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Honors Capstone Project

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Determination of taxonomic placement of falsely-branched taxa in soils of San Nicolas Island and reassessment of the Tolypothrichaceae.

Abstract:

This study was conducted to determine the taxonomic placement of falsely-branched taxa in the soil crusts of San Nicolas Island, which is the largest of the Channel Islands lying off the coast of California. After microscopic analysis of the strains collected from the island, a phylogenetic analysis of 16S rRNA genes, and an analysis of the 16S-23S ITS region, we have identified that these strains belong to the soil genus *Spirirestis*, which is in the Tolypothrichaceae family. The Tolypothrichaceae is a well-characterized monophyletic lineage of non-attenuated, false-branching heteropolar types containing the genera *Spirirestis, Hassallia, Tolypothrix, Coleodesmium,* and *Rexia*. The strains analyzed specifically belonged to the genus *Spirirestis*, which is characterized by having heterocyte formation, false branching, presence of sheath, and tightly spiraled trichomes. In addition to determining the taxonomic placement of the falsely- branched taxa in the soils of San Nicolas Island, we reassessed the Tolypothichaceae family. Based on our results, there was a clear distinction between the soil and the aquatic clades in the family. However, the family should not be split into two families due to high similarity of the 16S rRNA genes. The Tolypothrichaceae needs revision based on the presence of many polyphyletic genera.

Introduction:

Biological soil crusts consist of water stable aggregates composed of cyanobacteria, eukaryotic algae, fungi, lichens, and mosses. As the name implies, they are a living soil that creates a crust over the landscape. Biological soil crusts can be found worldwide, but they are best developed in desert ecosystems. They play an important role in preventing soil erosion, increasing soil fertility, improving water relations, etc. (Johansen 1993).

The taxonomy of components of crusts have long been of interest. Recently, with the advent of molecular methods, many taxa, particularly cyanobacteria, have been described from biological soil crusts (Boyer et al. 2002). The taxonomy of cyanobacteria is rapidly expanding. In fact, there have been 80 new genera described since 2000.

Scientists have employed molecular techniques to answer questions about cyanobacterial taxonomy. The ribosomal RNA (rRNA) operon, which consists of three rRNA molecules (16S, 23S, and 5S) are separated by internal transcribed spacer (ITS) regions and can serve as a popular target for sequence analysis. Sequence determination of the entire 16S rRNA gene has provided insight into the phylogenetic relationships of genera within the different families. The ITS region is particularly interesting because there are sequences present that encode structural genes for tRNA and intervening sequence (IS) regions that do not encode products incorporated into the ribosomes (Flechtner et al. 2002). Within these latter sequences, the greatest incidence of sequence divergence exists. The ITS regions are designated as 16S-23S ITS (between the small subunit or 16S and the large subunit or 23S) and as the 23S-5S ITS (between the 23S and 5S subunits). The 16S-23S ITS has been used extensively both for phylogeny of species within a single genus and for generating thresholds based on sequence dissimilarity for recognizing species. We have used a polyphasic approach that combines extensive morphological characterization with DNA sequence data from the 16S rRNA gene and the 16S-23S ITS region to determine the taxonomic position of genera within the family Tolypothrichaceae.

In 2021, Dr. Johansen secured a contract from the US Navy to study all algae occurring in biological soil crusts of San Nicolas Island. This work is meant to build on the study by Flechtner et al.

(2008), which described several new algal species, but did not include any molecular characterizations. The Navy wants to use metagenomic approaches to characterize soil crust communities, and needs molecular barcodes for all algae to conduct this work. So far, the lab has characterized 78 strains of cyanobacteria isolated from the soils of San Nicolas Island. Early sequencing was conducted on simple filamentous taxa (Synechococcus and Oscillatoriales) and unbranched heterocystous taxa (Nostocaceae in the Nostocales). The false branching strains were most recently sequenced as part of the present project. However, based upon previous work (Hauer et al. 2014, Johansen, personal communication), it appears that the soil forms of false branching taxa (*Hassallia, Spirirestis, Tolypothrix*) may be phylogenetically separate from the aquatic forms (*Tolypothrix, Rexia, Hassallia, Coleodesmium*). All these taxa are presently in Tolypothrichaceae. However, this group needs revision based upon previously observed polyphyly in most genera. The goal of the present work is to examine the soil forms from San Nicolas Island and determine to which taxa they are most closely related. Then we will examine the combined phylogeny of the soil and aquatic Tolypothrichaceae to see if they form a monophyletic group. A monophyletic group is a group of taxa or strains that all have a common ancestor, and includes all descendents of that ancestor. If the Tolypothricaceae is shown to be monophyletic, then it will be preserved intact. However, if members fall into two different clades that lack a common ancestor but contain other taxa as part of the descendants of that ancestor, it is polyphyletic, and may need to be revised so that the soil forms will comprise a new family in the Nostocales. Resolution of the genera in *Tolypothrix sensu lato* will also be undertaken.

Objectives:

The goal of this study was to classify the falsely-branched taxa on San Nicolas Island as well as reassess the family Tolypothrichaceae and determine if it should be retained as presently understood, or split into two families, one for aquatic forms and one for soil forms. This project had several objectives:

1). Sequencing the 16S rRNA gene and associated ITS region for all species of false-branching cyanobacteria in the soils of San Nicolas Island.

2). Compiling an alignment of the taxa currently assigned to Tolypothrichaceae, including the sequences obtained from San Nicolas Island

3). Completing phylogenetic analyses of the 16S rRNA gene and ITS region for the strains of interest

4). Interpreting these analyses and making taxonomic decisions regarding taxonomic placement of our strains.

Research Methods:

Cultivation and characterization of strains. Eight cyanobacterial strains were used in this study and obtained from San Nicolas Island. The strains were grown on nitrogen-free media to stimulate production of heterocytes. The strains were then observed under a microscope to characterize the morphology. This characterization included measurements of all cell types, characterization of branching types, color of sheath material, polarity of trichomes, tapering of trichomes, end cell morphology, and any other distinctive morphological feature not presently anticipated. All strains were photographed, and photographic plates were assembled.

Molecular analyses. Total genomic DNA was extracted from cultures using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA; analog to the modern day DNeasy UltraClean Microbial Kit, Qiagen, Hilden, Germany) employing vortex cell disruption and following the manufacturer's protocols. A partial 16S rRNA sequence (1162 nucleotides) with associated 16S-23S ITS region was amplified using polymerase chain reaction (PCR). The forward primer used was primer VRF2 5'-GGG-GAA-TTT-TCC-GCA-ATG-GG-3' and reverse primer VRF1 5'-CTC-TGT-GTG-CCT-AGG-TAT-CC-3' (Wilmotte et al. 1993, Nübel et al. 1997, Boyer et al. 2001, 2002). Amplification

reactions were as follows: 50 lL PCR reaction mix containing 19 reaction buffer, 1.5 mM MgCl2, 2.5U of Taq DNA polymerase, 0.2 lM of each primer, 0.2 mM dNTPs (ThermoFisher Scientific, Waltham, MA, USA), and 10 ng of genomic DNA. PCR was run in a Bio-Rad PCR Thermocycler with a 3-min incubation at 94°C to minimize non-specific DNA amplifications. Following PCR, products were cloned with the StrataClone PCR cloning kit (La Jolla, CA, USA) according to manufacturer recommendations. At least two *E. coli* colonies per strain were picked. Plasmid DNA was extracted and purified with QIA-Prep Miniprep Spin kit (Qiagen, Carlsbad, CA, USA). EcoRI digestion was performed to select successful clones. Selected clones were sequenced using Sanger sequencing technology with five internal sequence primers including M13 forward, M13 reverse, primer 5 (5'-TGT-ACA-CAC-CGG-CCC-GTC-3'; Wilmotte et al. 1993), primer 7 (5'-AAT-GGG-ATT-AGA-TAC-CCC-AGT-AGT C-3') and primer 8 (5'-AAG-GAG-GTG-ATC-CAG-CCA-CA-3'; Nübel et al. 1997) by Functional Biosciences, Inc. (Madison, Wi, USA). For each submitted clone, associated raw sequence reads were aligned, error proofed, and assembled to contigs using Chromas. Replicate clones for each strain were sequenced so that sequences could be proofed for PCR error and yield consensus sequences. Assembled clone sequences were submitted to NCBI's GenBank database.

Sequence analysis. To find sequences phylogenetically close to our strains, BLAST searches were conducted for each strain as well as for known aquatic and subaerial strains purportedly in the family Tolypothrichaceae. The related sequences identified on the NCBI GenBank database were then aligned using CLUSTAL-W. Once we had an alignment, we used the SHOWDIST command in PAUP to determine p-distance for both the 16S alignment and the ITS alignment so that percent similarity and percent dissimilarity could be determined, respectively. We then conducted phylogenetic analyses (Bayesian Inference Analysis = BA, and Maximum Likelihood analysis = ML) of the 16S rRNA sequence alignment to obtain phylogenetic trees. For ITS, we conducted Bayesian Inference Analysis coding indels as standard data, and Maximum Parsimony analysis (= MP) with indels counted as a fifth base. Once the trees were made, we determined the relationships among strains and to what family the falsely-branched

taxa belonged. Using the phylogenetic trees made, we provided an assessment of the Tolypothrichaceae family, including which species could be recognized as belonging to the family. Assessment of the genera was also conducted.

Results:

The phylogenetic analyses based on 16S rRNA sequence (Fig. 1) both showed clear separation of the desert soil clade Tolypothrichaceae (top polygon, red circle) and the aquatic/wet subaerial clades of Tolypothrichaceae (second polygon, blue and yellow circles). The strains from desert soils, including those from San Nicolas Island, were mostly separated from the clades with aquatic forms. The family Tolypothrichaceae was monophyletic with high support. A few soil forms fell out of the Tolypothrichaceae clade (*Roholtiella* sp., *Halotia wernerae, Halotia longispora,* and *Calothrix* sp.), and so likely belong to a different family along with strains from aquatic and subaerial habitats. *Godleya,* also from soils, was clearly distinguished by a long branch, but is still in the Tolypothrichaceae. Most genera in the phylogeny showed polyphyly. This is evidence that this family needs revision.

The ecological signature of the expanded soil clade is very strong because all of the strains were from desert crusts (Fig. 2). Despite all of these strains belonging to desert soils, this group was also polyphyletic as it was a mix of *Tolypothrix* sp., *Hassallia* sp., *Spirirestis* sp., and unidentified Microchaetaceae cyanobacteria (the former family containing members of Tolypothrichaceae). However, all strains in this clade had percent similarity of 16S rRNA sequence ≥ 97.0, well above the 94.5% threshold accepted for recognizing different genera. Thus, the desert forms all belong to the same genus. *Hassallia* and *Tolypothrix* are found in wet subaerial and aquatic habitats, respectively, so should not be used for this clade of terrestrial forms. *Spirirestis* was described from desert soils, and its reference strain, *S. rafaelensis* SRS70, is in this clade. Consequently, it is the genus epithet that should be used for all members of this clade in order to have a monophyletic taxon. Only two strains in the clade have the

characteristic spiral coiling for which this genus was named, SRS70 and WJT71-NPBG6, so this group of Tolypothricoid species (*Spirirestis)* should be defined by ecology rather than by morphology.

The aquatic clade, when expanded, contains a mix of *Dactylothamnos, Kryptousia, Tolypothrix,* and Tolypothrichaceae cyanobacteria (Fig. 3). In order for this clade to be monophyletic, all these taxa would have to be either placed in *Tolypotrhix,* or a new genus would be needed for the clade containing Tolypothrichaceae cyanobacterium, *Kryptousia macronema, Tolypothrix* HanysB and *Tolypothrix* preslic8. In either scenario, it appears that *Dactylothamnos antarcticus* should be combined into *Tolypothrix* as *Tolypothrix distorta* is the type species of the genus and its reference strain is *T. distorta* ACOI 731. While the family is well-defined, the genera are problematic. As a result, this group requires a great deal of revision. Below this clade are a number of paraphyletic strains, including *Hassallia, Tolypothrix, Coleodesmium*. *Rexia, Godleya,* and *Toxopsis* do not require revision based on our analysis, but the other three aquatic and subaerial taxa require redefinition.

Molecular definition of cyanobacterial species has recently been achieved using a combination of phylogenetic analyses of aligned ITS regions as well as percent dissimilarity of ITS sequences. Dissimilarities below about 3.0% can be taken as evidence that strains are in the same species, while percent dissimilarities above 7% can be taken as strong evidence that strains are in different species. Dissimilarities between 3 and 7% are ambiguous, although recently values above 4 have been used to distinguish species with different ecologies or morphology as supporting evidence. For example, in this study, the three strains from San Nicolas Island highlighted in light orange are considered to be the same species (Table 1). Likewise, the pairs of strains highlighted in green and blue are considered to be two species, respectively. The eight strains highlighted in darker orange, which contain five strains from San Nicolas Island represent another species and the largest group of strains highlighted in yellow represent *Spirirestis rafaelensis,* as that set contains the reference strain for that species. The five unhighlighted strains in the soil clade that are not represented by multiple strains, and each represents a species distinct from the rest. Consequently, there are nine species represented in our phylogeny, including the named

type of *Spirirestis*, and nine *Spirirestis* needing to be described, which include four new species from San Nicolas Island. Phylogenetic analyses based on the 16S-23S ITS region showed evolutionary relationships among species in the genus (Fig. 5). There was agreement between this phylogeny with the percent dissimilarity of ITS sequences. For example, *Spirirestis* sp. SNI-TA17-ML2 and *Spirirestis* sp. SNI-TA31-BJ5 formed a supported clade. *Hassallia* sp. ATA2-3-CV2 and *Tolypothrix* sp. ATA2-1-CV also formed a supported clade.

Discussion:

Determination of taxonomic placement of falsely-branched taxa in soils of San Nicolas Island: Our results showed that the stains from San Nicolas Island belonged to the genus *Spirirestis,* which is part of the family Tolypothrichaceae. After a phylogenetic tree analysis was performed, all of the San Nicolas Island strains belonged in the soil clade. Based on the 16S rRNA phylogeny and ITS dissimilarity matrix, the soil clade should all be a single genus. In terms of naming this clade, *Spirirestis* has priority. *Tolypothrix* and *Hassalia* were described a long time ago but are aquatic and wet subaerial. *Tolypothrix* is typically aquatic and *Hassalia* is typically subaerial. Thus, we can conclude that the strains are from the genus *Spirirestis* as they are from the desert soils.

Reassessment of the Tolypothrichaceae family: The phylogenetic analysis performed in our study supported that there was a clear separation between the soil and aquatic forms. However, there was a mix of *Hassalia*, *Tolypothrix*, *Dactylothamnos*, *Kryptousia* and *Coleodesmium* in the tree, which leads to the conclusion that these genera were not monophyletic. All of these genera are in the aquatic clade. When they appear in the soil clade, they are misidentified, and they will need to be transferred to *Spirirestis*. Even after excluding the soil representatives in these incorrect genera, it is apparent that the aquatic and wet subaerial taxa need revision as well. The reference strain for *Tolypothrix distorta*, the type species of that genus, is in the *Dactylothamnos* clade, and consequently, *Dactylothamnos* is a later synonym of *Tolypothrix* and should be subsumed into that genus. Other *Tolypothrix* are confused with

Kryptousia, and *Kryptousia* itself is not monophyletic. This can solved in one of two ways. The *Tolypothrix* species confused with *Kryptousia* could be transferred into that genus, thus very narrowly defining the genus *Tolypothrix*. The more likely solution is that one or both species of *Kryptousia* will be transferred into *Tolypothrix*.

Conclusion: The phylogenetic analysis performed in our study supports that the falsely-branched taxa on San Nicolas Island can be classified as *Spirirestis*. The phylogenetic tree also depicts that there is a clear separation between the soil and the aquatic clade. However, the family Tolypothrichaceae cannot be split into two families—one for the aquatic forms and one for the soil forms. While nine species need to be represented in *Spirirestis*, the other members of the Tolypothrichaceae family clade need revision. The percent similarity of the 16S sequences for all strains in the family are very high and indicate that the Tolypothrichaceae should remain a single family.

Acknowledgements:

I thank the researchers which sequenced the strains used in this study, especially Brian Jusko. I would also like to thank John Carroll University's Biology Department and the US Navy for research funding.

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Table 1. Percent dissimilarity among ITS regions of Tolypothricaceae. Part 1A. Pairwise comparisons among strains from desert soils (all belong in *Spirirestis* based on this metric). Strains in same color highlight belong to the same species based on low (generally less than 3.5%) dissimilarity. The type species of *Spirirestis* (*Spirirestis rafaelensis*) is highlighted in yellow. Aquatic forms (blue highlight) are shown for comparison.

	Hassallia byssoidea KZ-7-1-5 MK211232	*Spirirestis* sp. KH22-PS OK083626	*Tolypothrix* JOH20C KY563664	*Spirirestis* sp. SNI-TA17-ML2 clone 1	*Spirirestis* sp. SNI-TA17-ML2 clone 2	*Spirirestis* sp. SNI-TA31-BJ5 operon 1	*Spirirestis* sp. SNI-TA23-BJ7 clone 1	*Spirirestis* sp. SNI-TA23-BJ7 clone 2	*Hassallia* sp. ATA2-3-CV2 KF934148	*Tolypothrix* sp. ATA2-1-CV KY411158	*Spirirestis* sp. SNI-TA17-BJ34 cons	*Spirirestis* sp. SNI-TA31-BJ5 operon 2	*Spirirestis* sp. SNI-TA1-JJ1 cons	*Spirirestis* sp. SNI-TA34-BJ1 clone 1
Hassallia byssoidea KZ-7-1-5 MK211232														
Spirirestis sp. KH22-PS OK083626	12.7													
Tolypothrix sp. JOH20C KY563664	11.8	6.7												
Spirirestis sp. SNI-TA17-ML2 clone 1	13.3	7.1	5.0											
Spirirestis sp. SNI-TA17-ML2 clone 2	13.1	7.5	5.4	1.5										
Spirirestis sp. SNI-TA31-BJ5 operon 1	12.7	6.9	5.2	2.3	1.5									
Spirirestis sp. SNI-TA23-BJ7 clone 1	10.5	10.4	11.2	9.9	10.2	9.7								
Spirirestis sp. SNI-TA23-BJ7 clone 2	10.5	10.4	11.2	9.9	10.2	9.7	0.4							
Hassallia sp. ATA2-3-CV2 KF934148	9.7	9.8	10.2	9.3	10.1	9.9	3.6	4.0						
Tolypothrix sp. ATA2-1-CV KY411158	10.3	9.6	10.6	9.5	10.3	9.7	3.8	4.2	2.2					
Spirirestis sp. SNI-TA17-BJ34 cons	11.5	12.3	12.0	10.1	10.0	9.0	5.2	5.2	4.6	4.2				
Spirirestis sp. SNI-TA31-BJ5 operon 2	11.7	11.5	12.0	9.3	9.2	8.1	4.8	4.8	4.6	3.8	1.0			
Spirirestis sp. SNI-TA1-JJ1 cons	10.9	11.6	11.4	9.8	9.8	9.2	4.2	4.2	4.0	4.0	2.2	2.0		
Spirirestis sp. SNI-TA34-BJ1 clone 1	10.7	11.3	11.7	10.1	10.1	9.4	5.0	5.0	3.8	3.2	2.2	1.8	2.6	
Tolypothrix distorta Mon65 MK478704	10.1	11.1	10.8	9.3	9.2	9.0	3.4	3.4	3.0	3.4	2.8	2.8	2.2	2.2
Tolypothrix distorta Mon65 MK478703	10.1	11.1	10.8	9.3	9.2	9.0	3.4	3.4	3.0	3.4	2.8	2.8	2.2	2.2
Tolypothrix distorta LSB87 MW403965	7.8	8.0	8.8	5.5	5.9	6.1	2.9	2.9	2.7	2.7	2.9	2.3	2.5	2.7
Spirirestis sp. SNI-TA17-BJ30 clone 2	8.4	8.3	8.8	5.1	6.3	6.5	3.2	3.1	2.9	2.9	3.1	2.5	2.7	2.9
Spirirestis sp. SNI-TA2-AZ3 cons	8.7	9.7	9.9	9.5	10.1	9.5	4.7	4.7	3.6	4.2	5.0	4.8	4.8	4.4
Hassallia sp. CM1-HA09 JQ083649	7.9	9.0	8.6	9.3	9.9	9.6	5.2	5.2	4.3	4.5	5.1	5.1	5.3	5.1
Tolypothrix tenuis f. *terrestris* UFS-BI-NPMV-1A2-F06 JQ083651	7.7	8.8	8.4	9.1	9.6	9.4	5.0	4.9	4.1	4.3	4.9	4.9	5.1	4.9
Hassallia sp. CM1-HA08 JQ083648	7.7	8.8	8.4	9.1	9.6	9.4	5.0	4.9	4.1	4.3	4.9	4.9	5.1	4.9
Hassallia sp. CM1-HA11 JQ083650	8.2	9.2	8.8	9.1	10.0	9.8	5.4	5.4	4.5	4.7	5.3	5.3	5.5	5.3
Tolypothrix tenuis f. *terrestris* UFS-BI-NPMV-1A2-F06 JQ083652	8.1	8.9	8.8	8.8	9.4	9.2	4.9	4.9	4.3	4.1	4.9	4.9	5.3	4.9
Microchaetaceae cyanobacterium CMT-3SWIN-NPC18 KF934145	10.1	10.5	10.5	11.5	12.1	11.9	7.1	7.1	6.5	6.7	7.3	7.2	7.5	7.2
Hassallia sp. EM2-HA1 HQ847555	10.1	10.5	10.5	11.3	11.9	11.5	6.8	6.8	6.3	6.5	7.0	7.0	7.3	7.0
Microchaetaceae cyanobacterium CMT-3SWIN-NPC18 KF934144	10.1	10.5	10.5	11.5	12.1	11.9	7.1	7.0	6.5	6.7	7.3	7.2	7.5	7.2
Microchaetaceae cyanobacterium WJT2-NPBG8 KF934147	9.7	10.1	10.1	11.1	11.7	11.5	6.7	6.7	6.1	6.3	6.8	6.8	7.1	6.8
Microchaetaceae cyanobacterium CMT-1BRIN-NPC34 KF934135	9.9	10.3	10.3	11.3	11.9	11.7	6.9	6.8	6.3	6.5	7.0	7.0	7.3	7.1
Tolypothrix campylonemoides FI5-MK38 JQ083654	8.6	9.4	9.3	9.0	9.2	9.1	5.7	5.7	5.3	5.3	5.5	5.5	6.1	5.9
Tolypothrix campylonemoides FI5-MK38 JQ083653	9.1	9.9	9.8	9.4	9.6	9.6	6.2	6.2	5.7	5.7	5.9	5.9	6.6	6.3
Hassallia sp. CNP3-B3-C04 HQ847556	8.6	8.8	9.5	8.9	9.5	9.3	6.0	6.0	5.9	5.7	6.3	5.9	6.5	6.7
Spirirestis rafaelensis WJT71-NPBG6 JQ083656	9.3	10.8	11.0	10.2	11.1	10.7	6.6	6.6	6.3	6.1	6.2	6.2	7.3	6.6
Spirirestis rafaelensis WJT71-NPBG6 JQ083655	9.3	10.8	11.0	10.2	11.1	10.7	6.6	6.6	6.3	6.1	6.2	6.2	7.3	6.6
Microchaetaceae cyanobacterium CMT-2BRIN-HLNPC9 KF934143	9.8	10.7	10.4	10.1	10.6	10.2	6.1	6.1	5.5	5.5	5.3	5.3	5.9	5.7
Tolypothrix distorta var. symplocoides UTEX-B-424 KY488019	13.4	12.7	13.2	12.5	13.0	13.0	8.9	8.9	6.5	7.6	8.4	8.5	8.6	8.6
Dactylothamnos antarcticus CENA412 MN626663	14.2	16.3	15.9	16.6	16.9	16.9	13.6	14.0	14.5	14.1	15.2	15.0	14.9	15.0
Dactylothamnos antarcticus CENA433 MN626664	14.2	16.3	15.9	16.6	16.9	16.9	13.6	14.0	14.5	14.1	15.2	15.0	14.9	15.0
Dactylothamnos antarcticus CENA410 KM199732	17.4	19.8	20.0	20.2	20.2	20.1	16.7	17.2	17.9	17.4	18.4	18.4	18.7	18.2
Tolypothrichaceae cyanobacterium BACA0441 OL847351	14.9	17.4	16.6	17.3	17.6	17.6	14.1	14.5	14.8	14.6	15.9	15.7	15.5	15.7
Tolypothrichaceae cyanobacterium BACA0066 MT176722	14.9	17.4	16.6	17.3	17.6	17.6	14.1	14.5	14.8	14.6	15.9	15.7	15.5	15.7
Tolypothrichaceae cyanobacterium BACA0098 OL847350	14.2	17.0	16.0	16.6	17.0	17.0	13.4	13.8	14.1	13.9	15.2	15.0	14.8	15.0
Tolypothrix fasciculata ACOI3104 HG970653	14.8	17.4	16.5	17.8	17.5	17.7	14.8	14.8	15.5	15.0	16.0	15.9	15.8	15.8
Tolypothrix tenuis CCALA197 HG970655	14.6	16.7	16.5	17.4	17.7	17.3	14.5	14.9	16.1	15.5	16.4	16.0	15.8	16.4
Tolypothrix sp. HA4964-CV2 KU161666	15.7	14.0	16.2	15.6	15.8	16.2	15.6	16.1	16.4	16.0	16.8	16.9	16.5	16.7
Tolypothrichaceae cyanobacterium BACA0722 OM732263	16.7	18.0	19.4	20.1	20.2	19.8	17.8	18.2	18.2	17.8	18.7	18.9	18.7	18.1
Tolypothrix sp. CNP3-B1-C1 JQ083657	13.0	12.5	15.1	14.9	14.9	14.7	16.0	16.0	15.8	15.4	16.5	16.3	17.2	16.0
Tolypothrix sp. CNP3-B1-C1 JQ083658	12.6	12.3	14.8	14.7	14.6	14.5	15.8	15.8	15.6	15.2	16.3	16.1	17.0	15.8
Coleodesmium sp. HINDAK 2000/24 HE797727	9.7	17.3	17.1	18.5	18.6	18.5	15.8	15.8	14.9	14.6	16.0	16.4	16.2	15.6
Tolypothrix sp. HanysB LM992903	15.8	18.4	19.3	20.1	19.8	19.7	16.1	16.6	17.4	17.4	17.5	17.8	17.5	17.8
Tolypothrix sp. HA4266-MV1 JN385291	15.6	19.1	19.4	21.0	20.7	20.7	17.2	17.6	17.6	18.0	18.3	18.7	18.6	18.5

Table 1 (Continued). Part 1B.

	Tolypothrix distorta Mon65 MK478704	*Tolypothrix distorta* Mon65 MK478703	*Tolypothrix distorta* LSB87 MW403965	*Spirirestis* sp. SNI-TA17-BJ30 clone 2	*Spirirestis* sp. SNI-TA2-AZ3 cons	*Hassallia* sp. CM1-HA09 JQ083649	*Tolypothrix tenuis* f. *terrestris* UFS-BI-NPMV-1A2-F06 JQ083651	*Hassallia* sp. CM1-HA08 JQ083648	*Hassallia* sp. CM1-HA11 JQ083650	*Tolypothrix tenuis* f. *terrestris* UFS-BI-NPMV-1A2-F06 JQ083652	Microchaetaceae cyanobacterium CMT-3SWIN-NPC18 KF934145	*Hassallia* sp. EM2-HA1 HQ847555	Microchaetaceae cyanobacterium CMT-3SWIN-NPC18 KF934144	Microchaetaceae cyanobacterium WJT2-NPBG8 KF934147	Microchaetaceae cyanobacterium CMT-1BRIN-NPC34 KF934135
Tolypothrix distorta Mon65 MK478703	0.0														
Tolypothrix distorta LSB87 MW403965	2.3	2.3													
Spirirestis sp. SNI-TA17-BJ30 clone 2	2.1	2.1	1.0												
Spirirestis sp. SNI-TA2-AZ3 cons	4.2	4.2	3.4	3.6											
Hassallia sp. CM1-HA09 JQ083649	4.3	4.3	4.0	4.2	3.7										
Tolypothrix tenuis f. *terrestris* UFS-BI-NPMV-1A2-F06 JQ083651	4.1	4.1	3.8	4.0	3.5	0.2									
Hassallia sp. CM1-HA08 JQ083648	4.1	4.1	3.8	4.0	3.5	0.2	0.0								
Hassallia sp. CM1-HA11 JQ083650	4.5	4.5	4.2	4.4	3.9	0.6	0.4	0.4							
Tolypothrix tenuis f. *terrestris* UFS-BI-NPMV-1A2-F06 JQ083652	4.2	4.2	3.9	3.9	3.6	0.4	0.2	0.2	0.6						
Microchaetaceae cyanobacterium CMT-3SWIN-NPC18 KF934145	6.4	6.4	5.4	5.6	5.9	1.6	1.4	1.4	1.8	1.7					
Hassallia sp. EM2-HA1 HQ847555	6.2	6.2	5.2	5.4	5.7	1.6	1.4	1.4	1.8	1.5	0.8				
Microchaetaceae cyanobacterium CMT-3SWIN-NPC18 KF934144	6.4	6.4	5.4	5.6	5.9	1.6	1.4	1.4	1.8	1.7	0.8	0.8			
Microchaetaceae cyanobacterium WJT2-NPBG8 KF934147	6.0	6.0	5.0	5.2	5.5	1.2	1.0	1.0	1.4	1.3	0.4	0.4	0.4		
Microchaetaceae cyanobacterium CMT-1BRIN-NPC34 KF934135	6.2	6.2	5.2	5.4	5.7	1.4	1.2	1.2	1.6	1.5	0.6	0.6	0.6	0.2	
Tolypothrix campylonemoides FI5-MK38 JQ083654	4.6	4.6	4.3	4.1	4.2	1.5	1.3	1.3	1.7	1.3	1.9	1.7	1.9	1.5	1.7
Tolypothrix campylonemoides FI5-MK38 JQ083653	5.1	5.1	4.8	4.6	4.7	1.9	1.7	1.7	2.1	1.8	2.3	2.1	2.3	1.9	2.1
Hassallia sp. CNP3-B3-C04 HQ847556	5.5	5.5	4.6	4.4	5.1	2.2	2.0	2.0	2.4	2.1	2.0	1.8	2.0	1.6	1.8
Spirirestis rafaelensis WJT71-NPBG6 JQ083656	5.4	5.4	5.2	4.6	5.0	2.7	2.4	2.4	2.9	2.3	3.8	3.6	3.8	3.4	3.6
Spirirestis rafaelensis WJT71-NPBG6 JQ083655	5.4	5.4	5.2	4.6	5.0	2.7	2.4	2.4	2.9	2.3	3.8	3.6	3.8	3.4	3.6
Microchaetaceae cyanobacterium CMT-2BRIN-HLNPC9 KF934143	4.1	4.1	4.2	4.0	4.5	2.9	2.7	2.7	3.1	2.8	4.7	4.4	4.7	4.2	4.4
Tolypothrix distorta var. symplocoides UTEX-B-424 KY488019	7.7	7.7	6.1	6.3	7.0	5.9	5.7	5.7	5.7	5.5	7.6	7.4	7.6	7.4	7.6
Dactylothamnos antarcticus CENA412 MN626663	14.3	14.3	12.4	12.6	13.8	11.6	11.4	11.4	11.8	10.7	12.4	12.2	12.4	12.0	12.2
Dactylothamnos antarcticus CENA433 MN626664	14.2	14.2	12.3	12.6	13.8	11.6	11.4	11.4	11.8	10.7	12.4	12.2	12.4	12.0	12.2
Dactylothamnos antarcticus CENA410 KM199732	17.7	17.7	15.9	15.9	17.2	15.3	15.0	15.0	15.5	15.2	16.4	16.6	16.9	16.4	16.7
Tolypothrichaceae cyanobacterium BACA0441 OL847351	14.7	14.7	12.6	13.0	14.0	12.0	11.8	11.8	12.2	11.1	12.2	12.1	12.3	11.8	12.1
Tolypothrichaceae cyanobacterium BACA0066 MT176722	14.7	14.7	12.6	13.0	14.0	12.0	11.8	11.8	12.2	11.1	12.2	12.1	12.3	11.8	12.1
Tolypothrichaceae cyanobacterium BACA0098 OL847350	14.0	14.0	11.8	12.2	13.3	11.2	11.0	11.0	11.4	11.1	11.5	11.3	11.5	11.1	11.3
Tolypothrix fasciculata ACOI3104 HG970653	14.8	14.8	13.3	14.0	14.5	12.5	12.3	12.3	12.7	11.7	13.4	13.2	13.4	13.0	13.2
Tolypothrix tenuis CCALA197 HG970655	15.4	15.4	13.8	14.0	15.1	12.7	12.5	12.5	12.9	12.1	13.4	13.2	13.4	13.0	13.2
Tolypothrix sp. HA4964-CV2 KU161666	15.2	15.2	13.7	14.0	16.1	14.3	14.1	14.1	14.5	13.3	15.2	15.0	15.2	14.8	15.0
Tolypothrichaceae cyanobacterium BACA0722 OM732263	17.3	17.3	15.8	15.8	17.7	15.4	15.2	15.2	15.6	14.6	15.5	15.7	15.9	15.5	15.7
Tolypothrix sp. CNP3-B1-C1 JQ083657	15.5	15.5	14.7	15.1	15.2	14.0	13.8	13.8	14.2	13.9	14.8	14.6	14.8	14.4	14.6
Tolypothrix sp. CNP3-B1-C1 JQ083658	15.3	15.3	14.5	14.9	15.0	13.8	13.6	13.6	14.0	13.7	14.6	14.4	14.6	14.2	14.4
Coleodesmium sp. HINDAK 2000/24 HE797727	14.6	14.6	12.9	13.4	14.3	12.9	12.7	12.7	12.7	12.5	14.3	14.1	14.3	13.9	14.1
Tolypothrix sp. HanysB LM992903	16.6	16.6	16.4	16.7	16.8	14.9	14.7	14.7	15.1	14.3	15.2	15.4	15.4	15.0	15.2
Tolypothrix sp. HA4266-MV1 JN385291	17.5	17.5	17.3	17.5	17.0	15.8	15.6	15.6	16.0	15.2	16.3	16.3	16.3	15.9	16.1

Table 1 (continued). Part 1C. *Tolypothrix sensu stricto*, *Coleodesmium sensu stricto*, and *Dactylothamnos sensu stricto* are all in the aquatic clade (blue highlight).

	Tolypothrix campylonemoides FI5-MK38 JQ083654	*Tolypothrix campylonemoides* FI5-MK38 JQ083653	*Hassallia* sp. CNP3-B3-C04 HQ847556	*Spirirestis rafaelensis* WJT71-NPBG6 JQ083656	*Spirirestis rafaelensis* WJT71-NPBG6 JQ083655	Microchaetaceae cyanobacterium CMT-2BRIN-HLNPC9 KF934143	*Tolypothrix distorta* var. *symplocoides* UTEX-B-424 KY488019	*Dactylothamnos antarcticus* CENA412 MN626663	*Dactylothamnos antarcticus* CENA433 MN626664	*Dactylothamnos antarcticus* CENA410 KM199732
Tolypothrix campylonemoides FI5-MK38 JQ083653	0.4									
Hassallia sp. CNP3-B3-C04 HQ847556	1.5	1.9								
Spirirestis rafaelensis WJT71-NPBG6 JQ083656	1.5	1.9	2.0							
Spirirestis rafaelensis WJT71-NPBG6 JQ083655	1.5	1.9	2.0	0.0						
Microchaetaceae cyanobacterium CMT-2BRIN-HLNPC9 KF934143	2.5	3.0	2.7	2.4	2.4					
Tolypothrix distorta var. symplocoides UTEX-B-424 KY488019	6.3	6.3	6.9	7.6	7.6	7.3				
Dactylothamnos antarcticus CENA412 MN626663	11.7	12.1	11.2	11.6	11.6	12.6	16.0			
Dactylothamnos antarcticus CENA433 MN626664	11.6	12.1	11.2	11.6	11.6	12.6	16.0	0.2		
Dactylothamnos antarcticus CENA410 KM199732	15.6	15.8	15.2	15.7	15.7	15.8	19.8	6.7	6.7	
Tolypothrichaceae cyanobacterium BACA0441 OL847351	12.3	12.7	11.8	12.5	12.5	13.2	15.9	3.4	3.6	9.0
Tolypothrichaceae cyanobacterium BACA0066 MT176722	12.3	12.7	11.8	12.5	12.5	13.2	15.9	3.4	3.6	9.0
Tolypothrichaceae cyanobacterium BACA0098 OL847350	11.5	12.0	11.0	11.8	11.8	12.5	15.2	3.3	3.5	9.0
Tolypothrix fasciculata ACOI3104 HG970653	12.7	13.1	12.6	13.2	13.2	13.5	16.6	4.9	5.1	11.4
Tolypothrix tenuis CCALA197 HG970655	12.9	13.3	12.6	12.8	12.8	13.7	17.0	3.9	4.1	9.8
Tolypothrix sp. HA4964-CV2 KU161666	12.9	13.3	14.2	14.6	14.6	15.1	18.4	14.1	13.9	17.8
Tolypothrichaceae cyanobacterium BACA0722 OM732263	13.8	14.2	15.4	15.8	15.8	16.5	20.1	14.2	14.2	17.1
Tolypothrix sp. CNP3-B1-C1 JQ083657	12.5	13.0	14.7	14.6	14.6	14.4	17.5	16.8	16.8	19.2
Tolypothrix sp. CNP3-B1-C1 JQ083658	12.3	12.8	14.5	14.4	14.4	14.2	17.3	16.4	16.4	19.0
Coleodesmium sp. HINDAK 2000/24 HE797727	11.8	12.2	13.3	13.5	13.5	14.4	18.0	16.7	16.7	20.2
Tolypothrix sp. HanysB LM992903	14.2	14.7	15.2	14.4	14.4	15.0	18.8	8.3	8.1	10.8
Tolypothrix sp. HA4266-MV1 JN385291	15.1	15.5	16.2	15.2	15.2	16.0	19.4	8.8	8.6	12.6

Table 1 (continued). Part 1D. *Tolypothrix sensu stricto, Coleodesmium sensu stricto*, and *Dactylothamnos sensu stricto* are all in the aquatic clade (blue highlight).

	Tolypothrichaceae cyanobacterium BACA0441 OL847351	Tolypothrichaceae cyanobacterium BACA0066 MT176722	Tolypothrichaceae cyanobacterium BACA0098 OL847350	*Tolypothrix fasciculata* ACOI3104 HG970653	*Tolypothrix tenuis* CCALA197 HG970655	*Tolypothrix* sp. HA4964-CV2 KU161666	Tolypothrichaceae cyanobacterium BACA0722 OM732263	*Tolypothrix* sp. CNP3-B1-C1 JQ083657	*Tolypothrix* sp. CNP3-B1-C1 JQ083658	*Coleodesmium* sp. HINDAK 2000/24 HE797727	*Tolypothrix* sp. HanysB LM992903
Tolypothrichaceae cyanobacterium BACA0066 MT176722	0.0										
Tolypothrichaceae cyanobacterium BACA0098 OL847350	0.0	0.0									
Tolypothrix fasciculata ACOI3104 HG970653	4.7	4.7	4.5								
Tolypothrix tenuis CCALA197 HG970655	5.9	5.9	5.9	3.9							
Tolypothrix sp. HA4964-CV2 KU161666	15.1	15.1	15.5	15.1	15.1						
Tolypothrichaceae cyanobacterium BACA0722 OM732263	14.8	14.8	15.2	14.1	14.5	8.3					
Tolypothrix sp. CNP3-B1-C1 JQ083657	17.8	17.8	17.6	17.7	17.5	15.8	18.0				
Tolypothrix sp. CNP3-B1-C1 JQ083658	17.4	17.4	17.2	17.3	17.1	15.8	18.0	0.6			
Coleodesmium sp. HINDAK 2000/24 HE797727	16.8	16.8	16.2	15.9	16.9	18.6	20.3	15.1	14.7		
Tolypothrix sp. HanysB LM992903	9.1	9.1	9.1	9.9	9.9	14.7	13.7	18.8	18.4	15.6	
Tolypothrix sp. HA4266-MV1 JN385291	9.6	9.6	9.6	10.3	10.1	14.5	13.7	18.8	18.4	16.7	4.4

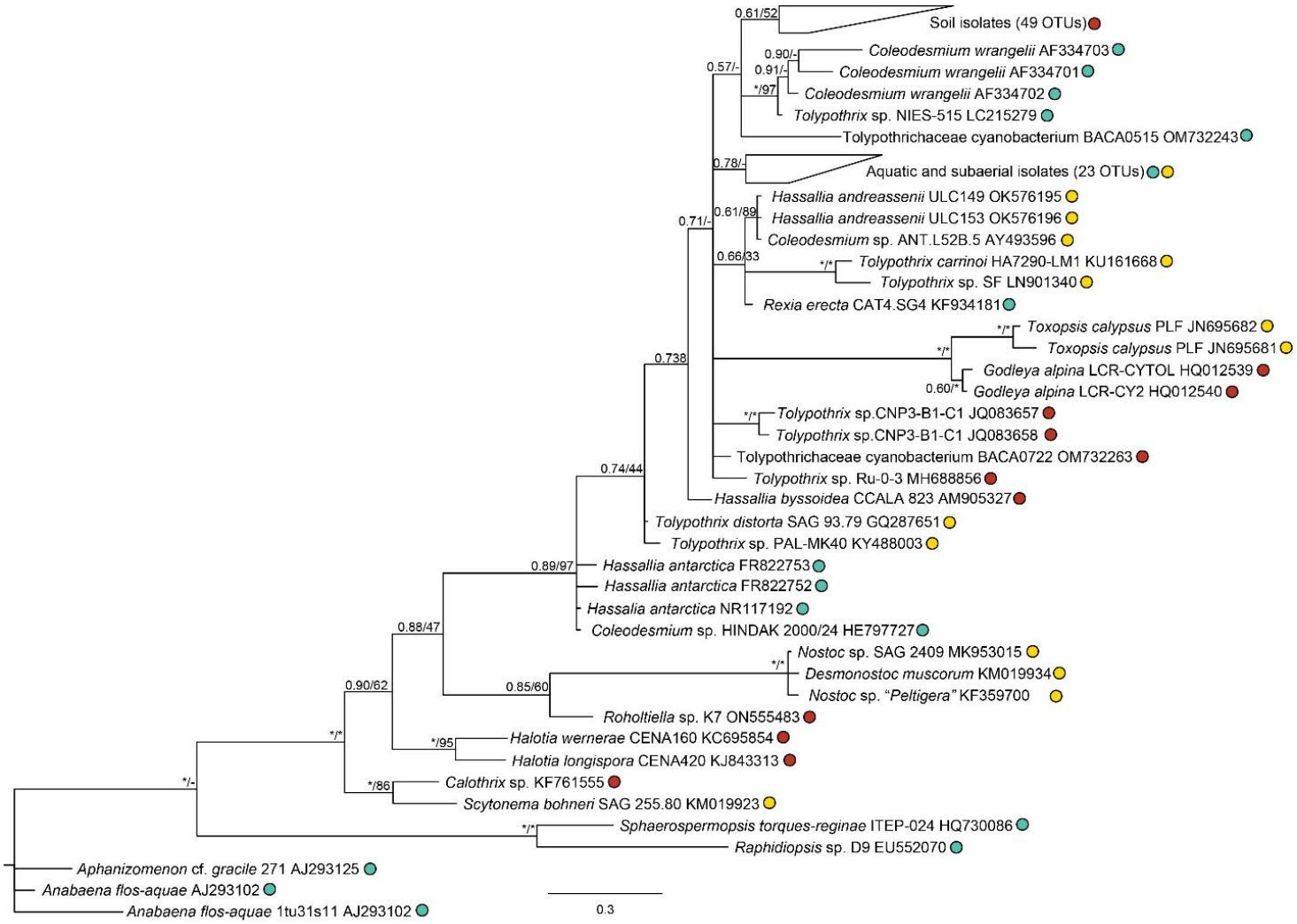

Figure 1. Bayesian Inference analysis (BA) with posterior probabilities at the nodes, and bootstrap support values from the Maximum Likelihood analysis mapped to the nodes after posterior probabilities. Strains from soils have red dots, strains from aquatic habitats have blue dots, and strains from wet subaerial habitats have yellow dots.

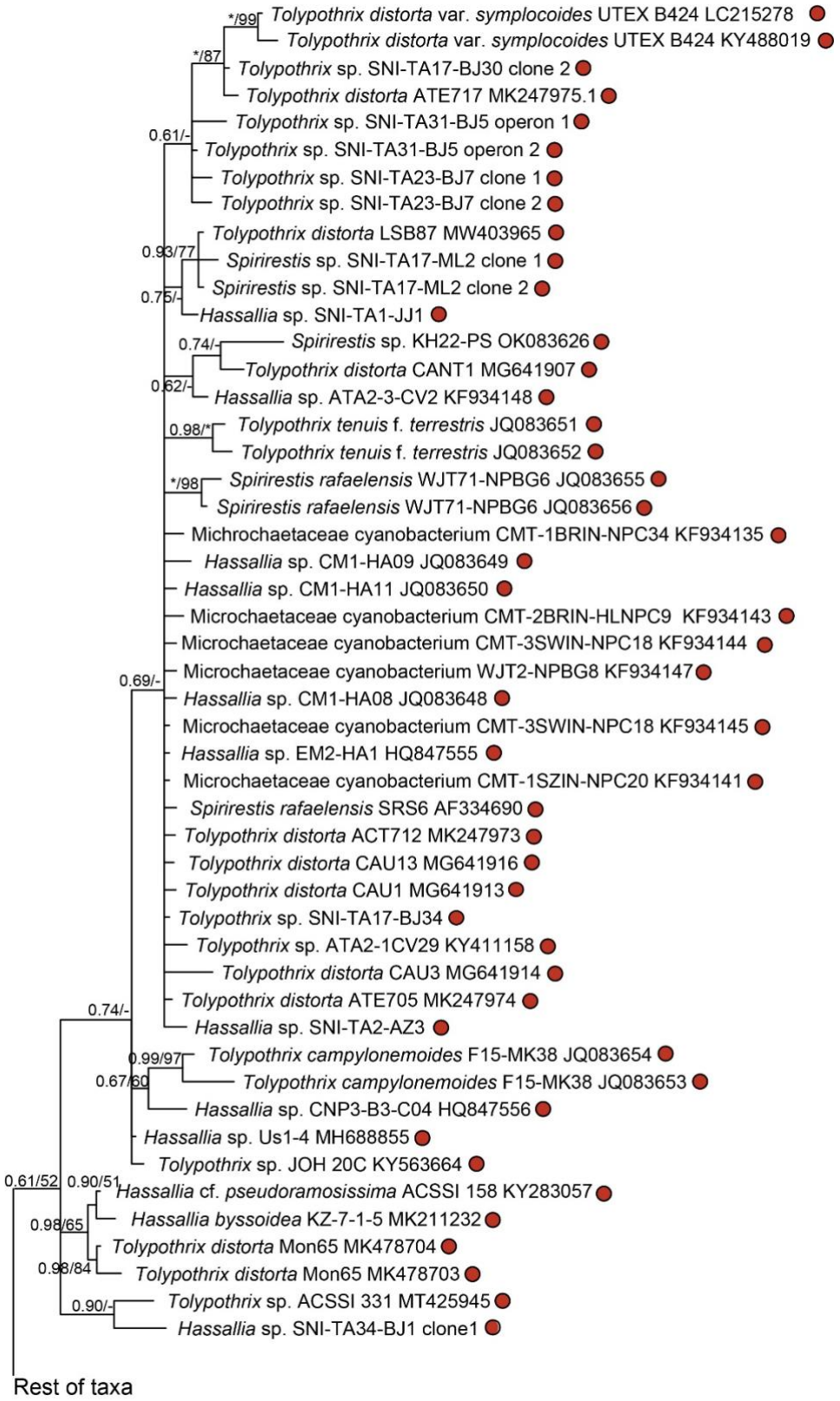

Figure 2. Expanded node from Fig. 1 showing strains from desert soils.

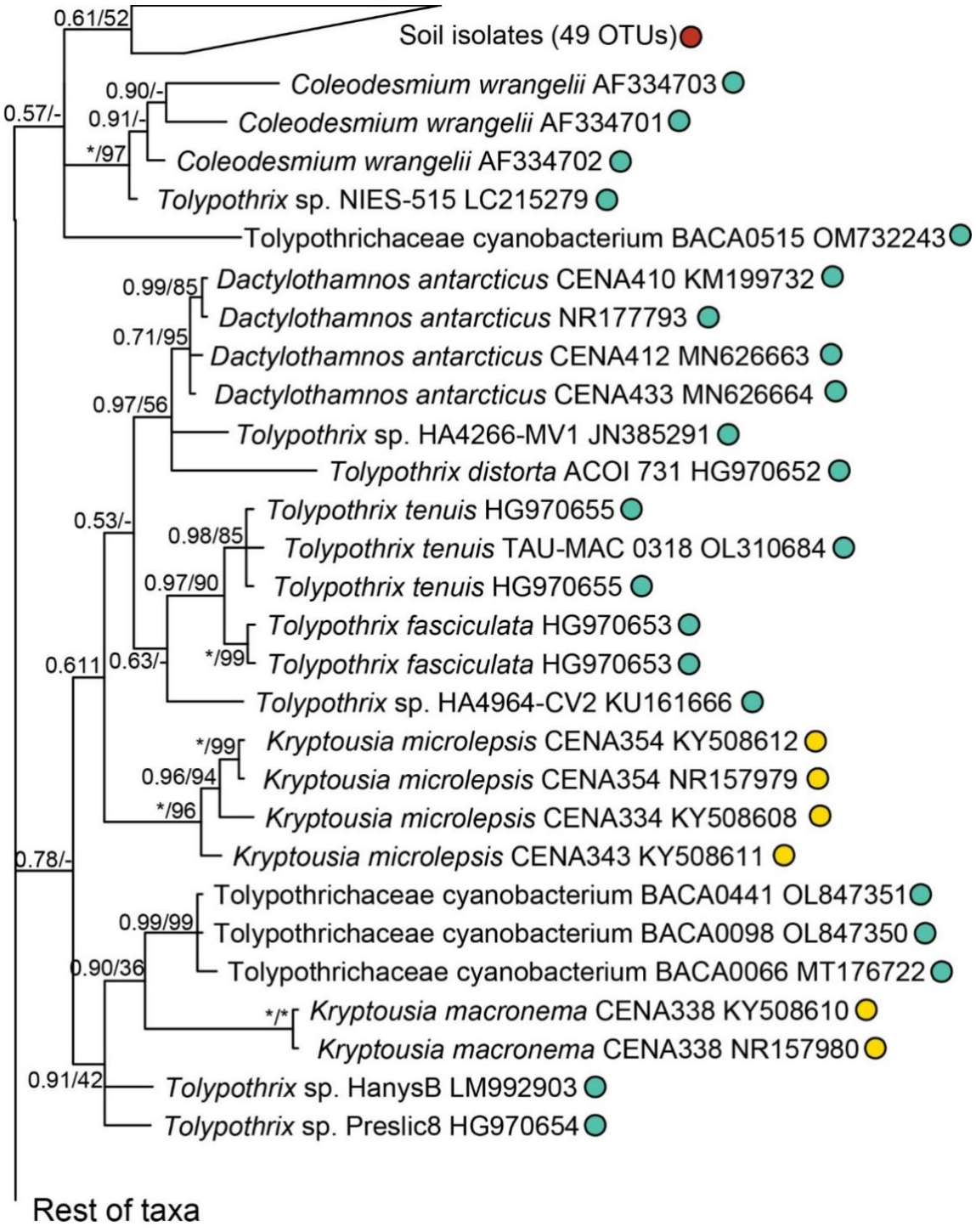

Figure 3. Expanded node of strains from aquatic and wet subaerial habitats. The collapsed node of soil forms is shown for reference.

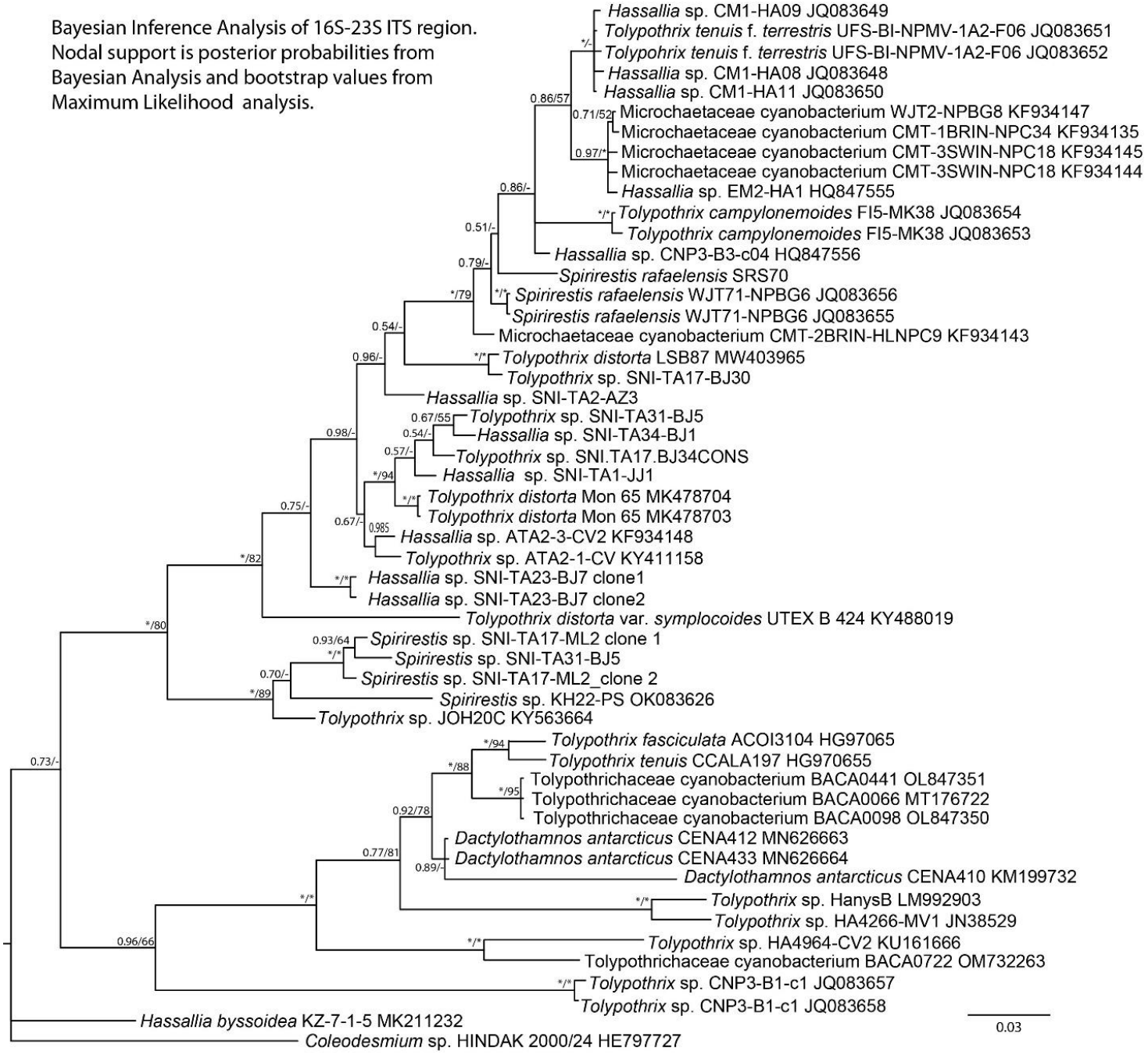

Figure 4. Baysian inference analysis based on an alignment of ITS regions for available sequences of that region, with Maximum Parsimony bootstrap values mapped to nodes. The reference strain for *Spirirestis rafaelensis* is SRS70. The two major nodes are for soil forms (top) and aquatic and wet subaerial forms (bottom).