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First record of *Coelastrella* species (Chlorophyta: Scenedesmaceae) in Bulgaria

by

Blagoy A. UZUNOV, Maya P. STOYNEVA, Georg GÄRTNER & Werner KOFLER *)

Synopsis: The present paper provides information on the first finding of two green algal species of the genus *Coelastrella* (*C. terrestris* (REISIGL) HEGEWALD et HANAGATA and *C. aeroterrestrica* TSCHAIKNER, GÄRTNER et KOFLER) from different soils of Pirin Mt (Bulgaria) with data on their morphology in light microscope (LM) and in scanning electron microscope (SEM). Comparison with the same species from alpine soils in Tyrol (Austria) was done and the establishment of *C. aeroterrestrica* as a good species was proved. It is supposed that further careful investigations of algal cultures from soil will reveal the broader distribution of both species in Bulgaria and other countries in Europe.

Keywords: soil algae, *Coelastrella terrestris*, *Coelastrella aeroterrestrica*, green algae, Pirin Mt, Bulgaria

1. Introduction:

The earliest investigation of the soil algae in Bulgaria could be traced back to the end of 19th century, when *Botrydium granulatum* (GREV.) FISCHER was recorded by PETKOFF (1898-1899). Since then 228 species, 17 varieties and 10 forms of soil algae have been reported for the country by different authors (detailed list of references and taxa is available in UZUNOV et al. 2007, 2008). In the lists of algae published on Bulgarian edaphophyton, cyanoprokaryotes prevailed and are followed by green algae (Chlorophyta and Streptophyta), which were represented by 61 species and 7 varieties. Among them the representatives of the peculiar green algal genus *Coelastrella* CHODAT were not mentioned. Some soil algae have been reported from different Bulgarian mountains (Stara Planina, Vitosha, Vlahina, Rouy, Rila and Rodopi) but yet there are no records of this interesting ecological group from Pirin Mt – National Park and UNESCO Monument of Cultural and

*) Addresses of the authors: Assist. Mag. Blagoy Uzunov and Assoc. Prof. Dr. Maya P. Stoyneva, Department of Botany, Faculty of Biology, Sofia University 'St Kliment Ohridski', 8 bld Dragan Zankov, 1164-Sofia, Bulgaria; Univ.-Prof. Dr. Georg Gärtner and Werner Kofler, Institut für Botanik der Leopold-Franzens-Universität Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria. E-Mails: blagoy_uzunov@abv.bg; mstoyneva@abv.bg; georg.gaertner@uibk.ac.at; werner.kofler@uibk.ac.at

Natural Heritage. The present paper provides information on the first finding of two *Coelastrella* species (*C. terrestris* (REISIGL) HEGEWALD et HANAGATA and *C. aeroterrestrica* TSCHAIKNER, GÄRTNER et KOFLER) from soils of Pirin Mt with data on their morphology in light microscope (LM) and in scanning electron microscope (SEM).

2. Material and methods:

The study is based on 24 samples collected in August 2006 from 12 localities in Pirin Mt (Table 1). The soil types were characterized after DONOV (1973), BONDEV (1990) and BOZHILOV (1999) - Table 1. From each soil locality two samples were collected (at 2 and at 10 cm below the soil surface) in sterile plastic tubes (10 cm^3 in volume). In the laboratory, after removal of extraneous materials (e.g. stones, worms, parts of leaves or roots) a liquid soil solution was prepared (1 part soil with 2 parts distilled water) and its pH measured with pHep 3 Hanna pH-meter (Table 1). The algal cells from the soil solution were inoculated onto agar plates by using of 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 in 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'.

Tab. 1: Sampling sites and localities in Pirin Mt (Bulgaria), their coordinates in Military Grid Reference System (MGRS), altitude (Alt. - meters above sea level), vegetation dominant and soil type (LMMS – leached mountain meadow soils, MFS – maroon forest soil, MFDS – mountain forest dark soil, MMPS – mountain meadow peaty soil, MMS – mountain meadow soil, RS – rendsina soil) with pH value in brackets.

Site	Locality	MGRS	Alt.	Soil type	Vegetation dominant
1	Kresna Gorge	34TGM7967324232	227	MFS (7)	*Juniperus excelsa* BIEB.
2	Nearby hut 'Yavorov'	34TFM9768932914	1740	MFDS (6.5)	*Pinus heldreichii* CHRIST.
3	Above hut 'Yavorov'	34TFM9656931459	2206	RS (8)	*Pinus mugo* TURRA
4	Below peak Razlozhki Souhodol	34TFM9604630139	2569	MMPS (6)	*Thymus pirinicus* (VEL.) JALAS
5	Peak Vihren	34TFM9940526715	2915	MMS (8)	-
6	Below peak Vihren	34TFM9974327196	2583	MMS (8)	*Leontopodium alpinum* (TEN.) TUTIN
7	Nearby hut 'Bunderitsa'	34TGM0140626772	1872	MFDS (7.5)	*Pinus heldreichii* CHRIST.
8	Above hut 'Vihren'	34TGM0074024479	2132	MFDS (5)	*Pinus mugo* TURRA
9	Above hut 'Demyanitsa'	34TGM0459424192	2090	MFDS (5)	*Pinus peuce* GRSEB.
10	Gazeyski Cirque	34TGM0574020733	2241	LMMS (5)	*Bruckenthalia spiculifolia* (SALISB.) RCHB.
11	Near to lake Bezbog	34TGM0997523149	2249	LMMS (6)	*Pinus mugo* TURRA
12	Below hut 'Bezbog'	37TGM1065624687	1960	LMMS (5.5)	*Pinus peuce* GRSEB.

Observations of colony characteristics and isolation of single cells or colonies for axenic cultures were made with Motic stereomicroscope SFC 11 at a magnification 10x and 30x. Light microscopic investigations were made with 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 processing of the photos 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 in 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.

3. Results and discussion:

The coccal green algal genus *Coelastrella* CHODAT is peculiar by its sculptured cell wall with well pronounced ribs. There are several other green coccoid terrestrial algae with meridional ribs, which belong to the genera *Scotiella* FRITSCH, *Scotiellopsis* VINATZER and *Graesiella* KALINA et PUNČOCHÁŘOVÁ (KALINA & PUNČOCHÁŘOVÁ 1987, ETTL & GÄRTNER 1995). The modern taxonomy of these genera with partial rearrangement of taxa is based mainly on the studies by HANAGATA (1998) and HEGEWALD & HANAGATA (2000, 2002) and was most recently discussed by TSCHAIKNER et al. (2007ab, 2008), who established a new species – *C. aeroterrestrica* in addition to the already wellknown *C. terrestris* (REISIGL) HEGEWALD et HANAGATA (with basionym *Scotiella terrestris* REISIGL and synonyms: *Scotiellocystis terrestris* (REISIGL) FOTT, *Scotiellopsis terrestris* (REISIGL) PUNČOCHÁŘOVÁ et KALINA and *Scenedesmus terrestris* (REISIGL) HANAGATA). The both species are clearly separated according to their morphology in LM and SEM studies (GÄRTNER & INGOLIĆ 1993, TSCHAIKNER et al. 2007a).

During the recent investigations *Coelastrella terrestris* was found to grow separately in the enrichment culture from one locality (Site 6 from Table 1), while in the cultures from sites 2 and 4 it was found to grow together with *C. aeroterrestrica* (Fig. 7). In the text below their morphological features and mode of reproduction are described consecutively.

Coelastrella terrestris is characterized by broadly ellipsoidal or lemon-shaped cells, 7-9.5 µm broad and 9 .5-13.5 µm long (Figs. 1a-d, 3, 4). The meridional ribs on the cell wall of living cells are hardly visible in LM (mainly after staining with Methylene Blue) and were better and easier seen on dead, empty cells (Fig. 3). The ribs (8-14 in number) are well visible in SEM studies (Figs. 7-12). Some of the ribs do not end at the cell poles (Fig. 8) but this could depend on culture conditions, as it was earlier proposed by TSCHAIKNER et al. (2007a). Chloroplast is parietal, single in young cells and divides in fragments with cell ageing. Pyrenoid is one, well visible, with a sheath of two or three starch plates (Figs. 1, 3, 4). Additional fine starch grains could be seen in the chloroplast (Fig. 1a). Reproduction by 2-4-(8) autospores has been observed (Fig. 1e, 3).

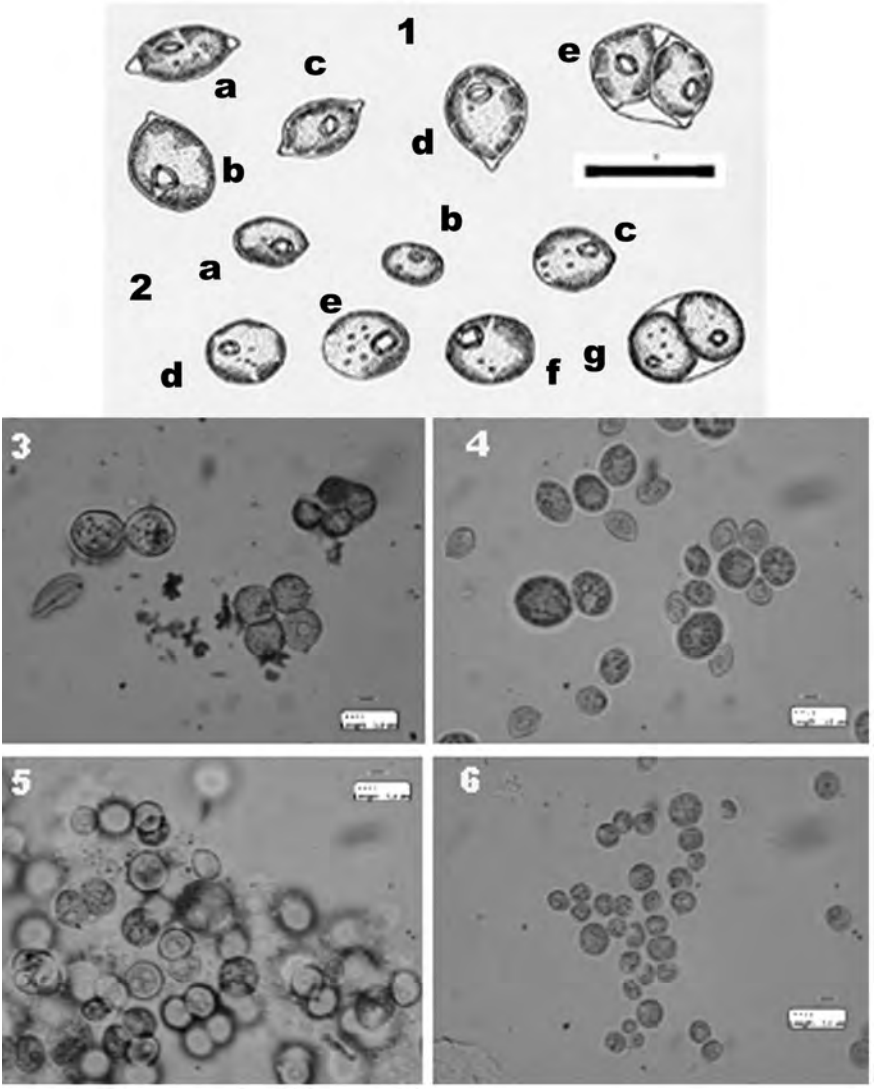

Fig. 1: *Coelastrella terrestris*, clonal culture from Site 2 (Pirin Mt, Bulgaria). Scale bar: 10 µm.
Fig. 2: *Coelastrella aeroterrestrica*, clonal culture from Site 4 (Pirin Mt, Bulgaria). Scale bar: 10 µm.
Fig. 3: LM photos of *Coelastrella terrestris* after staining with Methylene blue, clonal culture from Site 4 (Pirin Mt, Bulgaria). Scale bar: 5 µm.
Fig. 4: LM photos of *Coelastrella terrestris*, clonal culture from Site 2 (Pirin Mt, Bulgaria). Scale bar: 5 µm.
Fig. 5: LM photos of *Coelastrella aeroterrestrica*, clonal culture from Site 4 (Pirin Mt, Bulgaria). Scale bar: 5 µm.
Fig. 6: LM photos of *Coelastrella aeroterrestrica*, clonal culture from Site 4 (Pirin Mt, Bulgaria). Scale bar: 5 µm.

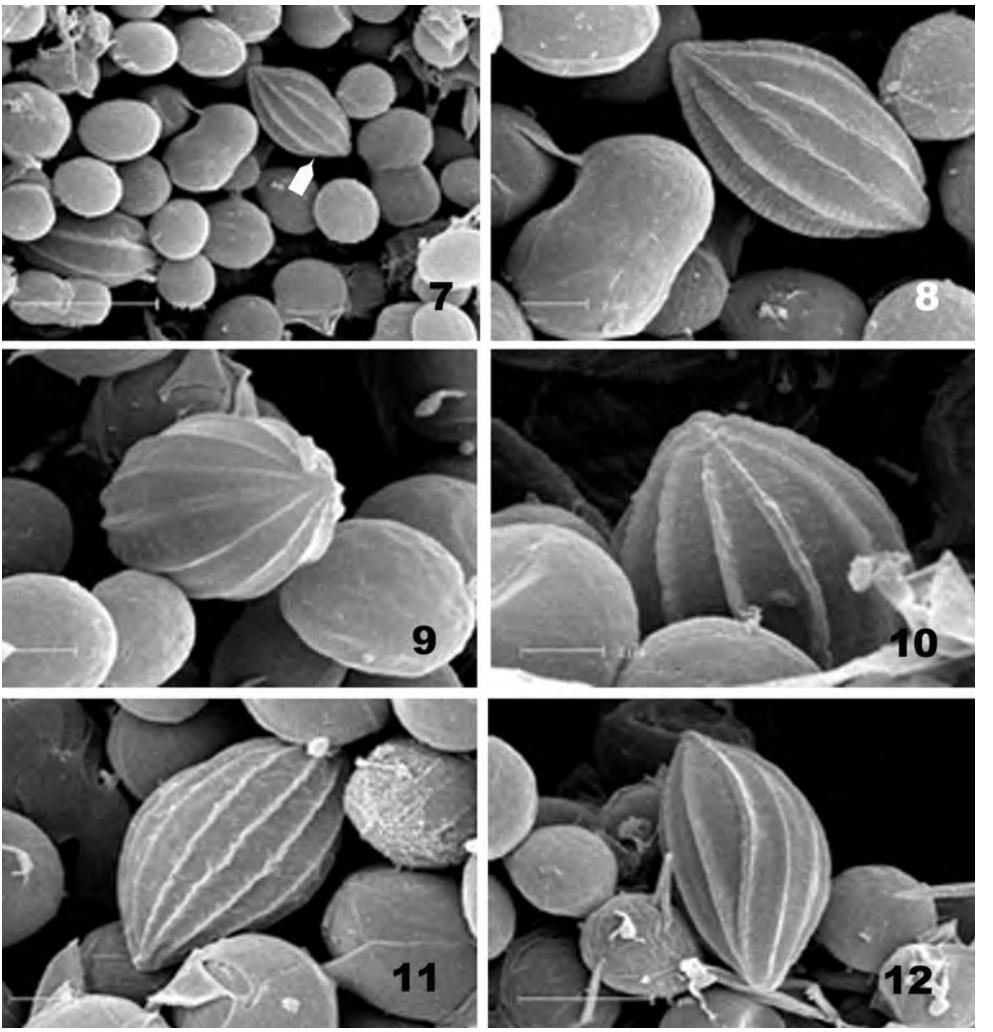

Fig .7-12: SEM photos of *Coelastrella terrestris* (indicated by the arrow) and *Coelastrella aeroterrestrica*, non-clonal culture from Site 2 (Pirin Mt, Bulgaria). 7 - *C. terrestris* and *C. aeroterrestrica*; 8-12 - single cells of *Coelastrella terrestris* with visible meridional ribs on the cell walls. Scale bars indicated on the photos: 5 μm.

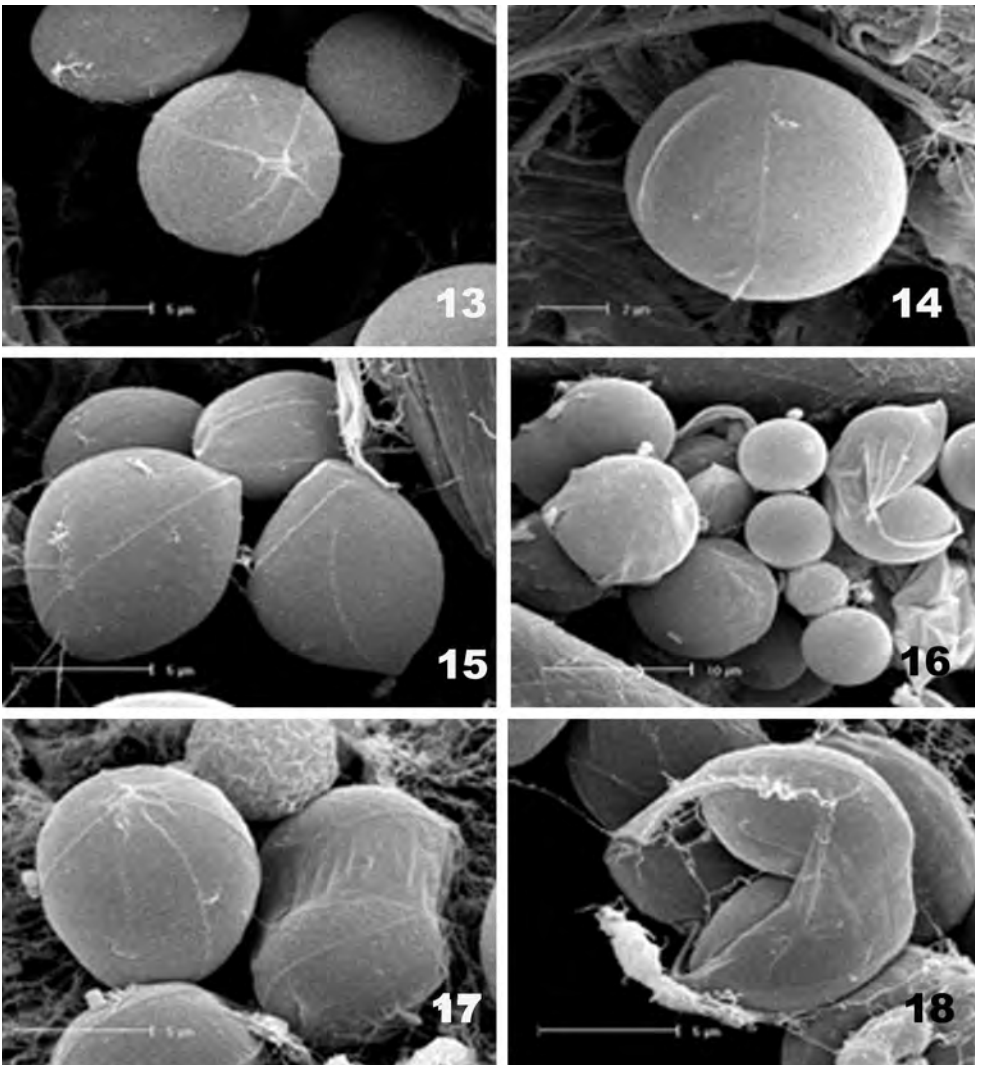

Fig. 13-18: SEM photos of *Coelastrella aeroterrestrica*, clonal culture from Site 4 (Pirin Mt, Bulgaria). 13-15 - single cells of *C. aeroterrestrica* with fine irregularly situated ribs on the cell walls; 16-17 - autosporangia with two autospores and single cells of *C. aeroterrestrica;* 18 - autosporangium with four autospores (3 visible) of *C. aeroterrestrica* before liberation. Scale bars indicated on the photos: 5 µm.

Coelastrella aeroterrestrica is characterized by globular (9.5-13.5 µm in diameter) and rarer broadly ellipsoidal cells (5.5-7 x 5.5-8 µm) – Figs. 2a-f, 5, 6. Sometimes slight thickenings of the cell walls were observed (Fig. 2c). The longitudinal ribs on the cell wall of living cells are very fine, almost invisible in LM (the same valid even after staining with Methylene blue). However, the fine network of ribs (up to 6) is well visible in SEM (Figs.13-18). Chloroplast is parietal, single, cup-shaped and deeply incised in old cells (Fig. 2). The single prominent pyrenoid is surrounded by two to five starch plates (Figs. 2a-g). Additional fine starch grains could be seen in the chloroplast (Fig. 2). Reproduction by 2-(4)-8 autospores has been observed (Figs. 2g, 16-18).

The materials studied coincide well with the former isolated strains of both species as they are described in TSCHAIKNER et al. (2007ab) from alpine soils in Tyrol (Austria) and confirm the establishment of the second species *Coelastrella aeroterrestrica* TSCHAIKNER et al. (2008). The present study of different soils from Pirin Mt is the first documentation of the genus and it's both species for Bulgaria, but it is to be expected that further careful investigations of culture material will reveal its broader distribution in other soils of Bulgaria and other countries in Europe.

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