Review of the European *Microcystis*-morphospecies (Cyanoprokaryotes) from nature

Přehled evropských přírodních druhů r. *Microcystis* (Cyanoprokaryota)

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Abstract

The cyanobacterial genus *Microcystis* has been delimited according to genetic criteria (molecular sequencing by 16S rRNA), but the subgeneric classification is unclear, and the existence of traditional morphospecies is doubtful. However, populations of this genus form heavy water-blooms in eutrophic waters over the world, numerous populations produce toxins, and therefore the orientation in the natural subgeneric diversity is important. The article reviews the main morphospecies recognised in European waters with their phenotype diacritical markers. The review was elaborated with a support of the EU-grant MIDI-CHIP EVK-2 as a basis for further studies.

Introduction

The coccoid genus *Microcystis* is one of the most important cyanobacteria. The different populations cause heavy water blooms in water bodies, and therefore they are intensely studied in the last years. They develop in stagnant freshwaters with increasing eutrophication all over the world, and several species produce toxins (CARMICHAEL 1992, CARMICHAEL & FALCONER 1993, CODD 1995, WOITKE et al. 1997, CHORUS & BARTRAM 1999).

The genus *Microcystis* is characterised by colonies with irregularly agglomerated spherical cells in common, not stratified, colourless mucilage. The cells divide mainly in three planes in successive generations according to the type species (KOMÁREK & ANAGNOSTIDIS 1998, KOMÁREK 1999). Colonies are micro- up to macroscopic, they live in freshwater plankton and form morphologically different stages during the vegetation cycle (REYNOLDS & al. 1981, BITTENCOURT-OLIVEIRA 2000, KOMÁREK & al. 2002). The cells of all the species are able to produce gas vesicles gathered in aerotopes, which are always

present in vegetative stages. The gas vesicles are reversible and they control buoyancy of colonies in the water column (REYNOLDS et al.1981, FAY 1983).

Taxonomic classification of *Microcystis* is difficult. Several traditional species have been described according to morphological characters (KOMÁREK 1958, 1991), however, the variability of colonies is very wide, and the features of many populations overlap the limiting criteria (CRONBERG & KOMÁREK 1994, OTSUKA et al. 2000). It is very difficult to define the limits between traditional species. Unidentifiable colonies, atypical stages or transient forms of *Microcystis* commonly occur in planktic samples. Such taxonomically unclear colonies arise usually in the beginning and at the end of vegetation period, in the overwintering stages or in slightly or atypically developed populations. The sequencing data proved a clear genotype delimitation of the genus *Microcystis* (sensu stricto, only species with gas vesicles), but inside the genus any subgeneric units were not detected, that could be characterised as the "species" (Fig.1; CASTENHOLZ 2001). The detailed analyses yielded enormous number of populations and strains, which all differ one from another, but it is very difficult to recognize natural, well delimited clusters (Fig. 2; KONDRATEVA 1968, KATO & al. 1991).

The "species" category in cyanobacteria is now problematic. Recently, the meaning exists, that the species category is not justifiable not only in the genus *Microcystis* (OTSUKA & al. 2000, 2001), but also in other genera of cyanobacteria (CASTENHOLZ 2001). However, several characteristic *Microcystis*-morphotypes, that were usually classified as the traditional species, really exist and repeatedly occur in different regions. They can be characterised at present only conventionally as morphotypes (morphospecies), that belong to one genotype and have the similar ecology. Such traditional species with distinct phenotypic and ecophysiological features cannot be completely omitted; their identification is useful and necessary for ecological research, ekotoxicological studies, etc. They are stable also in culture. The taxonomic unification of all main morphospecies (*M. aeruginosa, M. ichthyoblabe, M. viridis, M. novacekii, M. wesenbergii;* OTSUKA et al. 2001) seems to be therefore premature, till the reasons of their physiological and morphological diversity will be explained (e.g., *M. wesenbergii* vs. *M. ichthyoblabe*; Tab. 14 –15). It is also unclear, why the classification of *Microcystis* under the rules of the Bacteriological Code should be different from any other classification (OTSUKA et al. 2001). The taxonomy is always conventional in a certain degree, and any taxonomic classification should be uniform, and as simple and practical for common use as possible.

The present paper yields therefore a review of the main traditional *Microcystis*-morphospecies, occurring repeatedly and commonly in European freshwaters (compared with similar types described from tropical and other regions). They are identifiable in typical stage (which is unique for particular morphospecies), but, of course, there occur also atypical and therefore

unidentifiable colonies in natural populations. For reliable identification of the morphotype the knowledge of whole life cycle is necessary.

Results

The following phenotype features are used to the classification of *Microcystis*-morphospecies:

- Form of colonies (with holes, flattened, lobate, composed of compact subcolonies).
- Mucilage structure (width of mucilaginous margin around colonies, delimited/diffuse).
- Diameter of cells (limits, average).
- Density and organisation of cells in colonies (distinctly in three-dimensional paketts, partly in rows, very densely and irregular, constantly scarcely).
- Pigment content (PC:PE ratio).
- Life cycles (absence/presence of certain stages, identification of atypical – dormant stages, limits of morphological variability). The examples: Figs 3,4.

The main European species are characterised in Fig. 5 and Tables 1 to12. Table 13 contains review of main tropical morphospecies. All drawings and photos are derived from KOMÁREK (1958), KOMÁREK et al. (1992) and after different authors from KOMÁREK & ANAGNOSTIDIS (1998).

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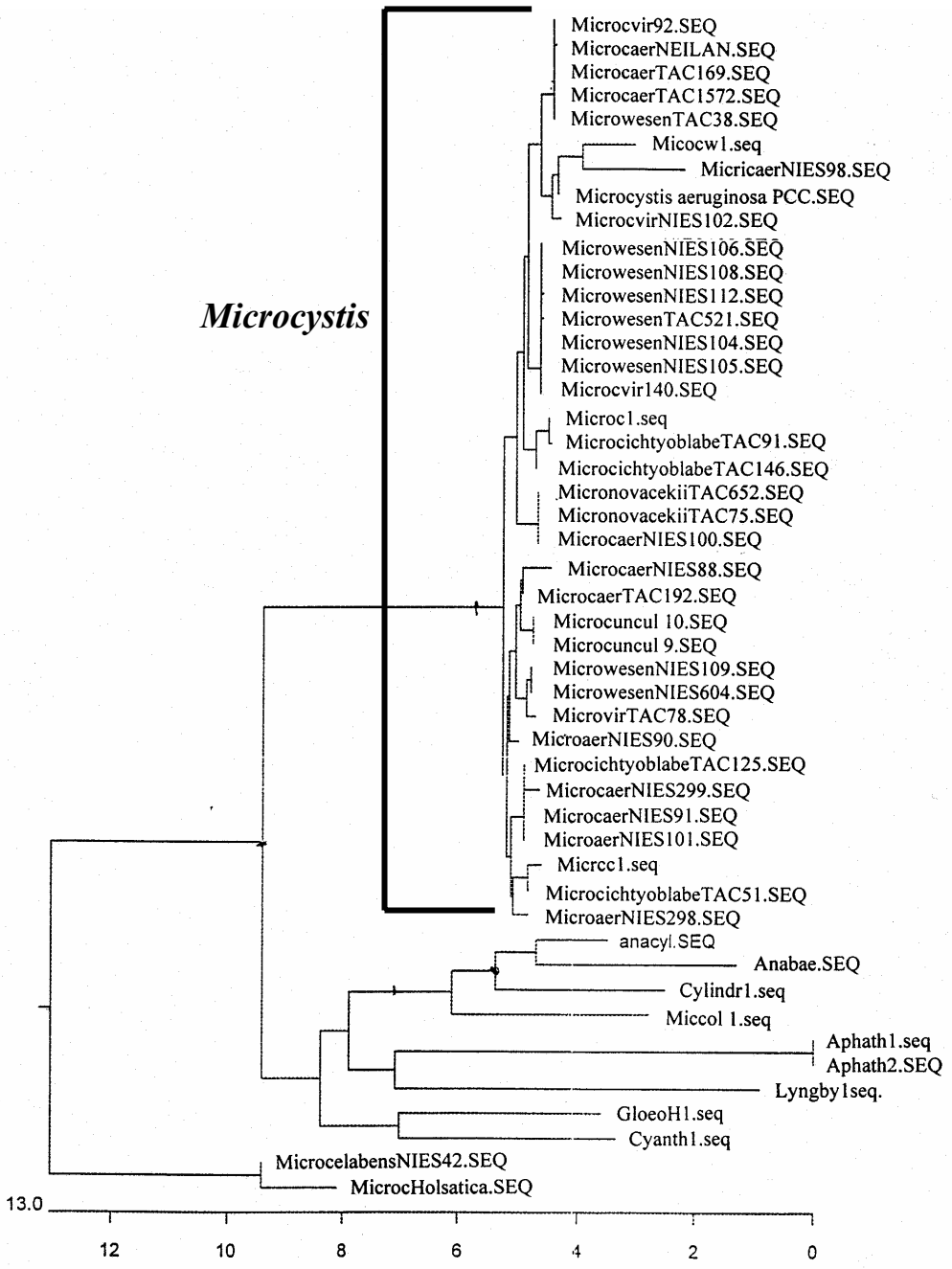


Fig.1. Part of a phylogenetic tree indicating the uniformity of the genus *Microcystis* and the negligible differences between traditional species (International Gene Bank).

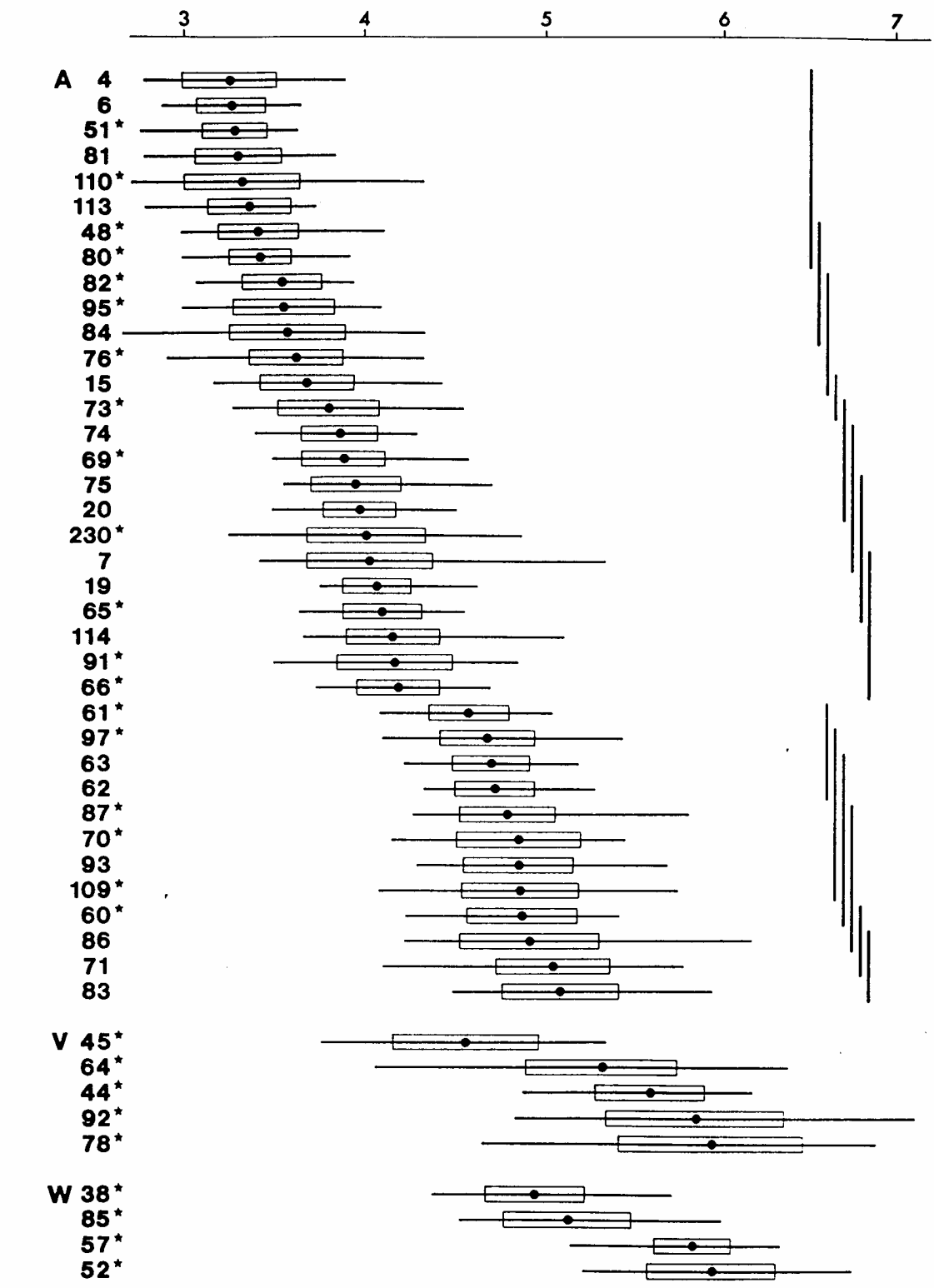


Fig. 2. Morphological variability (diameter of cells, μm) of *Microcystis* populations from central Japan; **A** = *M. aeruginosa* complex, **V** = *M. viridis* complex, **W** = *M. wesenbergii* complex (KATO et al. 1991).

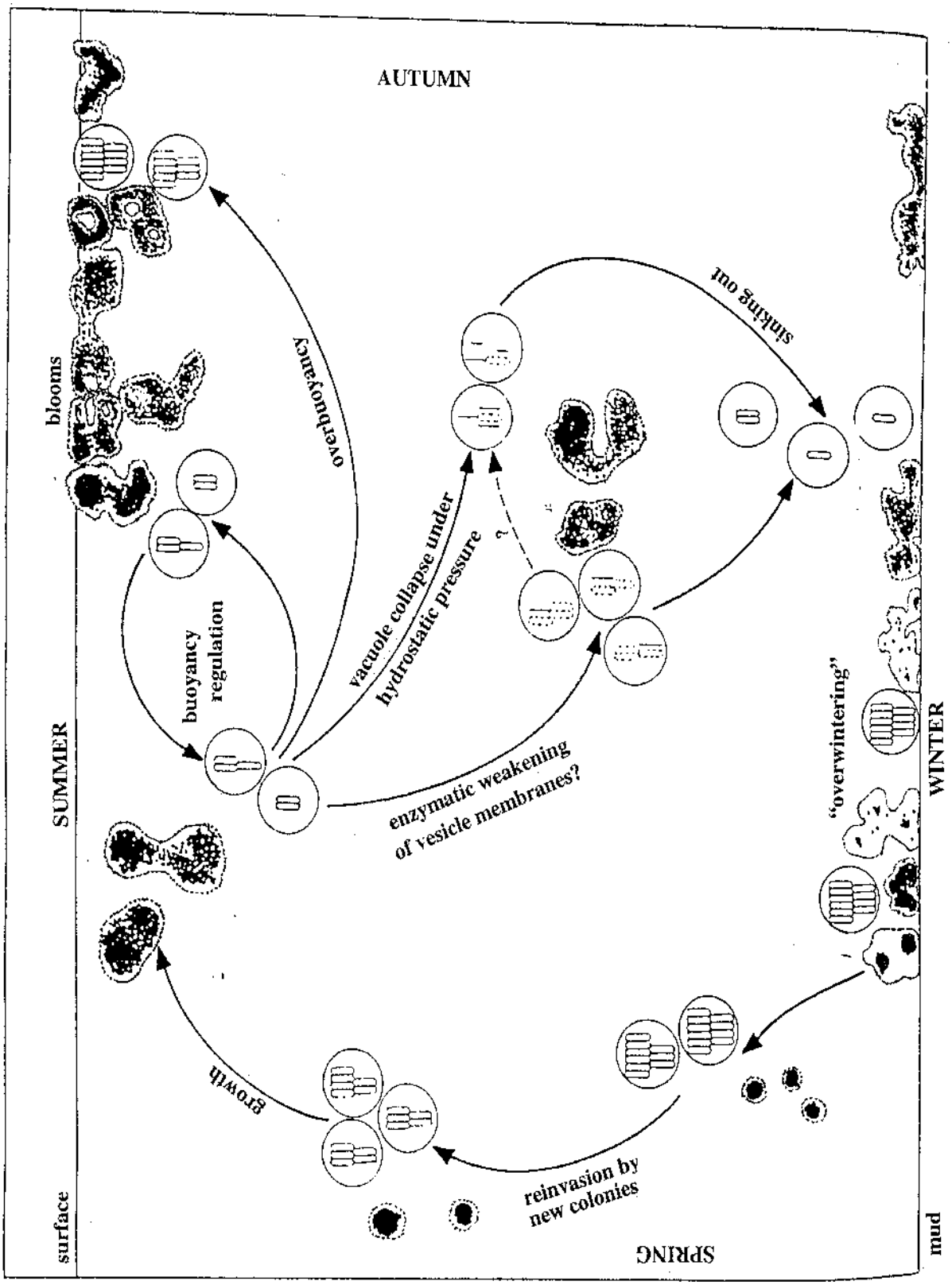


Fig. 3. Vegetation cycle of *Microcystis aeruginosa*. (After REYNOLDS et al. 1981)

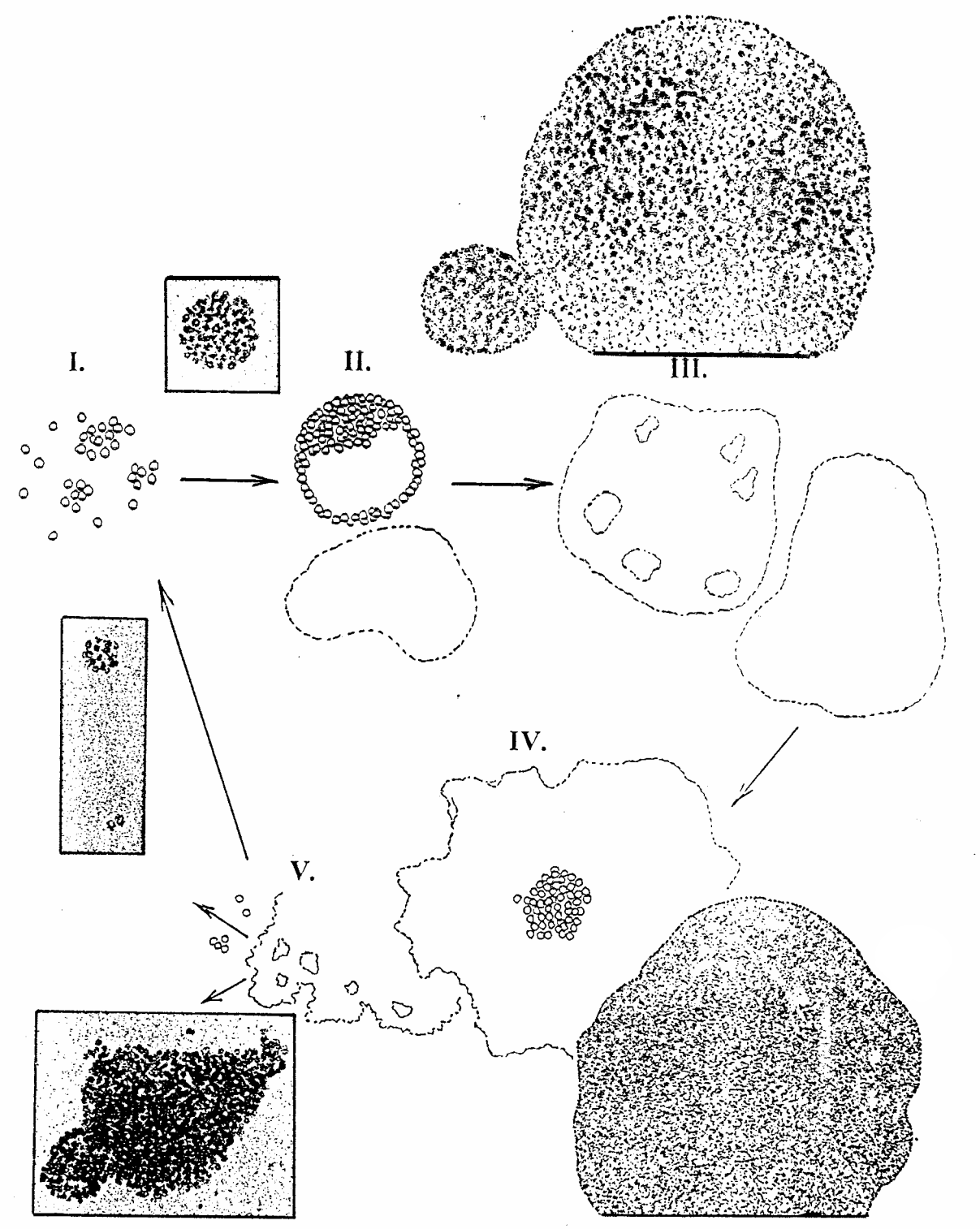


Fig. 4. Vegetation cycle of *Microcystis panniformis.* (After KOMÁREK et al. 2002.)

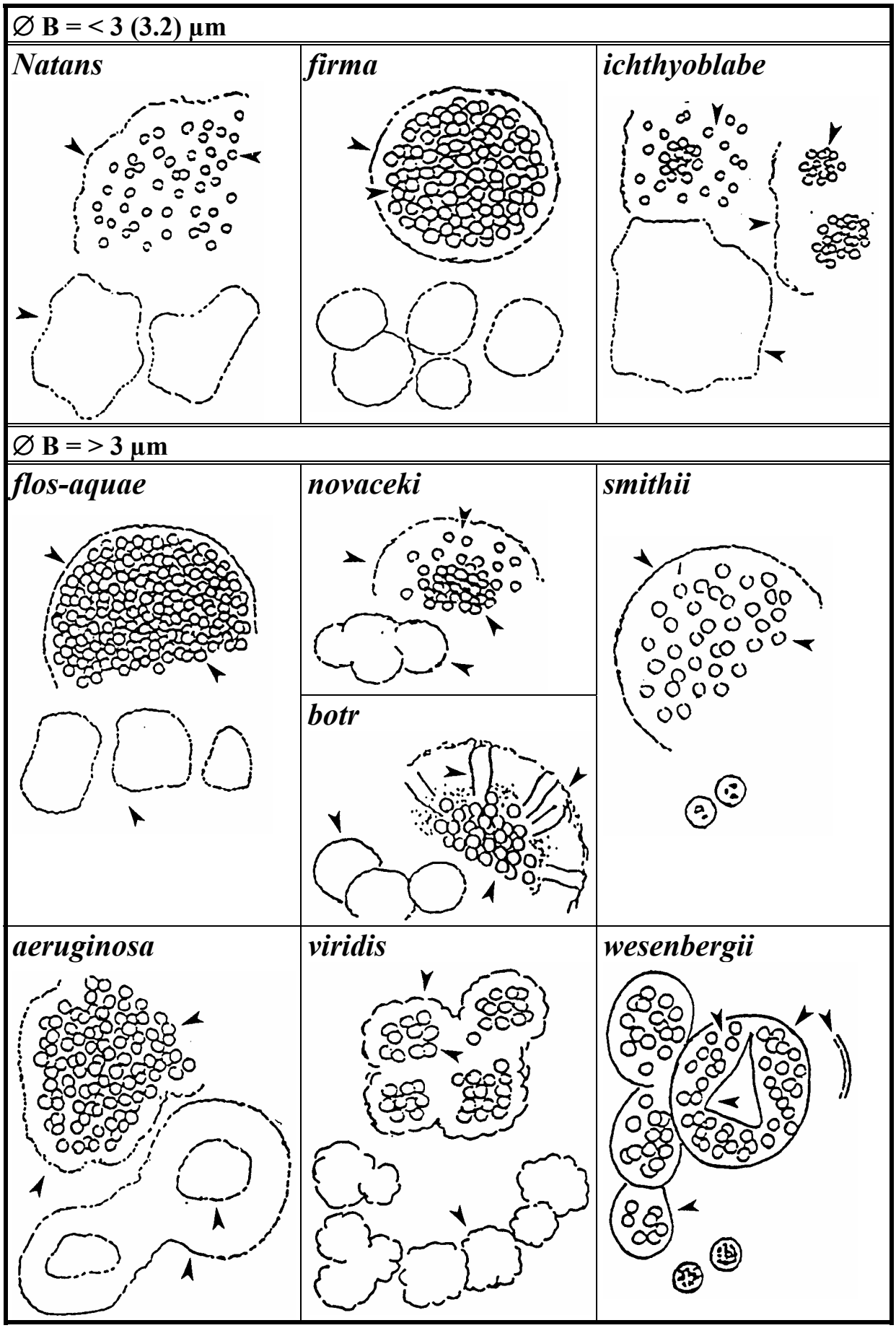


Fig. 5. Schematic drawings of typical colonies of main European *Microcystis*-morphospecies.

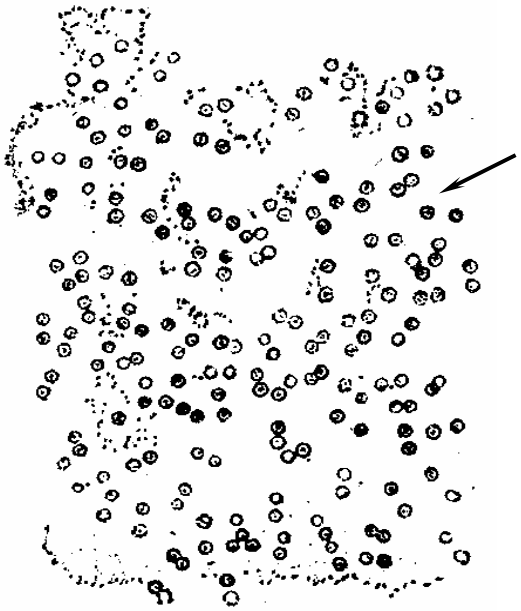
Tab. 1. Review of the main European *Microcystis*-morphospecies.

cells mostly < 3.2 µm	cells loosely situated, colonies without holes, slime very diffuse, wide; cells 1-2(3) µm in diameter			***natans*** **(Tab.2)**
	slime does not overlap distinctly the cells	cells densely agglomerated	colonies without holes	***ichthyoblabe*** **(Tab.3)**
			irregular colonies with small holes	***panniformis*** **(Tab.4)**
			spheroidal colonies without holes	***flos-aquae*** **(Tab.7)**
cells mostly > 3 µm	slime distinctly overlap the clusters of cells	colonies irregular or ± spheroidal, without holes, ± with wide slime margin (sometimes more colonies agglomerated together)	cells densely agglomerated, mucilaginous margin delimited, slightly overlapping the cells	***firma*** **(Tab.5)**
			cells densely agglomerated in colon. centre, wide mucilaginous envelopes with solitary cells	***novacekii*** **(Tab.6)**
			cells scarcely distributed, margin of mucilage ± distinct	***smithii*** **(Tab.8)**
			cells densely agglomerated, mucilage diffluent but distinct, often with radial structure, solitary cells in mucilage	***botrys*** **(Tab.11)**
			packet-like subcolonies, slime with wavy, ± refractive margin, distinct	***viridis*** **(Tab.10)**
		old colonies irregular, with holes, often composed from subcolonies	colonies irregular, later with distinct holes; slime diffuse, slightly overlapping the cells	***aeruginosa*** **(Tab.9)**
			colonies lobate, with holes, mucilage distinctly delimited, smooth, refractive	***wesenbergii*** **(Tab.12)**

Tab. 2.

***Microcystis natans* LEMMERMANN ex SKUJA**
Acta Horti Bot. Univ. Latv. 7: 45, 1934

Form of colonies: irregular, without distinct holes, to 200 μm in diameter

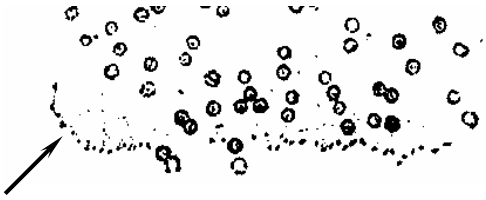


young stages: clusters of sparcely situated cells in diffluent mucilage

Cell diameter: 1-2(3?) μm

Mucilage (margin of colonies):
fine, indistinct, wide, very diffuse, colourless, distinctly overlapping cell clusters

Cell density (and organization):
irregular, evenly, usually (mainly) loosely situated



Diagnostical characters:

- size of cells
- density of cells
- mucilaginous envelopes
- 1-3 aerotopes/cell

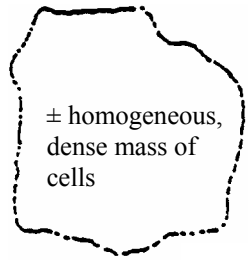
Life cycle:

Distribution: colder regions of temperate zones

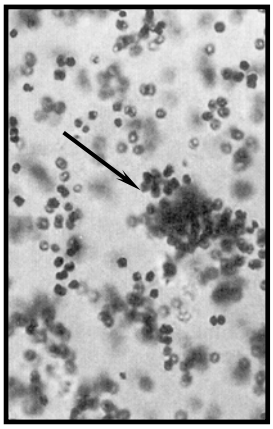
Tab. 3.

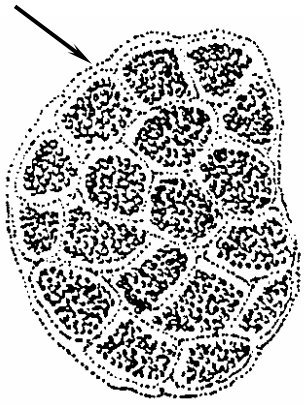
***Microcystis ichthyoblabe* KÜTZING**
Phyc. Gener., p. 170, 1843

Form of colonies: irregular, without holes, often flattened, often composed from subcolonies (cell-clusters) in common mucilage, up to large irregular compact colonies; later disintegrating in masses of solitary cells (with small groups of aggregated cells)

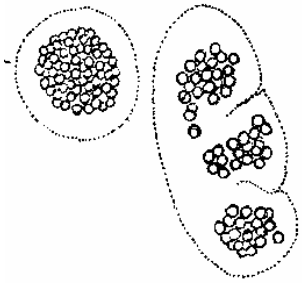


form of colonies





young stages:
small clusters of cells, ± spherical



Cell diameter: 2-3.2(3.8) µm

Mucilage (margin of colonies):
very irregular outline,
slime indistinct, diffuse,
irregulalry overlapping cells

Cell density (and organization):
cells densely regularly (homogeneously) and evenly agglomerated

Diagnostical characters:

- structure and disintegration of colonies
- sometimes composed subcolonies
- toxic compounds
- cell size

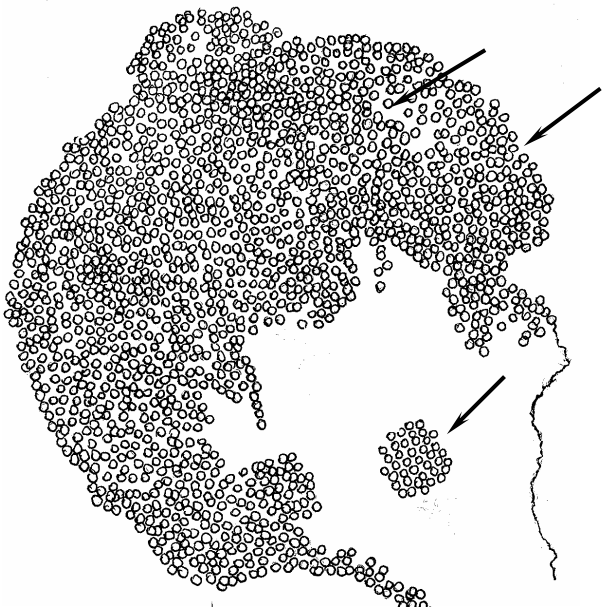
Life cycle:

Distribution: commonly in temperate zones, in eutrophic waters, up to northern regions

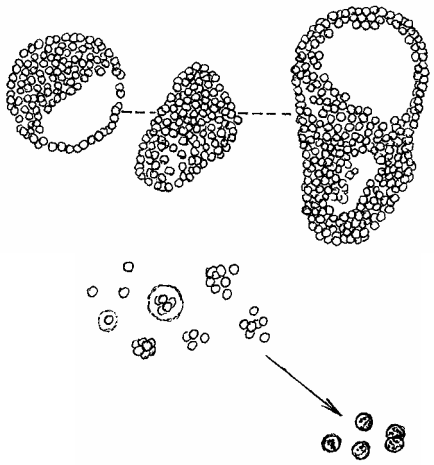
Tab. 4.

***Microcystis panniformis* KOMÁREK et al.**
Cryptogamie/Algologie, 2002

Form of colonies: flattened irregular up to monolayers, with small holes (in old colonies), later disintegrating in small groups



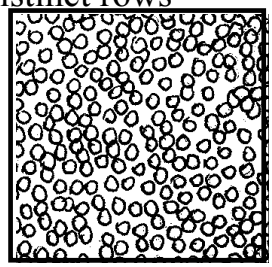
young stages: small clusters of cells, flat or circular in outline, sometimes spheroidal and ± hollow



Cell diameter: (2.5)3-4.6(4.8) µm

Mucilage (margin of colonies): diffuse, not overlapping cells; margin of colonies smooth or (in old colonies) irregular

Cell density (and organization): cells regularly densely and evenly agglomerated, sometimes in indistinct rows



Diagnostical characters:

- flat colonies with small holes
- homogeneously arranged cells
- toxicity
- life cycle

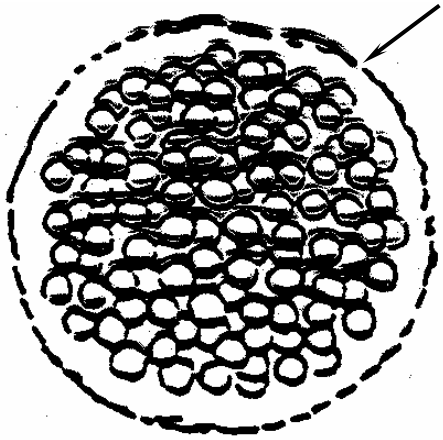
Life cycle: KOMÁREK et al. 2002

Distribution: tropical, probably pantropical species (S. Africa, N. Australia, S. America, Africa), probably invading in regions with mediterranean climate

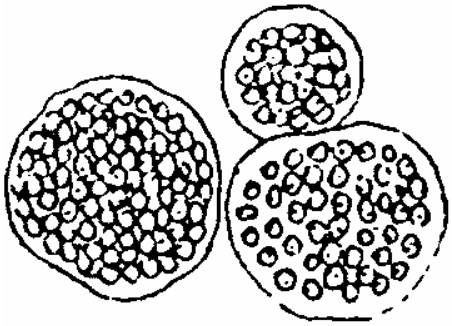
Tab. 5.

Microcystis firma (KÜTZING) SCHMIDLE
Engler Bot. Jahrb. 23: 57, 1902

Form of colonies: spheroidal to slightly irregular, sometimes aggregated together without holes



young stages:



Cell diameter: (0.8?)2-3.7(4.8?) µm

Mucilage (margin of colonies): slightly overlapping the cell clusters, delimited or diffuse (? old colonies)

Cells (density and organization): densely agglomerated in the colonial center

Diagnostic characters:

- form of colonies

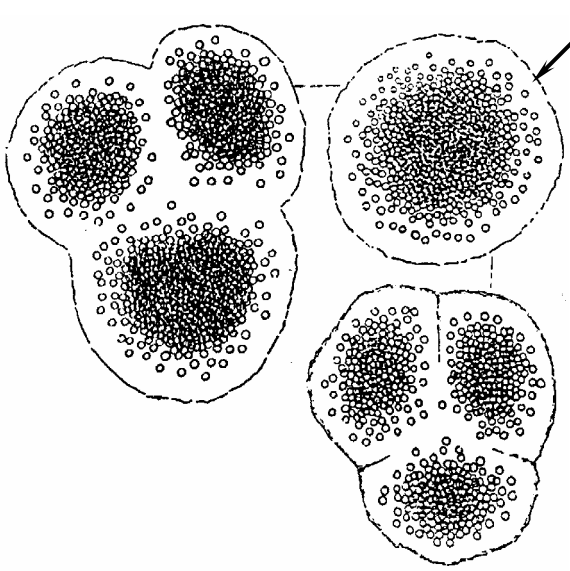
Life cycle:

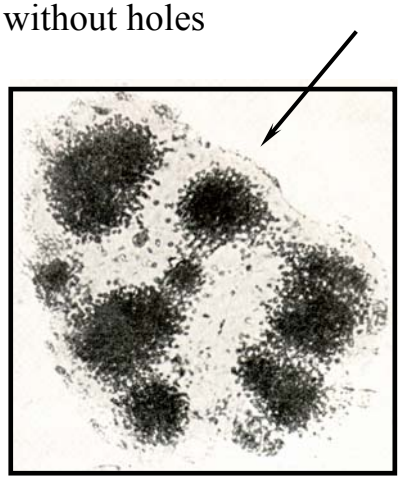
Distribution: known only from northern Europe (Baltic region), possibly slightly halophilic; not common; other localities should be revised

Tab. 6.

***Microcystis novacekii* (KOMÁREK) COMPÈRE**
Cah. O.R.S.T.O.M., Hydrobiol. 8(3-4), 1974

Form of colonies: in outline ± spheroidal and slightly flattened, sometimes (old colonies) aggregated together, without holes





young stages: small clusters of cells

Cell diameter: 2.4-6 µm

Mucilage (margin of colonies):
wide, delimited (rarely diffuse), homogeneous or indistinctly concentrically lamellated

Cell density (and organization):
± densely concentrated in the centre of colony, few solitary cells in enveloping mucilage

Diagnostic characters:

- form of colonies
- delimited mucilaginous margin
- solitary cells in slimy margin

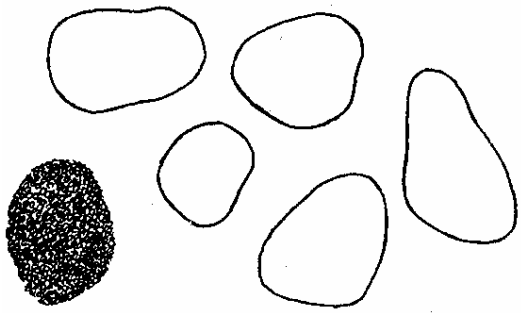
Life cycle:

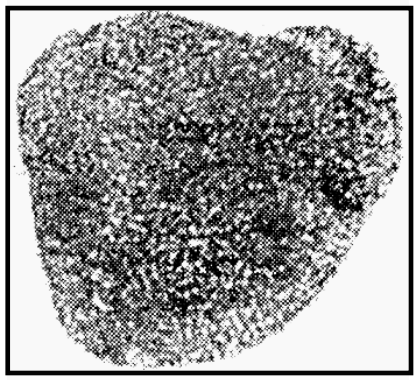
Distribution: in mesotrophic or eutrophic reservoirs; tropical, facultatively (rarely) in warmer areas (or in summer season) of temperate zones

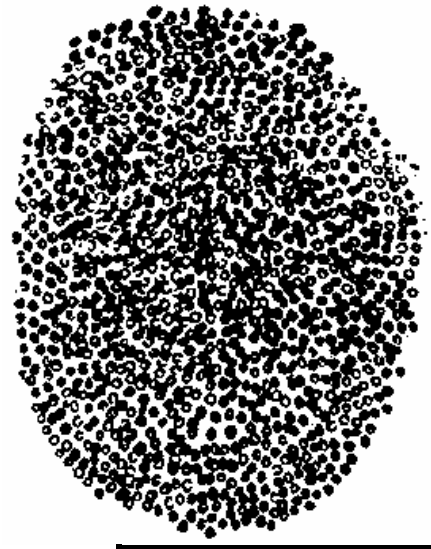
Tab. 7.

***Microcystis flos-aquae* (WITTROCK) KIRCHNER ex FORTI**
Syll. Myxophyc., p. 86, 1907

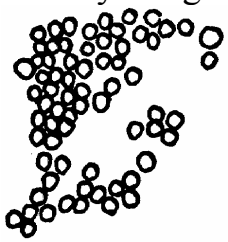
Form of colonies: solitary, irregularly spheroidal, compact, not lobate, without holes (only indistinct in old colonies)





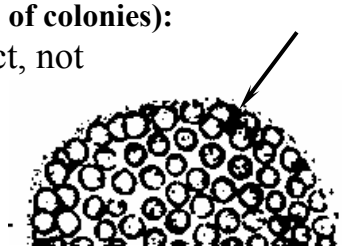


young stages: small irregular to spheroidal clusters of densely agglomerated cells, without visible slimy margin



Cell diameter: (2.5?-3)3.5-4.8(5.6?) µm

Mucilage (margin of colonies): diffuse, indistinct, not overlapping the clusters of cells



Cell density (and organization): very densely and homogeneously agglomerated cells

Diagnostic characters:

- form of colonies
- content of isopropylthio-compounds
- does not contain neurotoxins

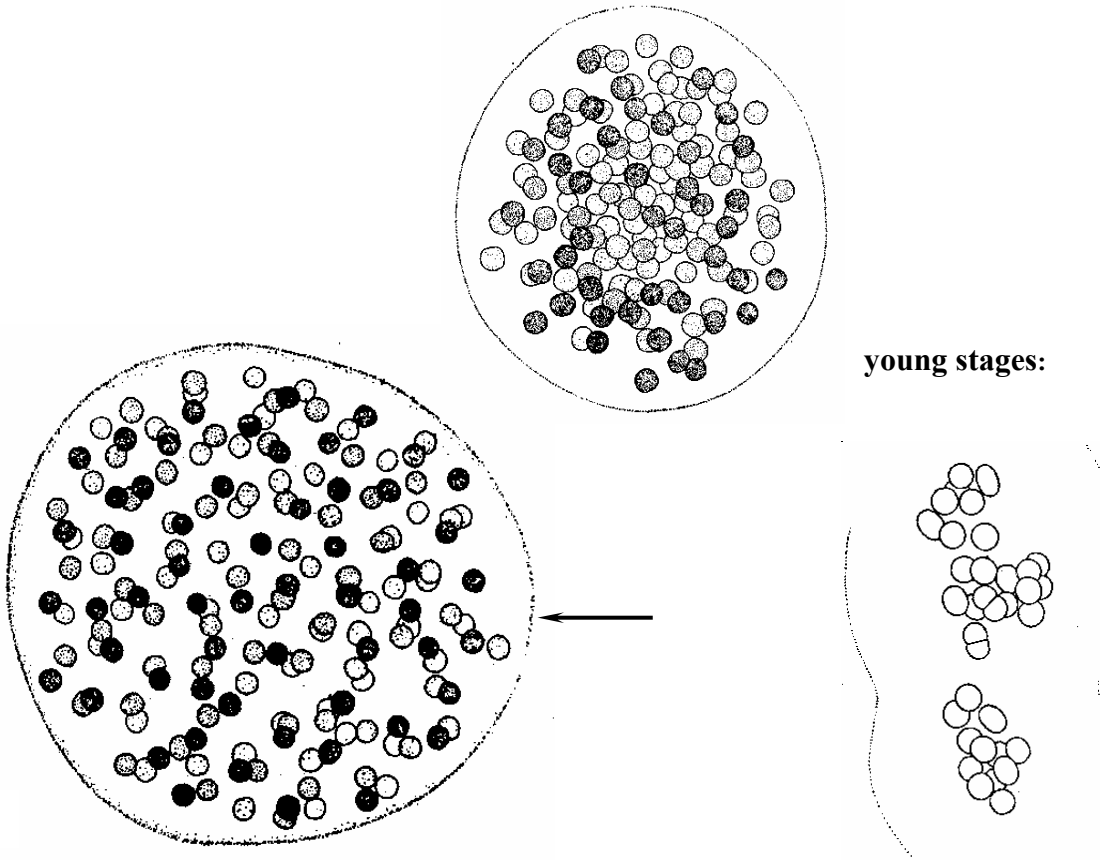
Life cycle:

Distribution: temperate zones, not very common, usually subdominant of other water blooms; tropical populations are problematic

Tab. 8.

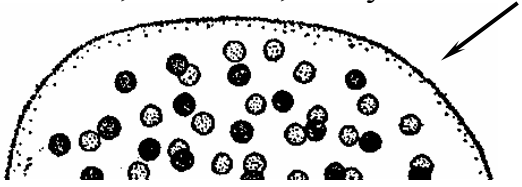
Microcystis smithii *KOMÁREK & ANAGNOSTIDIS*
Preslia, Praha, 67: 21, 1995

Form of colonies: spherical or spheroidal, rarely slightly elongated, without holes



Cell diameter: 3.2-5.6 µm

Mucilage (margin of colonies):
slime fine, clearly overlapping the cells, delimited, rarely diffuse



Cell density (and organization):
cells ± loosely and evenly situated in colonies

Diagnostic characters:

- in cells usually only (0)1-3 aerotopes (rarely more)
- spherical colonies
- density of cells

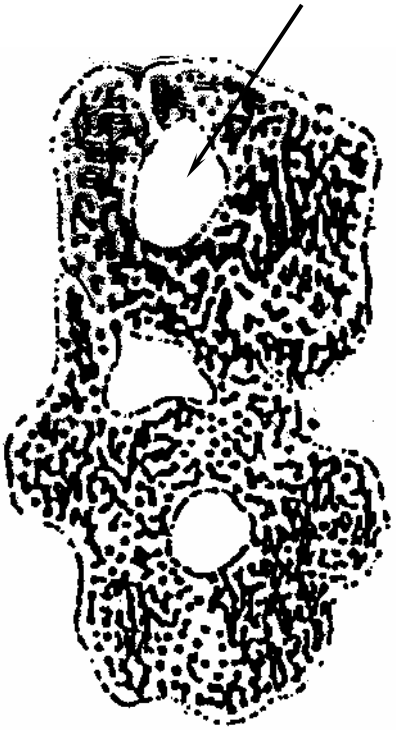
Life cycle:

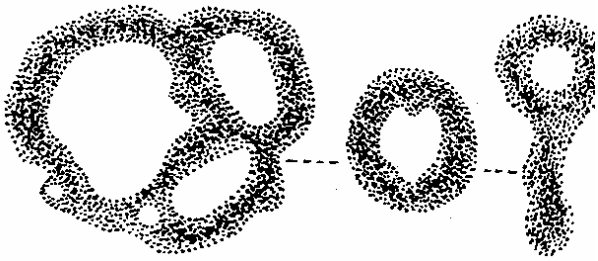
Distribution: clear, mesotrophic to eutrophic, usually not very alkaline lakes connected with peaty areas, in temperate zones; rarely

Tab. 9.

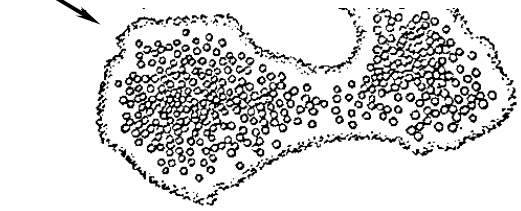
***Microcystis aeruginosa* (KÜTZING) KÜTZING**
Tab. Phycol. 1: 6, 1846

Form of colonies: irregular in outline, lobate and with distinct holes (old colonies), up to macroscopic





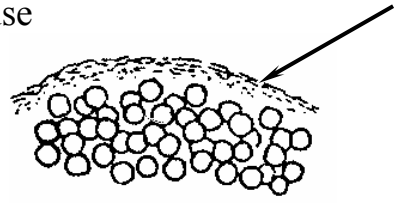
young stages: small ± spherical or irregular colonies (various density of cells)



Cell diameter: (3.5)4-6.5(9.4? before division) µm

Mucilage (margin of colonies): slightly overlapping cell agglomerations (to 5 µm wide), diffuse

Cell density (and organization): cells ± densely and irregularly agglomerated



Diagnostic characters:

- production of neuro- and hepatotoxins
- form of old colonies

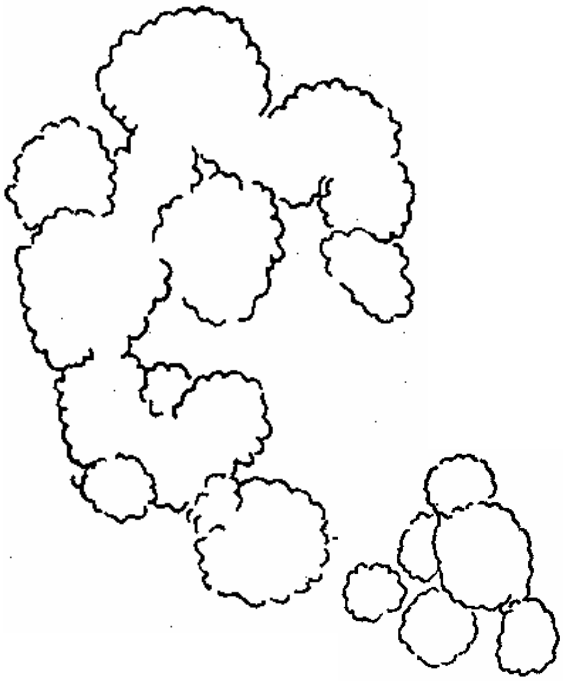
Life cycle: REYNOLDS & al. (1981)

Distribution: cosmopolitan; populations from distant areas (esp. from tropical regions) should be compared

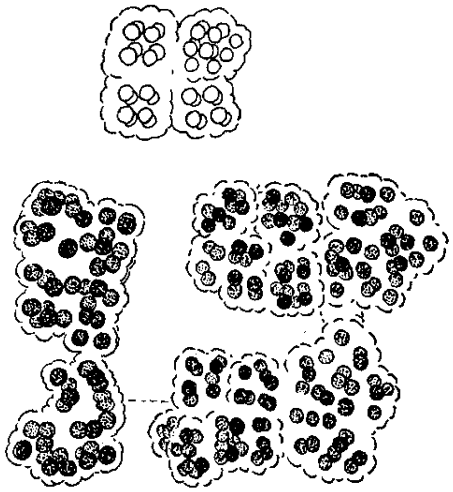
Tab. 10.

***Microcystis viridis* (A.BRAUN in RABENHORST) LEMMERMANN**
Abh. Nat. Ver. Bremen 17: 342, 1903

Form of colonies: colonies composed from typical packet-like subcolonies, irregularly agglomerated together and then usually elongated



young stages: small groups of cells in distinct mucilage



Cell diameter: (3)4-7.9 µm

Mucilage (margin of colonies): mucilage distinct, slightly overlapping cells, at the margin wavy and ± refractive

Cell density (and organization): irregularly, not very densely (rarely densely), sometimes clearly three-dimensionally (almost cubic), indistinctly in perpendicular rows

Diagnostic characters:

- ± cubic arrangement of cells
- margin of mucilaginous envelopes
- strong toxicity

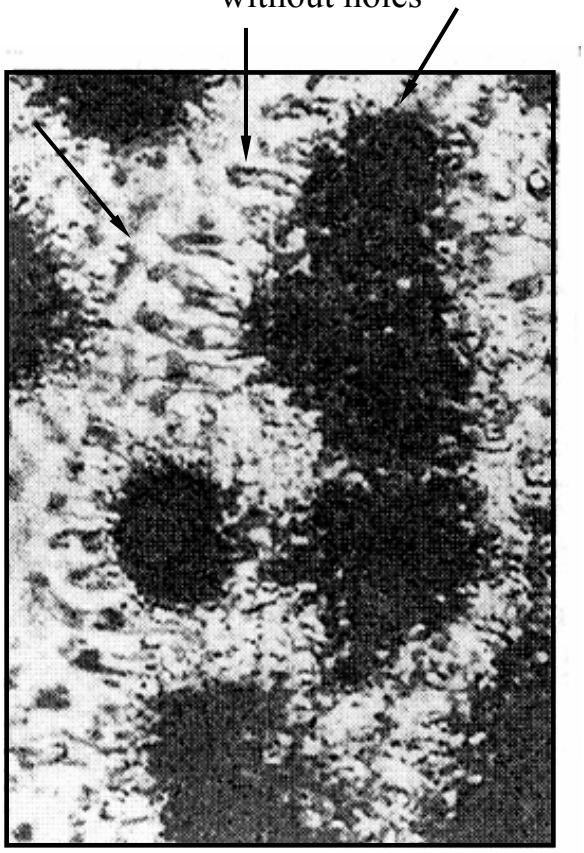
Life cycle:

Distribution: cosmopolitan; mainly in temperate zone (more mesotrophic to slightly eutrophic waters), tropical populations should be revised

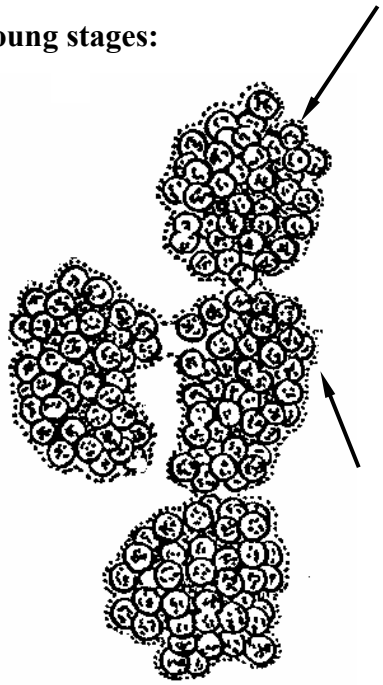
Tab. 11.

***Microcystis botrys* TEILING**
Bot. Notiser 1942: 63, 1942

Form of colonies: ± spherical subcolonies, often joined irregularly together, without holes



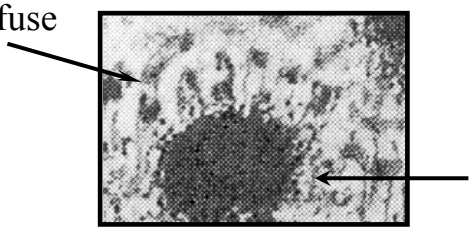
young stages:



Cell diameter: 4.9-6(7) µm

Mucilage (margin of colonies): envelopes distinct, usually wide, with radial semiglobose or tubular structures, with irregular, not refractive margin, later diffuse

Cells (density and organization): densely agglomerated in the colonial center, few ("expulsing") cells in enveloping slime



Diagnostic characters:

- radially structured enveloping slime
- solitary cells in enveloping slime
- toxic strains

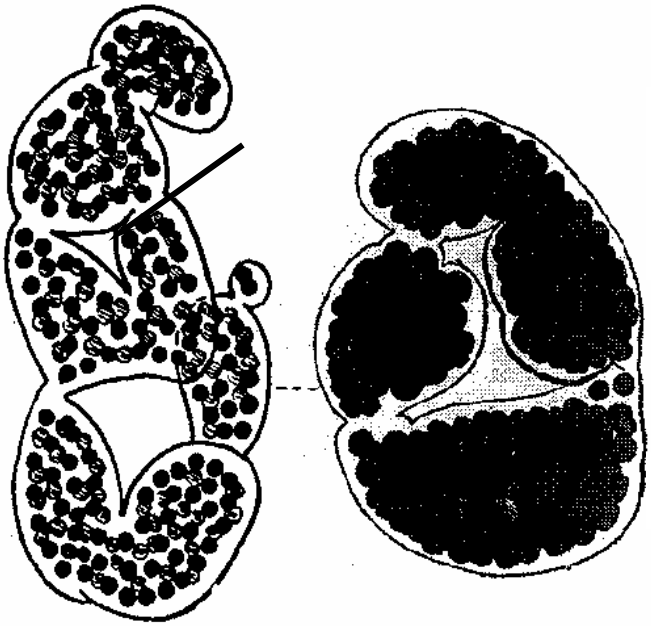
Life cycle:

Distribution: cosmopolitan, but primarily distributed in colder and northern areas of temperate zone; tropical populations should be revised (identity?)

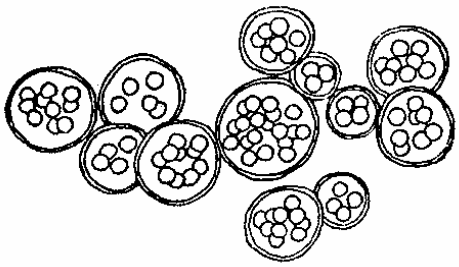
Tab. 12.

***Microcystis wesenbergii* (KOMÁREK) KOMÁREK in KONDRATEVA**
Cvetenie vody, Naukova Dumka Kiev, p. 32, 1968

Form of colonies: irregular, spheroidal to lobate or elongate, with holes when old; often composed with connected spheroidal subcolonies

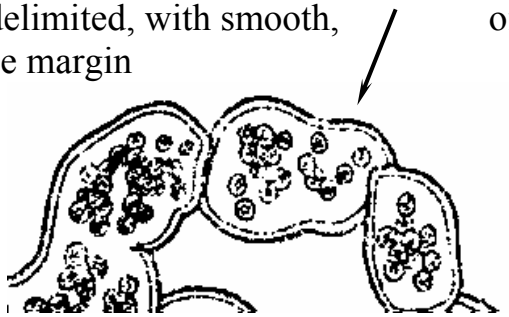


young stages: spherical colonies



Cell diameter: **4-8.5(10) µm**

Mucilage (margin of colonies):
mucilage overlapping cells, clearly delimited, with smooth, refractive margin



Cell density (and organization):
sparsely to densely agglomerated, often near the surface of subcolonies

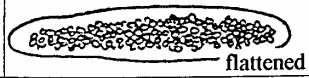
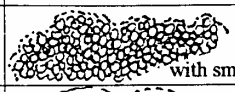
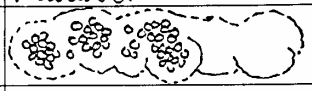
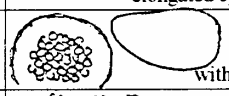
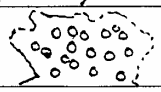
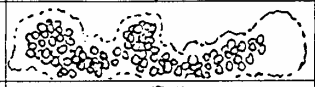
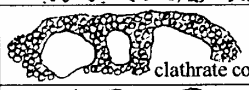
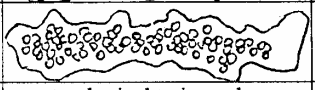
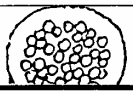
Diagnostic characters:

- form of colonies
- delimited margin of mucilage
- majority of populations without toxic compounds
- many small aerotopes

Life cycle:

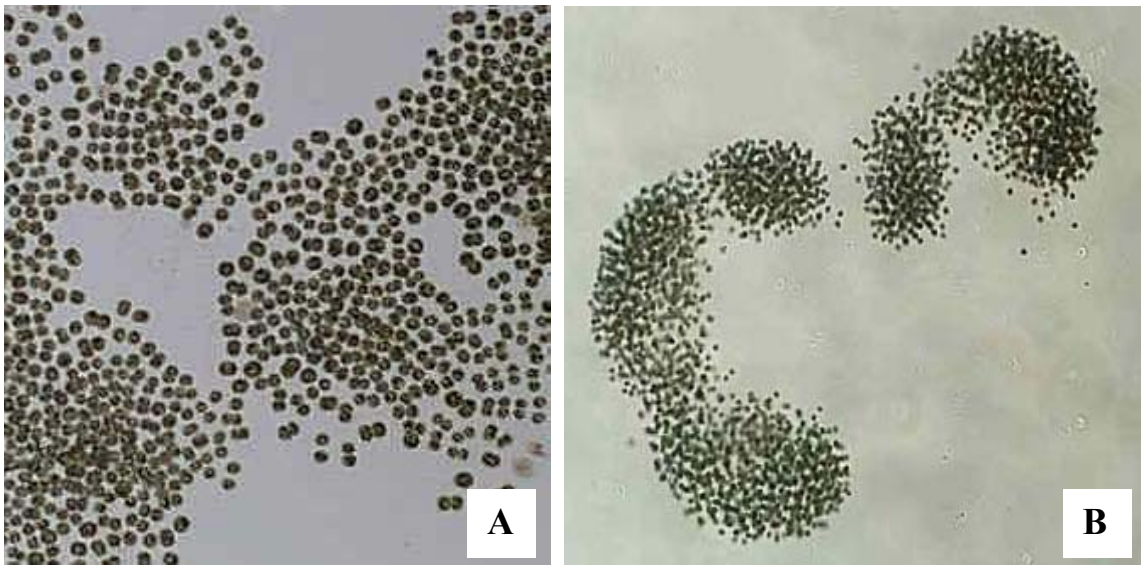
Distribution: maybe cosmopolitan, but with special morphotypes in tropical and nordic regions (CRONBERG & KOMÁREK 1994)

Tab. 13. List of main tropical *Microcystis*-morphospecies (from CROW 1923, STEPHENS 1949, DESIKACHARY 1959, KOMÁREK 1984 and KOMÁREK et al. 2002).

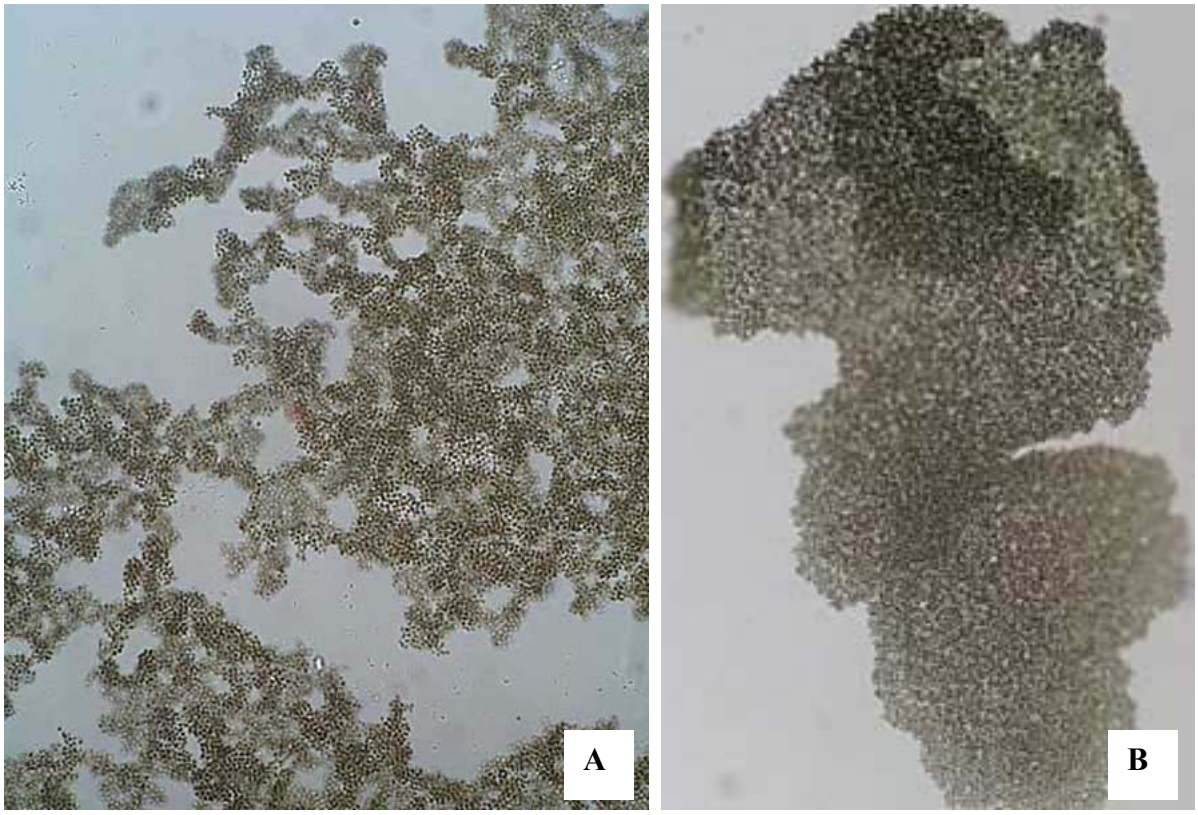
Species	Diameter of cells [µm]	Form of colony	Cell density	Mucilaginous margin	Distribution
lamelliformis HOLSINGER 1954	3-4(5)	 flattened	loosely	narrow to wide, fine, delimited	Srí Lanka
panniformis KOMÁREK et al. 2002	(2.5)3-4.6(4.8)	 with small holes	± densely	very narrow, diffuse	pantropical ?
ramosa 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	 without holes	± densely	wide, fine, but distinctly delimited	Cuba
protocystis CROW 1923	(3)3.5-6.5(7.2)	 without holes	always loosely	wide, fine, irregular, diffuse	pantropical
pseudofilamentosa CROW 1923	3-7		densely	narrow, but distinct, ± diffuse	India, Srí Lanka
toxica STEPHENS 1949	3.4-7	 clathrate colonies	± densely	thin, diffuse (attached to cells)	S. Africa
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

From „cosmopolitan" species occur in tropical regions morphotypes of *M. aeruginosa*, *M. novacekii*, *M. wesenbergii*, *M. viridis* and probably also *M. botrys*.

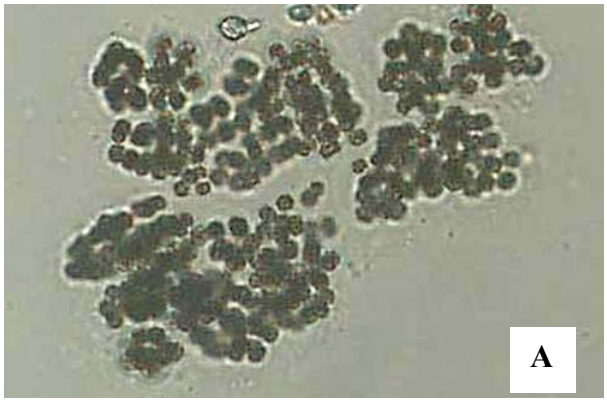
M. aeruginosa

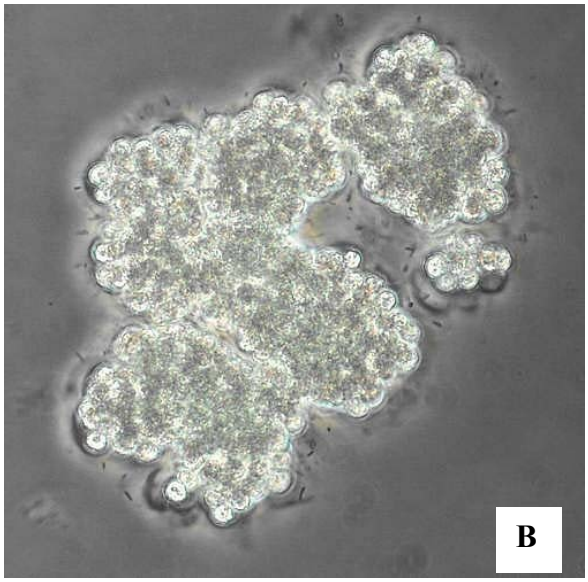


M. ichthyoblabe



M. viridis





M. wesenbergii