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Ultrastructure and taxonomic position of two species of the cyanobacterial genus *Schizothrix*

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Abstract – The cyanobacterial genus *Schizothrix* is traditionally classified in the special family Schizotrichaceae (order Oscillatoriales) according to the structure of the filaments and thallus: one, two, or more ensheathed and fasciculated trichomes are enveloped by common sheaths. The fine structure of cells and filaments of two natural populations of typical *Schizothrix*-species (*S. facilis, S. lacustris*) were investigated in our study. The ultrastructure of trichomes was found to be similar to the pseudanabaenacean types (thylakoid arrangement, inclusions, cell wall), and indicates the close relationship to this group of simple filamentous cyanobacteria. The special life form, which was considered as the most important phenotypic intergeneric (and interfamilial) differentiating character was proven: Fasciculated trichomes are enveloped by their own sheaths, and form (usually heteropolar) filaments enveloped by another common sheath. However, in spite of the fact that the ultrastructure and morphology of trichomes were found to be similar to other pseudanabaenacean types, the relationship to *Pseudanabaenaceae* must await detailed molecular studies to be more completely evaluated. The first molecular results concerning a few *Schizothrix*-like strains from Antarctica show that most belong to a cluster, which is separated from the other oscillatorian clusters. This could support the genetic basis of the *Schizothrix* genus.

Conservation / Cyanobacteria / cyanoprokaryotes / *Schizothrix* / ultrastructure / taxonomy

Résumé – Ultrastructure et position taxinomique de deux espèces du genre *Schizothrix* (Cyanobactéries). Le genre *Schizothrix* est traditionnellement classé dans la famille des *Schizotrichaceae* (ordre des *Oscillatoriales*) sur la base de la structure des filaments et des thalles : un, deux ou plusieurs trichomes engainés et fasciculés sont enveloppés par

des gaines communes. La structure fine des cellules et des filaments de deux populations naturelles d'espèces typiques de *Schizothrix* (*S. facilis, S. lacustris*) est étudiée ici. L'ultrastructure des trichomes apparaît très similaire à celle de type « *Pseudanabaenaceae* » (arrangement des thylacoïdes, inclusions, paroi cellulaire), et indique une étroite relation avec ce groupe de cyanobactéries filamenteuses simples. La forme de vie particulière qui a été considérée comme le caractère phénotypique discriminant au niveau intergénérique (et interfamilial) le plus important est démontrée : des trichomes fasciculés sont enveloppés par leur propre gaine, et forment des filaments (habituellement hétéropolaires) enveloppés par une autre gaine commune. Toutefois, bien que l'ultrastructure et la morphologie des trichomes soient similaires à celles d'autres types « pseudanabaenaceéens », la relation avec les *Pseudanabaenaceae* doit attendre des études moléculaires détaillées pour être complètement évaluée. Les premiers résultats moléculaires concernant quelques souches antarctiques ressemblant à *Schizothrix* montrent que la plupart d'entre elles appartiennent à un cluster séparé des autres clusters d'Oscillatoriales. Ceci pourrait constituer un soutien génétique en faveur du genre *Schizothrix*.

Cyanobacteria / cyanoprokaryotes / *Schizothrix* / ultrastructure / taxinomie / conservation

INTRODUCTION

Schizothrix [Kützing 1843] ex Gomont, 1892, *Ann. Sci. Nat., Bot.*, Ser. 7, 15: 192, is an oscillatorialean cyanobacterial genus with a complicated taxonomic position. It is heterogeneous, as shown from a re-evaluation of morphological characters (Anagnostidis & Komárek, 1988). Perhaps the greatest problem hampering a modern taxonomic evaluation of the genus is that isolation into monospecific culture is difficult and only a few strains exist in culture collections. As a consequence, well-defined and revised *Schizothrix* strains have not yet appeared in the molecular literature and phylogenetic trees, except for a few *Schizothrix*-like strains studied recently (Taton *et al.*, submitted). In addition, until now, no ultrastructural studies have been published from which relationships to known clusters of filamentous, oscillatorialean cyanobacteria could be deduced. The lack of molecular and ultrastructural information is apparently also the reason why the genus was not included in the last edition of Bergey's Manual (Castenholz, 2001).

The traditional genus *Schizothrix* (Geitler, 1932) is based on the type-species *S. fuscescens* Kützing ex Gomont 1892, characterised by filaments enveloped by more-or-less firm, wide, yellow-brown sheaths that are attenuated ("pointed", "closed") at the ends. Sheaths are a little wider than trichomes and can contain one, two, or more trichomes, usually with their own sheaths. The thallus is regarded as polarised (*i.e.* divided into basal and apical parts); trichomes parallel situated in one sheath are particularly visible in the lower parts of filaments.

In Geitler (1932), *Schizothrix* was divided into four morphologically characteristic clusters (taxonomic "sections"). The first two morphotypes, corresponding more or less to the original generic diagnosis, were classified in two groups that probably differed only in the ability of colonies to calcify ("typical"

substantially from the *Schizothrix* = *Hypheothrix/Inactis* complex both morphologically and in the structure of trichomes and cells, and must be classified as a separate genus *Symplocastrum* (Gomont) Kirchner 1898 in the family *Phormidiaceae* (see Anagnostidis & Komárek, 1988). The last section *Chromosiphon* Gomont 1890, *J. Bot. (L. Morot)* 4: 352, is based on a taxonomically worthless character (not justifiable at the generic level in the corresponding group of cyanobacteria): facultatively coloured sheaths also occur in different types of *Schizothrix*-like cyanobacteria, without correlation with other generic (or other phenotypic) markers. However, from the nomenclatural point of view the section *Chromosiphon* should be called section *Schizothrix* since it includes the type species of the genus *Schizothrix*, *S. fuscescens*, which also has coloured sheaths, but clearly belongs to the group of "typical" *Schizothrix* by all other morphological markers.

Natural populations of two traditional species from the typical *Schizothrix*-group were investigated in this study, using transmission electron microscopy, and their taxonomic position is discussed.

MATERIAL AND METHODS

The following morphospecies were examined:

1) A cultured strain of *Schizothrix facilis*, isolated from a sample collected from a snow-fed stream, flowing from a snow field near Ny Ålesund, Western Svalbard (Figs 1-4), in the Western part of West Brøgger glacier morain. This species was originally described by Skuja 1964 from Swedish Lappland, from a similar habitat.

2) A natural population of *Schizothrix lacustris*, sampled from submerged wooden and stony substrata in the littoral of the oligotrophic lake Lunzer Mittersee near Lunz am See, Austria (August 1969). It was preserved in 3% formalin and the sample was studied after almost 35 years of preservation (see Discussion).

The morphological descriptions were derived from these living natural materials.

For the ultrastructural studies the material was fixed with *ca* 3% formaldehyde (unbuffered) and kept in this solution for several days at room temperature (*Schizothrix facilis*). The material of *Schizothrix lacustris*, which was collected in 1969, had been kept in a cyanobacteria sample collection for almost 35 years.

The sample of *S. facilis* was washed with 0.05 M phosphate buffer (pH 7.2) and postfixed with 2% osmium tetroxide in the same buffer for 2 hours at room temperature. At the onset of the postfixation, the cells were briefly irradiated in a microwave oven (80 Watts, 60 seconds). After washing with 0.05 M phosphate buffer, the cells were dehydrated with a graded isopropanol series and embedded in Spurr's resin (Spurr, 1969) using propylene oxide as an intermediate stage. Thin sections were stained with uranyl acetate and lead citrate and observed in a transmission electron microscope Jeol JEM 1010 at 80 kV.

prepare it for ultrastructural investigation. It was transferred into 2% formaldehyde (prepared from paraformaldehyde) buffered with a 0.05 M phosphate buffer and kept in it for several days (at room temperature). Thereafter, the material processed similarly to the cells of *Schizothrix facilis*. The only difference was that the cells of this species were irradiated in a microwave oven at the onset of each step of the specimen preparation. The relatively good preservation of cell ultrastructure of many cells in this material was a surprising outcome.

RESULTS

***Schizothrix facilis* (Skuja) Anagnostidis, 2001, *Preslia* 73: 368** (Figs 1-10)

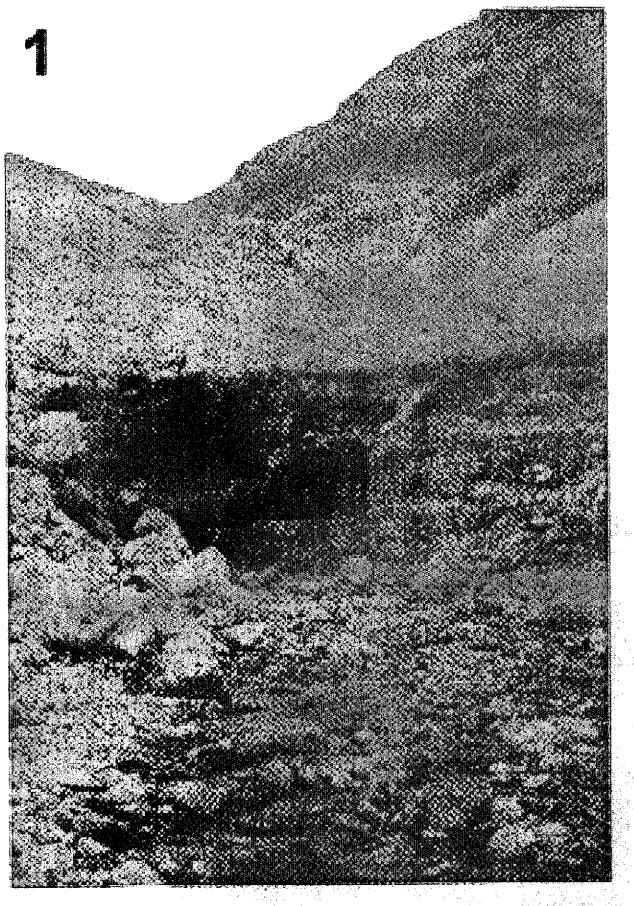
Morphology: Mucilaginous biofilms, clusters and mats, occasionally forming microscopic to macroscopic, ± hemispherical yellowish-brownish colonies on submerged stones and rocks in flowing waters, cascades and waterfalls; colonies composed of ± parallel and fasciculated filaments. Filaments are slightly irregularly coiled and contain usually (1)2 or more trichomes. Sheaths have a fine, yellowish-brown colour and narrowed apical parts. Trichomes inside sheaths are solitary, two, several to many together, later densely fasciculated (Figs 6-10), slightly constricted at cross-walls. Cells cylindrical, ± isodiametric, 6-7 × 4-5 µm, apical cells rounded. Necridic cells are present, similarly as in *Leptolyngbya*-species "with short, isodiametric cells" (Albertano & Grilli-Caiola, 1988). – Common species in streams, flowing from the melting glaciers near Ny Ålesund, Svalbard.

Ultrastructure: Sheaths are composed of distinct, firm, polysaccharidic layers with fibrous structure; sheaths are composed of several layers, and form their own sheaths around solitary trichomes, enveloped by a common sheath (Fig. 9). Cells have (2)3-4 parietally located thylakoids. Inside the cells nucleoid regions, numerous small granules of cyanophycin and polyphosphates, as well as carboxysomes, are visible.

***Schizothrix lacustris* [A. Braun in Kützing, 1849] ex Gomont, 1892, *Ann. Sci. Nat., Bot.*, Ser. 7, 15: 301** (Figs 11-23)

Morphology: Microscopic to macroscopic (up to 2 cm long), fine, greyish, fasciculated colonies, attached to stony and wooden, submerged substrata in stagnant water. Sheaths firm, thick, colourless. Trichomes parallely arranged, 1-4 in a sheath, with their own sheaths, slightly constricted at cross-walls. Cells cylindrical, 2-3 times longer than wide, 1.3-3.5 × 0.8-1.2 µm. Apical cells slightly narrowed and rounded. Necridic cells not observed. – Collected in littoral parts of Lunzer Mittersee, Austria.

Ultrastructure: Sheaths are very thick, firm and fibrous, slightly distant from trichomes, with a rough surface. If several trichomes are ensheathed, they have their own sheaths (Figs 11-15, cross sections). Cells have (2)3-4(5) parietal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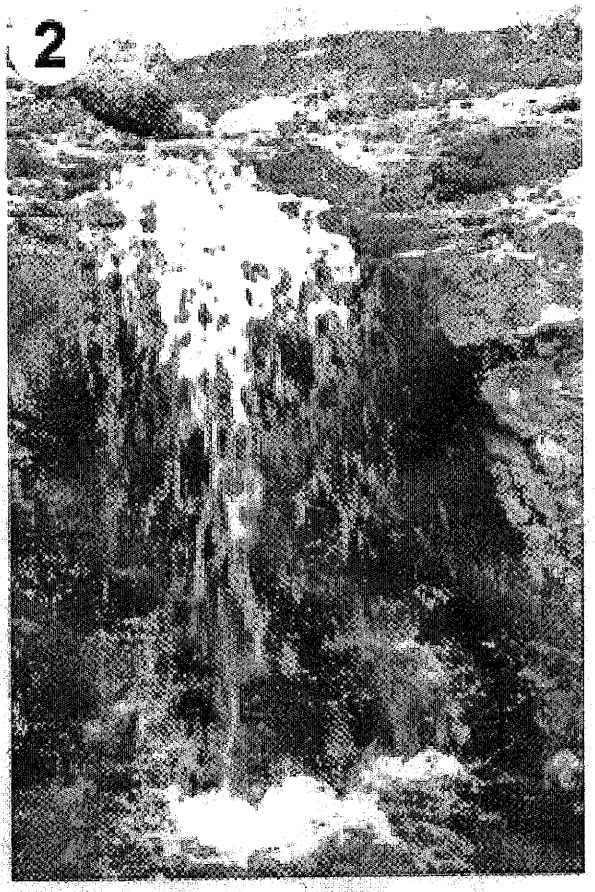
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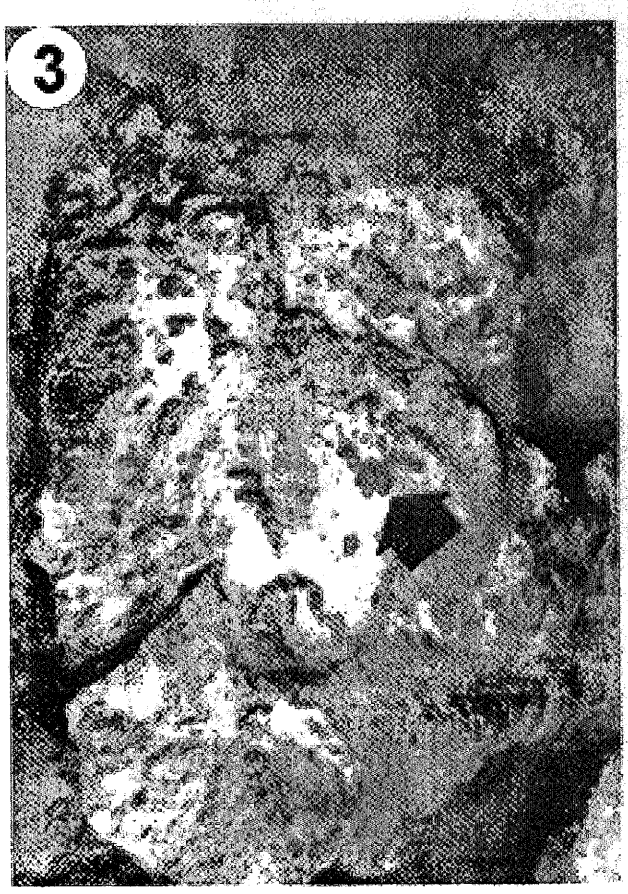
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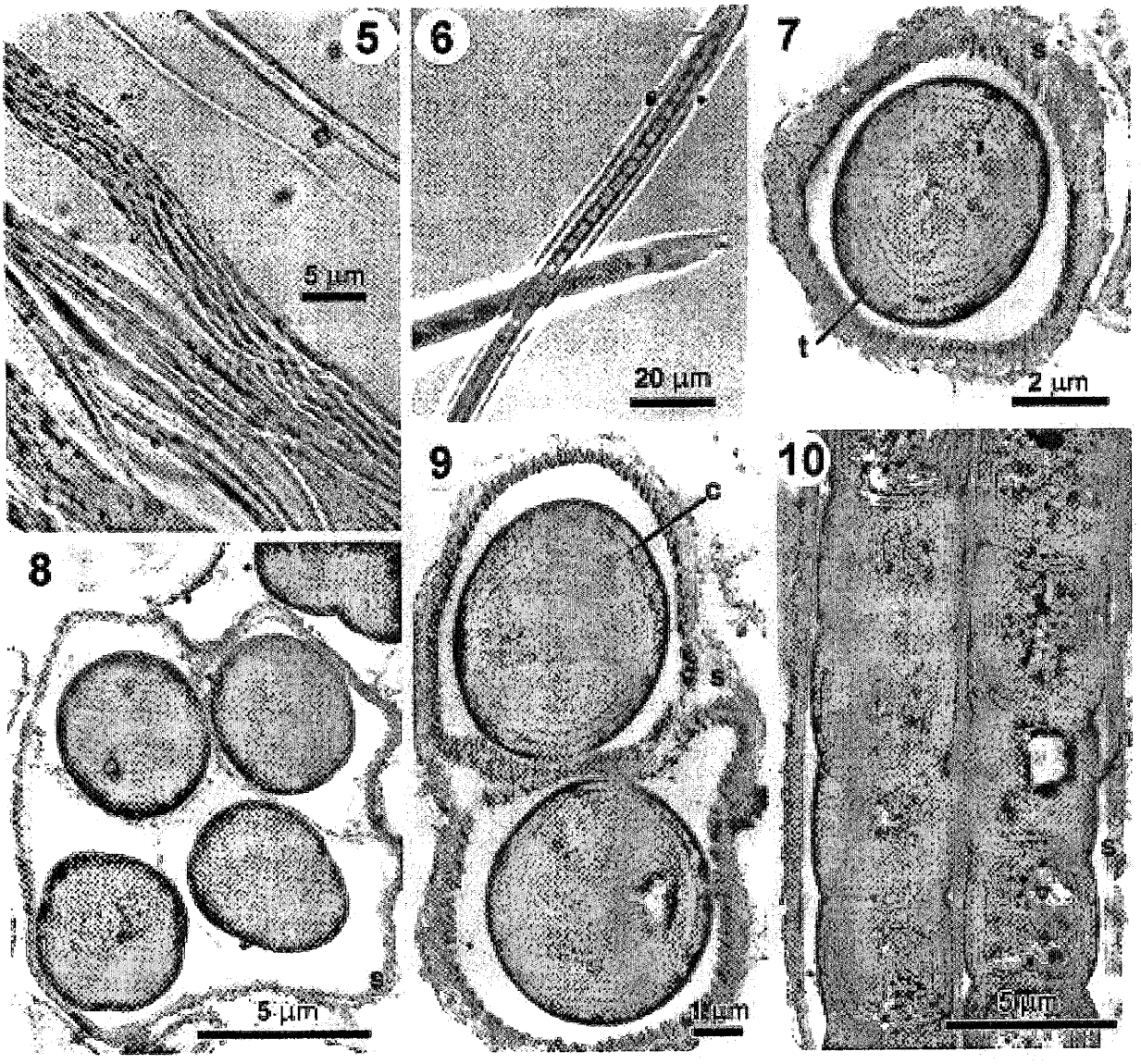
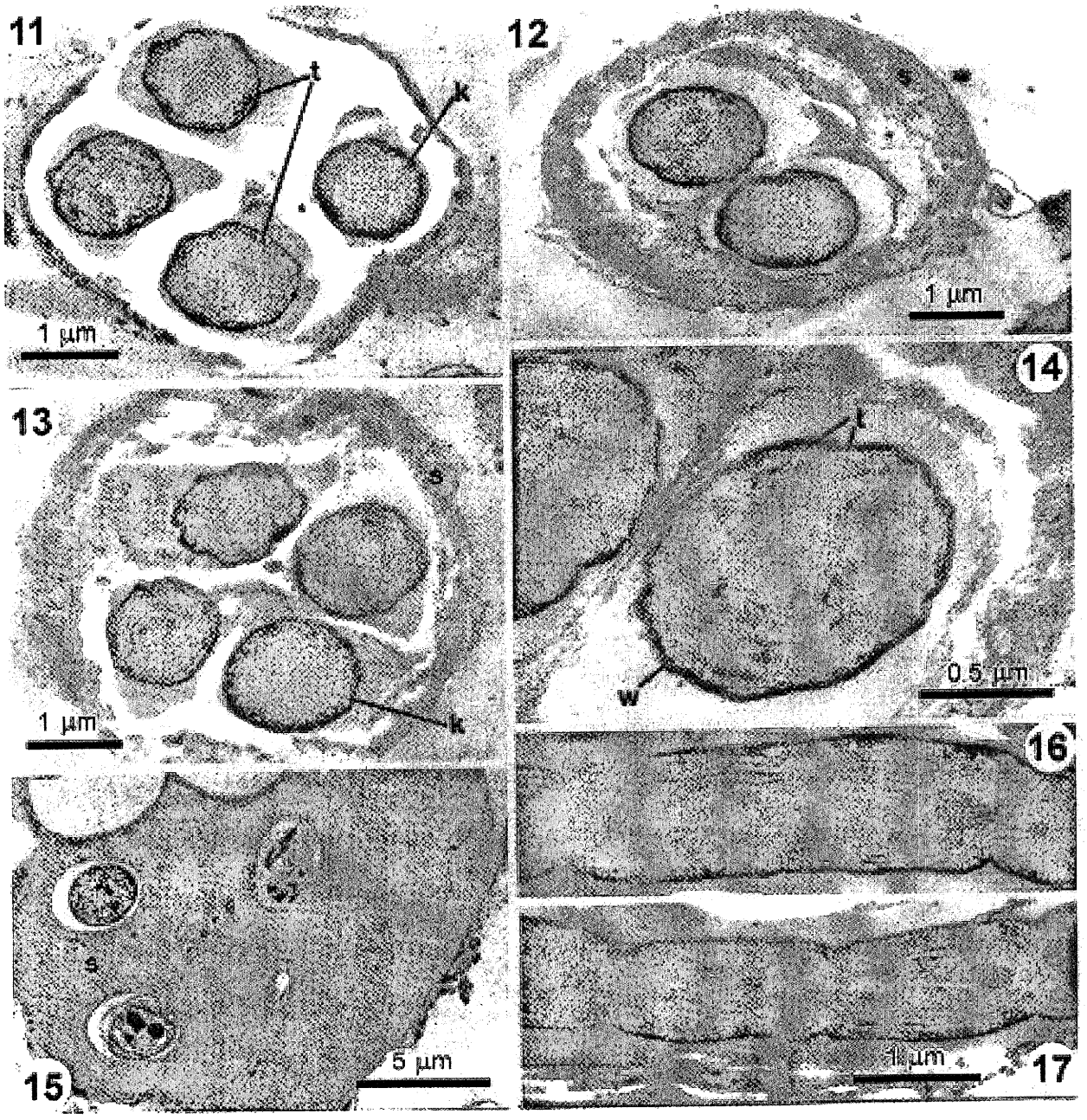


Figs 5-10. *Schizothrix facilis*: **5.** Fasciculated trichomes in common, divaricated sheaths; **6.** Filaments with details of solitary trichomes; **7.** Cross section of solitary filament with one trichome in a sheath; **8-9.** Cross sections of filaments with four and two ensheathed trichomes; **10.** Lengthwise section of a filament with two parallel trichomes in a sheath. [**s.** Sheaths, **t.** Thylakoids, **n.** Necridic cells, **k.** Carboxysomes, **c.** Cyanophycin granules, **w.** Cell wall].

DISCUSSION

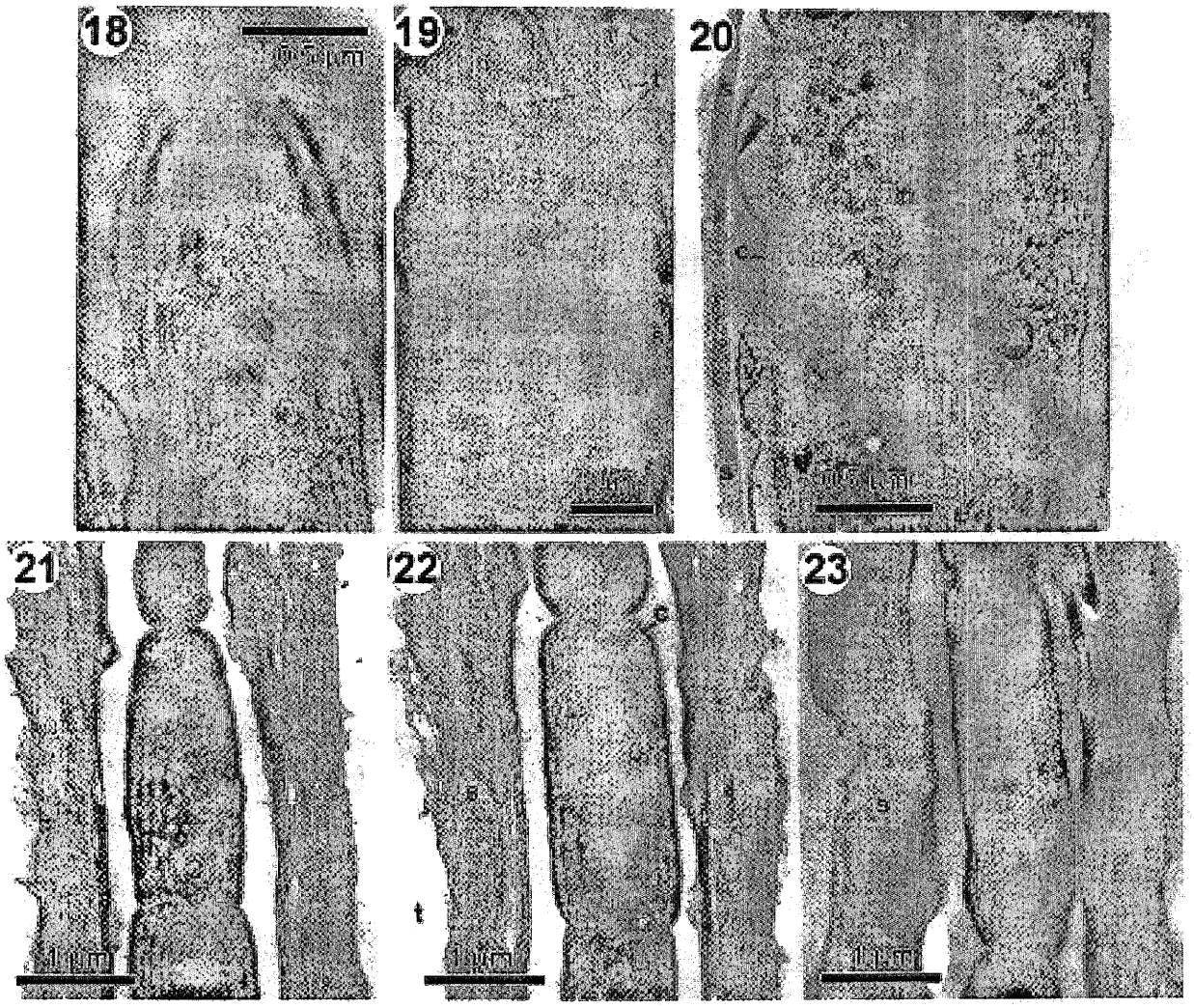
The results of our study confirm the generic morphological characters of the traditional genus *Schizothrix* Kützing *ex* Gomont, primarily the fasciculation of ensheathed trichomes enveloped by a common sheath, which is closed at the apex (Figs 18-19). It opens only during the formation and separation of hormogonia. The ultrastructural features (mainly thylakoid patterns) are similar to pseudanabaenacean cyanobacteria (Komárek & Čelakovská, 1991; Komárek &



Figs. 11-17. *Schizothrix lacustris* (from preserved sample): **11-13.** Cross sections of filaments with 4 and 2 trichomes with their own sheaths; **14.** Cross section through a trichome; **15.** Cross section through the filament near the basis with two living and other dead trichomes; **16-17.** Lengthwise section of trichomes. [**s.** Sheaths, **t.** Thylakoids, **n.** Necridic cells, **k.** Carboxysomes, **c.** Cyanophycin granules, **w.** Cell wall].

classification as a separate family *Schizotrichaceae* could be justified according to phenotypic and ultrastructural criteria.

The genetic (molecular) background of *Schizothrix* has not yet been studied. However, results obtained by Taton *et al.* (submitted) using a polyphasic approach, which includes 16S rRNA analysis, show the existence of a genetic cluster composed of 5 *Schizothrix*-like strains in which the morphology resembles *Schizothrix* or *Pseudanabaenidium* (Fig. 24). The characteristic features of these



Figs. 18-23. *Schizothrix lacustris* (from preserved sample): **18-19.** Apical parts of filaments with closed sheaths; **20.** Lengthwise section of a filament with two trichomes (note the separate cell wall with initiating centripetal cross-walls - arrow); **21-23.** Different parts of a filament with thick fibrous sheath and one trichome. [**s.** Sheaths, **t.** Thylakoids, **n.** Necridic cells, **k.** Carboxysomes, **c.** Cyanophycin granules, **w.** Cell wall].

and false branching. These features appear to be influenced by the culture's age and conditions, but were observed frequently enough to differentiate these strains from other thin Oscillatoriales. However, the sequences within this cluster were only more than 2.5% dissimilar to the other cyanobacterial sequences available in the databases. This result, therefore, indicates the close relatedness to cyanobacterial *Leptolyngbya*- or simple *Phormidium*- (e.g., *Ph. priestleyi*) cluster, and possibly also supports a novel lineage (on the level of a special generic entity). The genetic basis of the *Schizothrix* genus should be confirmed by the sequences of other *Schizothrix* strains as well as a combined molecular and electron microscopy analysis of the same strains.

It will surely be necessary to remove from the genus *Schizothrix* (and from the family Schizotrichaceae, respectively) all the types that do not exactly correspond to the generic characters of the typical *Schizothrix* species (genetic uniformity and several phenotypic characters, e.g., fasciculated and enclosed

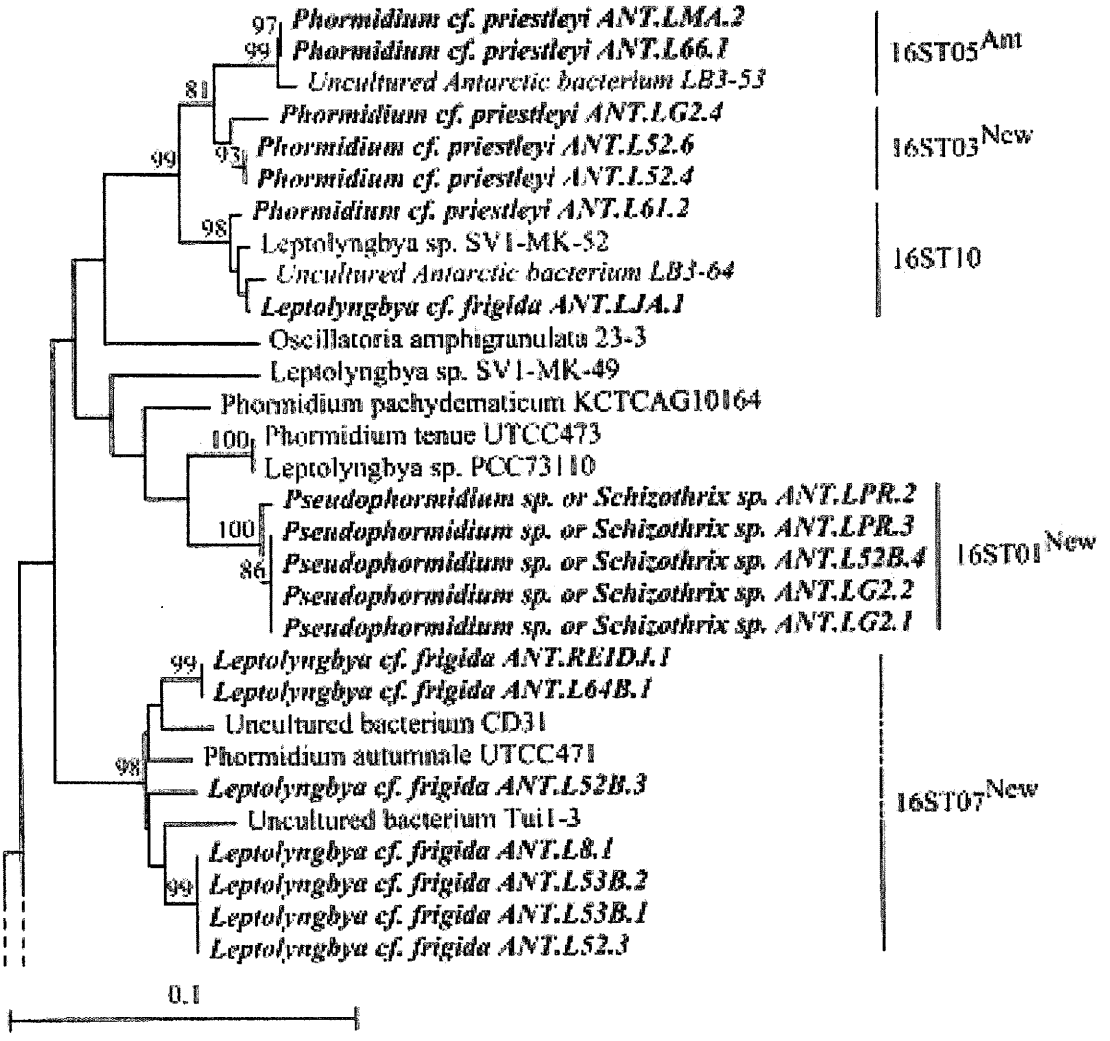


Fig. 24. Part of a neighbor-joining tree based on partial 16S rRNA gene sequences corresponding to *E. coli* positions 405 to 780 (Taton *et al.* submitted for publication). Bootstrap values equal or greater than 70% are indicated at the nodes. The evolutionary distance between two sequences is obtained by adding the lengths of the horizontal branches connecting them and using the scale bar (0.1 mutation per position).

characterisation of the subgenus (or "section") *Symplocastrum* sensu Geitler (1932; = form-genus *Symplocastrum* (Gomont) Kirchner 1898), and the delimitation of *Schizothrix* and *Pseudophormidium*.

This study was also used for the comparison of ultrastructural patterns recognisable in one sample extraordinarily preserved by formalin for a long time (almost 35 years, kept more or less at room temperature). The results were surprisingly positive. It was possible to observe the number and position of thylakoids within the cells in this sample. The cyanophycin granules and carboxysomes were also distinguishable in the cells, as well as the structure of sheaths being recognisable (Figs 11-23). From our results, we can conclude that the long preservation mostly destroyed the cell-wall (Figs 18-20). However, this deformation yielded interesting new information about the simultaneous formation of cross-walls in *Schizothrix* (Fig. 20 – arrow), which is known only in more complicated oscillatorialean types from the family Oscillatoriaceae

modern techniques. The separate position of *Schizothrix*-like types from other oscillatorialean families is indicated by the preliminary results of Taton *et al.* (Fig. 24; Taton *et al.*, submitted).

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