2023

A BIODIVERSITY SURVEY OF THE SOIL CRUSTS OF THE GEOGRAPHICALLY ISOLATED SAN NICHOLAS ISLAND, CALIFORNIA, USA AND DESCRIPTION OF SPECIES IN THREE GENERA (ATLANTICOTHIX, PYCNACRONEMA, KONICACRONEMA) PREVIOUSLY RESTRICTED TO BRAZIL USING A POLYPHASIC APPROACH TO CYANOBACTERIAL TAXONOMY

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A BIODIVERSITY SURVEY OF THE SOIL CRUSTS OF THE GEOGRAPHICALLY ISOLATED SAN NICHOLAS ISLAND, CALIFORNIA, USA AND DESCRIPTION OF SPECIES IN THREE GENERA (*ATLANTICOTHIX, PYCNACRONEMA, KONICACRONEMA)* PREVIOUSLY RESTRICTED TO BRAZIL USING A POLYPHASIC APPROACH TO CYANOBACTERIAL TAXONOMY

A Thesis Submitted to the

Graduate School of

John Carroll University

in Partial Fulfillment of the Requirements

for the Degree of Master of

Science

By

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2023

Chapter 1: A Biodiversity Survey of the Biological Soil Crusts from the Geographically Isolated San Nicholas Island, California, USA

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ABSTRACT

San Nicholas Island, California, USA is a geographically-isolated island that experiences a semiarid climate and exhibits significant topographic and geologic diversity. Access to the island is restricted to the public and, as a result, only one previous study has been done on the algal biodiversity of its biological soil crusts. The previous study used morphology as the sole basis of species identification, and it was the aim of this study to corroborate and expand upon the results by including molecular data. Using 16S rRNA and 16S–23S ITS sequences and phylogenetic analyses, a diverse set of taxa were identified and are presented herein. Multiple taxa were determined to be common to both studies based on morphological similarity, and several putative new genera and species were identified based on molecular analyses. Several genera were identified that had not been observed in the Northen Hemisphere, raising questions about the distribution of taxa at the genus and species level.

KEY WORDS: Cyanobacteria, San Nicholas Island, Polyphasic Approach, Taxonomy, Biological Soil Crust, Endemism, Biodiversity

INTRODUCTION

Biological Soil Crusts

Soils are highly variable and complex ecosystems that are ecologically critical and are thought to hold a quarter of all biodiversity on earth. Whereas knowledge of soil biodiversity is presently growing, researchers recognize that only roughly 2% of soil microbes have been discovered (Orgiazzi et al. 2016). One subset of soil diversity particularly underrepresented in literature is that of biological soil crusts found in dryland ecosystems, which make up around 40% of earth's land area (Prăvălie 2016). New species continue to be named in most studies of soil crust diversity, indicating there are many new species yet to be discovered. Arid ecosystems present many challenges to microbes due to the lack of UV protection from plant cover, irregular precipitation, and significant diurnal temperature variation (Flechtner et al. 2008). As a result of these challenges, dryland ecosystems were historically thought to lack significant microbial diversity. However, studies of soil crust biodiversity performed in the deserts of western North America (Baldarelli et al. 2022, Becerra-Absalón et al. 2018, Flechtner et al. 1998 & 2008, Johansen et al. 2001, Patzelt 2014, Pietraskiak et al. 2014, 2019, Ward et al. 2021) have provided evidence that diversity in these areas is higher than previously believed. These biological soil crusts (BSCs), composed of basal plants, microalgae, fungi, and heterotrophic bacteria, play several important roles in processes such as soil stabilization and erosion resistance in arid environments lacking plant cover (Belnap 2003, Belnap & Lange 2013). In addition to supporting soil stability, algae and other microbes are thought to provide benefits to soil quality, including an increase in overall biomass as well as organic carbon (Jeffries et al. 1993) and nitrogen via atmospheric fixation (Evans & Johansen 1999, Harper & Marble 1989, Kleiner & Harper 1972, Jeffries et al. 1993, Pietrasiak et al. 2013, West 1990). Whereas BSCs in semiarid

to arid environments have been studied more extensively in recent years (Johansen et al. 2001, Pietrasiak et al. 2011 & 2013, Williams & Büdel 2012), they are still underrepresented in new research, setting up a need for further investigation of the complex relationships among the constituent microorganisms.

Although soil crusts are biologically diverse, with representatives from all three domains of life (Nielsen et al. 2015), they are thought to be dominated in most cases by filamentous cyanobacteria, especially for crusts early in the colonization process. The filaments of cyanobacteria interweave with other microorganisms to form a web-like structure that serves to bind soil particles together and to prevent erosion in regions with geology susceptible to hydraulic or aeolian erosion (Johansen 1993). In addition to the weaving function of filaments, cyanobacteria secrete extracellular polysaccharides (EPS). These external polymers found in many bacteria are thought to provide the additional benefits of adhesion and desiccation resistance (Christensen 1989). The ability to retain moisture and prevent erosion benefits all members of the crusts, which would otherwise be subject to complete drying in arid environments. Whereas soils in more temperate regions are likely bound by the root systems of higher plants, much of the surface area in regions with arid and semi-arid climates is completely devoid of plant cover. Without microbes acting to bind soil particles, the soil would readily erode during precipitation events (Harper & St. Clair 1985). Another important function of cyanobacteria in soil crusts is their ability to photosynthesize and, in some cases, fix atmospheric nitrogen (Evans & Johansen 1999). Desert systems are generally nitrogen-limited, and some cyanobacteria can create specialized cells, called heterocytes, containing the nitrogen-fixing nitrogenase enzyme. The process of atmospheric nitrogen fixation by cyanobacteria may be one

of the most significant sources of new bioavailable nitrogen entering the system (Büdel et al. 2016, Johansen 1993).

It is of increasing importance to identify which species of cyanobacteria are present in BSCs and to determine their niche role in crusts, as large portions of Earth's biocrusts have been negatively affected by human activity, such as grazing by livestock, fires, vehicles, as well as tilling by farmers (Johansen 1993, Marble & Harper 1989). This understanding appears to be crucial to future restoration efforts; organisms adapted to local conditions have the best chance of successfully restoring damages BSCs (Chiquoine et al. 2016). Evidence suggests that niche differentiation is common and that even geographically continuous crusts in close proximity can vary greatly in community composition (Grondin & Johansen 1993, Johansen et al. 1993, Pietrasiak 2011, Wheeler et al. 1993). Therefore, in order to gain a clearer understanding of both global and biome-specific biodiversity, the goal of this study was to further categorize the diversity of cyanobacteria found in desert soils as well as to make inferences about distributions at the genus and species level.

San Nicholas Island

San Nicholas Island (SNI) is part of an eight-island archipelago off the coast of Santa Barbara, California, USA. The island chain, known as the Channel Islands, is a US national park; however, SNI serves as a naval base for the US Navy. SNI experiences a semiarid climate receiving around 200 mm precipitation per year, albeit with a relatively high humidity resulting from consistent sea sprays from the Pacific Ocean. Although the island is relatively small (58.93 km^2), it exhibits significant topographic and geological diversity. Found on the island are stabilized sand dunes, steep cliffs, natural springs, caliche, gypsum soil, and salty crusts. The

restricted access has left the island with relatively undisturbed BSCs relative to the other islands and has reduced the likelihood of human-introduced, non-native species. SNI is 98km off the coast from mainland California and is quite geographically isolated from other western deserts, which has led to the evolution of unique endemic species. The combination of these characteristics made the island a particularly interesting location for a biodiversity study.

Because public access is restricted to SNI, there have been relatively few research opportunities on its biological soil crusts. In fact, the only such study done on the island prior to this study was by Flechtner et al. (2008). This study served as an important starting point for future research on SNI crust diversity. Whereas this study characterized the soil features and provided an idea of the types of soil microalgae that are present on the island, it didn't involve the use of molecular methods for species identification. Although the authors were able to provide tentative identifications of the soil algae based on morphological characteristics, it remained necessary to reevaluate their results with the addition of modern genetic sequencing and molecular analysis to confirm and expand on the results. Flechtner et al. (2008) determined that SNI exhibited the same microheterogeneity found in mainland deserts, and that it likely shared fairly little overlap with mainland US deserts at the species level.

Species Concepts, Geographic Isolation, and Taxonomic Recognition

Although studies have found several North American deserts' soil crust to possess evidence of heterogeneous distribution of species (Pietrasiak et al. 2011), most of these regions are not geographically isolated. Because most desert regions are formed as a result of major geological features (such as mountain ranges) on large, geographically continuous continents, there are few opportunities to study true instances of distinctly isolated populations of

microalgae in soil crusts. In studies of arid ecosystems, new endemic soil algae species continue to be found with variation in nearby crusts which refutes the idea of cosmopolitan distribution first popularized by Becking (1934). Consequently, it is safe to assume that populations of microalgae long isolated by hard-to-transverse geographical features may be especially likely to have evolved into endemic species as a result of genetic separation and unique environmental pressures. The ultimate goal of systematic biology is to gain, through investigation of phylogenetic relationships, the most accurate and complete understanding of evolutionary history and the emergence of biodiversity. While there exists a long history of cyanobacterial taxonomy, accurately delineating species has proven to be challenging. Early investigations of biocrust diversity and the resulting taxonomy were based solely on morphological characteristics and failed to consider other factors such as intraspecific plasticity and development (Johansen & Casamatta 2005). Because cyanobacteria are in many cases morphologically simple and subject to convergent evolution, using morphological characteristics alone to differentiate them has led to polyphyletic taxonomic placements and other issues up to the family level (Mai et al. 2018). Some groups are especially problematic, such as the Leptolyngbyaceae in the Synechococcales, due to their simplicity and lack of clear morphological differentiation among species (Becerra-Absalón et al. 2018). The advent of molecular methods, such as the sequencing of the 16S rRNA gene provided evidence that the previous taxonomic groupings were inaccurate, vastly under-representative of biodiversity, and completely unable to recognize cryptic species (Boyer et al. 2001). Despite the fact that this method was definitively better than morphology-based taxonomy alone, researchers later determined that the 16S rRNA gene sequence was insufficiently variable to resolve to the species level (Fox et al. 1992). These issues have led to the adoption of a total evidence, polyphasic approach to cyanobacterial taxonomy that considers morphological,

ecological, biogeographical, and molecular characteristics to delineate new species, and this is the method that was used in this study (Flechtner et al. 2002, Komàrek 2017, Osorio-Santos et al. 2014). An important addition to the molecular aspect of cyanobacteria taxonomy is the popularization of sequencing of the 16S–23S internal transcribed spacer (ITS) region of the rRNA. This region is conserved enough to be useful at the level of genus while variable enough in length and secondary structure of helices to resolve species (Boyer et al. 2002, Johansen et al. 2011, Osorio-Santos et al. 2014). Differentiation in the ITS region has now become a standard criterion in the field of cyanobacterial taxonomy. Although the ITS is of utmost importance in determining which lineages are species, it remains important to provide a complete picture that includes morphological, biochemical, and ecophysiological factors (Johansen & Casamatta 2005). Including a variety of criteria bolsters the case for delineating new species.

Although determining criteria for species recognition is important, such criteria are impossible to apply outside the framework of a theoretical species concept. Though some popular species concepts, such as biological species concept (Mayr 1942), may be sufficient to classify sexually reproducing organisms, it is impossible to apply to the prokaryotic and asexual cyanobacteria. There has been much debate about which species concept applies best to prokaryotes, and some have argued for a prokaryotic-specific species concept (Colwell et al. 1995). Because this concept is similarity-based and not universally applicable, it (along with other phenetic concepts) is not ideal and would tend to underestimate biodiversity in prokaryotes. Because all life is related and originates from a universal common ancestor, the ideal species concept should be universal and nonspecific to any variety of lifeform. Therefore, the evolutionary species concept proposed by Simpson (1951), revised by Wiley (1978), and applied to cyanobacteria by Johansen & Casamatta (2005), is the framework under which new species

were identified in this study. This concept defines species as “a single lineage of ancestral descendant populations of organisms which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate” (Wiley 1978). Additionally, this concept is the most justifiable because it lacks necessity for specific, non-universal criteria and meshes well with a polyphasic approach to species identification (Johansen & Casamatta 2005). As a result, a lineage-based model was chosen as the theoretical framework under which the criteria mentioned above were applied for species delineation in this study.

Although an evolutionary species concept provides the most accurate framework for defining what a species is, there are some challenges that present themselves when such a concept is taken to its logical extreme. Most notably, genetically identical cyanobacterial populations even just meters apart would be considered separate species as a result of their lack of gene flow, making them distinct lineages. Consequently, a subcategory of the evolutionary concept, called a phylogenetic species concept, was later applied in a number of papers in order to resolve this problem (Becerra-Absalón et al. 2018, 2020, Pietrasiak et al. 2019). The phylogenetic concept delineates only those lineages that are far enough along in the speciation process (as evidenced by the criteria previously mentioned, i.e., genetic variation and morphology) as worthy of taxonomic recognition as new species (Osorio-Santos et al. 2014). At its core, the practice of cataloging species was developed to gain understanding of diversity on earth in a way that is accurate and useful, and thus only lineages that have developed repeatable, diagnosable differences should be considered for recognition as separate lineages. To avoid the potential issue of overrepresentation of diversity, the phylogenetic subcategory of the evolutionary concept was applied in this study, and only lineages that exhibit tangible evidence of differentiation were recognized as unique.

The present study sought to further an understanding of general and SNI-specific algal diversity by applying a modern polyphasic approach to taxonomy to a floristic study. Previously described species found on SNI are reported with special attention given to those taxa which were isolated by Flechtner et al. (2008) and the present study. Putative new species and genera across several orders of cyanobacteria are presented, although these will be formally described in future publications.

METHODS

Field Collection

A total of 36 soil samples were collected on SNI (Fig. 1) with one reference sample collected from the nearby Santa Barabara Island (SBI). Samples SBI and SNI-TA1–TA5 were collected 11 February 2021, and samples SNI-TA6–TA36 were collected 25 May 2021. All samples were collected with a metal spoon and were transferred into Whirl-Pak bags for shipping and storage (Baldarelli 2022). Samples were taken from sites that varied topographically and geologically across the island (Table 2) to ensure the maximum extent of diversity was sampled while accounting for environmental heterogeneity and patchy species distributions.

Strain Isolation and Culture

From each sample representing a sampling site, 1.0 g subsamples were taken by selecting small portions of larger soil crust pieces. These subsamples were diluted 10^{-3} in sterile Erlenmeyer flasks containing 99 mL sterile Z8 media (Carmichael 1986) and were agitated on a rotary shaker for at least 30 minutes to liberate microalgae from the soil matrix. Afterwards, 1.0 mL of the soil slurry was further diluted 10^{-2} in 9.0 mL sterile Z8 media. Agar solidified Z8 enrichment plates were then inoculated with 0.1 mL of the now 10^{-4} diluted samples, with 5

replicates plated from each. The following day, plates were sealed with Parafilm and placed under a 12h light-dark cycle at approximately 20°C until macroscopic algal colonies were visible (4–6 weeks). Algal strains were isolated by choosing well-isolated colonies under an Olympus SZ40 stereoscope using sterile Pasteur pipettes pulled to a fine point. Cultures were placed in test tubes containing 5.0 mL sterile liquid Z8 media and returned to the 12h light-dark cycle until significant growth was observed (3–5 weeks). Developed cultures were transferred to test tubes containing sterile agar solidified Z8 slants for storage and further downstream use.

Morphological Characterization, Tentative Identification, and Vouchering

After significant growth developed, wet mounts were made and strains were observed, photographed, and morphologically characterized using an Olympus BH-2 microscope equipped with Nomarski DIC optics, an Olympus DP25 camera and cellSens software. A minimum of 20 photographs were taken of each strain with care given to capture all specialized cell types and life-cycle stages. When nitrogen-fixing cyanobacteria taxa were observed, these strains were transferred to sterile, nitrogen-free Z8 media to stimulate growth of heterocytes and were photographed again when development was observed. Length and width measurements were taken for vegetative cells, filament width, apical cells, and heterocytes and akinetes, when applicable. Other relevant morphological features such as apical cell shape, presence of calyptra, and necridia were noted.

Vouchers were made by applying material from each strain to glass fiber filters via vacuum flask. At least two filters were made per strain, and each was allowed to dry completely under a fume hood inside unsealed glass petri dishes. New species were also preserved on permanent slides as potential holotype material by fixing them in Spurr's resin using the

manufacturers protocol. These materials will be submitted to the herbarium collection at the Santa Barbara Botanical Garden, and living strains on Z8 slants will be stored in the John Carroll University (University Heights, Ohio, USA) culture collection for storage and maintenance. In total, 462 cyanobacterial strains and 135 strains of eukaryotic algae were isolated and processed.

Molecular Characterization

From each sampling site, a number of strains (78 total) were chosen for sequencing and molecular characterization. Effort was given to select strains across the fullest range of diversity at the order and family level based on preliminary visual identifications: morphologically diverse coccoid, filamentous, and heterocytous taxa were chosen to ensure the maximum representation of diversity. Strains chosen were transferred to agar-solidified Z8 plates and placed under the 12h light-dark cycle until a microbial lawn was grown to increase biomass. Genomic DNA was extracted from strains using Qiagen DNeasy Powersoil Pro Kits following the manufacturer's protocol and eluted in 50 µL of elution buffer. DNA presence was confirmed with a 1% TBE agarose/ethidium bromide gel and stored at -20°C. Genomic DNA samples were used to amplify the 16s rRNA gene and the 16S–23S internal transcribed spacer (ITS) region using primers VRF1R (5' – CTC TGT GTG CCT AGG TAT CC -3') and VRF2F (5'- GGG GAA TTT TCC GCA ATG GG -3'; Boyer et al. 2002) in polymerase chain reaction (PCR) to amplify the gene encoding the16S ribosomal RNA along with the 16S–23S ITS region. The reaction mixture containing 1 µl of each primer at 0.01 mM concentration was combined with 12.5 µl of LongAmp™ Taq 2x Master Mix (NEB, Ipswitch MA), 1 µl template DNA (50 ng/ml) and 9.5 µl nuclease free water. This PCR mix was subjected to 35 cycles of denaturing (94º for 45 sec), annealing at 57º for 45 sec, an extension at 72º for 135 sec, and a final extension for 5 minutes.

The PCR reaction was performed using both 1 µL and 2 µL samples of genomic DNA (varying the amount of dH_2O), and the stronger of the two reactions was chosen for further downstream use via visualization on a 1% TBE agarose gel. PCR products were inserted into plasmid pSC-A-amp.kan and cloned into the LacZ gene of StrataClone competent *Escherichia coli* cells via heat shock following the manufacturer's protocol. *E. coli* cells were plated on agar-solidified LB-amp media plates with 40 µL 2% X-Gal and three properly transformed colonies were picked via blue-white screening. Overnight cultures were grown, and plasmid DNA was isolated with Qiagen QIAprep® Miniprep (Qiagen, Carlsbad, CA) kits following the manufacturer's protocol. Insertions were confirmed by EcoR1 restriction enzyme digest followed by visualization on 1% TBE agarose gels. Two or three clones of each strain were ultimately sent to Functional Biosciences, Inc (Madison WI, USA) for Sanger sequencing. Plasmid primers M13 forward and M13 reverse and internal primers VRF5 (5' –TGT ACA CAC CGG CCC GTC- 3'), VRF7 (5'-AATGGG ATT AGA TAC CCC AGT AGT C -3'), and VFR8 (5'- AAG GAG GTG ATC CAG CCA CA -3'; Wilmotte et al. 1993) were used to obtain partial overlapping sequences. Sequences were error proofed using Chromas software (version 2.6.6) and assembled into contigs by alignment with ClustalW (Larkin et al. 2007).

Phylogenetic Analyses

Tentative identifications were obtained by entering assembled sequences into GenBank BLAST search and comparing them to highly similar sequences present in the database. The data were used to gather sequences of related organisms from the database to complete further phylogenetic analyses. The 16s rRNA gene was subjected to analysis by both Bayesian inference (BI) and maximum likelihood (ML) analysis using the CIPRES Science Gateway (Miller et al.

2010) to obtain posterior probability and bootstrap support values for each node in the tree. BI analyses were performed using MrBayes on XSEDE 3.2.6 (Ronquist et al. 2012), and ML analyses were performed using RAxML-HPC2 on XSESE 8.2.10 (Stamatakis 2014). In both cases, the GTR + I + G evolutionary model was used. BI analyses were run for 80 million generations with the first 25% of samples discarded as burn-in. ML analyses were run using the same alignments used for BI with 1000 bootstrap iterations. 16S gene analyses were performed at the family or order level with several representatives from each related genus for which sequences were available in GenBank to produce robust phylogenetic trees. Values from both the BI and ML analyses were considered in the analysis and superimposed on the phylogenetic trees at the appropriate nodes. The 16S–23S ITS genes were phylogenetically analyzed at the genus level with BI to determine relationships to closely related strains. Phylogenetic trees were visualized with Fig Tree (Rambaut 2009) and reconstructed in Adobe Illustrator (Adobe Systems, San Jose, California).

RESULTS

Of the 37 samples plated on enrichment plates, 31 produced agal colonies that could be isolated. In total, 492 strains of cyanobacteria and 135 strains of eukaryotic algae (Chlorophyta and Eustigmatophyta) were isolated and cultured (Table 2). For all isolated strains photographs were taken and tentative identifications were made based on morphological characteristics. Of the 492 cyanobacterial strains isolated, 78 were chosen for molecular analysis (Table 1) and successfully sequenced for 16S rRNA and 16S–23S ITS rRNA. Strains were chosen with care given to cover the maximum amount of diversity and to cover each sampling site; morphologically variable strains were chosen with at least one strain sequenced per site when

possible. Among these, at least 19 were determined to be putative new species. Furthermore, six potentially new genera were found with at least one representative strain; however, more analysis needs to be done to confirm this result. Several previously-described species were also found in the process. These results will be outlined below.

As a note, an attempt was made to obtain 18S rRNA and rbcL gene sequences for a number of eukaryotic algae isolates by directly sequencing purified PCR products; however, this attempt was unsuccessful. As a result, only morphology-based tentative identifications were able to be made. Future attempts will be made to obtain genetic data with the addition of cloning to ensure sequences are clean and complete. Because all eukaryotic algae identified were coccoid, and thus difficult to identify on morphology alone, tentative identifications are not presented in this paper.

Taxa in Common with the Previous Study

Six previously-described species were found on SNI via sequencing efforts and molecular analysis. Among these are *Microcoleus vaginatus, Myxacorys californica, Roholtiella edaphica, Scytonema hyalinum, Symplocastrum flechtneri.* and *Symplocastrum torsivum.* However, the only definitively identified species common to both this study and those in Flechtner et al. (2008) was *M. vaginatus,* which is unsurprising given the abundance and wide distribution of the species (Dvořák et al. 2012). This taxon is quite easy to identify based on morphology alone, so we are confident that the identification by Flechtner et al. (2008) was correct.

Although no other species was common between the two studies with certainty, there was significant overlap among the genera. The genera confirmed with certainty to have been observed in both studies are: *Hassallia; Nostoc; Tolypothrix; Scytonema;* and *Trichocoleus*.

Several common taxa found in both studies were identified based on other criteria, such as morphological similarity and consideration of recent taxonomic revision. An SNI strain of *Hassallia* (SNI-TA1-JJ1) fits the description of *Hassalia pseudoramosissima* well and, given that both were isolated from the island, they are likely a match (Fig. 2). It can also be said with certainty that *Oculatella* was found in both studies. Flechtner et al. (2008) described an isolated strain (identified only as *Leptolyngbya* sp. 4), as a thin, filamentous taxon with distinctive red apical granules, and *Oculatella* (although not described until 2012 by Zammit et al.) is the only taxon for which this feature is diagnostic (Fig. 2). Four strains in this genus were found in the present study. This study identified several strains in the Leptolyngbyaceae, two of which likely represent a new genus and two of which were confirmed to be *Myxacorys californica.* Flechtner et al. (2008) observed another *Leptolyngbya*-like taxon (identified to the genus level as *Leptolyngbya* sp. 3), and descriptions and line drawings presented in the original paper match the description of *M. californica* remarkably well, and it is possible (if not likely) that they are the same species (Fig. 2). Given that the genus was not described until 2019 (Pietrasiak et al. 2019), this determination could not have been made at the time. Because morphology was the sole taxonomic criterion applied in Flechtner et al. (2008), and because of significant taxonomic revisions made in the following years, there is likely additional overlap at the species level that has yet to be determined.

Other Taxa

Several strains sequenced in this study represent putative new taxa across several orders. These taxa will not be formally described directly in this paper; however, they will be listed below. In some cases, it has not yet been determined if strains represent new taxa although these findings

will also be presented. Isolation locations and strain designations for each strain sequenced can be found in Table 2. Site descriptions, as well as the total number of strains isolated and sequenced from each site can be found in Table 1.

Coccoid Cyanobacterial Taxa

Aliterella

Three strains (SNI-TA17-BJ5, SNI-TA17-BJ17, SNI-TA17-BJ26) sequenced in this study from one sampling site (TA17) represent one new species (Fig. 3) of *Aliterella,* a recently described genus in the Chroococcidiopsidales (Rigonato et al. 2016). Interestingly, the genus was originally described with a species isolated from the South Ocean, near Antarctica. It is quite unusual for a cyanobacterial genus to exist in both ocean waters and on soil crusts. This is the first record of the genus in the USA,

Gloeocapsopsis

Also in the Chroococcidiopsidales is the coccoid genus *Gloeocapsopsis.* Two study sites (TA-17, TA-20) produced strains (SNI-TA17-BJ23, SNI-TA20-JG1) (Fig. 3) in this genus, although the species-level placement of these strains is yet to be determined.

Pleurocapsa

Four strains (SNI-TA17-BJ15, SNI-TA17-BJ20, SNI-TA25-BJ13, SNI-TA31-BJ8) from three sampling sites (TA17, TA25, TA31) on SNI represent one new putative species (Fig. 3) of *Pleurocapsa* in the Chroococcales.

Pseudoacaryochloris

One strain (SNI-TA23-BJ45) was found to be a putative new species of *Pseudoacaryochloris* (Fig. 3). Currently, there is only one described species (*P. sahariense)* which was originally isolated in a hyperarid area in the Sahara Desert receiving <30 mm precipitation per year (Mehda et al. 2022).

Non-Heterocytous Filamentous Taxa

Coleofasciculaceae

Two strains (SNI-TA4-BJ9, SNI-TA19-BJ8) from two sampling sites (TA4, TA19) on SNI represent a likely new genus and species (Fig. 3) in the Coleofasciculaceae. Whereas the data strongly corroborates this, more work needs to be done in order to correctly place this taxon.

Kastovskya

One SNI strain (SNI-TA36-BJ6) (Fig. 3) was determined to be a putative new species of *Kastovskya,* a genus described by Mühlsteinova et al. (2014) from the Atacama Desert in Chile. Notably, this is the first observation of this genus in the United States.

Konicacronema

Two strains (SNI-TA6-AZ30, SNI-TA14-AZ14) from two sites (TA6, TA14) were determined to represent one new species of *Konicacronema*, a genus recently with only one species (*K. caatingensis*) described from the Caatinga habitat in Brazil (Machado-de-Lima & Branco 2020). This is one of several genera found on SNI which were previously restricted to Brazil.

Leptolyngbyaceae

Two strains (SNI-TA16-ML1, SNI-TA16-BJ25) from one sampling site (TA16) (Fig. 3) were determined to be a likely new genus in the Leptolyngbyaceae. More molecular analysis needs to be done to properly delineate this taxon, although preliminary data suggests this is a new genus.

Microcoleus

Four strains (SNI-TA6-AZ25, SNI-TA7-BJ1, SNI-TA8-AZ3, SNI-TA17-BJ7) from four sampling sites (TA6, TA7, TA8, TA17) were determined to be *Microcoleus vaginatus.* This is an unsurprising result given the abundance and large geographical distribution on the taxon. This is the only taxon definitively found in common between the 2008 study and the current study.

Myxacorys

Two strains (SNI-TA20-BJ3, SNI-TA20-JG10) from one study site (TA20) were determined to be *M. californica*. It appears likely that one of the strains listed as "*Leptolyngbya* sp." in Flechtner et al. (2008) was this species based on morphological similarity (Fig. 2).

Nodosilinea

Nodosilinea (Perkerson et al. 2011) appears to be quite common on SNI. Seven strains (SNI-TA2-BJ11, SNI-TA15-JRJ2, SNI-TA2-JJ1, SNI-TA15-JRJ1, SNI-TA15-AZ4, SNI-TA15-AZ5, SNI-TA23-BJ18) representing at least 3 putative new species were found; however, several strains have yet to be analyzed at the species level.

Nodosilineaceae

In addition to the *Nodosilinea* species mentioned above, two additional strains (SNI-TA3-BJ15, SNI-TA25-BJ8) in the Nodosilineaceae appear to represent a new genus and species in the

family. More analysis needs to be done to confirm this; however, the preliminary data strongly suggest a new genus.

Oculatella

Four strains (SNI-TA6-AZ3, SNI-TA14-AZ10, SNI-TA24-BJ1, SNI-TA14-AZ8) from three sampling sites (TA6, TA14, TA24) represent two new species of *Oculatella* which will be described in a future publication. Although the genus was not described until after Flechtner et al. (2008) by Zammit et al. (2012), it is clear this genus was found based on the description of a filamentous taxon with the distinctive red apical granules which is diagnostic for this genus (Fig. 2).

Oculatellaceae

Three strains (SNI-TA9-AZ1, SNI-TA14-AZ1) from two sampling sites (TA9, TA14) represent a likely new genus and species in the Oculatellaceae. Again, further molecular analysis needs to be done to confirm this.

Prochlorotrichaceae

One strain (SNI-TA8-AZ4) from one study site (TA8) likely represents a new genus and species in the family Prochlorotrichaceae. This family currently has only one genus *Prochlorothrix* (Burger-Wiersma et al. 1989), which is a planktic taxon originally isolated from a shallow eutrophic lake in the Netherlands. The type species *P. hollandica* contains gas vacuoles at its polar ends and has exclusively been found in freshwater environments in Europe.

Pycnacronema

Two strains (SNI-TA3-BJ5, SNI-TA19-BJ1) from two sampling sites (TA3, TA19) represent two putative new species of *Pycnacronema*, a large filamentous genus in the Wilmottiaceae with eight currently accepted species. Like *Konicacronema*, this genus was described from and previously restricted to Brazil (Machado-de-Lima & Branco 2020).

Stenomitos

Stenomitos is a thin filamentous genus originally described by Miscoe et al. (2016), and five strains (SNI-TA2-BJ10, SNI-TA2-BJ16, SNI-TA6-AZ9, SNI-TA6-AZ26, SNI-TA-33-BJ10) from SNI were found (Fig. 3) that represent three putative new species: "S. maritimus," "S. brittonii," & "S. hoyerii." This genus is morphologically uninformative, and the species tend to be cryptic. Consequently, the diversity of this taxon can only be determined with molecular methods.

Symplocastrum

Three strains (SNI-TA8-AZ5, SNI-TA35-BJ2, SNI-TA35-BJ6) representing two previously described species (*S. torsivum, S. flechtneri*) were isolated. Phylogenetic analyses on this group revealed that a recently-described genus *Arizonanema* (Fernandes et al. 2021) is a later synonym of *Symplocastrum* and requires taxonomic revision.

Trichocoleus

Two (SNI-TA9-AZ2, SNI-TA17-BJ3) from two sampling sites (TA9, TA17) were determined to be at least one new putative species of *Trichocoleus* and will be described in a later publication.

Heterocyte-Forming Taxa

Atlanticothrix

Atlanticothrix is a nostocalean genus recently described from strains isolated in the Atlantic Forest in Brazil (Alvarenga et al. 2021). Currently, there is only one species, *A. silvestris.* This genus proved to be the most diverse taxon sampled on SNI. At least 10 strains (SNI-TA1-JJ4, SNI-TA4-BJ2, SNI-TA5-JJ1, SNI-TA5-JJ3, SNI-TA18-ML7, SNI-TA23-BJ18, SNI-TA23-BJ35, SNI-TA23-BJ41, SNI-TA26-BJ1, SNI-TA26-BJ7) were isolated and sequenced from the island, and these represent at least three putative new species (Fig. 3). Along with *Konicacronema* and *Pycnacronema,* this genus was also previously restricted to Brazil. The three putative new species will be described in a future publication.

Hassallia

Four strains (SNI-TA1-JJ1, SNI-TA12-AZ3, SNI-TA23-BJ7, SNI-TA34-BJ1) determined to be in the genus *Hassallia* were isolated from SNI. One of these strains (SNI-TA1-JJ1) appears to match the morphological description of *H. pseudoramosissima* (Fig. 2) which was isolated and described in Flechtner et al. (2008).

Nostoc

One strain (SNI-TA23-BJ46) sequenced was determined to be in the common genus *Nostoc.* Species level placement of this strain is yet to be determined; however, the preliminary analyses suggest this is a potential putative new species.

Unknown Nostocales

Two strains (SNI-TA18-ML2, SNI-TA18-ML4) were isolated from the island which may represent a new genus and species in the Nostocales (Fig. 3). However, some preliminary

investigation suggests that these may be a new species in the genus *Cyanocohniella*, which was originally isolated from thermal springs in the Czech Republic (Kaštovský et al. 2014).

Roholtiella

Roholtiella is a tapering and branching nostocalean genus described by Bohunická et al. (2015). One strain (SNI-TA28-BJ7) from SNI was determined to be *R. edaphica.* Interestingly, the SNI strain exhibits a purplish coloration which was not observed when the species was first described (Fig. 3).

Scytonema

One strain (SNI-TA29-BJ21) in the genus *Scytonema* was sequenced in this study. This strain was determined by molecular analyses to be *S. hyalinum* (Fig. 3).

Tolypothrix

Four strains (SNI-TA17-BJ30, SNI-TA17-BJ34, SNI-TA31-BJ5, SNI-TA31-BJ14) from two study sites (TA17, TA31) were determined to fall in the genus *Tolypothrix* (Fig. 3). More work needs to be done to place these strains at the species level, but they will later be used in an analysis which attempts to revise the genera *Hassallia, Tolypothrix,* and *Scytonema.*

DISCUSSION

The aim of this study was to better understand algal diversity, and to corroborate and expand upon the findings of the only previous floristic study on San Nicholas Island. Much like Flechtner et al. (2008), care was given when sampling the island to account for environmental heterogeneity and potentially patchy species distribution. Although it remains difficult to compare the results of a morphology-based study to one which incorporates molecular data,

some of the previous results were able to be confirmed using a polyphasic approach. Although only the presence of *Microcoleus vaginatus* was definitively confirmed in both studies, there is good evidence that several other taxa were present over time. At the genus level, *Hassalia, Tolypothrix, Scytonema, Trichocoleus, Oculatella,* and *Nostoc* were definitively recognized in both this study and the previous one. Additionally, it is probable that *Myxacorys* was present in Flechtner et al. (2008) based on morphological evidence (Fig. 2); however, this taxon was not yet described at the time. Although the island was extensively sampled in both studies, it is likely that further isolation and sequencing efforts would yield additional taxonomic overlap with Flechtner et al. (2008).

Taxonomic revision over time has made comparing the results somewhat challenging; however, morphology alone was able to be used as the sole criterion for this purpose in some cases. This highlights the continued importance and utility of morphology as a criterion for species delineation. Taxa with diagnosable morphological features remain identifiable via light microscopy, and often this is the most useful technique for recognizing species isolated from the field. However, some challenges still present themselves when morphology is used as the sole characteristic taxonomically. Several new putative species found in this study (such as those in genus *Stenomitos*) are morphologically uninformative and can only be resolved with the use of molecular methods. For these reasons, the use of a total evidence, polyphasic approach to taxonomy continues to prove important to gain a complete understand of diversity, while also being useful to researchers who wish to confidently identify taxa with minimal effort and cost.

It is significant that the same taxa (in some cases) were able to be isolated from the same area over time. Biogeography has been used as an additional criterion for species delineation,

and this study serves as evidence that some taxa are endemic and not globally distributed. It was previously thought that microbial species experienced a cosmopolitan distribution (Becking 1934), but this study has provided further evidence that isolations from a given area are often repeatable and reliable over time. The number of new putative species found in this study further suggests that although species are often restricted by geography, genera can have widespread distributions across a variety of habitats (Jung et al. 2020). New species representing the genera *Aliterella, Pseudoacaryochloris,* and *Atlanticothrix,* for example, were found on SNI which is vastly isolated from the sites from which these genera were first described. However, evidence suggests that each has bifurcated at the species level from its common ancestor in a diagnosable way due to the environmental pressures of varying habitats. A minimal number of taxa analyzed in this study belonged to previously described species; however, almost all belonged to previously described genera.

SNI proved to be quite diverse, being home to a wide variety of cyanobacterial species across several orders and families. It remains unclear if this is a consequence of its relatively undisturbed biological soil crusts or some other factor; however, it is certain that further investigation will lead to additional recognition of new species previously unknown to science. Future floristic studies on SNI and the other Channel Islands are a unique and interesting opportunity to observe the effects of geographic isolation on endemism and species distributions.

Although many taxa in this study were determined to be new species and genera based on molecular analysis, further work needs to be done to provide sound taxonomic descriptions using a polyphasic approach to taxonomy. The putative new species outlined in this study will be formally described in future papers with consideration given to morphological, molecular,

ecological, and biogeographical factors to provide a holistic characterization and justification of each distinct lineage.

ACKNOWLEDGMENTS

I would like to thank the John Carroll University Biology department for their support throughout this project. Special thanks are owed to the professors in the department who have provided input relating to this project, as well as personal and professional guidance and support. In particular I would like to thank Dr. Ralph Saportio, Dr. James Watling, Dr. Jennifer Murphy, Dr. Cari Hickerson, and Dr. Carl Anthony. I would also like to thank my committee members, Dr. Christopher Sheil and Dr. Rebecca Drenovsky for their guidance. I would especially like to thank my advisor, Dr. Jeffrey Johansen for his continued professional and personal support and his assistance with this project and my development as a researcher. Thanks to Amanda Szinte and Chelsea Villanueva for helping me to learn the molecular methods necessary for the sequencing aspect of this project. Several graduate and undergraduate students also provided assistance with isolating cultures, taking microscope pictures, and making herbarium vouchers. In particular, I would like thank Mathew Luknis, Emily Masluk, Salvador Peron, Elliot Cho, Angela Burns, and Braden Lau. The US Navy provided the funding for this project as part of a larger metagenomic study to preserve endangered snails on San Nicholas Island. Thanks to Bill Hoyer and Kristen Lehman for their help with sample collection.

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Table 1. The 78 cyanobacterial isolates for which 16S rRNA and 16S-23S ITS sequence data were obtained. If the species name is followed by a number, it was determined by molecular analysis that the strain belongs to a new species. Any relevant notes relating to each strain are listed, including putative species names if determined.

Site	Strain designation	Identification	Notes
TA17	SNI-TA17-BJ5	*Aliterella* sp. 1	Recent genus, first record in USA
TA17	SNI-TA17-BJ17	*Aliterella* sp. 1	Recent genus, first record in USA
TA17	SNI-TA17-BJ26	*Aliterella* sp. 1	Recent genus, first record in USA
TA18	SNI-TA18-ML7	*Atlanticothrix* sp. 1	Recent genus, first record in USA New putative species “A. crispata”
TA23	SNI-TA23-BJ41	*Atlanticothrix* sp. 1	Recent genus, first record in USA New putative species “A. crispata”
TA26	SNI-TA26-BJ1	*Atlanticothrix* sp. 1	Recent genus, first record in USA New putative species “A. crispata”
TA26	SNI-TA26-BJ7	*Atlanticothrix* sp. 1	Recent genus, first record in USA New putative species “A. crispata”
TA23	SNI-TA23-BJ18	*Atlanticothrix* sp. 1	Recent genus, first record in USA New putative species “A. crispata”
TA23	SNI-TA23-BJ35	*Atlanticothrix* sp. 1	Recent genus, first record in USA New putative species “A. crispata”
TA5	SNI-TA5-JJ3	*Atlanticothrix* sp. 2	Recent genus, first record in USA New putative species “A. nostocoides”
TA1	SNI-TA1-JJ4	*Atlanticothrix* sp. 2	Recent genus, first record in USA New putative species “A. nostocoides”
TA4	SNI-TA4-BJ2	*Atlanticothrix* sp. 3	Recent genus, first record in USA New putative species “A. testacea”

TA18	SNI-TA18-ML7	*Atlanticothrix* sp. 1	Recent genus, first record in USA New putative species “A. crispata”
TA5	SNI-TA5-JJ1	*Atlanticothrix* sp. 2	Recent genus, first record in USA New putative species “A. nostocoides”
TA4	SNI-TA4-BJ9	Coleofasciculaceae	Likely a new genus and species
TA19	SNI-TA19-BJ8	Coleofasciculaceae	Likely a new genus and species
TA17	SNI-TA17-BJ23	*Gloeocapsopsis*	Putative new species
TA20	SNI-TA20-JG1	*Gloeocapsopsis*	Putative new species
TA1	SNI-TA1-JJ1	*Hassallia*	Possibly *Hassallia pseudoramosissima*
TA12	SNI-TA12-AZ3	*Hassallia*	Species-level placement yet to be determined
TA23	SNI-TA23-BJ7	*Hassallia*	Species-level placement yet to be determined
TA34	SNI-TA34-BJ1	*Hassallia*	Species-level placement yet to be determined
TA36	SNI-TA36-BJ6	*Kastovskya* sp. 1	Recent genus, first record in USA
TA6	SNI-TA6-AZ30	*Konicacronema* sp. 1	Brazilian genus, first record in USA New putative species “K. haraasengensis”
TA14	SNI-TA14-AZ14	*Konicacronema* sp. 1	Brazilian genus, first record in USA New putative species “K. haraasengensis”
TA16	SNI-TA16-ML1	Leptolyngbyaceae	Likely a new genus and species
TA16	SNI-TA16-BJ25	Leptolyngbyaceae	Likely a new genus and species
TA6	SNI-TA6-AZ25	*Microcoleus vaginatus*	Also isolated in Flechtner et al. (2008)
TA7	SNI-TA7-BJ1	*Microcoleus vaginatus*	Also isolated in Flechtner et al. (2008)
TA8	SNI-TA8-AZ3	*Microcoleus vaginatus*	Also isolated in Flechtner et al. (2008)
TA17	SNI-TA17-BJ7	*Microcoleus vaginatus*	Also isolated in Flechtner et al. (2008)
TA20	SNI-TA20-BJ3	*Myxacorys californica*	Likely isolated by Flechtner et al. (2008)
TA20	SNI-TA20-JG10	*Myxacorys californica*	Likely isolated by Flechtner et al. (2008
TA2	SNI-TA2-BJ11	*Nodosilinea* sp. 1	ITS region quite unique
TA15	SNI-TA15-JRJ2	*Nodosilinea* sp. 1	ITS region quite unique
TA2	SNI-TA2-JJ1	*Nodosilinea* sp. 2	ITS region quite unique

TA15	SNI-TA15-JRJ1	*Nodosilinea* sp. 3	ITS region quite unique
TA15	SNI-TA15-AZ4	*Nodosilinea*	Recently sequenced, not determined
TA15	SNI-TA15-AZ5	*Nodosilinea*	Recently sequenced, not determined
TA23	SNI-TA23-BJ18	*Nodosilinea*	Recently sequenced, not determined

TA3	SNI-TA3-BJ15	Nodosilineaceae	Likely a new genus and species
TA25	SNI-TA25-BJ8	Nodosilineaceae	Likely a new genus and species
TA23	SNI-TA23-BJ46	*Nostoc*	Species-level placement undetermined
TA18	SNI-TA18-ML2	Nostocales	Likely a new genus and species
TA18	SNI-TA18-ML4	Nostocales	Likely a new genus and species
TA6	SNI-TA6-AZ3	*Oculatella* sp. 1	Putative new species
TA14	SNI-TA14-AZ10	*Oculatella* sp. 1	Putative new species
TA24	SNI-TA24-BJ1	*Oculatella* sp. 1	Putative new species
TA14	SNI-TA14-AZ8	*Oculatella* sp. 2	Putative new species
TA9	SNI-TA9-AZ1	Oculatellaceae	Likely a new genus and species
TA9	SNI-TA9-AZ3	Oculatellaceae	Likely a new genus and species
TA14	SNI-TA14-AZ1	Oculatellaceae	Likely a new genus and species
TA17	SNI-TA17-BJ15	*Pleurocapsa* sp. 1	Putative new species
TA17	SNI-TA17-BJ20	*Pleurocapsa* sp. 1	Putative new species
TA25	SNI-TA25-BJ13	*Pleurocapsa* sp. 1	Putative new species
TA31	SNI-TA31-BJ8	*Pleurocapsa* sp. 1	Putative new species
TA8	SNI-TA8-AZ4	Prochlorotrichaceae	Likely a new genus and species
TA3	SNI-TA3-BJ5	*Pycnacronema* sp. 1	Brazilian genus, first record in USA New putative species “P. juana-mariae”
TA29	SNI-TA29- BJ1	*Pycnacronema* sp. 2	Brazilian genus, first record in USA New putative species “P. aeruginosum”
TA23	SNI-TA23-BJ45	*Pseudoacaryochloris* sp.1	Recent genus, first record in North America
TA28	SNI-TA28-BJ7	*Roholtiella edaphica*	Unusual purple coloration
TA29	SNI-TA29-BJ21	*Scytonema hyalinum*	To be used in Tolypothrichaceae revision
TA17	SNI-TA17-ML2	*Spirirestis* sp. 1	To be used in Tolypothrichaceae revision
TA2	SNI-TA2-BJ10	*Stenomitos* sp. 1	New putative species *“*S. maritimus*”*
TA2	SNI-TA2-BJ16	*Stenomitos* sp. 1	New putative species *“*S. maritimus*”*
TA6	SNI-TA6-AZ9	*Stenomitos* sp. 2	New putative species *“*S. brittonii*”*
TA6	SNI-TA6-AZ26	*Stenomitos* sp. 2	New putative species *“*S. brittonii*”*

TA33	SNI-TA33-BJ10	*Stenomitos* sp. 3	New putative species “S. hoyerii”
TA8	SNI-TA8-AZ5	*Symplocastrum flechtneri*	To be used in taxonomic revision of genus
TA35	SNI-TA35-BJ2	*Symplocastrum torsivum*	To be used in taxonomic revision of genus
TA35	SNI-TA35-BJ6	*Symplocastrum torsivum*	To be used in taxonomic revision of genus
TA17	SNI-TA17-BJ30	*Tolypothrix*	To be used in Tolypothrichaceae revision
TA17	SNI-TA17-BJ34	*Tolypothrix*	To be used in Tolypothrichaceae revision
TA31	SNI-TA31-BJ5	*Tolypothrix*	To be used in Tolypothrichaceae revision
TA31	SNI-TA31-BJ14	*Tolypothrix*	Partial 16S only
TA9	SNI-TA9-AZ2	*Trichocoleus* sp. 1	Putative new species
TA17	SNI-TA17-BJ3	*Trichocoleus* sp. 1	Putative new species

Table 2. A brief description of each sampling site used in this study. Coordinates and a brief description are provided. The number of cyanobacterial strains isolated and sequenced, as well as the number eukaryotic algae isolated from each site are listed and totaled at the bottom.

Site	Coordinates	Description	Cyanobacterial Strains Isolated	Cyanobacterial Strains Sequenced	Eukaryotic Strains Isolated
SBI	Composite	Diverse Composite	0	0	14
SNI-TA1	33.24761, -119.45800	Steep N-facing slope	5	2	0
SNI-TA2	33.22352, -119.44159	Badlands, Canyon	15	4	5
SNI-TA3	33.23795, -119.44460	Slope, W-facing drainage	20	3	3
SNI-TA4	33.26087, -119.49422	Endemic snail habitat	9	2	7
SNI-TA5	33.26087, -119.49422	Under shrubs near TA4	4	2	19
SNI-TA6	33.236632, -119.504441	Lichenized hilltop	23	5	10
SNI-TA7	33.2351101, -119.5056080	Slickrock near TA6	3	1	4
SNI-TA8	33.2349085, -119.5063286	Lichenized rugose crust	19	3	6
SNI-TA9	33.2304903, -119.5135552	Shallow depressions in slickrock	5	3	1
SNI-TA10	33.2253370, -119.5133181	Salt crust hoodoo, sloped	0	0	0
SNI-TA11	33.2304425, -119.5193350	Spring, near sea level	0	0	0
SNI-TA12	33.230516, -119.519103	Hummock above stream bed	2	1	2
SNI-TA13	33.230548, -119.518883	Hummock above stream bed	12	0	0
SNI-TA14	33.230320, -119.518651	Hill, covered in BSC	14	4	0
SNI-TA15	33.230701, -119.518852	Upslope from TA11	65	4	0
SNI-TA16	33.232866, -119.517705	Spring origin	50	2	2
SNI-TA17	33.232864, -119.517807	Cliff upslope of spring origin	33	11	6
SNI-TA18	33.232864, -119.517807	Damp rock above spring	12	4	0
SNI-TA19	33.2242076, -119.5069044	Gypsiferous soil	8	1	0
SNI-TA20	33.224336, -119.506701	Gypsiferous soil, highly crusted	22	3	0
SNI-TA21	33.224705, -119.506520	Fluffy gypsum soil with algal and chemical crust	30	0	0

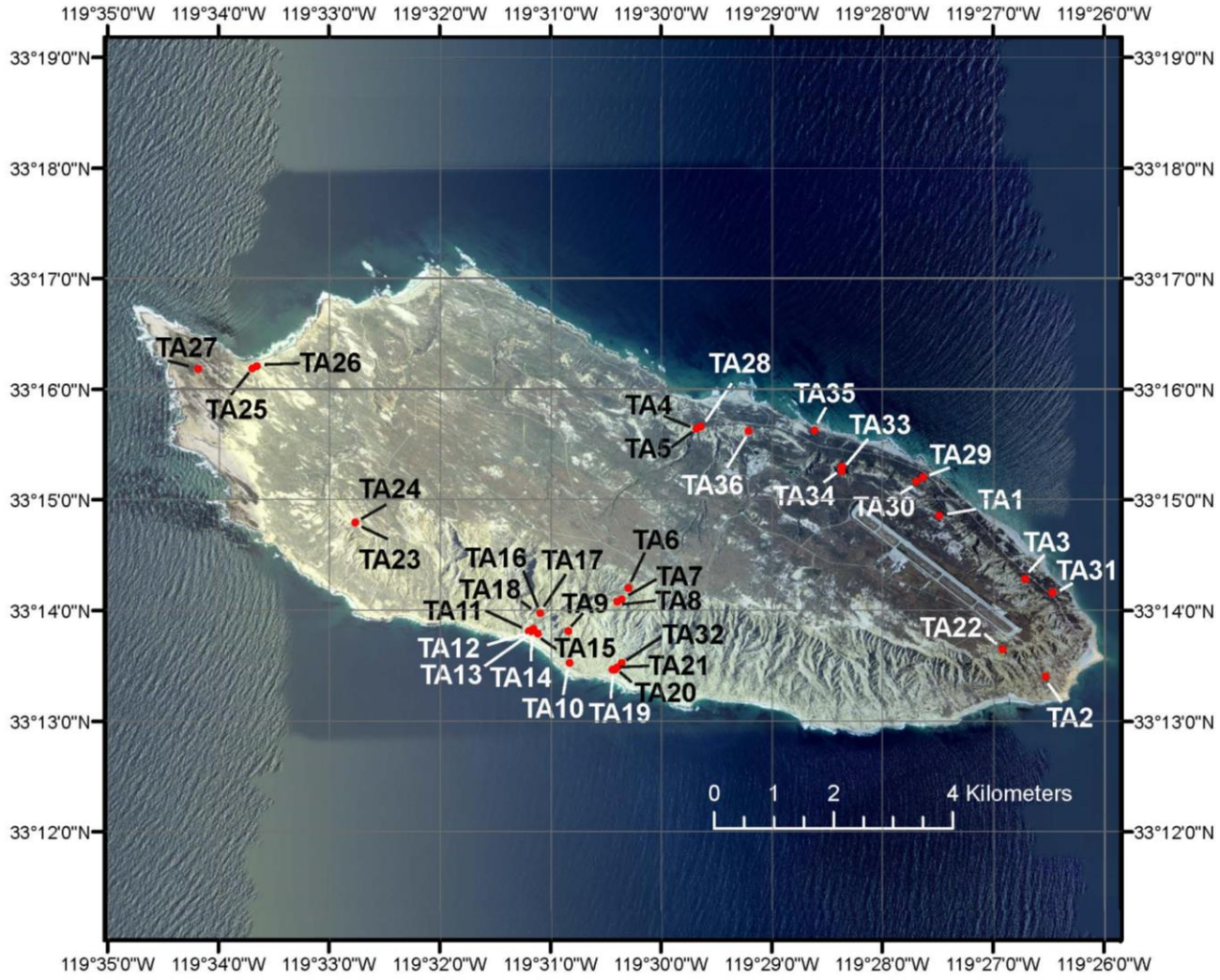
SNI-TA22	33.22740, -119.44797	Weathered lichenized soil on bluff	0	0	0
SNI-TA23	33.24653. -119.54604	Exposed caliche in small hollow	45	7	1
SNI-TA24	33.24653, -119.54604	Thin layer of topsoil on caliche	1	1	0
SNI-TA25	33.270189, -119.560364	Stabilized dunes	14	2	16
SNI-TA26	33.27037, -119.56095	Stabilized dunes	7	2	0
SNI-TA27	33.27007, -119.56968	Poorly developed yellow lichen crust	0	0	0
SNI-TA28	33.26110, -119.49345	Uncrusted soil near TA5	10	1	17
SNI-TA29	33.25362, -119.46002	Poorly crusted soil near road	19	2	5
SNI-TA30	33.25270, -119.46111	Lichenized soil upslope of TA29	0	0	0
SNI-TA31	33.23583, -119.44076	Fragile crust from Buckwheat dunes	17	3	2
SNI-TA32	33.225513, -119.505910	Wet soil in spring bed	0	0	0
SNI-TA33	33.25386, -119.47226	Vertical rock face, soil veneer	8	1	10
SNI-TA34	33.25398, -119.47222	Developed, pedicled crust on slope	8	1	1
SNI-TA35	33.25490, -119.47245	Developed, pedicled crust on slope	6	2	4
SNI-TA36	33.26033, -119.48643	Rugose, blackened, pedicled crust in canyon	6	1	0
Total			492	78	135

CHAPTER 1 FIGURE DESCRIPTIONS

Figure 1. Map of San Nicholas Island with sampling site locations superimposed.

Figure 2. A comparison of taxa that are likely common between the current study and Flechtner et al. (2008) based on morphological similarity. The top row are images of strains isolated from SNI, and the bottom row are micrographs and line drawings taken from Flechtner et al. (2008); scale bar = 10µm in all cases. The scale on the top left applies to the entire top row. A: Likely *Hassalia pseudoramosissima* (strain SNI-TA1-JJ1). B: *Oculatella* sp.1 (strain SNI-TA24-BJ1). C: *Myxacorys californica* (strain SNI-TA20-JG10). D–E: Micrograph and line drawing of *Hassalia pseudoramosissima* isolated in 2008. F: Micrograph of "*Leptolyngbya* sp.4" isolated in 2008. Note the red granules diagnostic of *Oculatella.* G: Line drawing of "*Leptolyngbya* sp.3" from 2008. Note the morphological similarity to *Myxacorys californica.*

Figure 3. A non-exhaustive collection of photos from some taxa isolated in this study. The top row are coccoid taxa, the middle row are non-heterocytous filamentous taxa, and the bottom row are heterocytous taxa. (Scale bar = 10µm, applies to entire row. A–B: *Aliterella sp. 1* (SNI-TA17-BJ5, SNI-TA17-BJ17). C: *Gloeocapsopsis* sp.1 (SNI-TA17-BJ17). D: *Pleurocapsa* sp.1 (SNI-TA17-BJ15). E: *Pseudoacaryochloris* sp.1 (SNI-TA23-BJ45). F: *Kastovskya* sp.1 (SNI-TA36-BJ6). G: New putative genus in the Coleofasciculaceae (SNI-TA19-BJ8). H: New putative genus in the Leptolyngbyaceae (SNI-TA16-BJ25). I: Putative new genus in the Nodosilineaceae (SNI-TA25-BJ8) J: New putative species "Stenomitos hoyerii" (SNI-TA33-BJ10). K: *Tolypothrix* sp. (SNI-TA17-BJ30). L: *Scytonema hyalinum* (SNI-TA29-BJ21). M: *Roholtiella edaphica* (SNI-TA28-BJ7). Note the purplish coloration not observed when this taxa was described. N: A new putative genus in the Nostocaceae (SNI-TA18-ML4). O: Putative new species "Atlanticothrix crispata" (SNI-TA23-BJ41).


119°35'0"W
119°34'0"W
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119°32'0"W
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119°29'0"W
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119°26'0"W
33°19'0"N
33°18'0"N
33°17'0"N
33°16'0"N
33°15'0"N
33°14'0"N
33°13'0"N
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Figure 1.

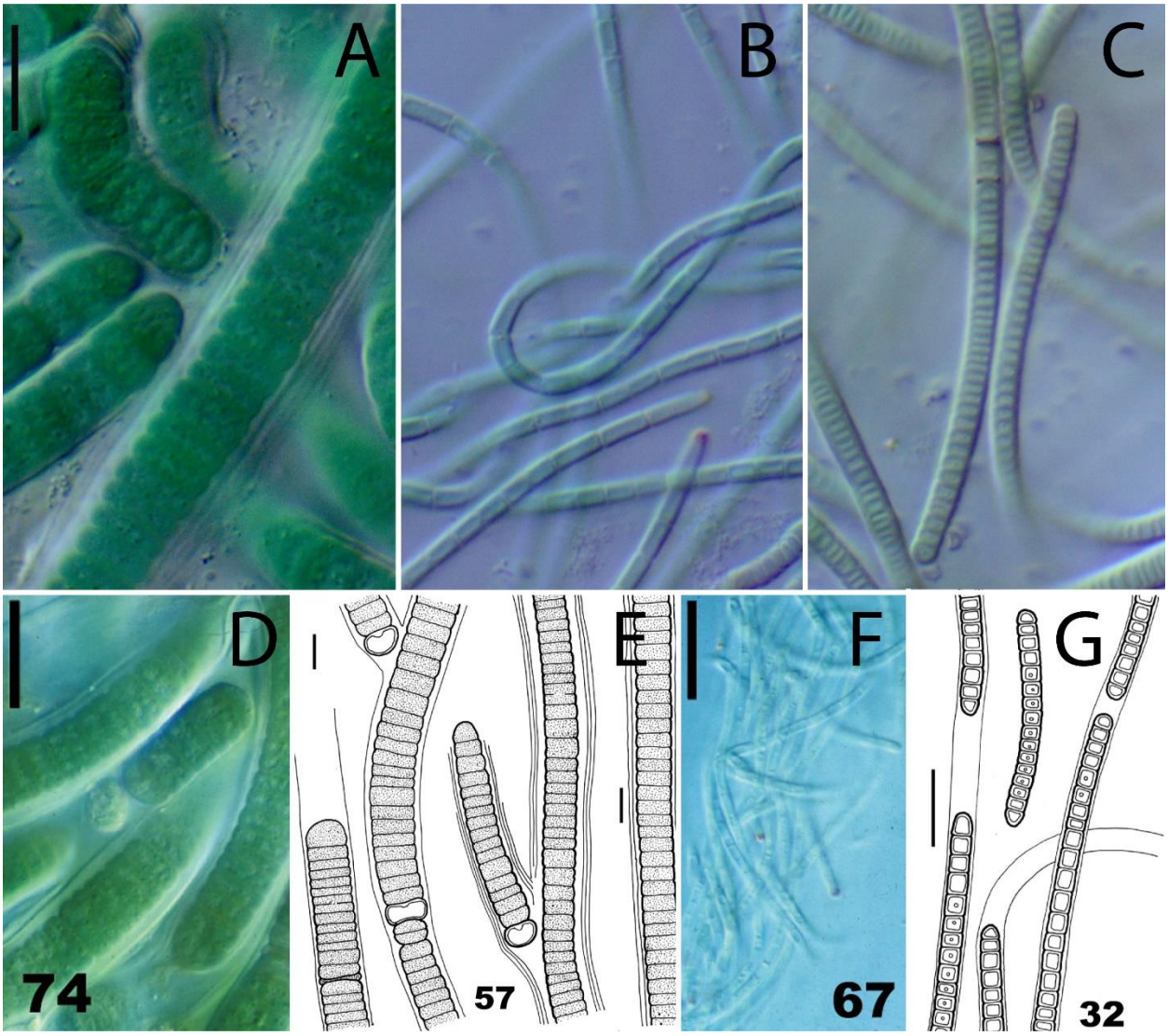

Figure 2.

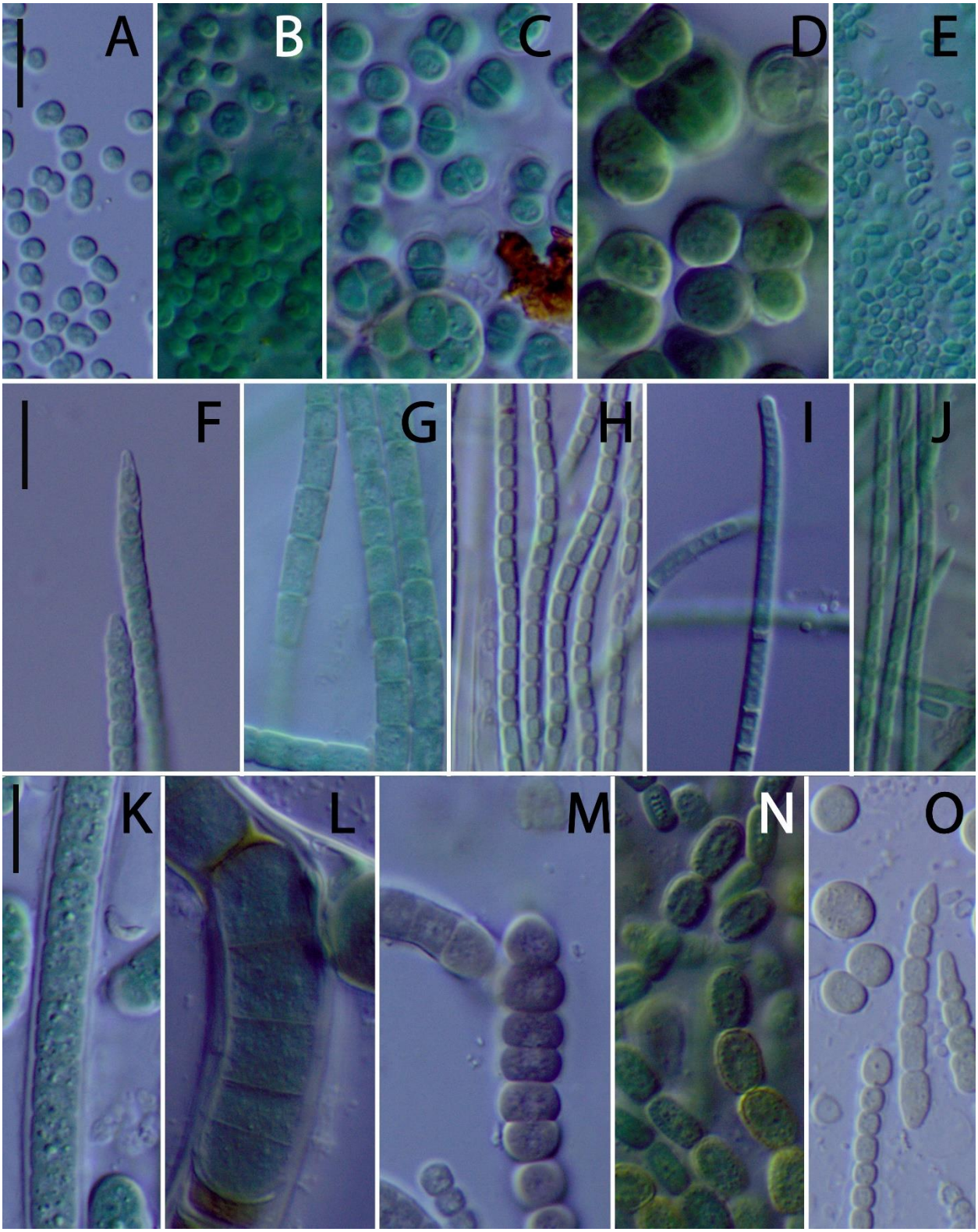

Figure 3.

Chapter 2: Description of Six New Cyanobacterial Species from Genera Previously Restricted to Brazil Using a Polyphasic Approach

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ABSTRACT

As the taxonomic knowledge of cyanobacteria from terrestrial environments increases, it remains important to analyze biodiversity in areas that have been understudied to fully understand global and endemic diversity. This study was done as part of a larger algal biodiversity study of the soil crusts of San Nicholas Island, California, USA. Among the taxa isolated were several putative new species in three genera (*Atlanticothrix, Pycnacronema,* and *Konicacronema,)* which were described from, and previously restricted to, Brazil. New putative taxa are described herein using a polyphasic approach to cyanobacteria taxonomy that considers morphological, molecular, and biogeographical factors. Morphological data corroborated by molecular analysis including sequencing of 16S rRNA and the 16S–23S ITS region was used to delineate three new putative species of *Atlanticothrix,* two putative species of *Pycnacronema,* and one putative species of *Konicacronema.* The overlap of genera from San Nicholas Island and Brazil suggests that cyanobacteria genera may be widely distributed in some cases, whereas the presence of distinct lineages may indicate that this is not true at the species level.

KEY WORDS: *Atlanticothrix, Pycnacronema, Konicacronema*, San Nicholas Island, Brazil, Species Distribution, Polyphasic Approach, Taxonomy, Cyanobacteria

INTRODUCTION

Although soils hold a significant portion of global microbial diversity, they are vastly understudied taxonomically. In particular, the biodiversity present in soils of arid environments is not well understood. In these areas, biological soil crusts (BSCs) consisting of a consortium of microorganisms (cyanobacteria, green algae, lichens, fungi, and heterotrophic bacteria) form on the top layer of the soil surface (Belnap 2003). BSCs are ecologically significant for their ability to prevent erosion, to prevent desiccation, and to increase soil fertility via atmospheric fixation of carbon and nitrogen ((Evans & Johansen 1999, Harper & Marble 1988, Kleiner & Harper 1972, Jeffries et al. 1993, Pietrasiak et al. 2013, West 1990). Among the most significant and abundant members of these crusts are cyanobacteria, which can bind soil particles and can increase biogeochemical cycling in an otherwise limited environment (Evans & Johansen 1999). Studies have shown cyanobacteria present in BSCs to be diverse, and new taxa continue to be described from studies in these environments (Baldarelli et al. 2022, Pietrasiak et al. 2021, Becerra-Absalón et al. 2018 & 2020, Flechtner et at. 2002).

As taxonomic knowledge of cyanobacteria increases, species representing genera previously thought to be geographically restricted have been found in distant locations (Brown et al. 2021, Casamatta et al. 2019, Hentschke et al. 2017, Miscoe et al. 2016 & Osorio-Santos et al. 2014). While some terrestrial cyanobacterial species such as *Microcoleus vaginatus* are known to have widespread distributions, most species have often been found only in relatively isolated areas (Dvořák et al. 2012 & Sherwood et al. 2015). It was previously hypothesized that microbial organisms might have cosmopolitan distributions (Becking 1934), but there has been little evidence to support this claim (Garcia-Pichel et al. 2001). Genera on the other hand can often be found in vastly different and isolated environments (Jung et al. 2020). Although the criteria that

group species into the taxonomic category "genus" are subjective, the evolutionary relationships among lineages are objective. Genetic relatedness suggests a more recent common ancestor, and the distribution of closely related species across vast geographical space raises questions about the speed and ease with which terrestrial algal taxa can become relocated and diversify.

San Nicholas Island (SNI), 98 km off the coast of Santa Barbara, California, USA is part of an eight-island archipelago known as the Channel Islands. Several of the Channel Islands comprise a national park; however, SNI serves as a Naval Base for the US Navy with public access restricted, leaving it relatively undisturbed compared to other habitats in the western USA. SNI experiences a semiarid climate receiving about 200 mm precipitation per year, albeit with relatively high humidity relative to other arid systems due to sea sprays from the Pacific Ocean. Although the island is small and primarily composed of limestone (58.9 km^2), it has significant topographic and geological variation. The northwest side of the island is characterized by stabilized sand dunes and a gentle slope, while the south side of the island is gypsiferous with steep cliffs. Also found on the island are natural springs, salty chemical crusts, and lichenized crusts at various stages of development. Due to the restricted access, only one previous soil crust study (solely using morphological data) was done on the island (Flechtner et al. 2008), making SNI an ideal site for a new taxonomic study integrating genetic data.

In this paper, three putative new species of *Atlanticothrix*, two putative new species of *Pycnacronema*, and one putative new species of *Konicacronema* are described with a polyphasic approach (Mühlsteinová et al. 2014) considering morphological, genetic, and biogeographical characteristics. *Atlanticothrix* is genus in the family Nostocales with one currently described species, *A. silvestris* (Alvarenga et al. 2021). *Pycnacronema* and *Konicacronema* are large filamentous genera recently moved to Wilmottiacea and Konicacronematacea, respectively

(Strunecký et al. 2023). There are currently eight recognized species of *Pycnacronema* and one species of *Konicacronema*, *K. caatingensis*. Currently, all species representing these three genera have been described from terrestrial environments in Brazil (Alvarenga et al. 2021, Machado-de-Lima & Branco 2020 & Martins et al. 2019). However, multiple strains representing new species have been found on SNI. The overlap among these genera (but not species) between North and South America may shed light on cyanobacterial distributions at the genus level.

METHODS

Field Collection

A total of 36 soil crust samples were collected from sites on SNI (Fig. 1) with one additional reference sample from nearby Santa Barbara Island (SBI). Samples SBI and SNI-TA1 through SNI-TA5 were collected 11 February 2021 and samples SNI-TA6 through SNI-TA36 were collected 25 May 2021. Samples were collected with a metal spoon and transferred into bags for storage and shipping.

Isolation and Culture

From each sample, 1.0 g subsamples were taken from various larger pieces of crusted soil, diluted in Erlenmeyer flasks with 100 mL sterile liquid Z8 media (Carmichael 1986) and agitated for 30 minutes on a rotary shaker to release cyanobacteria from the soil matrix. Soil slurries were further diluted 10^{-2} with 1.0 mL subsamples added to 9.0 mL sterile Z8 media. Enrichment plates of agar solidified Z8 were inoculated with 0.1mL representing each sampling site with 5 replicates. Plates were sealed with Parafilm and placed under a 12h light-dark cycle at 20°C until macroscopic agal colonies were visible (about 5 weeks). Uniagal cultures were obtained by picking well-isolated colonies under an Olympus SZ40 stereoscope using sterile

Pasteur pipettes pulled to a fine point. Cultures were placed in test tubes containing 5.0 mL sterile liquid Z8 media and returned to the 12h light cycle until significant growth was observed. Developed cultures were transferred to test tubes containing sterile agar solidified Z8 slants.

Morphological Characterization

Wet mounts were made by placing a small amount of biological material from each strain in liquid culture onto a glass microscope slide with a coverslip and immersion oil. Strains were observed, photographed and morphologically characterized using an Olympus BH-2 microscope equipped with Nomarski DIC optics and cellSens software. A minimum of 20 photographs were taken of each strain with care given to capture all specialized cell types and life-cycle stages. Length and width measurements were taken for vegetative cells, filament width, apical cells, and heterocytes and akinetes when applicable. Other relevant morphological features such as cell shapes and color were noted. *Atlanticothrix* strains were later transferred to nitrogen free Z8 media to promote further heterocyte growth and were again photographed.

To determine if *Atlanticothrix* strains could be separated in species based on cell size, a principal component analysis was performed using mean measurements (vegetative cell, akinetes, heterocytes, and apical cell) and length-width ratios obtained from *Atlanticothrix* strains in R (4.2.1). To obtain each average measurement value, 30 individual length and width measurements were taken for each cell type, averaged, and assigned to the respective strain.

Molecular Characterization

Genomic DNA was extracted from strains using Qiagen DNeasy Powersoil Pro Kits following the manufacturer's protocol and eluted in 50 µL of elution buffer. DNA presence was confirmed with a 1% TBE agarose gel and stored at -20°C. DNA samples were used to amplify

the 16s rRNA gene and the 16S–23S internal transcribed spacer (ITS) region using primers VRF1R and VRF2F (Wilmotte et al. 1993). The reaction mixture containing 1 µl of each primer at 0.01 mM concentration was combined with 12.5 µl of LongAmp™ Taq 2x Master Mix (NEB, Ipswitch MA), 1 µl template DNA (50 ng/ml) and 9.5 µl nuclease free water. This PCR mix was subjected to 35 cycles of denaturing (94º for 45 sec), annealing (at 57º for 45 sec), an extension (at 72º for 135 sec), and a final extension (for 5 minutes). The PCR reaction was performed using both 1 µL and 2 µL samples of genomic DNA (varying the amount of dH_2O), and the stronger of the two reactions was chosen for further downstream use via visualization on a 1% TBE agarose/ ethidium bromide gel. PCR products were inserted into plasmid pSC-A-amp.kan and cloned into the LacZ gene of StrataClone (Agilent, Santa Clara, CA) competent *Escherichia coli* cells via heat shock following the manufacturer's protocol. *Escherichia coli* cells were plated on agar-solidified LB-ampicillin plates with 40 µL X-Gal, and three properly transformed colonies were picked via blue-white screening. Overnight cultures were grown, and plasmid DNA was isolated with Qiagen QIAprep Miniprep kits following the manufacturer's protocol. Insertions were confirmed by EcoR1 restriction enzyme digest followed by visualization on 1% TBE agarose gels. Two or three clones of each strain were sent to Functional Biosciences, Inc (Madison WI, USA) for Sanger sequencing. Primers M13 forward and M13 reverse and internal primers VRF5 (5' –TGT ACA CAC CGG CCC GTC 3'), VRF7 (5'- AAT GGG ATT AGA TAC CCC AGT AGT C -3'), and VFR8 (5'- AAG GAG GTG ATC CAG CCA CA -3') (Nübel et al. 1997, Wilmotte et al. 1993) were used to obtain partial overlapping sequences. Sequences were error proofed using Chromas software (version 2.6.6) and assembled into contigs by alignment with ClustalW (Larkin et al. 2007). When possible, two or three clones were used to construct consensus sequences.

Phylogenetic Analyses

The 16s rRNA gene was subjected to analysis by both Bayesian inference (BI) and maximum likelihood (ML) analysis using the CIPRES Science Gateway (Miller et al. 2010) to obtain posterior probability and bootstrap support values for each node in the tree. BI analyses were performed using MrBayes on XSEDE 3.2.6 (Ronquist et al. 2012), and ML analyses were performed using RAxML-HPC2 on XSESE 8.2.10 (Stamatakis 2014). In both cases, the GTR+I+G evolutionary model was used. The BI analysis was run for 80 million generations with the first 25% of samples discarded as burn-in. The ML analysis was run on the same alignments with 1000 bootstrap iterations. 16S rRNA gene analyses were performed at the family or order level with several representatives from each related genus for which sequences were available in GenBank to produce robust phylogenetic trees. Posterior probability and bootstrap support values (from BI and ML analyses, respectively) were considered in the analysis and superimposed on the phylogenetic trees at the appropriate nodes. ITS phylogenetic analyses were performed at the genus level with BI with the parameters listed above to determine relationships to closely related strains. Phylogenetic trees were visualized with Fig Tree (Rambaut 2009) and reconstructed in Adobe Illustrator (Adobe Systems, San Jose, California).

Percent similarity of 16S rRNA sequences was determined using the SHOWDIST command in PAUP (Swofford 1998). Percent dissimilarity of 16S-23S ITS region sequences were calculated in PAUP. ITS dissimilarity values were determined with alignments of sequences from strains related at the genus level. Hypothetical ITS secondary structures for the D1-D1', Box-B, V2 and V3 helices were identified based on conserved basal clamps. Location of basal clamps for SNI strains were determined by creating multiple alignments of closely related taxa with ClustalW (Larkin et al. 2007) and using existing taxa with known helix locations as a

guideline. Secondary structures were determined via folding in Mfold (Zuker 2003). Drawing mode was set to untangle with loop fix and all other settings were set to default. Secondary structures derived from Mfold were reconstructed in Adobe Illustrator (26.4.1) by manually adding base pairs. Lines connecting bases were used to represent canonical pairings and dots were used to represent noncanonical U-G pairings.

RESULTS

Atlanticothrix

Putative species "Atlanticothrix crispata"

Fig. 2 L–Q

Description: Colonies on agar soft, mucilaginous, not shiny, becoming mounded, blue-green, purplish, or brownish depending on stage of life cycle. Filaments with slight diffluent mucilage, not forming *Nostoc*-like colonies, often with mucilage not evident, consisting of vegetative cells early in life cycle, but soon producing long continuous series of purplish to brownish akinetes. Trichomes of vegetative cells thin, with conical vegetative cells at ends, or with bluntly-conical and flame-shaped terminal heterocysts, constricted at crosswalls, blue-green or purple in color when composed of vegetative cells, brown in color when composed of maturing-to-mature akinetes, 1.8–3.4 μm wide (2.6 μm average). Vegetative cells not constricted to bead-like series, but sharing connections at the crosswalls so that cells barrel-shaped or cylindrical, sometimes slightly constricted in mid-cell, mostly non-granular, with some being minutely granular, 1.9–4.9 μm long (2.9 μm average). Heterocytes mostly terminal with a single polar nodule, rounded, becoming elongated and bluntly conical 2.0–3.1 μm wide and 3.0–5.3 μm long, rare intercalary heterocytes oval, with paired polar nodules 2.0–4.0 μm wide, 2.4–4.4 μm long. Akinetes develop

continuously from vegetative cells by increase in both length and width, when mature having thickened outer wall, before germination transitioning from brown to blue-green in color, revealing multiple vegetative cells immediately before germination when akinete can become enlarged to accommodate curled germinating trichomes, 3.2–7.4 μm wide, 3.1–7.4 μm long. Discarded cell walls of akinetes persist following release of hormogonia.

Etymology: L. *crispatus* = curly, referring to the tightly curled hormogonia produced in akinetes before germination.

Putative species "Altanticothrix nostocoides"

Fig. 2 G–K

Description: Colonies on agar soft, mucilaginous, somewhat shiny, becoming mounded, blue-green, purplish, or brown depending on stage of life cycle. Filaments with slight diffluent mucilage, occasionally with mucilage not evident, consisting of vegetative cells early in life cycle, but soon producing long continuous series of compressed blue-green to purplish brown akinetes. Filaments often form large *Nostoc*-like colonies with evident diffluent mucilage. Trichomes of vegetative cells thin, bluntly-conical apical cells or bluntly flame-shaped heterocysts at one or both ends, constricted at cross walls, blue-green to purplish in color, blue-green or purplish or brownish when akinetes develop, 2.2–4.4 μm wide. Vegetative cells barrel-shaped, occasionally minutely granular, 2.0–4.4 μm long. Heterocytes mostly terminal with a single polar nodule, bluntly rounded to bluntly flame-shaped, 2.4–4.2 μm wide, 2.4–5.2 μm long. Rare intercalary heterocytes oval with paired polar nodules, 3.6–3.8 μm wide, 3.6–5.2 μm long. Akinetes develop continuously from vegetative cells by increase in length and width, when mature having a thickened outer wall, forming in long continuous compressed series before

dissociating at maturity, becoming blue-green before germination, revealing multiple vegetative cells immediately before germination when akinetes become enlarged to accommodate germinating trichomes, 3.6–7.6 μm wide, 2.6–7.4 μm long. Discarded akinete cell walls persist following release of hormogonia.

Etymology: L. *nostocoides* = *Nostoc*-like, so named because this is the only species in the genus observed thus far that can produce *Nostoc*-like mucilaginous colonies.

Putative species "Atlanticothrix testacea"

Fig 2. A–F

Colonies on agar soft, mucilaginous, shiny when composed of vegetative cells and not shiny when akinetes develop, becoming mounded, shifting from blue-green to golden brown as vegetative cells become akinetes. Filaments with evident diffluent mucilage, often with mucilage absent, consisting of vegetative cells early in life cycle, but soon producing long continuous series of compressed blue green to golden brown akinetes. Trichomes of vegetative cells thin, with rounded vegetative cells at ends, or with rounded or bluntly flame-shaped heterocytes at one or both ends, constricted at crosswalls, blue-green or purplish in color when consisting of vegetative cells, purplish to golden brown in color when composed of maturing to mature akinetes, 2.2-4.0 μm wide. Vegetative cells constricted to bead-like series or barrel-shaped, mostly non-granular but occasionally minutely granular, 1.6–3.6 μm long. Heterocytes mostly terminal with a single polar nodule, rounded or bluntly flame shaped, 2.8–4.3 μm wide and 2.4–4.4 μm long, rare intercalary heterocytes rounded to oval, with paired polar nodules, 4.8 μm wide and 3.6 μm long. Akinetes develop continuously from vegetative cells by increase in length and width, when mature having a thickened outer wall, before germination transitioning from golden

brown to blue-green, revealing multiple vegetative cells immediately before germination when akinete can become enlarged to accommodate germinating trichomes, 4.2–8.0 μm wide, 2.8–7.2 μm long. Discarded cell walls of akinetes persist following release of hormogonia.

Etymology: L. *testaceus* = brownish yellow, named for the golden brown color of the mature akinetes.

Phylogenetic Analyses

The 16S rRNA analysis (Fig. 3) performed with all SNI *Atlanticothrix* strains and all *A. silvestris* strains formed a well-supported (0.99 BI posterior probability, 77 ML bootstrap support) monophyletic generic clade sister to *Roholtiella.* The *A. silvestris* strains formed a monophyletic clade within the genus with a posterior probability of 0.96 suggesting that the Brazilian strains form a species that is a different lineage from all SNI strains. The 16S rRNA analysis at the species level among the SNI strains was, however, uninformative. All *Atlanticothrix* strains are >98.7% similar in 16S rRNA sequence (Table 1), and the BI/ML analyses were unable to clearly group together SNI strains at the species level.

Analysis of 16S–23S ITS

The 16S–23S ITS analysis (Fig. 4) was more informative at resolving species. The ITS phylogenetic analysis performed with available *Atlanticothrix* strains produced a tree with three well-supported (all 100% bootstrap support) species among the SNI strains. One operon of strain SNI-TA23-BJ35 fell sister to the *A. silvestris* clade, whereas the other operon fell clearly within the "A. crispata" clade. All SNI species were at least 8.0% dissimilar from each other in the ITS region except one operon of *Atlanticothrix* sp. SNI-TA23-BJ35, which differed at 4.9% from *A. silvestris* and >9.5% from the "A. crispata" clade. However, the other operon differed <1% from

"A. crispata" and >8.8% from *A. silvestris* (Table 2). Within each species with multiple strains, ITS dissimilarity was <1% except for the operon mentioned above. Dissimilarity values above 7% are strong evidence that strains are different species, suggesting strongly that the SNI strains form three distinct lineages, none of which are *A. silvestris.*

Analysis of ITS Secondary Structures

Hypothetical secondary structures of conserved domains of the 16S-23S ITS region varied among each species. The D1-D1' helix (Fig. 5 A–C) varied only slightly with all three SNI strains (except one operon) sharing identical helices. The SNI structure varied slightly from the *A. silvestris* structure in the penultimate internal bulge with an additional adenine on either side, and one fewer set of paired bases before the next bulge. The SNI Box-B helices (Fig. 5 D–G) were all somewhat different from the *A. silvestris* structure and were six base pairs longer. The V3 helices (Fig. 5 H–K) were also somewhat similar among all species, although some base pair differences were observed among SNI strains and *A. silvestris* strains. The V2 structure (Fig. 6) was the most informative at resolving lineages to the species level. The V2 representing "A. testacea" (Fig. 5–A) was very different from all other structures with one large internal bulge not observed in any other strain. The strains representing "A. nostocoides" (Fig 5 B–D) had in common four internal bulges with the top and bottom bulges terminating in a non-canonical U-G pairing at one end. The "A. crispata" helices (Fig. 5 E–F) were the most similar to *A. silvestris* (Fig. 5 H–I); however, they shared in common a terminal loop sequence and an unpaired uracil three base pairs upstream of the terminal loop. One operon of SNI-TA23-BJ35 had unique structures from all other SNI and *A. silvestris* strains (Fig. 5–G). The V2 helix was the portion of the ITS region with the highest proportion of overall dissimilarity among strains and is the best evidence of separate lineages.

Morphological Analysis

All strains were grown in identical conditions and distinctive morphological features were observed among species. “A. crispata” was distinctive in the formation of curled hormogonia with conical end cells inside germinating akinetes (Fig. 2–P). This feature was observed regularly in multiple strains of this species and never observed in strains of other species. The akinetes of this species formed in uncompressed series and were often longer than wide which was unlike the highly compressed wider-than-long series observed in the “A. nostocoides” and “A. crispata” (Fig. 2 C–F, I–J, N–O). “A. testacea” was unique in its formation of golden brown akinetes (Fig. 2-C).

Strains of “A. nostocoides” were the only strains to exhibit formation of large distinctive *Nostoc*-like colonies (Fig. 2–K). Colonies with obvious diffluent mucilage formed consistently in all strains representing the species and were never observed in other species, including *A. silvestris*.

Vegetative cells and apical cells also varied among species. “A. crispata” often developed rounded end cells that were indistinguishable from other vegetative cells in the filament (Fig. 2-A). This was also the only species to develop rounded vegetative cells in a bead-like series. In most cases, “A. nostocoides” developed bluntly conical apical cells that were longer than wide (Fig. 2–G). On the other hand, “A. testacea” developed distinctly conical end cells coming to a fine point (Fig. 2–L). This type of apical cell was not observed in the other species.

There was overlap in many of the length and width measurements for each cell type among species, so a principal component analysis (PCA) was performed (Fig. 7) using mean length and width measurements for vegetative cells, apical cells, heterocysts, and akinetes as

well as length-width ratios for each measurement (based on 30 measurements for each parameter). The PC1 and PC2 axes explained 54.6% and 21.3% of variation in the data, respectively, for a total of 75.9% variation explained. Vegetative cell length, width, and length-width ratio, as well as apical cell length-width ratio were the traits most strongly associated with PC1. Heterocyte width and akinete diameter were the traits most strongly associated with PC2. Heterocyte length and length-width ratio contributed to both axes. In general, strains were separated into species groups along PC1. "A crispata" was isolated from all other strains in the analysis on the far left of the graph. Strains representing "A. nostocoides" formed a group in the center of the graph. "A. testacea" strains were the most ambiguous with two on the far right and one at both the top and bottom of the PCA plot. Although some overlap exists in cell measurements among species, the PCA along with the previously mentioned distinctive features serves as evidence that the lineages have evolved diagnosable morphological traits.

Pycnacronema

Putative species "Pycnacronema juana-mariae"

Fig. 8 A–E

Filaments solitary or forming non-entangled bundles, without trichomes sharing common sheath, 7.0–8.2 µm wide. Sheaths firm, hyaline, thin, occasionally extending beyond trichome. Trichomes with gliding motility, minutely-constricted at crosswalls, cylindrical, tapered slightly when conical apical cell present, lacking necridia, 4.6–7.0 µm wide. Cells wider than long or isodiametric, green to blue-green, with visible crosswalls, occasionally with granular contents, with trichomes sometimes appearing distinctively fasciculated, 2.6–5.8 µm

long. Apical cells bluntly rounded when hormogonia form but becoming elongated and bluntly conical, yellow in color, 4.8–6.8 µm wide, 5.0–10.6 µm long.

Etymology: Named for Juana Maria, the last Native American inhabitant of San Nicolas Island.

Putative species "Pycnacronema aeruginosum"

Fig. 8 F–K

Filaments either solitary or forming bundles of parallel filaments, without trichomes sharing a common sheath, 4.6–6.6 µm wide. Sheaths firm, hyaline, thin, occasionally extending beyond trichome, sometimes appearing wavy or lamellated. Trichomes with gliding motility, minutely constricted at crosswalls, occasionally constriction not evident, cylindrical, tapering slightly when conical end cell present, lacking necridia, 4.2–5.2 µm wide. Cells approximately isodiametric but can be longer or shorter than wide, blue-green, sometimes slightly yellow near apices, often granular, with trichomes appearing distinctively fasciculated, 3.4–6.5 µm long. Apical cells bluntly-rounded after hormogonia form, but becoming elongated and conical, yellowish in color, 3.6–5.2 µm wide, 4.2–9.4 µm long.

Etymology: L. *aeruginosus* = bright blue-green, named for the intense blue-green color of the trichomes.

Phylogenetic Analysis

Based on 16S rRNA analysis (Fig. 9), *Pycnacronema* forms a well-supported generic clade (0.91/50 BI posterior probability/ ML bootstrap support) sister to *Symplocastrum,* albeit with two taxa assigned "Microcoleus paludosus" SAG 1449-1a and "*Parifilum solicrustae*"

SON57 suggesting some revision is necessary. Both “P. juana-mariae” and “P. aeruginosum” fall inside the genus as separate lineages from previously described *Pycnacronema* species.

Analysis of 16S–23S ITS

Based on phylogenetic analysis of the 16S–23S ITS (Fig. 10), “P. juana-mariae” and “P. aeruninosum” are distinct lineages from any of the previously described species. The species level nodes were highly supported in most cases. Both “P. aeruginosum” and “P. juana-mariae” had overall ITS dissimilarity values >10% from other species which is good evidence that both putative species are different lineages from each other and all other described species.

Analysis of ITS Secondary Structures

There was significant variation in the secondary structures among all *Pycnacronema* species (Fig. 11). All species shared a conserved basal clamp in the D1-D1' helix; however, variation in both size and sequence was observed in the terminal loop and other bulges along the helix. “P. aeruginosum” was the most different from the others with a significantly longer stem and a 3 bp bulge before the terminal loop. Variation was observed among species in the Box-B helix as well. Differences in terminal loop sizes and sequences were observed. Both “P. juana-mariae” and “P. aeruginosum” were unique to all other helices, and *P. arboriculum* had a non-canonical U-G pairing near the terminal loop not observed in any other species. Perhaps the most variation was observed in the V3 helix. Species varied in the number and size of bulges, at the terminal loops, non-canonical base pairings, and overall size of the helix. “P. aeruginosum” had a notably shorter V3 helix than another other species at 36 base pairs. “P juana-mariae” differed from other species in the sequence of its terminal loop, as well as the presence of a bulge with one unpaired adenine on the upstream side of the terminal loop and three unpaired adenines on

the downstream side. Differences in the ITS secondary structures serve as good evidence of distinct lineages at the species level, suggesting the two new putative species are distinct from other *Pycnacronema* lineages.

Konicacronema

Putative species "Konicacronema haraasnagensis"

Fig. 12

Filaments either solitary, entangled, or forming twisted bundles, without trichomes sharing a common sheath, 4.4 - 6.6 µm wide. Sheaths thin, hyaline, occasionally extending beyond trichome, sometimes not evident. Trichomes with gliding motility, distinctly constricted at crosswalls, cylindrical, tapering slightly at one to several terminal cells when conical end cell present, lacking necridia, 3.8-4.9 µm wide. Cells wider than long to isodiametric, blue-green in color, slightly yellow at crosswalls, granular, chromoplasm often restricted to parietal region of cell with trichomes sometimes appearing fasciculated, 2.6-4.4 µm long. Apical cells bluntly rounded when hormogonia form but becoming bluntly to distinctly conical, 3.0-4.6 µm wide, 3.6-11.2 µm long. Rare multiple *Kastovskya*-like protrusions sometimes form from apical or penultimate cell in filament (Fig. 12-E).

Phylogenetic Analysis

Based on 16S rRNA analysis (Fig. 9), *Konicacronema* forms a well-supported (1.0/96 BI posterior probability/ ML bootstrap support) generic clade, although containing strains *Funiculus tenuis* HSN023 and *Trichocoleus sociatus* SAG 26 92 which should be revised. *Funiculus* is a later synonym of *Konicaronema*, and therefore *F. tenuis* will require transfer to *Konicacronema.* *Trichocoleus sociatus* is a validly described taxon but is outside of the *Trichocoleus* clade most

likely containing the type species, which is not yet sequenced (Mühlsteinova et al. 2014). The 16S rRNA analysis produced a well-supported (0.9, 89 BI/ML) species level clade consisting of all three "K. haraasnagensis" strains serving as evidence of a lineage unique from the type species. "K. haraasengensis" 16S rRNA gene sequences were 98.2-98.8% similar to *K. caatingensis* strains. Although this does not meet the 98.7% threshold in some cases, it is quite close even at the high range of similarity. This is ambiguous evidence of a distinct species; however, high dissimilarity in the ITS region provides strong evidence that these are distinct lineages.

Analysis of 16S-23S ITS and Secondary Structures

All strains of "K haraasnagensis" were >11.8% dissimilar from *K. caatingensis* which serves as good evidence that the lineage is distinct from the type species. Additionally, variation was observed in the secondary ITS structures between the type species and "K. haraasnegensis (Fig. 13). The D1-D1' terminal loops varied significantly in size, with 11 and 4 base pairs respectively. "K. haraasnagensis" also showed a U-G pairing directly before the terminal loop that was not observed in the type species. Significant variation was also observed in the Box-B and V3 helices terminal loops and presence of U-G pairings between the species .

DISCUSSION

The use of a polyphasic approach (Johansen & Casamatta 2005, Mühlsteinová et al. 2014) to cyanobacterial taxonomy continues to be important in determining lineages worthy of taxonomic recognition. The use of both morphological and molecular characteristics for species delineation were integral to developing holistic arguments for species recognition in this study. The use of 16S rRNA similarity thresholds for prokaryotic taxonomy has been shown to be

inadequate as the sole criterion for species diagnosis due to insufficient variability in this region (Fox 1993), and this is highlighted especially in the case of *Atlanticothrix*. While none of the putative species meet the <98.7% 16S similarity threshold historically used to differentiate prokaryotic species in this genus, there are repeatable, diagnosable morphological characteristics that may demonstrate lineage differentiation. The formation of *Nostoc*-like colonies in "A. nostocoides" (never observed in other species), along with variation in vegetative cell, heterocyte, and akinete size and shape among species suggests that these groups are unique and could be confidently reidentified at the species level should they be isolated again in the future. Morphological evidence is strongly corroborated by differences in the ITS region, with species being >8% dissimilar in this region and varying in their secondary structures. The ITS region, unlike the 16S rRNA, is highly conserved at the species level but can vary significantly among closely related species at the genus level (Boyer 2001 & 2002, Johansen et al. 2011). The V2 helices are especially informative in this case, as they vary significantly among species but do not vary significantly when strains representing a species are considered. Additionally, biogeographical and ecological considerations serve as further evidence of unique lineages. The type species was originally isolated from the Atlantic Forest in Brazil, which has conditions significantly different from those found San Nicholas Island, and which is several thousand kilometers separated geographically. Because these data sets are aligned, the total evidence is strong that the three new putative species are cohesive lineages separate from each other and also from *A. silvestris*.

In the case of *Pycnacronema* and *Konicacronema*, the genetic evidence more strongly suggests the new putative species are distinct from all previously described species. The new species range from 10.4–27.2% overall dissimilarity in the ITS region which is significantly

higher than the 7% threshold, suggesting very strong evidence of distinct species lineages (Erwin & Thacker 2008, Osorio-Santos et al. 2014, Pietrasiak et al. 2014 & 2019). Again, the ITS region is conserved at the species level but varies significantly among members of a genus, and both congeneric groups show significant differences in this area in sequence and secondary structure.

The presence on SNI of species in multiple genera originally described from Brazil raises questions about the distribution of closely-related taxa and the vectors responsible for their dispersal. Unlike species which are real biological entities, genera are an arbitrary (yet useful) taxonomic classification that serves to group monophyletic sets of similar species sharing a relatively recent common ancestor. The criteria and genetic similarity thresholds that researchers have developed to determine which taxa constitute a genus do not describe objective biological reality; however, the close evolutionary relationships among the species assigned to a genus are objective. Consequently, it is worthwhile to investigate distribution patterns at the genus taxonomic level to further understand the sources of endemism common in terrestrial cyanobacterial taxa.

Whereas microbes in aquatic environments have numerous potential distribution vectors such as waterfowl and water currents, terrestrial algae are generally limited to wind as a significant driver of dispersal (Marshall & Chambers 1997). Cyanobacteria have been found in atmospheric collections (Després et al. 2012), and it has been shown that biological soil crusts can become eroded such that they can become aerosolized and be carried by wind currents (Büdel et al. 2004). A potential common origin to the lineages which separated into the endemic species in Brazil and SNI may exist in Africa. North Africa (the Sahara, specifically) is the Earth's largest producer of dust, and particles originating from this region have been detected in both North America and the Amazon in appreciable numbers (Engelstaedter et al. 2006, Prospero

et al. 2021). Although the three genera in this paper have not been isolated in Africa, it would be unsurprising if they were in future studies. With further investigation, it is probable that more overlap will be found between genera found in North and South America, and possibly Africa. Among the cyanobacteria in general, there is a phenomenon of hemispherically restricted genera (Mühlsteinová et al. 2014, Pietrasiak et al. 2019 & 2021); however, species in a genus may be widely distributed in respective hemispheres. This again may be a consequence of global wind patterns that are in most cases unlikely to carry microbial organisms across the equator due to the Coriolis Effect.

While it is unclear when the Brazilian and SNI *Atlanticothrix, Pycnacronema,* and *Konicacronema* species last shared a common ancestor, the genetic and morphological divergence among them suggests evolutionarily significant time has passed since the lineage was cohesive. This provides further evidence that species of terrestrial algae (especially in BSCs) disperse slowly regardless of which vectors are responsible for their distribution, and that cosmopolitan distributions in these taxa are unlikely. Future floristic studies on San Nicholas Island and elsewhere need to be undertaken to understand the full extent of cyanobacterial diversity, and thus to better understand distributional patterns. Although the taxa in this study come from environments with some overlapping attributes, it is likely that sampling bias (to some extent) has played a role in the apparent distribution of these taxa. In recent years, a significant number of taxa have been described form Brazil due to a concerted effort by researchers (Alvarenga et al. 2021, Machado-de-Lima & Branco 2020, Martins et al. 2018) , and it is possible that this has led to the assumption that the region is particularly diverse. While it is likely that Brazil is diverse given its size and wide range of environmental conditions, other regions across the globe remain under sampled due to lack of funding and researchers in the

field. Further sampling efforts in understudied and underfunded regions are necessary to make this determination.

ACKNOWLEDGMENTS

I would like to thank the John Carroll University Biology department for their support throughout this project. Special thanks are owed to the professors in the department who have provided input relating to this project, as well as personal and professional guidance and support. In particular I would like to thank Dr. Ralph Saportio, Dr. James Watling, Dr. Jennifer Murphy, Dr. Cari Hickerson, and Dr. Carl Anthony. I would also like to thank my committee members, Dr. Christopher Sheil and Dr. Rebecca Drenovsky for their guidance. I would especially like to thank my advisor, Dr. Jeffrey Johansen for his continued professional and personal support and his assistance with this project and my development as a researcher. Thanks to Amanda Szinte and Chelsea Villanueva for helping me to learn the molecular methods necessary for the sequencing aspect of this project. Several graduate and undergraduate students also provided assistance with isolating cultures, taking microscope pictures, and making herbarium vouchers. In particular, I would like thank Mathew Luknis, Emily Masluk, Salvador Peron, Elliot Cho, Angela Burns, and Braden Lau. Additionally, I would like to thank the John Carroll Graduate School for funding throughout the master's program. The US Navy provided the funding for this project as part of a larger metagenomic study to preserve endangered snails on San Nicholas Island. Thanks to Bill Hoyer and Kristen Lehman for their help with sample collection.

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Table 1. *Atlanticothrix* 16S rRNA similarity matrix. Highlighted values indicate similarity among strains within a putative species.

	A. silvestris CENA590 MW326977	*A. silvestris* CENA564 MW326973	*A. silvestris* CENA357 NR172568	*A. Silvestris* CENA368 KR137589	*A. silvestris* CENA585 MW326976	*A. silvestris* CENA576 MW326974	*A. silvestris* CENA5 9 MW326975	A. testacea SNI-TA4-BJ2 OQ780331	A. nostocoides SNI-TA1-JJ4 OQ780330	A. nostocoides SNI-TA5-JJ1 OQ780333	A. nostocoides SNI-TA5-JJ3-1 OQ780334	A. nostocoides SNI-TA5-JJ3-2 OQ780335	A. crispata SNI-TA23-BJ35-1 OQ780337	A. crispata SNI-TA23-BJ35-2 OQ780338	A. crispata SNI-TA23-BJ35-3 OQ780339	A. crispata SNI-TA23-BJ41-1 OQ780340	A. crispata SNI-TA23-BJ41-2 OQ780341	A. crispata SNI-TA23-BJ41-3 OQ780342	A. crispata SNI-TA26-BJ1 OQ780343	A. crispata SNI-TA26-BJ7 OQ780344
A. silvestris CENA590																				
A. silvestris CENA564	99.7																			
A. silvestris CENA357	99.8	99.7																		
A. silvestris CENA368	99.9	99.8	99.9																	
A. silvestris CENA585	99.8	99.7	99.8	99.9																
A. silvestris CENA576	99.8	99.7	99.8	99.9	99.8															
A. silvestris CENA579	99.7	99.6	99.7	99.7	99.7	99.7														
"testacea" SNI-TA4-BJ2	99.7	99.6	99.7	99.7	99.7	99.7	99.5													
"nostocoides" SNI-TA1-JJ4	99.8	99.7	99.8	99.9	99.8	99.8	99.7	99.8												
"nostocoides" SNI-TA5-JJ1	99.7	99.7	99.7	99.8	99.7	99.7	99.6	99.7	99.9											
"nostocoides" SNI-TA5-JJ3-1	99.7	99.6	99.7	99.7	99.7	99.7	99.5	99.7	99.8	99.7										
"nostocoides" SNI-TA5-JJ3-2	99.6	99.5	99.6	99.7	99.6	99.6	99.4	99.6	99.7	99.7	99.6									
"crispata" SNI-TA23-BJ35-1	99.5	99.4	99.5	99.6	99.5	99.5	99.3	99.5	99.7	99.6	99.5	99.4								
"crispata" SNI-TA23-BJ35-2	99.6	99.5	99.6	99.7	99.6	99.6	99.4	99.6	99.7	99.7	99.6	99.5	99.9							
"crispata" SNI-TA23-BJ35-3	99.6	99.5	99.6	99.7	99.6	99.6	99.4	99.6	99.7	99.7	99.6	99.5	99.4	99.5						
"crispata" SNI-TA23-BJ41-1	99.7	99.6	99.7	99.7	99.7	99.7	99.5	99.7	99.8	99.7	99.7	99.6	99.5	99.6	99.9					
"crispata" SNI-TA23-BJ41-2	99.5	99.4	99.5	99.6	99.5	99.5	99.3	99.5	99.7	99.6	99.5	99.4	99.3	99.4	99.7	99.8				
"crispata" SNI-TA23-BJ41-3	99.7	99.6	99.7	99.7	99.7	99.7	99.5	99.7	99.8	99.7	99.7	99.6	99.5	99.6	99.9	100	99.8			
"crispata" SNI-TA26-BJ1	99.6	99.5	99.6	99.7	99.6	99.6	99.4	99.6	99.7	99.7	99.6	99.5	99.4	99.5	99.8	99.9	99.7	99.9		
"crispata" SNI-TA26-BJ7	99.7	99.6	99.7	99.7	99.7	99.7	99.5	99.7	99.8	99.7	99.7	99.8	99.7	99.7	100	100	99.9	100	99.9	

Table 2. *Atlanticothrix* 16S-23S ITS percent dissimilarity matrix.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	"A testacea" SNI-TA4-BJ2 clone 2																
2	"A testacea" SNI-TA4-BJ2 clone 1&3	0.00															
3	"A. nostocoides" SNI-TA1-JJ4	6.61	6.61														
4	"A. nostocoides" SNI-TA5-JJ3	6.63	6.63	0.37													
5	"A. nostocoides" SNI-TA5-JJ1	6.44	6.44	0.19	0.19												
6	"A. crispata" SNI-TA18-ML7	2.23	2.23	7.23	7.26	6.87											
7	"A. crispata" SNI-TA26-BJ1 clone 1	2.23	2.23	7.23	7.26	6.87	0.00										
8	"A. crispata" SNI-TA26-BJ7 clone 1	2.23	2.23	7.23	7.26	6.87	0.00	0.00									
9	"A. crispata" SNI-TA23-BJ41 clone 2	2.42	2.42	7.43	7.45	7.07	0.19	0.19	0.19								
10	"A. crispata" SNI-TA23-BJ41 clone 3	2.23	2.23	7.23	7.26	6.87	0.00	0.00	0.00	0.19							
11	"A. crispata" SNI-TA23-BJ35 clone 3	2.60	2.60	7.23	7.26	6.87	0.37	0.37	0.37	0.56	0.37						
12	"A. crispata" SNI-TA23-BJ35 clone 1	10.17	10.17	15.16	15.21	14.82	9.68	9.68	9.68	9.87	9.68	10.05					
13	*A. silvestris* CENA590	8.96	8.96	13.73	13.77	13.38	8.44	8.44	8.44	8.63	8.44	8.82	4.91				
14	*A. silvestris* CENA585	8.96	8.96	13.74	13.78	13.39	8.44	8.44	8.44	8.63	8.44	8.81	4.73	0.18			
15	A. silvestris CENA579	8.96	8.96	13.74	13.78	13.39	8.44	8.44	8.44	8.63	8.44	8.81	4.73	0.18	0.00		
16	*A. silvestris* CENA576	9.15	9.15	13.93	13.97	13.58	8.63	8.63	8.63	8.82	8.63	9.00	4.91	0.36	0.18	0.18	
17	*A. silvestris* CENA564	9.14	9.14	13.92	13.97	13.57	8.63	8.63	8.63	8.82	8.63	9.00	4.91	0.36	0.18	0.18	0.36

CHAPTER 2 FIGURE DESCRIPTIONS

Figure 1. A map of San Nicholas Island. Study sites are superimposed on the map.

Figure 2. Images showing diagnostic morphological features for each putative *Atlanticothrix* species (Scale bar = 10μm, applies to the entire figure). All photos are 1000x magnification. A-F: "Atlanticothrix testacea" (SNI-TA4-BJ2). G-K: "Atlanticothrix nostocoides" (G & J: SNI-TA5-JJ1, H & I: SNI-TA1-JJ4, K: SNI-TA5-JJ3). L-Q: "Atlanticothrix crispata" (M: SNI-TA23-BJ41, N, P & Q: SNI-TA26-BJ1, M & O: SNI-TA26-BJ7).

Figure 3. Nostocaceae bayesian inference 16s rRNA phylogeny with maximum likelihood bootstrap support values added to nodes. "*" indicates full support for nodes (1.0 posterior probability, 100 bootstrap support). "-" indicates bootstrap support <50. All available *Atlanticothrix sivestris* and San Nicholas Island *Atlanticothrix* sequences were included in the analysis. The genus level *Atlanticothrix* clade had high support (0.99 BI, 77 ML), although 16S rRNA data was less informative at the species level.

Figure 4. Maximum parsimony phylogenetic tree based on 16S-23S ITS sequences. All available *A. silvestris* and San Nicholas Island *Atlanticothrix* sequences were used in the analysis.

Figure 5. Hypothetical ITS secondary structures for the D1-D1'. Box-B, and V3 helices for all strains representing "A. crispata, " "A. testacea, " "A. nostocoides," and *Atlanticothrix silvestris.* A: Common D1-D1' helix for all strains representing "A. crispata, " "A. testacea," and "A. nostocoides" except SNI-TA23-BJ35 clone 1. B: D1'D1' helix for SNI-TA23-BJ35 clone 1. C: *Atlanticothrix silvestris* D1-D1'. D: Common Box-B helix among all "A. testacea" and "A. nostocoides" strains. E: Common Box-B helix for all "A. crispata" strains except SNI-TA23-BJ35 clone 1. F: Box-B helix for SNI-TA23-BJ35 clone 1. G: *A. silvestris* Box-B helix. H:

Common V3 helix among all "A. testacea" and "A. nostocoides" strains. I: Common V3 helix for all "A. crispata" strains except SNI-TA23-BJ35 clone 1. J: V3 helix for SNI-TA23-BJ35 clone 1. K: *A. silvestris* V3 helix.

Figure 6. Hypothetical ITS structures for the V2 helix for all strains representing "A crispata," "A. testacea," "A. nostocoides" and *A. silvestris*. Representative strains for each helix are listed on the figure. A: "A. testacea" V2. B-D: A. nostocoides V2. E-G: "A. crispata" V2 helices. H-I: *A. silvestris* V2 helices.

Figure 7. Principal component analysis on *Atlanticothrix* strain cell measurements. Analysis was run on mean length and width measurements and length-width ratios for vegetative cells, apical cells, heterocytes, and akinetes. Each average measurement was obtained by taking 30 individual measurements for each cell length and width. Principal component axes 1 and 2 are displayed.

Figure 8. Micrographs of diagnostic morphological features for putative species "Pycnacronema juana-mariae" and "Pycnacronema aeruginosum." Scale bar = 10µm and applies to the entire figure. All photos are 1000x magnification. A-E: "Pycnacronema juana-mariae" SNI-TA3-BJ5.F-J: "Pycnacronema aeruginosum" SNI-TA29-BJ1.

Figure 9. Bayesian inference phylogenetic tree for *Pycnacronema Konicacronema,* and closely related genera. Maximum likelihood bootstrap support values were added to the nodes. "*" indicates full support for nodes (1.0 posterior probability, 100 bootstrap support). "-" indicates bootstrap support <50. All available *Pycnacronema* and *Konicacronema* 16S sequences were used from San Nicholas Island strains and those from previously described species in both genera.

Figure 10. Maximum parsimony phylogeny based on *Pycnacronema* 16S-23S ITS sequences with Bayesian inference posterior probability values added to the nodes. Sequences from previously described species and those from "P. juana-mariae" and "P. aeruginosum" were included in the analysis.

Figure 11. Hypothetical secondary ITS structures for the D1-D1', Box-B, and V3 helices in previously described Pycnacronema species, as well as 'P. juana-mariae" and "P. aeruginosum." A-I: D1-D1' helix structures. J-R: Box-B helix structures. S-Z: V3 helix structures. The species for which each helix represents are included in the figure.

Figure 12. Photographs of diagnostic morphological features for putative species "K. haraasengensis" (Scale bar = 10µm, applies to entire figure). A-D: Strain SNI-TA14-AZ4. E: SNI-TA6-AZ20.

Figure 13. Hypothetical secondary ITS structures for the D1-D1', Box-B, and V3 helices in putative species "Konicacronema haraasengensis" and the type species of the genus *K. caatingensis.* A-B: D1-D1' structures. C-D: Box-B structures. E-F: V3 structures.

CHAPTER 2 FIGURES

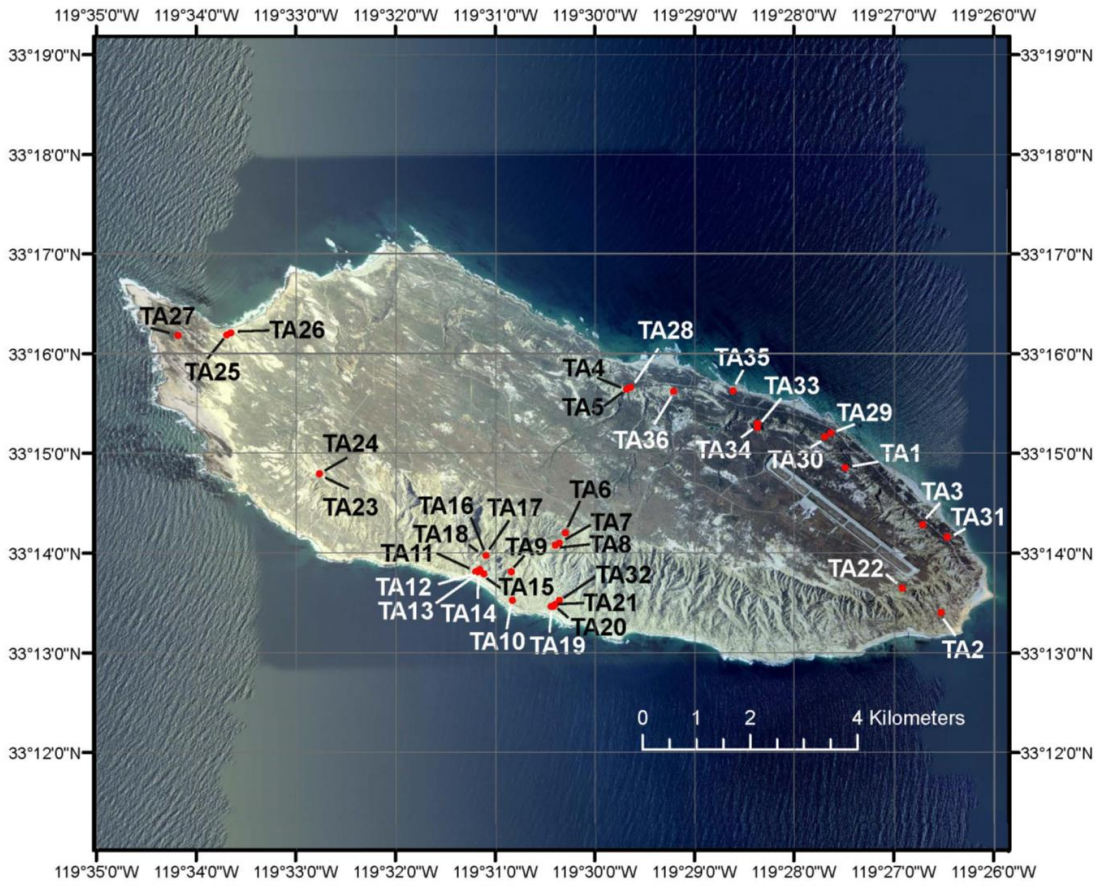

Figure 1.

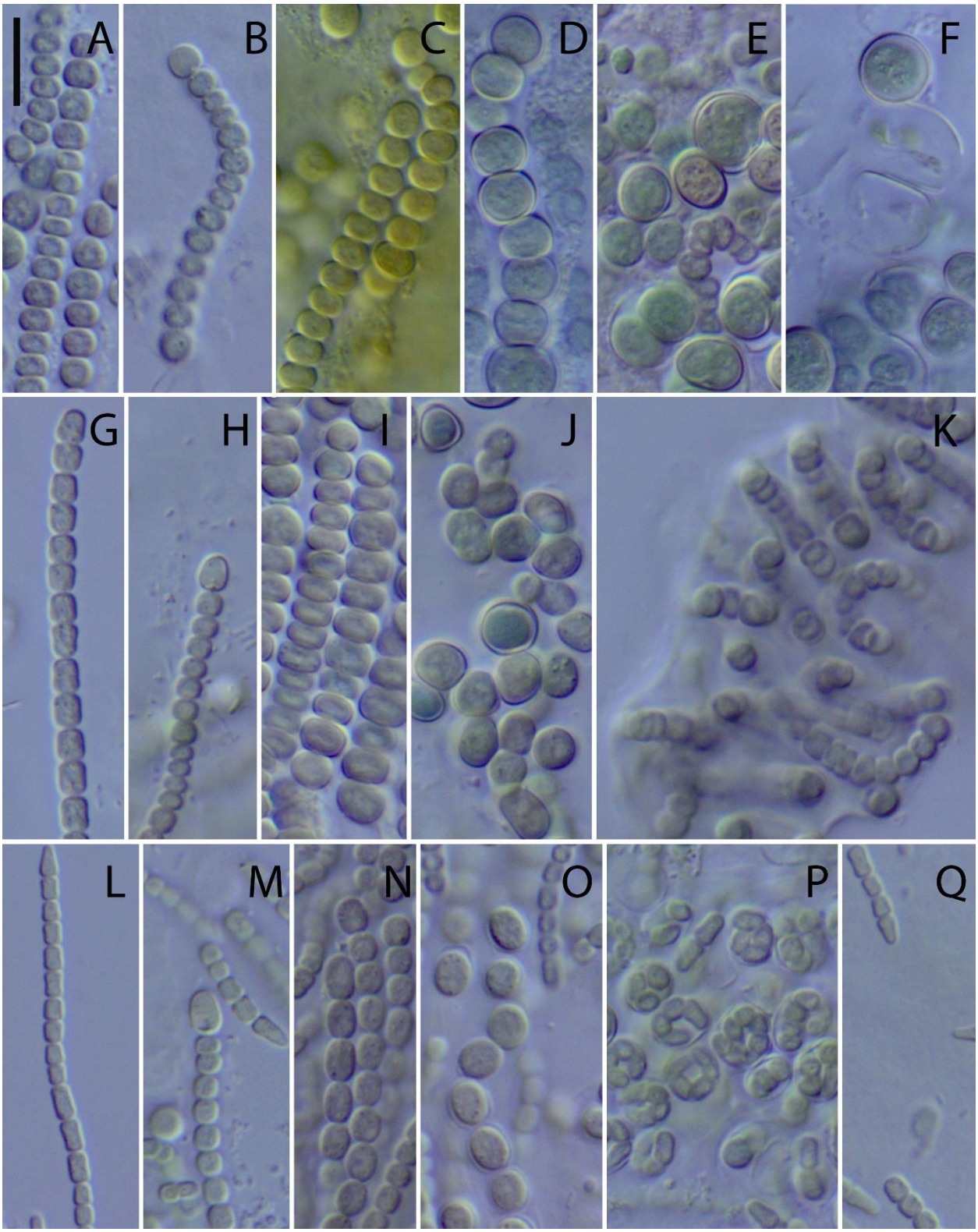

Figure 2.


"Dendronalium phyllosphericum" CENA88 GQ259207
"Nostoc" sp. CENA535 KX458486
Allinostoc morphoplasticum NOS NR_158066 T
Allinostoc alkaliphilum CENA513 KX458483
"Nostoc" sp. CENA543 KX458489
Allinostoc sp. SA43 MK503794
Allinostoc sp. HA4241-MV4 MW344116
Allinostoc magnakinetifex SA18 MK503791
"Nostoc elgonense" TH3S05 AM711548
Purpureonostoc tibetica CHAB5880 NR_172592 T
"Nostoc" sp. 8941 AY742448
Halotia longispora CENA420 KJ843313
Halotia branconii CENA392 KJ843312 T
Halotia wernerae CENA158 KC895852
Cyanocohnniella calida CCALA 1049 clone operon 1 KJ737427 T
Nostocales cyanobacterium SNI-TA18-ML4 OQ784090
Nostocales cyanobacterium SNI-TA18-ML2 OQ784091
Goleter apudmare HA4356-MV2 p8GH JN385288
Goleter apudmare HA4356-MV2 p8I JN385289
Goleter apudmare HA4340-LM2 KF417425 T
Komarekiella atlantica MTQ17-MM2 MW344113
Komarekiella atlantica MTQ8-MM1A MW344112
Komarekiella atlantica CCIBt 3483 KX635487 T
Komarekiella atlantica CCIBt 3307 KX638483
Cylindrospermum stagnale PCC 7417 CP003642
Cylindrospermum majus CCALA996 NR_125687 T
Cylindrospermum alatosporum SAG 43.79 GQ287650
Cylindrospermum pellucidum CCALA869 KF052600
Nostocaceae cyanobacterium HA4266-MV3 MW344094
Atlanticothrix silvestris CENA590 MW326977
Atlanticothrix silvestris CENA564 MW326973
Atlanticothrix silvestris CENA357 NR_172568 T
Atlanticothrix silvestris CENA368 KR137589
Atlanticothrix silvestris CENA585 MW326976
Atlanticothrix silvestris CENA576 MW326974
Atlanticothrix silvestris CENA579 MW326975
"Nostoc calcicola" AM50C MG641901
Atlanticothrix SNI-TA26-BJ1 OQ780343
Atlanticothrix SNI-TA18-ML7 OQ780336
Atlanticothrix SNI-TA26-BJ7 OQ780344
Atlanticothrix SNI-TA23-BJ41 OQ780340
Atlanticothrix nostocoides SNI-TA1-JJ4 OQ780330
Atlanticothrix nostocoides SNI-TA5-JJ3 OQ780334
Atlanticothrix nostocoides SNI-TA5-JJ1 OQ780333
Atlanticothrix SNI-TA23-BJ35 OQ780337
Atlanticothrix SNI-TA4-BJ2 OQ780331
Rohoitiella mojaviensis WJT36-NPBG58 KM268892
Rohoitiella sp. K7 ON555483
Rohoitiella bashkiriorum RU6 KM268883
Rohoitiella fluviatilis RU7 KM268884
Rohoitiella edaphica JOH39 KM268878 T
Rohoitiella mixta Py23 MK990636
"Nostoc" sp. "Azolla cyanobiont" AY742450
Calochaete cinnmanii CCALA 1012 HF912386 T
Nostoc commune WY1KK1 clone 4 EU586733 T
Nostoc cf. commune 257-16 HQ877826
Nostoc sp. 07115_00001 clone 7115B2 KC854767
Nosloc sp. "Peltigera malacea DB3992 cyanobiont" JX219463
Nostoc sp. SNI-TA23-BJ46 OQ784100
Nostoc sp. "Peltigera membranacea cyanobiont N6" JX975209
Nostoc sp. HA6929-AV1 clone 1 MW344114
Nostoc aromo ETH. 2.4. M.5 MH427659
Nostoc lichenoides CNP-AK1 clone B MH427689
Nostoc sp. 8901:1 AM711539
Nostoc cf. punctiforme Bashkir clone 6A EU586732
Nostoc desertorum CM1-VF14 MH427691
Nostoc indistinguendum CM1-VF10 MH427692
Nostoc cf. indistinguendum FI5-VF4 MH427693
Mojavia aguilerae ATA2-1-KO23 KU161650
Mojavia dolomitestris CMT-3FDIN-NPC3 KU161675
Mojavia pulotva strain JT2-VF2 AY577534 T
Mojavia sp. AR1 MF002044
Desmonostoc magnisporum AR6_PS MH497086
Desmonostoc sp. Ds1 HG004579
Desmonostoc punense MCC 2741 KT166436
"Nostoc" sp. HA4356-MV1 clone p12B HQ847577
"Nostoc linckia" var. arvense IAM M-30 AB325907
Desmonostoc sp. 111_CR4_BG11N KF781564
Desmonostoc muscorum Lukesova 1/87 AM711523
Desmonostoc muscorum Lukesova 2/91 NR_176981 T
Desmonostoc geniculatum HA4340-LM1 clone 37A KU161660
Desmonostoc sp. 7N clone NC3C KF934182
"Nostoc" sp. UAM 307 HM623782
Compactonostoc shennongjiaense CHAB5781 MH598843 T
Nostocaceae cyanobacterium HA4199-MV4 clone 1 MW344103
Nostocaceae cyanobacterium MTQ15-MM2 clone 1 MW344102
Nostocaceae cyanobacterium HA5028-AV1 clone 1 MW344101
Dendronalium phyllosphericum CENA358 KR137579
Nostocaceae cyanobacterium HA4193-MV3 clone 1 MW344097
Dendronalium phyllosphericum CENA389 KR137610
Nostocaceae cyanobacterium HA7590-KK11 clone 1 MW344107
"Nostoc calcicola" TH2S22 AM711529
Violetonostoc minutum CHAB 5841 MN400070
Violetonostoc minutum CHAB 5840 MN400069
"Desmonostoc vinosum" HA7617-LM4 KF417429
Amazonocrinis nigriterrae CENA67 MN551902 T
Amazonocrinis nigriterrae CENA238 MN551905
Nostocaceae cyanobacterium HA4962-CV1 clone 2 MW344096
Camptylonemopsis sp. HA4241-MV5 JN385292
Camptylonemopsis sp. HA4241-MV1 MW344104
Camptylonemopsis sp. HA4241-MV6 clone 1 MW344106
"Nostoc carneum" IAM M-35 AB329906
Pseudoallinostoc tiwarii LI_PS MH497064
Pseudoallinostoc constrictum SA30 MK503793
Nostocaceae cyanobacterium HA4233-MV7 clone 1 MW344110
"Allinostoc soli" ZH1(3)_PS MH497065
Pseudoallinostoc sejongens ACKU 594 MT000691 T
Nostocaceae cyanobacterium HA4196-MV1 clone 1 MW344109
Trichormus sp. ATA116B_CV3_AU4_RT KF761566
Trichormus variabilis NIES23 AF247593
Trichormus variabilis AB016520
"Nostoc" sp. PCC 7120 X59559
Trichormus variabilis ATCC 29413 HF67850 T
Trichormus variabilis HA4236-MV5 clone 1 MW344108
Minunostoc cylindricum CHAB5843 MH918062 T
Minunostoc cylindricum CHAB5844-1 MH918063
Minunostoc cylindricum CHAB 5844-1 clone 2 MK045294
Minunostoc cylindricum CHAB 5844-1 clone 3 MK045295
Minunostoc cylindricum CHAB 5843 clone 2 MK045296
Minunostoc cylindricum CHAB 5843 clone 3 MK045297
Nostocaceae cyanobacterium HA4199-MV3 clone 1 MW344091
"Trichormus anomalus" HA4352-LM2 KF417426
Nostocaceae cyanobacterium HA4218-MV1 clone 1 MW344115
Nostocaceae cyanobacterium HA4218-MV3 clone 1 MW344099
"Nostoc" sp. HK-01 AB085687
Nostocaceae cyanobacterium HA4266-MV2 clone 1 MW344093
Desikacharya nostocoides BHU1-PS MH036167 T
Desikacharya soli BHU2-PS MH036168
Desikacharya thermotolerans 9C-PS KX252675
Desikacharya constricta SA10 MK354274
"Nostoc piscinale" CENA21 AY218832
"Trichormus azollae" Kom BAU1983 AJ630454
"Nostoc ellipsosporum" CCAP 1453/2 HF678488
Chroococcidiopsis sp. BB82.3 SAG 2024 AJ344553
Chroococcidiopsis cubana SAG 39.79 AJ344558
Chroococcidiopsis sp. 9E-07 FR798923
Chroococcidiopsis thermalis PCC 7203 AB039005 T

Figure 3.

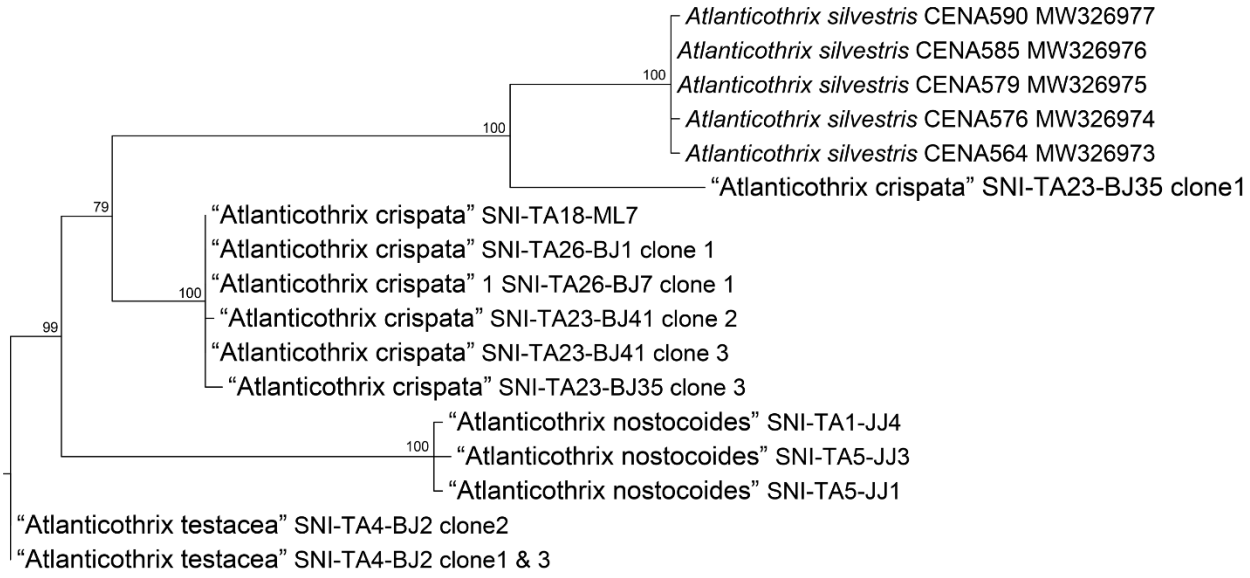

Figure 4.

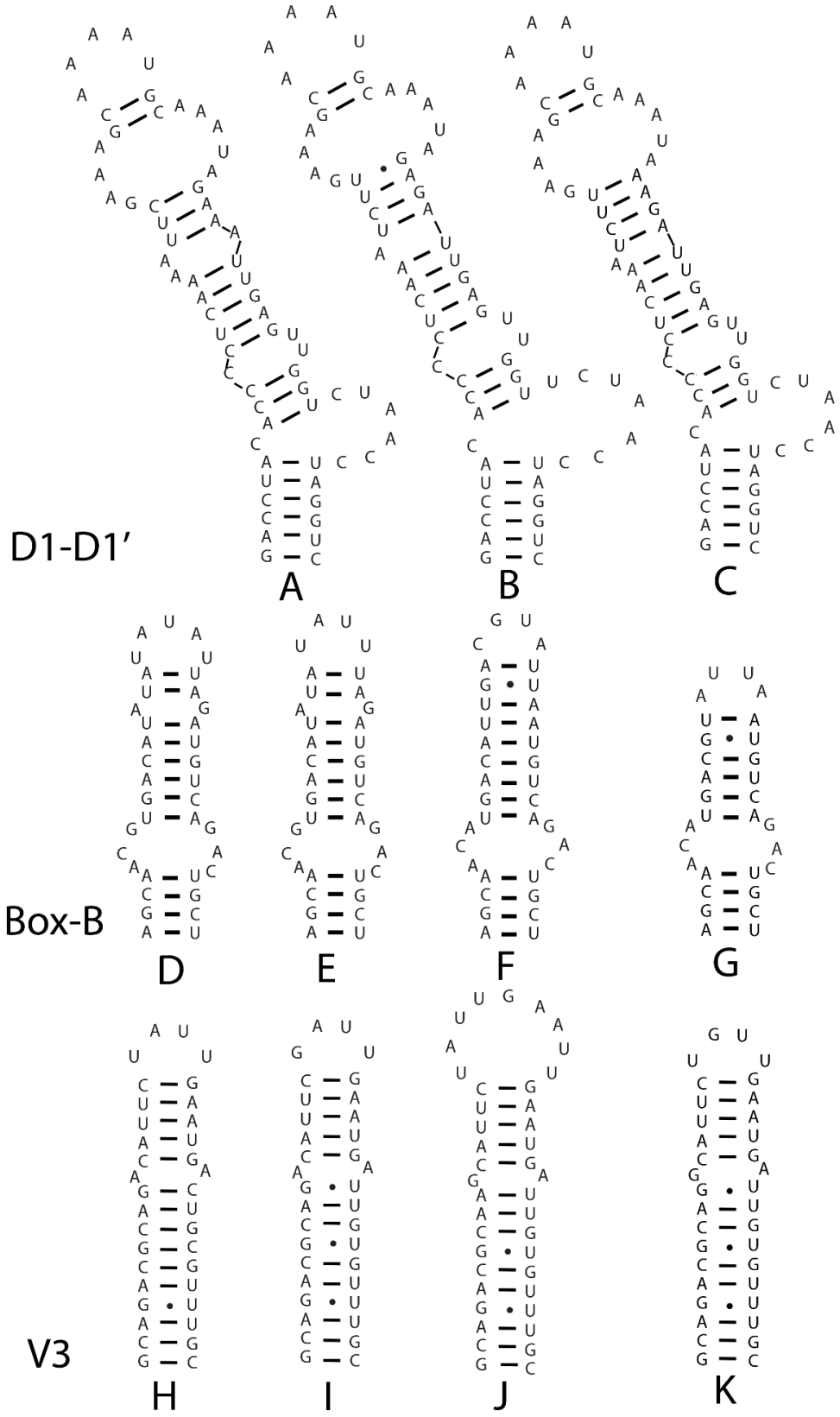

Figure 5.

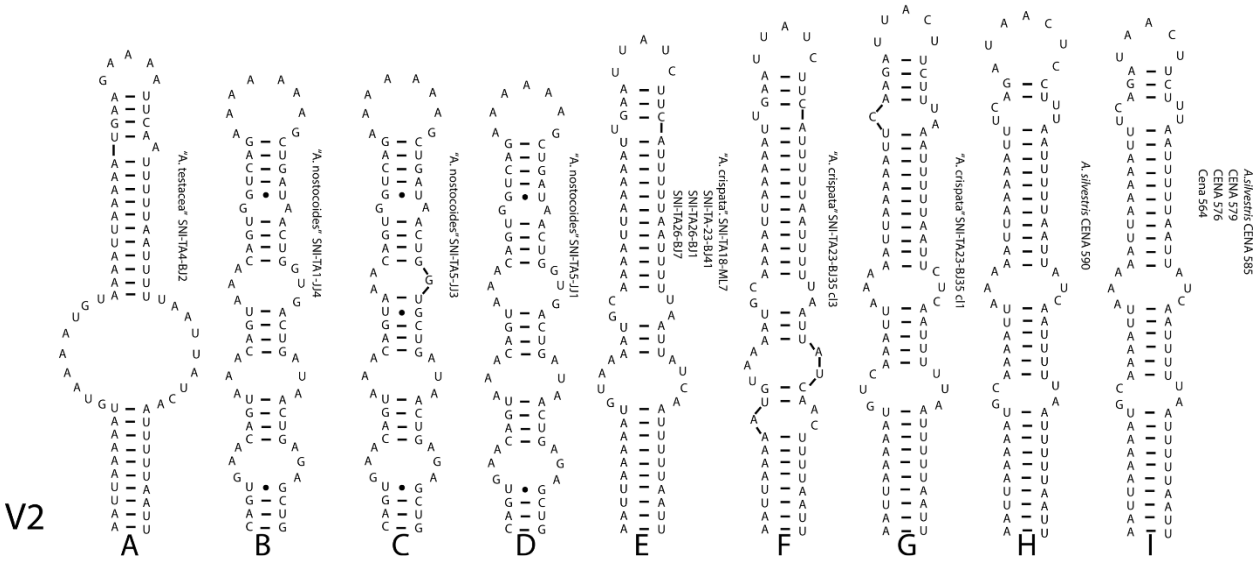
V2
A
"A. testacea" SNI-TA4-BJ2
B
"A. nostocoides" SNI-TA1-JJ4
C
"A. nostocoides" SNI-TA5-JJ3
D
"A. nostocoides" SNI-TA5-JJ1
E
"A. crispata" SNI-TA18-MJ7
SNI-TA-23-BJ41
SNI-TA26-BJ1
SNI-TA26-BJ7
F
"A. crispatar" SNI-TA23-BJ35 cl3
G
"A. crispata" SNI-TA23-BJ35 cl1
H
A. silvestris CENA 590
I
A.silvestris CENA 585
CENA 579
CENA 576
Cena 564

Figure 6.

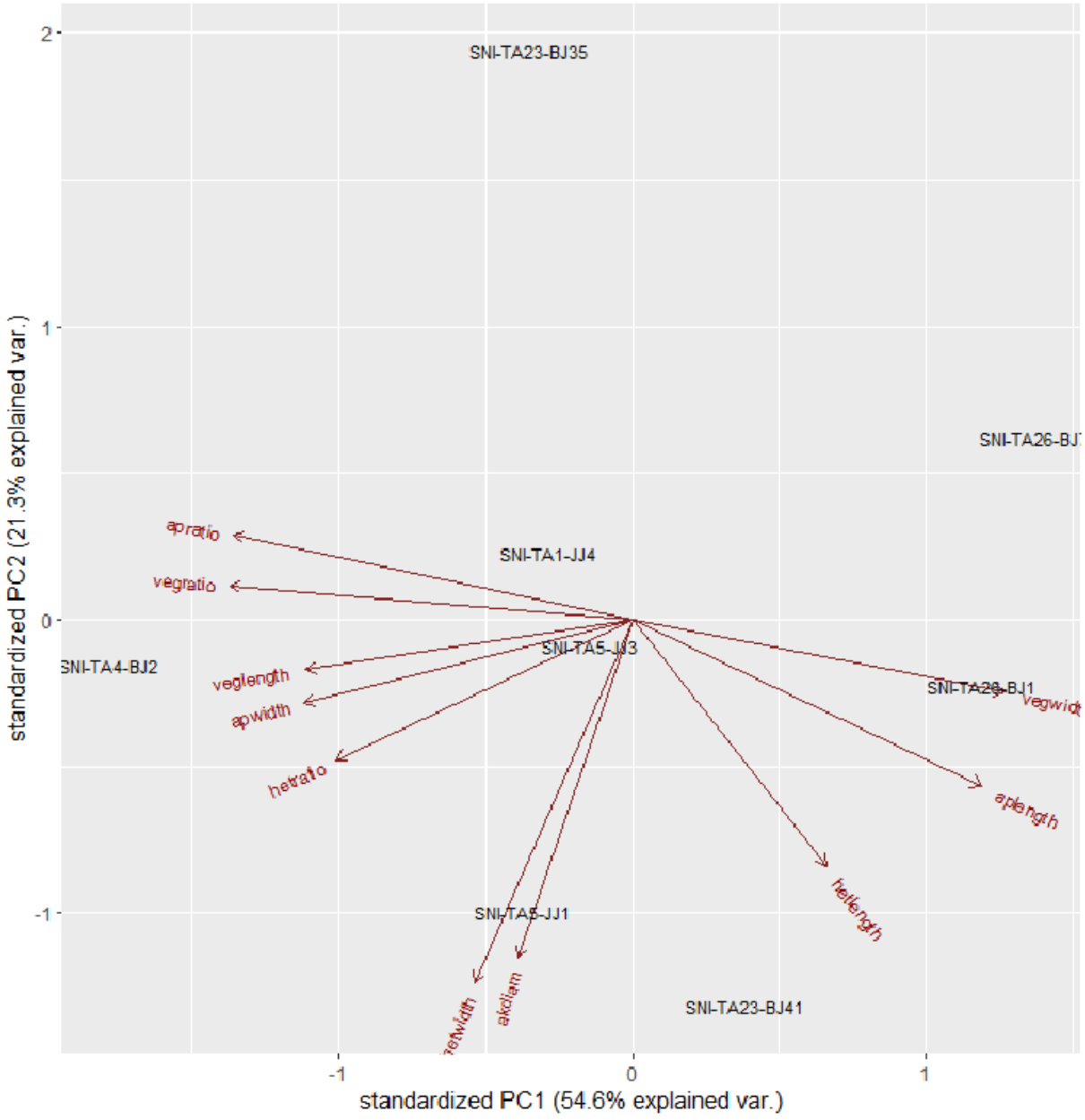
SNI-TA23-BJ35
SNI-TA26-BJ
SNI-TA1-JJ4
SNI-TA4-BJ2
SNI-TA5-JJ3
SNI-TA28-BJ1
SNI-TA5-JJ1
SNI-TA23-BJ41
apratio
vegratio
veglength
apwidth
hetratio
aplength
hetlength
akdiam
standardized PC2 (21.3% explained var.)
standardized PC1 (54.6% explained var.)
2
1
0
-1
-1
0
1

Figure 7.

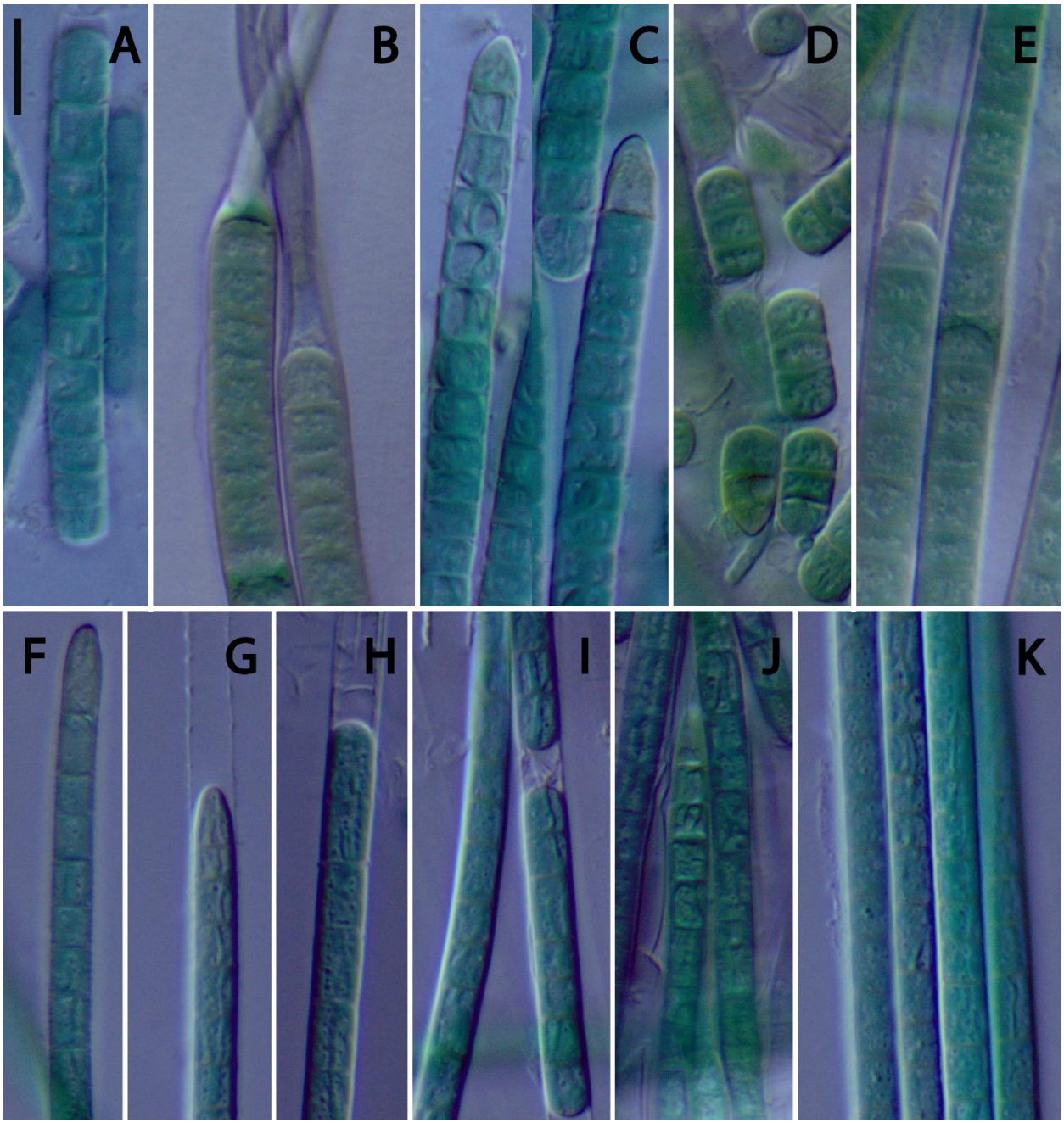

Figure 8.


Pycnacronema aeruginosum SNI-TA29-BJ1 OQ780350
"Microcoleus paludosus" SAG 1449-1a EF654090
"Parifilum solicrustae" SON57 NR_176557
Pycnacronema edaphicum CATCA7 NR_172608
Pycnacronema juana maniae SNI-TA3-BJ5 OQ780349
Pycnacronema conicum 7PC MF581656
Pycnacronema arboriculum 41PC MF581657
Pycnacronema brasiliense 46PC MF581662
Pycnacronema brasiliense 45PC MF581661 T
Pycnacronema brasiliense 44PC MF581660
Pycnacronema savannense 49PC MF581663
Pycnacronema rubrum 43PC MF581659
Pycnacronema marmoreum 42PC MF581658
Desertifilacaeae cyanobacterium SNI-TA4-BJ9 OQ784056
Desertifilacaeae cyanobacterium SNI-TA19-BJ8 OQ784093
Symplocastrum flechtnerae WJT32-NPBGF NR_172670
Symplocastrum flectnerae SNI-TA8-AZ5 OQ784063
"Arizonema commune" HS024 NR_176554 T
"Arizonema" sp.HS003 MT664813
Oscillatoriales cyanobacterium HS041.1 MT667354
Symplocastrum sp. SNI-TA35-BJ2 OQ784112
Symplocastrum sp. SNI-TA35-BJ6 clone 1OQ784113
Symplocastrum sp.SNI-TA35-BJ6 clone 2 OQ784114
Symplocastrum torsivum CPER-KK1 NR_172658
"Arizonema" sp. FB15 MT664812
Symplocastrum californicum WJT9-NPBG9 NR_172671
Potamolinea aerugineo-caerulea 1PC KX001786
Potamolinea aerugineo-caerulea 38PC KX001788
Potamolinea magna 47PC KX001789 T
Wilmottia murrayi FBCC-A401 MN473877
Wilmottia murrayi 24PC KY288989
Wilmottia stricta 31PC KY288996
Wilmottia koreana FBCC-A812 NR_172594
Konicacronema haraasnagensis SNI-TA17-BJ3 clone1 OQ780347
Konicacronema haraasnagensis SNI-TA17-BJ3 clone2 OQ780348
Konicacronema haraasnagensis SNI-TA6-AZ20 OQ780345
Konicacronema haraasnagensis SNI-TA14-AZ4 OQ780346
"Funiculus tenuis" HSN023 NR_176558
Konicacronema caatingensis CATCB6 MT311249
Konicacronema caatingensis CATCB1 MT311248 T
"Trichocoleus sociatus" SAG 26.92 EF654080
Marmoreocelis xerophila CATCB5 MT311247 T
Porphyrosiphon notarisii ACT693 clone 1 MK247998 T
Desertifilum tharense PD2001/TDC4 FJ158995 T
Crassifilum sonorensis SON62 NR_176555 T
Crustifilum sp. SON60 MT664821
Crassifilum sp. BN27 MT664818
Allocoleopsis sp. DW001 MT664811
Allocoleopsis franciscana PCC 7113 NR_176473 T
Gracilinea arenicola CATCC10 MT311245 T
Gracilinea arenicola CATCB9 MT311246
Cephalothrix komarekiana CCIBt 3277 KJ994514 T
Cephalothrix komarekiana SAG 75.79 KJ994517
Cephalothrix lacustris CCIBt 3261 KJ994513
Phormidium irriguum CCALA 759 EU196638
Kastovskya adunca ATA3-4Q-CV17 clone AC KF312342
Kastovskya adunca ATA3-5Q-LB05 clone AB KF312341
Kastovskya sp. SNI-TA36-BJ6 OQ784115
Kastovskya adunca ATA6-11-RM11 clone AC KF312347 T
Coleofasciculus chthonoplastes SAG 2209 EF654055 T
Coleofasciculus chthonoplastes WW5 EF654053
"Kamptonema formosum" BDU 92022 KU958133
Symploca atlantica PCC 8002 AB039021
Symploca sp. HBC5 16S EU249122
Moorena producens NAC8-48 GU724200 T
Geitlerinema sp. WUC1581 ON685824
Microcoleus vaginatus KZ-23-1 MK211228
Microcoleus vaginatus LSB45 clone 1 MW403930
Microcoleus vaginatus SNI-TA8-AZ3 OQ784062
Microcoleus vaginatus SAG 2502 MK953007
Microcoleus vaginatus SEV1-KK3 EF654076
Microcoleus vaginatus S43 JQ712634
Microcoleus vaginatus CNP3-KK2 EF654064
Microcoleus vaginatus SNI-TA6-AZ25 OR257829
Microcoleus vaginatus SNI-TA7-BJ1 OQ784059
Microcoleus vaginatus SNI-TA17-BJ7 OQ784080
Microcoleus vaginatus S48 JQ712637
Microcoleus vaginatus CJI-U2-KK2 EF654063
"Oscillatoria nigro-viridis" PCC 7112 NR_102469
Kamptonema animale SAG 1459-6 EF654087
Phormidium irriguum f. minor ETS-02 FN813342
Kamptonema laetevirens NIES-31 LC455672
Oscillatoria sancta PCC 7515 NR_112112
Planktothrix agardhii NIES-1258 LC455655 T
Planktothrix sp. PCC 7811 GQ351564
Planktothrix pseudagardhii 2LT34S02 FM177501
Ancylothrix terrestris BEA0843B ON803474
Ancylothrix terrestris BEA 1480B ON803465
Ancylothrix rivularis 9PC KT819198 T
Planktothricoides raciborskii FBCC-A1472 ON775546 T
Arthrospira platensis PCC 7345 NR_125711
Arthrospira jenneri EB 9604 GQ184185 T
Geitlerinema sp. CENA556 MF084984
Trichocoleus desertorum CMT-2SZIN-NPC9 MH427711
Trichocoleus desertorum ATA4-8-CV3 cl2 KY498224
Trichocoleus desertorum WJT40-NPBG1 KF307611
Trichocoleus badius CRS-1 operon3 EF429298
Trichocoleus badius CRS-1 operon 2 EF429297
Trichocoleus desertorum CXA025 clone 2 MH463396
Trichocoleus desertorum SIK114 clone 3 MZ677350
Oscillatoria acuminata PCC 6304 NR_102463
Gloeobacter violaceus PCC 7421 NR_074282 T
0.07

Figure 9.

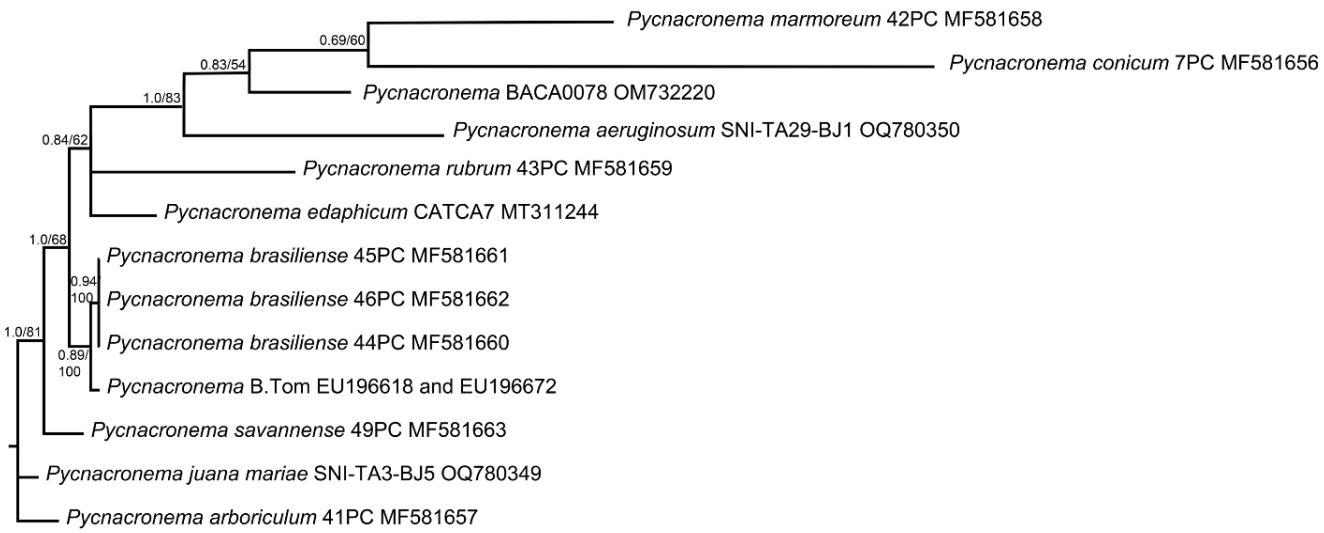
Pycnacronema marmoreum 42PC MF581658
0.69/60
Pycnacronema conicum 7PC MF581656
0.83/54
Pycnacronema BACA0078 OM732220
1.0/83
Pycnacronema aeruginosum SNI-TA29-BJ1 OQ780350
0.84/62
Pycnacronema rubrum 43PC MF581659
Pycnacronema edaphicum CATCA7 MT311244
1.0/68
Pycnacronema brasiliense 45PC MF581661
0.94/
100
Pycnacronema brasiliense 46PC MF581662
1.0/81
Pycnacronema brasiliense 44PC MF581660
0.89/
100
Pycnacronema B.Tom EU196618 and EU196672
Pycnacronema savannense 49PC MF581663
Pycnacronema juana mariae SNI-TA3-BJ5 OQ780349
Pycnacronema arboriculum 41PC MF581657

Figure 10.

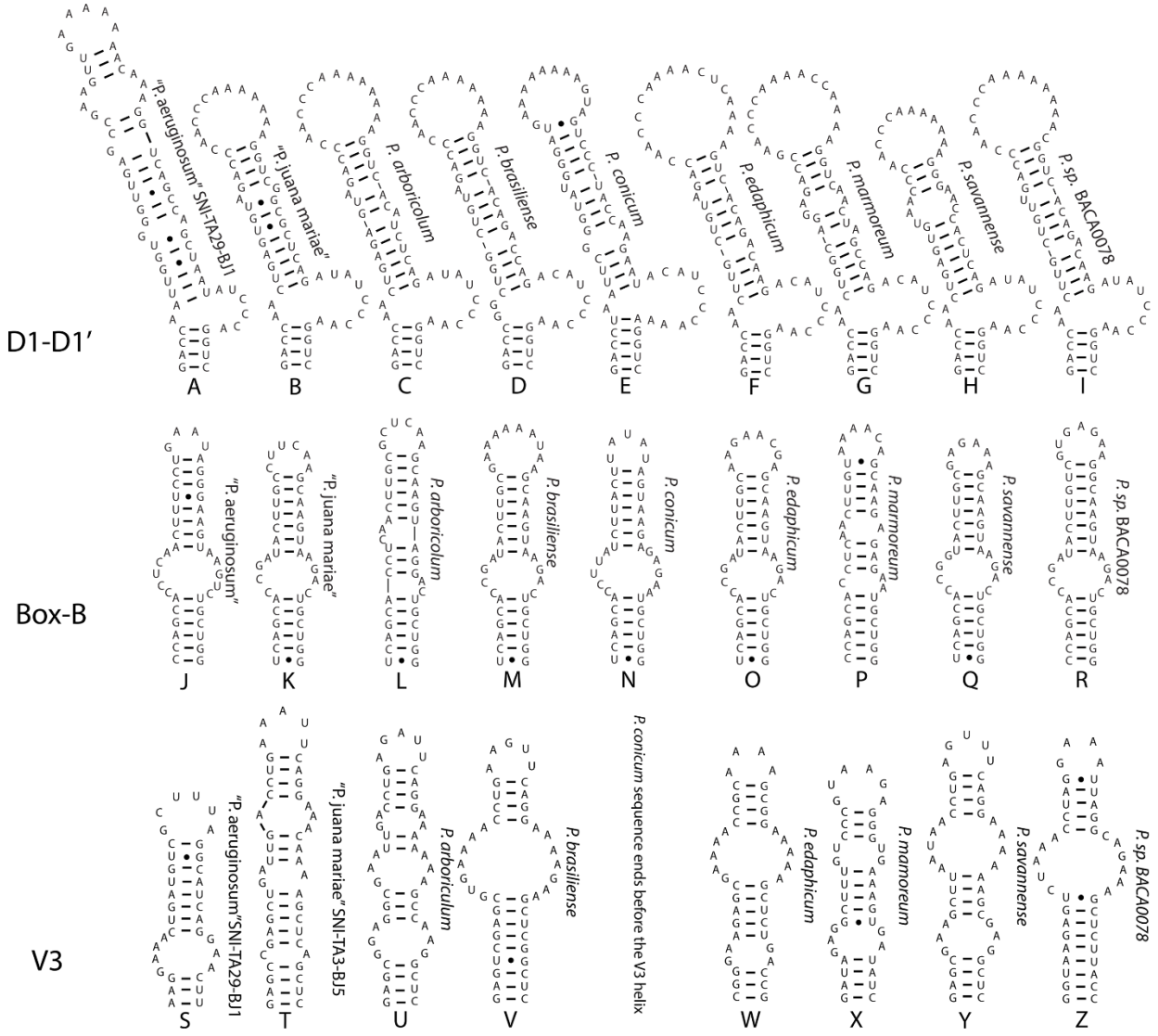
D1-D1′
Box-B
V3
"P. aeruginosum" SNI-TA29-BJ1
"P. juana mariae"
P. arboricolum
P. brasiliense
P. conicum
P. edaphicum
P. marmoreum
P. savannense
P. sp. BACA0078
"P. aeruginosum"
"P. juana mariae" SNI-TA3-BJ5
P. conicum sequence ends before the V3 helix
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z

Figure 11.

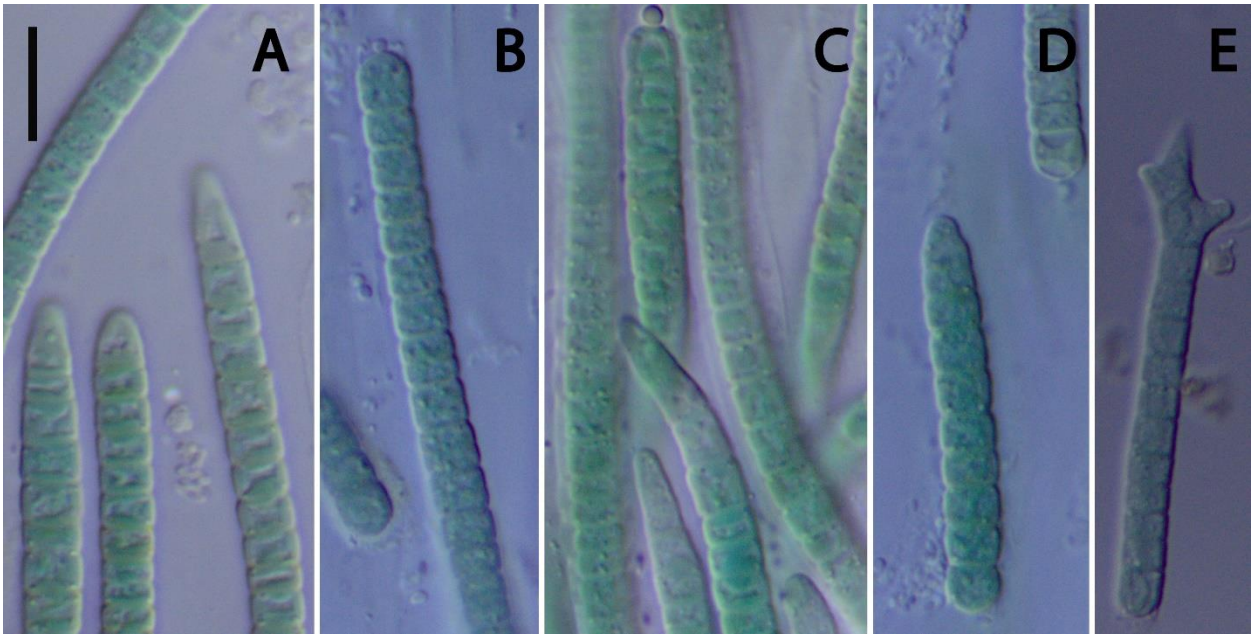

Figure 12.

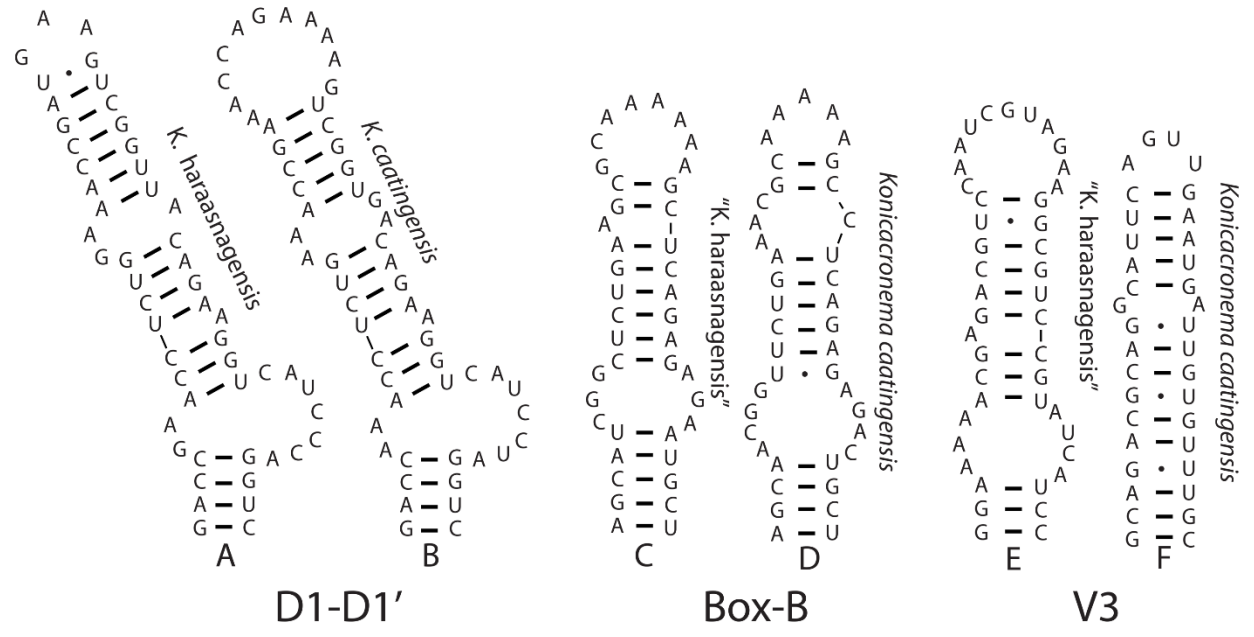

Figure 13.