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A revision of the genus *Geitlerinema* and a description of the genus *Anagnostidinema* gen. nov. (Oscillatoriophycidae, Cyanobacteria)

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Abstract: The simple filamentous cyanobacterial genus *Geitlerinema* is heterogeneous. At least two distinct phylogenetic clades can be derived from the set of most common freshwater *Geitlerinema* species. Our revision is based on the original description of the type species *G. splendidum* aka *Oscillatoria spendida* and on molecular sequencing of morphologically relevant strains. The revised *Geitlerinema* contains only one species according to morphological similarity with its original description. Consequently, the majority of other freshwater species inferred from molecular sequencing of 16S rRNA gene and 16S–23S ITS analysis (related both genetically and morphologically to *G. pseudacutissimum*) must be classified as a special taxon on the generic level. The name *Anagnostidinema* is proposed for this genus, which was selected in memory of the prominent late cyanobacterial specialist Konstantinos Anagnostidis. The genetic position, short review and characteristics of the newly defined genus *Anagnostidinema* is presented in this paper. The taxonomy of the rest of species (including the marine taxa), which are currently unable to be classified taxonomically with certainty, remain to be resolved in future studies.

Key words: 16S rRNA; *Anagnostidinema*; Cyanobacteria; ecology; *Geitlerinema*; 16S–23S rRNA ITS; molecular sequencing; morphology; new genus

Abbreviations: ITS, Internal Transcribed Spacer situated between 16S and 23S rRNA; CCALA, Culture Collection of Autotrophic Organisms, Třeboň, Czech Republic; CBFS, České Budějovice Faculty of Science Herbarium; PCC, Pasteur Culture Collection of Cyanobacteria, Paris, France; SAG, Culture Collection of Algae at the University of Göttingen, Germany

Introduction

The thin simple filamentous cyanobacteria from the orders Synechococcales and Oscillatoriales have very low morphological variability. Historical reliance on only few markers, e.g., lack of visible sheath or its pigmentation, filament tapering, length of cells, etc., led to the erection of genera that subsequently have been demonstrated to be polyphyletic according to 16S rDNA sequence analyses (e.g. Komárek & Anagnostidis 2005; Perkerson et al. 2011; Chatchawan et al. 2012; Komárek et al. 2014). As a consequence of findings based on the 16S rRNA gene, re–evaluation of the corresponding cytomorphological and ecological features is necessary.

The various species of *Geitlerinema* were classified in the past mainly into the genus *Oscillatoria* in respect to the absence of all types of derived cells (heterocytes etc.) and lack of visible sheaths (e.g., Gomont 1892; Geitler 1956). The genus *Geitlerinema* was separated from the LPP–B "Oscillatorian" group sensu

Rippka et al. (1979) by Anagnostidis (1989). The main diacritical features of this genus that differentiate it from other similar simple filamentous genera are thin, cylindrical trichomes (< 6 μm), intense gliding motility with oscillation and rotation and lack of sheath material. The relatively thin trichomes with various types of attenuation and bending of terminal cells are never firmly attached to any substrate. The position of thylakoids in cells is in principle parietal, but with numerous irregularities and specificities.

The type species of *Geitlerinema* is *G. splendidum* (Greville ex Gomont) Anagnostidis (Anagnostidis 1989), which possesses apical cells strikingly attenuated and hooked (when fully developed), and more or less spherically capitate at the ends. The trichomes are thin (1.8–3 μm wide), intensely motile, gliding with oscillation and rotation, not constricted at the cross–walls, attenuated towards the ends. Cells are 2–4 times longer than wide, sometimes with prominent cyanophycin granules near the cross–walls (Komárek & Anagnostidis 2005). The type locality of the taxon is a freshwater water tube in the Botanical Garden at Edinburgh, Scotland.

Recent phylogenetic analyses demonstrated that the genus *Geitlerinema* in its current concept is polyphyletic (Margheri et al. 2003; Stringfellow et al. 2007; Bittencourt–Oliveira et al. 2009; Perkerson et al. 2010) and revision of the genus is required. The combined molecular and morphological description of a characteristic representative of the type species, *G. splendidum,* has not yet been published, although images and phylogeny were documented in Hašler et al. (2012). In this study, we describe both phylogenetic and cytomorphological features of this species. Using a total–evidence approach we characterize and taxonomically transfer the former *Geitlerinema* species *G. pseudacutissimum* and *G. amphibium* into *Anagnostidinema* gen. nov. We also transfer nine additional *Geitlerinema* species into *Anagnostidinema* based on morphological data. Some *Geitlerinema*, such as the marine species, likely do not belong to either *Geitlerinema* or *Anagnostidinema*, and consequently we regard this study as only the first step in revision of the genus; we anticipate further fragmentation in the future.

Material and Methods

The strains in this study were isolated from freshwaters and soils, sampled in various localities worldwide. A few strains were obtained from the CCALA Culture Collection of Autotrophic Organisms (Třeboň, Czech Republic). Recently obtained strains designated as morphologically confirmed reference strains in this study were newly included into this collection. Accession numbers and origin of the strains are provided in Table 1. The strains were maintained on agar solidified Z8 medium (Carmichael 1986) at 18 °C and dim light in 12/12 photoperiod.

Morphological Evaluation and Ultrastructure. Cultured strains were examined by both light microscopy (LM) and transmission electron microscopy (TEM). Light microscopy observations were performed using an Olympus BX 51 light microscope equipped with Nomarski DIC optics and an Olympus DP71 digital camera.

For TEM, young cultures in exponential growth phase were prepared by fixation in glutaraldehyde: 3% (w/v) glutaraldehyde in 100 mM cacodylate buffer, pH 7.3, was applied for 3 hours at 4 °C, followed by postfixation by 1% (w/v) osmium tetroxide in the same buffer for 2 hours at 4 °C. The material was then washed with the buffer overnight at 4 °C. Thereafter, it was instilled in 2% (w/v) agar and, within it, dehydrated by a series of ethanol at gradually increasing concentrations. Following the dehydration, it was infiltrated with metacrylate LR White – in ethanol 1:1 for 30 min, then in ethanol 2:1 for 60 min, in ethanol 3:1 for 2 hours and, finally, in LR White pure at 4 °C for 3 days. Afterwards, it was encapsulated and polymerized by UV rays at 4 °C for 3 days. The material was then cut to ultrathin sections by a Reichert–Jung ultramicrotome Ultracut E. All the final sections were placed on supporting grids and contrasted by 2.5% (w/v) uranyl acetate. The final sections were photographed at various magnifications using a digital transmission electron microscope JEOL JEM–1010.

16S rRNA Gene Sequencing and Phylogenetic Analysis. Total genomic DNA was isolated following the modified xanthogenate–SDS buffer extraction protocol with addition of 3% PVPP and PEG–$MgCl_2$ precipitation (Mareš et al. 2013). A section of the rRNA operon containing the partial 16S rRNA gene and the 16S–23S intergenetic spacer (ITS) was amplified using the primers 359F (5'–GGG GAA TYT TCC GCA ATG GG–3'; Nübel et al. 1997), and 23S30R (5'–CTT CGC CTC TGT GTG CCT AGG T–3'; Wilmotte et al. 1993). The template DNA was mixed with 5 pmol of each primer in 50 μl of commercial PCR mix with Taq polymerase (Plain PP Master Mix, Top Bio, Czech Republic). The amplification was run using the following settings: a starting denaturation step (94 °C, 5 min); 40 cycles of 30 s at 94 °C, 30 s at 53 °C, and 3 minutes at 72 °C; final extension for 7 minutes at 72 °C and cooling to 4 °C. The success of the PCR was verified by running a sub–sample on a 1.5 % agarose gel stained with ethidium bromide. Sequencing was done by Macrogen (Amsterdam, Netherlands) using primers 359F, 23S30R, 1492R (5'–TAC GGY TAC CTT GTT ACG ACT T–3') and 810R (5'–GTT ATG GTC CAG CAA AGC GCC TTC GCC A–3') (Strunecky et al. 2014).

Sequences of the 16S rRNA gene were aligned in MAFFT (mafft.cbrc.jp; Katoh & Toh 2010). Minor changes were done manually with BioEdit 7.0.1 (Hall 1999). A fragment of ~1081 nt was used for the phylogenetic analysis of the 16S rDNA (starting at *E. coli* ATCC 11775 16S rRNA residue 302). The obtained sequences were blasted against GenBank and sequences of similar organisms were added into the alignment together with other cyanobacterial strains previously published as *Geitlerinema*. The rest of the sequences of cyanobacterial strains were added as outgroup taxa for a congruent phylogeny (cf. Mares et al. 2013; Bohunická et al. 2015).

The phylogenetic tree topology was based on Bayesian inference analysis computed in MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003). For the Bayesian analysis, four runs of four Markov chains were calculated for 12 million generations, sampling every 1,000 generations. The initial 25%

Table 1. Characterization of the strains used in taxonomic evaluation of genera *Geitlerinema* and *Anagnostidinema*, including accession numbers of the sequences obtained within the study. CCALA = Culture Collection of Autotrophic Organisms, Třeboň, Czech Republic; strains labelled with other strain numbers were not included into any official culture collection.

Species	Strain number	Original designation	Habitat	Locality	Collector/isolator	Accession #
Geitlerinema splendidum	P014	*G. splendidum*	epipelon	Italy, Lake Monbino	Poulíčková/Hašler	JQ712598
	P017	*G. splendidum*	epipelon	Italy, Lake Tovel	Poulíčková/Hašler	JQ712599
	CCALA1004	*G. splendidum*	wet wall	USA, Utah, Grand Staircase–Escalante NM	Bohunická	KP412630
Anagnostidinema pseudacutissimum	CCALA142	*G.* cf. *pseudacutissimum*	meadow soil	Czech Republic, river Lužnice valley	Klabouchová	KT315934
	CCALA151	*G.* cf. *splendidum*	meadow soil	Czech Republic, river Lužnice valley	Klabouchová	KT315935
	P03	*G. pseudacutissimum*	epipelon	Italy, Lake Tovel	Poulíčková/Hašler	JQ712614
	JR16S117	*G. deflexum*	seepage	Antarctica, James Ross Island	Elster & Šnokhousová	KT315936
	LD26	*Geitlerinema* sp.	soil	India, Ladakh	Čapková	KT315937
	LD27	*Geitlerinema* sp.	soil	India, Ladakh	Čapková	KT315938
	JR23S123	*Leptolyngbya* sp.	seepage	Antarctica, James Ross Island	Elster & Šnokhousová	KT315939
	CCALA150	*G.* cf. *splendidum*	water plants basin	Czech Republic, Třeboň, Institute of Botany	Kašpárková	KT315940
	M2	*G. lemmermannii*	epipelon	Czech Republic, Pond Javorníček	Hušáková	KT315941
	P005	*G. pseudacutissimum*	epipelon	Italy, Lake Monbino	Poulíčková/Hašler	JQ712608
	P004	*G. pseudacutissimum*	epipelon	Italy, Lake Monbino	Poulíčková/Hašler	JQ712617
	GSE–PSE04–08G	*G. amphibium*	seep wall	USA, Utah, Grand Staircase–Escalante NM	Bohunická	KT315942
	LD9	*Geitlerinema* sp.	soil	India, Ladakh	Čapková	KT315931
	JR18S118	*Leptolyngbya* sp.	seepage	Antarctica, James Ross Island	Elster & Šnokhousová	KT315943
Anagnostidinema amphibium	JR21S121	*Leptolyngbya* sp.	seepage	Antarctica, James Ross Island	Elster & Šnokhousová	KT315945
	RO–MK72	*G. acutissimum*	wet rock	Romania, Apuseni NP	Veselá	KT315946
	HA4216–MV1	*Leptolyngbya* sp.	stream	USA, Hawaii, Oahu, Laie Falls	Johansen & Vaccarino	KC525097
	MK80	*Geitlerinema* sp.	mat on tree roots	Czech Republic, Vidnava	Bohunická	KT315947
	P013	*G. carotinosum*	epipelon	Austria, Lake Untersee Lunz	Brablcová/Hašler	JQ712598

of generated trees were discarded as burn–in. The tree was validated by maximum likelihood method in RAxML 7.0.4 (Stamatakis 2006) under a GTR model with 1,000 bootstrap repetitions and neighbor joining under maximum composite likelihood model with uniform rates among sites in MEGA 6.06 (Tamura et al. 2011) with 1,000 bootstrap repetitions. The secondary structures of different ITS regions (D1–D1′ helix and Box–B helix) were predicted with the Mfold web server version 3.2 (Zuker 2003) with temperature set to default conditions (37 °C) and draw mode at untangle with loop fix. Secondary structures were then drawn in Adobe Illustrator (CS–3).

Results

A total of 23 strains were studied (Table 1). Morphological evaluation of freshwater and soil strains confirmed taxonomical designation into the genus *Geitlerinema* according to Komárek & Anagnostidis (2005). The strains P014, P017, CCALA 1004 morphologically matched the typical *G. splendidum* sensu stricto. Other strains were originally classified as *G. pseudacutissimum, G. amphibium, G. carotinosum*, or *Geitlerinema* sp. The sequence data of the 16S rRNA gene (for accession numbers see Table 1) grouped the strains into three major clades. One clade was formed by three morphologically distinct strains of *G. splendidum* sensu stricto (Figs 1, 2A–F, 3A–D). The rest of the freshwater strains clustered into another clade that was both phylogenetically and morphologically distinct, and which we here designate as *Anagnostidinema* gen. nov. (Figs 1, 2G–V, 3E–I). A third clade of marine *Geitlerinema* strains was situated near to *Planktothrix agardhii* and was phylogenetically very distant from either freshwater clade (Fig. 1), which is consistent with previous reports (Perkerson et al. 2010). As these species are clearly not in *Geitlerinema* sensu stricto or *Anagnostidinema* gen. nov., they will not be treated further here.

Examination of sequence identities of the strains of interest based on their 16S rRNA gene sequence clearly supported the separation of the three clades. The gene identity of *G. splendidum* representatives was internally high (>97%), but was lower than 93% in comparison to the species formerly classified in *Geitlerinema* that we now assign to *Anagnostidinema*. The members of the marine *Geitlerinema* clade were less than 89% identical to the two freshwater clades (Table 2). *Anagnostidinema* gen. nov. formed an isolated clade with 16S rRNA sequence identity always less than 93% to representatives of adjacent clades (Fig. 1; Table 2). Within *Anagnostidinema*, at least two species were evident (Fig. 1). One group of strains was highly morphologically consistent with the original description of *G. pseudacutissimum* (Figs 2G–N, 3E–I), and we place all of these strains into *Anagnostidinema pseudacutissimum* comb. nov., regardless of original strain designation (Table 1). The other group of strains was morphologically consistent with either *G. amphibium* or *G. carotinosum* (Figs 2O–V), two species whose separation has been questioned by other workers. We place almost all of these strains into *Anagnostidinema amphibium* comb. nova in this paper, although we recognize that further study may show this lineage has genetically diverse members (i.e. cryptic species, probably with *A. carotinosum*).

The cellular ultrastructure of *Geitlerinema* and *Anagnostidinema* is similar. In both taxa thylakoids are irregularly parietal, with 4–6 thylakoid layers cylindrically and peripherally positioned along the cell walls (Fig. 3). In *G. splendidum*, thylakoids are characteristically wavy and sometimes separated from a strictly parietal position (Figs 3A–D). Thylakoids constitute the majority of the cytoplasm, with granules visible within the centroplasm.

Examination of the 16S–23S ITS region, which was found only as a single operon per studied strain according to raw sequences, showed evidence of deep genetic separation of *Geitlerinema* and *Anagnostidinema* (Fig. 4; Table S1). The lengths of the domains of the ITS region were remarkably consistent within both species and genera. *Geitlerinema splendidum* was notably longer in sequence length for the D1–D1' helix, spacer with D2 and D3, V2, and spacer to end of BoxB, while its D4 to V3 domain and V3 helix were both smaller. The differences in lengths between *A. pseudacutissimum* and *A. amphibium* were less marked, but fairly consistent within species grouping (Table S1).

Secondary structure of the 16S–23S ITS region was also congruent with the morphological and phylogenetic evidence. *G. splendidum* had markedly different D1–D1', boxB, and V3 helices in comparison to the two *Anagnostidinema* species (Fig. 4). Specifically, the D1–D1' helix of *Geitlerinema* (Figs 4F,G) had a short basal stem of only 4 bp, a large bilateral bulge only 2 bp from the basal unilateral bulge, and a large terminal loop (15–16 nt as compared to 4 nt in *Anagnostidinema*; Figs 4A–E). The BoxB helices in *Geitlerinema* were characterized by a basal mismatch that was C:AA rather than A:CC (Figs 4H–N). Finally, the V3 helices in *Geitlerinema* were highly divergent from those in *Anagnostidinema* in both sequence and structure (Figs 4O–T).

Within *Anagnostidinema*, the differences in secondary structures of the two species were striking, and their separation was well supported. The structures within *A. pseudacutissimum* were more consistent between strains than in *A. amphibium* (Figs 4A–B, H–I, O–P). The structures were sufficiently divergent within *A. amphibium* to conclude that cryptic diversity may exist in this cluster (Figs 4C–E, J–L), and that with further study, more species may be recognized in the clade. More sequences of existing taxa are needed, as the diversity may have already been taxonomically circumscribed. In particular, *A. carotinosum* is similar

to *A. amphibium* and may be represented in our phylogeny together with *A. amphibium* (they differ only slightly in dimensions and by the composition of granules in cells). We consider resolution and combination of cryptic species within *Anagnostidinema* to be beyond the scope of this study.

From the combined molecular analyses and congruent detailed morphology, it is evident that *Geitlerinema* sensu stricto currently includes only the type species, *G. splendidum*. The other species previously assigned to it are, based on morphology and our limited molecular study, in other genera. At this time we more narrowly circumscribe the genus *Geitlerinema*, give a species description based on the strains we used for sequencing, and describe *Anagnostidinema*, as well as transfer the majority of noncapitate species to the latter genus. Sequenced strains assigned to *Geitlerinema* that fall outside of both *G. splendidum* and *Anagnostidinema* clades require further study and eventual taxonomic circumscription. Two previously described taxa, *G. apolloniae* Anagnostidis and *G. sandbergii* (Skuja) Anagnostidis, most probably do not belong to either genus based on their very divergent morphology.

The holotype specimen of *Geitlerinema splendidum* probably resides in the Herbarium Greville at the Royal Botanic Garden Edinburgh, Scotland, but have not been catalogized yet. *Oscillatoria splendida* was originally collected in tubs of water in a stove in the Botanic Garden in Edinburgh in 1824. This genus and species were correctly typified and validly published under the ICN and are well established in a recent work (Komárek & Anagnostidis 2005).

We have studied and sequenced the strains CCALA 1004, P014, and P017 (Hašler et al. 2012), which all fully correspond to the description of *G. splendidum* (Komárek & Anagnostidis 2005, p. 129, fig. 137). The original description and illustrations of *Oscillatoria splendida* were also examined (Gomont 1892, p. 224, plate 7, figs. 7, 8) and found to be in strict agreement with later circumscriptions of the species. This species is easily recognized by its translucent trichomes which exhibit rapid gliding as well as very distinctive capitate end cells. Below we characterize our strain material to document the morphology of our sequenced strains. The European strains P014 and P017 were unfortunately lost after morphological characterization and sequencing (Hašler et al. 2012). We are consequently proposing North American CCALA 1004 be considered a reference strain until European material again becomes available, recognizing that this reference strain has no nomenclatural standing in either nomenclatural code governing cyanobacteria. Because the holotype material is not accessible and supposed to be deformed after nearly 200 years of existing in a dried state in a herbarium, we consider it to be "demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name to a taxon" (Article 9.20, ICN), and consequently establish an unambiguous type based upon dried material deposited in the CBFS Herbarium in České Budějovice, Czech Republic. That material (CBFS A–056) is based on the strain CCALA 1004, which was chosen because it was the only taxonomically confirmed strain present in an actively curated public culture collection, which can be obtained by other workers for molecular or biochemical study.

Geitlerinema (Anagnostidis et Komárek) Anagnostidis 1989

Description: Thallus thin, delicate, mostly bright blue–green, diffluent, sometimes fascicle–like, usually forming thin mats; occasionally isolated trichomes. Sheaths absent. Trichomes ± parallel–arranged, cylindrical, straight, slightly flexuous, not constricted at the cross–walls, bent and spherically capitate at the ends, motile, with intense gliding in the direction of the longitudinal axis, sometimes accompanied by a distinctive rotation, 1.5–3 μm wide. Cells rarely short, up to 2–4(6) × longer than wide, 3.5–8 μm long, pale grayish, blue–green or bright blue–green, without evident chromatoplasma or centroplasma, sometimes with translucent cyanophycin granules at the cross–walls, without aerotopes. Apical cells in developed trichomes bent or/and apparently spherical–capitate. Thylakoids concentrically arranged, peripheral and more or less parallel to the longitudinal cell walls, forming a wavy concentric inner cylinder. Centroplasma includes droplets of polyphosphates, starch and carboxysomes (Fig. 3, A–D). The main diacritic features are trichomes lacking sheaths and particularly the morphology of the end cells that are bent, spherically capitate, and elongated up to 15 μm when mature. Reproduction by disintegration of trichomes into motile hormogonia, without necridic cells.

Habitat: The taxon occurs commonly in shallow stagnant freshwaters, thermal water and wet rocks.

Iconotype: *Geitlerinema splendidum* (Greville ex Gomont) Anagnostidis 1989, based on *Oscillatoria splendida* Greville ex Gomont, Annales des Sciences Naturelles, Botanique, Série 7 16:224, pl. VII: figs 7, 8, 1892.

Geitlerinema splendidum (Greville ex Gomont) Anagnostidis 1989 (Figs 2A–F, 3A–D)

Colony (thallus) spreading, with bundles of filaments, sometimes forming coils, bright green to blue–green, olive when old, not mucilaginous. Trichomes up to 1 mm long, intensely motile, slightly bent and entangled, slightly constricted at the distinct cross–walls, 2–2.3 μm wide. Cells 2–4× longer than wide, pale grayish, blue–green or bright blue–green, without evident chromatoplasma or centroplasma, with translucent cyanophycin granules at the cross–walls, (3)3.5–8 μm long. The main diacritic feature is morphology of trichomes without sheaths and particularly the shape of the end cells, which are in developed stages attenuated and

elongated, narrowed, bent, spherically capitate, up to 15 μm long.

Habitat: The taxon occurs commonly in shallow stagnant freshwaters and wet rocks.
Epitype: dry material CBFS A–056 is based on strain: *Geitlerinema splendidum* CCALA 1004.

Species excluded from *Geitlerinema* according to morphologic evaluation

G. apolloniae Anagnostidis
This species has trichomes too wide (up to 6.5 μm) to be considered *Geitlerinema*, as well as cells that are shorter than wide to isodiametric. It requires revision, and at this point in time without sequence data or ultrastructure it cannot be reliably assigned to any genus.

G. sandbergii Skuja
This species was described from soil in Sweden. It is also too wide (5–6.5 μm) and has cells shorter than wide to isodiametric. It is also calyptrate. It conforms in both morphology and ecology to *Microcoleus vaginatus*, and we consider it to be a later synonym of that species.

***Anagnostidinema* Strunecký, Bohunická, Johansen et Komárek gen. nov.**

Description: Colonies spreading, green to blue–green, mucilaginous, amorphous. Sheaths absent. Trichomes thin, mostly bright blue–green (aquamarine), usually straight or slightly bent, dispersed evenly and loosely without mat formation, unconstricted or only indistinctly constricted at cross–walls. Sheaths absent. Trichomes cylindrical, straight, not capitate, motile with intense gliding in the direction of the longitudinal axis, sometimes accompanied by bending and distinctive rotation, 1–3 μm wide. Cells usually 2–4 longer than wide, 4–8 μm long, sometimes with visible peripheral chromatoplasma, sometimes with evident carotenoid granules, without aerotopes, after division growing to the size of the mother cells before the next cell division. Apical cells in developed trichomes bent, narrowed and pointed–rounded, never calyptrate or capitate. Thylakoids parietal, with triangle or rhombic organization in transversal section. Reproduction by disintegration of trichomes in hormogonia without necridic cells.

Etymology: named in memory of the prominent late cyanobacterial specialist Konstantinos Anagnostidis, combined with the Greek word *nema* – a cable.

Type species: *Anagnostidinema pseudacutissimum* (Geitler) comb. nov.

Anagnostidinema morphologically differs from *Geitlerinema* in the absence of capitate apical cells, phylogenetic placement, and in secondary structure of conserved domains of the 16S–23S ITS region.

***Anagnostidinema pseudacutissimum* (Geitler) Strunecký, Bohunická, Johansen et Komárek comb. nov. (Figs 2G-N, 3E-I)**
Basionym: *Oscillatoria pseudacutissima* Geitler, Österr. Bot. Zeitschr. 103(2–3): 343, pl. 1, figs. c, d, 1956.
Synonym: *Phormidium pseudacutissimum* (Geitler) Anagnostidis et Komárek, Arch. Hydrobiol. Suppl./Algol. Stud. 50–53: 404, 1988; *Geitlerinema pseudacutissimum* (Geitler) Anagnostidis, Plant. Syst. Evol. 164: 43, 1989; (Figs 2G–N, 3E–I).

Description: Colonies (thallus) spreading, mounded, in cultures penetrating the agar, with rings of trichomes visible as minute coils in the colony, blue–green, dark green, pale olive–green to orange. Sheaths absent. Trichomes thin, bright blue–green, usually straight or slightly bent, dispersed evenly and loosely without mat formation, unconstricted or only indistinctly constricted at the cross–walls, 1.3–2.2 μm wide, slightly attenuated and bent at the ends, motile with gliding motility. Sheaths absent. Cells pale olive–green to bright blue–green, sometimes with orange granules at the cross–walls, 2–4× longer than wide, 4–8 μm long. Apical cells (developed) cylindrical rounded and elongated, conical, sometimes bent or hooked, never calyptrate. Necridic cells not observed. Thylakoids parietal with generally straight four to five layers of thylakoid membranes. Thylakoids in transversal section with triangle or rhombic organization (Fig. 3G). Numerous polyphosphates of variable size were found, mainly at the peripheral part of the cells (e.g., Fig. 3I).

Habitat: Freshwater, in littoral of lakes, periodically flooded meadows, thermal springs, isolated pools.
Holotype: *O. pseudacutissima* specified by Geitler (1956) in the original publication as an exsicate in the Herbarium of the Botanischen Institut der Universität Wien. Due to the ambiguous nature of dried material, we here designate an epitype.
Epitype: dry material CBFS A–057 is based on the strain CCALA150. Other strains available for comparison which we consider also to be this species are CCALA150 and CCALA 151.

***Anagnostidinema amphibium* (Agardh ex Gomont) Strunecký, Bohunická, Johansen et Komárek comb. nov. (Figs 2O-V)**
Basionym: *Oscillatoria amphibia* Agardh ex Gomont, Ann. Sci. Nat.–Bot. 16: 221, pl. VII, fig. 6, 1892.
Synonym: *Phormidium amphibium* (Agardh ex Gomont) Anagnostidis et Komárek, Arch. Hydrobiol. Suppl./Algol. Stud. 50–53: 404, 1988; incl. *Oscillatoria amphibia* f. *contorta* G.S. West, J. Bot. 67: 243, 1909a ; *Oscillatoria amphibia* f. *circinata* Anagnostidis, Ereynai Kyanoph. Therm. Ellados, p. 233, 1961; *Geitlerinema amphibium* (Agardh ex Gomont) Anagnostidis, Plant. Syst. Evol. 164: 38, 1989).

Description: Colonies (thallus) spreading, mounded, in cultures penetrating the agar, expanding, with rings of trichomes visible as minute coils in the colony, dark

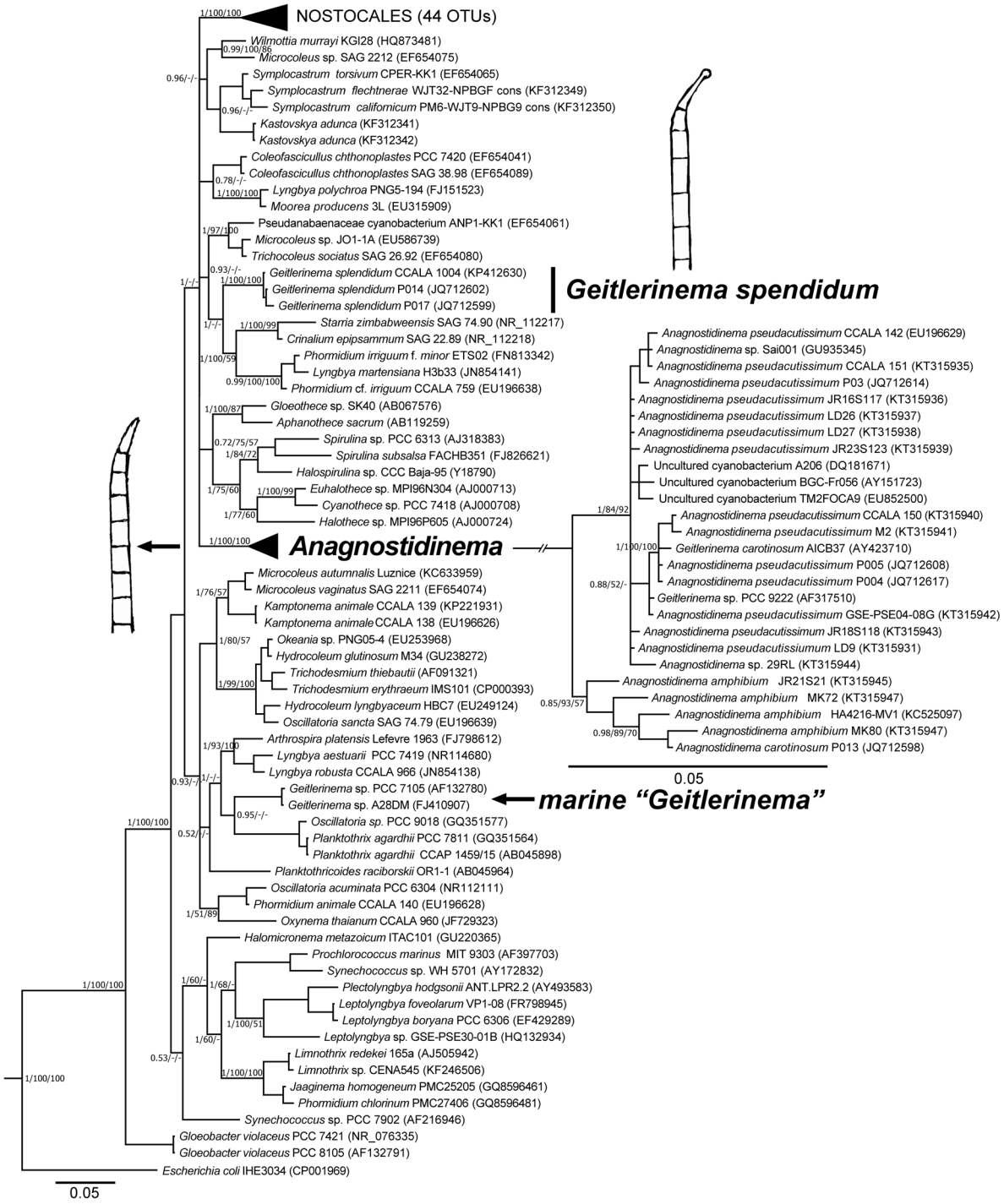

Fig. 1. Phylogenetic analysis based on 126 sequences of the 16S rRNA gene showing position of the genera *Geitlerinema* and *Anagnostidinema*. 1,081 bp of the 16S rRNA gene (starting at *E. coli* ATCC 11775 16S rRNA residue 302) were used for phylogenetic comparisons. Branch support values are shown as Bayesian posterior probability, Maximum Likelihood, and Neighbor Joining bootstrap values, respectively. Values smaller than 0.5 or 50 percent are not shown.

green, blue–green, pale olive–green to orange. Trichomes usually straight or slightly curved and long, ± parallel arranged, frequently also screw–like coiled or circled, motile with gliding motility (oscillation or rotation), forming thin mats or dispersed evenly and loosely without mat formation, unconstricted at the cross–walls, (1)1.5–3(4) μm wide, slightly attenuated and bent at the ends. Cells pale olive–green to blue–green, sometimes greenish to yellow–green, without evident chromatoplasma or centroplasma, often with orange granules at the cross–walls, 2–3×, sometimes up to 4 times longer than wide, 3–9 μm long (Figs 2O–V). Thylakoids in cross–section concentrically arranged, peripheral, parallel oriented, in transverse section with triangular orientation. Apical cells (in natural material) cylindrically rounded or elongated and

Table 2. Nucleotide similarity of 16S rRNA gene of *Geitlerinema* and *Anagnostidinema* with phylogenetically relevant strains. The values are given in percentage of similarity of 1,081 bp of the 16S rRNA gene (starting at *E. coli* ATCC 11775 16S rRNA residue 302).

	Strain name	1	2	3	4	5	6	7	8	9	10	11
1	*Geitlerinema splendidum* CCALA 1004											
2	*Crinalium epipsammum* SAG 22.89	93										
3	*Starria zimbabweensis* SAG 74.90	91	97									
4	*Wilmottia murrayi* KGI28	92	93	91								
5	*Trichocoleus sociatus* SAG 26.92	93	93	90	93							
6	*Coleofasciculus chthonoplastes* SAG 38.89	91	91	90	93	92						
7	*Kamptonema animale* CCALA 138	92	93	91	93	93	93					
8	*Geitlerinema* sp. PCC 7105	89	88	88	89	89	91	91				
9	*Limnothrix redekei* 165a	88	88	87	87	89	89	90	90			
10	*Gloeobacter violaceus* PCC 7421	88	88	87	87	89	88	88	88	87		
11	*Anagnostidinema pseudacutissimum* CCALA 142	90	90	88	92	91	91	91	87	88	87	
12	*Anagnostidinema amphibium* RO–MK72	89	90	88	92	92	91	91	88	88	87	98

gradually attenuated at the ends, sometimes bent or hooked, never calyptrate or capitate (Fig. 2O–V).

Habitat: Freshwater, benthic, usually in periphyton of stagnant waters, in greenhouses, on wet soils, etc.
Holotype: Gomont (1892), as a specimen authenticated in the Agardh Herbarium, housed in the Lund University Botanical Museum. Due to the ambiguous nature of old dried material, we here designate an epitype.
Epitype: dry material CBFS A508 is based on the strain RO–MK72.

Species belonging to *Anagnostidinema* according to morphology

Anagnostidinema acutissimum (Kufferath) Strunecký, Bohunická, Johansen et Komárek comb. nov. – Basionym: *Oscillatoria acutissima* Kufferath, Ann. Biol. Lac. 7: 264, 1914

Anagnostidinema carotinosum (Geitler) comb. nov. (basionym: *Oscillatoria carotinosa* Geitler, Österr. Bot. Zeitschr. 103(2–3): 342, pl 1, figs. a, b, 1956; syn: *Phormidium carotinosum* (Geitler) Anagnostidis et Komárek, Arch. Hydrobiol. Suppl./Algol. Stud. 50–53: 404, 1988; *Geitlerinema carotinosum* (Geitler) Anagnostidis, Plant. Syst. Evol. 164: 39, 1989)

Anagnostidinema deflexum (W. et G.S. West) comb. nov. – Basionym: *Oscillatoria deflexa* W. et G.S. West, Brit. Antarct. Exped. 1(7): 295, 1911

Anagnostidinema epiphloeophyticum (Anagnostidis) comb. nov. – Basionym: *Geitlerinema epiphloeophyticum* Anagnostidis, Preslia 73: 364, 2001

Anagnostidinema exile (Skuja) comb. nov. – Basionym: *Oscillatoria exilis* Skuja, N. Acta Reg. Soc. Sci. Upsal. Ser. 4, 18(3): 51, 1964

Anagnostidinema ionicum (Skuja) comb. nov. – Basionym: *Oscillatoria ionica* Skuja, Hedwigia 77: 30, 1937

Anagnostidinema lacus–solaris (Campbell et Golubic) comb. nov. – Basionym: *Oscillatoria lacus–solaris* Campbell et Golubic, Algol. Stud. 38/39: 324–325, 1985

Anagnostidinema lemmermannii (Woloszynska) comb. nov. – Basionym: *Oscillatoria lemmermannii* Woloszynska, Bull. Int. Acad. Sci. Cracovie, mat.–nat., Ser. B, 1911: 689, 1912

Anagnostidinema tenue (Anisimova) comb. nov. – Basionym: *Oscillatoria amphibia* f. *tenuis* Anisimova in Elenkin Monogr. Alg. Aquidulc., Pars spec. 2: 1326, 1949. = *Geitlerinema tenue* (Anisimova) Anagnostidis, Preslia 73: 364, 2001

The overview of the genera *Geitlerinema* and *Anagnostidinema* after revision is included in Table S2. The generic position of other *Geitlerinema* species (cf. Komárek & Anagnostidis 2005) must be solved by other studies, together with their morphological characterization.

Discussion

Our combined morphological and phylogenetic analyses confirmed the polyphyletic nature of the genus *Geitlerinema* reported previously by Perkerson et al.

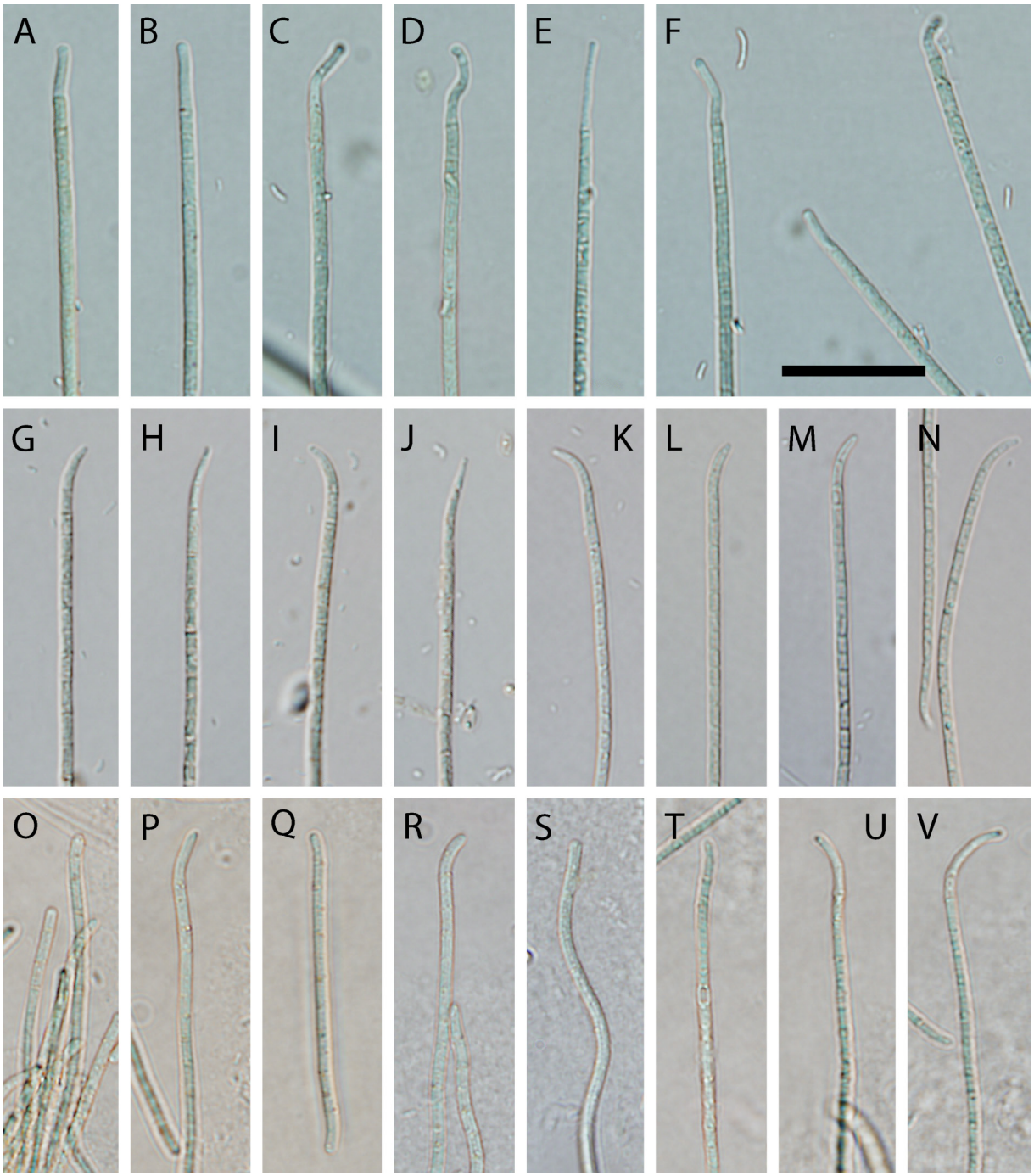

Fig. 2. Morphology of the members of the genera *Geitlerinema* and *Anagnostidinema*: (A–F) *G. splendidum* CCALA 1004, (G–J) *A. pseudacutissimum* CCALA 150, (K–N) *A. pseudacutissimum* CCALA 142, (O–V) *A. amphibium* RO–MK72. Scale bar = 20 µm, applies to all figures.

(2010) and Hašler et al. (2012). The genus *Geitlerinema* was validly established by Anagnostidis (1989) with the type species designated as *G. splendidum,* which can be clearly characterized by the capitate, spoon–like ends of apical cells. Although the shape of the apical cells is characteristic and easily recognizable, and the taxon is widely reported, we have not found this species in any culture collection prior to reports of the three strains given here. Our three strains corresponding to *G. splendidum* fully agree with the morphological description of Anagnostidis (1989) and with the original description of Greville ex Gomont (1892). We recommend using *Geitlerinema splendidum* CCALA1004, the basis of the epitype we designate here, as a morphologically confirmed reference strain. We also use the sequence of the widely cited bacterial marker, the 16S rRNA gene of the strain *G. splendidum* CCALA 1004 (KP412630), as a reference sequence of the genus *Geitlerinema*. At present, all strains from the genus *Geitlerinema* are of freshwater origin.

Other species that were recognized as *Geitle-*

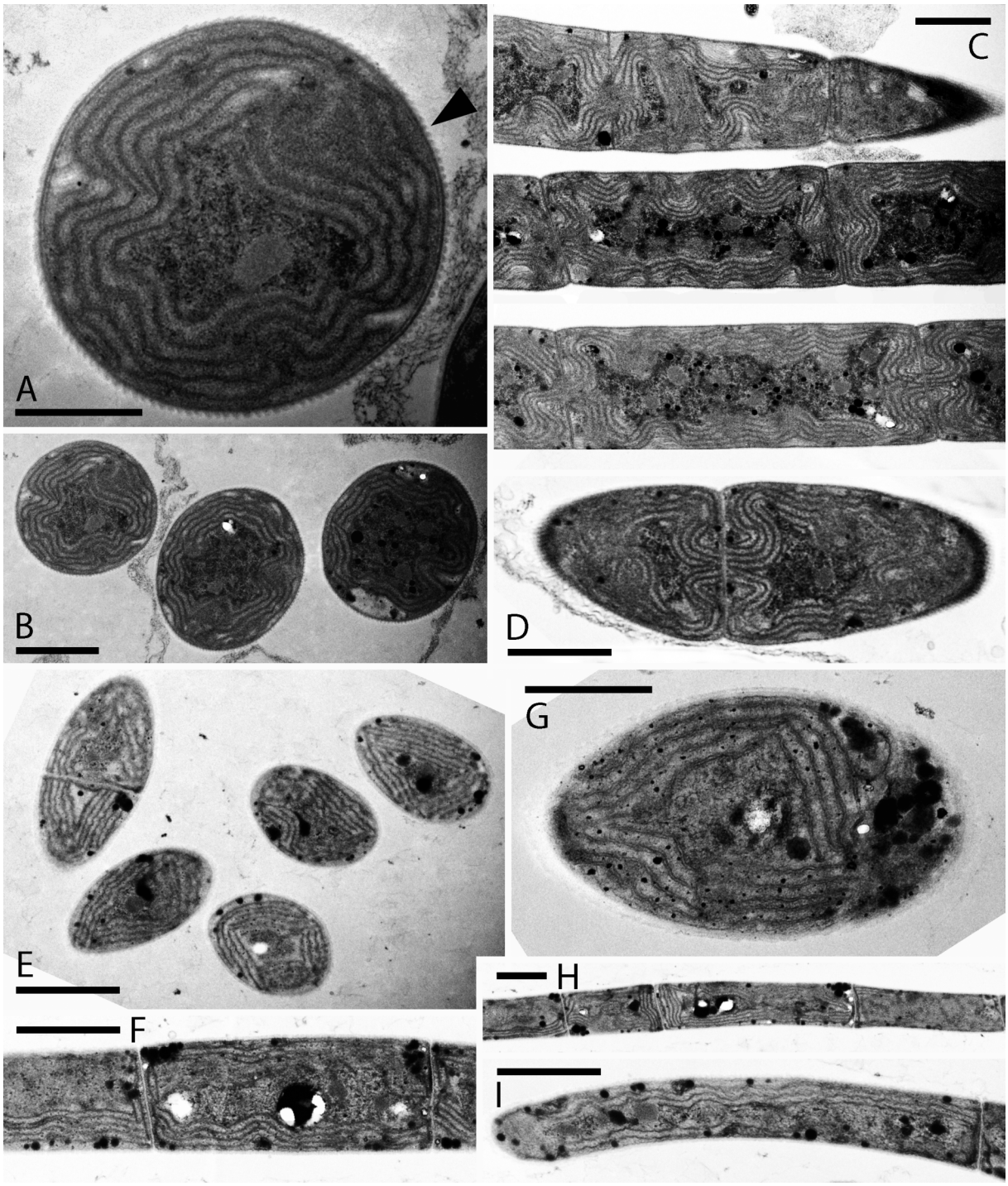

Figure 3. Ultrastructure the members of the genera *Geitlerinema* and *Anagnostidinema* examined in TEM: (A–D) *G. splendidum* CCALA 1004, (A, B) transversal sections of the trichome, arrow points to its the typical mucilaginous cover, (C, D) longitudinal sections; (E–I) *A. pseudacutissimum* GSE–PSE04–08G, (E, G) transversal sections of the trichome, (F–I) longitudinal sections of the trichome. Scale bar (A, G) 500 nm, (B–D, E–F, H–I) 1 μm.

rinema according to Komárek & Anagnostidis (2005) are morphologically and presumably also phylogenetically distant from *G. splendidum*; thus they do not belong to the genus *Geitlerinema* and must be taxonomically reevaluated. We have transferred a number of non–capitate species into *Anagnostidinema* gen. nov. (Table S2). However, several others, e.g. *G. acuiforme, G. nematodes*, or *G. acuminatum* probably belong in close vicinity of the genus *Oxynema* (Chatchawan et al. 2012), whereas *G. jasorvense* possibly belongs in *Kamptonema* (Strunecky et al. 2014). The taxonomic position of these species must await definitive resolution in future studies.

In the most recent taxonomic system proposed

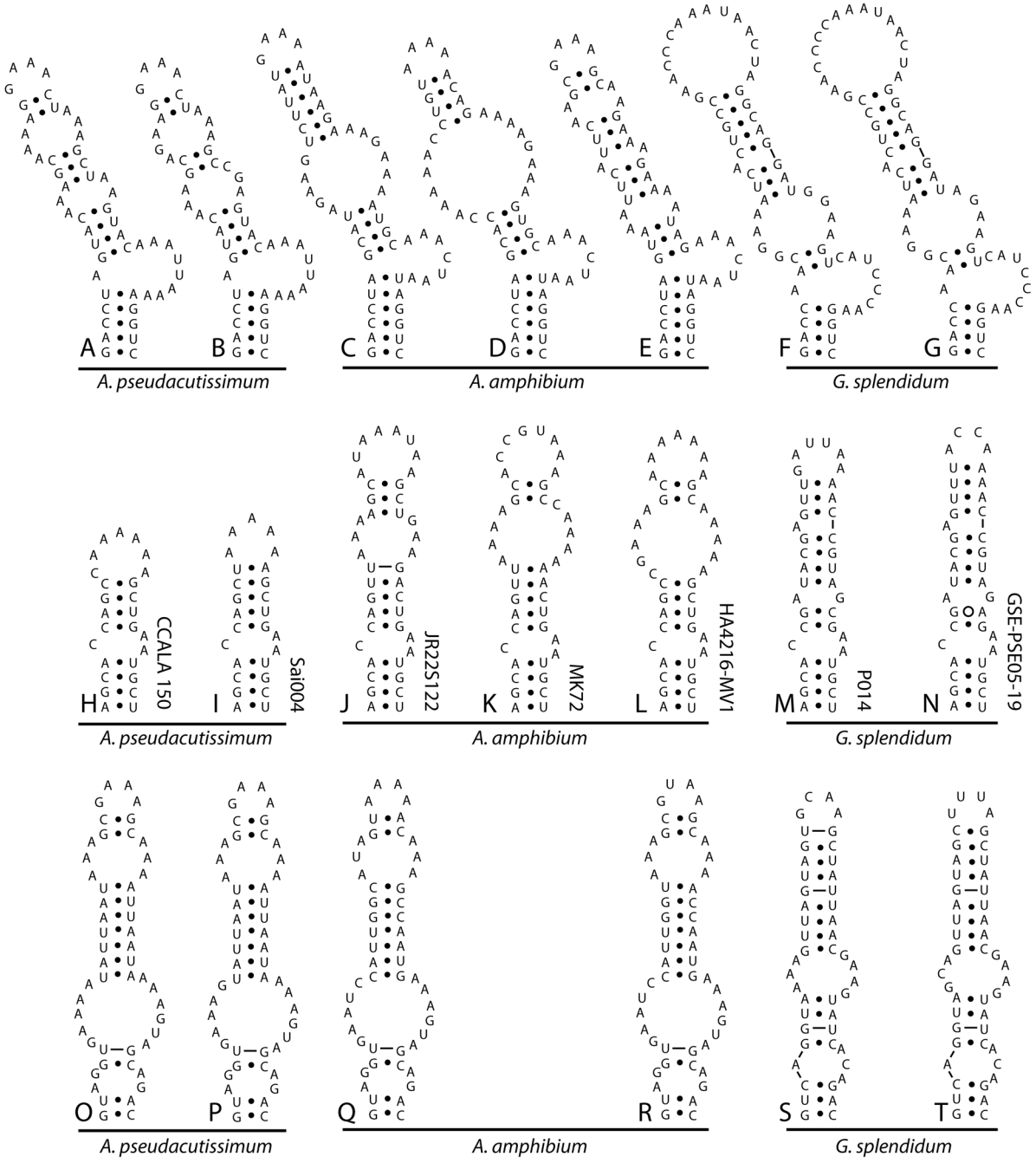

Figure 4. Characterization of the secondary structures of the ITS regions in studied strains of *Geitlerinema* and *Anagnostidinema*: (A–G) D1–D1' helix, (H–N) boxB helix, (O–T) V3 helix; (A–B, H–I, O–P) *Anagnostidinema pseudacutissimum*, (C–E, J–L, Q–R) *A. amphibium*, (F–G, M–N, S–T) *G. splendidum* (GSE-PSE05-19 = CCALA 1004).

by Komárek et al. (2014), *Geitlerinema* was assigned to the Coleofasciculaceae. However, in our study the phylogenetic position of *G. splendidum* (and therefore of the genus *Geitlerinema* after revision) is sister to the cellulose and cylindrospermopsin producing clade Gomontiellaceae (cf. Fig. 1; Bohunická et al. 2015), which is rather curious. Species of the family Gomontiellaceae have distinctively constricted cross walls and they possess special morphology (trichomes not circular in cross–section) among the non–heterocytous, filamentous cyanobacteria (Bohunická et al. 2015). *Geitlerinema* will likely need to be reassigned to a different family in the future, as its placement based on our analysis is currently ambiguous.

Anagnostidinema forms a new very isolated clade of cyanobacteria and their detailed phylogenetic relationships to other filamentous cyanobacteria could be better resolved using a multiple loci or whole genome phylogenetic approach in the future. Yet, the creation of the new genus *Anagnostidinema* for freshwater

representatives with more or less unique morphology is supported by the abundance of accessible strains (Table 1). The high number of sequences based on either cultured or uncultured material that are deposited in GenBank suggests wider distribution of the genus *Anagnostidinema* than that of *Geitlerinema* sensu stricto.

Our phylogenetic analysis based on the 16S rRNA gene clearly separated also other highly motile species with similar morphologies that were previously included in the genus *Geitlerinema* and originated from a marine environment. These species did not possess the rounded–capitate apical cells and were phylogenetically distant from *Anagnostidinema*, therefore they must be separated into other genera in future studies. This is true in the case of the marine strain *Geitlerinema* sp. PCC 7105, recognized as a reference strain for the genus *Geitlerinema* according to Bergey's Manual (Castenholz et al. 2001). In Bergey's Manual the concept of the genus *Geitlerinema* is wider than that given in the original description (Anagnostidis 1989). It includes filamentous cyanobacteria with straight to slightly coiled cylindrical trichomes without necridia, less than 5–6 μm in diameter with active (gliding) motility accompanied by trichome rotation. Cells are longer than broad or isodiametric, dividing by binary fission; constrictions between adjacent cells are absent or very shallow. Apical cells are rounded, conical or distinctly pointed, tapered, and often bent (Castenholz et al. 2001). This concept of the genus *Geitlerinema* has led subsequent workers to identify many other marine strains as *Geitlerinema* based on BLAST searches or sequence similarity, and such strains have been reported from tropical oceans in coral reefs or associated with stromatolites (Richert et al. 2006; Myers et al. 2007). Our study clearly demonstrates that the marine types recognized as *Geitlerinema* are phylogenetically distant from freshwater *Geitlerinema* (Fig. 1). These marine "*Geitlerinema*" have a basal paraphyletic relationship to all Oscillatoriophycidae and Nostocophycidae according to the 30–loci phylogeny in Komárek et al. (2014), and likely represent a separate, yet undescribed order of cyanobacteria.

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Supplementary material

the following supplementary material is available for this article:

Table S1. Sequence lengths for the recognized domains of the 23S-16S ITS region.

Table S2. List of traditional *Geitlerinema* species according their former morphologic designation.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)