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Background of the Caruaru tragedy; a case taxonomic study of toxic cyanobacteria

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With 9 figures and 3 tables in the text

Abstract: We describe phenotype characters of cyanobacterial populations, dominating in the Tabocas reservoir, State of Pernambuco, Brazil, in the period February-June 1996. Massive development of cyanoprokaryotic species in the reservoir was then responsible for cyanotoxin production into the water. As a consequence, over 50 people died after intoxication by cyanotoxins in the hemodialysis center in Caruaru, Brazil. All the dominating cyanobacterial species from Tabocas reservoir belong exclusively to the tropical, not well known species. The basic phenotype descriptions and ecological characters of *Aphanothece stratus, Romeria caruaru* (spec. nova), *Aphanocapsa* cf. *cumulus, Microcystis protocystis, M. panniformis, Chroococcidiopsis* cf. *indica* and *Aphanizomenon manguinii* are included in the article. - Two important conclusions can be drawn from our results: (i) Numerous important specific and little known cyanobacterial types occur in tropical regions, different from these ones known from temperate zones, and (ii) the "picoplanktic" populations can be formed by cells liberated from macroscopic colonies of benthic and periphytic species. Our results support also the hypothesis about the toxicity of cyanobacteria from various taxonomic groups and various biotopes, and urge further study of the natural populations from little known tropical biotopes by combined traditional and modern molecular methods.

Key words: Cyanobacteria, Cyanophytes, taxonomy, ecology, plankton, picoplankton, toxicity, tropical regions, Brazil.

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Introduction

From February 17th to May 26th, 1996, 116 of 130 patients (89%) experienced visual disturbances, nausea and vomiting following routine treatment at a hemodialysis center in Caruaru city, State of Pernambuco, Brazil. As of August 4th, fifty-five of the patients died having a common syndrome associated with liver hemorrhage or liver failure. Microcystins (hepatotoxins produced by cyanobacteria) were detected in water from Tabocas reservoir from which the water for the town Caruaru (and for the dialysis center) was supplied, and in serum and liver tissues of case patients. The intoxication by microcystin was found to be responsible for this tragedy (SANTA CRUZ & FERNANDES 1996, JOCHIMSEN et al. 1998).

Toxins produced by cyanobacterial water blooms in eutrophized reservoirs and ponds (CARMICHAEL 1992, 1997, CODD 1995) could not be dangerous in simple contact with skin of people and animals, and can cause only allergies and minor intoxications. However, under special conditions, they can lead to death if they are accumulated in drinking water for warm-blooded animals, and then, of course, become to be dangerous for men in water supplies for human communities, as was just the case of Caruaru (CARMICHAEL & FALCONER 1993, CODD 1995, and others). For this reason, many projects, symposia and studies have been focused on cyanotoxins recently. The importance of this subject in Brazil follows from numerous studies concerning the trophic level and development of cyanobacterial water-blooms in Brazilian reservoirs (AZEVEDO et al. 1994, HUSZAR et al. 1998, NASCIMENTO et al. 1998, DOMINGOS et al. 1999, SILVA et al. 2001 etc.). The toxicity among almost all ecological groups of cyanobacteria was already detected (CODD 1995). However, the data about facultative (or permanent) toxicity or non-toxicity in various populations and species are still not satisfactory. The poor knowledge of cyanobacterial diversity in nature is complicating factor in this research.

The wide diversity and ecological specificities of cyanobacteria were proved. In spite of morphological simplicity and similarity, the cyanoprokaryotic phenotype taxonomic units ("species") are not ubiquitous and do not have cosmopolitan distribution; the cyanoprokaryotic diversity from tropical regions is particularly insufficiently known. The correct identification of field samples from various biotopes is difficult and its importance underestimated, and lot of various concepts and arbitrary used names cause the misleading ideas about ecology, ecophysiology and distribution of various cyanobacterial types.

Therefore, the taxonomic evaluation of cyanobacterial samples and strains from Tabocas reservoir was complicated. Moreover, the samples were collected and cultures were isolated with a certain delay, so that they do not correspond exactly with cyanobacterial community from the time of tragedy. The *Microcystis*- and *Aphanizomenon*-types were recognized in the first samples but their identification on the species level was difficult in both cases. Toxicity of the morphologically similar *Microcystis flos-aquae* (from temperate zone) is not known with certainty; the best known *Aphanizomenon*-species *(A. flos-aquae)* is ecologically and geographically delimited in temperate zones, and was never found with certainty in tropical countries. Interesting strains containing the "picoplanktic" solitary cells (about 1 µm in diameter) and mucilaginous colonies with cells of the similar type were isolated, but their identification with known species was impossible.

In spite of problems with evaluation of taxa within cyanobacteria, the identification of field samples according to traditional phenotype characters is important for the toxicological studies. The cytomorphological evaluation of cyanoprokaryotic taxa, found in samples and strains from Tabocas reservoir near Caruaru, is therefore the content of this article.

Methods

All natural samples from the Tabocas reservoir were collected in the sub-surface. In 1996, the sampling was done from March until May. In 1997, a scientific project aims to study limnological characteristic of the reservoir, and relationship with cyanobacteria dominance was started (Nascimento et al. 1998); since then phytoplankton samples were collected fortnightly.

Tabocas reservoir is located approximately 40 km from Caruaru (08°06'15"S; 36°13'23"W). The dam was built in 1969 to retain water for irrigation and drinking purposes. Its direct catchment area covers a surface of 384 km^2 and the maximum capacity is 13.6×10^6 m^3 (theoretical volume). At this optimal capacity, the maximum depth is 23 m in front of the dam. A dry season lasts from August to February in this region, with precipitation average of 407 mm. This region is semi-arid with temperatures over 18° year-round.

The analyses of phytoplankton before 1996 performed by Water Treatment Company from Pernambuco State (COMPESA) showed that cyanobacteria were dominant in the reservoir since 1990 with *Microcystis, Anabaena* and *Cylindrospermopsis* identified as the most common genera (Nascimento et al. 1998).

The following samples were studied by light microscopy:

(1) Nine natural samples collected in March and May 1996, preserved by Lugol solution. Ten additional samples, containing planktic populations from years 1997 and 1998 were available.

(2) Cultures of natural samples collected during 1996, containing mixtures of several types (species).

(3) 41 more or less monospecific (but not bacteria-free) strains, isolated from natural samples.

According to the observations of first natural samples under optical microscope, three basic cyanoprokaryotic types occurred abundantly: one filamentous *Aphanizomenon*-species, two *Microcystis*-species and small-celled morphotypes with cells about 1 µm in diameter. Unfortunately, only one type of *Microcystis* and "picoplanktic" strains were isolated in culture. Cultivation was performed in liquid cultures using ASM-1 medium (Gorham et al. 1964), at 23 °C with a photoperiod of 12h and 45 μmol $photon.m^{-2}.s^{-1}$. The isolated strains were studied by electron microscopy as describes in KOMÁREK & CEPÁK (1998).

From the small-celled colonial strains containing three different morphotypes (colonies of the *Aphanothece*-, *Aphanocapsa*- and *Romeria*-types) two morphological deviations with rod-like cells were selected for sequencing to prove their taxonomic relationships. For comparison, one phenotypically distinctly different strain of *Chroococcidiopsis* was used. For 16S rDNA sequencing, cultures of monospecific isolates were harvested by centrifugation and genomic DNA was isolated by phenol extraction, chlorophorm extraction and sodium acetate/ ethanol precipitation. The almost complete 16S rDNA from the genomic DNA was amplified by PCR by *Pfu* DNA polymerase using primers corresponding to nucleotides 8–27 of the RNA-like strand and 1458–1437 of the complementary strand of *Synechococcus* PCC 67301 (TOMIOKA & SUGIURA 1983). The PCR product was directly cloned into pGATA positive selection vector (Takara) and trasformed into *E. coli* strain DH5α. Sequencing of plasmid DNA was carried on ABI PRISM 310 sequencer (Perkin-Elmer). Sequence comparisons were made with the help of the RDP database (MAIDAK et al. 2000).

Results and discussion

The samples from the Tabocas reservoir contained rich community of planktic microflora, characteristic for freshwater tropical, eutrophic water bodies, in which numerous coccal green algae, mainly from the genera *Ankistrodesmus, Coelastrum, Monoraphidium, Pediastrum, Scenedesmus, Tetraedron*, etc. were commonly present, accompanied by several Diatom species (e.g., from the genus *Nitzschia*). Cyanobacteria were another dominant group in phytoplankton. We do not have the data about abundancy of various species from the critical time of the Caruaru tragedy, but the cyanobacteria were commonly present in later samples, and their tendency to form water blooms was evident.

Seventeen cyanoprokaryotic morphotypes ("species" in traditional sense) were recognized according to the phenotype features (Table 1), from which six appeared in higher biomass: *Aphanocapsa* cf. *cumulus, Aphanothece* cf. *stratus, Leptolyngbya* sp., *Microcystis protocystis, M. panniformis* and *Pseudanabaena* cf. *galeata.* The tropical *Aphanizomenon manguinii* was condominant among water bloom forming species. Later, characteristical strain of *Synechococcus* sp. appeared in cultures, which could be, however, a contaminant.

The common presence of two picoplanktic cyanobacteria (with spherical and rod-like cells) was remarkable. However, cultivation of strains indicated that these solitary cells were liberated from massive benthic mucilaginous colonies, corresponding morphologically well to the genera *Aphanothece* and, possibly, *Aphanocapsa*, namely to *Aphanothece stratus* and *Aphanocapsa cumulus* recently

Table List of cyanoprokaryotic types in the Tabocas reservoir (collected in 1996).

Aphanocapsa cumulus KOM. et CRONB.*
picoplanktic small spheres, less than 1 µm in diam.*
Aphanothece cf. *stratus* KOM. et CRONB.*
picoplanktic rods (liberated from previous species)*
Aphanizomenon manguinii BOURR. forma*
Chroococcidiopsis cf. *indica* DESIK.*
Jaaginema sp.
Leptolyngbya sp.
Microcystis panniformis KOM. et al.*
Microcystis protocystis CROW*
Oscillatoria - trichome fragments
Phormidium sp.
Pseudanabaena cf. *galeata* BÖCHER*
Pseudanabaena sp.
Rhabdogloea cf. *subtropica* HIND.
Romeria caruaru spec. nova*
Synechococcus sp. (long, cylindrical cells)

* = with comments in our text

discovered by KOMÁREK & CRONBERG (2001) from Botswana, southern Africa. The name of *Aphanocapsa cumulus* was used originally for the identification of all picoplanktic specimens from Tabocas reservoir (comp. DOMINGOS et al. 1999). Picoplanktic cyanobacteria are usually considered to be unicellular types of the genera *Cyanobium, Synechococcus* and *Synechocystis* (KOMÁREK 1999). Typical picoplanktic species from these genera surely exist, but obviously some distinct freshwater picoplankters from Tabocas reservoir were liberated cells from colonies (and regular growth stages) of benthic and periphytic cyanobacteria living primarily in mucilaginous colonies.

Comments to studied species

(1) "Picoplanktic species" (*Aphanothece stratus, Aphanocapsa* cf. *cumulus, Romeria caruaru* spec. nova)

In phytoplankton samples (unfiltered water) two types of picoplanktic cells were found: fine rods with cells 0.8–1.5 × 0.4–0.6 µm, and spheres about 0.5–1.0 µm in diameter. Similar cells were found in mucilaginous colonies from benthic samples. The solitary cells corresponded to the genera *Cyanobium* and *Synechocystis* respectively, and were originally identified, together with benthic colonies, by KOMÁREK [in litt.] as "*Aphanocapsa cumulus* + solitary living spherical cells". This designation was used also in the first study about the Caruaru picoplanktic cyanobacteria (DOMINGOS et al. 1999).

It was recognised, that in cultures the most common clones belonged to the "*Cyanobium*-type" with very short rod-like to oval cells. This type occurred frequently in five natural samples and was isolated in 12 subcultures. Solitary cells were rarely agglomerated into small mucilaginous clusters with several, loosely dispersed cells, enveloped by fine, colorless and diffuse slime. In older cultures (especially at the stationary phase of growth), the fine macroscopic, mucilaginous colonies of irregular shape, to 2 cm in diameter, appeared on the bottom of cultivation vessels, resembling the colonies of *Aphanothece* (subg. *Anathece*) *stratus*. The size, morphology and ultrastructure of cells were the same in solitary cells and within colonies (Fig. 1).

Apparently, the solitary picoplanktic *"Cyanobium"* cells (rod-like) and colonial "*Aphanothece*-type" represent the same genotype with a complicated life cycle (Fig. 2), in which both morphotypes, unicellular and colonial, can regularly occur dependent on the environmental factors. As the morphologically and ecologically almost identical species is *Aphanothece status*, recently described from southern Africa, Botswana, with rod-like cells 0.6–2.0 × 0.5–0.8 µm, we designate this species by this name. The corresponding strain is in culture collection (CCALA, Třeboň). Several similar, very fine species known mainly from temperate zone (*A. minutissima, A. bachmannii, A. clathrata*) live exclusively planktic in small colonies in mesotrophic lakes and ponds, the benthic and occasionally planktic *A. nebulosa* is known only from cold and oligotrophic northern lakes of Scandinavia. *Aphanothece stratus* is evidently a tropical, colonial species, living benthic in shallow reservoirs, in which solitary *Cyanobium*-like cells can release and live periodically picoplanktic in tropical water bodies. The population from Tabocas reservoir was studied in 12 subcultures.

Cytomorphologically slightly different species from *Aphanothece stratus*, forming short, wavy and irregular rows (filamentous formations) of elongated, slightly coiled cells, was found in our picoplanktic cultures (Fig. 3). This strain has very similar internal structure of cells like *Aphanothece* (Fig. 4), and it is different only a little by cell size, but distinctly by life cycle from *Aphanothece* cf. *stratus* (Fig. 2). Its solitary cells were hardly distinguishable from liberated picoplanktic unicells of *Aphanothece* cf. *stratus*. The possible genetic identity with *Aphanothece* was therefore also possible. The partial 16S rDNA sequencing indicated that these strains are really related, but not identical (Table 2), with the difference corresponding more or less with that between other cyanobacterial morphotypes (often considered as traditional species and/or genera). Therefore, the morphotype forming pseudofilamentous formations in the life cycle is described as a new species, *Romeria caruaru* spec. nova (Figs 3–4). However, both strains clearly belong to the *Prochlorococcus marinus*/marine *Synechococcus* cluster ("branch A" by WILMOTTE 1994).

***Romeria caruaru* spec. nova**

Diagnosis:

Cellulae solitariae vel in trichoma curta; trichoma libere natantia, brevissima, 1–4(12)-cellularia, contorta, evaginata, ad dissepimenta paucim constricta, solitaria vel in aggregationibus mucilaginosis libere implexa; cellulae cylindricae, 1–12,8 × 0.7–1.0 µm, apicibus ro-

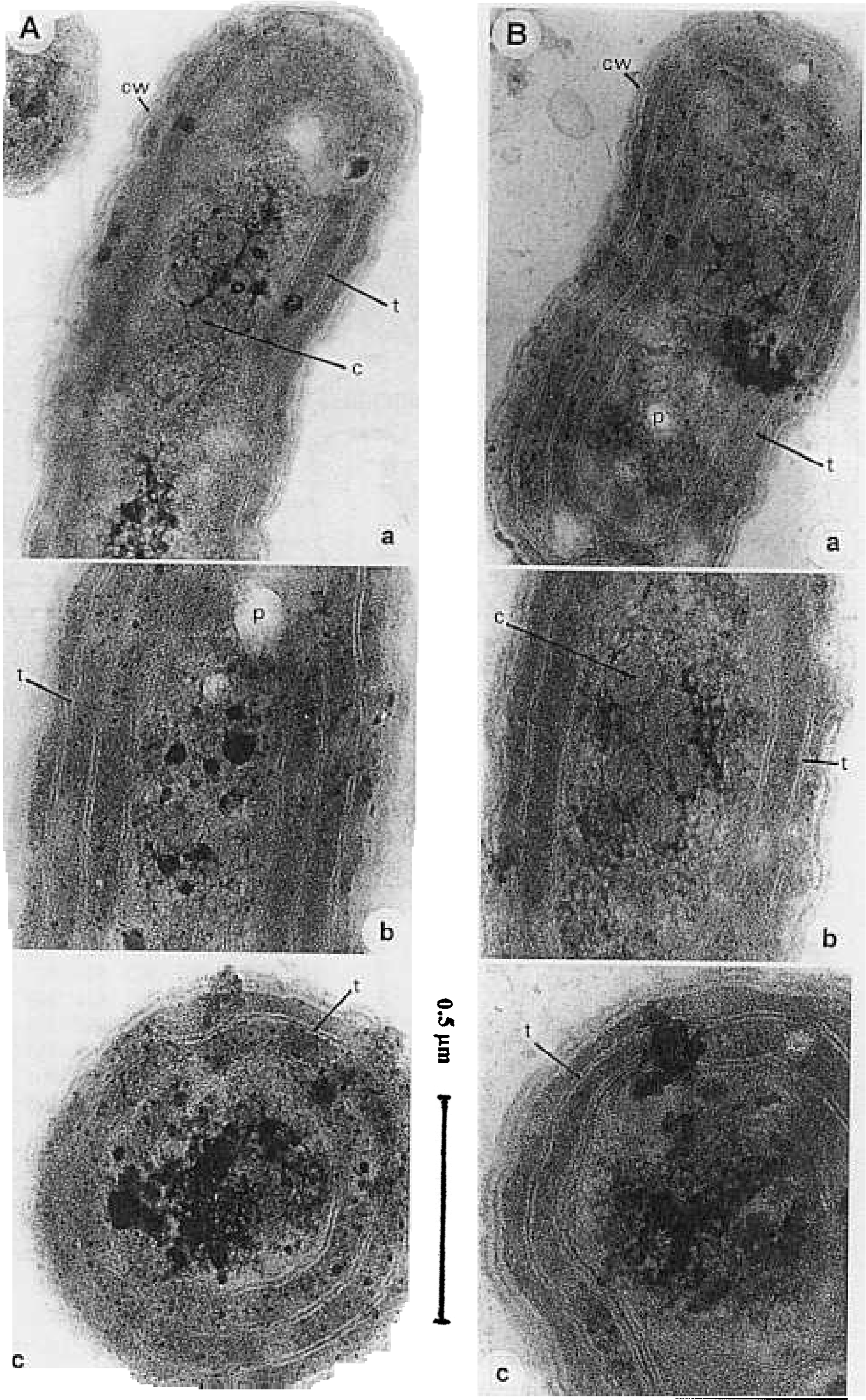

Fig. 1 Fine sections of picoplanktic *Cyanobium*-like cells (**A**) and cells from colonies of *Aphanothece stratus* (**B**). Explantations: **t** = thylakoids, **cw** = cell wall, **c** = carboxysomes, **p** = polyphosphates.

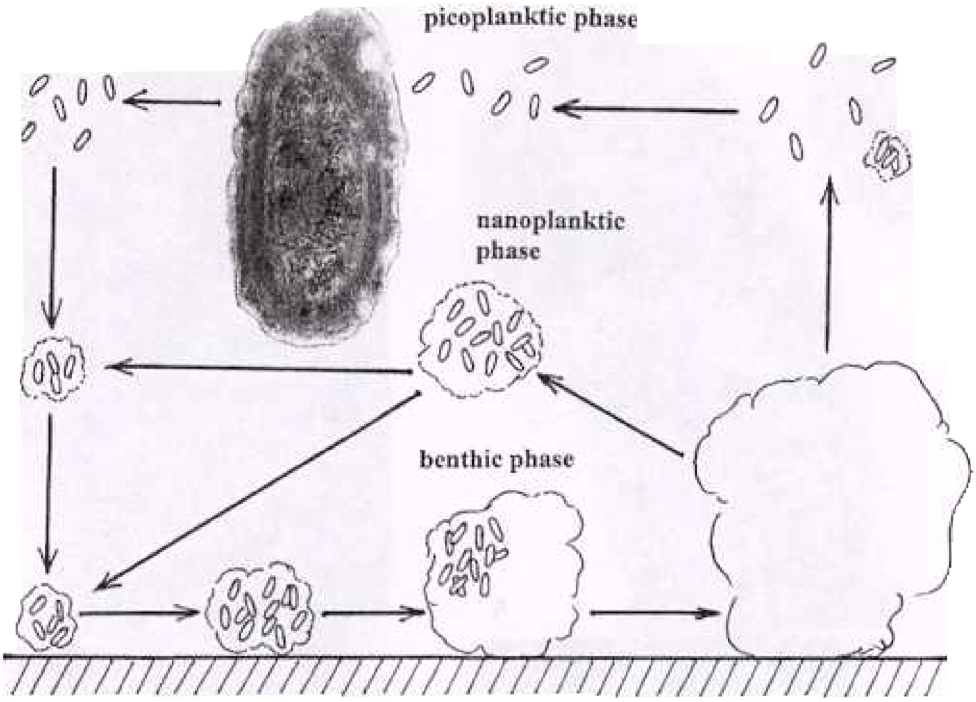

Fig. 2 Scheme of the life cycle of *Aphanothece stratus*. - Orig.

Table 2. Pairwise similarities between 16S rDNA sequence (818 bp compared) of *Aphanothece stratus, Romerica caruaru* and related cyanobacterial strains.

Strain		Pairwise similarity							
		2	3	4	5	6	7	8	9
Aphanothece stratus	1	.998	.998	.985	.967	.926	.922	.907	.786
Romeria caruaru	2		1.00	.974	.965	.915	.914	.893	.778
Synechococcus PCC 6307	3			.974	.965	.915	.914	.893	.778
Synechococcus sp. PCC 7001	4				.963	.911	.907	.897	.770
Prochlorococcus marinus PCC 9511	5					.904	.903	.887	.778
Synechococcus sp. PCC 7942	6						.997	.901	.784
Synechococcus sp. PCC 6301	7							.898	.777
Synechococcus sp. PCC 7335	8								.766
Escherichia coli	9								

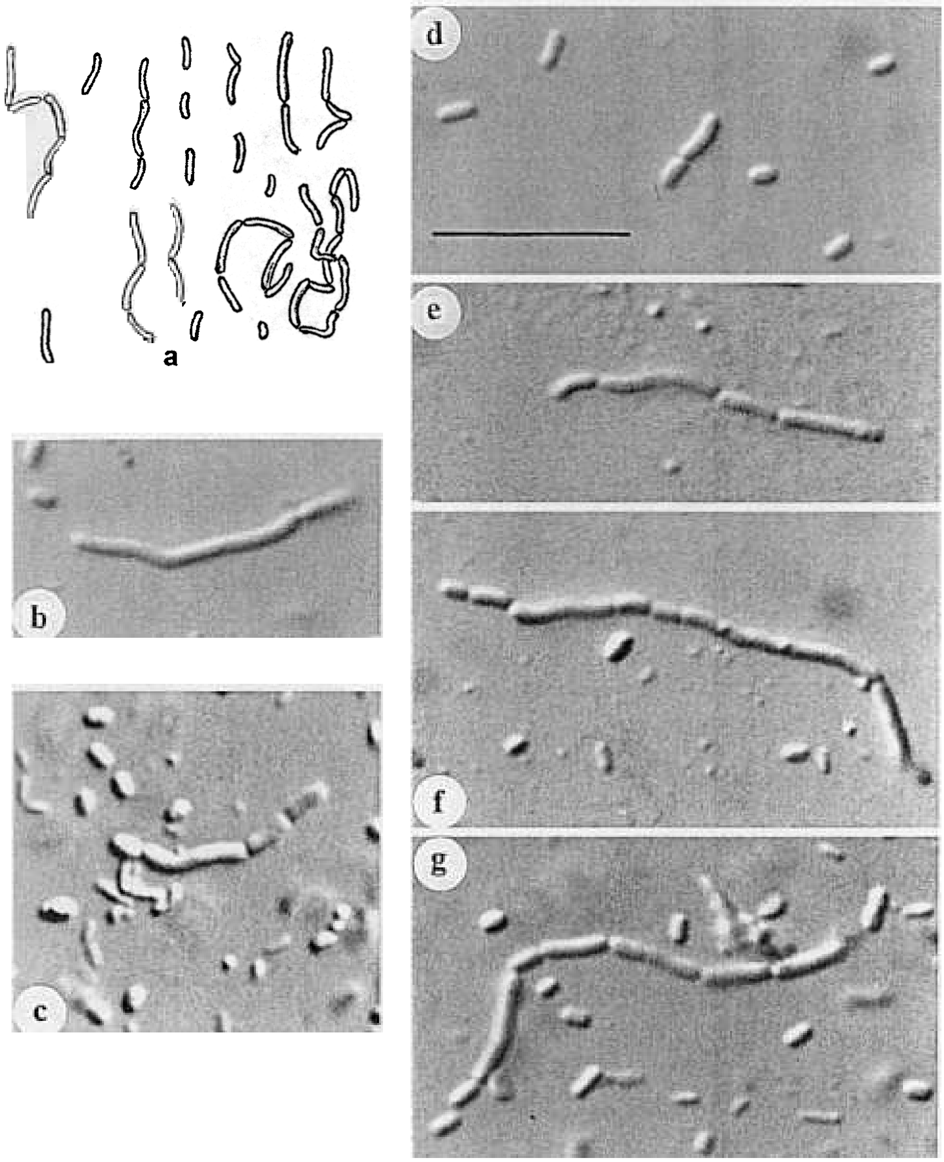

Fig. 3 *Romeria caruaru* from cultures from Tabocas reservoir; solitary cells and short, irregular trichomes; **b-g** = Nomarski contrast. [Bar = 10 μm.]

tundatis, contentu pallide aerugineo, homogeneo, irregulariter conjuctae; mucilago homogenea, incolora, diffluens. Thylakoidae (2)3(4), parietales.
Habitatio: Planktice in stagno eutrophico artificiali prope oppido Caruaru, Brasilia orientalis (locus classicus).
Typus: specimen no. BRNM-HY 1201; icona typica: figura nostra 3.

Another cyanobacterium with similar size of cells, which were, however, of spherical shape and about 1 μm in diameter, was abundant in other four samples

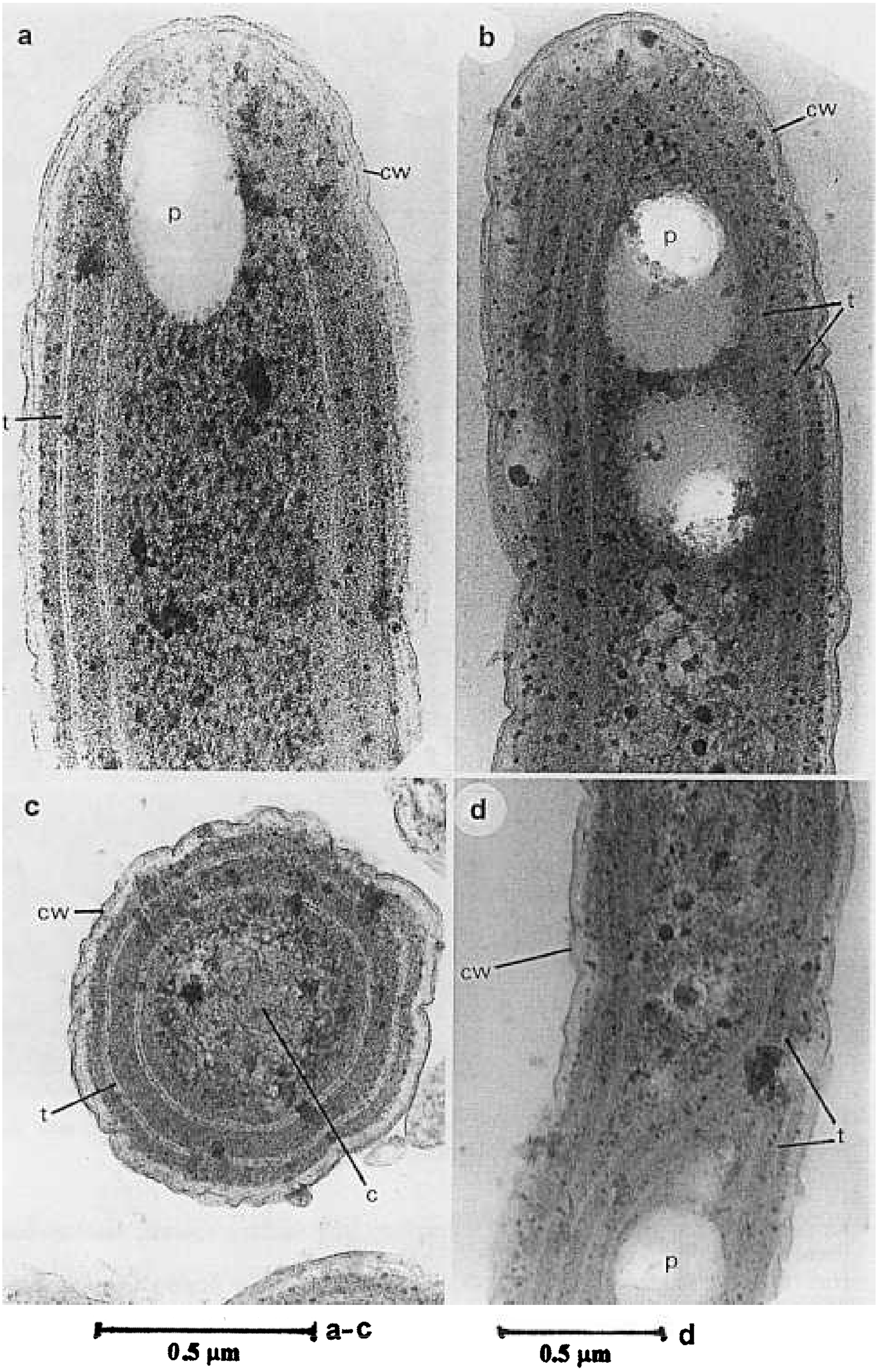

Fig. 4 *Romeria caruaru*, fine sections of cells from culture. Explanations as in Fig. 1.

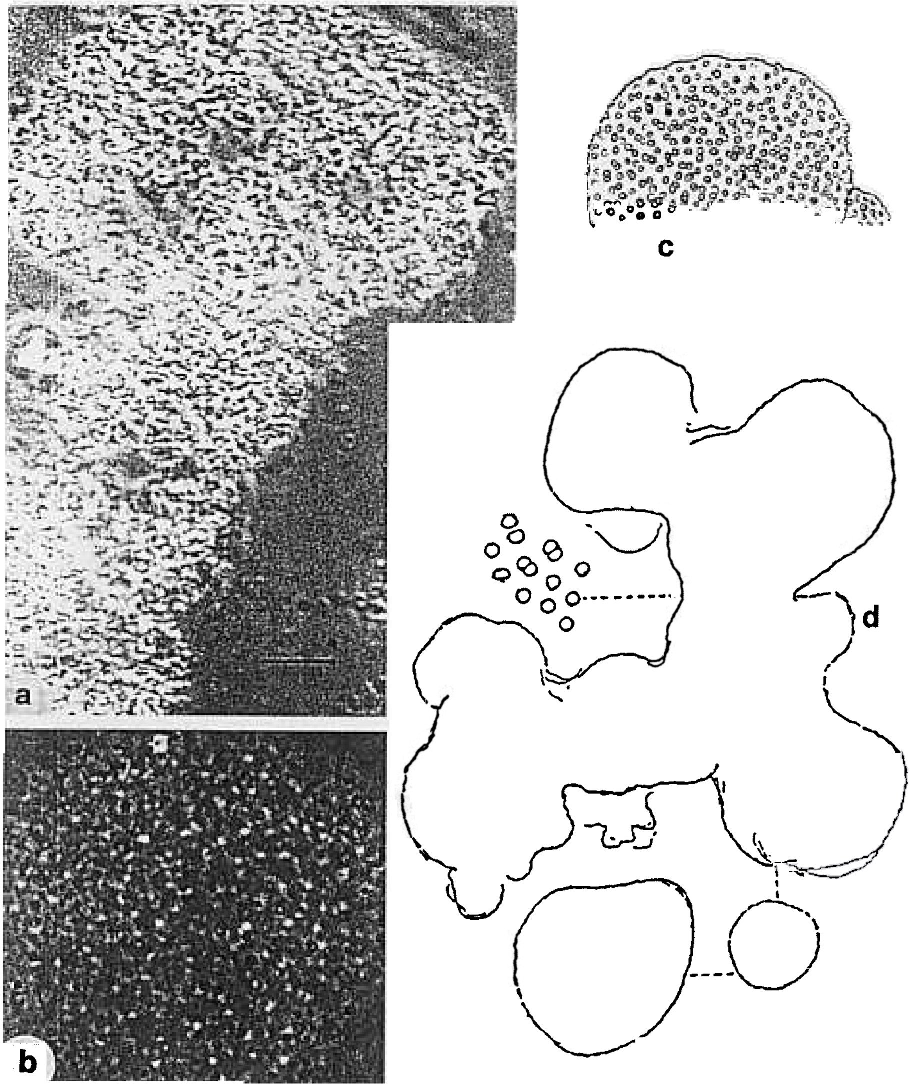

Fig. 5 *Aphanocapsa* cf. *cumulus*; from KOMÁREK & CRONBERG (2001).

and in two cultures (Fig. 5). This type formed large and irregular macroscopic colonies with very densely aggregated cells in colorless slime, up to more than 2 cm in diameter. The colonies were dark green, yellowish green or blue-green. This type with spherical cells resembles morphologically closely another species described recently (*Aphanocapsa cumulus* KOMÁREK & CRONBERG 2001) from benthos of swampy littoral of ponds in Botswana, southern Africa. Spherical picoplanktic solitary cells were found together with *Aphanocapsa* colonies in two cultures, from which follows, that they also liberated from *Aphanocapsa* colonies.

To summarize, the both rod-like and spherical picoplanktic cells from Tabocas reservoir do not represent themselves special species, but they are only the stages of benthic, colonial cyanobacteria. The solitary "*Cyanobium*-like" or "*Synechocystis*-like" cells can be released occasionally into the water from some benthic, cyanobacterial, colonial species, and have in this case a function of picoplanktic cyanobacteria (Fig. 2). From our two types, one with spherical cells resembles the cells of the colonial *Aphanocapsa cumulus* known originally from Botswana. The second type, corresponding to the *Aphanothece* (subg. *Anathece*) and joined in the paper of DOMINGOS et al. (1999) with *Aphanocapsa cumulus*, corresponds phenotypically and by morphology of colonies to another species, *Aphanothece stratus*. The genotype resemblance of both mentioned types must be proved in future. The newly defined *Romeria caruaru* has rather the nanoplanktic type of life, and must be defined as a special phenotype species different distinctly from *Aphanothece*, with similar rod-like cells, which join, however, into pseudofilamentous formations.

There is little known about toxicity of picoplanktic, nanoplanktic and small-celled colonial cyanobacteria. Distinct cytotoxic and immunotoxic effects in several picoplanktic strains were detected by BLÁHA & MARŠÁLEK (1999). The potential toxin production of *Aphanocapsa* cf. *cumulus* from Tabocas reservoir is discussed by DOMINGOS et al. (1999). The responsibility of common fine *Aphanocapsa, Aphanothece* and *Romeria* populations for intoxication of Caruaru water is therefore very probable.

(2) *Microcystis protocystis* and *M. panniformis*

Two *Microcystis* morphotypes were found in natural samples from Caruaru reservoir, distinctly different from populations known from temperate zones. Morphology of the genus *Microcystis* is very simple with wide infraspecific variation (KONDRATEVA 1968, KATO et al. 1991), and the up to date molecular analyses indicated genotype uniformity within this genus, so that species identification is rather difficult. However, characteristic form of colonies, cell size and life cycle are different and recognisable in numerous natural *Microcystis* populations, and used for their identification. Their phenotype taxonomy is important for investigations of toxic effect of various *Microcystis* populations and can not be underestimated.

The Brazilian *Microcystis* populations are intensely studied during past years. The specimens from Caruaru were found identical with two species, recently characterized by KOMÁREK et al. (2001) from São Paulo State, and identified as *M. protocystis* CROW 1923 (Fig. 6) and *M. panniformis* KOM. et al. 2001 (Fig. 7). Both these species are probably common pantropical species, forming often heavy water blooms. Particularly *M. protocystis* is known from reservoirs of the whole tropical zone. There are indications of a strong toxicity of both mentioned species strains (M.T.P. AZEVEDO, in litt.). Both species deserve therefore further study for their ecological and sanitary importance.

The cell structure of both species *M. protocystis* and *M. panniformis* was

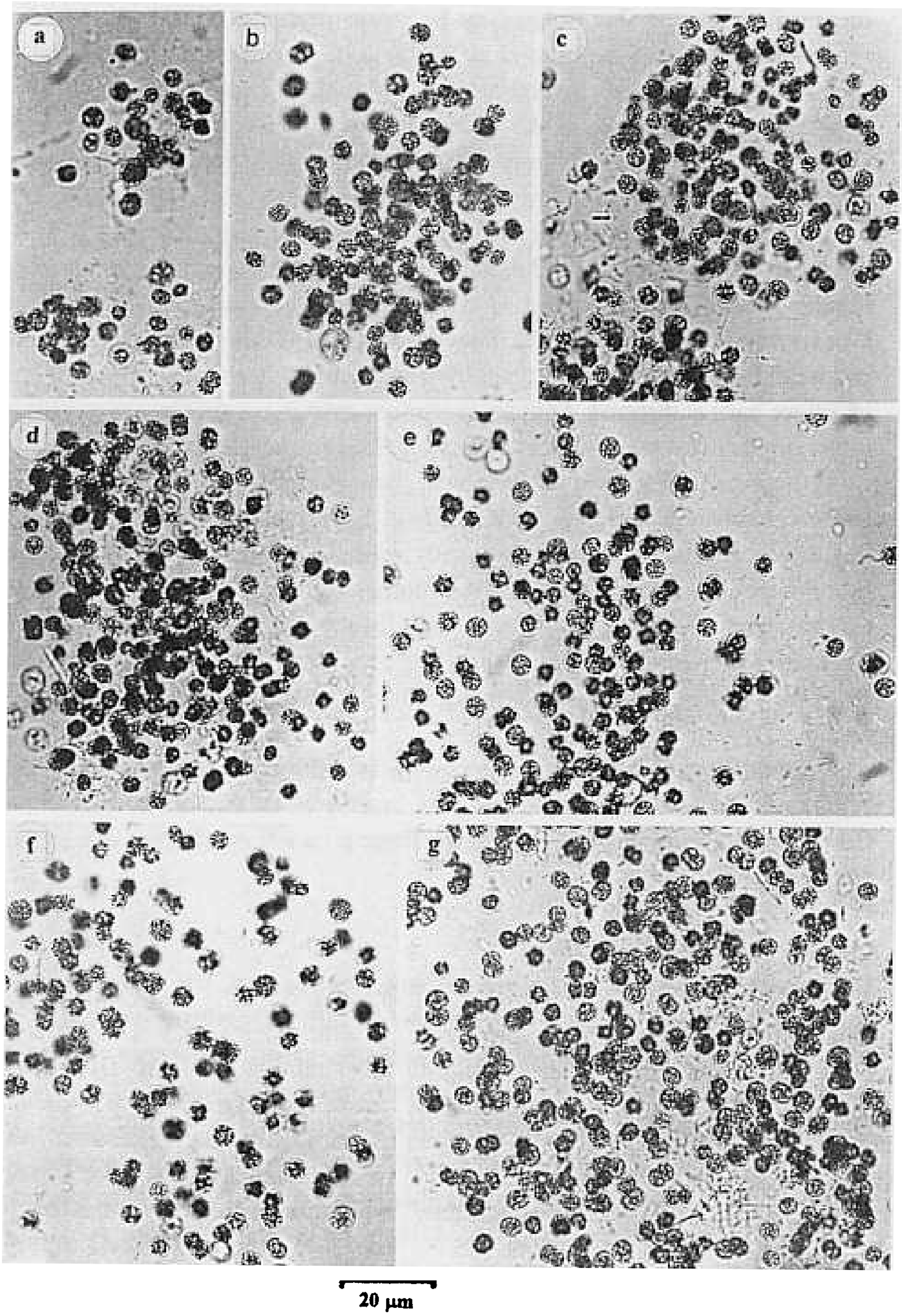

Fig. 6 *Microcystis protocystis*; different form of colonies from field samples (Tabocas reservoir).

investigated from strains isolated by P. DOMINGOS and M.T.P. AZEVEDO respectively (comp. in KOMÁREK et al. 2001). Both species were surely found and mentioned in literature several times, but probably misinterpreted with *M. aeruginosa* (young stages of *M. protocystis* and *M. aeruginosa* are particularly similar). *M. panniformis* with smaller and densely agglomerated cells was usually designated as *M. flos-aquae* (this species is similar, but with different life cycle, with slightly larger cells, and occurs probably only in temperate zones). However, the most similar is toxic *M. ichthyoblable*, known mainly from temperate zones. Similar species is also tropical *M. lamelliformis*, described from Srí Lanka by HOLSINGER in 1954 (ROTT 1983). The type material of this species does not exist, and we did not found identical types in recent samples from lakes from Srí Lanka, which we have at disposal. Another specimens exist there, that better correspond to the original HOLSINGER's description of *M. lamelliformis*.

M. protocystis (Fig. 6) is characteristic particularly by the obligatory sparsely and irregularly arranged cells in very diffuse, homogeneous, colorless slime over the whole vegetation period, and with cells 3.5–7.2 μm in diameter (under suboptimal culture conditions are the mother cells up to 8.5 μm in diameter). Colonies are irregular in outline and easily disintegrate to small clusters of cells. Clathrate colonies with densely aggregated cells were never observed. For ultrastructure of the strain isolated from Tabocas reservoir see KOMÁREK et al. (2001).

M. panniformis (Fig. 7) has more complicated life cycle (see KOMÁREK et al. 2001) and evenly, densely arranged cells, mainly in surface layers of colonies. Cells are 2.8–4.8 μm in diameter, the mucilage does not overlap the cell agglomerations.

(3) *Chroococcidiopsis* cf. *indica*

Our population of *Chroococcidiopsis* is not planktic and was not detected in natural samples, but it appeared commonly in cultures, and was isolated in one monospecific strain (Fig. 8). The toxicity of this strain is unknown. The strain corresponded phenotypically and ecologically mostly to *Chroococcidiopsis indica* DESIK. 1959, described from India, but the proof of identity is problematic. The 16S rDNA sequencing indicated close genotype relationship to *Chroococcidiopsis* sp. PCC 7203 (Table 3). It corresponds phenotypically also to the type-species of this genus, *C. thermalis* (see KOMÁREK & HINDÁK 1975, strain GREIFSWALD/149) with different ecology, and commonly to the genus *Chroococcidiopsis*. *Chroococcidiopsis* strains are related also to coccoid, non-baeocytic *Cyanothece*, to other baeocytic species, but also to cluster of nostocalean strains, although the mentioned coccoid morphospecies are aheterocytous (Table 3). Interestingly, all these types have similar type of thylakoidal patterns within cells. Their phenotype identification on the modern generic level (KOMÁREK & ANAGNOSTIDIS 1998) is without doubts and fully in coincidence with up to date molecular sequencing.

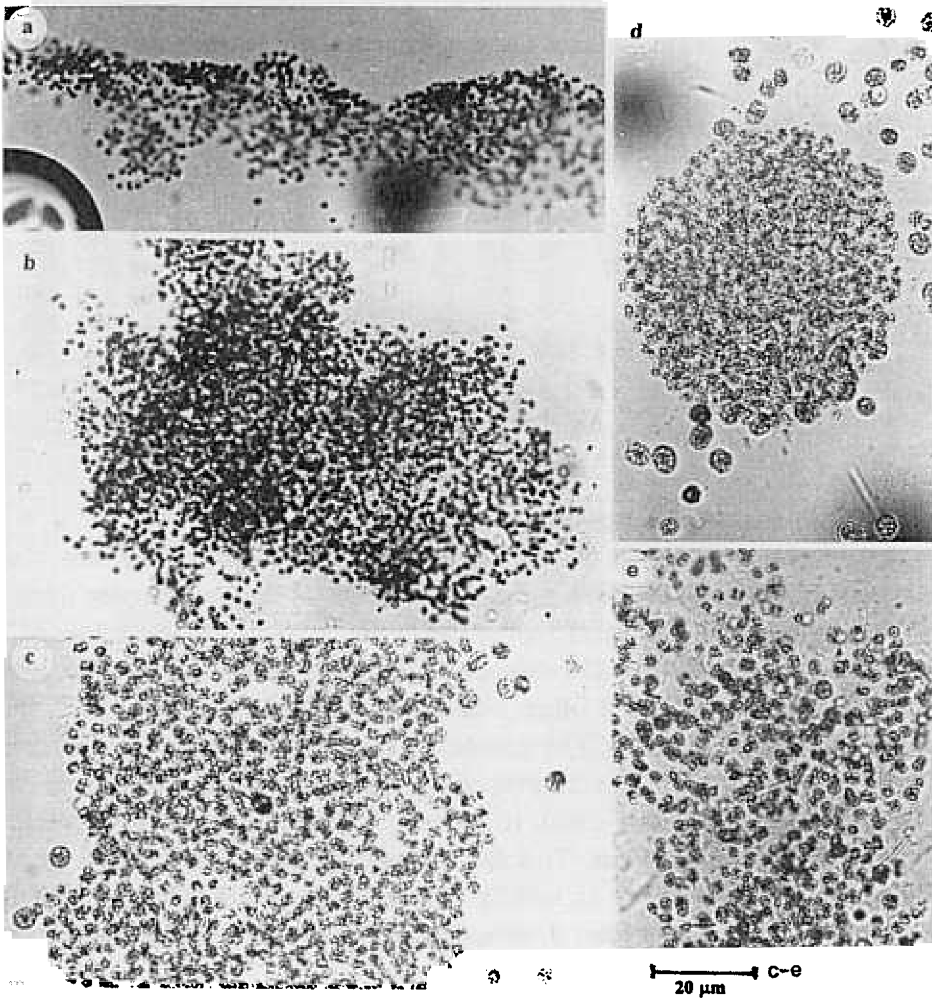

Fig. 7 *Microcystis panniformis*; **a-b** - colonies from São Paulo State; c-e - colonies from Tabocas reservoir (in Figs **c** and **d** are solitary, large cells of *Microcystis protocystis*).

Description of population from our isolated strain (Fig. 8): Cells solitary or in microscopic to macroscopic irregular aggregations, irregular spheroidal to spherical, 1.8–7.2 µm in diameter, enveloped by thick, firm, colorless sheaths tightly enveloping cells and clusters of young cells. Cell content slightly granular, olive-green. Thylakoids coiled, not fasciculated, but densely clustered and irregularly spread over the cell volume, mainly near the periphery. Reproduction by irregular cell division in various planes, which sometimes repeat within the sheath (baeocytes). The sheaths develop around the baeocytes already during the division and become thicker during growth. They are composed from two main distinct layers in old cells: from the inner thicker of layered and filamentous material, and outer homogeneous, electron dense. Daughter cells liberate from sheaths after their rupture.

Table 3. Pairwise similarities between 16S rDNA sequence (1389 bp compared) of *Chroococcidiopsis* cf. *indica* and most related filamentous, particularly heterocytous cyanobacterial strains.

Strain		Pairwise similarity 2	3	4	5	6	7	8	9	10	11
Chroococcidiopsis* cf. *indica	1	.983	.905	.904	.897	.896	.898	.894	.891	.890	.755
Chroococcidiopsis sp. PCC 7203	2		.908	.903	.899	.897	.898	.894	.892	.888	.750
Microcoleus sp. PCC 7420	3			.909	.909	.898	.907	.903	.901	.917	.761
Nostoc muscorum PCC 7120	4				.968	.946	.950	.949	.899	.905	.773
Cylindrospermum sp. PCC 7417	5					.949	.952	.948	.899	.907	.770
Anabaena cylindrica PCC 7122	6						.946	.940	.907	.909	.760
Nodularia BCNOD 9427	7							.939	.897	.897	.764
Nostoc punctiforme PCC 73102	8								.892	.893	.756
Chamaesiphon subglobosus PCC 7430	9									.900	.765
Trichodesmium contortum	10										.744
Escherichia coli	11										

(4) *Aphanizomenon manguinii*

Aphanizomenon manguinii BOURR. in BOURR. et MANGUIN 1952 was a condominant, water-bloom forming cyanobacterium in natural samples from the Tabocas reservoir. This characteristic freshwater or slightly brackish species was originally described from Guadeloupe (BOURRELLY & MANGUIN 1952), now is known only from several other localities in tropical America (Brazil, Cuba, Mexico; KOMÁREK 1984). The population from Tabocas reservoir had several morphological specificities in comparison with other populations (particularly morphology of trichome ends). It grows in solitary trichomes and sometimes forms slight water blooms. This species was not yet isolated in culture, and nothing is known about its toxicity, ultrastructure and detailed ecology. It is difficult to estimate, whether *A. manguinii* participated to the toxin production in Tabocas reservoir.

A manguinii belongs to few tropical *Aphanizomenon*-species living in solitary trichomes with terminal cells morphologically different from vegetative cells and rounded at the apex, but not intensely elongated. The majority of *Aphanizomenon* species has delimited areas of distribution in dependence on the ecological demands. It concerns also the best known *A. flos-aquae*, which grows in fascicles and its distribution is restricted to temperate zones.

Description of our population (Fig. 9): Trichomes solitary, free floating, almost straight or slightly irregularly curved, in the middle 5–5.6 μm wide, slightly constricted at cross walls, at the ends sometimes not constricted. Cells cylindrical to very slightly barrel-shaped, with pale greyish blue-green content, often with scattered, distinct granules and probably facultative aerotopes (but parts of trichomes are very pale, without any granulation), (5)6–8.5 μm long, towards ends narrower, sometimes hyaline and up to 14.5 μm long; apical cells cylindrical or slightly barrel-shaped and rounded at the end. Heterocytes solitary, intercalar,

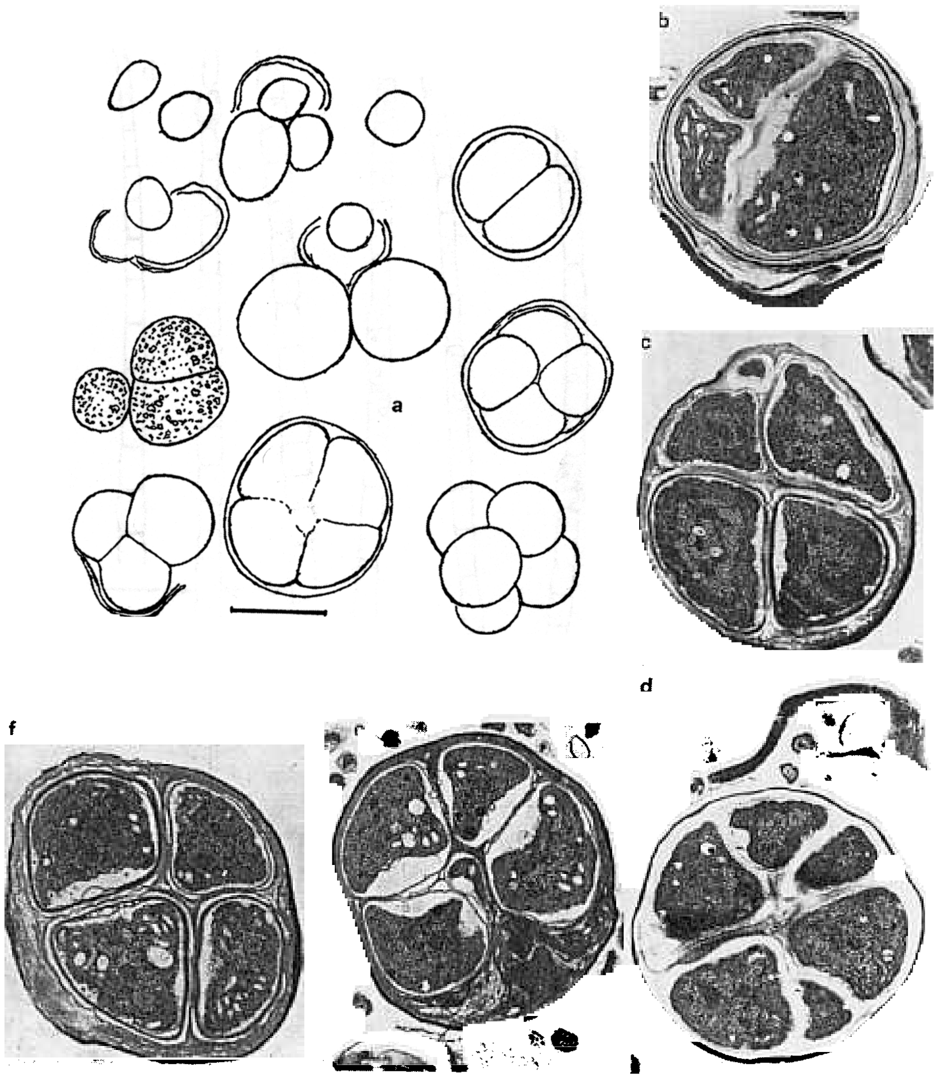

Fig. 8 Morphology and fine structure of cells of *Chroococcidiopsis* cf. *indica*, isolated from Tabocas reservoir; [bars = 5 µm.] - Orig.

usually 1–2, rarely up to 6 in one filament, more or less rounded in outline or slightly elongated, with hyaline content, 5.6–8.5 × 5.2–6.8 µm. Akinetes solitary or (less frequently) in pairs, intercalar, separated from heterocytes, oval or ellipsoidal in outline, later to almost spherical, with smooth, yellowish-brown exospore, with granular content, 11.8–15.9 µm in diameter.

(5) Accessoric species

One *Synechococcus* morphotype and species with fine filaments (especially *Leptolyngbya* sp. and *Pseudanabaena* sp.) occurred in numerous subcultures from Tabocas reservoir. They were never found in distinct populations in planktic

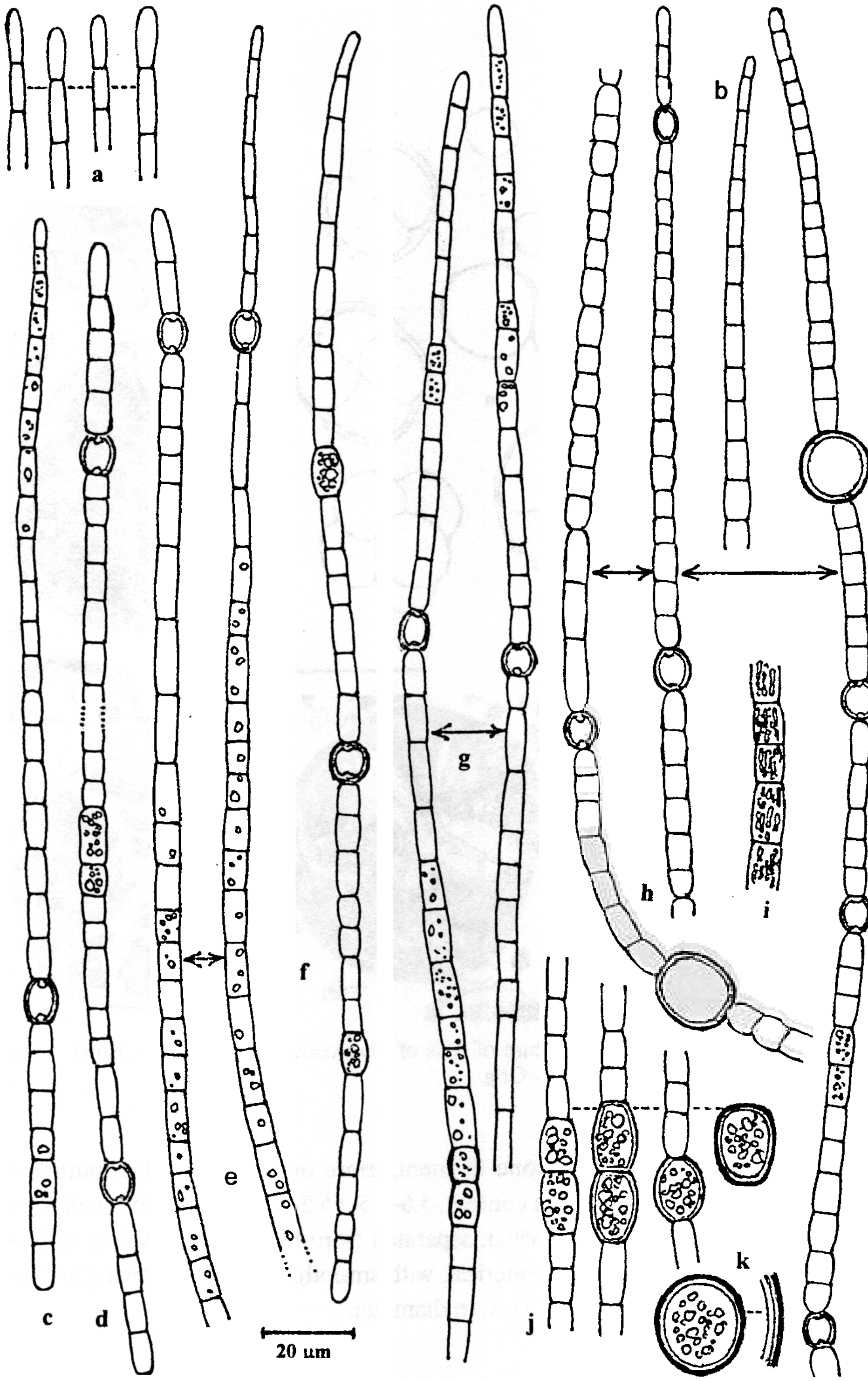

Fig. 9 *Aphanizomenon* cf. *manguinii*; filaments from field samples in Tabocas reservoir; **a-b** – details of trichome ends; **c-h** – solitary filaments, **i** – detail of cells with aerotopes; **j** – development of akinetes; **k** – spherical, ripe akinete with detail of the cell wall. – Orig.

samples from the reservoir, probably occur mostly in littoral and benthic algal communities. There is nothing known about their toxicity. However, in spite of it, they can participate on the production of cyanotoxins into the water. Their role in reservoirs is generally very little known.

Almost all populations from Tabocas reservoir were phenotypically slightly different from currently defined corresponding species, and their detailed taxonomic evaluation is open.

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