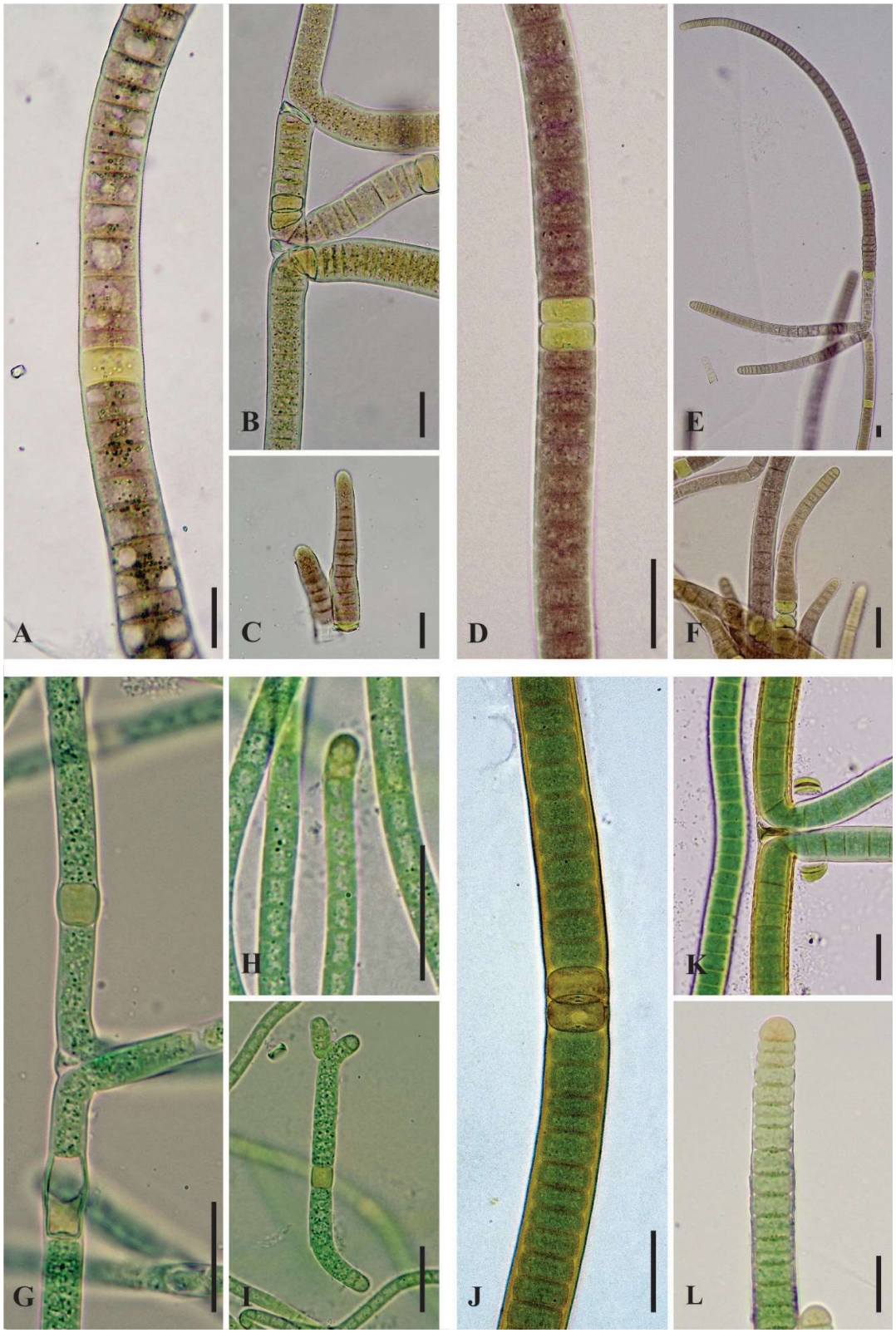

Fig. 10. Light photomicrographs of scytonenatacean strains. A-C: *Brasilonema octagenarum*, strain T001; D-F: *Brasilonema* sp., strain T076; G-I: *Scytonema* sp. type 1, strains T051 and T056; J-L: *Scytonema* sp. type 2, strain T063. Scale bar = 20µm

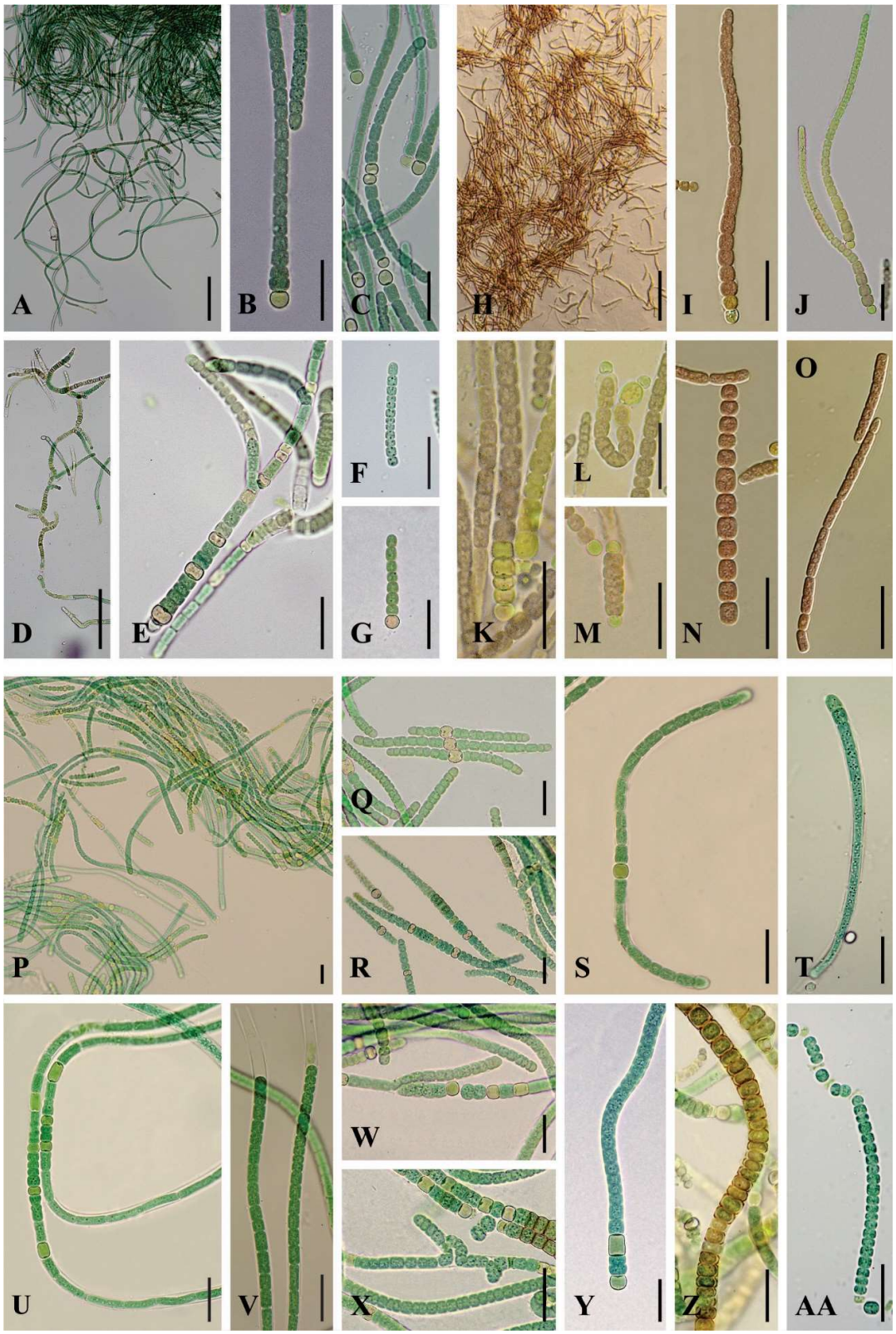

Fig. 11. Light photomicrographs of *Microchaete, Calochaete*, and *Camptylonemopsis* strains. A-G: *Microchaete* sp. type 2, strain T060; H-O: *Calochaete* sp., strain T037; P-AA: *Camptylonemopsis* cf. *pulneyensis*, strain T054. Scale bar = 100 µm, for A, D and H; 20µm for B, C, E-G, I-AA.

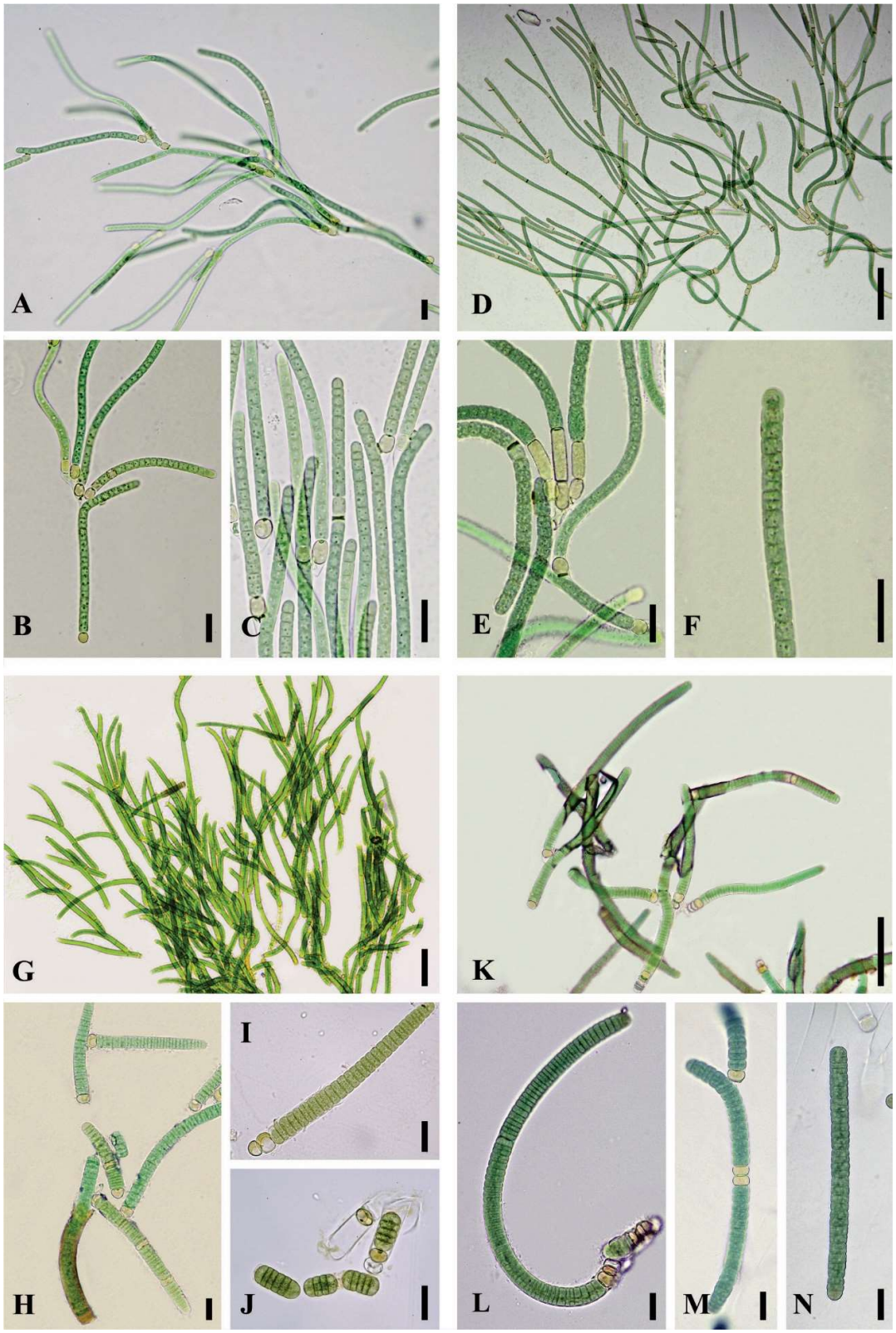

Fig. 12. Light photomicrographs of tolypothrichacean strains. A-C: *Tolypothrix* cf. *helicophila*, strain T002; D-F: *Tolypothrix* sp., strain T079; G-J: *Hassallia californica*, strains T061 and T081; K-N: *Hassallia* sp., strain T075. Scale bar = 20µm

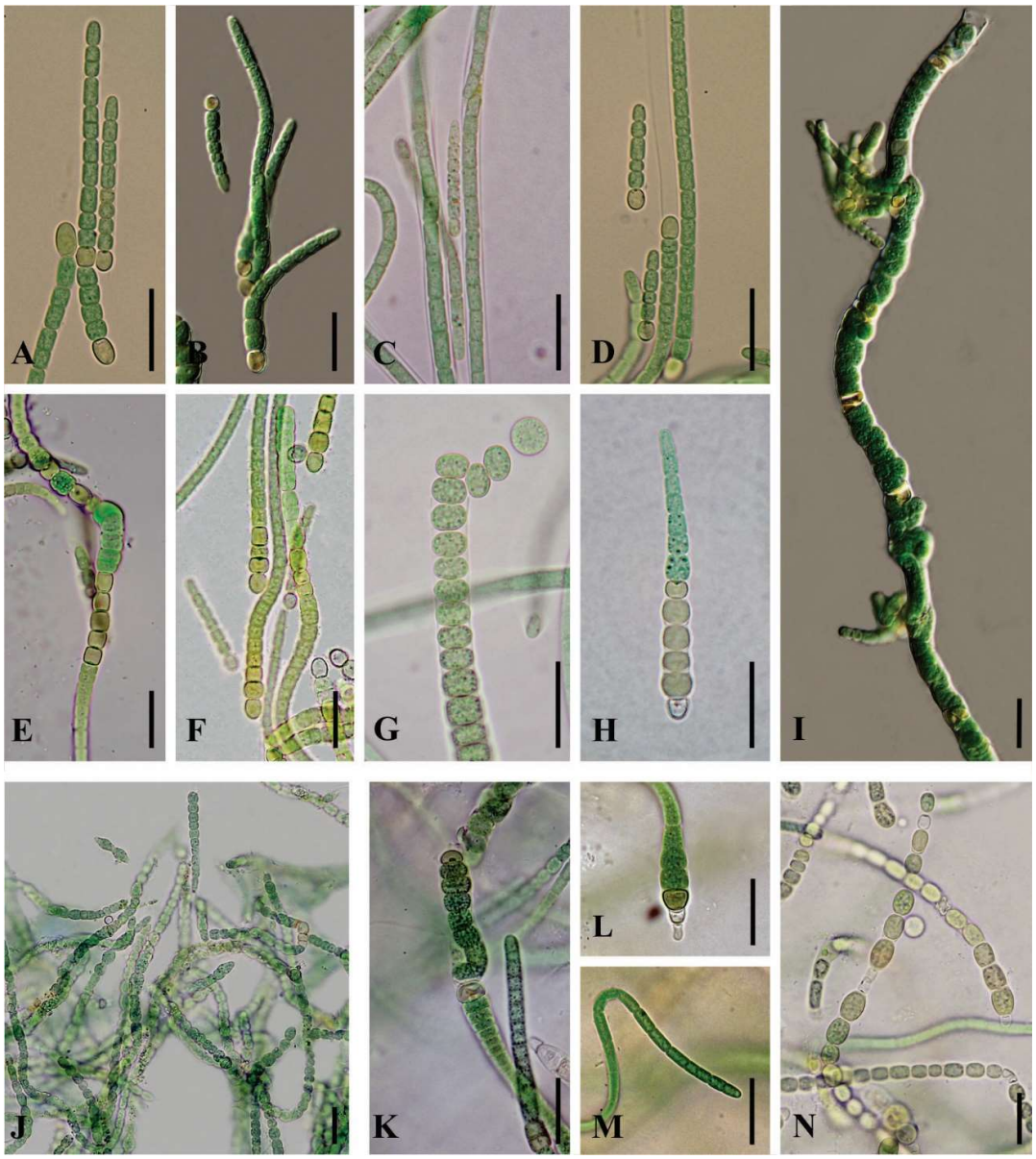

Fig. 13. Light photomicrographs of "*Microchaete*"-like strains. A-I: *Microchaete* sp. type 1, strains T042 and T047; J: strain JOH06; K-N: strain JOH42. Scale bar = 20µm

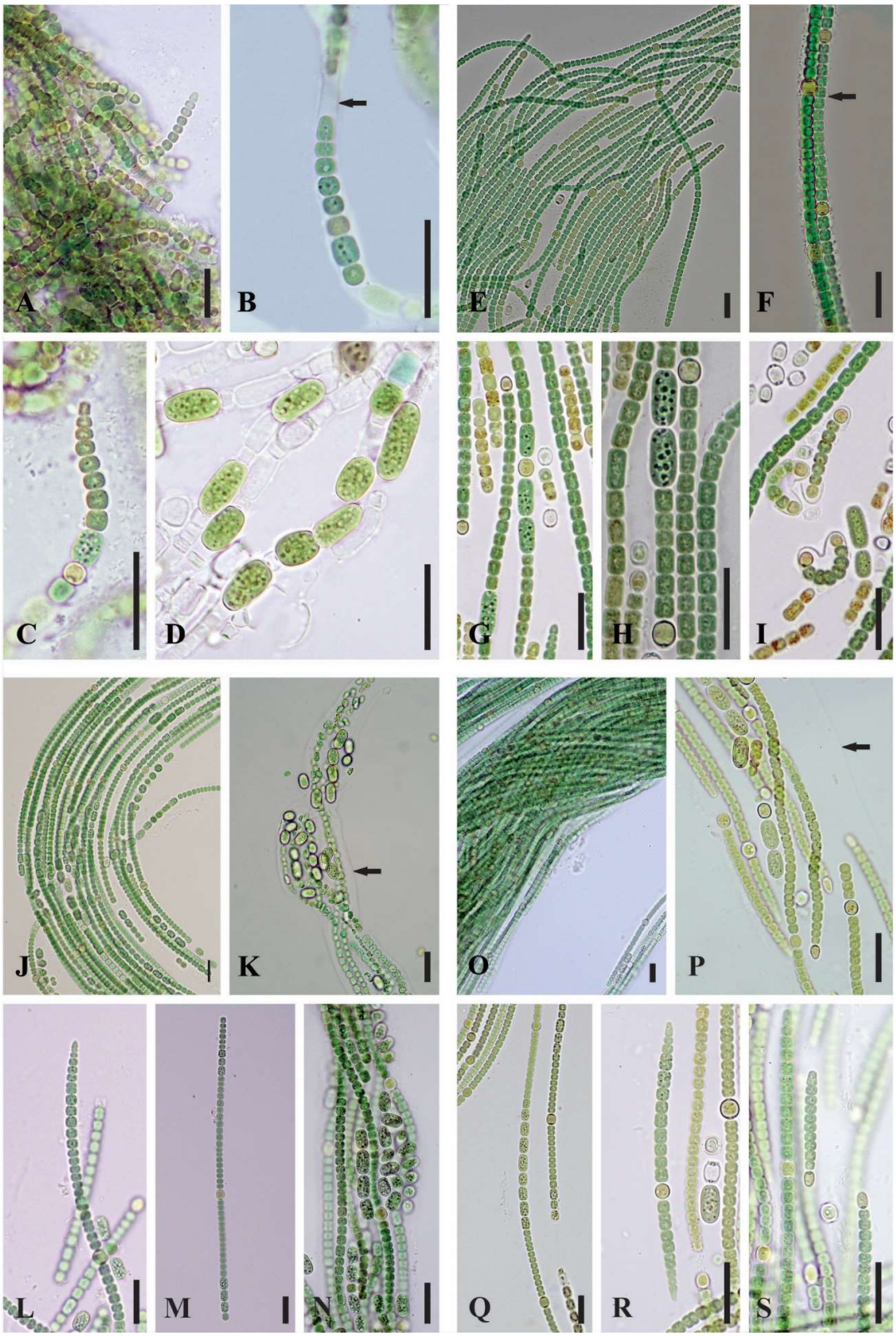

Fig. 14. Light photomicrographs of nostocacean strains. A-D: *Anabaena* sp. type 1, strain T065; E-I: *Anabaena* sp. type 2, strain T033; J-N: *Trichormus variabilis*, strain T020; O-S: *Trichormus* cf. *indicus*, strain T082. Arrows indicate the mucilaginous sheaths. Scale bar = 20µm

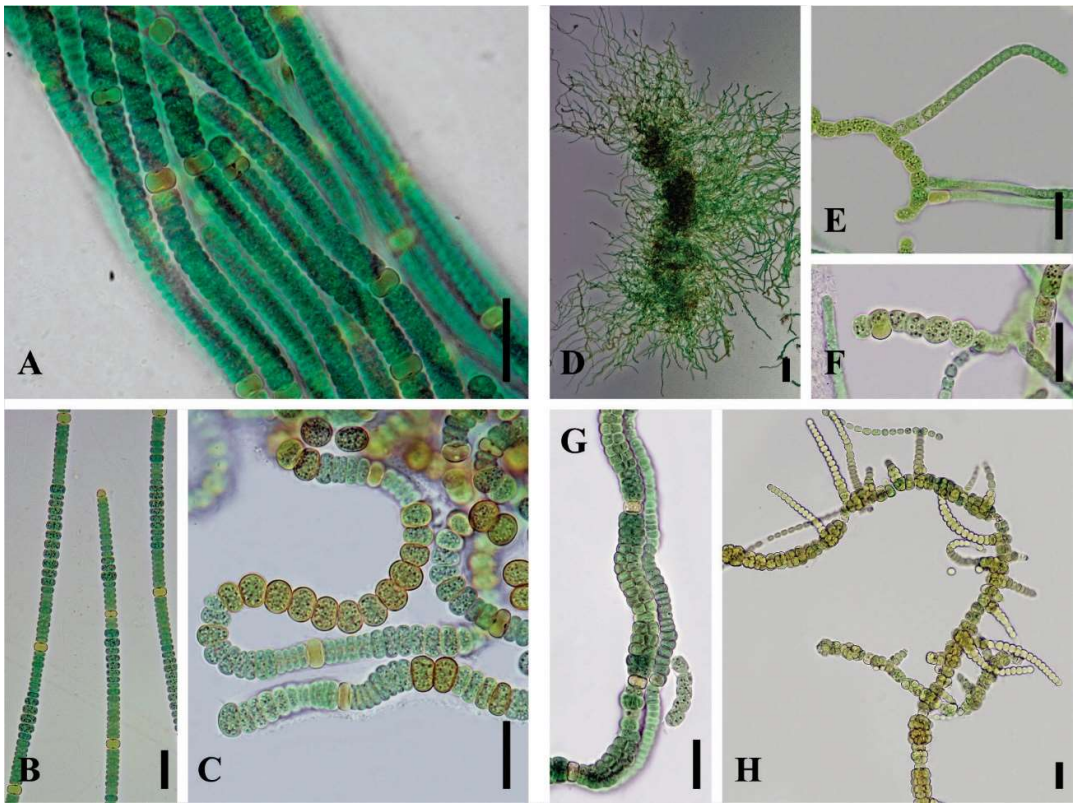

Fig. 15. Light photomicrographs of *Nodularia* and *Westiellopsis* strains. A-C: *Nodularia* sp., strain T049; D-H: *Westiellopsis* sp., strain T028. Scale bar = 20µm

B. **List of sequences used for the elaboration of phylogenetic trees, including their accession numbers.** Codes for the phylogenetic trees: A = Rivulariaceae Tree, B = Scytonemataceae Tree, C = "Fortieaceae", Tolypothrichaceae Tree, D = Aphanizomenonaceae, Nostocaceae, Hapalosiphonaceae Tree.

N°	Strain	Genbank Acc. number	Phylogenetic trees			
			A	B	C	D
1	*Gloeobacter violaceous* PCC 7421	NC005125	X	X	X	X
2	*Chroococcidiopsis thermalis* PCC 7203	CP003597	X	X	X	X
3	*Chroococcidiopsis cubana* SAG 39.79	AJ344558	X		X	X
4	*Phyllonema aviceniicola* CENA326	KT731147	X			
5	*Phyllonema aviceniicola* CENA330	KT731150	X			
6	*Mastigocoleus testarum* BC008	LMTZ01000039	X			
7	*Rivularia* sp. 1PA3	FJ660973	X			
8	*Rivularia* sp. 7PA9	FJ660991	X	X		
9	*Rivularia* sp. 7PA6	FJ660990	X	X		
10	*Rivularia* sp. PUNA NP3 PCI185B	KY296608	X			
11	*Calothrix* sp. CCMEE 5093	AY147029	X			
12	*Rivularia biasolettiana* ERIVALH2	EU009142	X			
13	*Rivularia atra* environmental colony BIR MGR1	AM230675	X			
14	*Rivularia atra* environmental colony BIR KRIV1	AM230674	X	X	X	X
15	*Rivularia* sp. XSP25A	AM230665	X			
16	*Rivularia* sp. XP3A	AM230672	X			
17	*Rivularia* sp. BECID10	AM230673	X			
18	*Rivularia* sp. BECID12	AM230666		X	X	X
19	*Calothrix* sp. ANT LPR2 4	AY493597	X			
20	*Rivularia* sp. XP16B	AM230676	X			
21	*Calothrix* sp. CCAP 1410/5	HF678513	X			
22	*Kyrtuthrix huatulcensis* C708	KT936560	X	X	X	X
23	Nostocales cyanobacterium HI14	FJ660994	X	X		
24	Nostocales cyanobacterium HI15	FJ660993	X	X		
25	*Calothrix parasitica* NIES-267	NZ_AP018227	X			
26	*Rivularia* sp. PCC 7116	NC_019678	X	X	X	X
27	*Rivularia* sp. LEGE 07159	KC989702	X			
28	Uncultured *Rivularia* sp. PCHap18	KR150510	X			
29	Uncultured *Rivularia* sp. PCHap35	KR150502	X			
30	*Nunduva biania* C708 M10	not yet released	X			X
31	*Nunduva fasciculata* C694 M8 CL2CL3	not yet released	X		X	
32	*Nunduva kania* C458	not yet released	X		X	X
33	*Nunduva britannica* CCAP 1445/1	HE797730	X	X	X	X
34	*Calothrix* sp. UAM 374	HM751856	X		X	
35	Uncultured cyanobacterium clone D3B06	EU753653	X			
36	Uncultured cyanobacterium clone D3F11	EU753654	X			
37	Uncultured cyanobacterium clone D1H05	EU753648	X			
38	Uncultured cyanobacterium clone D1E07	EU753645	X			
39	*Calothrix* sp. CYN89	JQ687339	X			

40	*Calothrix* sp. UAM 342	HM751842	X		X	
41	*Scytonematopsis contorta* HA4267-MV1	HQ847557	X	X	X	X
42	*Scytonematopsis contorta* HA4292-MV4	HQ847560	X	X	X	X
43	*Macrochaee psychrophila* CCALA 1092	KR350577	X	X	X	X
44	*Macrochaete santannae* CCALA 1093	KT336440	X	X	X	X
45	*Macrochaete lichenoides* SAG 32.92	KU559618	X	X	X	X
46	*Calothrix elsteri* CCALA 953	NR_117190	X			
47	*Calothrix* sp. AHLA9	AM230694	X			
48	*Calothrix* sp. BECID9	AM230688	X			
49	*Calothrix* sp. CAL3361	AM230697	X			
50	*Calothrix parietina* CCAP 1410/11	HE974991	X			
51	*Calothrix* sp. XP4B	AM230692	X			
52	*Calothrix* sp. PCC 8909	AM230693	X			
53	Cf. *Calothrix* sp. Muscicolous cyanobiont 5	AF506237	X			
54	*Calothrix* sp. BECID1	AM230680	X			
55	*Calothrix* sp. UKK3412	AM230681	X			
56	*Calothrix* sp. BECID16	AM230682	X			
57	*Calothrix* sp. HA4860-CV1	KT336444	X			
58	*Calothrix* sp. BECID33	AM230683	X			
59	*Calothrix* sp. PCC 7715	KM019959	X			
60	*Calothrix* sp. PCC 7103	AM230700	X			
61	*Calothrix parietina* CCAP 1410/10	HF678479	X			
62	*Calothrix* sp. SEVV5-4-C5	KT336446	X	X	X	X
63	*Calothrix* sp. MU27 UAM 315	EU009152	X			
64	*Calothrix* sp. TJ12 UAM 372	EU009154	X			
65	*Calothrix*sp. UAM 373	HM751855	X	X	X	X
66	*Calothrix desertica* PCC 7102	AM230699	X			
67	*Calothrix parietina* SRS-BG14	AF334695	X			
68	*Calothrix parietina* 2T10	FR798917	X			
69	*Calothrix* sp. PCC 7714	AJ133164	X	X	X	X
70	*Calothrix* sp. HA4395-MV3	HQ847571	X			
71	*Calothrix* sp. HA4186-MV5	HQ847580	X			
72	*Rivularia* sp. VP4-08	FR798919	X			
73	*Rivularia haematites* MU24 UAM 305	EU009149	X			
74	*Rivularia* sp. IAM M-261	AB325536	X			
75	*Cyanomargarita melechinii* APA-RS9	KY296605	X	X	X	X
76	*Cyanomargarita calcarea* GSE NOS 12	KY296607	X	X	X	X
77	*Goleter apudmare* HA4340-LM2	KF417425	X	X	X	X
78	*Goleter apudmare* HA4356-MV2	JN385288	X	X	X	X
79	*Gloeotrichia echinulata* URA3	AM230705	X	X	X	X
80	*Gloeotrichia echinulata* PYH6	AM230703	X		X	
81	*Gloeotrichia pisum* SL6-1-1	KY296602	X	X	X	X
82	*Gloeotrichia longicauda* SAG 32.84	KM019918	X		X	
83	*Dapisostemon apicaliramis* CCIBt 3318	KJ566947			X	
84	*Dapisostemon apicaliramis* CCIBt 3536	KJ566945			X	
85	*Godleya alpina* LCR-CYTOL	HQ012539	X	X	X	X

86	*Godleya alpina* LCR-CY2	HQ012540	X		X	X
87	Uncultured cyanobacterium B107212D	HQ189094			X	
88	*Toxopsis calypsus* PLF clone 1	JN695681	X	X	X	X
89	*Toxopsis calypsus* PLF clone 2	JN695682	X		X	X
90	*Toxopsis calypsus* PLF clone 3	JN695683			X	
91	*Coleodesmium* sp. HINDAK 200/24	HE797727	X	X	X	X
92	*Coleodesmium* cf. *scottianum* ANT.L52B.5	AY493596			X	
93	*Coelodesmium wrangelii* MC-JRJ1	AF334701	X	X	X	X
94	*Hassallia antarctica* CCALA 956	FR822753		X		
95	*Hassallia antarctica* CCALA 957	FR822754	X		X	X
96	*Hassallia byssoidea* CCALA 823	AM905327	X	X	X	X
97	*Hassallia andreassenii* CCALA 954	FR822751			X	
98	*Hassallia littoralis*C76	KF017617			X	
99	*Hassallia* sp. ATA2-3-CV2	KF934148			X	
100	*Hassallia* sp. UB1-KK1 clone 1	KF934131			X	
101	*Hassallia* cf. *pseudoramosissima* ACSSI 158	KY283057			X	
102	*Dactylothamnos antarcticus* CENA410	KM199732			X	
103	Uncultured bacterium clone AK4DE2 01G	GQ397073			X	
104	*Spirirestis rafaelensis* WJT-71-NPBG6	JQ083655			X	
105	*Spirirestis rafaelensis* SRS70	AF334690			X	
106	*Rexia erecta* CAT4-SG4	KF934181			X	
107	*Tolypothrix distorta* SAG 93.79	GQ287651			X	
108	*Tolypothrix distorta* ACOI 3104	HG970653			X	X
109	*Tolypothrix distorta* ACOI 731	HG970652	X	X		
110	*Tolypothrix distorta* SEV2-5-BG	AF334694			X	
111	*Tolypothrix* sp. NIES-4075	BDUC01000001			X	
112	*Tolypothrix* sp. HA4266-MV1	JN385291	X	X		X
113	*Tolypothrix carrinoi* HA7290-LM1	KU161667			X	
114	*Tolypothrix fasciculata* ACOI 3104	HG970653	X	X	X	X
115	*Tolypothrix tenuis* CCALA 197	HG970655			X	
116	*Tolypothrix* sp. HanysB	LM992903			X	
117	*Tolypothrix* sp. Preslic8	HG970654			X	
118	*Tolypothrix* sp. NIES-515	LC215279			X	
119	*Tolypothrix campylonemoides* FI5-MK38	JQ083653			X	
120	*Tolypothrix* sp. CXA109-3-BZ	KF934130			X	
121	*Tolypothrix* sp. ACSSI 038	KY283049			X	
122	*Kryptousia macronema* CENA338	KY508610			X	
123	*Kryptousia microlepis* CENA354	KY508612			X	
124	*Scytonema mirabile* SAG 83.79	KM019943		X	X	
125	*Aulosira laxa* NIES-50	NZ_AP018307	X	X	X	
126	*Nostoc carneum* NIES-2107	AP018180			X	
127	*Calothrix brevissima* NIES-22	NZ_AP018207	X		X	
128	*Calothrix anomala* SAG 1410-4	KM019945			X	
129	*Calothrix membranacea* SAG 1410-1	KM019924			X	
130	*Calothrix* sp. Mk1-C1	AB275345			X	
131	*Camptylonemopsis* sp. HA4241-MV5	JN385292			X	X

132	*Camptylonemopsis* sp. MGCY355I	KY056812			X	X
133	*Microchaete* sp. CENA 541	KX458488			X	
134	*Microchaete diplosiphon* NIES-3275	NZ_AP018233			X	
135	*Microchaete diplosiphon* CCALA 811	JX827160			X	
136	*Microchaete diplosiphon* KSU-AQIQ-10	LN997859			X	
137	*Tolypothrix tenuis* SAG 94.79	KM019944			X	
138	*Tolypothrix tenuis* PCC 7101	NZ_AP018248	X	X	X	
139	*Tolypothrix* sp. PCC 7601	JX827161	X	X	X	
140	*Tolypothrix* sp. PCC 7504	AM230669	X	X	X	
141	*Tolypothrix* sp. NQAIF319	KJ636968			X	
142	*Tolypothrix* sp. TOL328	AM230706			X	
143	*Mojavia* sp. CMT-3FDIN-NPC3	KU161676	X	X	X	X
144	*Mojavia* sp. ATA2-1-KO23	KU161649			X	
145	*Mojavia pulchra* JT2-VF2	AY577534	X	X	X	X
146	*Nostoc commune* EV1-KK1	AY577536	X	X	X	X
147	*Nostoc lichenoides* CNP-AK1	AY577535	X	X	X	X
148	*Nostoc edaphicum* X	AJ630449	X	X	X	X
149	*Roholtiella edaphica* CCALA 1063	KM268878	X	X	X	X
150	*Roholtiella fluviatilis* UAM 337	HM751851	X	X	X	X
151	*Roholtiella bashkiriorum* CCALA 1057	KM268883	X	X	X	X
152	*Calochaete cimrmanii* CCALA 1012	HF912385	X	X	X	X
153	*Desmonostoc muscorum* Lukesova 1-87	AM711523	X	X	X	X
154	*Desmonostoc muscorum* Lukesova 2-91	AM711524	X	X	X	X
155	*Desmonostoc muscorum* CCAP 1453/22	HF678509			X	X
156	*Desmonostoc* sp. PCC 6302	HG004582	X	X	X	X
157	*Fortiea laiensis* HA4221-MV2	HQ847570	X	X	X	X
158	*Fortiea contorta* PCC 7126	KB235930	X		X	X
159	*Fortiea coimbrae* ACOI 1451	HE797732		X	X	
160	*Fortiea coimbrae* ACOI 2161	HE797731			X	
161	*Microchaete* sp. SAG 47.93	KM019922			X	
162	*Microchaete tenera* ACOI 630	HE797733			X	X
163	*Aulosira bohemensis* ISB-2	EU532189			X	X
164	*Cylindrospermum stagnale* PCC 7417	AJ133163	X	X	X	X
165	*Cylindrospermum maius* CCALA 998	KF052614	X	X	X	X
166	*Cylindrospermum muscicola* SAG 44.79	KM019946			X	
167	*Cylindrospermum pellucidum* CCALA 992	KF052605			X	
168	*Calothrix* sp. CCAP 1410/13	HF678491			X	
169	*Calothrix* sp. RSUAII 9BNC	KF761554			X	
170	*Calothrix* sp. RSUAII 9CNC	KF761555			X	
171	*Trichormus* sp. ATA115-CL34-AU3-P2RT	KF761559			X	
172	Nostocales cyanobacterium NapMSIm13	KM438192			X	
173	Microchaetaceae cyanobacterium CENA550	KX458494			X	
174	*Microchaete violacea* ACOI 3057	HE797734			X	
175	*Chakia ciliosa* 3	KC875343	X	X		X
176	*Chakia ciliosa* 5	KC875344		X		
177	*Scytonema* sp. WJT9-NPBG6A clone PS3C	KF934163		X		

178	*Scytonema* sp. CMT-1BRIN-NPC31 clone PI2	KF934154		X		
179	*Scytonema* sp. HAF2-B2-c1	HQ847553		X		
180	*Scytonema* sp. HTT-U-KK4	HQ847552		X		
181	*Scytonema* sp. WJT4-NPBG1 clone PS2A	KF934161		X		
182	*Scytonema* sp. CMT-1FBIN-NPC21 clone RTCS2P1	KF934158		X		
183	*Scytonema* sp. ATA-SAL-RM1 clone B	KF934169		X		
184	*Scytonema* sp. HA4185-MV1 clone p2	HQ847565		X		
185	*Scytonema javanicum* U41-MK36	HF911525		X		
186	*Scytonema* cf. *fritschii* UCFS23	JN565282		X		
187	*Scytonema* cf. *fritschii* UCFS22	JN565281		X		
188	*Scytonema* sp. BHUS-5	KU058658	X	X	X	X
189	*Scytonema hofmannii* PCC 7110	AF132781	X	X	X	X
190	*Scytonema* sp. CG23	KT222810		X		
191	*Scytonema* sp. 1f	KU668900		X		
192	*Scytonema* sp. 1F-PS	KT935473		X		
193	*Scytonema* sp. R77DM	KM063575		X		
194	*Scytonema* sp. IAM M-262	AB093483		X		
195	*Scytonema* sp. MGL002	KX951410		X		
196	*Scytonema* sp. U-3-3	AY069954	X	X	X	X
197	*Symphyonemopsis* sp. VAPOR1	AJ544085	X	X	X	X
198	*Iphinoe spelaeobios* L02-B1	HM748317	X	X	X	X
199	*Brasilonema* sp. CENA382	KR137603		X		
200	*Brasilonema* sp. CENA360	KR137581		X		
201	*Brasilonema* sp. RKST-322	KU161678		X		
202	*Brasilonema octagenarum* UFV-E1	EF150854		X		
203	*Brasilonema* sp. RKST-3291	KU161679		X		
204	*Brasilonema* sp. CENA381	KR137602		X		
205	*Brasilonema* sp. CENA347	KT731163		X		
206	*Brasilonema* sp. BZ-HDL-007	KY365502		X		
207	*Brasilonema* sp. KEN-MK50	KY365506		X		
208	*Brasilonema* sp. CR6-4B clone CT45-c2	KY365503		X		
209	*Brasilonema* sp. CDV2	MF423481		X		
210	*Brasilonema roberti-lamyi* str. los manatiales	GQ443308	X	X	X	X
211	*Brasilonema angustastum* HA4187-MV1	HQ847566	X	X	X	X
212	*Brasilonema* sp. CENA366	KR137587		X		
213	*Brasilonema* sp. M31-F20B	KY365507		X		
214	*Brasilonema* sp. TH04-Ema	KY365511		X		
215	*Brasilonema burkei* HA4348-LM4	KU161665		X		
216	*Brasilonema bromeliae* SPC 951	DQ486055	X	X	X	X
217	*Brasilonema* sp. CENA114	EF117246		X		
218	*Brasilonema* sp. P09-MK13	KY365508		X		
219	*Brasilonema* sp. PT5-MK70	KY365509		X		
220	*Brasilonema* sp. NQAIF325	KJ636963		X		
221	*Brasilonema tolantongensis* str. Tolantongo	JN676147		X		
222	*Brasilonema terrestre* CENA116	EF490447		X		
223	*Scytonema* sp. 00557 00001 clone 0557N1	KC854780		X		

224	*Scytonema* sp. SAG 67.81	KM019951		X		
225	*Petalonema alatum* CBFS A-035	KM047021	X	X		X
226	*Petalonema* sp. HA4277-MV1	HQ847568		X	X	
227	*Petalonema* sp. ANT LG2 8	AY493624		X	X	
228	*Ewamiania thermalis* TS0513	KX781314	X	X		X
229	*Scytonema* sp. CXA108-5-BZ clone 5BZ	KF934174		X		
230	*Scytonema* sp. WJT9-NPG6A clone PS3A	KF934171		X		
231	*Scytonema* sp. CMT-1BRIN-NPC32	KF934173		X		
232	*Scytonema* sp. HAF2-B2-c1 clone p11c	JQ083659		X		
233	*Scytonema* sp. YK-02	AB694929		X		
234	*Scytonema* sp. HK-05	AB694934		X		
235	*Scytonema hyalinum* FI-8A	AF334700		X		
236	*Scytonema arcangeli* CCIBt3134	KC682101		X		
237	*Symphyonema* sp. 1269-1	AJ544083		X		
238	*Symphyonema* sp. 1517	AJ544084		X		
239	*Scytonema stuposum* CCALA 1009	HF911528		X		
240	*Scytonema stuposum* CCALA 1008	HF911527		X	X	
241	*Scytonema* cf. *chiastum* UCFS19	JN565280		X	X	
242	*Scytonema crispum* U55-MK38	HF911526	X	X	X	
243	*Scytonematopsis maxima* LCR-FBC	HQ012541	X	X	X	
244	*Iningainema pulvinus* ES0614	KX620756	X	X	X	X
245	*Streptostemon* sp. clone JUR201	KJ566946	X	X	X	X
246	*Aetokthonos hydrillicola* B3 Florida clone NCp8a	KF934176	X	X	X	X
247	*Aetokthonos hydrillicola* B3 Florida clone NCp8b	KF934177	X	X	X	X
248	*Stigonema turfaceum* CBFS A-031	KM046995	X	X	X	
249	*Stigonema hormoides* WYS4-4	KT867142	X	X	X	X
250	*Scytonema* cf. *crispum* UCFS10	HM629428		X	X	
251	*Scytonema* cf. *crispum* UCFS15	JN565279		X		
252	*Scytonema* cf. *crispum* UCFS16	JN565276		X	X	
253	*Scytonema* cf. *crispum* UCFS17	JN565277		X	X	
254	*Scytonema* cf. *crispum* UCFS21	JN565278		X		
255	*Scytonema bohneri* SAG 255.80	KM019923		X		
256	Uncultured bacterium clone YF924	KF037922			X	
257	*Westiellopsis prolifica* SAG 23.96	AJ544087	X	X	X	X
258	*Westiellopsis* sp. 1590-1	AJ544088				X
259	*Westiellopsis* sp. 1590-2	AJ544089				X
260	*Westiellopsis* sp. 89-785 4	AJ544222				X
261	*Westiellopsis* sp. 985-1	AJ544090				X
262	*Fischerella muscicola* SAG2027	AJ544077	X	X	X	X
263	*Westiellopsis prolifica* str. El Farafra	KX863667				X
264	*Westiellopsis* sp. Ar73	DQ786168				X
265	*Westiellopsis* sp. HPS	KY020124				X
266	*Fischerella muscicola* PCC 73103	AB074505				X
267	*Fischerella muscicola* NDUPC001	JX8768898				X
268	*Fischerella* sp. Ind26	JN197409				X
269	*Hapalosiphon arboreus* 3OW05S02	LT745778	X	X	X	X

270	*Nostochopsis* sp. HA4207-MV1	JN385294				X
271	*Nostochopsis* sp. 07008-00001	KC854779				X
272	*Pelatocladus maniniholoensis* HA4357-MV3	JN385293				X
273	*Hapalosiphon hibernicus* BZ-3-1	EU151900				X
274	*Nostochopsis lobatus* 92.1	AJ544080				X
275	*Hapalosiphon* sp. SJRDC1	KF761556				X
276	*Fischerella* sp. NQAIF323	KJ636970				X
277	*Westiellopsis* sp. SAG 20.93	KM019953				X
278	*Westiellopsis* sp. SAG 19.93	KM019952		X		X
279	*Fischerella* sp. CENA161	EU840724				X
280	*Fischerella ambigua* UTEX 1903	KJ768871				X
281	*Fischerella muscicola* UTEX 1829	AB075984				X
282	*Westiellopsis prolifica* SAG 16.93	AJ544086	X	X	X	X
283	*Westiellopsis prolifica* CR L32	EF545643				X
284	*Hapalosiphon welwitschii* UH IC-52-3	KJ767019	X	X	X	X
285	*Westiella intricata* UH HT-29-1	KJ767016	X	X	X	X
286	*Mastigocladus laminosus* NQAIF328	KJ636971	X	X	X	X
287	*Mastigocladus* sp. CHP1	KX035101				X
288	*Mastigocladus laminosus* Kovacik 1987-7B	EU116034	X			X
289	*Fischerella* sp. RV14	DQ786172				X
290	*Fischerella* sp. MV11	DQ786171				X
291	*Mastigocladus laminosus* CCAP 1447/1	AB039003				X
292	*Fischerella muscicola* PCC 7414	AF132788	X	X	X	X
293	*Mastigocladus laminosus* Greenland 8	DQ431003				X
294	*Mastigocladus laminosus* SAG 4.84	EU116035		X	X	X
295	*Fischerella* sp. CENA19	AY039703				X
296	*Fischerella* sp. CENA298	KP701038				X
297	*Stigonema dinghuense* DHS0072	KJ786940				X
298	*Komarekiella atlantica* HA4396-MV6	KX646832	X	X	X	X
299	*Komarekiella atlantica* CCIBT 3483	KX638487	X	X	X	X
300	*Trichormus variabilis* NIES-2093	AB016520				X
301	*Trichormus variabilis* NIES-23	AP018216				X
302	*Trichormus variabilis* ATCC 29413	CP000117				X
303	*Trichormus variabilis* KCTC AG10064	DQ234827				X
304	*Trichormus* sp. HA7287-LM1	KF417428				X
305	*Halotia branconii* CENA392	KJ843312	X	X	X	X
306	*Halotia longispora* CENA420	KJ843313	X	X	X	X
307	*Halotia wenerae* CENA158	KC695852				X
308	*Chrysosporum ovalisporum* NIVA-CYA 802	LN846957	X	X	X	X
309	*Chrysosporum ovalisporum* APH035D	FJ234885				X
310	*Chrysosporum bergii* 09-02	JQ237772	X	X	X	X
311	*Chrysosporum bergii* ANA360D	FJ234888				X
312	*Cyanocohniella calida* Kastovsky 1996-2	EU116036	X	X	X	X
313	*Cyanocohniella calida* CCALA 1049	KJ737428	X	X	X	X
314	*Cyanospira rippkae*	AY038036	X	X	X	X
315	*Cyanospira capsulata* 9NAT	FR774776	X	X	X	X

316	*Anabaenopsis elenkinii* SAG 252.80	KM020015	X	X	X	X
317	*Anabaenopsis* sp. PCC 9215	AY038033	X	X	X	X
318	*Nodularia spumigena* Huebel 1987-311	AJ781133	X	X	X	X
319	*Nodularia spumigena* PCC 9350	AJ781131				X
320	*Nodularia* sp. Saline2	KC912782				X
321	*Nodularia* sp. Saline3	KC812783				X
322	*Nodularia harveyana* BECID27	AJ81145				X
323	*Nodularia harveyana* SAG 44.85	KC912773				X
324	*Nodularia sphaerocarpa* HBU22	KC912767				X
325	*Nodularia harveyana* PCC 7804	AF268019				X
326	*Nodularia* cf. *harveyana* HBU26	KC912770				X
327	*Nodularia sphaerocarpa* Fae19	AJ781144				X
328	*Nodularia sphaerocarpa* PCC 73104	AJ781139				X
329	*Nodularia harveyana* SOOS3	KC912777	X	X	X	X
330	*Nodularia spumigena* CCALA 115	KC912779				X
331	*Nodularia moravica* CCALA 797	KC912780				X
332	*Nodularia armorica* L1	KC912775				X
333	*Trichormus variabilis* KCTC AG10008	DQ234829				X
334	*Trichormus variabilis* GREIFSWALD	AJ630457	X	X	X	X
335	*Trichormus variabilis* ACSSI 154	KY283054				X
336	*Trichormus variabilis* CCAP 1403/12	KC242850				X
337	*Trichormus variabilis* NQAIF309	KJ636969				X
338	*Trichormus variabilis* HINDAK 2001/4	AJ630456	X	X	X	X
339	*Anabaena* sp. Ms2	HM573452				X
340	*Anabaena* sp. SAG 28.79	KT290328				X
341	*Anabaena* sp. Sai001	GU935344				X
342	*Anabaena* sp. BECID23	EF583859	X	X	X	X
343	*Anabaena oscillarioides* BECID22	AJ630426	X	X	X	X
344	*Anabaena* sp. XP6A	EF568902				X
345	*Anabaena oscillarioides* BECID32	AJ630427				X
346	*Anabaena* sp. BECID20	EF583858				X
347	*Anabaena* sp. 12-Soos10-CI	KT290362				X
348	*Cuspidothrix issatschenkoi* 0tu37s7	AJ630446	X	X	X	X
349	*Cuspidothrix issatschenkoi* PMC210-03	GQ859624	X	X	X	X
350	*Aphanizomenon* sp. PCC 7905	AJ133154	X	X	X	X
351	*Aphanizomenon flos-aquae* 1tu29s19	AJ630441	X	X	X	X
352	*Dolichospermum flos-aquae* PCC 9302	AY038032				X
353	*Dolichospermum mendotae* 04-06	FN691905	X	X	X	X
354	*Dolichospermum planctonicum* NIVA-CYA 659	LN846959	X	X	X	X
355	*Anabaena* sp. BECID8	EF583856				X
356	*Anabaena* sp. BECID31	EF583862				X
357	*Anabaena* sp. XP35A	EF583855				X
358	*Anabaena* sp. XPORK36C	EF568907				X
359	*Anabaena* sp. BECID19	EF583857				X
360	*Anabaena cylindrica* Bio33	KC333872				X
361	*Anabaena* sp. 5-Kutnar09	KT290348				X

362	Cyanobacterium enrichment culture CAWBG85	KC818283				X
363	*Anabaena cylindrica* PCC 7122	CP003659				X
364	*Anabaena cylindrica* UTAD A212	GQ443447				X
365	*Anabaena cylindrica* NIES-19	AF247592				X
366	*Anabaena cylindrica* DC-3	EU780157				X
367	*Anabaena* sp. HBU3	KT290379				X
368	*Anabaena vaginicola* ISC90	JN873351				X
369	*Anabaena* sp. HBU8	KT290375				X
370	*Anabaena augstumalis* SCMIDKE-JAHNKE 4a	AJ630458				X
371	*Anabaena* sp. 15-Soos11-BA	KT290365				X
372	*Anabaena* sp. 4-Vresova10-L1	KT290341				X
373	Cyanobacterium BECID34	AJ781150				X
374	*Hydrocoryne spongiosa* HA4387-MV2	JN385287	X	X	X	X
375	*Hydrocoryne* sp. CENA398	KC346266	X	X	X	X
376	*Hydrocoryne* sp. CENA393	KC346265				X
377	*Hydrocoryne* sp. UFV-ANT31	KC346267				X
378	*Hydrocoryne* sp. UFV-ANT32	KC346268				X
379	*Sphaerospermopsis kisseleviana* NIES-73	AP018314				X
380	*Sphaerospermopsis reniformis* 06-01	FM161348	X	X	X	X
381	*Sphaerospermopsis aphanizomenoides* 04-43	FM161350	X	X	X	X
382	*Sphaerospermopsis torques-reginae* ITEP-024	HQ730086				X
383	*Raphidiopsis curvata* HB1	AY763116	X	X	X	X
384	*Raphidiopsis mediterranea* 07-04	JQ237770	X	X	X	X
385	*Raphidiopsis raciborskii*	AF092504	X	X	X	X
386	*Raphidiopsis raciborskii* Brazil 2	AF516734	X	X	X	X
387	*Anabaena* sp. SSM-00	DQ364237				X
388	*Anabaena* sp. WH School st. Isolate	AB075979				X
389	"*Nostoc azollae*" 0708	CP002059				X
390	*Wollea salina* L38	KT290381				X
391	*Wollea saccata* Hindak 2000/22	KT290378	X	X	X	X
392	*Wollea saccata* Frant4	KX424415	X	X	X	X
393	*Wollea saccata* ACCS 045	GU434226				X
394	*Wollea ambigua* TrebK1-31	KX424668				X
395	*Wollea ambigua* SAG 1403-7	KT290326				X
396	*Anabaena oscillarioides* BO-HINDAK 1984/43	AJ630428				X
397	*Calothrix* cf. *elenkinii* strain T014	from this study	X			
398	*Calothrix* cf. *marchica* strain T064	from this study	X			
399	*Calothrix* cf. *marchica* strain T067	from this study	X			
400	*Calothrix* sp. type 1 strain T030	from this study	X			
401	*Calothrix* sp. type 2 strain T035	from this study	X			
402	*Calothrix* sp. type 3 strain T019	from this study	X			
403	*Calothrix* sp. type 4 strain T029	from this study	X			
404	*Calothrix* sp. type 5 strain T044	from this study	X			
405	*Skacelovkia peruviana* strain T029	from this study	X			
406	*Skacelovkia* sp. strain P3C2e	obtained by E. Berrendero	X			

407	"*Dichothrix*" sp. Seq 1 strains T008, T017, T048	from this study	X			
408	"*Dichothrix*" sp. Seq 2 strains T008, T017, T048	from this study	X			
409	*Brasilonema octagenarum* strain T001	from this study		X		
410	*Brasilonema* sp. Strain T076	from this study		X		
411	*Scytonema* sp. type 2 strain T063	from this study		X		
412	*Scytonema* sp. type 1 strain T051	from this study		X		
413	*Scytonema* sp. type 1 strain T056	from this study		X		
414	*Calochaete* sp. strain T037	from this study			X	
415	*Camptylonemopsis* cf. *pulneyensis* strain T054	from this study			X	X
416	*Hassallia californica* strain T061	from this study			X	
417	*Hassallia californica* strain T081	from this study			X	
418	*Hassallia* sp. strain T075	from this study			X	
419	*Microchaete* sp. type 1, strain T042	from this study			X	
420	*Microchaete* sp. type 1, strain T047	from this study			X	
421	*Microchaete* sp. type 2, strain T060	from this study			X	
422	JOH06	obtained by E. Berrendero			X	
423	JOH42	obtained by E. Berrendero			X	
424	*Tolypothrix* sp. strain T079	from this study			X	
425	*Tolypothrix* cf. *helicophila* strain T002	from this study			X	
426	*Anabaena* sp. type 1, strain T065	from this study				X
427	*Anabaena* sp. type 2, strain T033	from this study				X
428	*Nodularia* sp. strain T049	from this study				X
429	*Trichormus variabilis* strain T020	from this study				X
430	*Trichormus* cf. *indicus* strain T082	from this study				X
431	*Westiellopsis* sp. strain T028	from this study				X