Contribution to the microflora of chroococcalean cyanoprokaryotes from São Paulo State, Southeast Brazil

Maria Teresa de P. Azevedo¹, Celia Leite Sant'Anna¹, [†]Pedro Américo Cabral Senna², Jirí Komárek³ and Jaroslava Komárková⁴

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ABSTRACT - (Contribution to the microflora of chroococcalean cyanoprokaryotes from São Paulo State, SE, Brazil). Colonial, simple chroococcalean Cyanoprokaryota have been extensively studied recently (1996-2001) from freshwater (planktic and metaphytic) water biotopes in the State of São Paulo, Southeast Brazil. Five new species [*Epigloeosphaera brasilica*, *Rhabdoderma sancti-pauli, Eucapsis densa, Chroococcus nanoplankticus*, and *Asterocapsa submersa*] are included in this article with taxonomic evaluation and data concerning their morphological variation and ecology The little known species *Eucapsis parallelepipedon*, described from Africa in 1902, was first time found in South America and taxonomically revised.

Key words: cyanoprokaryotes, Cyanobacteria, taxonomy, new species, distribution, Brazil

RESUMO - (Contribuição à microflora de cianoprocariotas cocóides do estado de São Paulo, Brasil). No estado de São Paulo, as Cyanobacteria cocóides de águas continentais (plâncton e metafiton) têm sido estudadas intensivamente nos últimos anos (1996-2001). No presente trabalho são propostas cinco novas espécies *Epigloeosphaera brasilica, Rhabdoderma sancti-pauli, Eucapsis densa, Chroococcus nanoplankticus e Asterocapsa submersa*. As circunscrições destas espécies são discutidas e dados sobre a variablidade morfológica e ecológica são apresentados. Problemas taxonômicos de táxons próximos e/ou pouco conhecidos são solucionados. A espécie rara e pouco conhecida *Eucapsis parallelepipedon*, descrita para a África em 1902, é reportada pela primeira vez na América do Sul e revisada taxonomicamente. Palavras-chave: cyanoprocariotas, Cyanobacteria, taxonomia, espécies novas, distribuição, Brasil

Introduction

In spite of the existence of some cyanoprokaryotes species with cosmopolitan distribution, their diversity is very different in various regions and biotopes. Numerous specific, ecologically and geographically delimited types have been recognized up to now, but the cyanobacteria from many regions and many biotopes still remain almost unknown. In the last few years of the 20th century, a serious investigation of cyanoprokaryote diversity started in numerous tropical countries, although the percentage of recognized taxa is very low even today. This research is stimulated by the increasing significance of cyanobacteria in eutrophic water bodies and other biotopes throughout the world, particularly in cases of production of large biomass. Numerous species were recognized causing allergies and producing toxic compounds.

In the State São Paulo, Brazil, the study of cyanobacteria was focused mainly on freshwater phytoplankton and metaphyton. These studies yielded data about several interesting species, communities of cyanobacteria, and their ecology. Several features were recognized in Brazilian cyanoprokaryotic microflora, to which belong a significant number of simple chroococcalean colonial species, still unknown from other regions. Of course, their distribution can be found more widely in the future, because the cyanobacteria from very large areas of South America (and not only South America) are almost unknown. More and more new species will be surely revised and studied with increasing knowledge of cyanobacterial taxonomy.

^{1.} Seção de Ficologia, Instituto de Botânica, Caixa Postal 4005, 01061-970 São Paulo, SP, Brasil.

^{2.} Departamento de Ecologia, Universidade Federal de São Carlos, Via Washington Luís, São Carlos, SP, Brasil (in memorian).

^{3.} Institute of Botany, Czech Academy of Sciences, Dukelska, Czech Repuplic.

^{4.} Institute of Hydrobiology, Czech Budejovice, Czech Republic.

^{5.} Corresponding author: tekazev@uol.com.br

Several interesting planktic species have already been described from the State of São Paulo over the last few years, e.g., *Coelomoron tropicale* Senna et al. (1998), *Coelosphaerium evidentermarginatum* Azevedo & Sant'Anna (1999) and others. Taxonomic evaluation, remarks about variability, ecology and distribution of five other new colonial, nanoplanktic or metaphytic cyanobacterial species and one rare species from freshwaters of São Paulo State are included in this article.

Material and methods

The studied material is part of the samples collected by the authors during the taxonomic survey of the plankthic cyanobacteria from São Paulo State. For taxonomic delimitations of tropical species of the genus *Eucapsis*, an additional sample from a small eutrophic lake in Montevideo Uruguay (Rodó lake), collected in August 1999 by C. Zankarelli, and the strain SPC 826 were analysed. The strain SPC 826 was isolated from a fish pond in São Paulo State (SP 355745) and was studied in Petri dishes containing solid BG-11 medium. The strain was kept at 22±1°C, light intensities 15 to 20 µmol m⁻² s⁻¹, and 14-10 h light-dark cycle.

All samples are deposited in the Herbarium "Maria Eneyda P.K. Fidalgo" (SP), Instituto de Botânica, São Paulo, Brazil.

Results and Discussion

Family: Synechococcaceae

Sub family: Aphanothecoideae

Epigloeosphaera brasilica sp. nov. Figures 1-2; 19-20

Epigloeosphaera brasilica spec. nova diagnosis: Coloniae sphaericae vel compositae de subcoloniis sphaericis, mucilaginosae; mucilago distincta, incolora, delimitata, cum cellulis superficie irregulariter ordinatae. Cellulae bacilliformes, perpendiculariter ad axem unam longitudinem dividuntur in generationibus successivis, contentu pallide aerugineo, $1.6-4 \times 0.6-0.8 \mu m$.

Habitatio: Epilimnice in stagnis lacubusque arteficialis; locus classicus: lacus arteficialis Atibainha dicta, in regione São Paulo, Brasilia.

Typus: Holotypus, positus in Sancto Paulo, Brasilia (SP 355737).

Colonies spherical, sometimes composed from 2, 3 or few hemispherical subcolonies to about 60 μ m diameter; mucilaginous envelope homogeneous, colorless, delimited at the margin, but not refractive; cells rod-like, sparcely and irregularly distributed on the colony surface, 1.6-4 × 0.6-0.8 μ m; cell content pale blue-green, homogeneous. Cell division only perpendicularly to the long cell axis; daughter cells shift soon after division one from another on the colonial surface. Reproduction by fomation of secondary colonies, separating from the colonial surface. Habitat: epilimnion of freshwater reservoirs.

The genus *Epigloeosphaera* was established recently by Komárková-Legnerová (1991). It has oval to rod-like cells, separated one from another, dividing perpendicularly to the longer cell axis; the main generic diacritical feature is that the cells are located irregularly on the surface (from the outside) of the gelatinous spheres. The mechanism of mucilage production is not explained, but it must be lateral from the cells. The position of cells and formation of mucilaginous spheres is different from the most related genera *Aphanothece* (subg. *Anathece*), where cells are distributed irregularly within mucilage, and *Lemmermanniella* with cells situated in one layer in the periphery of spheroidal colonies, but always below the mucilaginous surface.

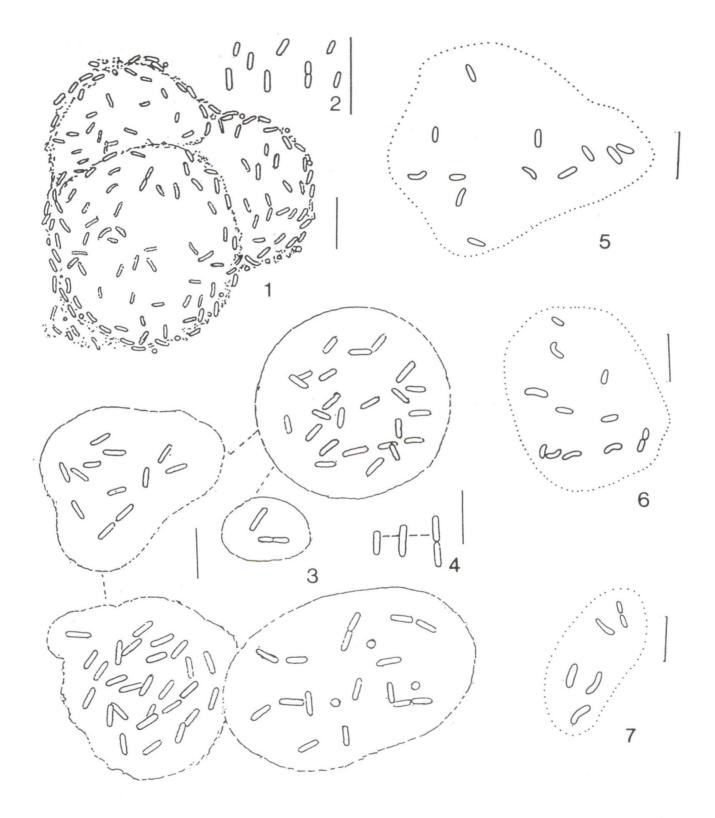
The type species of the genus, *E. glebulenta*, was described from benthos of northern, clear lakes, and it is known from Northern Russia, Sweden and Canada. The second species, *E. filamentosa*, in which the central mucilage forms long and sometimes divaricated formations of firm, refractive slime, was discovered in swamps in South Africa (Komárek & Cronberg 2001). In epilimnion of the reservoirs Atibainha, Cachoeira and Jacareí (Cantareira reservoirs system), State of São Paulo, was found an *Epigloeosphaera* population with spherical colonies, however, differing from *E. glebulenta* distinctly by form and width of cells. Because the character of reservoirs is also different from northern lakes, it is described as the new species, *E. brasilica*.

Family: Synechococcaceae

Sub family: Synechococcoideae

Rhabdoderma sancti-pauli sp. nov. Figures 5-7

Rhabdoderma sancti-pauli spec. nova - diagnosis: Coloniae microscopicae, subsphaericae, cum



Figures 1-2. *Epigloeosphaera brasilica*. 1. Composed colony. 2. Detail of cells. (Orig.) Figures 3-4. *Rhabdoderma* cf. *lineare*. 3. Stages of colony development. 4. Details of cells. (Orig.) Figures 5-7. *Rhabdoderma sancti-pauli*. (From Azevedo & al. 1996). Scale bars = 10 μm.

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cellulis solitariis sparsim irregulariter dispositis. Mucilago tenuis, incolora, inconspicua, homogenea, diffluens. Cellulae paucim elongatae, cylindricae et curvatae vel reniformes, rare sigmoideae, ad apices rotundatae, $2.9-5.7 \times 0.9-1.5 \mu m$, contentu homogeneo, aerugineo, sine aerotopis. Cellulae perpendiculariter dividuntur.

Habitatio: Planktice in piscinis lacubusque parvis, eutrophicis; locus classicus: Hortus Botanicus, São Paulo, Brasilia orientalis.

Holotypus: figure 27 in Azevedo et al. 1996, p. 32 (= fig. nostra 5-7).

Colonies subspherical with irregularly and sparsely arranged cells; mucilaginous envelope very fine, colorless, homogeneous, diffluent; cells cylindrical, usually slightly arcuated to sigmoid, $2.9-5.7 \times 0.9-1.5 \mu m$; cell content blue-green, homogeneous. Cell division perpendicular to the longer axis, and cells grow into original size before next division.

Habitat: plankthic in freshwater.

The genus *Rhabdoderma* is similar to the genus *Synechococcus* by the rod-like cell morphology and cell division type. It differs by life strategy: cells are unified into mucilaginous colonies, in which they are sparsely distributed. From about 15 described species, one was found as epiphytic on filamentous green algae, few others are known from saline biotopes or thermal and mineral springs, one species is cryosestic, one is known from periphyton of creeks, and one from peat bogs. However, the most known are three species occurring commonly in plankton of large oligotrophic to mesotrophic lakes. While *R. compositum* occurs only in large lakes of northern regions, *R. tenuissimum*, with very narrow cells, was described from large

African lakes and *R. lineare* is considered a cosmopolitan species, but it occurs mainly in temperate zones. In the eutrophic urban reservoir called "Lago das Garças" in São Paulo Botanical Garden, specimens of *R.* cf. *lineare* has straight, rod-like cells (figures 3-4) and cell dimensions were permanently at the lower limit of the type material $(3-22 \times 0.8-3.5 \,\mu\text{m})$.

Beside this type, other similar colonies, identified as Rhabdoderma sigmoidea Moore et Carter var. minor Moore et Carter (Azevedo et al. 1996, figures 5-7), occur in reservoirs in Botanical Garden in São Paulo. This taxon was originally described from one lake in North Dakota, USA; Brazilian specimens have similar form of arcuated cells, but differ by dimensions (table 1) and live in mucilaginous colonies of the Rhabdoderma type. The original Rhabdoderma sigmoidea (including var. minor) is explicitly unicellular, and recently classified into the genus Synechococcus (comp. descriptions in Geitler 1932, and Komárek & Anagnostidis 1999). The var. minor sensu Azevedo et al. 1996 therefore represents a separate species according to traditional criteria and the new species R. sancti-pauli is now proposed (table 1).

Family: Microcystaceae

Eucapsis parallelepipedon (Schmidle) Komárek & Híndak 1989 (Figures 8-9, 21-22)

Colonies free floating, with 4 to 32(64) cells,

distinctly distant one from another (with spaces mainly 2-8 µm large), arranged in cubic formations;

Table 1. Comparison of *Rhabdoderma* populations from the eutrophic lake Lago das Garças (Botanical Garden in the city of São Paulo) with the morphologically similar "*Rhabdoderma sigmoidea*" (= *Synechococcus sigmoideus*) and *Rhabdoderma lineare*.

	Rhabdoderma sigmoidea s. Moore et Carter (1923)	Rhabdoderma igmoidea var. minor Moore et Carter (1923)	<i>Rhabdoderma</i> <i>sigmoidea</i> sensu Azevedo et.al. (1996)*	Rhabdoderma cf. lineare (this study)	Rhabdoderma lineare Schmidle et Lauterb, in Komárek & Anagnostidis (1999)
Life form Shape of cells	solitary cells short cylindrical, usually sigmoid	solitary cells short cylindrical, usually sigmoid	colonies cylindrical, arcuated to sigmoid	colonies usually rod-like, straight, rarely slightly arcuated	colonies long cylindrical, rod-shaped, straight, slightly arcuate or sigmoid
Length of cells (µm)	4-13	5-10	2.9-5.7	3.6-7.4	3-22
Width of cells (µm)		± 1.5	0.9-1.5	1.0-1.8	0.8-3.5

* = Rhabdoderma sancti-pauli spec. nova

mucilaginous envelope wide, colorless, diffuse at the margin; cells spherical, 2.0-2.7 μ m diameter; cell content pale blue-green.

Habitat: in plankton of mesotrophic to eutrophic tropical and subtropical reservoirs.

Distribution: It is known in typical stages from Eastern Africa and from Uruguay, in South America.

Eucapsis parallelepipedon was described as "Chroococcus parallelepipedon" by Schmidle (1901) from large Eastern African lakes, with few cells within colony, which are 2-3 µm in diameter, distant from one another, but arranged in cubic formations (figure 8). The Schmidle's tropical material was later forgotten. The name (in the original form) was used only by Hansgirg (1892) for similar species from acidic and cold peat bogs in central Europe; this species was recognized as phenotypically different and especially by its ecology from Schmidle's original description, and described as Eucapsis starmachii by Komárek & Híndak (1989). According to these authors it occurs exclusively in cold stenotherm peat waters in central Europe, and its wider distribution is probable only in similar biotopes. The African planktic "Chroococcus parallelepipedon" sensu Schmidle was therefore never satisfactory characterized.

In slightly alkaline tropical, subtropical and warm temperate swamps occurs another metaphytic Eucapsis species, which was commonly identified as "Eucapsis alpina". It is known from several localities in Africa (e.g., Bourrelly 1961 a, b), Iran (Compère 1981), but also from warm Pannonian region in Europe [Komárek & Hindák 1989, figure 9, as "Eucapsis sp. (cf. E. parallelepipedon)"]. Both identifications do not correspond well to the types of original specimens. E. alpina is distributed in cold, acidic mountain and northern swamps, and differs substantially in its typical form from tropical populations as well as morphologically and by cell size (cells are 2.3-7.3 µm in diameter). The name "E. alpina" was used for tropical populations evidently because it was the single well described Eucapsis species in identification books at that time. Komárek & Hindák (1989) and Komárek & Anagnostidis (1999) classified this species as similar to the little known Eucapsis parallelepipedon, which was the only tropical Eucapsis species, but morphologically also distinctly different.

We have had opportunity to solve the morphological variability and ecological delimitation of both the tropical and subtropical *Eucapsis* species by the study of material from eastern regions of South America. We have found two different and well distinguishable morpho- and ecotypes existing here. They are planktic - subtropical, and metaphytic tropical. The first corresponds exactly to Schmidle's original description of *Chroococcus parallelepipedon* from Africa (comp. figures 8 and 9). The corresponding material was collected in August 1999 in a small eutrophic lake in Montevideo, Uruguay, by C. Zankarelli, and the correct name for it is *Eucapsis parallelepipedon* (Schmidle) Komárek & Hindák (figures 9, 21-22).

The second is a more common species of metaphyton from warm, slightly alkaline swamps, previously from tropical regions (Iran, W. Africa), and also known from warm areas of the temperate zone, in the backwaters of the Danube river in Pannonian region, by Komárek & Híndak [1989, as *Eucapsis* sp. (cf. *E. parallepipedon*)]. It was found in littoral of small lakes and swamps in São Paulo State in Brazil and differs evidently from *E. parallelepipedon*, as well as from *E. alpina*, and is newly described as *E. densa* (figures 10-14, 23-27).

Eucapsis densa sp. nov. Figures 10-14, 23-27

Synonym: *Eucapsis* sp. (cf. *parallelepipedon*) sensu Komárek & Hindák 1989

Eucapsis densa spec. nova - diagnosis: Coloniae microscopicae, multicellulares, irregulariter sphaericae cum cellulis dense agglomeratis, aggregatis plus minusve regulariter in grupos cubicos. Mucilago incolora, homogenea, ad marginem diffluens. Cellulae sphaericae, post divisionem hemisphaericae, contentu pallide aerugineo, cum chromatoplasmate visibili (positio thylakoidarum parietalis ?), 2.2-5.4 (-6) µm in diametro. Divisio cellularum evidenter in planis tres in generationibus successivis.

Habitatio: Metaphytice in paludibus, littorale piscinarum lacubusque inter plantas aquaticas in regionibus tropicalis vel subtropicalis (rare temperatis); locus classicus: piscina cum plantis aquaticis prope loco Broa, prope oppido São Carlos dicto, provincia São Paulo, Brasilia orientalis

Typus: Holotypus, positus in Sancto Paulo, Brasilia (SP 355744).

Colonies microscopic, with numerous densely arranged cells in more or less cubic formations, sometimes composed of subcolonies; mucilaginous envelope fine, colorless, diffuse at the edge, usually

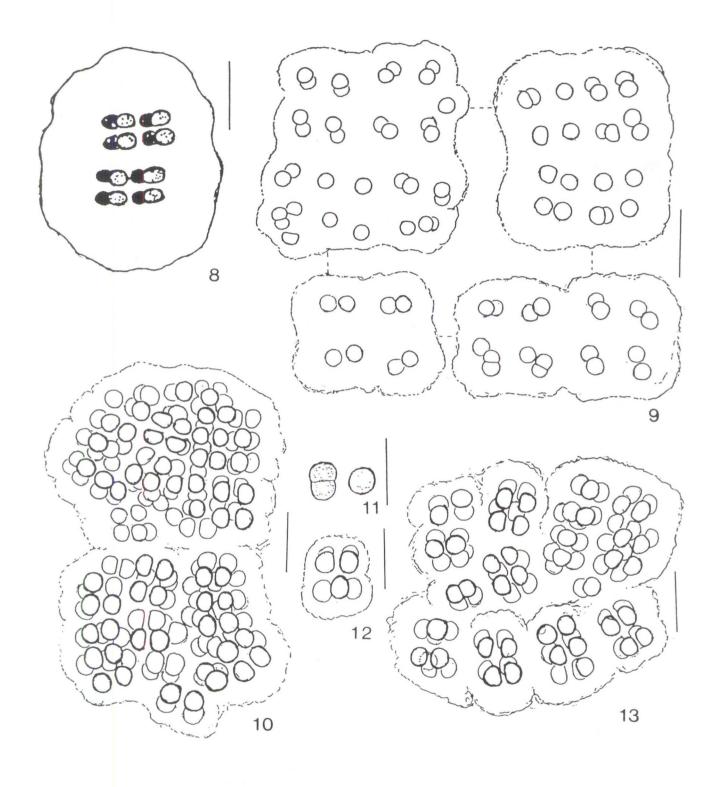


Figure 8. *Eucapsis parallelepipedon* (iconotype, sub "*Chroococcus parallelepipedon*" from E. Africa). (From Schmidle 1902.) Figure 9. *Eucapsis parallelepipedon*; variation of colonies from Uruguay. (Orig.). Figures 10-13. *Eucapsis densa*. 12. Young colony. 11. Detail of cells. 10, 13. Old colonies (Orig.). Scale bars = 10 µm.

forming 2-3 μ m wide margin around cell agglomerations; cells spherical, after divison hemispherical, 2.2-5.4 (-6) μ m diameter.; cell content blue-green, with distinct chromatoplasmic layer at the cell periphery. Cell division evidently in three perpendicular planes; cell content blue-green, with distinct chromatoplasmic layer at the cell periphery.

The genus *Eucapsis* now comprises nine well described species, but several others (particularly the multicellular forms and varieties of the widely conceived *E. alpina*) will be probably defined as separate taxonomic entities.

Family: Chroococcaceae

Chroococcus nanoplankticus sp. nov. Figure 15

Chroococcus nanoplankticus spec. nova diagnosis: Coloniae microscopicae, nanoplancticae, libere natantes, mucilaginosae, irregulares, cum cellulis irregulariter dispositis plus minusve in centro coloniae, in grupos iregulares. Cellulae sphaericae, post divisionem hemisphaericae, 1-3 µm in diametro, contentu pallide aerugineo vel griseo-aerugineo, plus minusve homogeneo, sine aerotopis. Divisio cellularum in planis perpendicularis. Reproductio disintegratione coloniis.

Habitatio: Planktice metaphyticeque in stagnis, paludis, lacubusque arteficialis eutrophicis, provincia São Paulo centralis, Brasilia orientalis; locus classicus: stagnum prope Valinhos.

Typus: Holotypus, positus in Sancto Paulo, Brasilia (SP 355746).

Colonies microscopic, mucilaginous, irregular, with cells arranged into irregular groups; mucilaginous envelope fine, homogeneous, diffuse; cell spherical or hemispherical after division, 1-3 μ m diameter; cell content pale blue-green, more or less homogeneous, without aerotopes.

Habitat: metaphyton and plankton of stagnant freshwaters.

Although the cell size differs in various populations, specimens exist in which almost all cells are about only 1 mm in diameter, or populations with 2-3 μ m large cells. Because other features are almost identical and because we have also observed transitional stages at the same locality, we suppose they are one and the same taxon. For our description it was used the type population from a small pond near Valinhos city; the populations with larger cells occur rather in larger reservoirs, e.g., in various parts of the

Guarapiranga reservoir.

The genus Chroococcus subg. Limnococcus contains planktic species, in which the cells are irregularly distributed within fine, hyaline slime, and only rarely produce their own, simple envelopes. Their type of cell division (in two or three planes in successive generations) and growth into the original shape before next division indicate the possible separate taxonomic position from the typical genus Chroococcus, but this question should be solved in the future. The subgenus is comprised of several species, mainly distinctly planktic in various freshwaters environments, differing mainly by cell size. Only two species, both described from Northern (Scandinavian) oligotrophic and cold lakes, C. microscopicus and C. aphanocapsoides, have cells less than 3 µm in diameter.

Numerous specific *Chroococcus*-types occur in tropical regions and are very little known (Komárek & Novelo 1994). In metaphyton and plankton of stagnant freshwaters in the central part of the State of São Paulo were found various populations of this subgenus with cells ranging from 1 to 3 µm in different localities, but with the same morphology and probably belonging to the same morphotype. Because the colonies were different from both Scandinavian species also in morphology (organization of cells within colony), we describe these populations as a new species (figure 15).

Asterocapsa submersa sp. nov. Figures 16-18

Asterocapsa submersa spec. nova - diagnosis: Coloniae solitariae vel in grupos aggregatae, sphaericae vel subsphaericae (adultae), ad 54 μ m in diametro, cum cellulis regulariter agglomeratis. Tegumenta coloniarum mucilaginosa firma, superficie laeves vel paucim verrucatae, paucim lamellosa, incolora vel subrosea ad rubro-fusca. Cellulae irregulariter sphaericae vel subsphaericae, atro-aerugineae, contentu plus minusve homogeneo, 6.4-9.1 μ m in diametro; cellulae in planis variis dividuntur. Reproduction disintegratione coloniis in cellulis solitariis, cum tegumentis propriis.

Habitatio: In coloniis cyanophycearum generis *Phormidium* in fossis periodicis; locus classicus: prope lacu arteficiali Atibainha dicto, provincia São Paulo, Brasilia orientalis.

Typus: Holotypus, positus in Sancto Paulo, Brasilia (SP355747).

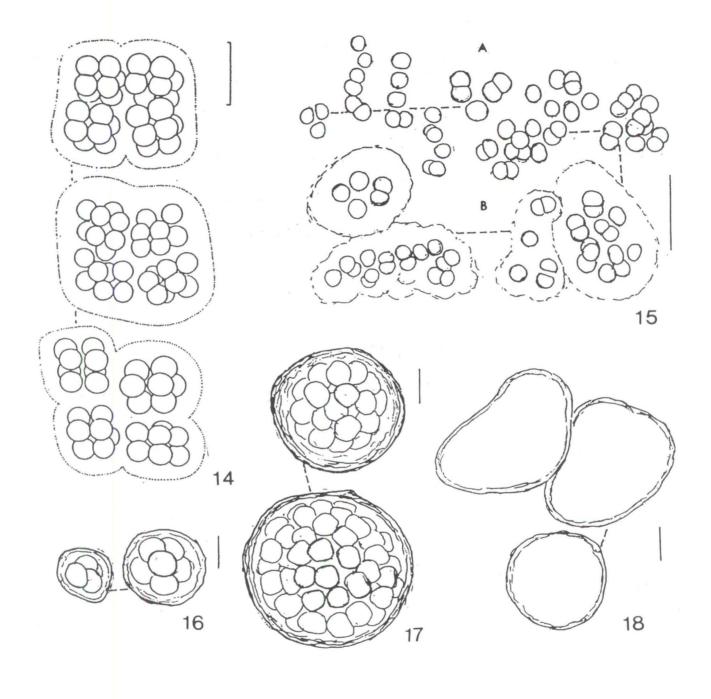
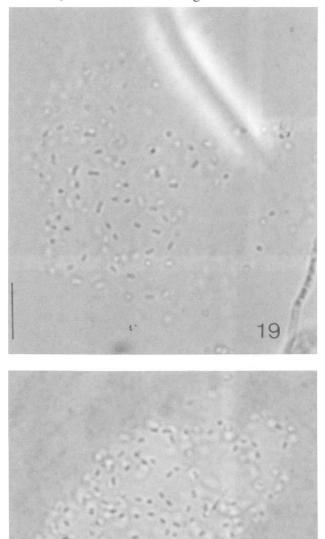


Figure 14: *Eucapsis densa*; colonies from Danube backwaters. (From Komárek & Hindák 1989.). Figure 15. *Chroococcus nanoplankticus*: a - young colonies, b - old colonies. (Orig.). Figures 16-18. *Asterocapsa submersa*. 16. Young colonies. 17. Old colonies. 18. Outline of old colonies. (Orig.). Scale bars = 10 µm.

Colonies solitary or agglomerated together, spherical or subspherical, up to 54 μ m in diameter, with cells aggregated in the center; mucilaginous envelope firm, colorless, pinkish up to reddish-brown, smooth or slightly granular on the surface, slightly lamellated; cells irregular sub-spherical, 6.4-9.1 μ m diameter; cell content dark blue-green. Cell division in



Figures 19-20. *Epigloeosphaera brasilica*, colonies from Atibainha reservoir. 20. With China ink. Scale bars = $10 \ \mu m$.

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different planes in successive generations. Reproduction by separation of the small, few-celled subcolonies, or by disintegration of the mother colony into solitary cells, which form very soon their own sheaths.

Habitat: with Phormidium.

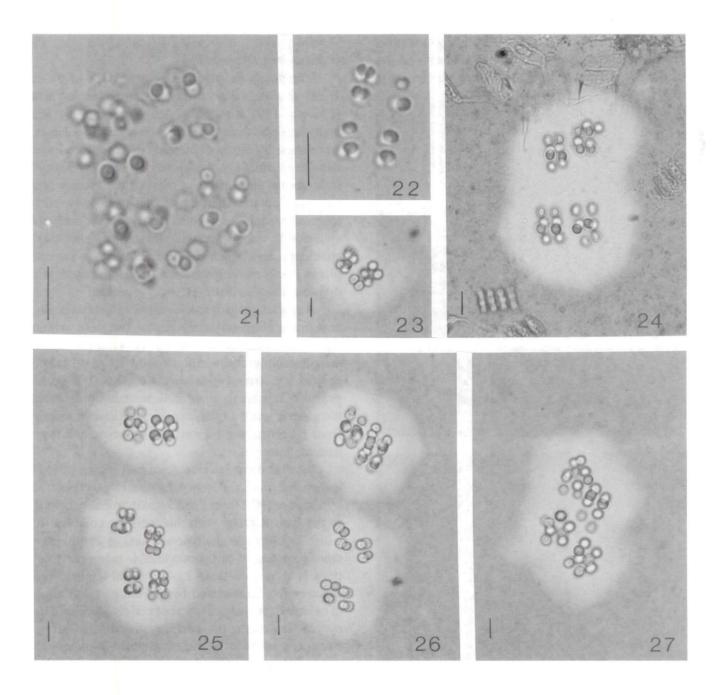
The genus *Asterocapsa* is comprised of about 15-20 species, mainly from subaerophytic habitats (Chu et al. 1991, Komárek & Anagnostidis 1999). The main diacritical features are: the sub-spherical colonies, enveloped by firm, delimited, often colored and lamellated mucilage, which has an usually "warty" structure on the surface in most of the species; the cells agglomerated in the center of colony, sub-spherical or slightly polygonal in shape (never perfectly spherical), sometimes with their own individual envelopes, and which divide irregularly in various planes in successive generations. The rapid nanocytic division was also observed in few species.

A special group of species exists within this genus, containing obligatory only one or two cells within envelopes, which always split after cell division and daughter cells escape and produce their own structured sheaths, e.g. *Asterocapsa badia* Komárek. This group probably represents a separate taxonomic entity at the generic level; all these species are known mainly from subaerophytic habitats in mountains and from extreme biotopes.

Several other species described under various names and from different localities (e.g., registered by Gardner 1927 from Puerto Rico) probably belong to the typical group with multicellular colonies (to which belongs also the type species). A comparison among species of this group and *A. submersa* spec. nova is shown on table 2. All typical *Asterocapsa* species are characterized by the same organization of colonies, form of cells, type of cell division, and life cycles. However, the surface structure of slimy envelopes seem to be more variable. *A. submersa* was found as accessory species in periodically flooded mats of benthic *Phormidium* in shallow channels near Atibainha reservoir (Cantareira reservoirs system), in October 1996 and with *Nostoc* sp.

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Figures 21-22. *Eucapsis parallelepipedon*, colonies from Uruguay. Figures 23-27. *Eucapsis densa*, colonies from São Paulo state (all with China ink). 24. Colony from Ibirapuera lake. 23 and 25-27. Colonies from fish ponds growing on solid BG-11 medium. Scale bars = $10 \mu m$.

	A. gloeotheciformis Chu (1952) (type species)	A. divina Komárek (1993)	A. submersa sp. nov.
Colonies	70-250 μm; multicellular	multicellular	up to 54 µm; multicellular
Colonial mucilaginous envelope	firm, lamellated or not, colorless, brownish or reddish, with warts on the surface	firm, lamellated, brownish, with warts on the surface	firm, slightly lamellated, pinkish up to reddish-brown, smooth or slightly granular on the surface
Cells	irregular, 12-16 × 7 μm	irregular, 6.2-14 µm in diameter	irregular, 6.4-9.1 µm in diameter
Cell content	_	blue-green	dark blue-green
Habitat	subaerial, China rocks, Mexico	subaerial, on wet limestone in shallow channels, Brazil	with benthic Phormidium

Table 2. Comparison among Asterocapsa gloeotheciformis, A. divina and A. submersa sp. nova.

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