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Trochisciopsis tetraspora f. *minor* forma nova (*Chlorophyceae-Chlorococcaceae*) – a New Terrestrial Green Algal Taxon from Pirin Mts. (Bulgaria) and its Ultrastructure

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Summary

Gärtner G., Uzunov B. A., Stoyneva M. P., Kofler W & Ingolić E. 2010. *Trochisciopsis tetraspora* f. *minor* forma nova (*Chlorophyceae-Chlorococcaceae*) – a new terrestrial green algal taxon from Pirin Mts. (Bulgaria) and its ultrastructure. – Phyton (Horn, Austria) 50(1): 127–136, with 18 figures.

The present paper describes the first finding of a member of the rarely recorded soil green algal genus *Trochisciopsis* Vinatzer in Bulgaria. Aspects of its morphology and reproduction were investigated under LM and SEM and details of its ultrastructure were revealed with TEM. Based on these findings, a new form of the species *Trochisciopsis tetraspora* Vinatzer is described – *T. tetraspora* f. *minor* Gärtner,

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Uzunov, Stoyneva, Kofler & Ingolić. The new form is characterized by i) smaller cells, ii) ribs of the cell walls interrupted towards the pole, not meeting at the pole.

Zusammenfassung

Gärtner G., Uzunov B. A., Stoyneva M. P., Kofler W. & Ingolić E. 2010. *Trochisciopsis tetraspora* f. *minor* forma nova (*Chlorophyceae-Chlorococcaceae*) – a new terrestrial green algal taxon from Pirin Mts. (Bulgaria) and its ultrastructure. [*Trochisciopsis tetraspora* f. *minor* forma nova (*Chlorophyceae-Chlorococcaceae*) – eine neue terrestrische Grünalgenform vom Pirin-Gebirge (Bulgarien) und deren Ultrastruktur]. – Phyton (Horn, Austria) 50 (1): 127–136, mit 18 Abbildungen.

Aus Bodenproben vom Pirin-Gebirge in Bulgarien wurde erstmals ein Vertreter der Grünalgengattung *Trochisciopsis* Vinatzer isoliert und in Kultur genommen. Untersuchungen der Morphologie und Reproduktion mittels Licht- und Elektronenmikroskopie (REM und TEM) führten zur Beschreibung einer neuen Form *Trochisciopsis tetraspora* Vinatzer f. *minor* Gärtner, Uzunov, Stoyneva, Kofler & Ingolić. Die neue Form ist durch folgende Merkmale charakterisiert: 1) kleinere Zellen; 2) Zellwandrippen an den Zellpolen unterbrochen, nicht zusammenschließend.

1. Introduction

The coccal green algal genus *Trochisciopsis* Vinatzer (*Chlorophyta-Chlorophyceae-Chlorococcaceae*) is peculiar by its sculptured cell wall with well pronounced ribs and a terrestrial mode of life (Vinatzer 1975; Trenkwalder 1975). There are several other green coccoid soil algae with meridional ribs, which belong to the genera *Coelastrella* Chodat incl. *Graesiella* Kalina & Punčochářová (*Chlorophyceae, Scenedesmaceae*), *Scotiella* Fritsch (*Chlorophyceae, Oocystaceae*) and *Scotiellopsis* Vinatzer (*Chlorophyceae-Scenedesmaceae*) (Kalina & Punčochářová 1987; Gärtner & Ingolić 1993; Ettl & Gärtner 1995; Hanagata 1998; Hegewald & Hanagata 2000, 2002; Tschaikner & al. 2007a, b, 2008). In spite of the general morphological similarity of the cell wall ornamentation, *Trochisciopsis* is clearly differentiated from the other mentioned genera by its mode of reproduction, in which both zoospores and autospores are involved (Komárek & Fott 1983; Ettl & Gärtner 1995) as well as by the absence of a pyrenoid. The genus *Trochisciopsis* consists of two species – *T. tetraspora* Vinatzer and *T. insignis* Trenkwalder, which have been studied by light microscopy (LM) and scanning electron microscopy (SEM). Until now these species are known only from a few mountain localities in Northern Italy and Ukraine (Vinatzer 1975; Trenkwalder 1975; Kostikov & al. 2001). They differ slightly in cell dimensions, in the number and shape of the cell wall ribs and in the asexual life cycle (Table 1). Peculiar for the asexual reproduction of the genus is the indirect formation of autospores (or zoospores) after obligatory division of the mother cell into one or more tetrads (Fig. 1).

The present paper reports on the first record of *Trochisciopsis* in Bulgaria from soils of Pirin Mts. and its ultrastructure by transmission electron microscopy (TEM). The results on cell morphology obtained by LM and SEM show clear differences to both other species of this genus and allow to describe the new taxon *Trochisciopsis tetraspora* f. *minor* GÄRTNER, UZUNOV, STOYNEVA, KOFLER & INGOLIĆ forma nova.

2. Material and Methods

The study is based on 24 samples collected in August 2006 from 12 localities in Pirin Mts., National Park and UNESCO Monument of Cultural and Natural Heritage, described in detail in UZUNOV & al. 2008. From each site two samples were collected (at 2 and at 10 cm below the soil surface) in sterile plastic tubes (10 cm^3 in volume). After removal of extraneous materials (e.g. stones, worms, parts of leaves or roots) in the laboratory a liquid soil solution was prepared (1 part soil with 2 parts distilled water) and its pH was measured with pHep 3 Hanna pH-meter. The algal cells from the soil solution were inoculated onto agar plates by using the atomized cell spray technique (PRINGSHEIM 1946; ETTL & GÄRTNER 1995; ANDERSEN 2005). The plates were incubated and after colony formation selected cells were removed and inoculated onto a new agar plate by the most common method for single-cells isolation by micropipette performed with Pasteur pipette or a glass capillary. After repeatedly proceeding, axenic clonal cultures were obtained and kept in sterile agar tubes. Bold's Basal Medium (BBM – BISCHOFF & BOLD 1963) was used routinely throughout the study. Soil samples and cultures are deposited in the Algal Collection of the Department of Botany of Sofia University 'St Kliment Ohridski' (ACUS, Bulgaria).

Observations of colony characteristics and isolation of single cells or colonies for axenic cultures were made with Motic stereomicroscope SFC 11 at 10x and 30x magnification. Light microscopic investigations were done using a Diapan Microscope Reichert with objectives 10x, 25x, 63x and 100x (oil immersion). Photomicrographs were taken with a Moticam 2000 camera attached to the Motic BA 400 or Reichert microscopes with special adaptors. For photoprocessing the computer software 'Motic Images Plus 2.0' was used. Cell walls were stained with Methylene Blue and starch was coloured with Lugol's solution (ETTL & GÄRTNER 1995).

For scanning electron microscopy (SEM) investigation algal cells were dehydrated in gradually increasing ethanol concentrations (up to 96% ethanol), transferred to formaldehyde-dimethyl-acetal (FDA, dimetoxymethane (GERSTBERGER & LEINS 1978) for 24 hours and 2 hours, critical-point dried with CO_2, sputter-coated with palladium/gold and examined with a Philips XL20 SEM microscope.

For transmission electron microscopy (TEM) algae were fixed in 3% glutaraldehyde in 0.1 mol cacodylate buffer pH 6.8–7.2 for 24 hours and postfixed with 1% OsO_4 in 0.1 mol cacodylate buffer for several hours. After dehydration in ethanol/acetone and embedding in Spurr's resin (SPURR 1969), ultrathin sections were cut with a diamond knife (Leica UCT microtome) and stained with 1% aqueous uranyl acetate and lead citrate (REYNOLDS 1963). Micrographs were taken with a Philips 300 transmission electron microscope.

3. *Trochisciopsis tetraspora* VINATZER f. *minor* GÄRTNER, UZUNOV, STOYNEVA, KOFLER & INGOLIĆ, forma nova

Diagnosis: differt a *T. tetraspora* VINATZER f. *tetraspora* cellulis minoribus (fere 10 μm diametro) et intermissione costae membranae apicalium.

Habitatio: in terra montis Pirin, 2090 m, Bulgaria.

Iconotypus: Figura nostra 2.

Cultura: deposita in thesaurum algarum universitatis Sofiensis (ACUS) sub numero ACUS-S 12.

Description: The new form differs from *T. tetraspora* VINATZER f. *tetraspora* only by constantly smaller dimensions, with an average cell diameter of 10 μm and by the fact that the line of ribs is interrupted towards the pole.

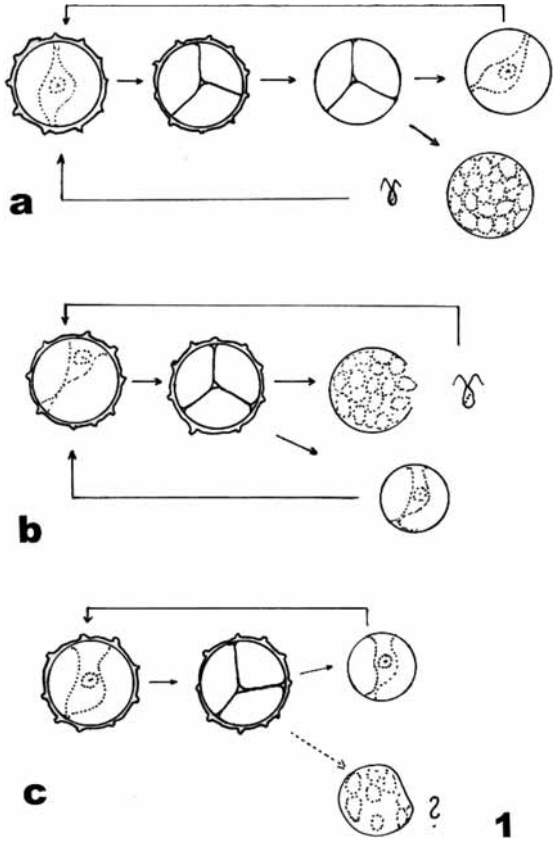

Fig.1. Comparative schema of reproduction of *Trochisciopsis insignis* (a), *T. tetraspora* f. *tetraspora* (b) and *T. tetraspora* f. *minor* (c), where a and b are organized according to the descriptions by TRENKWALDER 1975 and VINATZER 1975.

4. Observations and Discussion

During the recent investigations of soils in Pirin Mts. in Bulgaria a member of the genus *Trochisciopsis* was found to grow in the enrichment culture from one site underneath trees of *Pinus peuce* Griseb. at 2090 m a.s.l. It developed very well (as a pure culture) only when BBM drops were added to the agar plate. Colonies were seen by 'naked eye' as dark green spots with granular surfaces.

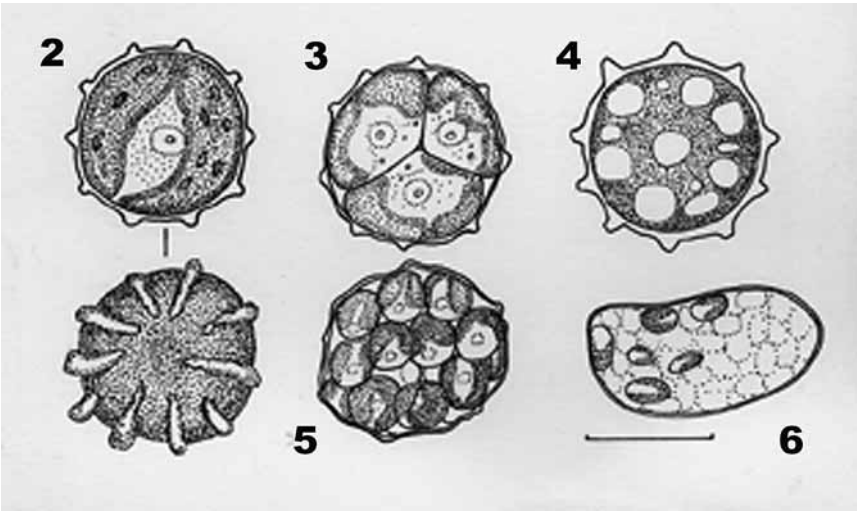

Fig. 2–6. – Fig. 2. *Trochisciopsis tetraspora* f. *minor* in axenic culture from Pirin Mts. (Bulgaria). – Fig. 3. First reproduction tetrad with ornamented cell wall. – Fig. 4. Old cell with vacuoles. – Fig. 5. Autosporangium with 16 autospores. – Fig. 6. Elongated cell with 32 immobile cells (?zoosporangium). – Scale bar 10 μm.

Cells are solitary, spherical, 9.5–12–18(–20) μm in diameter (Fig. 2, 7, 9, 12). The size depends on the age of the culture – young cells are up to 10–12 μm, while some of the old cells reach 18 (–20) μm. Even in 3-years old cultures the cell diameters do not exceed these dimensions.

The cell wall is thick, lamellated and ornamented with 10–12 coarse meridional ribs (Fig. 2, 7, 13), covered by the external cell wall layer (Fig. 14). The ribs are rough and knobby, especially in old cells, where they are clearly visible in LM without staining with Methylene Blue (Fig. 8, 12). The ribs do not join at their ends and therefore the cell pole region itself is free of them (Fig. 2, 10). In some cells the ribs are short, incomplete or interrupted (Fig. 11). The chloroplast is typical for the genus: parietal, cup-shaped and divided in two (or rarely four) wide lobes by deep fissures (Fig. 3, 15, 16). The lack of a pyrenoid, which is a generic feature, was proved by TEM investigations (Fig. 15, 16). Starch grains are lens-shaped,

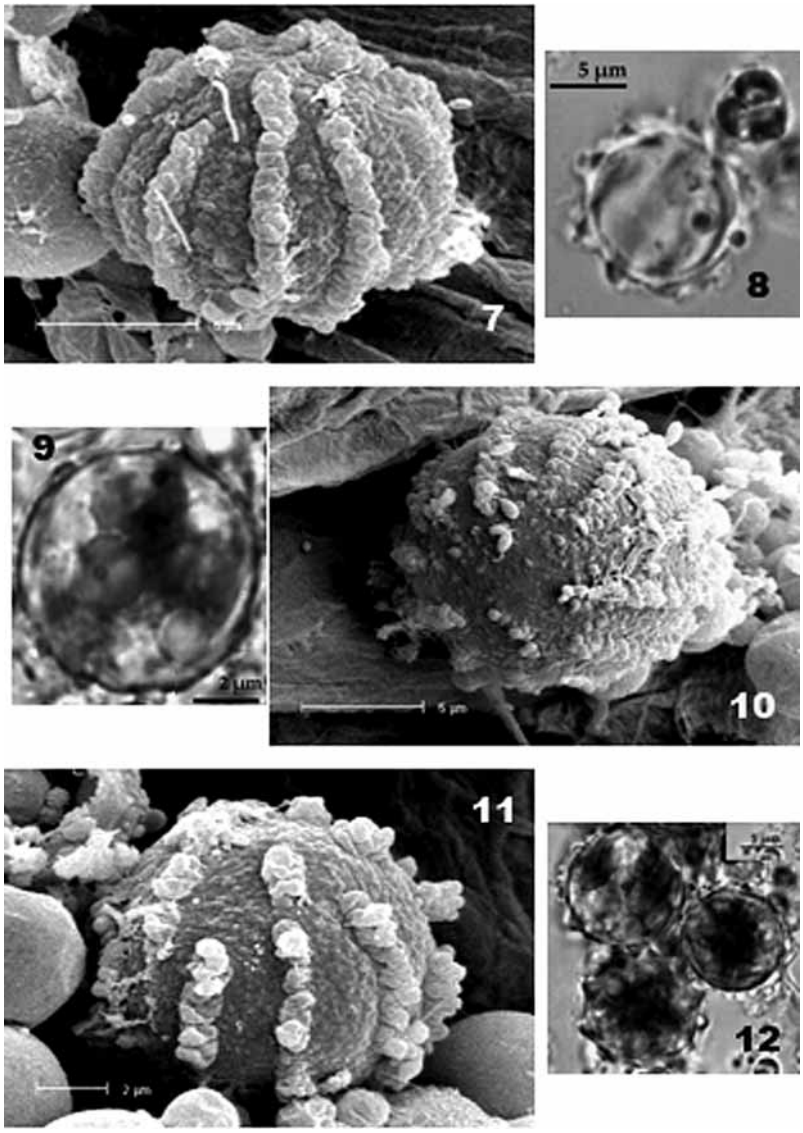

Fig. 7–12. LM and SEM photos of *Trochisciopsis tetraspora* f. *minor*: – Fig. 7. SEM photo of a cell with well developed coarse meridional ribs (scale bar 5 µm). – Fig. 8. LM photo of a cell with 10 ribs (scale bar 5 µm). – Fig. 9. LM photo of an old cell with well visible nucleus and vacuoles (scale bar 2 µm). – Fig. 10. SEM photo of a cell with well developed coarse meridional ribs and free polar area (scale bar 5 µm). – Fig. 11. SEM photo of a young cell with incomplete and interrupted meridional ribs (scale bar 2 µm). – Fig. 12. LM photo of group of cells in axenic culture from Pirin Mts. (Bulgaria) (scale bar 5 µm).

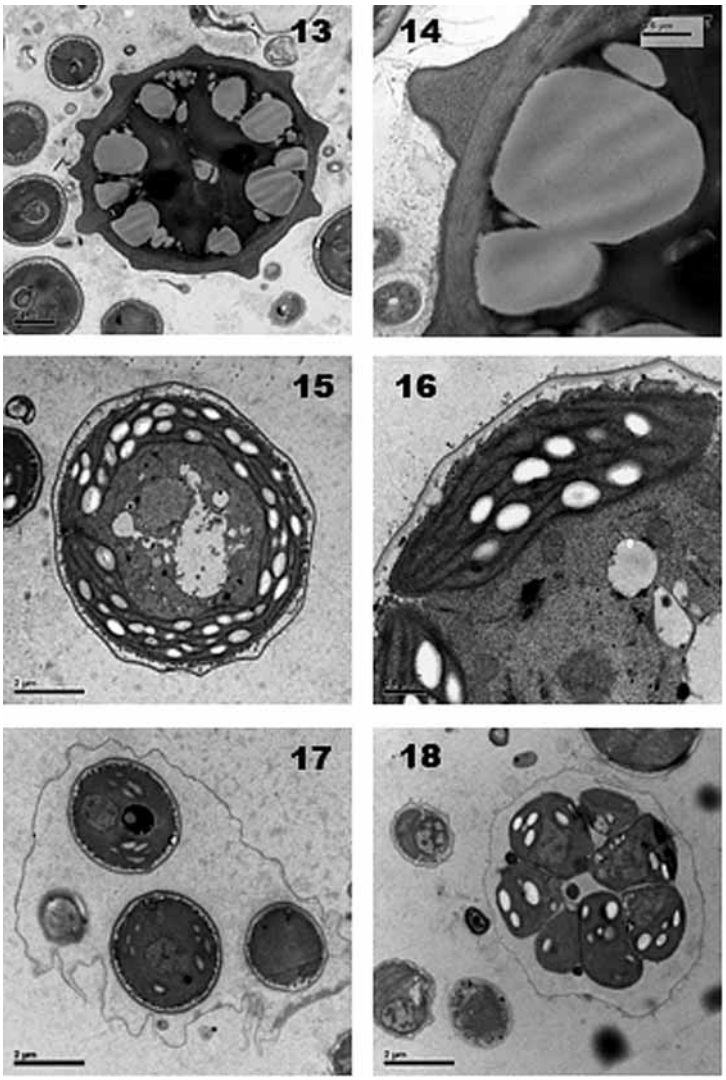

Fig. 13–18. TEM photos of *Trochisciopsis tetraspora* f. *minor* in axenic culture from Pirin Mts. (Bulgaria). – Fig.13. Old cell of *T. tetraspora* f. *minor* with 10 cell wall ribs and numerous vacuoles inside (scale bar 2 μm). – Fig. 14. Part of the same old cell (Fig. 13) with lamellated cell wall, one cell wall rib and three vacuoles (scale bar 0.5 μm). – Fig. 15. A young cell with beginning of rib formation, nucleus and two-lobed parietal chloroplast with many lens-shaped starch grains inside (scale bar 2 μm). – Fig. 16. A young cell with beginning of rib formation, nucleus and four-lobed parietal chloroplast with many lens-shaped starch grains inside (scale bar 0.5 μm). – Fig. 17. Autosporangium (scale bar 0.5 μm). – Fig. 18. Autosporangium with tetrad developed in 8 autospores (scale bar 0.5 μm).

lying in the chloroplast stroma between the thylakoids (Fig. 15. 16). The nucleus is large and easily visible between the chloroplast lobes (Fig. 9, 16). Old cells contain numerous vacuoles (Fig. 4, 9, 13, 14).

Asexual reproduction is by 2–16 autospores released after one tetrad formation (Fig. 1, 3, 5). Most of the autospores and young cells have cell walls without ribs (Fig. 5, 17). Ornamentation generally appears by aging but sometimes it is also visible inside the autosporangial wall. In spite of attempts to stimulate zoosporulation in liquid BBM medium, zoospores have not been observed. A single big elongated cell with 32 immobile daughter cells was detected once, which could be an incomplete zoosporangium (Fig. 6).

Table 1. Diagnostic features of *Trochisciopsis insignis, T. tetraspora* f. *tetraspora* and *T. tetraspora* f. *minor.*

TAXON/ DIAGNOSTIC FEATURE	***T. insignis*** (after TRENKWALDER 1975)	***T. tetraspora* f. *tetraspora*** (after VINATZER 1975)	***T. tetraspora* f. *minor***
Cell dimensions	20–35 (–60)	20–30 (–40)	9.5–12–18 (–20) μm
Ribs organization	16–18, knobby, not interrupted, meridional but join before the poles	10–12, knobby, not interrupted, meridional, most of which join at the poles	10–12, knobby, sometimes interrupted, meridional but not join at the poles
Asexual reproduction	Many (?) autospores and zoospores; autospores with smooth walls	Many (?) autospores and zoospores; autospores with smooth walls	2–16 autospores with smooth walls; zoospores not observed
Number of tetrad cell-formations and their cell wall ornamentation	More than one, only the first with ornamented wall	Only one, with sculptured cell wall	Only one, with sculptured cell wall

The comparison of our results with the diagnostic features of *Trochisciopsis tetraspora* and *T. insignis* (Table 1) clearly shows that the alga found in Pirin soil is very close to *T. tetraspora*, but differs in constantly smaller dimensions and by the fact that the line of ribs is interrupted towards the pole, which may justify its description as a new form.

5. References

ANDERSEN R. (ed.) 2005. Algal culturing techniques. 578 pp. – Phycological Society of America, Elsevier Academic Press, London.

BISCHOFF H. W. & BOLD H. C. 1963. Phycological studies. IV. Some soil algae from Enchanted Rock and related algal species. – Univ. Texas Publication No. 6318: 1–95.

ETTL H. & GÄRTNER G. 1995. Syllabus der Boden-, Luft- und Flechtenalgen. 721 pp. – Gustav Fischer, Stuttgart, Jena & New York.

GERSTBERGER P. & LEINS P. 1978. Rasterelektronenmikroskopische Untersuchungen an Blütenknospen von *Physalis philadelphica* (*Solanaceae*) – Anwendung einer neuen Präparationsmethode. – Ber. deutsch. bot. Ges. 91: 381–387.

GÄRTNER G. & INGOLIĆ E. 1993. Zur Morphologie und Taxonomie einiger Bodenalgen (Unterfamilie *Scotiellocystoideae, Chlorellaceae*) aus der Algensammlung in Innsbruck (ASIB, Austria). – Arch. Protistenk. 143: 101–112.

HANAGATA N. 1998. Phylogeny of the subfamily *Scotiellocystoideae* (*Chlorophyceae, Chlorophyta*) and related taxa inferred from 18S ribosomal RNA gene sequence data. – J. Phycol. 34: 1049–1054.

HEGEWALD E. & HANAGATA N. 2000. Phylogenetic studies on *Scenedesmaceae* (*Chlorophyta*). – Algol. Stud. 100: 29–49.

HEGEWALD E. & HANAGATA N. 2002. Validation of the new combinations of *Coelastrella* and *Neodesmus* and the description of the new subfamily *Desmodesmoideae* of the *Scenedesmaceae* (*Chlorophyta*). – Algol. Stud. 105: 7–9.

KALINA T. & PUNČOCHÁŘOVÁ M. 1987. Taxonomy of subfamily *Scotiellocystoideae* FOTT 1976 (*Chlorellaceae, Chlorophyceae*). – Algol. Stud. 45: 473–521.

KOMÁREK J. & FOTT B. 1983. *Chlorococcales*. – In: HUBER-PESTALOZZI G. (ed.), Das Phytoplankton des Süßwassers. 1043 pp. – Schweizerbart, Stuttgart.

KOSTIKOV I. Yu., ROMANENKO P. O., DEMCHENKO E. M., DARIENKO T. M., MIHAYLYUK T. I., RIBCHINSKIY O. V. & SOLONENKO A. M. 2001. Algae in soils of Ukraine. (History of the study and nomenclatural types of species). 300 pp. – Fitosotsiotsentr, Kiev (In Ukrainian).

PRINGSHEIM E. G. 1946. Pure cultures of algae, their preparation and maintenance. 119 pp. – Cambridge University Press, Cambridge.

REYNOLDS E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. – J. Cell Biol. 17: 208–212.

SPURR A. R. 1969. A low viscosity epoxy resin embedding for electron microscopy. – J. Ultrastruct. Res. 26: 31–43.

TRENKWALDER H. 1975. Neue Bodenalgen aus Föhrenwäldern im Raum von Brixen (Südtirol, Italien). – Ber. naturwiss.-med. Ver. Innsbruck 62: 7–19.

TSCHAIKNER A., INGOLIĆ E. & GÄRTNER G. 2007a. Observations in a new isolate of *Coelastrella terrestris* (REISIGL) HEGEWALD & HANAGATA (*Chlorophyta, Scenedesmaceae*) from alpine soil (Tyrol, Austria). – Phyton (Horn, Austria) 46(2): 237–245.

TSCHAIKNER A., INGOLIĆ E., STOYNEVA M. P. & GÄRTNER G. 2007b. Autosporulation in the soil alga *Coelastrella terrestris* (*Chlorophyta, Scenedesmaceae, Scenedesmoideae*). – Phytologia balcanica 13: 29–34.

TSCHAIKNER A., GÄRTNER G. & KOFLER W. 2008. *Coelastrella aeroterrestrica* n. sp. (*Chlorophyta, Scenedesmoideae*) – a new, obviously often overlooked aeroterrestrial species. – Algol. Stud. 128: 11–20.

UZUNOV B., STOYNEVA M. P., GÄRTNER G. & KOFLER W. 2008. First record of *Coelastrella* species (*Chlorophyta: Scenedesmaceae*) in Bulgaria. – Ber. naturwiss.-med. Ver. Innsbruck 95: 27–34.

VINATZER G. 1975. Neue Bodenalgen aus den Dolomiten. – Pl. Syst. Evol. 123: 213–235.

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