Tapinothrix clintonii sp. nov. (Pseudanabaenaceae, Cyanobacteria), a new species at the nexus of five genera

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Abstract: A new species was isolated and characterized from Grand Staircase–Escalante National Monument. This taxon shares morphological characteristics with five different genera, *Ammatoidea, Homoeothrix, Leptolyngbya, Phormidiochaete,* and *Tapinothrix*. An argument could be made to place it in any of these genera, however, we consider the most taxonomically correct genus for our pseudanabaenalean species to be *Tapinothrix*, and we accordingly describe it as *T. clintonii* sp. nov. Phylogenetically, the taxon is closest to *Leptolyngbya sensu stricto*, but it has very distinctive morphology and 16S–23S ITS sequence and secondary structure that support our conclusion to recognize *Tapinothrix* as a genus separate from *Leptolyngbya.*

Key words: *Ammatoidea, Homoeothrix, Leptolyngbya, Phormidiochaete, Tapinothrix*, Cyanobacteria, taxonomy, 16S rRNA, 16S–23S ITS, rRNA secondary structure

Introduction

In the course of study of the algal flora of Grand Staircase–Escalante National Monument (Krautová 2008), we isolated a distinctive non–heterocytous, filamentous strain of cyanobacteria. This strain is characterized by having isopolar filaments which taper at the apices and fragment in the center to form heteropolar tapering trichomes. The resulting "basal" portion of the heteropolar filament forms a mucilage pad, which likely serves to cement the widened trichome end to the substrate. The cell contents are rich in phycoerythrin, giving the trichomes a reddish coloration, with mature sheaths blackish. Cell organization (thylakoid arrangement and mode of cell division) is typical of the Pseudanabaenaceae.

Within the Pseudanabaenaceae there is a single genus with tapering trichomes whose species are attached to the substrate at the base – *Tapinothix* Sauvageau. Only two species were described in this genus, *T. bornetii* Sauvageau (1892) and *T. mucicola* Borge (1923). Another tapering genus, *Homoeothrix* (Thuret ex Bornet et Flahault) Kirchner (1898) was subsequently described, and many species were described within this taxon (Komárek & Anagnostidis 2005). *Tapinothrix bornetii* was transferred into *Homoeothrix bornetii* (Sauvageau) Mabille (1954), and *Tapinothrix* was forgotten for nearly half a century. In the most recent treatment of the Oscillatoriales, Komárek & Anagnostidis (2005) recognized that *Homoeothrix* was likely polyphyletic, with the type species, *H. juliana* (Bornet et Flahault) Kirchner clearly belonging to the Oscillatoriaceae, while the majority of described taxa apparently belong in the Pseudanabaenaceae. They concluded that these pseudanabaenacean species belong in *Tapinothrix*, but they did not make any taxonomic transfers.

In looking for species conforming morphologically to our isolate, the closest species is actually *Phormidiochaete fusca* (Starmach) Komárek in Anagnostidis (2001). *Phormidiochaete* was split out of *Homoeothrix* to accommodate the species within *Homoeothrix* that have a mode of cell division and thylakoid structure consistent with the Phormidiaceae (Anagnostidis 2001). The type species of *Phormidiochaete*, *P. nordstedtii* (Bornet et Flahault) Komárek et Anagnostidis, clearly fits into the Phormidiaceae, as does *P. balearica* (Bornet et Flahault) Komárek et Anagnostidis. This genus is in the subfamily Ammatoideoideae. However, *P. fusca* has much smaller diameter than the vast majority of

Phormidiaceae, and it may have been mistakenly placed in this genus. Interestingly, a feature of our taxon which separates it from *Phormidiochaete* is the common occurrence of isopolar trichomes tapering towards both apices that bend acutely in the center. Such isopolar forms are placed in the genus *Ammatoidea* WEST et G.S. WEST (1897), which includes mostly species with typical phormidiacean features as well as the apparently pseudanabaenacean marine species *A. murmanica* PETROV (1961).

Finally, we must mention the genus *Leptolyngbya*. This genus has become the home for a plethora of pseudanabaenacean species transferred from *Lyngbya, Plectonema*, and *Phormidium* because of their narrow, untapered trichomes forming simple sheaths. This genus has been said to be a polyphyletic assemblage (ALBERTANO & KOVÁČIK 1994; TURNER 1997; WILMOTTE et al. 1997; CASTENHOLZ 2001; WILMOTTE & HERDMAN 2001; TATON et al. 2003; CASAMATTA et al. 2005; KOMÁREK & ANAGNOSTIDIS 2005; JOHANSEN et al. 2008), but no revisionary work recognizing the separate clades as separate genera has been completed.

Our isolate is at the nexus of these five genera. It fits no described species in any genus, and some justification could be given to place it in any of the five genera discussed above. The intent of this paper is to describe our isolate as a new species in the genus which we consider to be most correct (*Tapinothrix*), determine the phylogenetic position of the isolate, and discuss the systematic and taxonomic ramifications of the discovery and characterization of this species, including reassignment of problematic species to what we consider to be the correct genera. While this may seem like an unusual case, it is likely representative of many similar stories that will be told as the revisionary process of the cyanobacteria continues.

Materials and Methods

Isolation and cultivation. A sample was collected in August 2006 from a sandstone seep wall adjacent to the cascade at Lower Calf Creek Falls in the Great Staircase–Escalante National Monument, Utah, USA (37°49'44.77" N, 111°25'12.58" W) and kept refrigerated until further processing in laboratory.

A small, unmeasured portion of the sample was dilution plated onto agar–solidified Z8 universal algal culture medium (KOTAI 1972). Enrichment was completed under 16:8 light:dark cycle and corresponding day and night temperatures of 15 °C and 10 °C. Colonies were picked 4–6 weeks later. The isolate discussed in this paper was a black cyanobacterial colony, and was isolated onto a Z8 slant. Morphological variability of the culture was determined by examining the culture as it aged from exponential growth phase through senescence (a period of about three months). Characterization was completed using an OLYMPUS BMAX 60 microscope with high resolution Nomarski DIC optics, equipped with a Spot digital camera. The cell dimensions were repeatedly measured using an ocular micrometer.

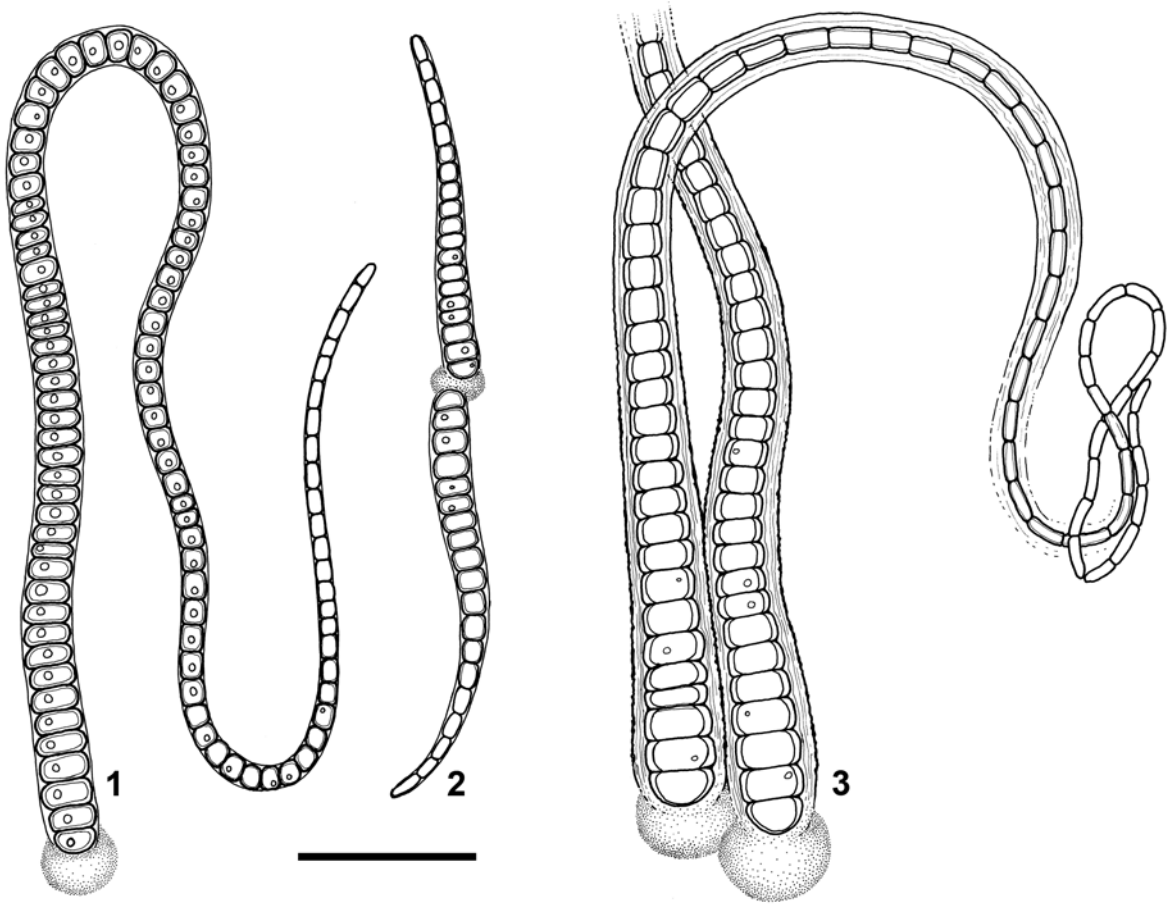
Molecular characterization. *Tapinothrix* was scraped from slants, and genomic DNA isolation was performed using the UltraClean Microbial DNA Isolation Kit from MO BIO (Carlsbad, CA, USA). DNA was eluted and stored at –20 °C.

A PCR product of 1600 bps containing the 16S–23S ITS was generated using primers 1 and 2 (BOYER et al. 2002). Fifty microliter reactions were performed in a Bio–Rad DNA Engine PTC200 (Hercules, CA, USA). Final concentrations of reagents in the reactions were 1X Taq polymerase buffer (USB, OH, USA), 1.5 mM $MgCl_2$, 2.5 pmol per µl of each primer, 1 µl of template DNA (100–200 ng total), 0.2 mM dNTPs (USB), and 1.25 units Taq polymerase (USB).Cycling conditions were 35 cycles of 94 °C for 45 sec, 57 °C for 45 sec, and 72 °C for 135 sec. A 5 min extension at 72 °C was performed, and the reactions were held at 4°C indefinitely. All PCR products were analyzed on 1% agarose gels before being TA–cloned into the pSC–amp/kan plasmid of the Stratagene Cloning Kit (La Jolla, CA, USA). Putative clones were isolated using QIAprep Spin kits (Qiagen, Carlsbad, CA, USA) with elution in 50 µl of sterile water. The presence of an insert was confirmed by *Eco*R I digestion.

Four 16S rRNA and 16S–23S ITS plasmids were sequenced by Functional Biosciences, Inc. (Madison, WI, USA) using the M13 forward and M13 reverse primers. Additionally, the 16S–23S ITS–containing plasmids were sequenced with internal primers 5 (5'–TGTACACACCGCCCGTC–3') and 8 (5'–AAGGAGGTGATCCAGCCACA–3') (FLECHTNER et al. 2002). Sequences were aligned and proofread using Sequencher software (version 4.8, Ann Arbor, MI, USA). Only one 16S–23S ITS operon was identified, the one that encodes both $tRNA^{Ile}$ and $tRNA^{Ala}$. Subsequently, the folded secondary structures were determined using Mfold version 3.2; the default conditions were used in this analysis except for draw mode: untangle with loop fix. Conserved domains within the 16S–23S ITS were identified through employment of comparative analysis with the ITS of other Pseudanabaenales, particularly with respect to the basal portions of each helix. Structures were drawn in Adobe Illustrator CS4 prior to publication.

Phylogenetic Analyses. In the first round of analyses reported in this paper, we selected a large group of non–heterocytous taxa to examine the phylogenetic position of our newly sequenced strain. A total of 272 sequences including representatives of Pseudanabaenaceae, Phormidiaceae, and Oscillatoriaceae were used in the first analysis. Sequences were aligned using CLUSTAL–W, and then proofed manually by eye. Secondary structure was consulted to align indels. Space does not permit listing the accession numbers of all strains used in this analysis, but single taxa shown in figures and tables are given with accession numbers. Based on the results of distance and parsimony analyses of this large data set, a smaller subset of taxa was selected to exclude taxa outside of the Pseudanabaenales as well as some isolates of uncertain affiliation (not identified at least to genus level). The final set for phylogenetic analysis contained 114 OTU's. Descriptions of the analyses using this data set are given below.

The GTR+I+gamma model was selected under the Akaike Information Criterion using PAUP* 4.0b10 (Swofford 2002) and Modeltest 3.7 (Posada & Crandall 1998) and was used for all model–based analyses. The software MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) was used for Bayesian inference. A Dirichlet (1,2,1,1,2,1) prior was used for the substitution rate parameters, a Dirichlet (2) prior was used for base frequencies, and a uniform (0,1 prior) was applied to the pinvar parameter. An exponential (1.0) prior was set on branch lengths and gamma shape parameters. Two runs were run for 10^7 generations, using one cold chain and three heated chains and sampling every 100 trees. The first 40,000 samples of each run were discarded as a burn–in phase. The stability of model parameters and the convergence of the two runs were confirmed using Tracer v1.4.1 (Rambaut & Drummond 2003) and AWTY (Wilgenbusch et al. 2004). A maximum likelihood (ML) analysis was performed using Garli (Zwickl 2006). A GTR+I+gamma model was applied and bootstrap support values obtained from 100 pseudoreplicate data sets. Additionally, an unweighted maximum parsimony analysis was carried out using PAUP* 4.0b10 (Swofford 2002), and bootstrap supports were obtained from 1000 pseudoreplicate data sets. Trees were prepared for publication using Adobe Illustrator CS4.



Figs 1–3. Morphological variability within *Tapinothrix clintonii*: (1) young tapered filament with thin sheath and mucilaginous pad at the base; (2) young filaments connected with common mucilaginous pad; (3) mature filaments with firm structured sheath and mucilaginous pad at the base. Scale bar 10 µm.

Results

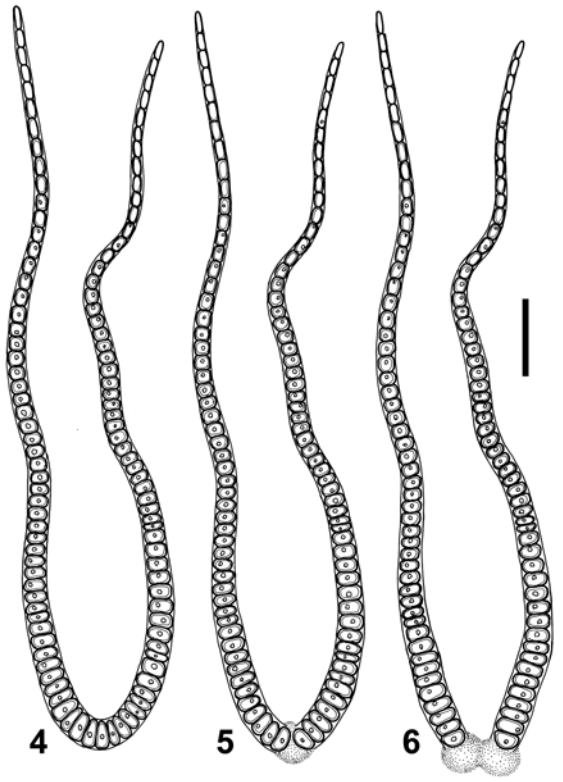
Tapinothrix clintonii BOHUNICKÁ et JOHANSEN, sp. nov. (Figs 1–29)

Diagnosis: Phormidiochaete fuscae maxime simile, a quo trichomate morina ad septum constricta, diametro latiore trichomae, habitatione paritum irrugarum in desertis differt. Homoeothrix violaceae (BORNET et FLAHAULT) KOMÁREK et KANN *simile, a quo diametro latiore trichomae, cellulis apicalibus elongatis et habitatione aquae dulcis differt.*

Most similar to *Phormidiochaete fusca*, from which it differs by the purplish color of the trichomes, the distinctly constricted crosswalls, the wider trichome diameter, and habitat preference of desert seep walls. Also similar to *Homoeothrix violacea*, from which it differs by the wider trichome diameter, the elongated cells in the apical region, and habitat preference of freshwater.

Descriptio: Colonia junior badia morinescens expansa. Filamento juniora isopolaria et ad apices ambo decrescentia, in centro rumpens heteropolarescentia, ad maturitatem tumida ad basim ad apices decresentia, muco basali incolorato, interdum pseudoramosa. Vagina firma aspera incolorata vel morina nigrescens, 0.5–1 μm lata. Trichomae ad basim et ad partem mediam constrictae ad septum, ad apices nonconstrictae ad septum, 3–4 μm latae ad basim, ad apices 0.75–1 μm latescentes, in pilum terminantes. Cellulae ad basim latitudone breviora, cytoplasmate granuloso badio, thylakoidibus parietalibus, saepe granulato centrali amplo, 0.75–1.5 μm longae. Cellulae ad apices cylindricae, sine vagina, pallide bruneorosae, latitudone longiora, 2–3 μm longae.

Colony brown when young, becoming mulberry–black, spreading. Filaments isopolar and tapering to both apices when young, fragmenting centrally to become heteropolar with maturity, swollen at the base, tapered towards the ends, with colorless mucilaginous pad at the base of mature filaments, occasionally pseudobranched. Sheath firm, rough, colorless to blackish–purple to black, 0.5–1 μm thick. Trichomes constricted at cross–walls in basal and median regions, not constricted towards the apices, 3–4 μm wide at the base, tapering to 0.75–1 μm wide at the apices, ending in a hair. Cells at the base shorter than wide, with brownish granulated content, parietal thylakoids, often with a large central granule, 0.75–1.5 μm long. Cells at the apices cylindrical, not enclosed in sheath, pale brownish–pink, longer than wide, 2–3 μm long.



Figs 4–6. Development of heteropolar filaments with mucilaginous pad at the base in *Tapinothrix clintonii*: (4) young filament tapered towards both ends, bent in the middle part; (5) filament breaks in the middle part, mucilaginous pad forms; (6) two young heteropolar filaments with mucilaginous pads. Scale bar 10 μm.

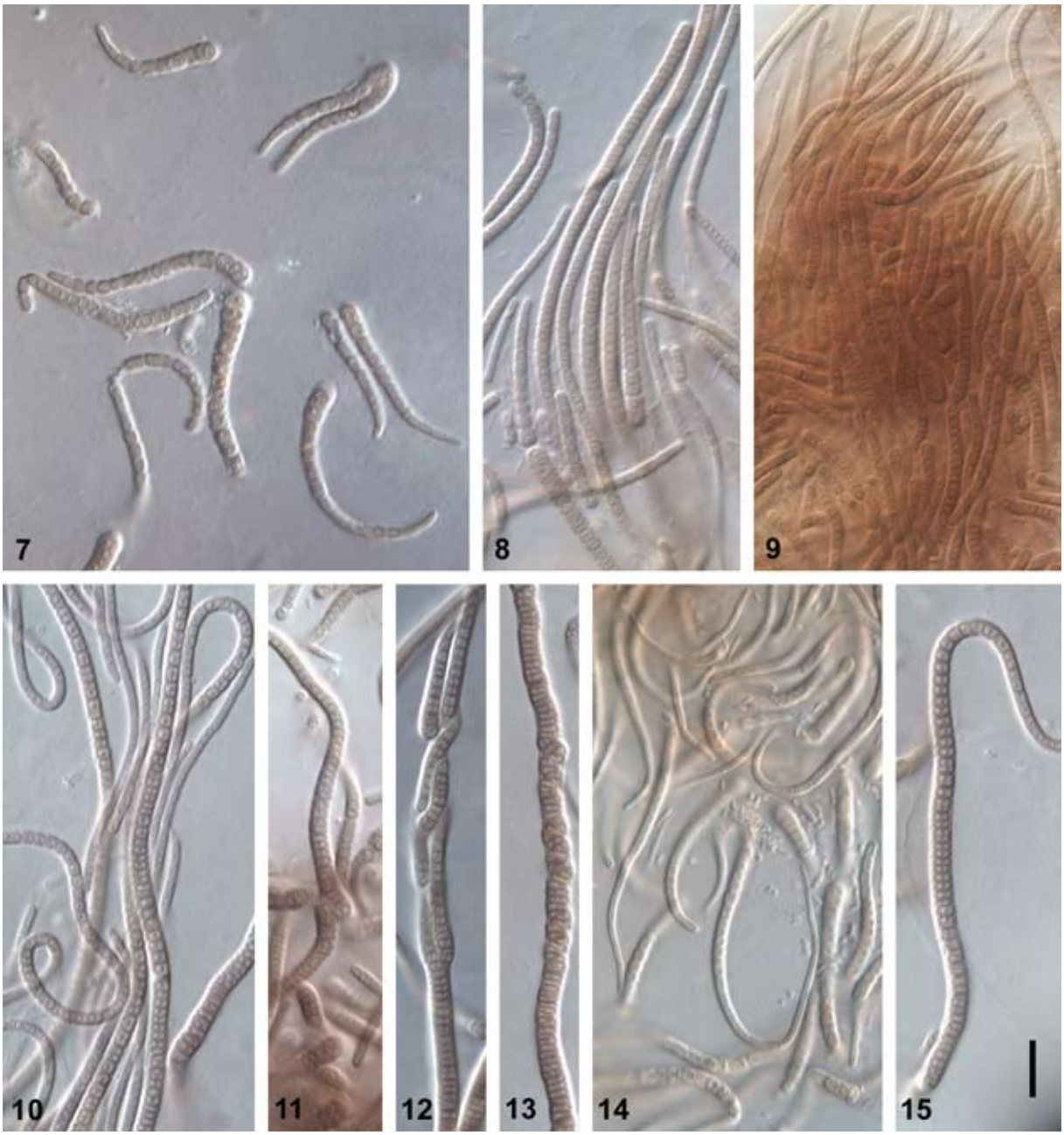
Holotype here designated: BRY37705 (unialgal population preserved in 4% formaldehyde), Herbarium of Nonvascular Cryptogams, Monte L. Bean Museum, Provo, Utah. Isotype materials included in the accession to BRY consisted of dried culture populations on filter paper (three filter papers, BRY37705).

Etymology: *Tapinothrix clintonii* = *Tapinothrix* named in honor of President William Jefferson Clinton, in recognition of his efforts to provide protection for the ecosystems and landscapes now set aside in the Grand Staircase–Escalante National Monument.

Reference strain: *Tapinothrix* GSE–PSE06–07G (UTEX temporary no. B ZZ872, CCALA 940).

Life cycle

Young filaments were observed to be tapered towards both ends, with a width of around 3.5 μm in the central, thickest part. These isopolar filaments apparently break in the middle to form two filaments attached to each other at the base by the mucilaginous disk (Figs 3, 4–6, 18, 20, 28).

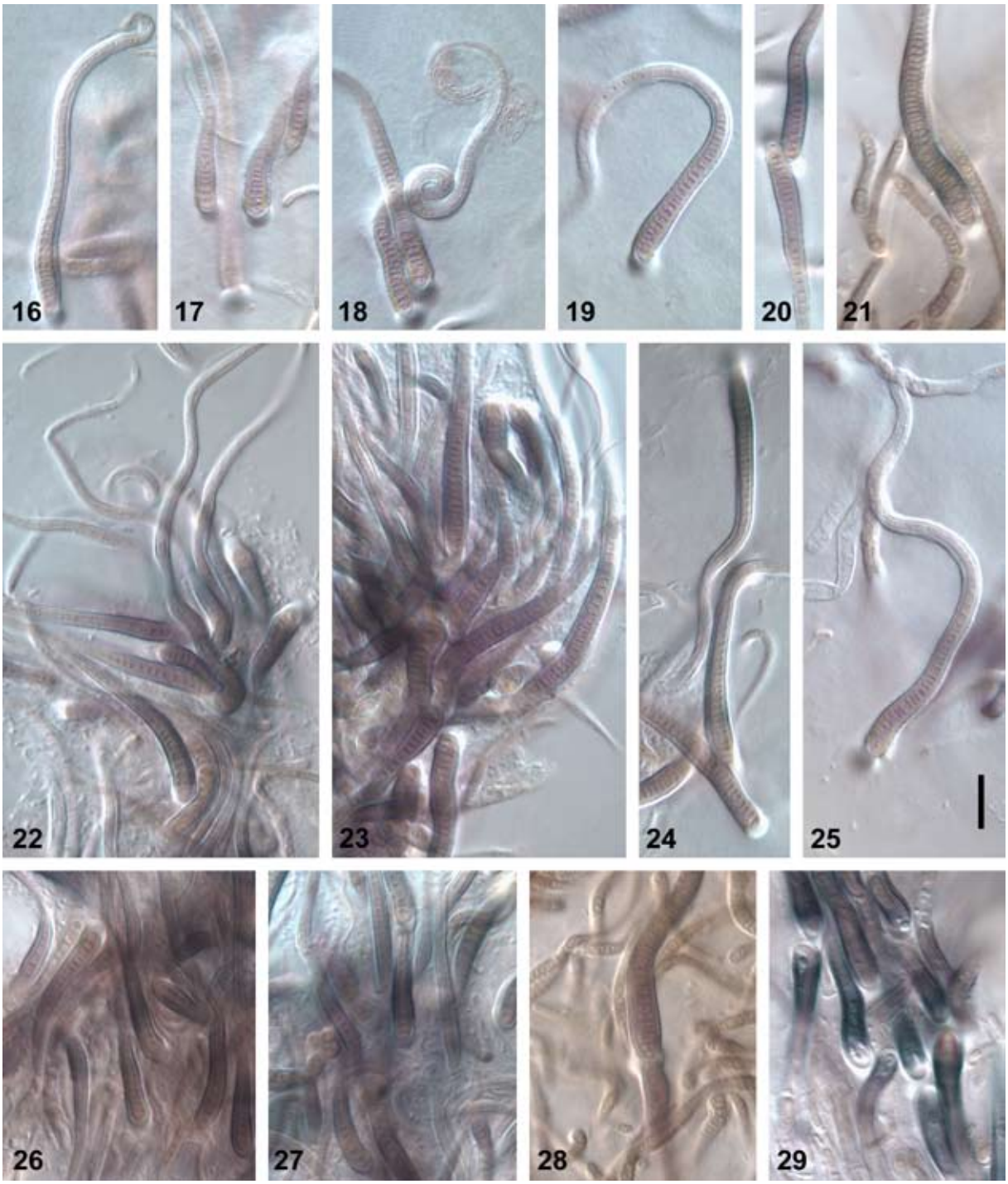


Figs 7–15. *Tapinothrix clintonii*, young filaments: (7) short young filaments breaking in the middle part; (8) young filaments swollen at the base, with no clear sheath; (9) very young *Leptolyngbya*–like filaments, not tapered; (10) middle part of long young filaments, central granules visible; (11) young filament breaking in the middle part; (12) middle part of long filament with irregular cells; (13) false branching; (14) tapering of young filaments; (15) young tapered filament. Scale bar 10 μm.

This process of fragmentation leads to formation of star–like colonies, with many swollen ends in the center and tapering ends extending out of the clump (Figs 22, 23). Young filaments have colorless or faintly purplish sheaths. The sheath becomes blackish–purple to black as the culture ages (Fig. 29). Colony on agar is brown to black.

Comparisons to other taxa

Most *Tapinothrix* taxa taper only very slightly (often not at all), and form grass–like turfs of upright filaments on hard surfaces (rocks). They are clearly members of the Pseudanabaenales in subclass Synechococcophycidae, based on cell diameter and peripheral thylakoid arrangement. Many are associated with a basal community of *Chroococcus* or *Pleurocapsa* species, and this association led to the incorrectly conceived and now abandoned genus *Amphithrix* (e.g. *Amphithrix janthina* Bornet et Flahault). Most species of *Tapinothrix* were originally described as members



Figs 16–29. *Tapinothrix clintonii*, mature filaments: (16–21) mature filaments swollen at the base, with thick, structured, blackish sheath; (22–23) star–like colonies, with many swollen ends in the center and tapering ends extending out of the clump; (24–25) long mature filaments with thick, structured, colored sheath; (26–27) old, mat–like colonies; (28) two mature filaments connected with one mucilaginous pad; (29) very old colony with black sheaths. Scale bar 10 μm.

of the genus *Homoeothrix*, but the type species of that genus, *Homoeothrix juliana* (BORNET et FLAHAULT) KIRCHNER, clearly belongs in the Oscillatoriaceae in subclass Oscillatoriophycidae (based on cell diameter and irregular thylakoid arrangement).

Our strain was growing on a rock surface, and likely had the typical heteropolar, basally attached filaments in nature. However, we did not see *Tapinothrix clintonii* in the natural material, and suspect that it was very rare in the original sample. Most species of *Tapinothrix* are pale greyish blue–green in color, and the brownish and purplish colors this strain possesses even in culture

are fairly distinctive. *Homoeothrix violacea* is one of the purplish species previously described, but is narrower at the base and has shorter cells in the terminal regions. Most importantly, *H. violacea* is a marine taxon. *Homoeothrix fusca* can be yellowish or blackish, but has blue–green trichomes, is not constricted or at most weakly constricted at the crosswalls, and is also narrower. Ecological separation for this taxon is also strong; *H. fusca* is reported to be xeno– to oligosaprobic in clear mountain waters, while *T. clintonii* was found in a warm desert seep wall associated with a stream flowing through cattle country (i.e., not oligosaprobic!).

Tapinothrix is likely most easily confused with species of *Heteroleibleinia*, which are heteropolar, attached at the base, but never tapering. The latter genus also seems consistently epiphytic rather than epilithic. Neither genus has been sequenced up until this time, and almost certainly confusion will surround these taxa until collection and analysis of sequence data is completed. There are no *Heteroleibleinia* species that could be confused with *T. clintonii*.

Phylogenetic placement

Tapinothrix clintonii and *Leptolyngbya sensu stricto* (the group of taxa associated with *Leptolyngbya boryana*, the type species of the genus) belong to the same clade (Clade A, Fig. 30). A strain possessing the morphology of *Leptolyngbya*, GSE–PSE30–01B may be sister to *Tapinothrix*, but there is poor support for this putative relationship. A number of *Pseudophormidium* strains belong to Clade B (Fig. 30). These strains are wider than most *Leptolyngbya*, and demonstrate multiple trichomes in a sheath with frequent false branching. Other clades contain mostly putative *Leptolyngbya* (Clades C, D, E and G). Clade F (Fig. 30) contains many taxa of uncertain placement in the LPP groups, along with some well established species and genera, such as *Halomicronema excentricum* Abed, Garcia–Pichel et Hernández–Mariné, *Prochlorothrix hollandica* Burger–Wiersma, Stal et Mur and *Leptolyngbya nodulosa* Li et Brand. Finally, Clade H contains a mixture of strains assigned to *Pseudanabaena* (10 OTU's), *Limnothrix redekei* (3 OTU's), and *Synechococcus* (1 OTU).

If the identification of the strains in this tree were made correctly (and we are not confident that they all were made consistent with Komárek & Anagnostidis 2005), then a number of genera are polyphyletic, including *Synechococcus* (Clades F and H), *Limnothrix redekei* (Clades F and H), *Pseudanabaena* (Clades D and H) and *Leptolyngbya* (Clades A, C, D, E, F, G). The strain identified as *Phormidium* (Clade F) is certainly incorrectly identified.

ITS structural comparisons

In cyanobacteria, the D1–D1' helix is the most conserved structure of the 16S–23S ITS region (Johansen et al. 2008). In all Pseudanabaenales examined to date, this helix contains a 5 bp basal helix (GACCU:AGGTC), a unilateral bulge of 5–8 nucleotides on the 3' side, followed by a 3 bp helix (ACC:GGU – except *Pseudanabaena* GSE–PSE20–05C and *Leptolyngbya appalachiana* GSM–SFF–MF60 which differ in sequence). A bilateral bulge or unilateral bulge comes next, with the rest of the helix being too variable to recognize a common pattern (Figs 31–43). Apart from these similarities, we did find further consistencies within the various LPP clades.

The clade consisting of 12 OTU's in *Leptolyngbya sensu stricto* includes *Leptolyngbya boryana, L. tenerrima,* and a number of unidentified species/strains. Of these 12, we have ITS sequences for eight strains, and all were markedly similar in secondary structure of the D1–D1' helix to the two strains chosen to represent this group (Figs 31, 32). This group of taxa all has sequences of 51 nucleotides, with a terminal loop of four unpaired nucleotides, a small irregularity caused by an adenosine residue that does not pair, and a basal unilateral bulge of only 5 nucleotides (Figs 31, 32). *Leptolyngbya crispata* which fell outside of *Leptolyngbya sensu stricto*, had a markedly similar D1–D1' helix to this clade (Fig. 33). The desert soil strains of *Leptolyngbya* had more variability in structure in this region than *L. sensu stricto*, and differed in several regards: a basal bilateral bulge of 7 nucleotides, the loss of the mid–helix bulge, and a larger terminal loop (Figs 34, 35).

The taxon sister to *T. clintonii* according to the phylogenetic analysis, *Leptolyngbya* GSE–PSE30–01B, had a strikingly different D1–D1' helix (Fig. 36). *T. clintonii* was similar to this strain in the basal part of the helix (Fig. 37), particularly with respect to the adenosine residue inserted opposite the unilateral bulge on the 3' part of the helix (a feature so far unique for this clade). The more terminal part of this helix was similar in length to those of *Leptolyngbya sensu stricto*,

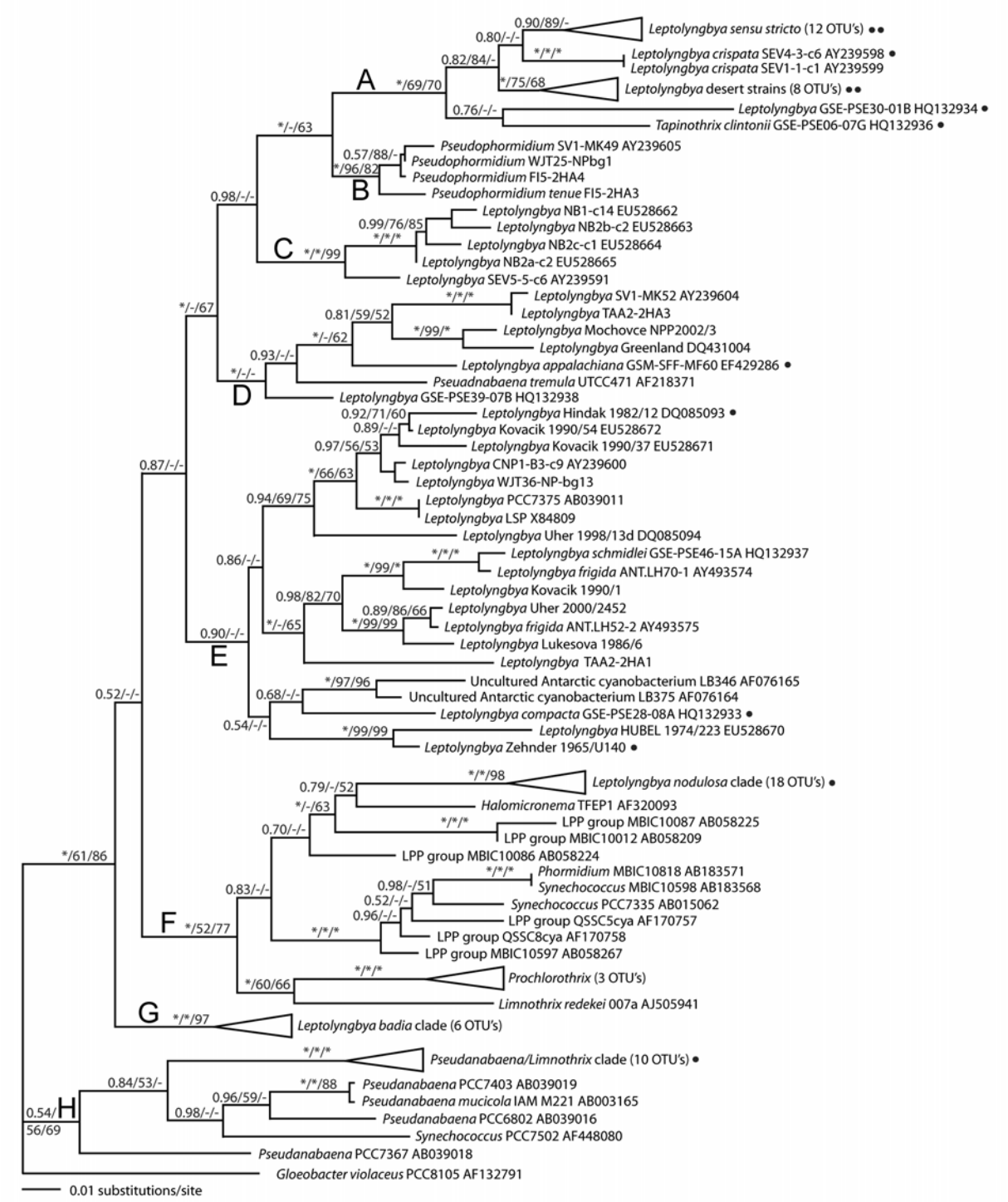
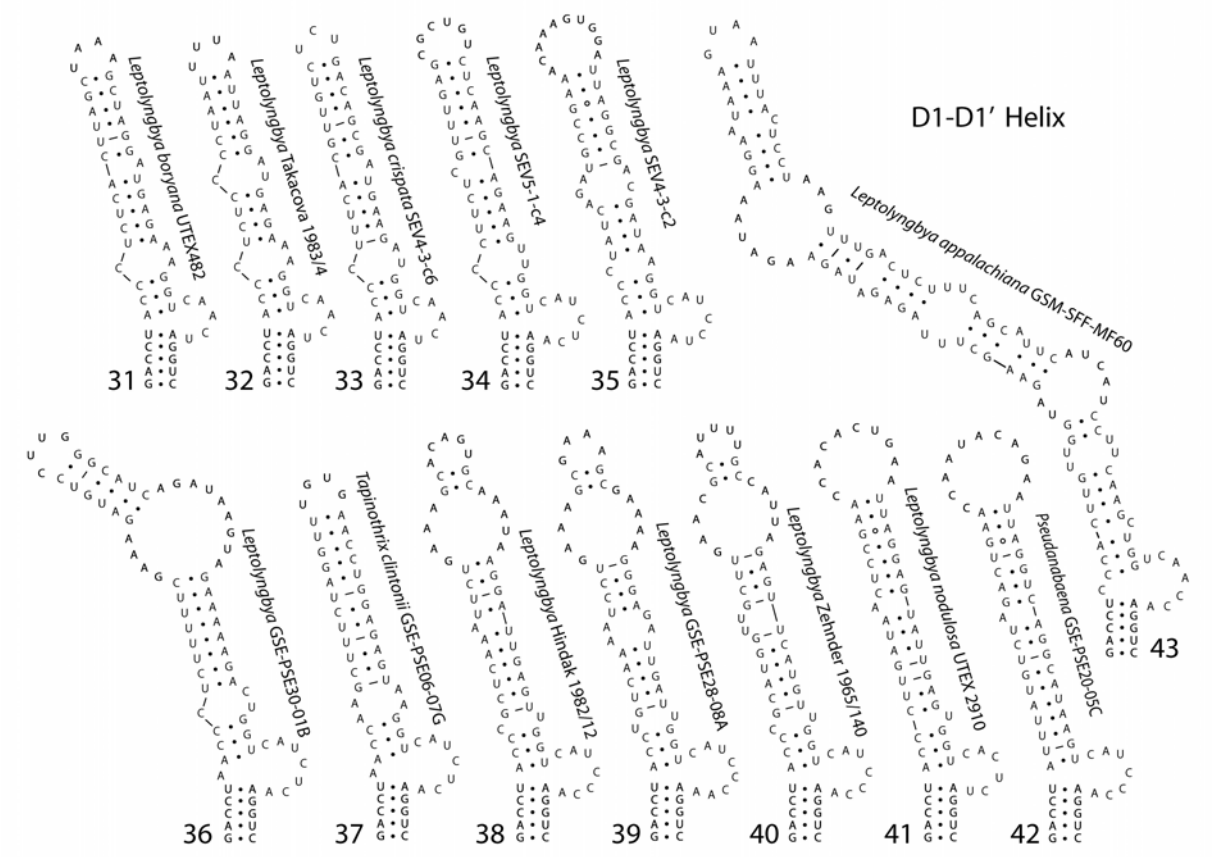


Fig. 30. Bayesian analysis of 113 OTU's of Pseudanabaenales plus *Gloeobacter violaceus* from the Gloeobacteriales. Node support is indicated as Bayesian posterior probabilities/bootstrap support from parsimony analysis/bootstrap support from likelihood analysis; an * means 1.0 or 100%, a – means support less than 0.50 or 50%. Major clades are indicated by letters (A–H). Black dots at the end of OTU names or sets of OTU's indicate that ITS structures were available and are shown in Figs 31–65.

but the sequence was markedly different in both the helix and terminal loop. The D1–D1' helix in strains more distantly related to *Leptolyngbya sensu stricto* had larger terminal loops, or a terminal loop subtended by a bilateral bulge (Figs 38–42). *Leptolyngbya appalachiana* had an unusually long D1–D1' helix (Fig. 43), and differed from all other taxa in the Pseudanabaenales sequenced thus far.

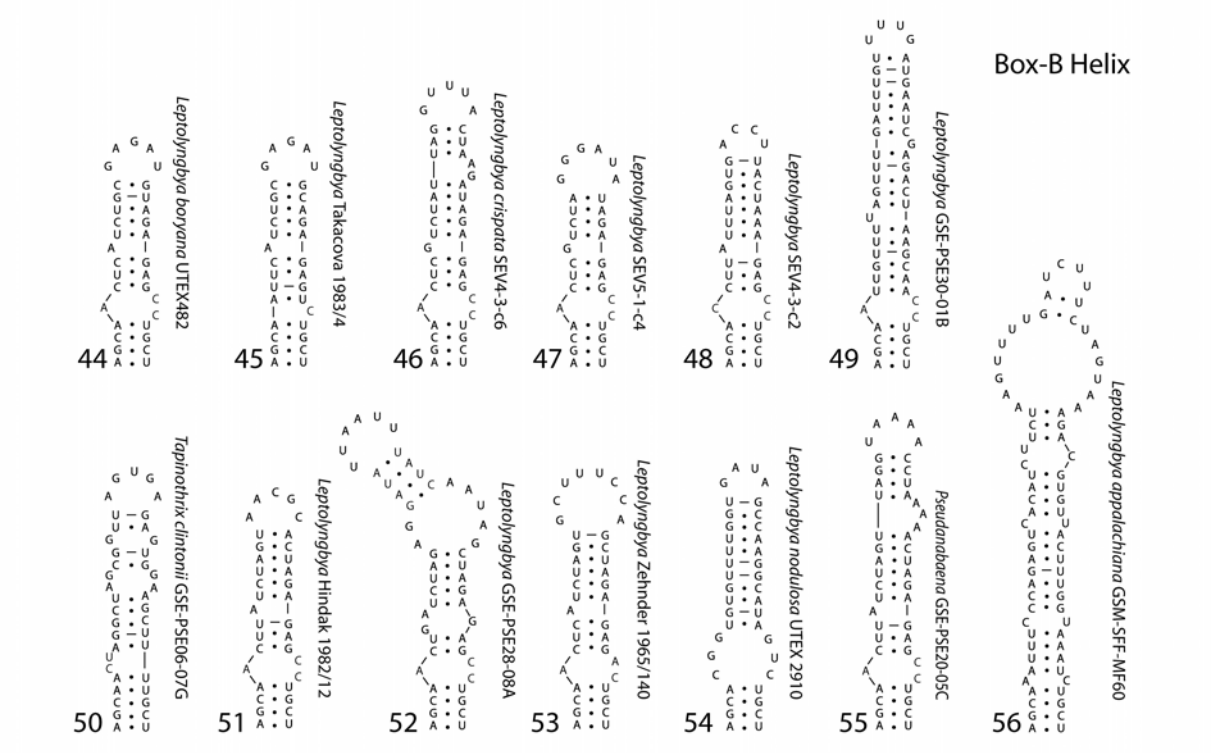
The Box–B antiterminator helix in *Tapino-*



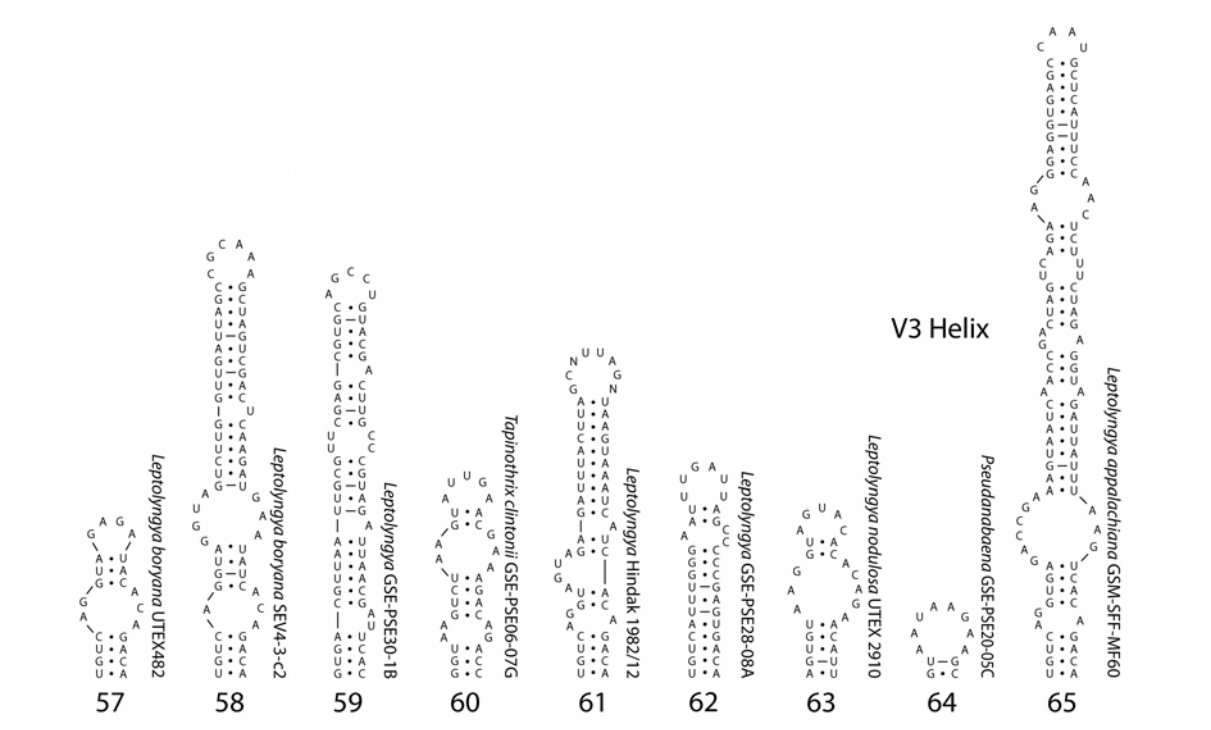
Figs 31–43. Secondary structure of the D1–D1' helix from the 16S–23S ITS region in several OTU's representing various Pseudanabaenalean clades, arranged in the order (top to bottom) they appear in the Bayesian analysis (Fig. 30): (31–32) *Leptolyngbya sensu stricto,* including the type species, *Leptolyngbya boryana*. This structure was seen in nearly all strains in this clade; (33–35) *Leptolyngbya* spp. from desert soils in New Mexico; (36) *Leptolyngbya* sp. with untapered, reddish colored trichomes from wet wall in Grand Staircase–Escalante National Monument; (37) *Tapinothrix clintonii*; (38–40) *Leptolyngbya* spp. from clade E (with cells longer than wide, see center, Fig. 30); (41) *Leptolyngbya nodulosa* UTEX2910, representing the *L. nodulosa* clade and clade F (Fig. 30); (42) *Pseudanabaena* sp. representing a clade of 10 OTU's (clade H, Fig. 30); (43) *Leptolyngbya appalachiana*, a species with exceptionally long helices in all conserved helices of the 16S–23S ITS (clade D, Fig. 30).

thrix clintonii was distinct from all other Box–B helices. This region is more variable than the D1–D1' helix, as evidenced by the different lengths and different placement of unilateral and bilateral bulges (Figs 44–56). The basal structure is more conserved, and this is the region different in *Tapinothrix* (Fig. 50). Most strains have a basal bilateral bulge above the initial helix (A–CC), whereas *Tapinothrix* has a unilateral double uracil insert on the 5' side of the helix. *Leptolyngbya* GSE–PSE28–08A and *Leptolyngbya appalachiana* had distinctively long helices that were also different than the Box–B regions of other strains.

The V3 helix is the most difficult structure to determine because its length is dependent on the length of the D4 and D5 helices. These latter two helices can only be determined with confidence when the 23S–5S ITS structure is sequenced and available. We did have this sequence for *Tapinothrix* so we are confident in this V3 structure (Fig. 60). The V3 for all taxa in *Leptolyngbya sensu stricto* (including *L. crispata* SEV4–3–c6) were identical in sequence and structure (Fig. 57). Surprisingly, the V3 helix for *L. crispata* SEV4–3–c2 (Fig. 58) was very different than *L. crispata* SEV4–3–c6 despite their close relationship inferred from the 16S rRNA phylogeny (Fig. 30). It seems possible this is due to sampling different ribosomal operons. *Tapinothrix* has a unique V3 among all taxa characterized (Fig. 60), with the base of the helix (the most conserved part of most helices) very distinct (5'GGU–ACC3') from the typical base (5'UGUC–GACA3'). Its putative sister taxon, *Leptolyngbya* GSE–PSE30–01B, also had a unique structure for the V3 among



Figs 44–56. Secondary structure of the Box–B helix from the 16S–23S ITS region in several OTU's representing various Pseudanabaenalean clades, arranged in the order (top to bottom) they appear in the Bayesian analysis (Fig. 30).



Figs 57–65. Secondary structure of the V3 helix from the 16S–23S ITS region in several OTU's representing various Pseudanabaenalean clades, arranged in the order (top to bottom) they appear in the Bayesian analysis (Fig. 30).

the sampled strains (Fig. 59). The remaining V3 helices were all unique (Figs 61–65), save for that of *Leptolyngbya nodulosa* UTEX2910. We have several sequences of strains in this clade, and they all have the same V3 helix (data not shown).

Nomenclatural revision of *Homoeothrix* species

We recommend that all of the *Homoeothrix* species clearly belonging to the Pseudanabaenaceae be transferred to *Tapinothrix*. While Komárek & Anagnostidis (2005) recommended that this revision occur, they felt that further work (cytological, morphological, and molecular) should be completed. The present work supports the hypothesis that these taxa are not in the Oscillatoriaceae, and further confusion will be experienced until the taxonomy is complete. Revisionary work is still needed in this genus, as many of the species overlap in morphology, and differentiation and identification are problematic. Currently, no living strains exist for any species of *Tapinothrix* or *Homoeothrix* except *T. clintonii*.

Based on its morphology, *Phormidiochaete fusca* (= *Homoeothrix fusca*) is the likely sister taxon of *T. clintonii*. It has similar cell measurements, but differs in the amount of constriction at the crosswalls, the trichome color, and habitat preference. This taxon should surely be in *Tapinothrix*, and the transfer is made below.

A few of the *Homoeothrix* species do not belong in the Pseudanabaenaceae or Oscillatoriaceae, and therefore correspond best with *Phormidiochaete* in the Phormidiaceae. *H. margalefii* Komárek et Kalina has trichomes up to 7 µm wide at the base and filaments up to 8.5 µm wide. This species is transferred below as well.

***Phormidiochaete crustacea* (Borzì ex Bornet et Flahault) Bohunická et Johansen, comb. nov.**
Basionym: *Leptochaete crustacea* Borzì ex Bornet et Flahault (1886–1888, ser. 3, p. 342).
Synonyms: *Homoeothrix crustacea* (Borzì ex Bornet et Flahault) Margalef (1953), *Homoeothrix margalefii* Komárek et Kalina (1965), non *Homoeothrix crustacea* Voronichin (1923).

***Tapinothrix articulata* (Starmach) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix articulata* Starmach (1960, p. 231).

***Tapinothrix batrachospermorum* (Skuja) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix batrachospermorum* Skuja (1964, p. 68).

***Tapinothrix calcarea* (Li) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix calcarea* Li (1994, p. 70).

***Tapinothrix crustacea* (Voronichin) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix crustacea* Voronichin (1923, p. 115).
Synonyms: *Homoeothrix globulus* Voronichin (1932), *Homoeothrix woronochinii* Margalef (1953); non *Homoeothrix crustacea* (Borzì) Margalef (1953).

***Tapinothrix fusca* (Starmach) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix fusca* Starmach (1934, p. 294).
Synonym: *Phormidiochaete fusca* (Starmach) Komárek et Anagnostidis.

***Tapinothrix gloeophila* (Starmach) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix gloeophila* Starmach (1960, p. 227).

***Tapinothrix gracilis* (Hansgirg) Bohunická et Johansen, comb. nov.**
Basionym: *Leptochaete crustacea* var. *gracilis* Hansgirg (1892b, p. 138).
Synonyms: *Leptochaete gracilis* (Hansgirg) Geitler (1925), *Homoeothrix gracilis* (Hansgirg) Komárek & Kováčik (1987).

***Tapinothrix janthina* (Bornet et Flahault) Bohunická et Johansen, comb. nov.**
Basionym: *Amphithrix janthina* Bornet et Flahault (1886–1888, ser. 3, p. 344).
Synonym: *Homoeothrix janthina* (Bornet et Flahault) Starmach (1959).

***Tapinothrix minuta* (Seckt) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix minuta* Seckt (1921, p. 425).

***Tapinothrix poljanskii* (Muzafarov) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix poljanskii* Muzafarov (1952, p. 84).
Synonym: *Homoeothrix schizotrichoides* Muzafarov (1952).

***Tapinothrix rivularis* (Hansgirg) Bohunická et Johansen, comb. nov.**
Basionym: *Leptochaete rivularis* Hansgirg (1892a, p. 54).
Synonym: *Homoeothrix rivularis* (Hansgirg) Komárek et Kann (1973).

***Tapinothrix simplex* (Voronichin) Bohunická et Johansen, comb. nov.**
Basionym: *Homoeothrix simplex* Voronichin (1932, p. 309).

***Tapinothrix stagnalis* (Hansgirg) Bohunická et Johansen, comb. nov.**

Basionym: *Leptochaete stagnalis* HANSGIRG (1892a, p. 54).
Synonym: *Homoeothrix stagnalis* (HANSGIRG) KOMÁREK et KOVÁČIK (1987).

***Tapinothrix subtilis* (SKUJA) BOHUNICKÁ et JOHANSEN, comb. nov.**
Basionym: *Homoeothrix subtilis* SKUJA (1964, p. 69).

***Tapinothrix varians* (GEITLER) BOHUNICKÁ et JOHANSEN, comb. nov.**
Basionym: *Homoeothrix varians* GEITLER (1927, p. 801).
Synonyms: *Homoeothrix simplex* var. *elegans* VORONICHIN (1932), *Homoeothrix simplex* f. *elegans* (VORONICHIN) ELENKIN (1949).

***Tapinothrix violacea* (BORNET et FLAHAULT) BOHUNICKÁ et JOHANSEN, comb. nov.**
Basionym: *Amphithrix violacea* BORNET et FLAHAULT (1886–1888, ser. 3, p. 344).
Synonym: Homoeothrix violacea (BORNET et FLAHAULT) KOMÁREK et KANN (1973).

Discussion

Tapinothrix clintonii shares characteristics with five different genera. To our knowledge, the unusual feature of forming trichomes which taper towards both apices is restricted to *Ammatoidea* (KOMÁREK & ANAGNOSTIDIS 2005). However, this taxon is based on a type species (*A. normannii* WEST et G.S. WEST) clearly belonging to the Phormidiaceae. *Phormidiochaete* possesses heteropolar trichomes that taper from the attached base to the apex, but it too belongs in the Phormidiaceae. The source of confusion between *Tapinothrix* and these two genera is that some species belonging to the Pseudanabaenaceae apparently have been transferred into these genera erroneously. While *T. clintonii* has features similar to these taxa, it clearly belongs in neither because of its phylogenetic placement in the Pseudanabaenales. *Phormidiochaete* can rarely form isopolar trichomes that are *Ammatoidea*–like, and these taxa may not be well separated.

Homoeothrix is a relatively species–rich taxon with a long nomenclatural history. No representative of *Homoeothrix juliana* has been sequenced to date, but it possesses the dimensions, cell–division pattern, and thylaloid arrangement consistent with the Oscillatoriaceae. The pseudanabaenalean taxa in *Homoeothrix* are likely very phylogenetically distant from the type species. We have placed the majority of these taxa in *Tapinothrix*, a genus which still needs considerable study and revision. These taxa are actually fairly common in unpolluted streams with rocky benthos, forming tufted fascicles of filaments.

Leptolyngbya GSE–PSE30–01B appeared to be the sister taxon to *Tapinothrix clintonii*. However, the two strains did not share high sequence similarity in the 16S rRNA gene (P = 91.1 %), did not have similar 16S–23S ITS secondary structures, and were dissimilar in morphology. We assume that this may be a case of long–branch attraction. *Tapinothrix* actually has highest sequence similarity to *Leptolyngbya sensu stricto* (P = 92.4–93.6 %), although this is still too low for it to be considered to belong to that genus since < 95 % similarity is often considered to be the cut–off for indication of different genera.

This paper reports the first sequence attributed to the genus *Tapinothrix*, a taxon fairly common in clean to slightly enriched mountain streams (KOMÁREK & KANN 1973). While common, the genus is not easy to obtain in culture as it often appears in an epilithic mat with many other taxa, and likely requires flowing water to develop its characteristic heteropolar, tapering morphology. In examination of other *Tapinothrix* in North America, JOHANSEN et al. (2011) found three species of *Tapinothrix*: *T. varians, T. janthina,* and a species new to science, *T. ozarkiana* JOHANSEN et ŘEHÁKOVÁ. They noted that species of *Tapinothrix* were difficult to differentiate and that more species might indeed be represented in the populations which they examined. Some species of *Tapinothrix* taper very little, and consequently resemble the epiphytic genus, *Heteroleibleinia*. The phylogenetic relationships of these taxa need determination. It seems possible that more than one tapering genus may be present within the Pseudanabaenaceae.

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