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Lobocystis michevii (Chlorophyceae) – a New Green Algal Species from the Bourgas Salines (Bulgaria)

By

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With 34 Figures

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Summary

Stoyneva M. P. 2008. *Lobocystis michevii (Chlorophyceae)* – a new green algal species from Bourgas Salines (Bulgaria). – Phyton (Horn, Austria) 48(1): 79–86, with 34 figures.

During the investigation of the phytoplankton of Bourgas Salines in the wetland complex Atanasovsko Ezero on Bulgarian Black Sea coast between March and May 1996 an abundant development of a coccal green alga from the genus *Lobocystis* was detected and described as a new species – *Lobocystis michevii* Stoyneva *(Botryococcaceae)*. The new species is characterized by: 1) very small ellipsoidal cells connected with a firm band-shaped mucilage; 2) cells arranged mostly in pairs and rarely in regular aggregates of 4 or 8, without common mucilage; 3) each cell contains a large parietal chloroplast with difficultly visible pyrenoid and two large refractive granules at the cell poles; 4) reproduction by transverse division of the protoplast and formation of two autospores within the gelatinizing mother cell wall; 5) planktonic mode of life in mixo-polyhaline shallow waters.

Zusammenfassung

Stoyneva M. P. 2008. *Lobocystis michevii (Chlorophyceae)* – a new green algal species from the Bourgas Salines (Bulgaria). [*Lobocystis michevii (Chlorophyceae)* – eine neue Grünalge aus den Burgas-Salinen (Bulgarien).] – Phyton (Horn, Austria) 48(1): 79–86, mit 34 Abbildungen.

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Im Rahmen von Planktonuntersuchungen in den Burgas-Salinen des Feuchtgebietskomplexes Atanasovsko Ezero an der bulgarischen Schwarzmeerküste wurden zwischen März und Mai 1996 reich entwickelte Grünalgen der Gattung *Lobocystis* gefunden, die als neue Art, *Lobocystis michevii* STOYNEVA *(Botryococcaceae)* beschrieben werden. Folgende Merkmale charakterisieren die neue Art: 1) Sehr kleine, ellipsoidische Zellen, durch sehr feste bandförmige Gallerte verbunden; 2) Zellen meist paarweise, seltener in Gruppen zu 4 oder 8 angeordnet, ohne gemeinsame Gallerte; 3) jede Zelle mit einem parietalen Chloroplasten mit sehr schwer sichtbarem Pyrenoid und 2 lichtbrechenden Körnern an den Zellpolen; 4) Fortpflanzung nach transversaler Protoplastenteilung durch 2 Autosporen welche einige Zeit in der verschleimenden Mutterzellwand verbleiben; 5) Planktonform in mixo-polyhalinem Seichtwasser.

1. Introduction

The genus *Lobocystis* has been described by THOMPSON 1952 with the type species *L. dichotoma* and is clearly delimited by its colonies, where pairs of cells retained in the expanded mother cell wall. The gelatinous threads between the cells are band-shaped and mostly firm. Each cell contains one or two parietal chloroplasts, each one with a single pyrenoid. The reproduction happens by autospores (THOMPSON 1952). Until now two distinct species have been recognized – *Lobocystis planctonica* (TIFF. & AHLSTR.) FOTT (syn. *L. dichotoma* THOMPSON) and *Lobocystis fottiana* COMAS, PÉREZ & NOVELLO (THOMPSON 1952, BOURRELLY 1966, FOTT 1975, KOMÁREK & FOTT 1983, COMAS GONZALEZ & PEREZ BALIERO 2002). The third species, *Lobocystis neodichotoma* IZAGUIRRE included two former varieties of *L. planctonica*: var. *mucosa* BOURRELLY and var. *minor* GUARR. & FERRARIO (IZAGUIRRE 1991). The two varieties are to be included within *L. planctonica* and thus *L. neodichotoma* should be be treated as nomen illegitimum for *L. planctonica* according to the discussion in COMAS GONZALEZ & PEREZ BALIERO 2002. In relation to ecology, the representatives of *Lobocystis* occur in mainly limnetic or oligohaline waters (e.g., THOMPSON 1952, BOURRELLY 1966, KOMÁREK & FOTT 1983, COMAS GONZALEZ & PEREZ BALIERO, 2002). COMIN & al. 1983 and KAWABATA & al. 1997 noted the occurrence and even mass development of *Lobocystis planctonica* in haline waters from N.E. Spain and Central Asia.

During the investigations carried out in the complex Black Sea coastal wetland 'Atanasovsko Ezero' (Bulgaria), an abundant development of peculiar small algal colonies in the spring phytoplankton found in the salt productive basins known as 'Bourgas Salinas' was detected. The material studied allowed us to refer them to the genus *Lobocystis* with a description of a new species – *Lobocystis michevii.*

2. Study Site

The Bourgas Salines are a part of the complex wetland Atanasovsko Ezero, which is situated on the Black Sea coast in the vicinity of the town

of Bourgas (Bulgaria – Fig. 1). It is a large (1700 ha – MICHEV & STOYNEVA 2007b) and diverse wetland complex formed by shallow water bodies with different size and halinity (18–280‰ – STOYNEVA 2000) used for industrial salt production, with a freshwater part and water supply canal. Atanasovsko Ezero is a managed reserve, a Ramsar and Natura 2000 Site, included in the Red List of Bulgarian Wetlands (MICHEV & STOYNEVA 2007a).

3. Material and Methods

In the period 1994–1996, a complex investigation of the wetland was carried out for preparation of its management plan (MICHEV 2001). Due to the complex character of the wetland, sampling was conducted in 20 salt production pools, which differ in morphometry and halinity (MICHEV 2001). Phytoplankton samples were collected in 1l plastic bottles, fixed in situ in 2–4% formalin and worked up after minimum sedimentation of 48 hours. Due to the extremely shallow character of the water bodies, the samples have been collected from the surface horizon (0–20 cm) between 10 a.m. and 2 p.m. Sampling for phytoplankton, phytobenthos, zooplankton and zoobenthos was combined with hydrochemical analyses and temperature measurements.

Phytoplankton determination and counting was done almost immediately after sampling in 64 squares of Thoma chamber in 8 reiterations on an Amplival microscope with magnification up to 1200x. Additionally, recently (2004 and 2008) detailed investigations of *Lobocystis* specimens have been carried out with a phase-contrast Motic 400 microscope and digital photographs were taken with a Moticam 2000 camera. The standard staining by indian ink, gentian violet and methylene blue for the mucilage (WILLIAMS 1972) and iodine solutions for pyrenoid revealing (ETTL & GÄRTNER 1988) was applied.

4. *Lobocystis michevii* STOYNEVA, spec. nova

Diagnosis: Cellulae minutae, ellipticae, interdum plusminusve globosae, saepe binae vel quaternae (raro octonae) in colonias per strato amylaceo firmoque aggregatae. Colonias pluricellulares et gelatinosas circumdatas desunt. Chloroplastus parietalis; pyrenoide parvo, saepe aegre visibili. Granula lucem refringentibus in cellulas locatis. Propagatio autosporis binatim intra cellulas matricales per divisiones transversales. Dimensiones cellularum: 3–4,6 µm longae, 1,5–3,5 µm latae.

Habitatio: Species planctonica, lacu Atanasovsko, Bulgaria.

Iconotypus: Figura nostra 2, ex collectione lacus Atanasovsko, Bulgaria.

Collectio conservata: Nr AE-58 deposita in herbaria autoris et Universitatis Sofiensis (SO).

Description: Cells very small, ellipsoid, sometimes globular, mainly in groups of 2 or 4, very seldom 8. Multicellular colonies surrounded by common mucilage are not formed. Pairs of cells are connected with band-like firm mucilage but are not included in common mucilage. The single chloroplast is parietal, nearly filling the central portion of the cell leaving

spaces at either end in which there is usually a refractive granule. The pyrenoid is small, delicate and poorly visible in LM (observed only after staining by iodine solutions and with immersion objective 100x). Reproduction is performed by two autospores, which are generated after transverse division of the protoplast. Autospores remaining in the mother cell wall, which later gelatinizes. Cell dimensions: 3–4,6 µm long, 1,5–3,5 µm wide (Fig. 2–32).

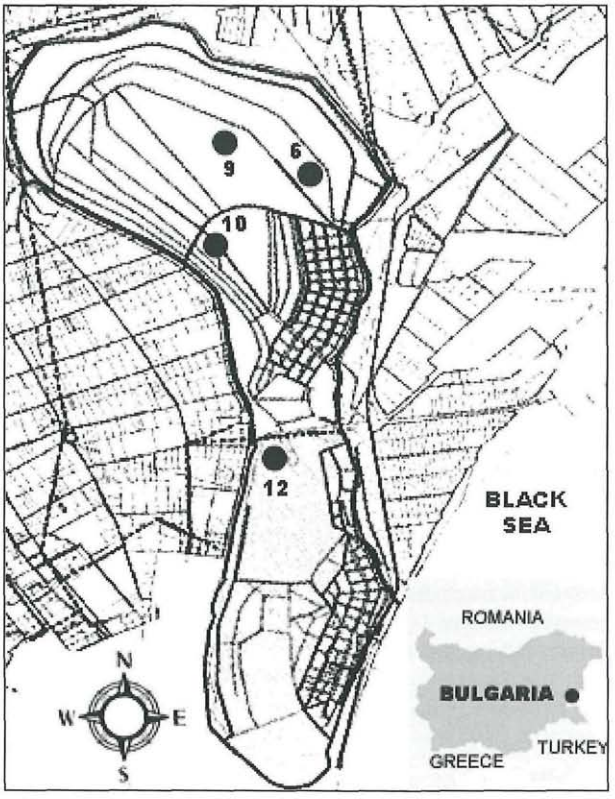

Fig. 1. Map of Atanasovsko Ezero with map of Bulgaria and situation of Atanasovsko Ezero on the Black Sea coast. Black dots indicate the sites where *Lobocystis michevii* was found.

Eponymy: The species epitheton *michevii* is given in honour of the life-work of the Bulgarian ornithologist and nature conservationist Assoc. Prof. Tanyo MICHEV, who devoted his life to the protection and restoration of the most significant Bulgarian wetlands and contributed notably to the designation of Atanasovsko Ezero as a Ramsar and Natura 2000 Site, as well as a Bulgarian managed reserve.

5. Observations and Discussion

The representatives of *Lobocystis* were found in the quantitative phytoplankton samples from the following dates and sites (Fig. 1): 1.03.1996 (site 6), 3.05.1996 (sites 9, 12) and 17.05.1996 (sites 9, 10). The alga occurred sporadically in March (in site 6 with halinity 19.83‰ and water temperature 18°C) but was more abundantly developed in May and on 3rd May 1996 dominated the phytoplankton in sites 9 and 12, reaching densities of

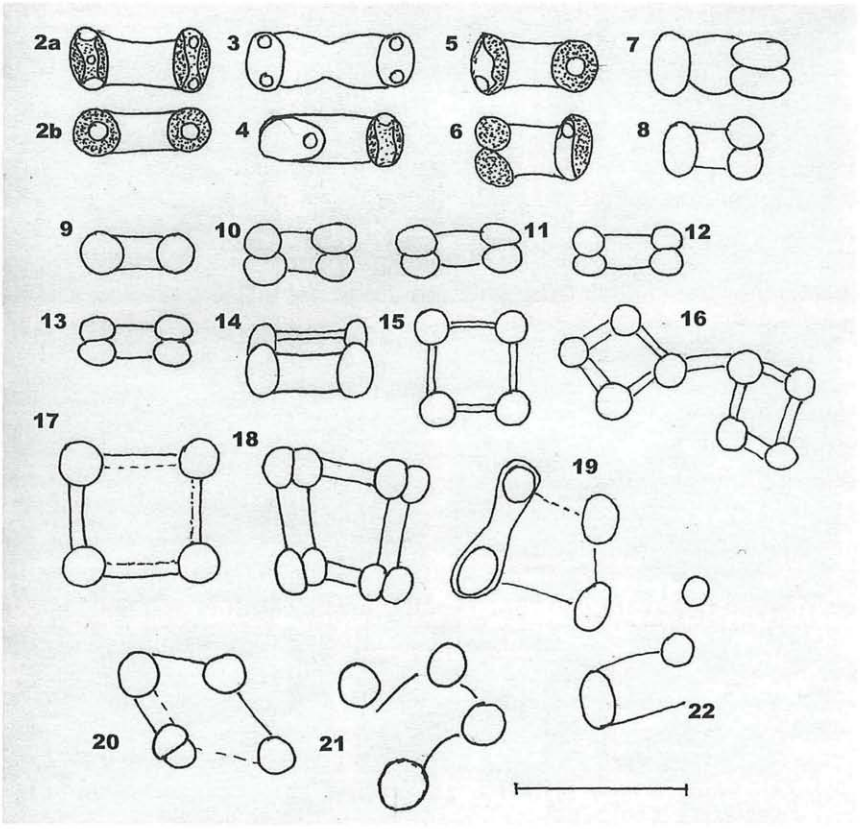

Fig. 2–22. *Lobocystis michevii*. – Fig. 2a. Pair of vegetative cells with parietal chloroplasts, pyrenoid and refractile granules at the poles and connecting firm band-like mucilage; 2b. view from above. – Fig. 3–5. Pairs of vegetative cells connected with mucilage. – Fig. 6–14. Division stages by autosporulation. – Fig. 15–18. Regular aggregates of 4 and 8 cells after repeated autosporulation. – Fig. 19–22. Dissolving cell aggregates with remnants of mucilage bands. – Scale bar: 10 μm.

$1.2x10^9$ cells l^{-1} and $6x10^6$ cells l^{-1}, respectively. At this time the waters of the shallow salt productive basins were mixo-polyhaline (22–26.2‰), slightly alkaline (pH-8.4–8.6) and eutrophic (PO_4-P – 0.059–0.062 mg l^{-1}) with a temperature of 21–21.5°C (Botev 1997).

Very important characteristics of the specimens found besides their firm mucilage connection are the small cell dimensions (1.5–4.6 μm), which were quite constant in the materials processed. Due to these small dimensions, at first sight under the light microscope without immersion, it is difficult even to see the chloroplast. However, after iodine staining and application of a 100x objective and immersion oil the presence of a large

parietal, cup-shaped chloroplast and two refractive granules becomes visible (Fig. 2–6, 23–25). The pyrenoids were extremely poorly, or not at all visible even after staining in spite of the fact that they have been indicated among the generic features.

The mucilage strand between the cells is very firm and could be seen in fixed material after 8 and 12 years held as museum samples (Fig. 22, 31, 32). The staining with indian ink did not reveal any presence of common mucilage around the colonies. The lack of a common matrix was described for the type species *Lobocystis dichotoma* by THOMPSON 1952. Staining by methylene blue and gentian violet did not reveal an individual mucilaginous envelope with a radiating structure (Fig. 30). This peculiarity is in contrast with the features of other *Lobocystis* species, where the individual mucilaginous envelope shows a radiating structure when stained by methylene blue (WILLIAMS 1972 for *L. planctonica* var. *mucosa* and STOYNEVA, unpubl. for *L. planctonica* – Fig. 33-34). The presence or absence of radiating structures in the mucilage after staining was not discussed for *L. fottiana* by COMAS GONZALEZ & PEREZ BALIERO 2002.

The pairs of cells connected with a mucilage thread represent the most typical appearance of this species (Fig. 2–32). After transverse autosporulation (Fig. 6–13, 19, 20) a peculiar arrangement of young daughter cells could be observed. They form more or less regular aggregates of four connected by one mucilaginous strand (Fig. 10–13). This was never described before and differs clearly from other *Lobocystis* species where irregular cell aggregates are formed (e.g., THOMPSON 1952, BOURRELLY 1966, KOMÁREK & FOTT 1983, COMAS GONZALEZ & PEREZ BALIERO 2002). Regular colonies of four or eight cells (Fig. 14–18) were rarely observed even in the cases when the alga monodominated the phytoplankton.

In conclusion, the abundant development of the alga in shallow mixopolyhaline waters in combination with peculiar morphology supports the description of a new species.

6. Acknowledgements

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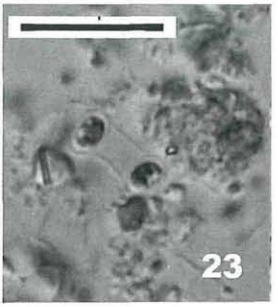
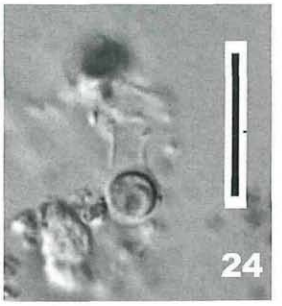
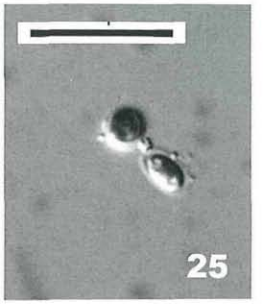
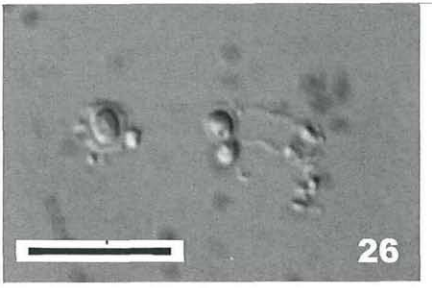
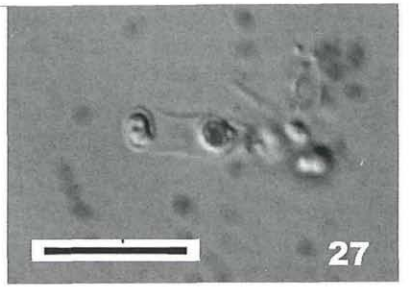
Fig. 23–32. Lobocystis michevii in the LM. – Fig. 23 and 25 stained with iodine solution; Fig. 26 autosporulation. – Fig. 30–32. Mucilage bands, stained with methylene blue.

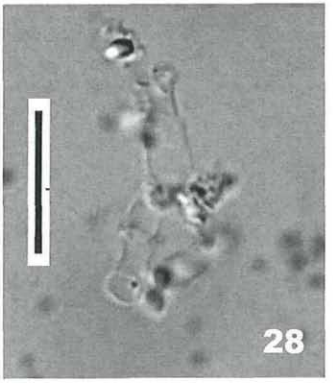


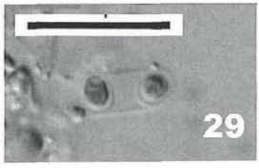


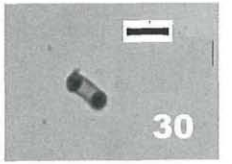


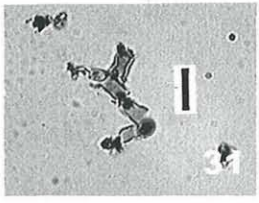


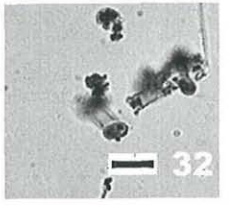


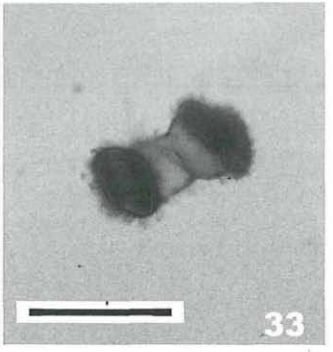


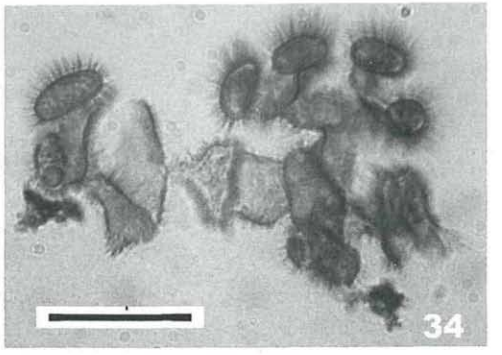












on light microscope during the 12th meeting of IAP in Canada (September 1999). Gratefulness has to be expressed to Univ.-Prof. Georg GÄRTNER (Innsbruck University) for his support and helpful comments during the preparation of the manuscript.

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