Phyton (Horn, Austria)	**Vol. 45**	Fasc. 2	237–247	30. 12. 2005

Closteriopsis petkovii – a New Green Algal Species from Lake Tanganyika (Africa)

By

Maya P. STOYNEVA*), Georg GÄRTNER**), Christine COCQUYT***) &

Wim VYVERMAN***)

With 27 Figures

Received May 9, 2005

Keywords: *Closteriopsis petkovii* spec. nova, *Trebouxiophyceae*. – Green algae, Phytoplankton. – Tanganyika, Africa.

Summary

STOYNEVA M. P., GÄRTNER G., COCQUYT Ch. & VYVERMAN W. 2005. *Closteriopsis petkovii* – a new green algal species from Lake Tanganyika (Africa). – Phyton (Horn, Austria) 45 (2):. 237–247, with 27 figures. – English with German summary.

During the recent (2002–2004) investigations of the phytoplankton in the large, ancient, tropical Lake Tanganyika an abundant development of peculiar fusiform cells was detected during the rainy season (October-May/June). They were referred to the trebouxiophycean genus *Closteriopsis* followed by a description of a new species – *Closteriopsis petkovii* STOYNEVA, GÄRTNER, COCQUYT & VYVERMAN (*Trebouxiophyceae*). This new species is characterized by: 1) symmetrically fusiform cells, at young stages twisted, with peculiar coiled ends, which are generally arranged in groups of two, four or, more rare eight cells due to rapid division; 2) one chloroplast per cell, which is parietal, band-like and sigmoidal, often additionally curved trough-like with 1–2 (-4) rounded pyrenoids, with a continuous starch sheath; 3) reproduction by two or four (more rare, eight) autospores, which are generated after

*) Assoc. Prof. Maya P. STOYNEVA, Department of Botany, Faculty of Biology, Sofia University "St Kliment Ohridski", 8 bld. Dragan Zankov, BG-1164, Sofia, Bulgaria; e-mail: mstoynev@techno-link.com

**) Univ. Doz. Dr. Georg GÄRTNER, Institut für Botanik, Universität Innsbruck, Sternwartestr. 15, A-6020 Innsbruck Österreich; e-mail: georg.gaertner@uibk.ac.at

***) Dr. Christine COCQUYT, Prof. Dr. Wim VYVERMAN, Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Krijgslaan 281-S8, B-9000 Ghent, Belgium; e-mail: christine.cocquyt@ugent.be, wim.vyverman@ugent.be

inclining cell division (simultaneous or successive) and gradually slip down, forming peculiar chains of fine, colourless mucilage strands, set up due to the gelatinization of the mother cell wall.

Zusammenfassung

STOYNEVA M. P., GÄRTNER G., COCQUYT Ch. & VYVERMAN W. 2005. *Closteriopsis petkovii* – eine neue Grünalge aus dem Tanganyika See (Afrika). – Phyton (Horn, Austria), 45 (2): 237–247, mit 27 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Im Rahmen von Phytoplanktonuntersuchungen in den Jahren 2002 – 2004 im Tanganyika See wurden während der Regenzeit (Oktober – Mai/Juni) reich entwickelte, eigenartige, spindelförmige Algenzellen aus der Gattung *Closteriopsis* (*Trebouxiophyceae*) entdeckt die als neue Art – *Closteriopsis petkovii* STOYNEVA, GÄRTNER, COCQUYT & VYVERMAN beschrieben werden. Die neue Art ist durch folgende Merkmale charakterisiert: 1) symmetrische, spindelförmige Zellen, in jungen Stadien verdreht und mit eigenartigen gewundenen Enden, meist in Zweier-, Vierer-oder selten Achtergruppen nach rascher Zellteilung; 2) in jeder Zelle ein parietaler bandförmiger, sigmoid und häufig zusätzlich zur gänze gebogener Chloroplast mit 1 – 2 (–4) abgerundeten Pyrenoiden mit einheitlicher Stärkehülle; 3) asexuelle Fortpflanzung durch 2 bis 4 (eher selten 8) Autosporen, welche nach schräger Zellteilung (simultan oder sukzedan) frei werden und sich zu eigenartigen Reihen in feiner, farbloser, aus Verschleimen der Mutterzellwand hervorgehender Gallerte anordnen.

1. Introduction

The genus *Closteriopsis* LEMMERMANN is delimited by its solitary, free-living, symmetrical, fusiform cells with 2–4 or more pyrenoids arranged lengthwise within the parietal band-shaped (more seldom screw-shaped) or axial plate-shaped chloroplast (HINDÁK 1970). The pyrenoid is covered by starch plates and its matrix is traversed by thylakoids (HEGEWALD & SCHNEPF 1986). The reproduction is by 2–8 autospores (HINDÁK 1970, KOMÁREK & FOTT 1983). The justification of the genus and its position were debatable for a long time (e.g. HINDÁK 1970, USTINOVA & al. 2001). According to the first description it was related to the genus *Closterium* RALFS within *Desmidiales* (LEMMERMANN 1899), but later on traditionally the genus was placed in *Chlorophyceae* among the families *Selenastraceae* (e.g. WEST & FRITSCH 1927) or *Ankistrodesmaceae* (e.g. HINDÁK 1970, KOMÁREK & FOTT 1983) depending on the family concept. Most recent molecular studies proved that *Closteriopsis acicularis* (G. M. SMITH) BELCHER & SWALE is closely related to *Parachlorella kessleri* (FOTT & NOVÁKOVÁ) KRIENITZ, HEGEWALD, HEPPERLE, HUSS, ROHR & WOLF from the class *Trebouxiophyceae* (KRIENITZ & al. 2001, 2004, USTINOVA & al. 2001).

In relation to ecology, within the genus *Closteriopsis* only one variety, *Closteriopsis acicularis* var. *africana* HINDÁK, was described from African soils (HINDÁK 1970), while most of the species are fairly common inhabitants of the plankton of various water bodies (KORSCHIKOFF 1953, KO-

MÁREK & FOTT 1983, USTINOVA & al. 2001, JOHN & TSARENKO 2002, SHUBERT 2003). *C. acicularis* was reported from the ancient large African Lake Tanganyika (COCQUYT & al. 1993).

During the recent investigations carried out in the Lake Tanganyika an abundant development of peculiar fusiform cells was detected. The material studied allowed us to refer them to the genus *Closteriopsis* with a description of a new species – *Closteriopsis petkovii.*

2. Study Site

The Lake Tanganyika is situated between 3° 30' and 8° 50' S and 29° 05' and 31° 15' E in a deep narrow trough of the western branch of the Rift Valley of East Africa (COULTER 1994). This tectonic lake with an area of 31 900 km^2 and a mean depth over 500 m is 650 km long and 50 km wide (HUTCHINSON 1957). It contains three distinct basins: the Kigoma basin in the north (max depth: 1310 m), the Kungwe basin in the centre (max depth: 885 m) and the Kipili basin in the south (max depth: 1410 m – PLISNIER & al. 1999). Lake Tanganyika is meromictic with anoxic monimolimnion, containing the second largest volume of anoxic water in the world after the Black Sea (PLISNIER & al. 1999). The main limnological characteristics (as yearly medians for the 0–100 m water column) of the lake at the both sampling stations Kigoma (Tanzania) in the north and Mpulungu (Zambia) in the south are provided according to PLISNIER & al. 1999:

1) water temperature – 25.7 °C at Kigoma and 24.5 °C at Mpulungu;
2) pH – ca. 8.9, generally similar at each station;
3) conductivity – 654 µS at Kigoma and 662 µS at Mpulungu;
4) turbidity – 0.25 NTU at Kigoma and 0.33 NTU at Mpulungu;
5) transparency – 12.8 m at Kigoma and 11.9 m at Mpulungu;
6) total phosphorus – 16 µg l^{-1} TRP in PO_4-P.

According to the same authors, nitrate had a maximum in concentration of ca. 0.1 mg l^{-1} NO_3-N at 60–80 m in the north and 100–140 m in the south.

3. Material and Methods

Since February 2002 a standard bi-weekly sampling was conducted in two pelagial and two littoral stations in the regions of Kigoma and Mpulungu. Additionally samples through a longitudinal transect of the lake were collected during the cruise in July 2003. The water samples were taken at each 20 m depth at the pelagial and from the surface at the littoral sites. For the quantitative investigation one liter of lake water from each depth was fixed in situ with an acid Lugol's solution, formalin and a 3% sodium thiosulphate solution (method after RASSOULZADEGAN in SHERR & SHERR 1993). Samples were settled during 48 h and then consecutively transferred to 100-ml bottles and to 10-ml sedimentation chambers. Plankton-net (10 µm mesh width) samples from the upper water column (0–50 m) in the same pelagial and littoral sites were collected also. All samples are stored in the Laboratory of Aquatic Ecology and Protistology of Ghent University.

A Zeiss Axiovert 135 inverted microscope was used to count phytoplankton (UTHERMÖHL 1931). Detailed investigations of *Closteriopsis* specimens on non-permanent slides were carried-out with a Leitz Diaplan microscope, equipped with Differential Interference Contrast at a magnification of 1000. The standard staining by Indian ink for the mucilage and iodine solutions for the pyrenoid revealing (ETTL & GÄRTNER 1988) was applied. Digital photographs were taken with an Olympus DP 50 camera.

4. *Closteriopsis petkovii* STOYNEVA, GÄRTNER, COCQUYT & VYVERMAN spec. nova

Diagnosis: Cellulae fusiformae symmetricae (rarissime arcuatae atque curvatae), juvenales distortae vel pone torquatae, (8–15)–16.6–28.2 (–38.2) µm longae, (1.2–1.5)–1.7–2.6(–3.2) µm latae, aut singulae aut plerumque binae et quaternae (raro octonae) in familias. Membrana cellulae glabra tenuisque. Cellulae singulo chloroplasto parietali, taeniato curvatoque, pyrenoide 1–2 (–4) globoso, strato amylaceo continuo. Multiplicatio fit autosporis binatim vel quaternatim (raro octonatim). Cellulae novellae intra integumentum mucosum in ordines consociatae. Cellulae adultae sine integumento mucoso.

Habitatio: Species planctonica vivens, laco Tanganyika, Africa.

Iconotypus: Figura nostra 19, ex collectione lacus Tanganyikae (Kigoma), Africa.

Collectio conservata: deposita in herbario universitatis Gandavae (GENT).

Description: Cells symmetrically fusiform (very rare – arcuately bend), at young stages twisted, with peculiar coiled ends, (8–15)–16.6–28.2–(38.2) µm long, (1.2–1.5)–1.7–2.6–(3.2) µm wide, generally arranged in groups of two, four or, more rare 8 cells due to rapid division and much more rare solitary, but even then appearing in groups of nearby located cells. Cell wall thin and smooth under LM, without mucilage in adult stages. Chloroplast one per cell, parietal, band-like and sigmoidal, often additionally curved trough-like with 1–2 (–4) rounded pyrenoids, with a continuous starch sheath under LM. Reproduction is by two or four (more rare, eight) autospores, which are generated after inclining cell division (simultaneous or successive) and gradually slip down, forming peculiar chains in fine, colourless mucilage strands, set up due to the gelatinization of the mother cell wall.

Etymology: The species epithethon *petkovii* is given in honour to the memory and life-work of the Bulgarian professor STEFAN PETKOV (1866–1951), who graduated at Ghent University in 1894 and then laid the fundamentals of Bulgarian phycology.

5. Observations and Discussion

The representatives of *Closteriopsis* were found in the quantitative (0–60 m) and semi-quantitative phytoplankton samples from the following dates: 26.11.2002, 11.03.2003, 25.03.2003, 08.04.2003, 22-23.04.2003, 06.05.2003, 20.05.2003, 10.06.2003, 24.06.2003, 16.09.2003, 11.11.2003, 25.11.2003, 11.12.2003, 23.12.2003 and 17.02.2004. The alga had more abundant development in the northern part (Kigoma site) and during the rainy season (October – May/June). In November and December 2003, as well as in February 2004 it appeared among the phytoplankton dominants.

The most striking peculiarity of the specimens studied is the seemingly rapid cell division due to which solitary cells were never observed, but the alga appears in chains of 2–4 (–8) cells or in groups of closely positioned cells, combined with the surrounding of the autospores and young cells by common colourless mucilage (Fig. 5–7, 12–22, 24). At the first sight the material resembles the colonies formed in the genera *Elakatothrix* WILLE, *Fusola* SNOW, *Quadrigula* PRINTZ, *Pseudoquadrigula* LACOSTE DE DIAZ and *Gregiochloris* MARVAN, KOMÁREK & COMAS (the taxonomy of which is still far away from being clear). However, in *Quadrigula, Pseudoquadrigula* and *Gregiochloris* the cell division is mainly along the cells with following contagious position of cells parallel to their longitudinal axis and in *Elakatothrix* and *Fusola* the division is transverse. In some species [e.g. *Gregiochloris chodatii* (TANNER-FULLMAN) MARVAN, KOMÁREK & COMAS, *G. lacustris* (CHODAT) MARVAN, KOMÁREK & COMAS] the formation of autospores has not been proved and it was suggested that cells divide crosswise into two parts (HINDÁK 1988). By contrast, in the material examined, after the inclining (and not longitudinal or cross) cell division (Fig. 3, 4, 9–11, 20–24) the autospores slip down and for some time remain contiguous by their ends, forming peculiar 2, 4 or, more rarely 8-celled chains in mucilage strands (Fig. 5–7, 9–17, 23, 24). In these chains the distance between the cells is different and increases with age (Fig. 12–17). The outline of the mucilage resembles the shape of the mother cell (Fig. 5, 12, 15). The cell division is not obligatory simultaneous, but can be also successive and then groups of three cells (one big and two small cells) appear (Fig. 20). Both types of successive and simultaneous cell division were observed in the material from another trebouxiophycean genus, *Oocystis* A. BRAUN, collected from the same lake (STOYNEVA & al., in prep.) The second difference to the five above mentioned genera is that the fine mucilage disappears with the cell growth and could not be seen around the adult cells even after staining with Indian Ink (Fig. 1, 2, 8, 18, 27). Until now the presence of a mucilage around the *Closteriopsis* representatives has not been mentioned, what is more – in all generic descriptions it was stressed that there is no mucilage around the cells, but its formation is a well-known phenomenon for most of the planktonic algae and in our opinion, it

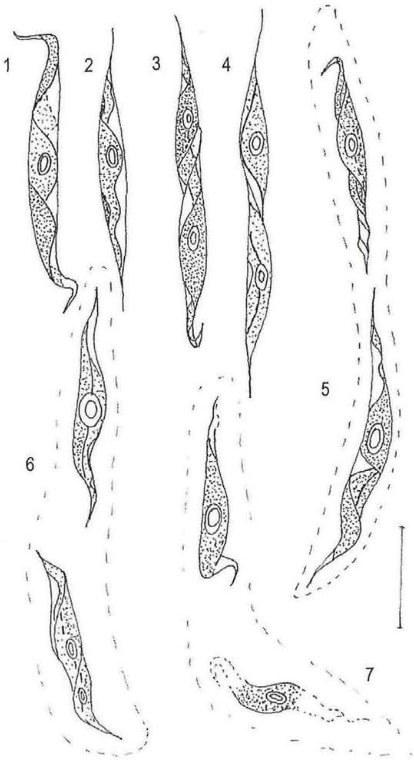

Fig. 1–7. *Closteriopsis petkovii*. – Fig. 1. Young cell. – Fig. 2. Adult cell. – Fig. 3–4. Autospores after inclining cell division. – Fig. 5–7. Autospores in mucilage strands. – Scale bar: 10 μm.

could have been simply overlooked by previous authors due to its fine, colourless character. On the other hand, the previous descriptions of mucilage presence or absence obviously concern only the adult cells and could not be accepted as a contradiction with the present observations. Moreover, taking into account the peculiar shape of the mucilage (which follows the outline of the mother cell), it could be proposed that it is a result of the gelatinization of the parental wall.

Another peculiarity of the *Closteriopsis*-cells is the shape of the young cells, which is symmetrically fusiform (very rare – arcuately bend), but twisted, with peculiar coils due to the very fine cell ends. These ends are in the juvenile stage in double or triple tight coils (Fig. 5, 22, 25) but with the age they become attenuated and more straight (Fig. 2, 12, 18, 27). However, very often the next cell division appears before the full attenuation of the cell ends. Commonly the both cell ends are twisted in different directions (Fig. 1, 18, 19, 24, 26) in an obvious relation with their autospore posi-

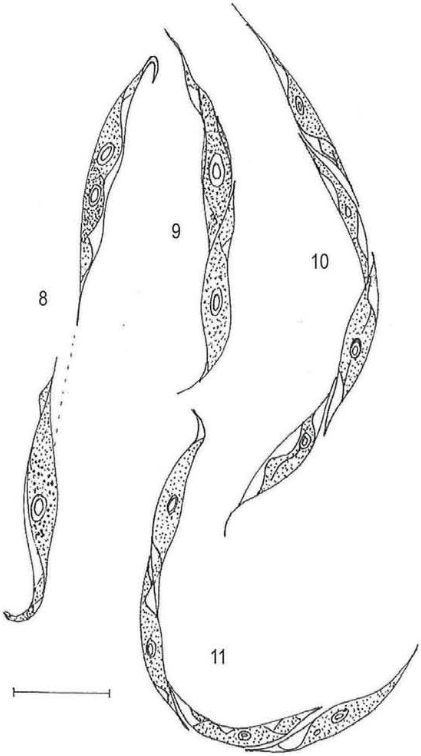

Fig. 8–11. *Closteriopsis petkovii*. – Fig. 8. Group of two closely situated cells. – Fig. 9. Two autospores. – Fig. 10–11. Four autospores. – Scale bar: 10 μm.

tioning (Fig. 3, 6, 20–25). Such type of twisting and coiling has not been noted for any described species of *Closteriopsis*. The fine coils of the cell ends in the material from Tanganyika are almost not to be seen at low magnifications (Fig. 13–17, 24), but are clearly observed with a 100x objective when immersion oil is applied (Fig. 1–11, 18–23, 25).

The fourth peculiarity of the specimens found is the chloroplast and pyrenoid organization, which resembles the features, described by HINDÁK 1970 for the edaphic taxon *Closteriopsis acicularis* var. *africana*. In this variety the autospores contain only one pyrenoid (as it is in the var. *acicularis*) but have a regularly sigmoid chloroplast (while in var. *acicularis* the chloroplast is band-shaped or through-shaped but always straight). In the planktonic material from Tanganyika each autospore has also one pyrenoid and a clear screw-shaped parietal chloroplast (Fig. 3–5, 7, 9–11, 21–23). The young cells until their complete development to the 'normal' cell shape also have one pyrenoid but with the cell growth the number could increase to 2 (–4) pyrenoids, while the chloroplast keeps its twisted, sig-

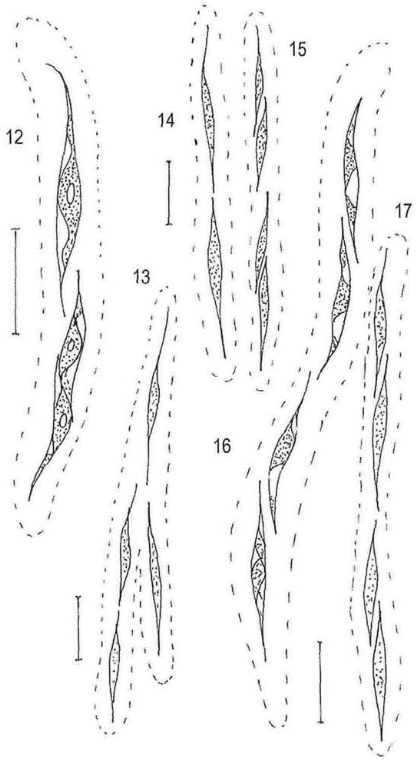

Fig. 12–17. *Closteriopsis petkovii*. – Fig. 12. Autospores in a mucilage strand after successive cell division. – Fig. 13–17. Autospores as seen on lower microscope magnifications. – Scale bars: 10 µm.

moidal character (Fig. 6, 8, 12, 18, 20, 25). Commonly the chloroplast becomes additionally bended and through-like, then seen with a pronounced lengthwise line (Fig. 4–6, 10, 23). The pyrenoids are relatively big, but not striking and could be easily overlooked without staining (Fig. 13–17, 24), especially at lower magnifications. According to the LM observations after staining with iodine solution, the starch sheath looks like a continuous ring (Fig. 3–9, 18–20, 22, 23, 25).

Sometimes relatively big oil droplets occur in the cells, a feature well-known for other members of *Trebouxiophyceae* (e.g. *Oocystis* A. Braun – Komarek & Fott 1983).

The dimensions of the cells in the material processed were the most variable characteristics being mainly dependent on the age. The autospores and very young cells were not only screwed, but were shorter and wider, while the adult cells were more thin and elongated. The dimensions measured were as follows: (1.2–1.5)–1.7–2.6–(3.2) µm width and (8–15)–16.6–28.2–(38.2) µm length (as straight line distance between the cell ends).

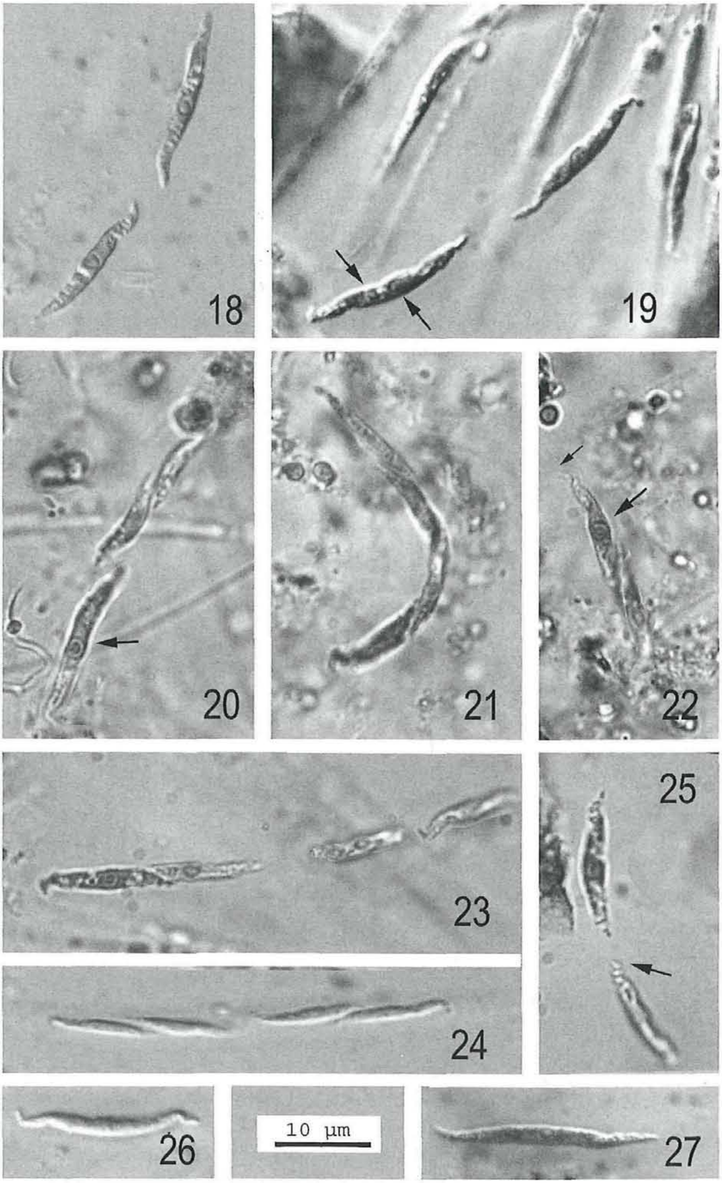

Fig. 18–27. *Closteriopsis petkovii*. – Fig. 18–19. Groups of cells (arrows indicate pyrenoids) stained with iodine solution. [Fig. 19 – iconotype]. – Fig. 20–23. Autospores (arrows indicate pyrenoid on Fig. 20 and coiled cell end on Fig. 22) stained with iodine solution. – Fig. 24. Autospores without staining. – Fig. 25. Young cells stained with iodine solution (arrow indicates the triple twisted cell end). – Fig. 26. Cell with ends twisted in different directions. – Fig. 27. Cell with attenuating ends. – Scale bar: 10 μm.

6. Acknowledgements

The study was carried out in the frame of the CLIMLAKE project EV/02/2C, financed by the Belgian Science Policy and in the frame of a research fellowship for the first author also financed by the Belgian Science Policy. The authors are grateful to the two local teams of scientists and technicians of TAFIRI (Tanzanian Fisheries Institute, Kigoma, Tanzania) and DOF (Department of Fisheries, Mpulungu, Zambia), who guaranteed the biweekly sampling in Lake Tanganyika.

7. References

COCQUYT C., VYVERMAN W. & COMPERE P. 1993. A check-list of the algal flora of the East African Great Lakes (Malawi, Tanganyika and Victoria). – Scripta bot. belg. 8: 1–55.

COULTER G. W. 1994. Lake Tanganyika. – Arch. Hydrobiol. Beih. Ergebn. Limnol. 44: 13–18.

ETTL H. & GÄRTNER G. 1988. Eine einfache Methode zur Darstellung der Struktur der Stärkehüllen von Pyrenoiden bei Grünalgen (*Chlorophyta*). – Archiv Protistenkunde 135: 179–181.

HEGEWALD E. & SCHNEPF E. 1986. Zur Struktur und Taxonomie spindelförmiger *Chlorellales* (*Chlorophyta*): *Schroederia, Pseudoschroederia* gen. nov., *Closteriopsis*. – Algol. Stud. 42: 21–48.

HINDÁK F. 1970. A contribution to the systematics of the family *Ankistrodesmaceae* (*Chlorophyceae*). – Algol. Stud. 1: 7–32.

— 1977. Studies of the chlorococcal algae (*Chlorophyceae*). I. – Biologické Práce 23 (4): 1–190.

— 1988. Studies of the chlorococcal algae (*Chlorophyceae*). IV. – Biologické Práce 34 (1–2): 1–262.

HUTCHINSON G. E. 1957. A treatise on limnology, I. Geography, physics and chemistry. 1015 pp. – New York.

JOHN D. & TSARENKO P. M. 2002. Chlorococcales. – In: JOHN D., WHITTON B.A. & BROOK A. J. (eds.), The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae, p. 327–408. – Cambridge.

KOMÁREK J. & FOTT B. 1983. Das Phytoplankton des Süsswassers. Systematik und Biologie. 7 Teil. *Chlorophyceae* (Grünalgen). Ordnung *Chlorococcales*. 1044 pp. – Stuttgart.

KORSCHIKOFF O. A. 1953. The Freshwater Algae of the Ukrainian SSR. V. Sub-class *Protococcineae*. *Vacuolales* and *Protococcales*. 412 pp. – Kiiv (in Ukrainian).

KRIENITZ L., USTINOVA I., FRIEDL T. & HUSS V. A. R. 2001. Traditional generic concepts versus 18S rRNA gene phylogeny in the green algal family *Selenastraceae* (*Chlorophyceae, Chlorophyta*). – J. Phycol. 37: 852–865.

— , HEGEWALD E., HEPPERLE D., HUSS V. A. R., ROHR T. & WOLF M. 2001. Phylogenetic relationship of *Chlorella* and *Parachlorella* gen. nov. (*Chlorophyta, Trebouxiophyceae*). – Phycologia 43 (5): 529–542.

LEMMERMANN E. 1899. Das Phytoplankton sächsischer Teiche. – Forschungsber. biol. Stat. Plön 7: 96–135.

PLISNIER P.-D., CHITAMWEBWA D., MWAPE L., TSIBANGU K., LANDENBERG V. & COENEN E. 1999. Limnological annual cycle inferred from physical-chemical fluctuations at three stations of Lake Tanganyika. – Hydrobiologia 407: 45–58.

SHERR E. B. & SHERR B. F. 1993. Preservation and storage of samples for enumeration of heterotrophic protists. – In: KEMP P. F., SHERR B. F., SHERR E. B. & COLE J. J. (eds.). Handbook of methods on aquatic microbial ecology, p. 207–212. – Boca Raton.

SHUBERT E. 2003. Nonmotile coccoid and colonial green algae. – In: WEHR J. D. & SHEATH R. G. (eds.), Freshwater Algae of North America. Ecology and classification, p. 253-309. – Amsterdam.

USTINOVA I., KRIENITZ L. & HUSS V. A. R. 2001. *Closteriopsis acicularis* (G. M. SMITH) BELCHER et SWALE is a fusiform alga closely related to *Chlorella kessleri* FOTT et NOVÁKOVÁ (*Chlorophyta, Trebouxiophyceae*). – Eur. J. Phycol. 36: 341–351.

UTERMÖHL H. 1931. Neue Wege in der quantitativen Erfassung des Planktons. – Verh. internat. Vereinigung theor. angew. Limnol. 5: 567–595.

WEST G. S. & FRITSCH F. E. 1927. A treatise on the British freshwater Algae. 534 pp. – Cambridge.