# Autosporulation in the soil alga *Coelastrella terrestris* (*Chlorophyta*, *Scenedesmaceae*, *Scenedesmoideae*)

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***Dedicated to Assoc. Prof. Dr Stefan Draganov in honour of his 75th anniversary***

**Abstract.** A strain of *Coelastrella terrestris* was isolated from alpine soil near the village Obergurgl (Tyrol, Austria) and taken in culture. Its morphology and reproduction were studied by light and electron microscopy. Special attention was paid to the asexual reproduction process by autospores, which are formed as true autospores. Their characteristic cell wall sculpture is formed during their development within the sporangium and is also known from other species of the genus.

**Key words:** autosporulation, electron microscopy, chlorophytes, morphology, *Scenedesmoideae*, soil algae

## Introduction

Among the chlorococcalean algae of the family *Chlorellaceae* (in the sense of Komárek & Fott 1983) some terrestrial taxa are known for the peculiar sculpture of their cell walls. Therefore, when members of the genera *Coelastrella* Chodat, *Scotiellopsis* Vinatzer, *Graesiella* Kalina & Punčoch., etc. should be identified with light microscope, cell morphology and especially wall sculptures can be very helpful. However, they are difficult for observation without a careful staining procedure. Many cytomorphological details (e.g. wall layers, chloroplast and pyrenoid structures), reproduction processes and, finally, the taxonomy of many strains – mainly based on cultivated material – have been elucidated so far with the help of the electron microscope (Punčochářová & Kalina 1981; Kalina & Punčochářová 1987; Gärtner & Ingolić 1993). Recently, the taxonomy of the above-named genera was partially changed, according to the phylogenetic molecular studies of Hanagata (1998) and Hegewald & Hanagata (2000, 2002) and, according to the latter, *Coelastrella terrestris* (Reisigl) E.H. Hegew. & Hanagata was placed within the subfamily *Scenedesmoideae* in the family *Scenedesmaceae*. During recent investigations of the soil algal flora in alpine regions near Obergurgl (Ötztal, Tyrol, Austria), some strains of *C. terrestris* were isolated by A. Tschaikner and taken into culture. Besides a more general investigation of the morphology and taxonomy of these strains by Tschaikner & al. (in press), this paper presents detailed observations of the asexual reproduction process by autosporulation, as compared with former studies on cultivated isolates from different European regions.

## Materials and methods

Soil samples were collected at 2350 m a.s.l. and 1 g from these samples was diluted in 99 ml distilled water, while aliquots of 0.2 ml were spread on agar plates solidified with Bold's Basal Medium (BBM, Bischoff & Bold 1963). Cultures were incubated under standard conditions at 10–13 °C, illuminated by fluorescent lamps at 20–30 µmol $m^{-2}sec^{-1}$ on a 12:12 h light:dark cycle. After 6–12 weeks of growth, axenic unialgal colonies were transferred into culture tubes with BBM-agar and deposited in the Algal Culture Collection of the Botanical Institute in Innsbruck, Austria (strains SWK 3:16, 24, 53; observations were made on strain SWK 3:53). Details of sampling procedure, locality and cultivation techniques are mentioned in Ettl & Gärtner (1995), Gärtner (1996) and Tschaikner & al. (in press).

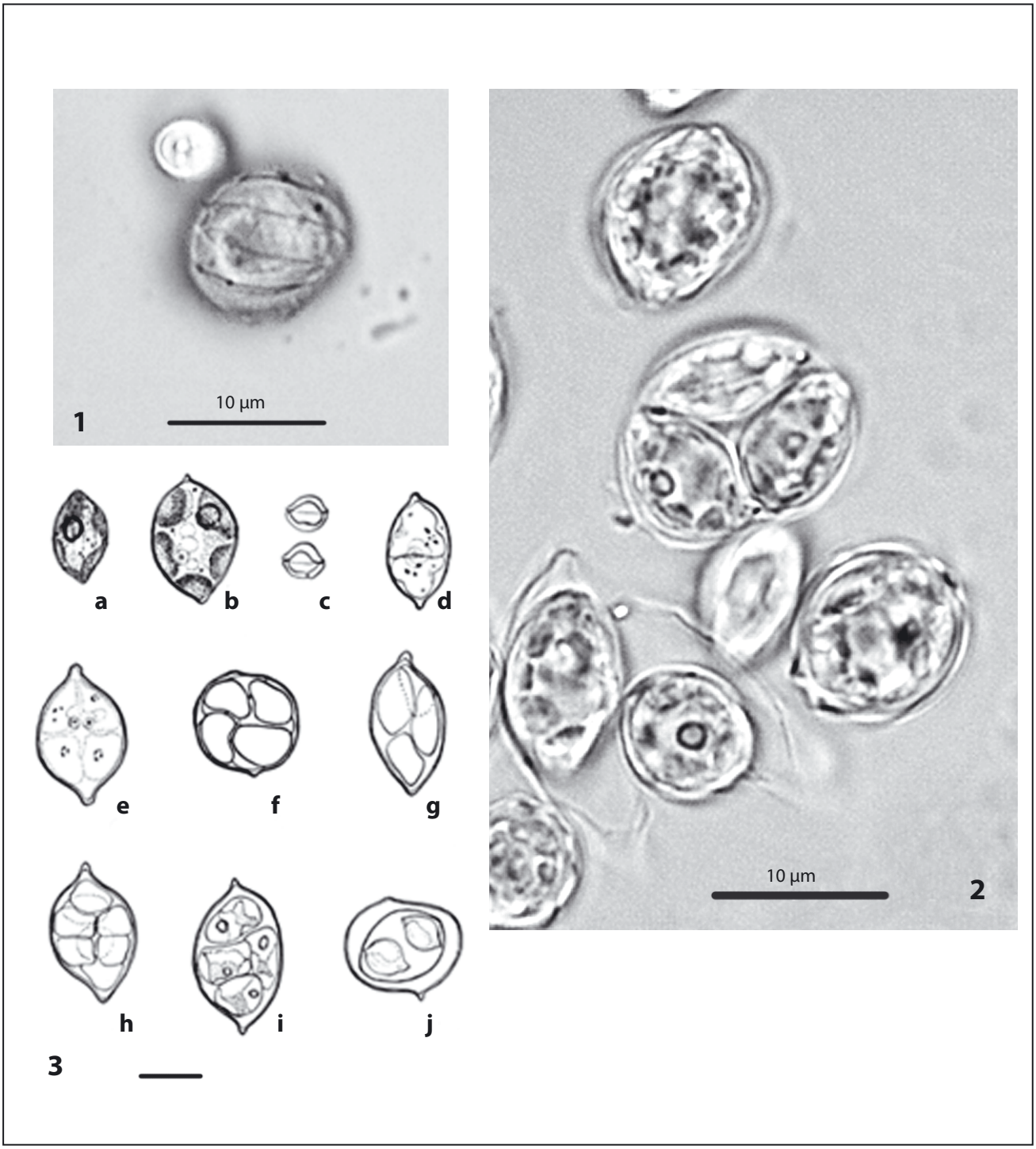
For light microscopy (LM), an Olympus BH–2 light microscope with Olympus PM–10 AK (Automatic Exposure Photomicrographic System) camera, or optionally a ProgRes C10 plus Jenoptic digital camera and PICed Cora image analysis software (Jomesa Meßsysteme GmbH) were used. Isolation and purification of algal colonies was done with a Wild M8 stereo microscope and an Olympus SZH 10 research stereo microscope. Cell walls were stained with methylene blue, starch with Lugol`s iodine solution and/or chlorine-iodine, while for pyrenoids the Azocarmine – G staining was applied. Additionally, neutral red for vacuoles and carmine acetic acid for nuclei were used. Identification was made on the basis of cell- and colony morphology, using standard authoritative references (e.g. Komárek & Fott 1983; Ettl & Gärtner 1995; John & al. 2002). All drawings were made of living material grown in culture, with light microscope at a magnification of 1000× (oil immersion). For transmission electron microscopy (TEM), colonies 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 alcohol/acetone and embedding in Spurr's resine (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. For scanning electron microscopy (SEM) investigation, algal cells were fixed in 3 % glutaraldehyde in 0.1mol cacodylate buffer pH 6.8–7.2, dehydrated in acetone, critical-point dried with $CO_2$ (after Anderson 1951), sputter-coated with gold-palladium, and examined with a Zeiss DSM 982 Gemini SEM microscope.

## Results

Vegetative cells of *C. terrestris* are solitary, broadly ellipsoidal or lemon-shaped, with a sculptured cell wall of 6–8–10 (-12) meridional ribs which join on both apices into wart-like thickenings (Plate I, Figs 1, 2, 3a, b). The parietal chloroplast divides during growth into several fragments, one of them containing a pyrenoid with a starch sheath consisting of 2 (3) dish-like starch plates (Plate I, Fig. 3c; Plate II, Fig. 2-ss). Vacuoles within the cell lumen are often formed. Dimensions: adult cells between 14–22 µm long, 6–15 µm wide.

Asexual reproduction runs in adult cells by the formation of autospores. The process is connected with a slight enlargement of the cells which function as sporangia. The cells, which originally were more or less regular lemon-shaped, or ellipsoidal, changed into sporangia with a broadly rounded irregular form. Before the first protoplast division the pyrenoid becomes indistinct, only some small starch grains are visible (seemingly the pyrenoid is formed *de novo* in the daughter cells (Plate I, Fig. 3d). After the following successive divisions, the daughter protoplasts are arranged tetrahedrically (Plate I, Fig. 2), or in a single plane when cruciately divided (Plate I, Fig. 3e). Their morphology was slightly angular, following the shape of the mother cell (Plate I, Fig. 3f, g, h). The number of daughter protoplasts could be 2–8 (even 16). After final division, in each daughter protoplast the chloroplast with pyrenoid appears in its full shape (Plate I, Fig. 3i). The starch sheath of the pyrenoid, consisting of two or three starch plates, is clearly visible with LM, especially when stained with Lugol's iodine solution (Plate I, Fig. 3i). Occasionally, subsequent autospore formation takes place within the original sporangium and two autospore generations can be found (Plate I, Fig. 3j). The secretion of the unique autospore cell wall with meridional ribs starts during the development phase within the sporangium but is hardly visible with LM, even after staining with methylene blue. Ultrathin sections across the autosporangia clearly show that autospores possess their complete cell wall

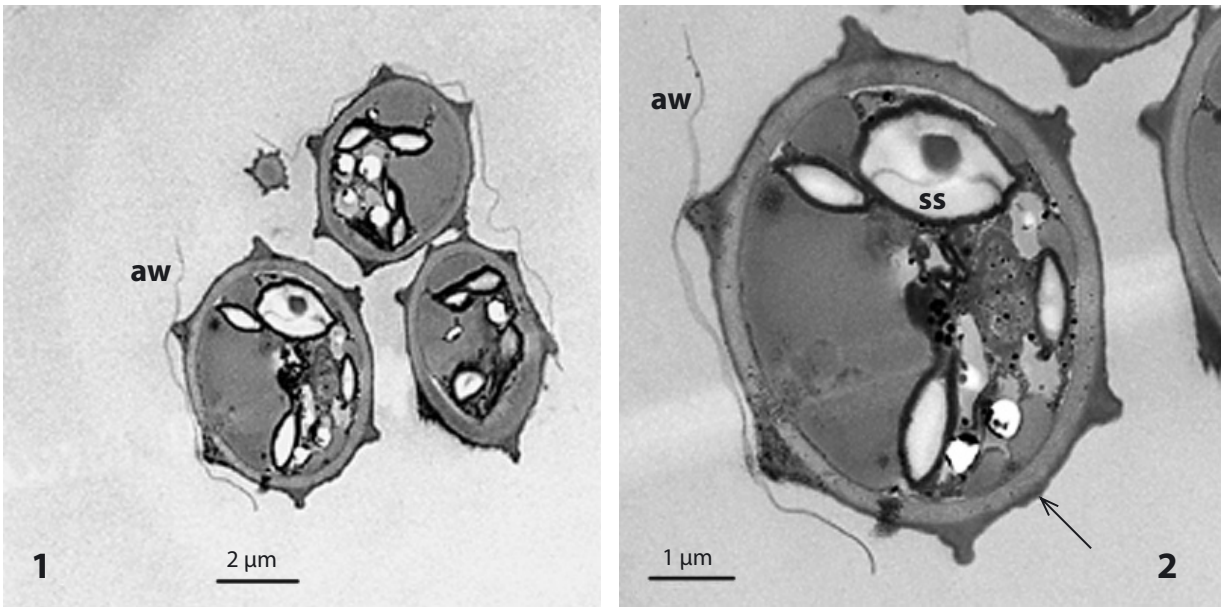
**Plate I**

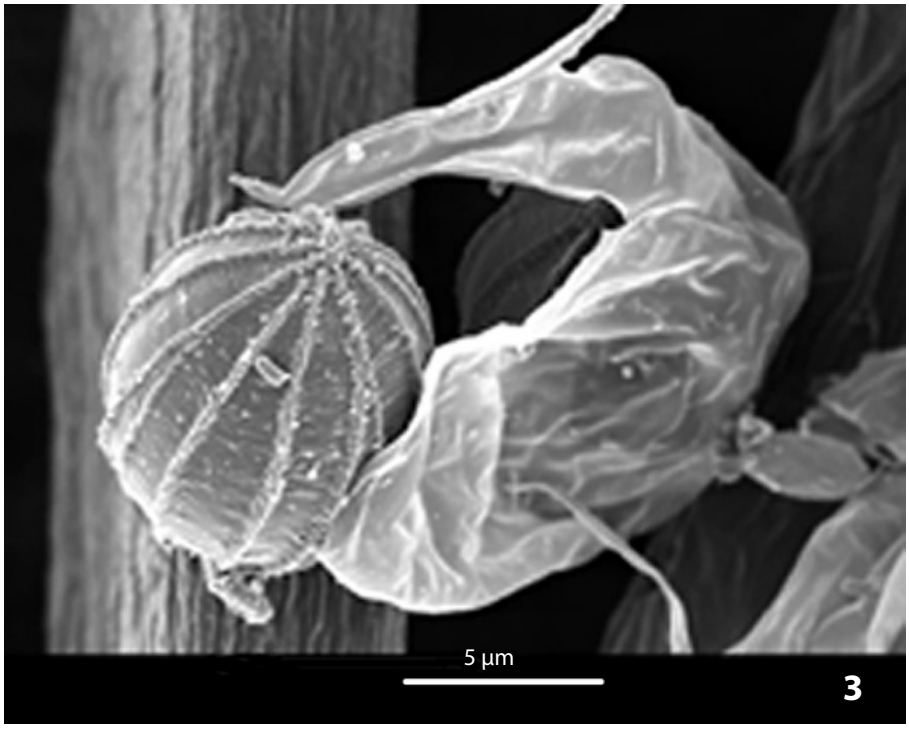



**Figs 1–3.** *Coelastrella terrestris*:

**1,** vegetative cells of *Coelastrella terrestris* strain SWK 3/53, meridional wall ribs stained with methylene blue; **2,** vegetative cells and autosporangium with autospores arranged tetrahedrically; **3a**, vegetative cell in juvenile stage; **3b**, adult vegetative cell with vacuoles; **3c,** starch sheath of the pyrenoid (enlarged); **3d,** first protoplast division, pyrenoid indistinct; **3e,** four daughter protoplasts (two nuclei visible) in tetrahedric arramngement; **3f, 3g, 3h,** daughter protoplasts after successive divisions in different positions within the sporangial wall; **3i,** young autospores with chloroplasts and pyrenoids; **3j,** 2 autospore generations within the primary sporangial wall (after the first division only one autospore divided further), autospores with delicate wall ribs. Scale bar = 10 µm (except **3c**).

**Plate II**




**Figs 1–3.** *Coelastrella terrestris*:

**1, 2,** ultrathin sections across the autosporangium of strain SWK 3/53 in TEM, autospores with wall ribs; **3,** release of autospores through the rupture of sporangial wall in SEM.
*Abbreviations*: **aw** – autosporangial wall; **ss** – starch sheath of pyrenoid; **arrow** – outer trilaminar cell wall component.

with ribs, when released. In most cases 6–8 (-12) meridional ribs cover the autospores before the release (Plate II, Figs 1, 2). The characteristic double-layered cell wall of *Coelastrella* cells, with an inner cellulose component and an outer trilaminar one, is visible even in autospores (Plate II, Fig. 2-arrow). The ruptured autosporangial wall is also visible (Plate II, Fig. 2-aw). Release of autospores takes place after rupture of the sporangial wall (Plate II, Fig. 3).

## Discussion

Cytomorphological features in the formation of autospores in the studied *C. terrestris*-isolate coincide with the earlier investigations by Punčochářová & Kalina (1981) and Gärtner & Ingolić (1993) of strains from soil and moist habitats from different European regions (Czech Republic, Germany, Austria, Italy, Tschaikner & al. in press). The number of autospores in most isolates is 2–8. When 16 autospores are formed, their wall ribs are almost invisible with LM within the sporangium, because of their small size. After staining with methylene blue, fine linear structures on the cell apices mark the beginning of the wall rib formation (Plate I, Fig. 3j). This was shown earlier in drawings by Punčochářová & Kalina (1981: 139), but the process of autospore formation was not described by these authors. Disappearance of the pyrenoid during the autospore formation process and its reappearance in the young autospores within the sporangium is very common in coccoid green algae and was discussed by Ettl (1988a, b). The remarkable processes of transformation of the protoplast compartments (simplification of chloroplast structures, dissolving of pyrenoid etc.) and reorganization after the autospore formation are typical characters of the asexual reproduction by sporulation and stand in contrast with the processes of cell division (Ettl 1988b). A subsequent autosporulation, where autospores in the sporangium divide again, which leads to the formation of two autospore generations was first observed in the culture of *C. multistriata* var. *multistriata* Kalina & Punčoch. by Kalina & Punčochářová (1987). It occurred also in our cultures but was mostly restricted to two autospores only (Plate I, Fig. 3j). Punčochářová & Kalina (1981) were the first to document the rupture of sporangia and the release of autospores by SEM on sporangia of *Scotiellopsis rubescens* Vinatzer (taxonomy and phylogenetic position of this terrestrial alga is still unclear), followed later by Gärtner & Ingolić (1993:105) on a strain determined as *Scotiellopsis terrestris* (Reisigl) Punčoch. & Kalina. Release of two autospores from the sporangium through a rupture in the sporangial wall could be seen on Plate II, Fig. 3. The autospores have all their meridional ribs and polar thickenings; they have developed completely within the sporangium and are real miniatures of vegetative cells (Ettl 1988b). Ultrathin sections across the autospores show that all cell wall components are developed before the realease. This includes also the double-layered cell wall with an inner cellulose component and an outer trilaminar one (Plate II, Figs 1, 2). Such composition is known from members of *Chlorellaceae* (Atkinson & al. 1972) and other chlorophytes (Rodriguez & al. 1999; Graham & Wilcox 2000). The outer trilaminar layer contains sporopollenin-like substances (algaenans ?) and this explains the great resistance to desiccation of the studied terrestrial alga and related species (Kalina & Punčochářová 1987; Gärtner & Ingolić 1993; Tschaikner & al. in press).

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