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Cytomorphological characters supporting the taxonomic validity of *Cyanothece (Cyanoprokaryota)*

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Key words: *Cyanophyta, Cyanobacteria, Cyanothece, Synechococcus, Cyanobium.* – Ultrastructure, nucleoids, taxonomy.

Abstract: The fine structure of the type species of the genus *Cyanothece* Komárek 1976, *C. aeruginosa*, is described and compared with the main cytological characteristics of morphologically related members of the genera *Cyanobium*, *Cyanobacterium* and *Synechococcus*. Several morphological features, such as cell walls with thick outer layers containing a special type of vesicles, position of thylakoids, "keritomy" (net-like appearance of protoplast caused by arrangement of thylakoids, net-like nucleoids and/or by tendency to form intrathylakoidal spaces) and a special structure of mucilaginous envelopes were found to be characteristic of this genus, supporting its separate position among coccal cyanoprokaryotes (cyanobacteria, cyanophytes). The taxonomic significance of ultrastructural features in all mentioned genera is discussed.

The cyanoprokaryotic genus *Cyanothece* (*Synechococcaceae, Chroococcales*) has been separated from the traditional genus *Synechococcus* Näg. 1849 by Komárek (1976). Characteristics of both genera are: (i) absence of slime, or its reduction to a fine, thin layer around single cells (the cells are either solitary in nature or form free aggregates in massive populations, but do not occur as mucilaginous colonies); (ii) shape of cells, which is oval (usually in *Cyanothece*) or cylindrical (usually in *Synechococcus*); (iii) the type of cell division, i.e. binary fission into two daughter cells, always perpendicular to the longitudinal cell axis (i.e. in a single plane in succeeding generations so that the daughter cells grow into the original shape and size before the next division).

In the original diagnosis, the main features differentiating *Cyanothece* and *Synechococcus* concern the shape of cells, the structure of cell protoplast (presence of "keritomy", i.e. the irregularly net-like appearance of cell content in *Cyanothece*), a modification of cell division, and a different type of "involution" cells (= atypical cells, growing under stress conditions) (Table 1). Both genera were reviewed by Komárek (1976) and Komárek & Anagnostidis (1976). In spite of the easy identification of both genera in samples from nature, their separation was not commonly accepted.

In 1983, RIPPKA & COHEN-BAZIRE defined two other genera of this cyanoprokaryotic type, *Cyanobium* and *Cyanobacterium*, and accepted also the generic name *Cyanothece* for another cluster of the morphologically similar strains (but used new diagnostic features, especially of biochemical character, e.g. G/C content). Therefore, their descriptions and differential characters are not quite comparable with the published cytomorphological variation ranges of *Synechococcus* and *Cyanothece* sensu KOMÁREK (1976). In spite of this, their whole cluster of three new genera types can be cytomorphologically also delimited and distinguished from characteristic members of the type species of *Synechococcus* and *Cyanothece* sensu KOMÁREK. The diagnostical features of the *Cyanobium/ Cynobacterium* clusters are as follows: cell division occurs always into two equal cells, small-sized cells are without any keritomy, long cylindrical cells never occur (compare KOMÁREK & ANAGNOSTIDIS 1996).

Four other studies are particularly relevant to the taxonomy of this group:

(i) CAMPBELL & GOLUBIC' (1985) described a new species, *Aphanothece shiloi*, from the hypersaline Solar Lake (Sinai), which does not form any delimited colonies with distinct gelatinous envelopes (= one from generic features of the genus *Aphanothece*), and possesses large, cylindrical-oval cells ($13 - 20 \times 6 - 8$ μm) with distinct net-like (keritomized) cell content (according to the micrograph which represents the iconotype). Because these characters correspond precisely with the diagnosis of the genus *Cyanothece*, this species was transferred into this genus by KOMÁREK & ANAGNOSTIDIS (1995).

Table 1. Comparison of diacritical features of the genera *Cyanothece* and *Synechococcus*

	Cyanothece	*Synechococcus*
Original diagnosis		
Shape of cells	mainly oval to cylindrical	± cylindrical
Cell content	granular, sometimes net-like or with lengthwise keritomy	± homogeneous or with distinct centro- and chromatoplasma
Involution cells	irregular	filamentous
Cell division	symmetrical, obligatory into 2 equal daughter cells	symmetrical or asymmetrical
EM features		
Cell walls	thick outer layer with vesicles	3-layered without thick outer layer
Thylakoid patterns	thylakoids numerous, irregular, radial, over the whole cell body, partly in fascicles	several, parietally localized
Nucleoids	net-like	compact, bend-like

(ii) CHOU & HUANG (1991) published data about the ultrastructure of the *Synechococcus*-like strain from paddy fields in Taiwan, with unusual irregular thylakoid pattern within cells. Because this thylakoid arrangement (distinctly parietally localized thylakoids) differs substantially from other typical *Synechococcus* species known from literature (*S. capitatus* – BAILEY-WATTS & al. 1968; *S. lividus* – EDWARDS & al. 1968; *S. nidulans* – GANTT & CONTI 1969, LANG & WHITTON 1973, and others), the authors discussed the probability of taxonomic separation of this type as a separate genus from *Synechococcus*.

(iii) ROUSSOMOUSTAKAKI & ANAGNOSTIDIS (1991) described a new species, *Cyanothece halobia*, from heliothermal saltworks in Greece, with morphological features corresponding well to the genus *Cyanothece* (keritomy, cell size and shape, type of cell division, type of involution cells). The authors studied the fine structure of their species and recognized that the keritomy in cells is caused by a net-like arrangement of thylakoids, together with the formation of large intrathylakoidal spaces, which occur in various cyanobacterial genera.

(iv) CEPÁK & al. (1991) and CEPÁK (1993) stained the nucleoids in various cyanoprokaryotic taxa by DAPI fluorochromic dye, and found several characteristic patterns in the nucleoid structure. Distinct differences between the genera *Cyanothece* (*C. aeruginosa*) and *Synechococcus* (*S. nidulans*, *S. bigranulatus*) were recognized, and the generic identity of *C. aeruginosa* and *C. halobia* was supported (CEPÁK 1996).

In the present paper, the fine structure of one strain, which corresponds morphologically to the type species of the genus *Cyanothece*, *C. aeruginosa* (NÄG.) KOM. 1976, is described and compared with the cytology of the related species *C. halobia*, described by ROUSSOMOUSTAKAKI & ANAGNOSTIDIS (1991). The diacritical differences between this genus and the related chroococcalean genera *Synechococcus*, *Cyanobium* and *Cyanobacterium* are discussed. The micrographs of several representative strains of these genera are presented for documentation of their differences.

Materials and methods

The strains were obtained from the Culture Collection of Autotrophic Organisms (CCALA) at the Institute of Botany at Třeboň. The experimental strain SAG 87.79 (originally from Göttingen, Germany) of *Cyanothece aeruginosa* was used for our study, other strains were selected for comparison of several features only; they are cited in figure legend (Figs. 4–6) and in the text. The cultures were grown in test tubes (10 ml) in a liquid medium Z8 according to ZEHNDER (STAUB 1961) at about 20°C. Irradiance was adjusted to about 20 $W.m^{-2}$. After 14 days of cultivation, the cells and the range of their variation corresponded fully with the description of this species (KOMÁREK 1976). The strain was not bacteria-free; it grew in axenic culture very poorly and formed anomalous forms. We preferred, therefore, the characteristic cell shape for our study.

Fluorescent DNA patterns were stained with DAPI as described earlier (ZACHLEDER & CEPÁK 1987, CEPÁK & al. 1991). In short, a drop of suspension was put on a slide and a drop of DAPI solution (concentration of 1 $\mu g.ml^{-1}$) in buffer S (KUROIWA & SUZUKI 1980) was added. A coverslip was placed on the sample and ringed with a nail varnish to retard drying. The preparations were examined with an epifluorescence microscope JENALUMAR (Carl Zeiss Jena, Germany). The fluorescence of the DNA-DAPI complex was excited by light from an HBO-202W mercury vapor lamp. Observation of the DAPI-stained samples was made using an UG-1 (UV transmitting) excitation filter and a 450 nm barrier filter.

The samples for electron microscopy of strains SAG 87.79 and SAG 88.79 were fixed with 2.5% glutaraldehyde, washed with 0.1 molar cacodylate buffer (twice for 15 min) and

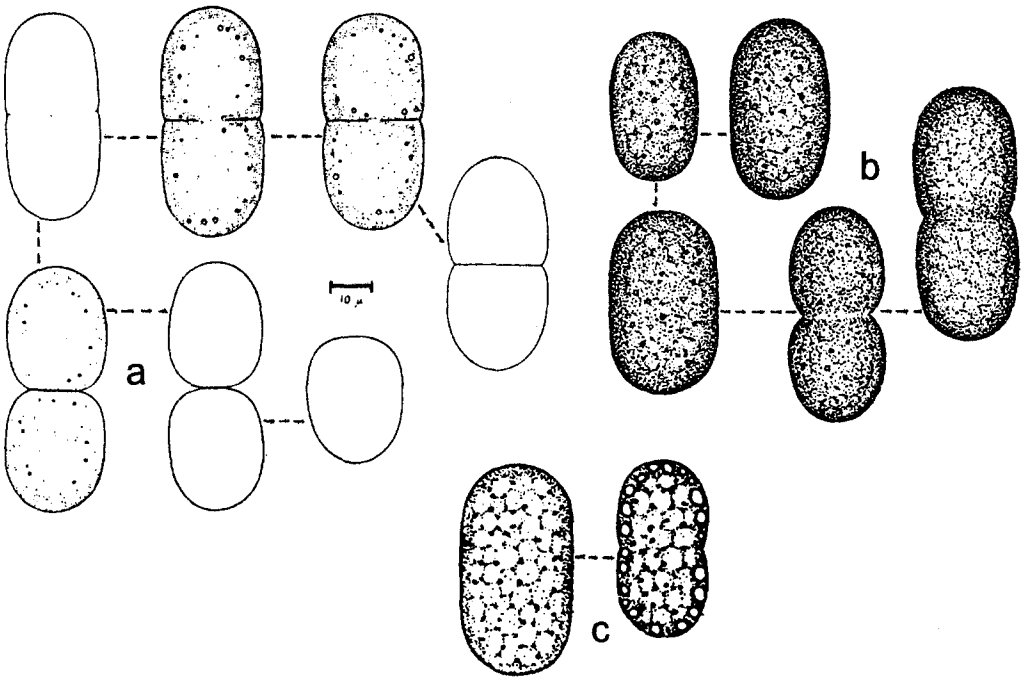

Fig. 1. Selected characteristic drawings of *Cyanothece aeruginosa* according to various authors, corresponding to the species description and to the strain SAG 87.79; *a* after GEITLER (1960), *b* after SKUJA (1956), *c* after GEITLER (1925)

transferred into agar. Postfixation was done by 2% OsO_4 in the same buffer and after 4 h a wash followed with cacodylate buffer plus glucose. The acetone row was used for dehydration. The sections were contrasted with concentrated uranylacetate solution in 50% ethanol, followed by an aqueous solution of Pb-citrate. For comparison, we have used micrographs of several strains produced by P. ALBERTANO (Fig. 5; for methods see ALBERTANO & LAPENTA 1994).

Results

Cyanothece aeruginosa grows as solitary cells, which are large in comparison with other unicellular cyanoprokaryotes ($9-50 \times 7-36$ μm; our strain SAG 87.79: $21.5-39.4 \times 21-25.2$ μm under our culture conditions), in cold peaty waters (swamps, moorland waters, oligotrophic wetland biotopes in northern and temperature zones, and in mountains), usually at pH lower than 7. Cells divide always perpendicularly to the longer axis of oval cells, i.e. in a single plane in successive generations. Daughter cells are joined together only for a short time after division. The morphological appearance of our strain was identical with that one described in literature. This identity follows also from the comparison of the best drawings from literature (Fig. 1) with our material.

Cells are surrounded by a narrow fine colourless mucilaginous envelope, about 0.21–0.32 μm thick. It appears two-layered in the electron microscope; it has an inner, dense filamentous and radially structured layer, which is embedded in a

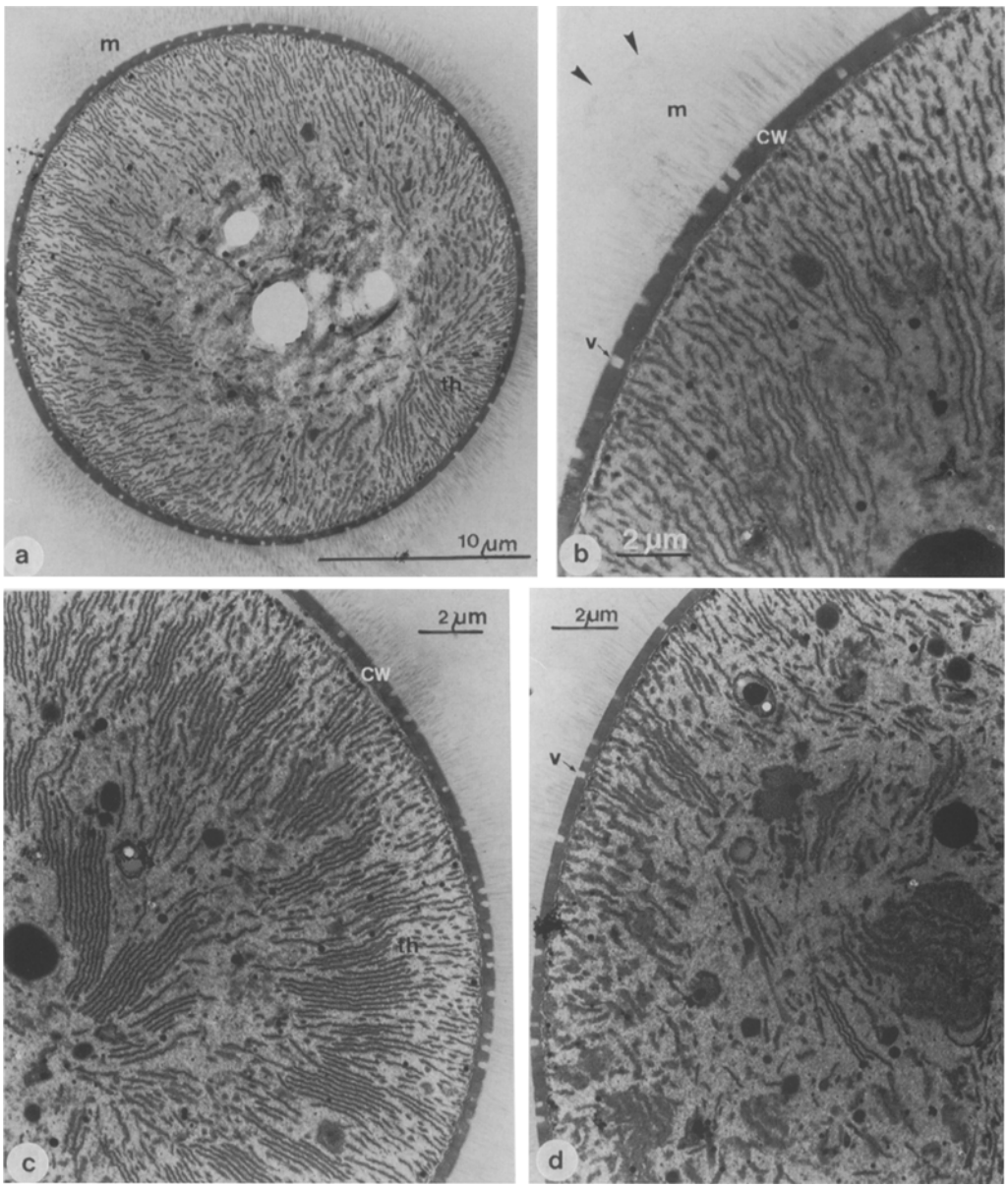

Fig. 2. Thin sections of cells of *Cyanothece aeruginosa*, strain SAG 87.79; *a* cross section through the cell; *b* detail of the peripheral part of a young cell; *c* part of a cell with fasciculated thylakoids; *d* part of an old cell with slightly widened thylakoids. *m* mucilaginous envelopes, arrowheads mark outer margin of amorphous slime, *cw* cell wall with hyaline vesicles *th* thylakoids, *c* carboxysomes; *v* vesicles within outer cell wall layer

hyaline, homogeneous outer matrix, delimited by a fine external continuous line (Figs. 2, 3).

The structure of the cell wall is slightly different from that of other cyanoprokaryotes by a remarkably thicker outer layer in which spherical, hyaline vesicle-like formations are distributed (Figs. 2b–d, 3a). They open into the surface

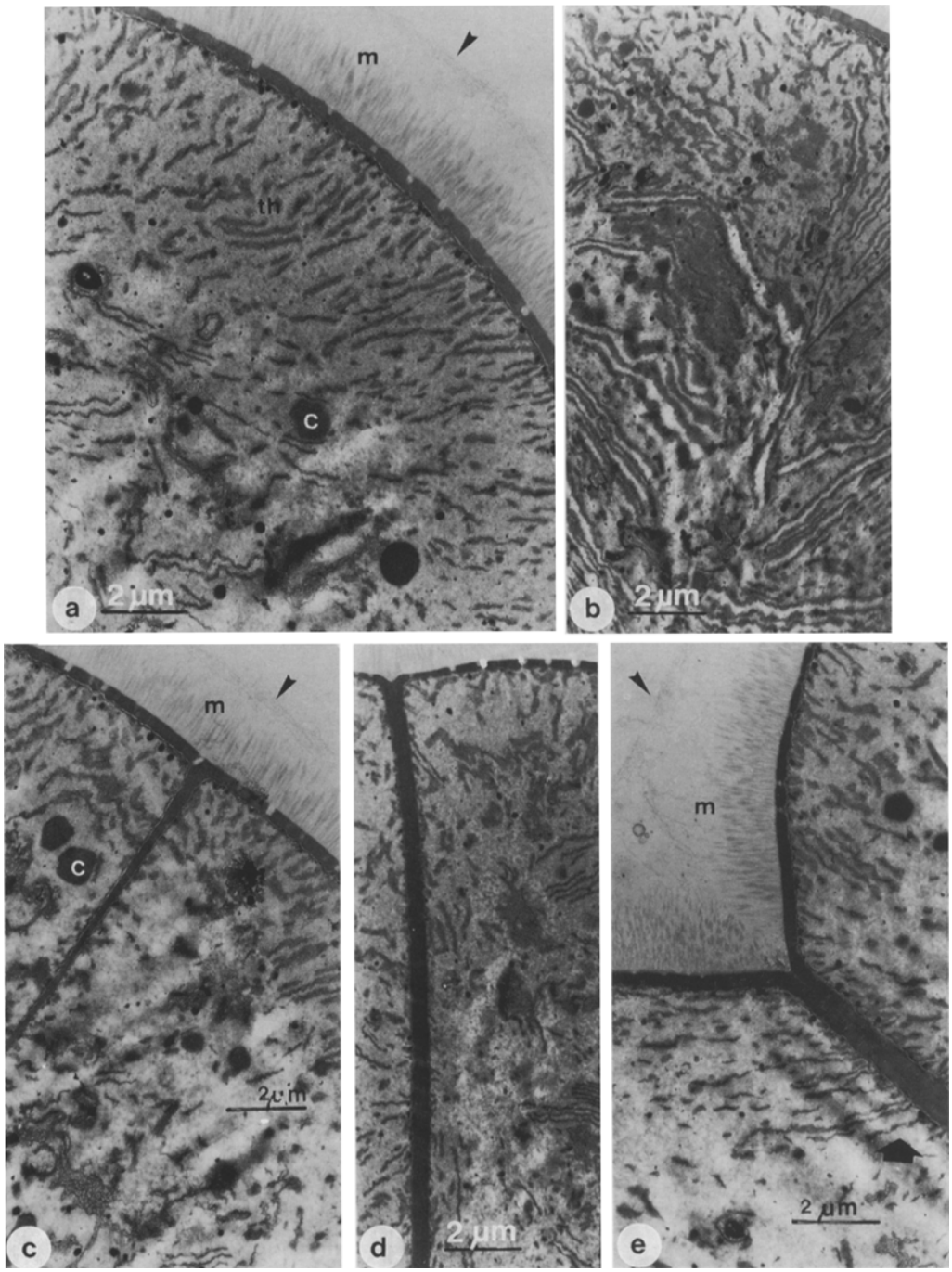

Fig. 3. Thin sections of cells of *Cyanothece aeruginosa*, strain SAG 87.79. *a* cross section of a margin of a cell before cell division; *b* detail of a dividing cell with widened thylakoids; *c–e* various stages of cell division (formation of the cross wall). Arrowheads mark margin of the mucilaginous envelope around cells, arrow marks new thylakoids; for labels see legend to Fig. 2

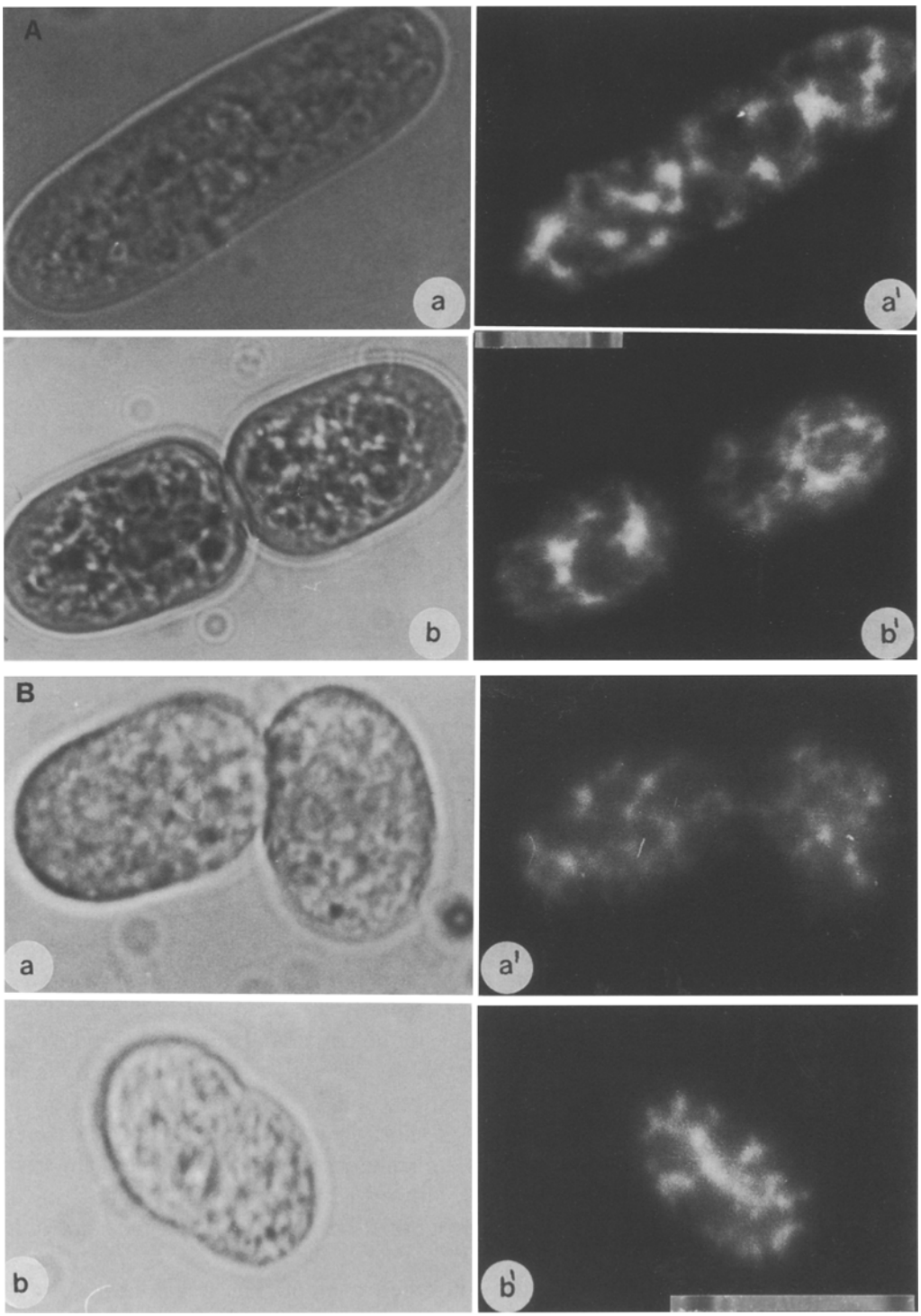

Fig. 4 A, B

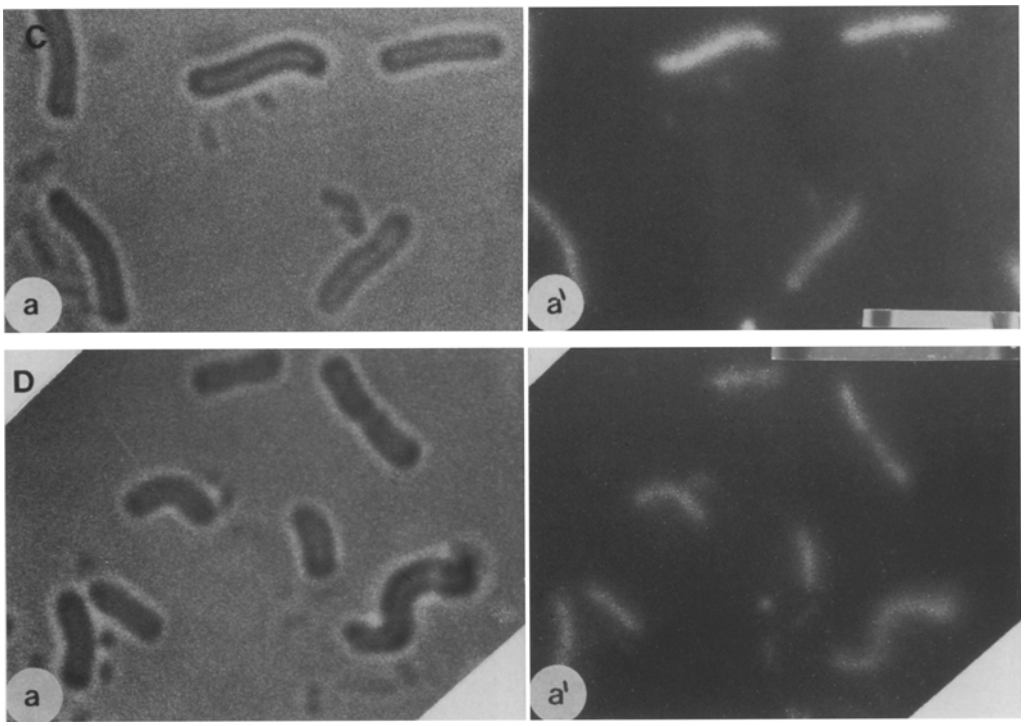

Fig. 4. Nucleoids stained with DAPI (the micrographs with DAPI-stained nucleoids marked a'–b' correspond to identical cells marked a–b); *A Cyanothece aeruginosa* (type species of the genus *Cyanothece*), strain SAG 87.79; *B Cyanothece halobia*, type strain cited in ROUSSOMOUSTAKAKI & ANAGNOSTIDIS (1991); *C Synechococcus nidulans*, strain UTEX 625; *D Synechococcus bigranulatus*, strain CCALA 187. – Bars: *A, B*: 10 μm, *C* 5 μm, *D* 10 μm

during the cell development. Their function is not solved; their connection with the production of the mucilaginous matter is possible. Inner cell wall layers do not differ from those in other unicellular cyanoprokaryotic species.

Thylakoids are numerous, more or less radially arranged and distributed over the whole cell volume, with a lower density in the cell center (Fig. 2a). Thylakoids are short, slightly wavy, and particularly in vegetative cells before division aggregated and parallel-arranged in indistinct and loose fascicles (Fig. 2b, c). Dividing cells possess a smaller number of short, irregularly distributed and wavy thylakoids (Fig. 3a, c–e). The thylakoids are sometimes slightly widened and form narrow intrathylakoidal spaces (Fig. 3b). However, widening of thylakoids never reaches such intensity as it was recognized, e.g. in *Cyanothece halobia* (ROUSSOMOUSTAKAKI & ANAGNOSTIDIS 1991).

DNA is spread unevenly over the entire cell content. The nucleoid has a net-like structure – numerous skeins of DNA are connected by thin DNA threads (Fig. 4). A mosaic of red (chlorophyll) and blue (DNA) areas is visible in *Cyanothece* cells under fluorescence optics. Other cellular inclusions (polyphosphate granules, carboxysomes, cyanophycin granules) are distributed irregularly over the whole protoplast (Figs. 2–4), and their character does not differ from other unicellular

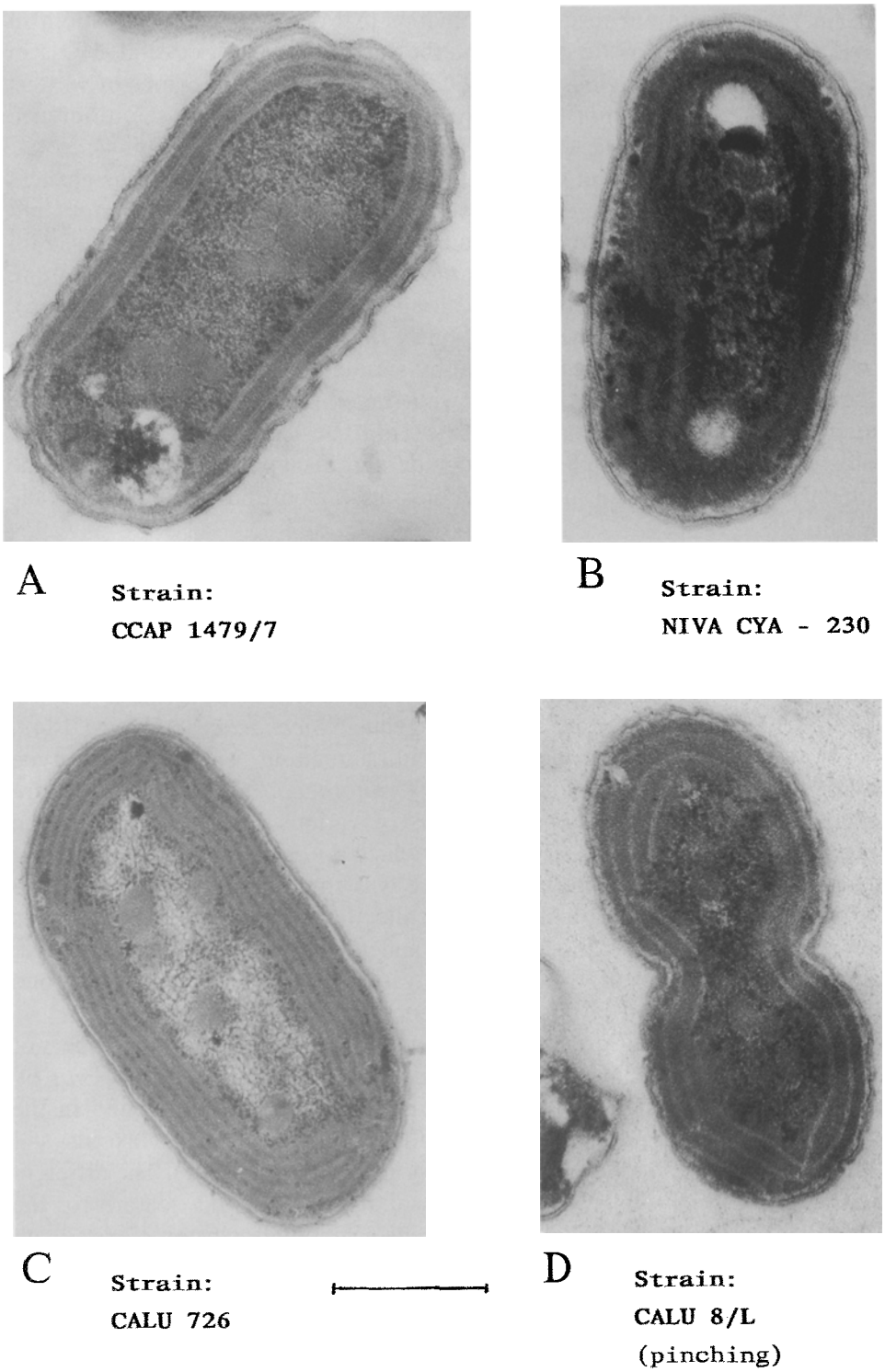

Fig. 5. Examples of cross sections of cells in various strains of *Cyanobium*-types (*A, B, D*), and one *Synechococcus*-type (*C*), with typical parietally localized thylakoids; (Photos by PATRIZIA ALBERTANO, Rome). – Bar: 0.5 µm

cyanoprokaryotes. From our results it is obvious that one type of DNA pattern in vegetative cells is characteristic of one and the same genus (CEPÁK & al. 1991; in our case in *Cyanothece aeruginosa* and *C. halobia*). A few micrographs of various strains from the genera *Cyanothece* and *Synechococcus*, illustrating the difference in DNA pattern on the generic level, are included for comparison (Fig. 4).

Evidently, the basic position of thylakoids is characteristic for various clusters of cyanoprokaryotes on the generic level. In all strains of *Cyanobium* and *Synechococcus* parietally localized thylakoids were found (Fig. 5), but in the strain SAG 88.79 of *Cyanothece* from the cluster *"minervae/cedrorum"* we found quite different, parallel position of thylakoids along the whole cell content (Fig. 6), which indicates the separate generic position of this cyanobacterium.

In *Cyanothece aeruginosa*, cells divide by simple binary fission; it is initiated by a centripetal invagination of cell wall layers in the middle of cells, perpendicular to the longitudinal cell axis (Fig. 3c–e). During this process, thylakoids slightly widen and partially degrade and disappear (Fig. 3). After the development of the cross wall, new thylakoids arise from the plasma membrane (Fig. 3e).

Discussion

Intergeneric features of the cyanoprokaryotic genera *Cyanothece* KOMÁREK 1976, *Cyanobium* RIPPKA & COHEN-BAZIRE 1983 and *Cyanobacterium* RIPPKA & COHEN-BAZIRE 1983, segregated from the traditional genus *Synechococcus* NÄGELI 1849, have never been completely defined. Major ultrastructural features of the type species of the cayanoprokaryotic genus *Cyanothece*, *C. aeruginosa*, are substantially different from the cell structures of various species (including the type species) of *Synechococcus*, the genus in which *C. aeruginosa* was originally classified (Table 1, Fig. 5C). The fine structure was studied only in one available strain of this species, from the SAG culture collection (SAG 87.79); this species was described originally by NÄGELI (1949), and the type strain does not exist. However, the morphology of the cells and variation of our strain correspond entirely with the description of this species (compare, e.g. KOMÁREK 1976).

Similar ultrastructural differences exist between *C. aeruginosa* and members of the genus *Cyanobium*, which was defined and separated from *Synechococcus* by RIPPKA & COHEN-BAZIRE (1983). The main differential features are found in the structure of cell walls, and particularly in the arrangement of thylakoids and intracellular inclusions, as follows from the EM fine sections (Fig. 5). The different nucleoid pattern within cells is another substantial distinguishing feature for the separation of *Cynothece* from the *Synechococcus/Cyanobium* cluster (CEPÁK & al. 1991, CEPÁK 1993) (Fig. 4). In contrast to our strains of *Cyanothece* (*C. aeruginosa*, *C. halobia*) with net-like nucleoids, all strains of *Synechococcus* and *Cyanobium* were distinguished since DNA was concentrated and evenly clustered in the cell centre (except for the narrow layer beneath the cell surface), in the form of homogeneous, compact and elongated band without any recognizable inner structure (Fig. 4C, D). The segregation of all these genera, therefore, seems justified. However, small differences between nucleoids in our *Cyanothece* strains and those described in the same genus by TSCHERMAK-WOESS & SCHÖLLER (1982)

Table 2. Main intergeneric features of the genera *Cyanobium*, *Synechococcus* and *Cyanothece*

	Cyanobium	*Synechococcus*	*Cyanothece* SAG 88.79	*Cyanothece* SAG 87.79
Cell form	oval to cylindrical	cylindrical	oval to widely oval	oval
Cell size	0.6–4.5 × 0.3–1.7(3)	(1.5)4–24(60) × (0.4)1.2–3.5(6)	(2)4–12(17) × (2.2)2.8–5(7–12)	(7)10–50(100) × (6)10–36(76)
Thylakoid arrangement	parietal	parietal	lengthwise	radial
Widened thylakoids	–	–	–	+
Involution cells	irregular	filamentous	irregular	irregular
Cell division	pinching	cleavage	cleavage	pinching
Nucleoids	plate-like	plate-like	plate-like with holes	net-like

must be explained before the final evaluation of this feature in the mentioned genera. The differential features of the genera *Cyanobium*, *Synechococcus* and *Cyanothece* (two cytomorphological types), are summarized in Table 2.

A corresponding cell structure to *Cyanothece aeruginosa* was found in the ecologically different *Cyanothece halobia* (Roussomoustakaki & Anagnostidis 1991, Cepák 1993), for which large intrathylakoidal spaces are particularly characteristic. The size of intrathylakoidal spaces, number of thylakoids and limits in cell dimensions are the main cytological differential features between these two species. The other characteristic *Cyanothece* species, that differ from each other mainly in quantitative features in cell morphology (but are still not studied by EM procedures), are *C. maior* (Schröt.) Kom. 1976 and *C. shiloi* (Campb. & Golubic') Kom. & Anagn. 1995. All these species evidently belong to one and the same genus *Cyanothece* according to their morphological similarity, and differ from each other in cell shape and cell size, in modifications in several intracellular structures, and in ecological characters.

The genera *Cyanobium* and *Synechococcus* differ from *Cyanothece* in cell shape and cell size, in other type of cells developing under suboptimal conditions ("involution cells"), and particularly in the position, number and form of thylakoids and type of nucleoids (Figs. 4, 5). The separation of both these genera *Cyanobium* and *Synechococcus* from each other, is a problem for a future study (shape of cells, type of cell division, type of involution cells).

In addition to the four large typical taxa of *Cyanothece* mentioned above, Komárek (1976) transferred from the genus *Synechococcus* and included into the genus *Cyanothece* also several species with smaller oval cells, the size of which vary more or less between "large" *Cyanothece* and the "small" *Synechococcus/Cyanobium* cluster (*Cyanothece diachloros*, *C. crassiuscula*, *C. notata*, *C. cedrorum*, *C. minervae*), and which are characterized by the structured protoplasts ("keritomy") with lengthwise striation of plasma (recognizable in light

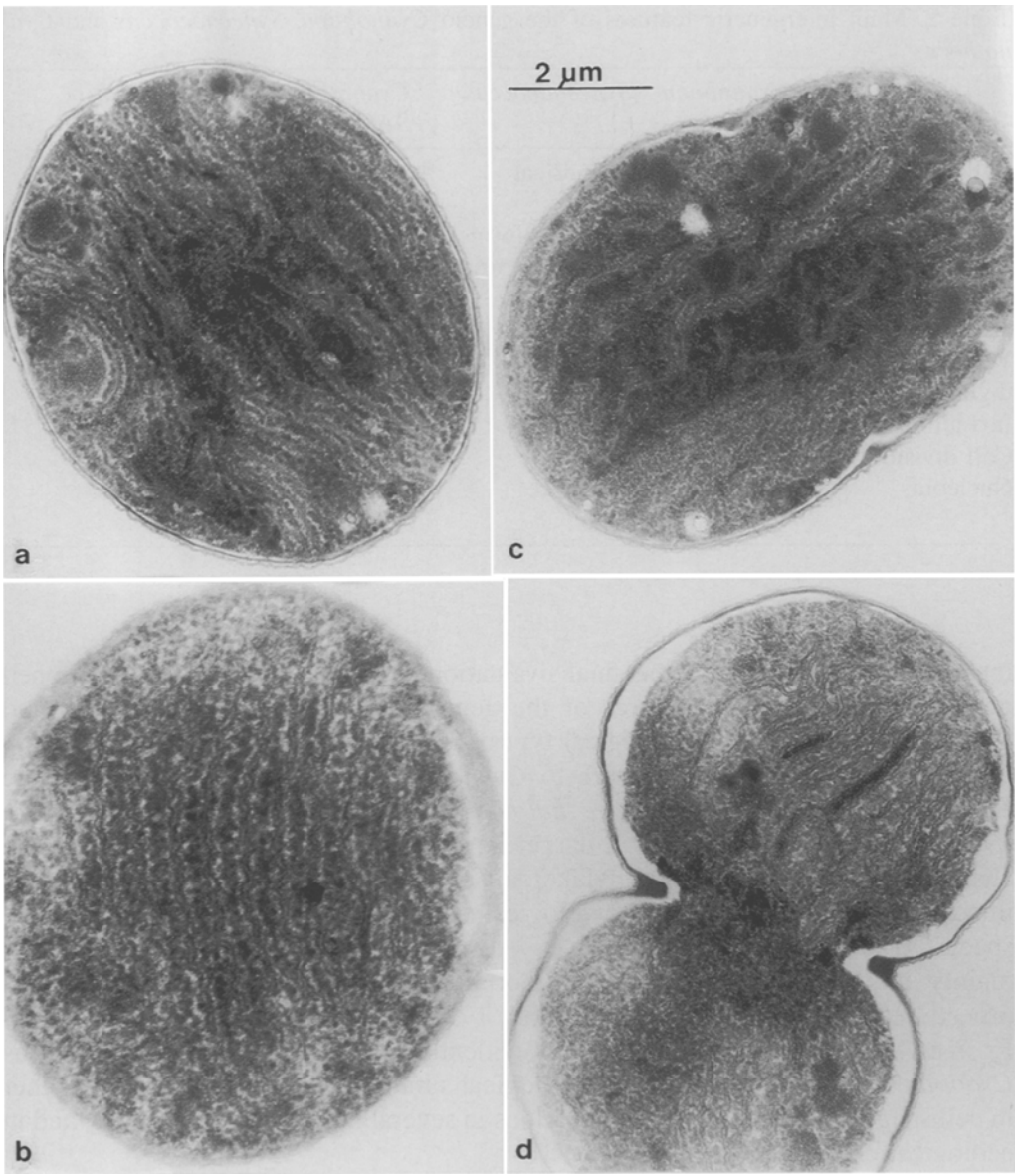

Fig. 6. Cross sections of cells of the *Cyanothece minervae/cedrorum* type (strain SAG 88.79), with radially and more or less lengthwise arranged thylakoids; *a* section through the cell; *b* tangential lengthwise section near the cell surface; *c* section through an old cell before division; *d* dividing cell (pinching)

microscope). From this group of species, we have studied the strain CCAP 88.79 of *Synechococcus/Cyanothece cedrorum* (Fig. 6). The species identity of this strain with the original *C. cedrorum* is not without doubt, but it represents evidently a member of this group of species with a lengthwise-striated cell content. It follows from our previous results that another type of thylakoid arrangement occurs in this

strain than it was found in *Cyanothece* or in the *Synechococcus/Cyanobium* cluster (Fig. 6). Therefore, this group of cyanobacteria evidently represents another well defined genus, which includes probably also the *Synechococcus* sp. of CHOU & HUANG (1991; see in our Introduction sub ii) and *Cyanothece* strains BH63 and BH68, the fine structure of which was studied by REDDY & al. (1993). (A generic relation of this not yet well defined genus with the genus *Cyanobacterium* RIPPKA & COHEN-BAZIRE 1983 is possible.) The last mentioned cluster of "small" *Cyanothece* species, included originally into this genus by KOMÁREK (1976), but without net-like or striated protoplasts and with distinctly recognizable chromatoplasmic peripheral layer in cells (*Cyanothece amethystina, C. eximia, C. gaarderi, C. rosea*), belong very probably into the genus *Cyanobium* according to cell morphology and cell content.

It is known that the position of thylakoids and their number vary within certain limits under the influence of environmental factors and changes during the life cycle (LANG & WHITTON 1973). However, this described variation concerns evidently mainly quantitative changes and have the character of modifications of one and the same structural type, like the number of thylakoids (which changes within certain limits in each species), their larger agglomeration in peripheral cell layers or over the whole protoplast, their density, etc. Similar changes were also found in our strain of *Cyanothece aeruginosa* during the life cycle. However, the basic pattern (parallel in peripheral or radial position, distribution over the cell volume, fasciculation, ability to form intrathylakoidal spaces, length and shape, very dense or free arrangement) is characteristic on the generic, or (in chroococcal types) on the species level (compare also KOMÁREK & ANAGNOSTIDIS 1996: introduction). For instance, the differences between the basic types of thylakoid arrangements in the *Synechococcus/Cyanobium* cluster (one or few parietal thylakoids), *Cyanothece* (free radial thylakoids over the whole cell volume, partly in fascicles), and *Cyanothece cedrorum*-type (irregularly lengthwise aggregated thylakoids over the cell volume, Fig. 6) represent different patterns, and transitional stages have never been found.

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