Tapinothrix ozarkiana sp. nov., with notes on distribution for the genus in North America

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Abstract: A total of 77 samples from streams and rivers were found to contain populations of *Tapinothrix* (Pseudanabaenaceae), a genus currently confused with *Homoeothrix* (Oscillatoriaceae). Three species within the genus were identified: *T. ozarkiana* sp. nov., *T. varians*. and *T. janthina*.

Key words: distribution, epilithon, *Homoeothrix,* lotic, North America, *Tapinothrix*

Introduction

Homoeothrix (Thuret ex Bornet et Flahault) Kirchner is defined as those nonheterocytous filamentous cyanobacteria that are polar, with basal cells of the filament wider than the apical portion of the trichome, which often tapers to a hair (Komárek & Anagnostidis 2005). The genus is currently polyphyletic. The type species, *H. juliana* (Bornet et Flahault) Kirchner, and at least four other taxa belong to the Oscillatoriaceae and possess widened discoid cells like other members of that clade. The majority of the species (over 20 taxa), however, have very thin trichomes, less pronounced tapering, and peripheral thylakoids, placing them in the Pseudanabaenaceae. This latter group has included taxa in *Amphithrix* Kützing ex Bornet et Flahault, *Leptochaete* Hansgirg, and *Tapinothrix* Sauvageau. The name that is considered correct for this group of taxa is *Tapinothrix* (Komárek & Anagnostidis 2005), but most of the taxa have remained in *Homoeothrix* pending transfer, which has occurred only very recently (Bohunická et al. 2011).

These taxa demonstrate the potential taxonomic confusion generated by having three different starting dates for cyanobacterial taxonomy (McNeill et al. 2006). *Homoeothrix* was mistakenly considered a member of the Nostocales, and so has its starting date with Bornet & Flahault (1886), although it has long been considered a member of the Oscillatoriales, starting with Gomont (1892, as *Lyngbya juliana* Meneghini ex Gomont). By the most recent standards, most of the species (*Tapinothrix*) are in the Synechococcineae, the subclass containing the coccoids, which start with Linneaus. The group is poorly known, requires revision, and is a taxonomic minefield with respect to the botanical code.

Homoeothrix, under any of its generic epithets, is scarcely reported from North America. *H. varians* and *H. janthina* have been reported from mountainous regions in both the western and eastern United States (Prescott 1951; Stein & Borden 1979; Komárek et al. 2003). Whitford & Schumacher (1969) report *H. stagnalis*, *H. crustaceae*, and *H. janthina* from North Carolina. We have seen *H. juliana* in samples from the Great Smoky Mountains National Park (Johansen et al. 2007).

Recently, as part of the NAQWA program, we realized that *Homoeothrix*, particularly those taxa allied with *Tapinothrix*, are actually quite abundant in North American streams. Their small, inconspicuous, and uncharismatic nature has likely led to them being ignored, or simply recorded in the past as *Oscillatoria* sp. or *Lyngbya*

Table 1. Site information for samples studied intensively for this work (Samples for which images are given).

Sample	Site Code	Locality	Coll. Date
GS162770	GS06927590	Woods Fork, near Hartville, Wright Co., MO	24 VII 06
GS162694	GS06928730	Big Piney River, at Simmons, Texas Co., MO	31 VII 06
GS162709	GS06928750	West Piney Creek, at Bado, Texas Co., MO	26 VII 06
GS162579	GS07053203	Long Creek, southeast of Denver, Boone Co., AR	08 VIII 06
GS162416	GS07053250	Yocum Creek near Oak Grove, Carroll Co., AR	25 VII 06
GS162662	GS07053250	Yocum Creek near Oak Grove, Carroll Co., AR	14 VIII 06
GS162610	GS07055893	Calf Creek, near Silver Hill, Searcy Co., AR	07 VIII 06
GS162700	GS07057280	North Fork White River, near Cabool, Douglas Co., MO	25 VII 06
GS162758	GS 070692655	Myatt Creek, east of Salem, Fulton Co., AR	07 VII 06

sp. We suspect that there may actually be many *Tapinothrix* taxa in North America, but that they are very difficult to separate with confidence. In this paper we document the most widespread and easily diagnosed species present in North America, transfer these taxa to *Tapinothrix* so that the genus will be more properly recognized, and describe *Tapinothrix ozarkiana* sp. nova.

Materials and Methods

Samples were collected for the NAQWA program, and included stream and river samples from around the United States. The 77 samples in which *Tapinothrix* populations were found to occur were a small portion of the total NAQWA set of collections examined, and were chosen because workers counting the soft algae in the samples had indicated the presence of either *Amphithrix* or *Homoeothrix*. Samples were preserved in formalin, and in most cases had been processed in a tissue grinder.

Analyses began with a set of collections from streams in the Ozark Mountains. A total of nine samples from Arkansas and Missouri made up this intensively sampled set (Table 1). Following study of these samples, a key to the North American species was constructed, and an additional 68 samples from various locations in the United States were studied and *Tapinothrix* populations were keyed to species using the key constructed from the Ozark Mountain populations.

Algae were examined at 1000 X magnification using Olympus and Zeiss high resolution photomicroscopes with Nomarski DIC optics at both the Academy of Natural Sciences in Philadelphia and at John Carroll University.

Results and Discussion

***Tapinothrix ozarkiana* JOHANSEN et ŘEHÁKOVÁ, sp. nov. (Fig. 1a–f)**

Diagnosis: T. gracili (HANSGIRG) BOHUNICKÁ et JOHANSEN maxime simile, sed trichomatibus angustatioribus et cellulis longioribus, praesertim in parte basali trichomatis.

Fila initio in fasciculis parvulis, demum in fasciculis magnis densis, calciocarbonate non incrustata, sine strato basali manifesto cyanobacteriarum. Vagina initio incolorata, postea distincte ochracescens, tenue vel lata, saepe ultra apex trichomatis extensa. Trichomata distincte constricta ad septum, contracta, 2.3–3.5 (4) µm lata, contracta ad pilum, qui est 1 µm latum. Cellulae doliiformes, isodiametrae vel saepe longiorae vel breviorae quam latiorae in 5 meditrichomate, septo tenui, nongranulares. Cellulae basales typice longiorae quam latiorae, 4–7 µm longae. Cellulae apicales longiorae quam latiorae in pilo.

Most similar to Tapinothrix gracilis (HANSGIRG) BOHUNICKÁ et JOHANSEN, but having narrower trichomes and longer cells, the latter particularly in the basal region of the trichome.

Filaments at first in small, loose fascicles, when mature in large dense fascicles, not incrusted with calcium carbonate, without an obvious base of coccoid cyanobacteria, 10–80 µm long. Sheath colorless when young, becoming distinctly yellowish with age, thin to wide, not uncommonly extending past tip of trichome. Trichomes distinctly constricted at the cross–walls, tapering, 2.3 – 3.5 (4) µm wide, tapering to a hair, which is 1 µm wide. Cells barrel–shaped, isodiametric or slightly longer or shorter than wide in midregion,

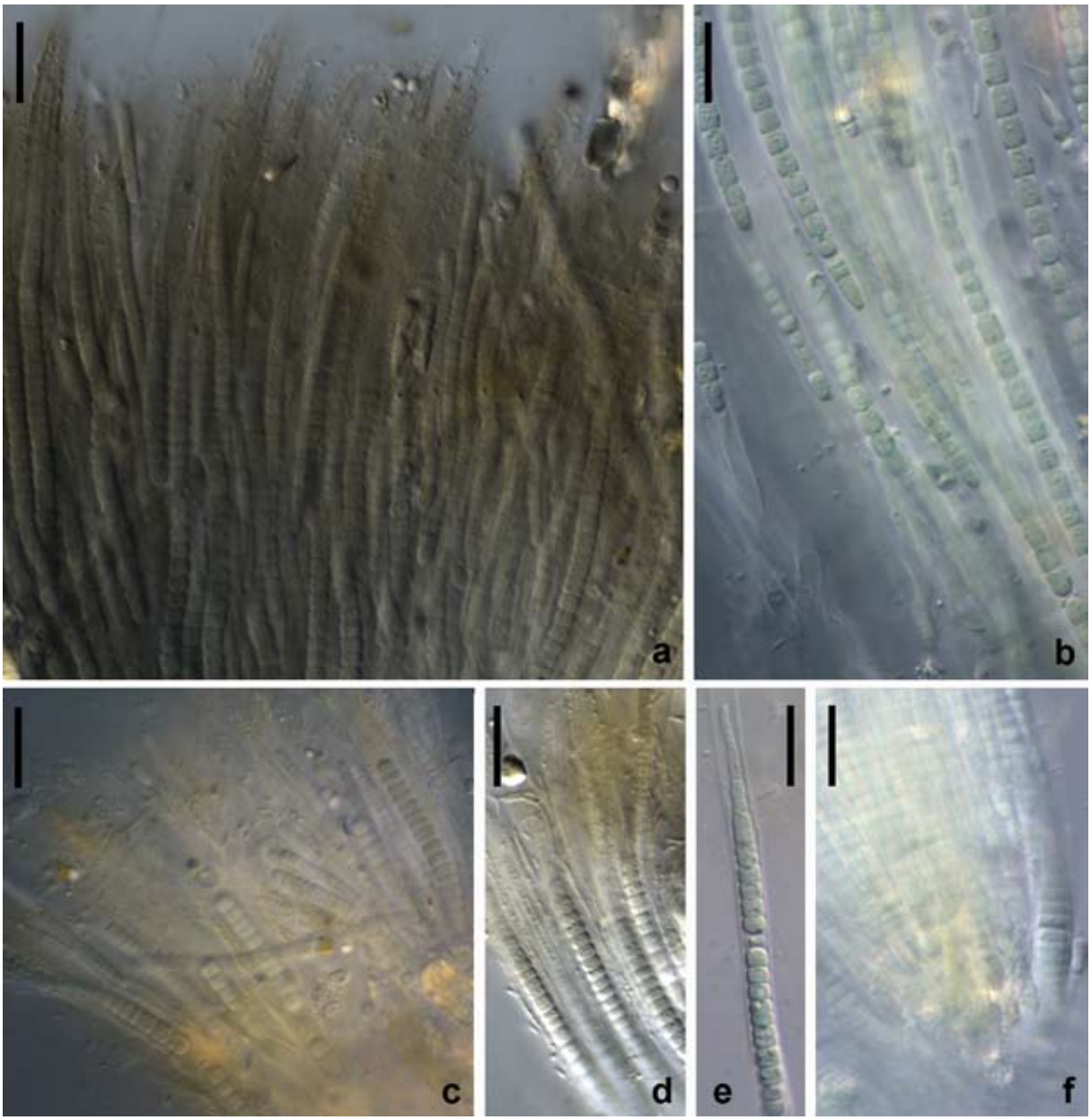

Fig. 1. Light micrographs of *Tapinothrix ozarkiana*: (a, c, d) type material, GS162709; (b, f) GS162694; (e) GS162579. Scale bars 10 µm.

with thin cross–walls, nongranular. Basal cells often longer than wide, 4 – 7 µm long, and can be thinner than midregion cells. Apical cells in hair longer than wide.

Holotype here designated: ANSP sample GS162709, collected 26 July 2006. Type locality: West Piney Creek, at Bado, Texas County, Missouri. Other reference materials: GS162416 Yocum Creek near Oak Grove, Carroll County, Arkansas, GS162579 Long Creek, southeast of Denver, Boone County, Arkansas. GS162694 Big Piney River at Simmons, Texas County, Missouri.

This taxon keys to *Homoeothrix gracilis* (Hansgirg) Komárek et Kováčik in Komárek and Anagnostidis (2005) (=*Tapinothrix gracilis* Bohunická et Johansen in Krautová et al. (in review), and indeed it is similar in many respects to that taxon. It is not calcium incrusted, it is distinctly constricted at the crosswalls, and it has a yellowish sheath. It differs by having narrower trichomes (2.3–3.5 µm wide as compared to 3–6 µm wide) and longer cells in the basal region of the trichome (4–7 µm long compared to rarely longer than wide). It also does not form the 6 telescopic sheaths reported for *T. gracilis* (Komárek and Anagnostidis 2005). While it forms large dense mats like *Tapinothrix incrustans* (Voronichin)

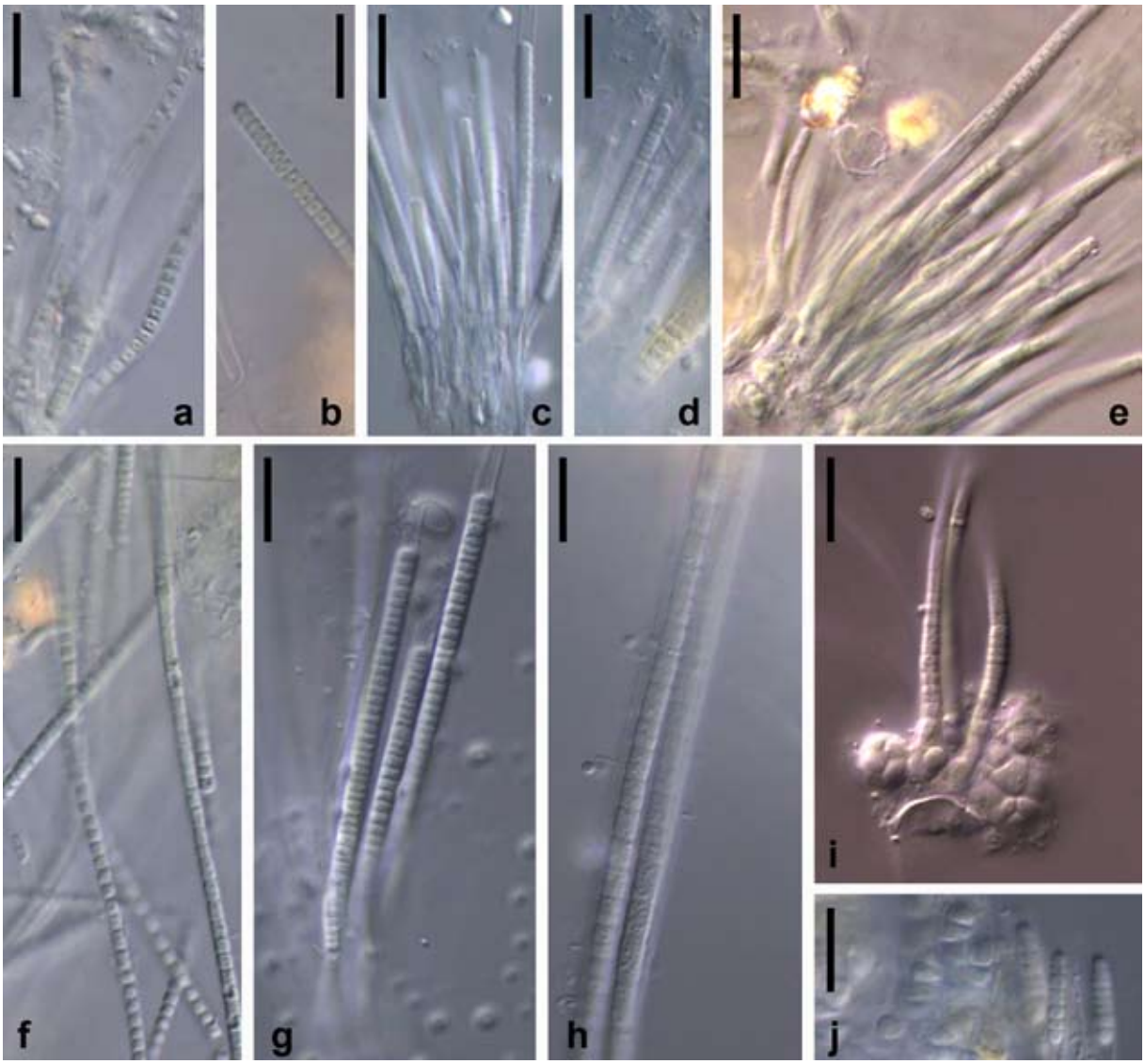

Fig. 2. Light micrographs of *Tapinothrix janthina* and *Tapinothrix varians*: (a–d) *T. janthina* forms overlapping with *T. simplex*, GS162610 (a), GS162758 (b), and GS162709 (c and d), respectively; (e) *T. janthina* GS162416; (f) *T. janthina* GS162610; (g, j) *T. varians* GS162770; (h) *T. varians* GS162700; (i) *T. varians* GS162709. Scale bars 10 µm.

BOHUNICKÁ et JOHANSEN, it is not calcium–incrusted like that taxon.

We first saw this taxon in samples from the Ozark Mountains where the best–developed populations occurred, but with further examination it was apparent in other regions of the United States. It is epilithic in circumneutral to slightly alkaline running waters.

Tapinothrix janthina **(BORNET et FLAHAULT) BOHUNICKÁ et JOHANSEN (2011), (Fig. 2a–f)**
Basionym: *Amphithrix janthina* BORNET et FLAHAULT 1886, 3: 344.

Filaments in loose fascicles, often with a basal layer of coccoid cyanobacteria. Sheath colorless, often extending past the end of the trichome. Trichomes only slightly constricted at the cross–walls, often cylindrical, 1.5–2.3 µm wide, but typically slightly thinner at apex than at base, sometimes tapering to a hair. Cells mostly isodiametric, or longer or shorter than wide, sometimes disarticulating.

This taxon includes populations that we originally referred to as *Homoeothrix simplex* VORONICHIN (Fig. 2a–d). This latter taxon is 2.0 µm wide, does not noticeably taper, and has cells consistently shorter than wide. *H. janthina* reportedly has isodiametric cells, which can be slightly longer or shorter than wide. However, in actively growing populations of *H. janthina*, trichomes can be seen which are dominated by cells distinctly shorter than wide. Thus, *H. simplex* could not consistently be separated from the widespread *H. janthina*, and at this time we

Table 2. North American Occurrences of *Tapinothrix* species (arranged alphabetically by state). Latitude and Longitude are reported in decimal degrees.

Site	° North	° West	State	*T. varians*	*T. janthina*	*T. ozarkiana*
GSN01182	60.0947	152.9106	AK	X		X
GSN91970	59.9183	151.6758	AK	X		
GSN91948	60.0344	151.6717	AK	X	X	
GSN91964	59.8572	151.6528	AK	X		X
GSN91856	61.7681	150.3369	AK		X	X
GSN92043	61.2053	149.8953	AK		X	
GSN20061	61.0875	149.8183	AK		X	
GSN01154	61.1672	149.7706	AK	X		
GSN20015	61.1672	149.7706	AK		X	
GSN92106	61.1672	149.7706	AK	X		
GSN17568	33.6075	86.7431	AL	X		X
GSN17529	33.7931	86.5172	AL	X	X	X
GSN17106	34.2683	85.8658	AL	X	X	
GSN17148	33.7797	85.7858	AL	X	X	
GSN00879	34.2903	85.5092	AL	X		X
GS162579	36.3681	93.2743	AR			X
GS162416	36.4539	93.3564	AR		X	X
GS162662	36.4539	93.3564	AR		X	
GS162610	35.9670	92.7757	AR		X	
GS162758	36.4233	91.6576	AR		X	
GSN00720	33.9686	117.4475	CA	X	X	X
GS025293	38.1839	107.7453	CO		X	
GS025423	38.1839	107.7453	CO	X		X
GS025283	38.5292	107.6483	CO	X		
GS025453	38.5175	106.9950	CO		X	X
GS025403	38.7842	106.8703	CO	X	X	
GS015103	39.6525	105.7069	CO	X		
GS017143	41.9140	72.5500	CT	X		
GSN28702	47.4917	115.9542	ID	X		
GSN29048	47.6569	115.8849	ID	X	X	
GSN29026	47.5256	115.8000	ID	X	X	
GSN01318	46.8319	114.0531	ID	X		
GSN20595	42.3853	88.3694	IL	X		
GS017343	42.5419	72.6942	MA	X		X
GS162770	37.2453	92.5678	MO	X	X	
GS162694	37.2418	92.0097	MO		X	X
GS162709	37.2721	92.1228	MO	X	X	X
GS162700	37.0549	92.1879	MO	X		

Table 2. Cont.

Site	° North	° West	State	*T. varians*	*T. janthina*	*T. ozarkiana*
GSN27734	47.3844	115.4007	MT	X		
GSN01350	47.0561	115.3522	MT	X	X	
GSN58747	45.5972	110.5653	MT	X		
GSN15534	45.0031	110.0011	MT	X	X	
GSN07258	46.2661	106.6900	MT	X	X	X
GSN15810	46.2661	106.6900	MT	X		
GSN58767	46.8047	105.2933	MT	X	X	
GSN58769	47.1000	104.7167	MT	X	X	
GSN00506	40.2217	74.7783	NJ	X		X
GSN87650	41.4772	74.9108	NY		X	X
GSN24359	41.8900	74.5903	NY		X	
GSN24490	40.6617	75.6272	PA	X		
GSN24536	40.0992	75.5556	PA			
GSN87731	41.2369	75.5011	PA			X
GSN87440	41.1636	75.2400	PA	X	X	
GSN87522	39.9678	75.1889	PA	X		
GSN87417	41.3189	74.8028	PA	X		X
GSN87449	41.3222	74.7956	PA	X	X	X
GSN00457	41.3706	74.6978	PA		X	
GSN18938	35.5850	86.5958	TN	X		
GSN23874	40.9817	111.8967	UT	X	X	
GSN00652	40.6144	111.8422	UT	X		
GSN24086	40.6186	111.7769	UT	X		
GSN00735	40.7975	111.7097	UT	X		
GSN23639	40.3139	111.6561	UT	X		
GSN24102	40.7928	111.4042	UT	X		
GS017023	43.7142	72.4186	VT	X		
GS030003	47.5144	123.3286	WA	X		X
GS030163	48.9458	122.4381	WA		X	
GS030153	48.9144	121.9911	WA		X	X
GS030083	47.4033	121.8980	WA		X	
GS030281	47.1819	121.3875	WA	X	X	
GSN63864	46.2500	120.3778	WA	X	X	
GSN28694	47.7031	116.9769	WA	X	X	X
GS004383	44.5244	89.3378	WI	X	X	X
GS004393	44.5244	89.3378	WI	X	X	X
GS004403	44.5244	89.3378	WI			
GS004593	45.7636	88.4631	WI	X	X	X
GSL00769	38.3789	80.4842	WV		X	X

Fig. 3. Geographic distribution of samples containing *Tapinothrix* species discussed in this paper. The 77 samples shown are from many hundreds of sampled and studied localities in streams in all regions of the United States. Black circles represent localities in which *T. ozarkiana* were found, and many contained either or even both *T. varians* or *T. janthina*. Hollow circles are additional sites in which either *T. varians* or *T. janthina* were found, but *T. ozarkiana* was absent.

recommend *H. simplex* not be used for populations in the USA. *H. borneti* is also confused with this taxon, having cells isodiametric or longer than wide, without any constriction at the cross–walls. We also recommend that this taxon not be used at present.

***Tapinothrix varians* (Geitler) Bohunická et Johansen (2011), (Fig. 2g–j)**

Basionym: *Homoeothrix varians* Geitler 1927, p. 801.

Filaments in loose fascicles, often with a basal layer of coccoid cyanobacteria. Sheath colorless, often extending past the end of the trichome. Trichomes only slightly constricted at the cross–walls, mostly cylindrical, 2.2 – 2.5 (3.6) µm wide, but sometimes tapering to a hair. Cells discoid (½ as long as wide) in meristematic regions, typically shorter than wide elsewhere, becoming isodiametric to longer than wide in distal part of trichome.

This taxon is most easily confused with *H. janthina*. When cylindrical trichomes with discoid cells are found (Fig. 2g), this is a clear indication of the presence of *T. varians*. Although diameter overlaps a small amount, cells of this species are mostly wider.

Biogeography

We were able to clearly differentiate three *Tapinothrix* species in our samples. Based on the keys and illustrations given in Komárek & Anagnostidis (2005), it seems possible more species exist in North America. Indeed, in a separate study, Bohunická et al. (2011) found a new *Tapinothrix* species in a desert stream in Utah, which they described as *Tapinothrix clintonii* Bohunická et Johansen. We considered *H. simplex*, *H. borneti*, and *H. margalefii* as possible additional species in our samples. These taxa are separated by very small morphological differences in size, as well as habitat preference and colony morphology. This difficult group would greatly benefit from molecular sequence analysis, but so far only *T. clintonii* has been sequenced. *Tapinothrix* usually grow tightly associated with other cyanobacteria (*Chroococcus, Calothrix, Phormidium, Pleurocapsa, Homoeothrix juliana*), have unique culture requirements, and no isolates of any species except *T. clintonii* exist, making sequence work difficult to undertake.

Taxonomy of *Tapinothrix* is additionally difficult due to the fact that these species often co–

Key for identification of *Tapinothrix* species in this study.

1a. Trichomes distinctly constricted at cross walls, with barrel shaped cells . . . . . *Tapinothrix ozarkiana*

1b. Trichomes slightly or not constricted at the cross walls 2

2a. Trichomes with regions of trichome with discoid cells (about ½ as long as wide) . . . . . *Tapinothrix varians*

2b. Trichomes with cells slightly shorter than wide to longer than wide, but not in regions of discoid cells 3

3a. Trichomes 2.2 – 3.6 µm wide, often cylindrical (not tapering), but can taper to a hair . . . . . *Tapinothrix varians*

3b. Trichomes 1.0 – 2.3 µm wide, usually tapering slightly, can taper to a hair . . . . . *Tapinothrix janthina*

occur in the same streams. This was true for many samples collected in different parts of the country (Table 2), and it appears at present that there are not geographic limitations on the distribution of any of the taxa. The 77 localities from which *Tapinothrix* were isolated demonstrate that the genus is present in rocky streams, typically in high energy mountain streams. *Tapinothrix* were found in the Pacific Coastal Range, Rocky Mountains, Ozark Mountains, and Appalachian Mountains (Fig. 3). The exceptional collections were two samples from Michigan, which has clean rocky streams but no mountains (Fig. 3). Our dichotomous key worked well on populations we examined, and we were able to identify species with fairly high levels of confidence. However, plasticity within populations was observed, and it may be that we would detect incorrect determinations if we had resolution from molecular data. We suspect that we may have underestimated the species diversity in the genus, and certainly further work on this inconspicuous but widespread genus is required.

Acknowledgements

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