Cryptogamie, Algol., 2002, 23 (2): 159-177

Two common *Microcystis* species (Chroococcales, Cyanobacteria) from tropical America, including *M. panniformis* sp. nov.

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(Received 28 March 2000, accepted 14 May 2001)

Abstract — Tropical populations of planktonic freshwater cyanoprokaryotes from the genus *Microcystis* are morphologically similar to species described from temperate zones, but differ in fine (but stable) phenotypic features and often in their life strategies. Morphological variation, life cycles and cell structure were studied in two species (both from natural populations and cultures) that are commonly distributed in tropical and subtropical regions of eastern and south-eastern Brazil. In the past they have been designated "*Microcystis aeruginosa*", "*Microcystis flos-aquae*" or "*Microcystis* cf. *lamelliformis*" in the floristic and hydrobiological literature, but, based on colony morphology and their life cycle, it is clear that they represent separate morphotypes. One corresponds phenotypically to *M. protocystis* originally described from Srí Lanka by Crow (1923) and later neglected; the other is newly described as *M. panniformis* sp. nov. They are both common "water-bloom"-forming, probably pantropical and toxic species.

Brazil / Cyanobacteria / cyanoprokaryotes / Cyanophyceae / ecology / freshwater plankton / *Microcystis panniformis* sp. nov. / taxonomy / tropical algae

Résumé — Deux espèces de *Microcystis* communes en Amérique tropicale. Certaines populations tropicales de cyanophytes planctoniques d'eau douce du genre *Microcystis* ressemblent morphologiquement à des espèces des régions tempérées mais en diffèrent par des caractères phénotypiques subtils mais stables et souvent par leurs cycles biologiques. Les variations morphologiques, les cycles vitaux et la structure cellulaire ont été étudiés à la fois dans des cultures et des populations naturelles de deux espèces communément

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distribuées dans les régions tropicales et subtropicales de l'est et du sud-est du Brésil. Elles ont été alternativement nommées « *Microcystis aeruginosa* », « *M. flos-aquae* » ou « *M. cf. lamelliformis* » dans la littérature floristique ou hydrobiologique, mais elles représentent clairement des morphotypes différents en fonction de la morphologie des colonies et des cycles vitaux. Une de ces espèces correspond phénotypiquement au *M. protocystis* originellement décrit du Srí Lanka par Crow en 1923 et oublié depuis lors tandis que la seconde est décrite ici comme *M. panniformis* sp. nov. Toutes deux appartiennent à un groupe d'espèces, probablement pantropicales et toxiques, formant des fleurs d'eau.

algues tropicales / Brésil / Cyanobacteria / cyanoprocaryotes / Cyanophyceae / écologie / *Microcystis panniformis* sp. nov. / plancton d'eau douce / taxinomie

INTRODUCTION

The genus *Microcystis* is one of the most important cyanoprokaryotes (cyanobacteria, Cyanophyceae) in freshwater ecosystems. It is delimited clearly at the generic level by molecular sequencing (Li *et al.* 1998; see also the internet sequencing databases), but in nature it occurs as numerous variable morphotypes (Kondrateva, 1968; Kato *et al.* 1991) in temperate and tropical freshwater reservoirs, and as a result the infrageneric taxonomy is problematic. However, several phenotypically distinguishable species have been described (see reviews in Crow, 1923; Geitler, 1932; Desikachary, 1959; Komárek & Anagnostidis, 1998) that develop in the plankton of eutrophic waters, sometimes forming dense "water blooms". The simple morphology, phenotypic diversity, range of variation, and difficulty in cultivation (with characteristic morphological stages) complicate identification of the various taxa.

Microcystis species (*i.e.* with identical genotypes) with a world-wide distribution probably occur, but comparisons from distant regions are difficult. In fact, no comparative studies have been reported on the genotypes of temperate and tropical populations of the common and highly variable *M. aeruginosa* (Kützing) Kützing. However, our own studies have indicated differences between various temperate and tropical *Microcystis* populations. Definitions of their characters and the determination of stable types are essential for progress in the study of cyanobacterial diversity in nature, because numerous populations have been recognized in reservoirs, both toxic and non-toxic. It is therefore important to establish their taxonomic delimitation and to enhance knowledge of their ecology and development. The natural *Microcystis* types can be distinguished from one another as distinct taxonomic units, if they are different by stable phenotype markers, life strategies and in their ecological features.

During our studies of planktic cyanoprokaryotes in the state São Paulo, Brazil, numerous *Microcystis* populations were collected, clearly belonging to various taxonomic types. Two little known and neglected species, which are nevertheless easy to distinguish from previously recognised taxa, are characterized herein.

METHODS

Samples were collected mainly during 1996 in reservoirs in the state São Paulo; several other randomly collected samples from other sites in Brazil are also included. The localities of species studied are listed in Results together with their ecology and distribution.

The life cycle of *Microcystis panniformis* sp. nov. was studied in several lakes in the state of São Paulo, during the period from 10 September to 25 October 1996. Developmental stages were measured and documented. Samples were concentrated using a 20-μm mesh plankton net, or by sedimentation of colonies and preservation in formaldehyde (1.5-4 % of final concentration), or Lugol's solution.

The strains isolated were cultivated in liquid media "BG 11", "ASM-1", or "Z" under more or less standard conditions: 18-24° C, and weak illumination (about 5 W m^{-2}), mainly in photoperiods 14^h L/10^h D. Two strains of *M. protocystis* Crow (SPC 618, isolated from Açúde de Bodocongó, Paraíba state, by M.T.P. Azevedo; CCALA 106-1, from Tabocas reservoir, Pernambuco state, by P. Domingos) and one strain of *M. panniformis* (SPC 702, isolated from the lake Lago das Garças, São Paulo, at 19 October 1999, by M.T.P. Azevedo) were used for our study (particulalry for characterization of cytomorphological features). The strains are available in the collections of the Institute of Botany, São Paulo, SP, Brazil (SPC) and partly (*M. protocystis*) in the Botanical Institute AS CR in Třeboň, Czech Republic (CCALA). *Microcystis panniformis* was studied also in mixed cultures, and in other monospecific strains from the SPC collection.

Ultrastructural sections were prepared by the method described in Komárek & Cepák (1998). The EM study of *M. protocystis* (Figs 7-10) was based on strain CCALA 106-1. That for *M. panniformis* (Figs 27-28) was based on strain SPC 702, which corresponds fully to the diagnosis and was selected as the reference strain.

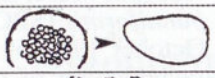
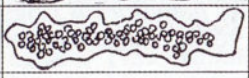
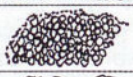
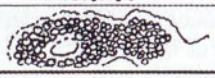
RESULTS

Two types from the genus *Microcystis* were recognized in eutrophic reservoirs of São Paulo state. These correspond in their cytomorphological characters to two species of *Microcystis*, but appear unusual in comparison with traditionally studied forms. One (*M. protocystis*) was described originally by Crow (1923) from Srí Lanka and later cited several times in identification manuals (Geitler, 1932; Desikachary, 1959), but it has otherwise been omitted almost entirely from floristic and hydrobiological papers dealing with tropical countries. The second species presented in this paper does not correspond to any described species.

Both these species do not occur in temperate zones; they probably belong among typical tropical cyanobacteria and have been cited under different names. They have usually been identified as "*Microcystis aeruginosa*" or "*Microcystis flos-aquae*" (Komárek, 1984; Peres & Senna, 1998; Senna *et al.*, 1998; Bittencourt-Oliveira, 2000). Our recent observations indicate that they are widely distributed in the tropics, forming prominent components of phytoplankton communities in many Brazilian reservoirs. As it seems likely that they are able to produce toxins (Komárek *et al.*, 2001), it is important to know their phenotypic variability. We have compared them with other tropical species (Tab. 1), and describe their morphological variability and life cycles.

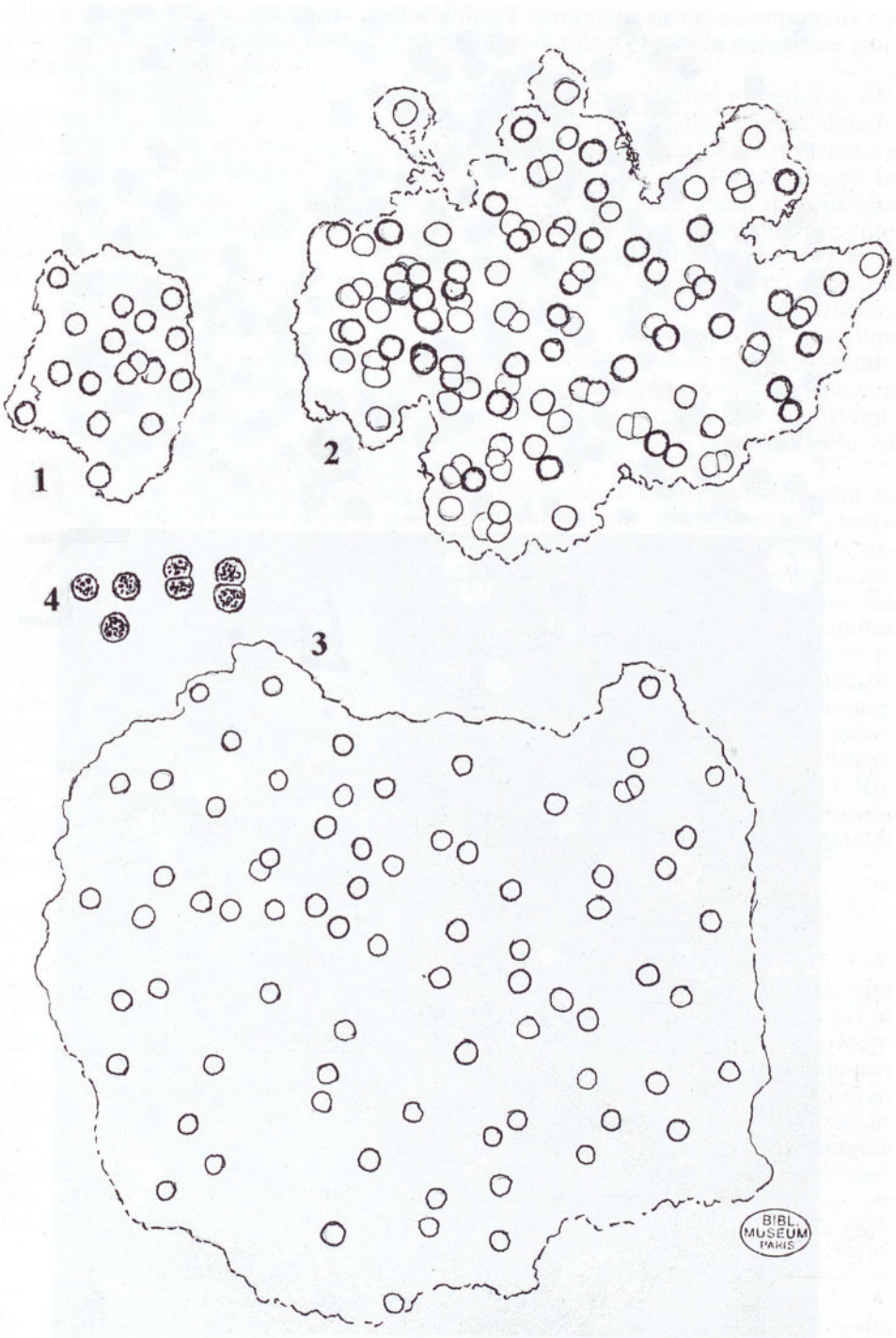
1. *Microcystis protocystis* Crow, 1923, New Phytol. 22:62 (Figs 1-13)
Syn.: *Microcystis aeruginosa* f. *protocystis* (Crow) Elenkin, 1938, Monogr. Alg. Cyanoph., pars spec. 1:106; *Anacystis cyanea* (Kützing) Drouet *et* Daily, 1952, *Butler Univ. Bot. Stud.* 10:221, *p.p.* (excl. typo).

Tab. 1. Comparison of main phenotype characters of *M. protocystis* and *panniformis* with main other tropical species and with morphologically similar species from temperate zones (*M. ichthyoblabe, M. flos-aquae* and *M. aeruginosa*).

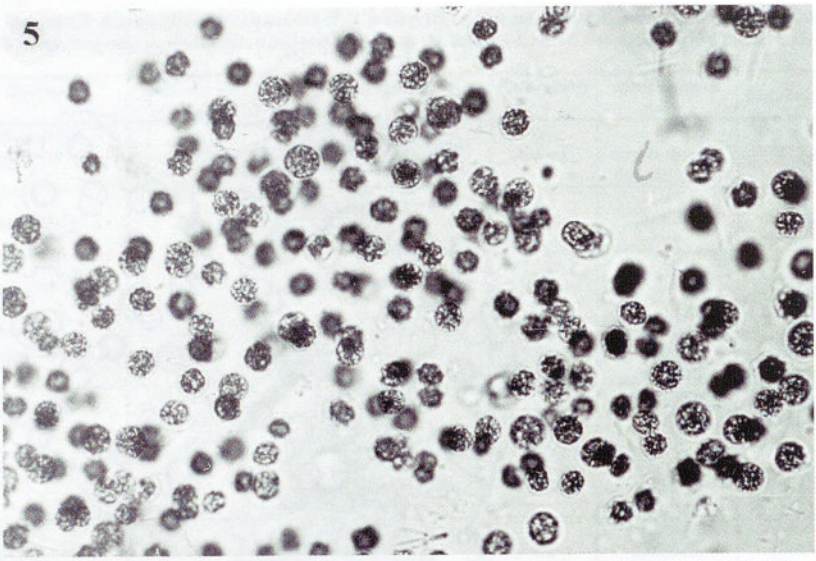
Species	Diameter of cells [μm]	Form of colony	Cell density	Mucilaginous margin	Distribution
Lamelliformis (1) Holsinger 1954	3-4(5)	 flattened	loosely	narrow to wide, fine, delimited	Srí Lanka
ramosa Bharadwaja 1935	3-5		separated	wide,diffuse	ponds India
densa G.S.West 1909	± 4	„elongated, cylindrical"	± densely		E. Africa
comperei Komárek 1984	4.5-5.2		± densely	wide, fine, but distinctly delimited	Cuba
protocystis Crow 1923	(3)3.5-6.5		very loosely	wide, fine, diffuse	India, Srí Lanka, prob. pantropical
pseudofilamentosa Crow 1923	3-7		densely	narrow, but distinct, ± diffuse	India, Srí Lanka
bengalensis Banerji 1936	3.5-6		densely	wide, distinct, stratified, delimited	India (Calcutta)
elongata Desikachary 1959	3.9-5.2		separated	wide, distinct, delimited, refractive	India (Madras)
maxima Bernard 1908	(3)4-5(8)	± spherical to irregular, wide, delimited mucilage	densely	narrow, diffuse	tropic. Asia
robusta Nygaard 1925	6-9		separated	± wide, delimited	India, Panama prob. pantropical
protocystis (2) (Brazilian populations)	(3.5)4-6(7.2)		loosely	wide, irregular, very fine, diffuse	Brazil
panniformis sp. nov.	(2.8)3-4.8		± densely	very narrow, diffuse	Brazil
ichthyoblabe (3) Kützing 1843	2-3.2		± densely	narrow to ± wide, fine, diffuse	temperate zones
flos-aquae (Wittrock) Kirchner 1898	(2.5)3.5-4.8		densely	thin (attached to cells on the colonial margin)	temperate zones ? cosmopolitan
Aeruginosa (Kützing) Kützing 1846	4-6(9.4)		densely	thin (about 5 μm wide)	cosmopolitan

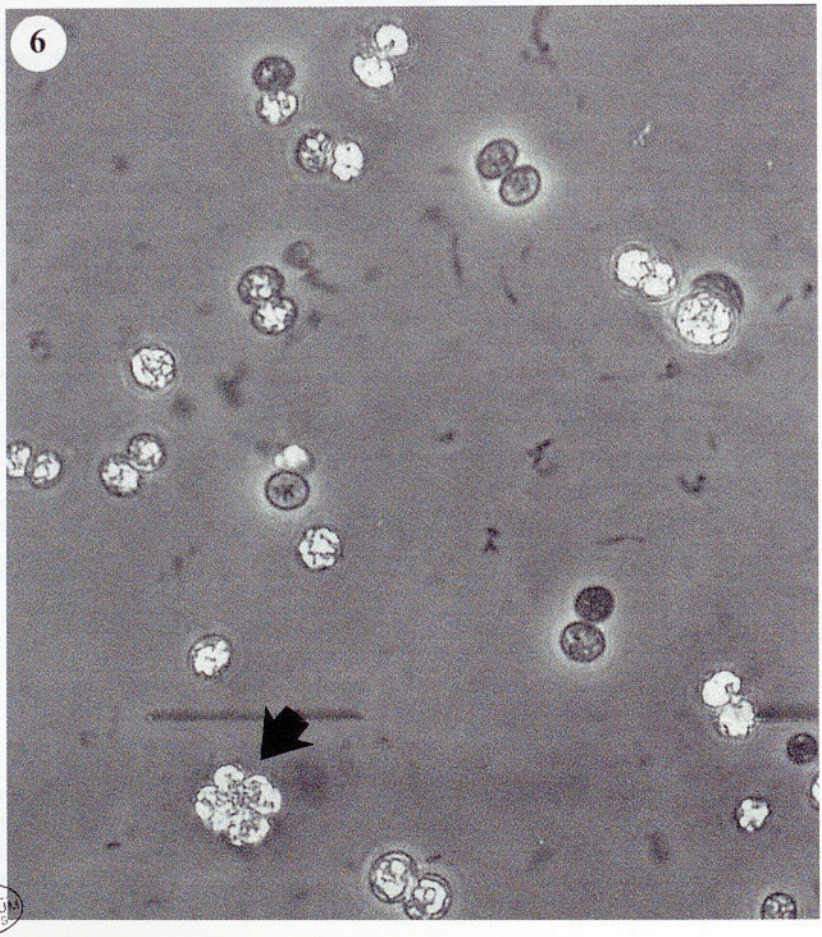
(1) Main tropical *Microcystis* species.
(2) *Microcystis*-species studied from São Paulo state.
(3) Three species from temperate zone, similar habitually to our Brazilian species.

Colonies (Figs 1-3, 5, 11-12) are free floating, usually microscopic, rarely up to macroscopically visible, irregular in outline, but without distinct lobes, and never with holes. Cells are distributed sparsely and irregularly within indistinct, colourless, homogeneous slime, which is diffuse at the margin. The slime overlaps widely the cells (Figs 11-12). Cells (Figs 4-6) are spherical, with aerotopes, slightly elongated before division, with very fine own (individual) envelopes (slightly visible, recognizable particularly after staining and in phase contrast; Fig. 13), (3.5)4-6(7.2) μm diameter, original data = (3)3.5-6.5 μm. Colonies disintegrate easily in



Figs 1-4. *Microcystis protocystis* Figs 1-3. Colonies of different age. Fig. 4. Detail of cells. (Orig.)





culture up to solitary cells. Cells under culture conditions grow exceptionally up to 8.5 µm diameter before division. Cell division in three planes in successive generations (Fig. 6, thick arrow).

The cellular fine structure was studied from the cultured material. It differs from *e.g. M. aeruginosa* (comp. Reynolds *et al.,* 1981) only in some details. Cells (Figs 7, 9-10) are enveloped with cell wall with finely granular substructure (*cw*). Numerous gas vesicles (*gv*), joined to aerotopes (*ae*), are always present in vegetative state (Figs 8, 9). Thylakoids (*t*) are mainly localized along the cell wall (Figs 7, 9), but in older cells and during division several wavy thylakoids are situated in central parts of the protoplast (Fig. 10). Numerous carboxysomes (*k*), scattered polyphosphate granules (*p*), phycobilisomes and ribosomes are recognizable.

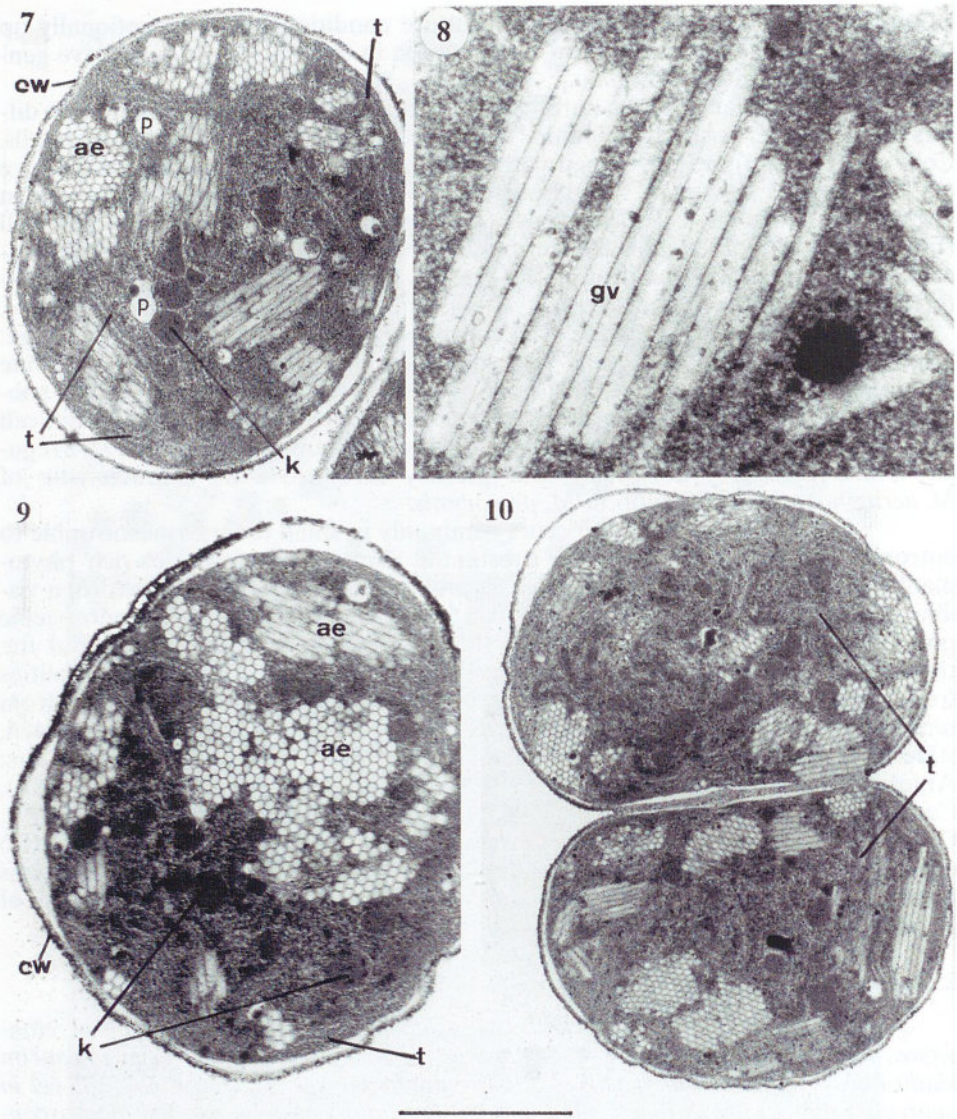
The life cycle is simple. The morphology of colonies does not change: large colonies disintegrate easily into small cell clusters enveloped by very fine and diffluent slime (Figs 2, 11-12). The organization of cells does not change substantially during vegetation period. Cell density within a colony is variable, but cell agglomerations never occur, as they do in *M. aeruginosa* (Figs 31-33). The irregular lobate colonies with holes and densely arranged cells characteristic of *M. aeruginosa* never develop in *M. protocystis.*

Microcystis protocystis occurs commonly in small to large mesotrophic to eutrophic reservoirs. It is often a substantial component of species-rich phytoplankton communities and sometimes forms a high enough biomass to form a visible water bloom; less frequently it can also form more or less monospecific populations. Our studies indicate that the species is widespread throughout the tropics. Komárek *et al.* (unpublished results) have registered numerous localities from tropical Asia, Africa and America, but relations to similar populations from temperate zones are unclear. *Microcystis protocystis* occurs commonly in Brazil, state of São Paulo. We have studied samples from the following reservoirs: Americana (8.1996), Barra Bonita (9. 1996), Billings (10. 1996), Broa (8. 1996), Cantareira reservoirs system (10. 1996), Clube de Penha in São Paulo (6. 1999), Guarapiranga (9. 1996), Lago das Garças (12. 1995), Paiva Castro (1. 1991), Rio Grande (10. 1996). In the case of other Brazilian states, we have studied strains from Açude de Bodocongó, Paraiba state, and from the reservoir Tabocas, state of Pernambuco, both in eastern Brazil.

2. *Microcystis panniformis* sp. nov. (Figs 14-28)

Diagnosis: *Coloniae juveniles subsphaericae, posterius irregulariter elongatae, lobatae, plus minusve complanatae, homogeneae vel posterius paucim clathratae, cum cellulis dispositis aequaliter praecipue sub superficie coloniis vel in aggregationibus intra coloniis inter cellularum alium; coloniae adultae macroscopice visibiles; mucilago incolora, homogenea, diffluens, indistincta, non marginem formans; cellulae sphaericae, post divisionem hemisphaericae, (2.5)3-4.6(4.8) µm in diametro, contentu luteo-viridi vel olivaceo, cum vesiculis gaseosis impletae. Reproductio cellulis solitariis liberantibus vel coloniis sphaeroideis, de margine coloniis adultis separantibus. - Habitatio: Libere (planktice) natans in aquis dulcibus, stagnis, piscinis, lacubusque eutrophicis in Brasilia subtropicali, in regione São Paulo dicto. – Holotypus*: specimen (preserved sample) SP336046 (SP-

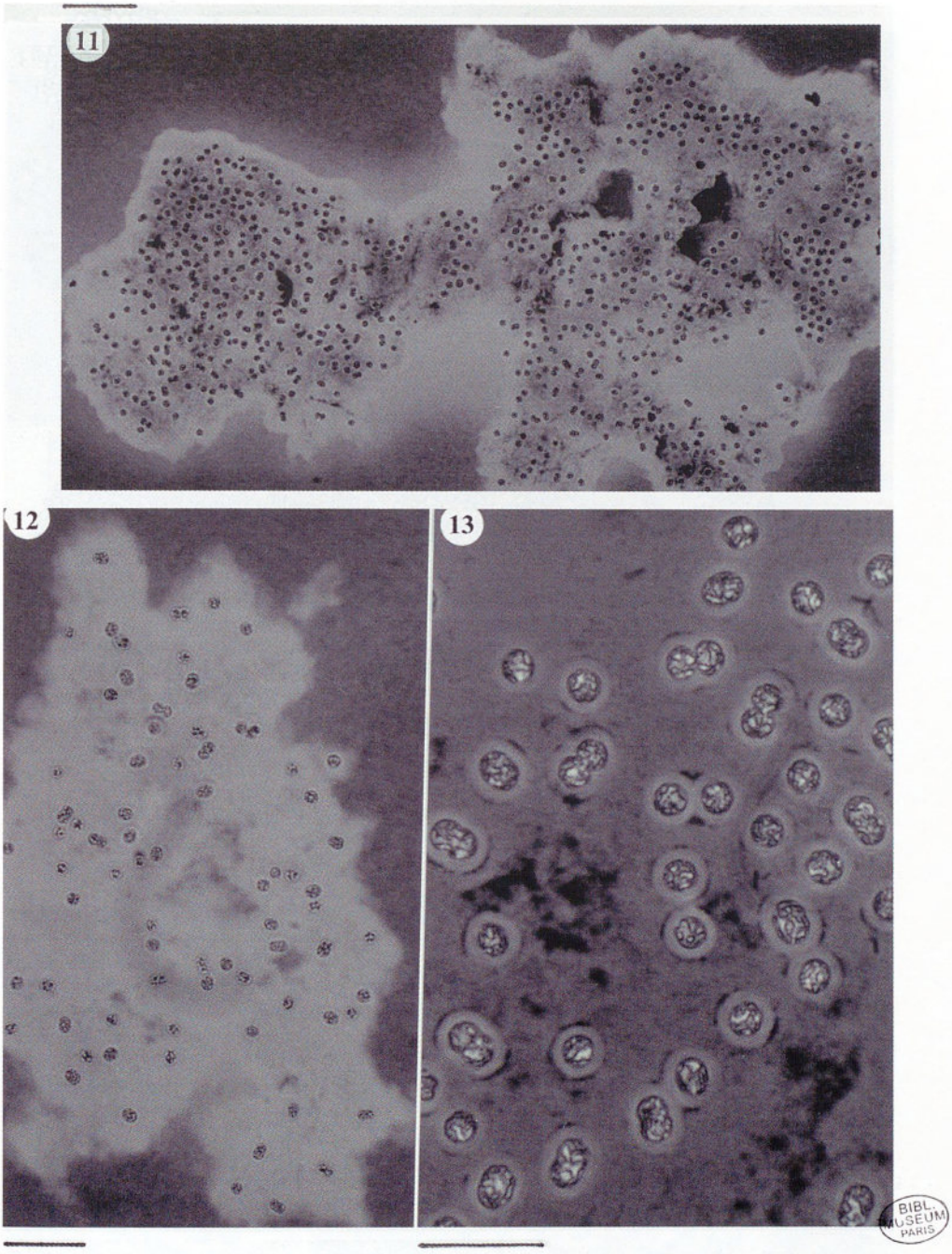
◀ Figs 5-6. *Microcystis protocystis.* Fig. 5. Detail of a colony from the culture CCALA 106-1 with characteristical arrangement of cells. Fig.6. Detail of liberated cells from the same culture (phase contrast); black arrow = group of dividing cells. (Photo J. Komárek.)



Figs 7-10. *Microcystis protocystis,* fine sections. Figs 7 and 9. Cross-sections of vegetative cells. Fig. 8. Detail of gas vesicles (lengthwise section). Fig. 10. Cross-section through dividing cell. Explanations: *cw* - cell wall, *gv* - gas vesicles, *ae* - aerotopes, *t* - thylakoids, *k* - carboxysome, *p* – polyphosphate granule. (Bars 7,9-10 = 2 μm; photo J. Komárek.)

Herbarium of Botanical Institute, São Paulo), collected from the eutrophic lake Pedreira, from the subtropical part of the state São Paulo, 15.3.2000.

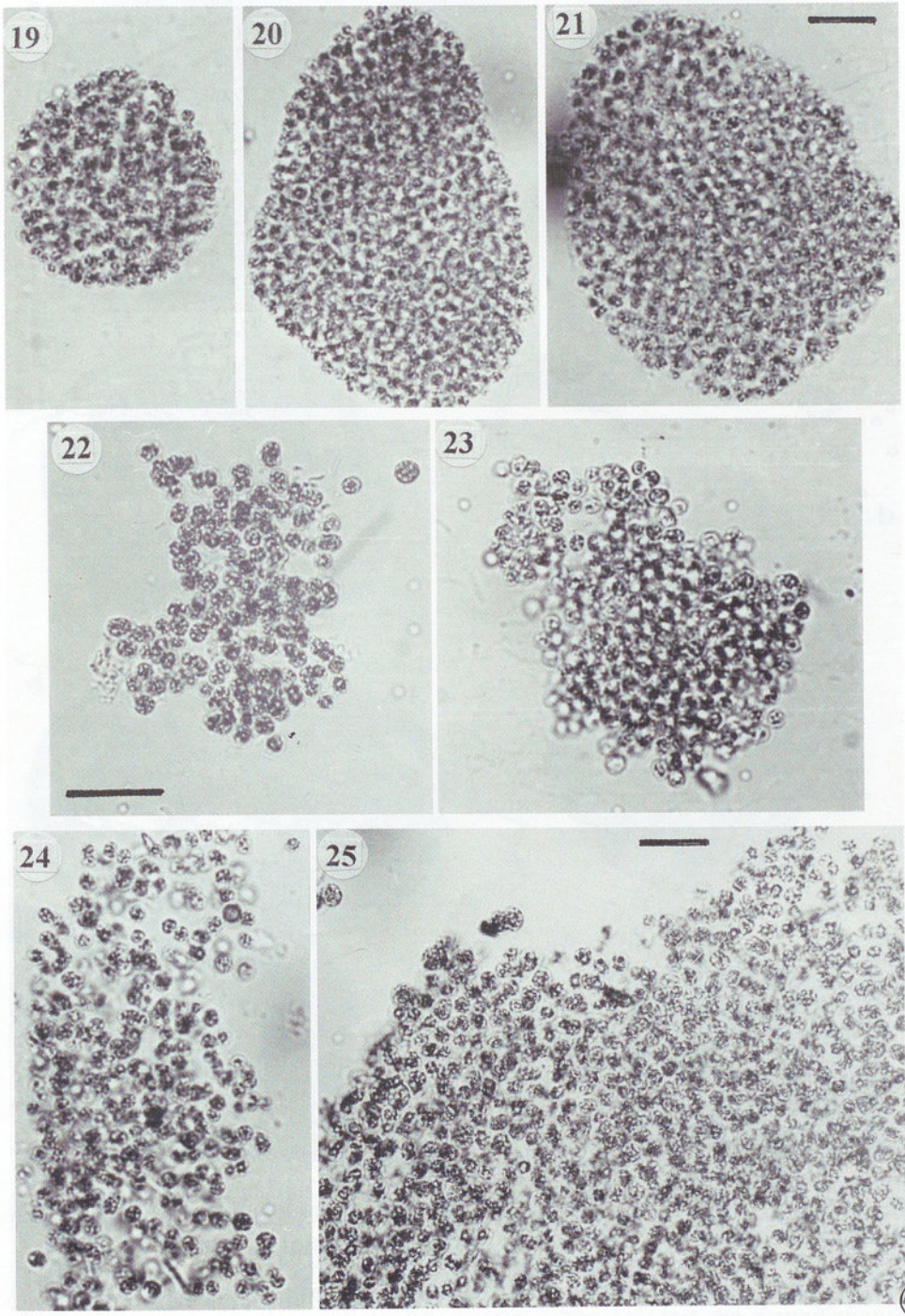
Figures 14-18 illustrate all the characteristic stages of this species. Colonies (Figs 14-25) are free floating, microscopic to macroscopic, initially in the form of irregular, tightly aggregated, few-celled, three-dimensional clusters of



Figs 11-13. *Microcystis protocystis*. Figs 11-12. Characteristical colonies from Brazilian reservoirs (mucilage stained by China ink). Fig. 13. Detail of cells in natural specimen with visible individual envelopes (phase contrast). (Bars in 11-12 = 50 μm, in 13 = 10 μm; photo M.A.P. Azevedo.)

Figs 14-18. *Microcystis panniformis*. Fig. 14. Initial stages with detail of cells. Fig. 15. Young hollow colonies. Figs 16-18. Old colonies; asterisk (*): details of cell arrangement in the surface layer. (Orig.)

cells, later more or less spherical or elongated with cells arranged evenly densely, mainly near the colonial surface (almost in a narrow layer), yellowish blue-green, olive-green, brownish to dark brown. The colonies sometimes appear "hollow"



Figs 19-25. *Microcystis panniformis*. Colonies of different age. (Bars = 20 µm; photo J. Komárek.)

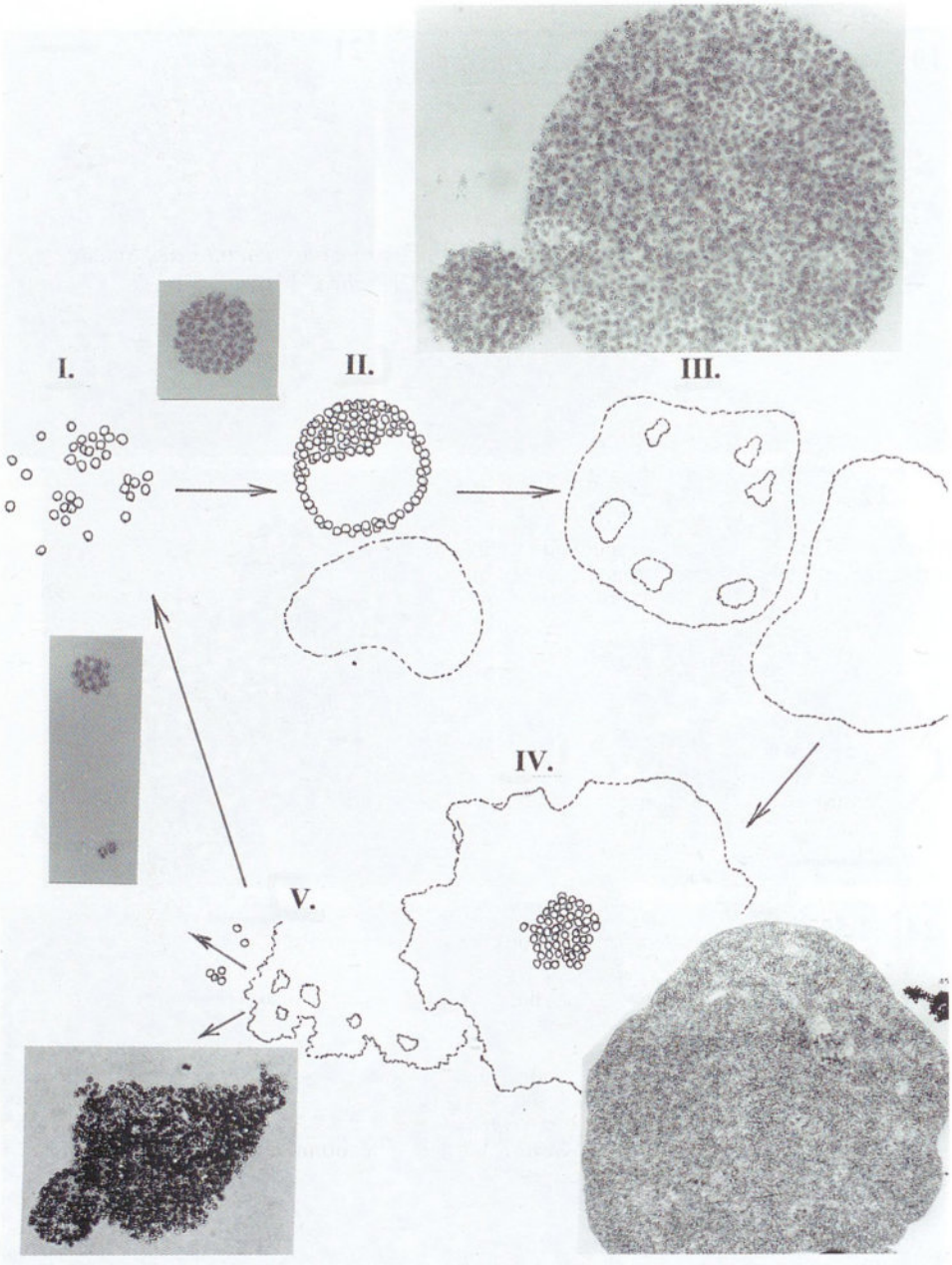
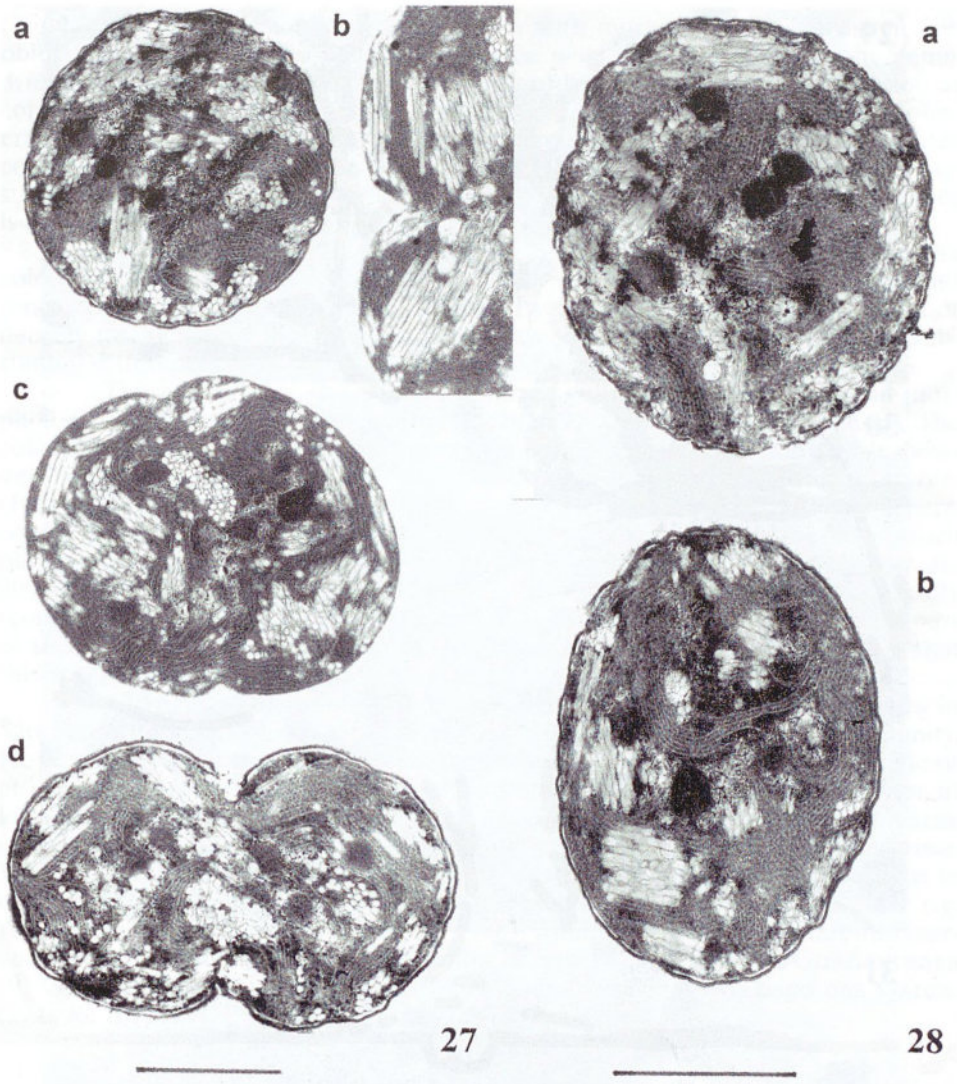


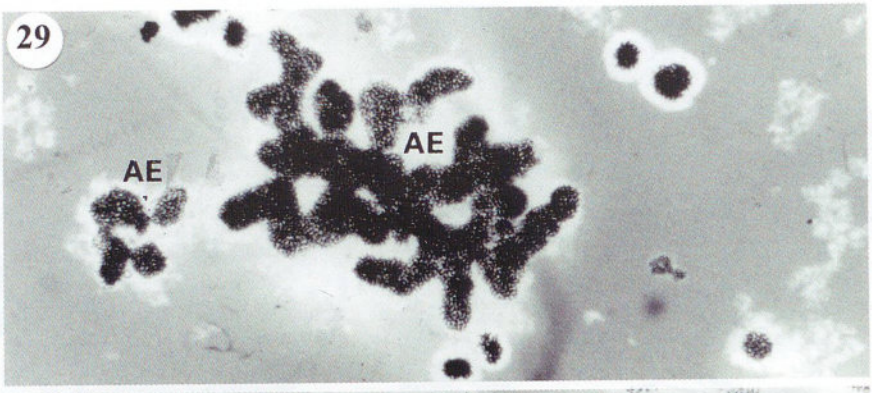
Fig. 26. *Microcystis panniformis*. Life cycle; morphological changes in colonies. Explanation see in text. (Orig., photo P.A.C. Senna.)

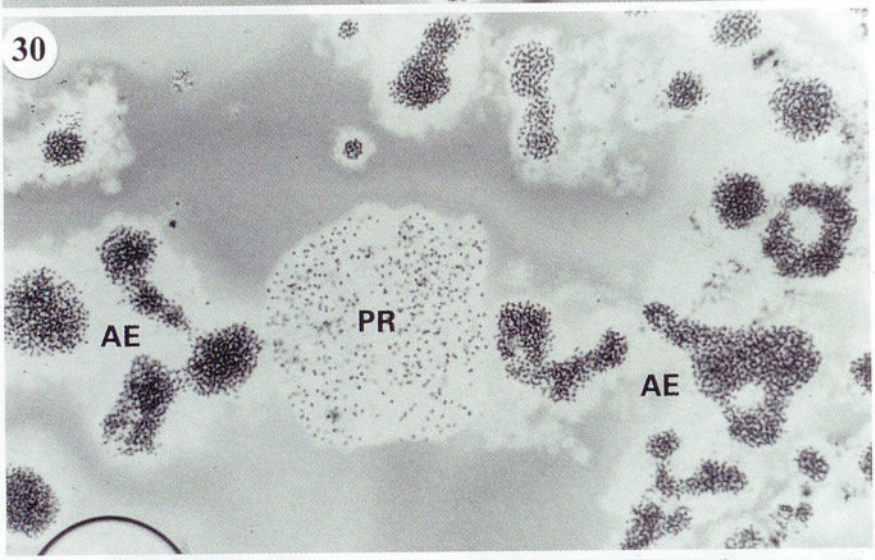
(with sparsely distributed cells inside the colonies). Old colonies are large, up to macroscopic, spherical-irregular to lobate with irregular indistinct holes, with cells arranged more or less evenly in flat, superficial formations, sometimes in indistinct,

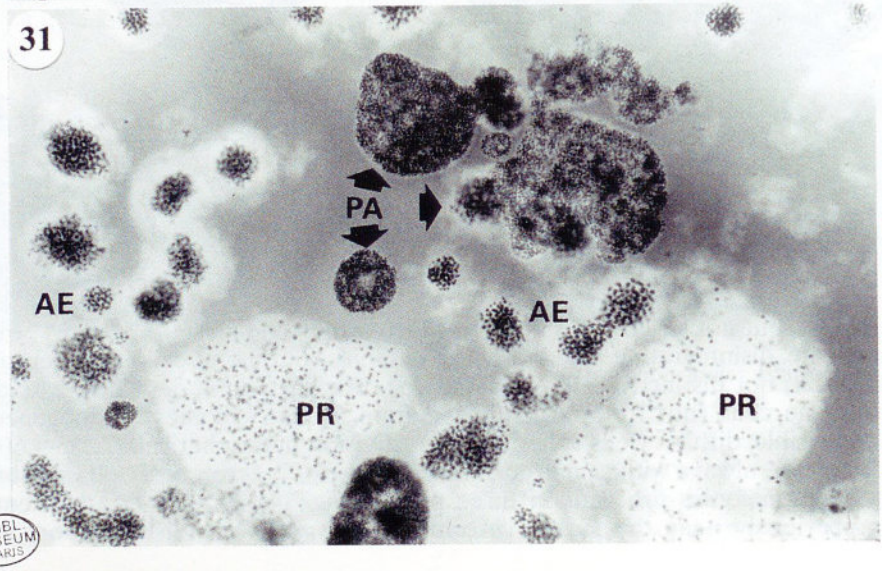


Figs 27-28. *Microcystis panniformis*, fine sections: 27b-d = dividing cell; 27b = detail of gas vesicles (lengthwise section); 28 = cells with partly degradated gas vesicles, but densely fasciculated thylakoids are visible. (Bars = 2 µm; photo J. Šulek.)

short rows, but not strictly in one layer. Macroscopic colonies are "cloudy", irregular, lobate, disintegrating. The margin of colonies (outline of cell agglomeration) is irregular, not distinctly delimited (Fig. 25). Mucilage colourless, not extending beyond the cells (not forming a slimy margin around colonies), homogeneous, slightly visible, diffuse. Cells spherical, after division hemispherical, (2.5)3-4.6(4.8) µm diameter, always with numerous, small ("dot-like") aerotopes. Cell division in very young colonies proceeds in three planes (Fig. 14), but the arrangement of cells previously near the surface in old colonies and in indistinct short rows







(Fig. 17, asterisks) resembles a combination with more simple cell division. The obligatory regularity of cell division in three perpendicular planes in the genus *Microcystis* should, therefore, be revised. Reproduction occurs by disintegration of colonies into small, mucilaginous clusters of several cells (Fig. 14), by solitary liberated cells enveloped by indistinct mucilaginous layer, or by spherical daughter colonies, liberated from the margin of large, old colonies (Figs 24-26). Reference strain: SPC 702. Etymology of the epithet "*panniformis*": the structure of colonies is similar to the cyanobacterial genus *Pannus*.

Examination of the fine structure of cells was difficult. We used the colonies from strain SPC 702, in which partly degraded gas vesicles were found repeatedly in all our specimens. However, Figs 27-28 clearly show the position of densely fasciculated thylakoids and numerous solitary gas vesicles. No other substantial differences from *M. protocystis* were found.

This species was also studied in mixed cultures, but the data about morphological variation and life cycle were obtained from natural populations. The colonies are agglomerated if they are developed in masses, and form visible, powder-like "blooms". The life cycle was studied in several Brazilian water reservoirs (Fig. 26). Some toxicity was detected in preliminarily assays (M.T.P.Azevedo, pers. comm.). This species was found also in samples from Tabocas Reservoir, which played an important role in the infamous Caruaru problems (Komarek *et al.*, 2001), but the species producing toxins there were not identified taxonomically (comp. Jochimsen *et al.*, 1998). Although *M. panniformis* represented only a part of the cyanobacterial community in that reservoir, it seems highly probable that this species contributed to the toxic effect.

Microcystis panniformis is widely distributed in eutrophic reservoirs in São Paulo state, Brazil, usually as a part of the rich phytoplanktonic community. However, populations of this species are known which are almost monospecific or at least the dominant. It is a common "water-bloom" forming cyanobacterium, at least in tropical America. *Microcystis aeruginosa* f. *flos-aquae* sensu Komárek (1984) from Cuba is probably also taxonomically identical with *M. panniformis*. We have found this species in ponds in the Zoological and Botanical Gardens in the city of São Paulo, and also in numerous reservoirs in São Paulo state e.g. Americana (8. 1996), Barra Bonita (9. 1996), Billings (10. 1996), Cantareira reservoirs system (10. 1996), Clube de Penha in São Paulo (6. 1999), Guarapiranga (9. 1996), Ibirapuera (1. 1989, 10.1996), Jurumirim (8. 1996), Lago das Garças (9. 1996, 10. 1999), Paraibuna (9. 1996), Pedreira (3. 2000).

DISCUSSION

This study is a step towards interpreting the wide morphological diversity of *Microcystis* populations in tropical America. Our methodological approach was the combine the study of phenotypic changes during their vegetation cycles in natural communities and the use of cultures. Comparison by sequencing procedures

◀ Figs 29-31. Comparison of colonies of *Microcystis aeruginosa* (AE), *M. protocystis* (PR) and *M. panniformis* (PA, thick arrows) at low magnifications, from the reservoirs Jurumirim (29) and Americana (30-31), São Paulo State. (Photo P.A.C. Senna.)

must follow the isolation of the corresponding number of strains. However, we considered the recognition and delimitation of various stable morphotypes from natural biotopes as essential to evaluate toxic populations in remote, especially tropical regions. The different stable types of *Microcystis* exist in natural communities, characterised according to phenotype, as well as biochemical and molecular characters (Kato *et al.*, 1991, Li *et al.*, 1998).

All molecular analyses indicate that the genus *Microcystis* is very uniform and well delimited in the modern sense (*i.e.* including only typical planktonic species with gas vesicles – Komárek & Anagnostidis, 1998, Li *et al.*, 1998; not sensu Geitler, 1932; Elenkin, 1938; Desikachary, 1959; and others). However, the separation of infrageneric species is difficult and disputable. Precise analyses are able to distinguish only morphospecies, defined by phenotypic characters (Kondrateva, 1968; Kato *et al.*, 1991; Komárek, 1991).

Li *et al.* (1998) did not observe distinct agreement of phenotype features with molecular characters in *Microcystis*. Otsuka *et al.* (2000) regard the specific differences as problematic, because in all species similar types of colony occur. In reality there exist numerous populations with "transitional characters", but if similar stages occur in the life cycle of various *Microcystis* morphotypes, it does not necessarily follow that they are taxonomically (genetically) identical. The different species have a "typical" development of their colonies; they occur repeatedly in various localities over the world in the same morphotypes, but different from other morphotypes. There is also little known about differences in the toxin production in various morphotypes. The suggestion of differences in toxins follows from the literature (Komárek in press: table 10), but a comparison is possible only with respect to the phenotypically distinguishable types, and based on many strains developed under various environmental conditions. The morphology of colonies (or the occurrence of analogous types of colonies in different species) is not the only critical character, and differences in life cycles, special carotenoid pigments, formation of specific morphological states, and in ecology are specific and stable in cyanobacteria (Kato *et al.* 1991; Skulberg & Skulberg 1985). Overlapping dimensions of cell diameters do not justify uniting the species. The definition of species (conventional?) in *Microcystis* is therefore still unclear, and we consider the recognition and definition of various stable morpho- and ecotypes as a better basis for future research, rather than working with the foggy jumble of various undefined populations, strains, morphotypes and names that are spread throughout the literature.

The broad account of the diversity within the genus *Microcystis* (*i.e.* the arbitrary use of names or designations by numbers, "spec.") is a reassuring, but problematic procedure, and not useful for ecologists and worthless for future detailed molecular studies. In cyanobacterial morphotypes, relationships between "classical" species and molecular differences are not always evident; in the variable *Microcystis* the phenotype "species" are difficult to recognise morphologically as well as by molecular sequencing. Proof that there is one variable genotype (and that the morphological changes appear quite accidentally) does not yet exist. The total independence of stable phenotypic (traditional interspecific) features from genotypes has not been proven and is highly improbable.

With respect to the morphospecies described in this paper, the common *M. protocystis* was described taxonomically by Crow (1923), but this species has evidently usually been identified as *M. aeruginosa* (see papers cited in Senna *et al.*, 1998). The cell size is very similar, and the colonies of *M. protocystis* correspond more or less to some stages of *M. aeruginosa*. However, as follows from the precise description of the life cycle of *M. aeruginosa* by Reynolds *et al.* (1981, figs. 3,

50), similar stages with sparsely distributed cells and diffuse slime occur in *M. aeruginosa* only "in samples of benthic populations", which "were assumed to be senescent or moribund" (*loc. cit.*, p. 427). Presumably these occur only in cold periods in the year (during "overwintering" in temperate zones), without distinct growth activity. In contrast, the typical morphological stages with sparsely located cells and diffuse slime are the main vegetative stages with numerous dividing cells found in populations of tropical *M. protocystis*. Typical "*aeruginosa*-stages" as characterized by Teiling (1941), Komárek (1958, 1991), Kondrateva (1968) and Reynolds *et al.* (1981: "status Ib to Vb") were never found in *M. protocystis*. The formation of special mucilaginous envelopes around individual cells (Fig. 13) seems to be species specific within *Microcystis*.

Microcystis protocystis had not been recorded previously in any paper from S. American reservoirs. It has evidently been neglected or considered and identified as initial or disintegrating stages of *M. aeruginosa* (less frequently as *M. flos-aquae* (Wittrock) Kirchner), with which it sometimes co-occurs. The main phenotype characters are the obligatory sparse arrangement of cells in colonies, the form of colonies and colonial mucilage. The differences between *M. protocystis* and other *Microcystis* species are comparable with all diacritical features used in *Microcystis* phenotype taxonomy.

Microcystis panniformis is morphologically similar to the temperate species *M. ichthyoblabe* Kützing, which has been dominant in numerous water bodies in central Europe in recent decades, but differs in its smaller cell size, details of colony morphology and particularly in their life cycles (compare data from Komárek, 1991; Komárek & Anagnostidis, 1998). The occurrence of typical *M. ichthyoblabe* in tropical regions has not yet been proven, and we did not find this species in numerous samples from different tropical regions (Komárek *et al.*, in press). Another similar species is the little known *M. lamelliformis* Holsinger, described insufficiently by Holsinger (1954) from Srí Lanka (see Desikachary, 1959). The type material of this species does not exist; however, we have found similar colonies that could be considered as *M. lamelliformis* in samples collected by Rott in Srí Lanka (Rott 1983). They differed from Brazilian populations, particularly in the unique shape of colonies, which was different from any stage of the life cycle of *M. panniformis*.

M. panniformis has been found several times in samples from Brazilian reservoirs. However, because of the difficulties with *Microcystis* taxonomy, old colonies with holes were usually designated as *M. aeruginosa*, even if the size of cells was smaller and the distribution of cells inside of colonies was different (Bittencourt-Oliveira, 2000). Moreover, young colonies with regularly and densely arranged cells were misinterpreted as *M. flos-aquae*, or, recently, as *M.* cf. *lamelliformis*.

Acknowledgements. The authors thank Prof. Dr Sandra M.F.O. Azevedo and Dr Patricia Domingos (Rio de Janeiro, Brazil) for the culture of *Microcystis protocystis*, isolated from the reservoir Rio Tabocas, state of Pernambuco, Brazil. They are also indebted to Prof. Dr Eugen Rott (Innsbruck, Austria) for the samples from Srí Lanka, from where *Microcystis lamelliformis* was described. The EM-studies were made in the Laboratory of Electron Microscopy of the Academy of Sciences in České Budějovice, Czech Republic (headed by Ing. Jana Nebesářová CSc. and performed by Dr Josef Šulek). We are thankful also for critical remarks of reviewers, particularly to Prof. Dr. Pierre Compère (Meise, Belgium). The study was finished in the frame of collaboration between Brazil and Czech phycological programmes, and supported by the Czech Grant Agency, project no. A6005704, by the project AS-CR no. 21-Biodiversity, and by grant no. 451858/96-4 from Conselho Nacional de Pesquisa – CNPq, Brazil.

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