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Phyton (Horn, Austria)	**Vol. 46**	Fasc. 2	237–245	11. 6. 2007

Observations in a New Isolate of *Coelastrella terrestris* (REISIGL) HEGEWALD & HANAGATA (*Chlorophyta, Scenedesmaceae*) from Alpine Soil (Tyrol, Austria)

By

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With 11 Figures

Received November 7, 2006

Key words: *Coelastrella terrestris, Scenedesmaceae.* – Green algae, soil algae.- Austrian Alps.

Summary

TSCHAIKNER A., INGOLIĆ E. & GÄRTNER G. 2007. 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, with 11 figures.- English with German summary.

During a recent investigation of the algal flora from alpine soils around the village Obergurgl (Tyrol, Austria), a coccoid green alga with peculiar wall ornamentation was isolated and taken into culture. The strain was assigned to the genus *Coelastrella* (syn. *Scotiellopsis*) and to the species *C. terrestris*. The cytomorphological characters revealed by light and electron microscopy agree with those of similar strains from different localities in Europe. It became obvious that *Coelastrella terrestris* is confined to terrestrial habitats and only occasionally a member of the kryoflora. More than 40 years after the first record, it is again reported from the area of the type collection. Comments on the current problems in taxonomy and nomenclature are included.

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Zusammenfassung

TSCHAIKNER A., INGOLIĆ E. & GÄRTNER G. 2007. Beobachtungen an einem neuen Isolat von *Coelastrella terrestris* (REISIGL) HEGEWALD & HANAGATA (*Chlorophyta, Scenedesmaceae*) aus alpinen Böden (Tirol, Österreich). – Phyton (Horn, Austria) 46(2): 237–245, mit 11 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Im Rahmen einer Untersuchung der Algenflora alpiner Böden im Gebiet von Obergurgl (Tirol, Österreich) wurde eine coccale Grünalge mit auffallender Zellwandskulptur isoliert und in Kultur genommen. Das Isolat ließ sich der Gattung *Coelastrella* (syn. *Scotiellopsis*) und der Art *C. terrestris* zuordnen. Licht- und elektronenmikroskopische Untersuchungen zeigten cytomorphologische Übereinstimmung mit ähnlichen Isolaten aus anderen Gebieten Europas. Es wurde deutlich, dass *Coelastrella terrestris* fast ausschließlich in terrestrischen Habitaten und nur gelegentlich im Artenspektrum der Kryoflora vorkommt. Über 40 Jahre nach der Entdeckung konnte die Art neuerlich im Gebiet der Typusaufsammlung nachgewiesen werden. Auch die derzeitigen Probleme der Taxonomie und Nomenklatur werden kommentiert.

1. Introduction

Among terrestrial coccoid green algae, taxa with a peculiar structure of the cell wall surface with meridional ribs have been known for a long time. In 1912, FRITSCH established the genus *Scotiella*, which was regarded as the only terrestrial genus with such a cell wall type for more than 50 years. Some species of *Scotiella* were later on considered to be cysts of, e.g., cryophilic flagellates (HOHAM & MULLET 1978). Reproduction and biology of nearly all *Scotiella* species are more or less unknown and their taxonomy is still not clear (KOMÁREK & FOTT 1983, HANAGATA 1998). REISIGL 1964 described *Scotiella terrestris*, with 6 – 12 meridional ribs and autosporulation, from alpine soil (Ötztal, Tyrol). The genus *Scotiellopsis* was established by VINATZER 1975, with the type strain *S. rubescens* VINATZER, isolated from alpine soil of the Dolomites (Italy). The significant differences to *Scotiella* are the more delicate ribs and the smaller dimensions of the cells. PUNČOCHÁŘOVÁ & KALINA 1981 investigated all species of *Scotiellopsis* by electron microscopy and transferred *Scotiella terrestris* REISIGL to this genus. In 1987, KALINA & PUNČOCHÁŘOVÁ studied *Scotiellopsis* again, as well as related taxa like *Coelastrella* CHODAT and *Graesiella* KALINA & PUNČOCHÁŘOVÁ, and placed them among others in a subfamily *Scotiellocystoideae* of *Chlorellaceae* (detailed information and chronology in KALINA & PUNČOCHÁŘOVÁ 1987, GÄRTNER & INGOLIĆ 1993 and HANAGATA 1998). Based on phylogenetic studies by HANAGATA 1998 and HEGEWALD & HANAGATA 2000, 2002, the taxa previously assigned to *Scotiellopsis, Coelastrella*, and *Graesiella* were partially rearranged. *Scotiellopsis terrestris* is now placed as *Coelastrella terrestris* (REISIGL) HEGEWALD & HANAGATA within the subfamily *Scenedesmoideae* (HEGEWALD & HANAGATA 2002).

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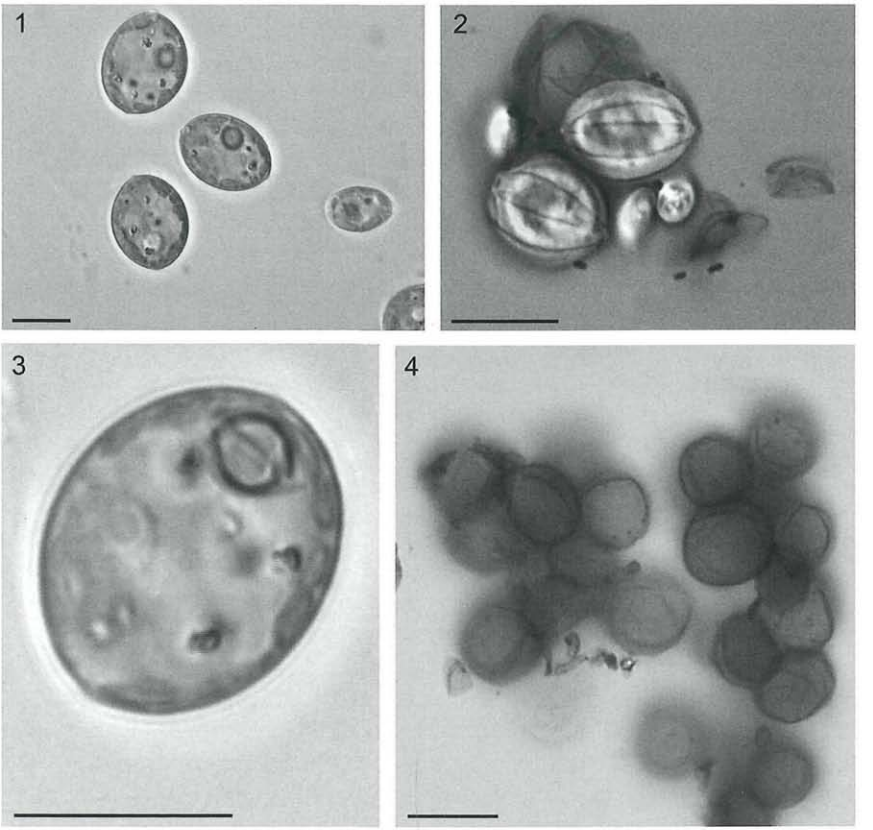

Fig. 1 – 4. *Coelastrella terrestris* isolates SWK 3 in LM. – Fig. 1. SWK 3:16, vegetative cells. Scale bar: 10 µm. – Fig. 2. SWK 3:53, vegetative cells stained with methylene blue, the wall ribs are visible in LM. Scale bar: 10 µm. – Fig. 3. SWK 3:53, vegetative cell with pyrenoid and starch sheath consisting of two starch plates. Scale bar: 10 µm. – Fig. 4. SWK 3:16, empty cell walls after acetolysis, stained with methylene blue. Scale bar: 10 µm.

During recent investigations of the algal flora of alpine soils near the village Obergurgl (Ötztal, Tyrol), strains of *Coelastrella terrestris* were isolated and taken into culture, more than 40 years after the species had been discovered (Reisigl 1964, as *Scotiella terrestris*). The cytomorphological characters observed by light (LM) and electron microscopy (EM) agree well with those of earlier isolates of this taxon. *Coelastrella terrestris* is apparently confined to soil, only once it was recorded from the kryoflora of red and green snow (Punčochářová & Kalina 1981).

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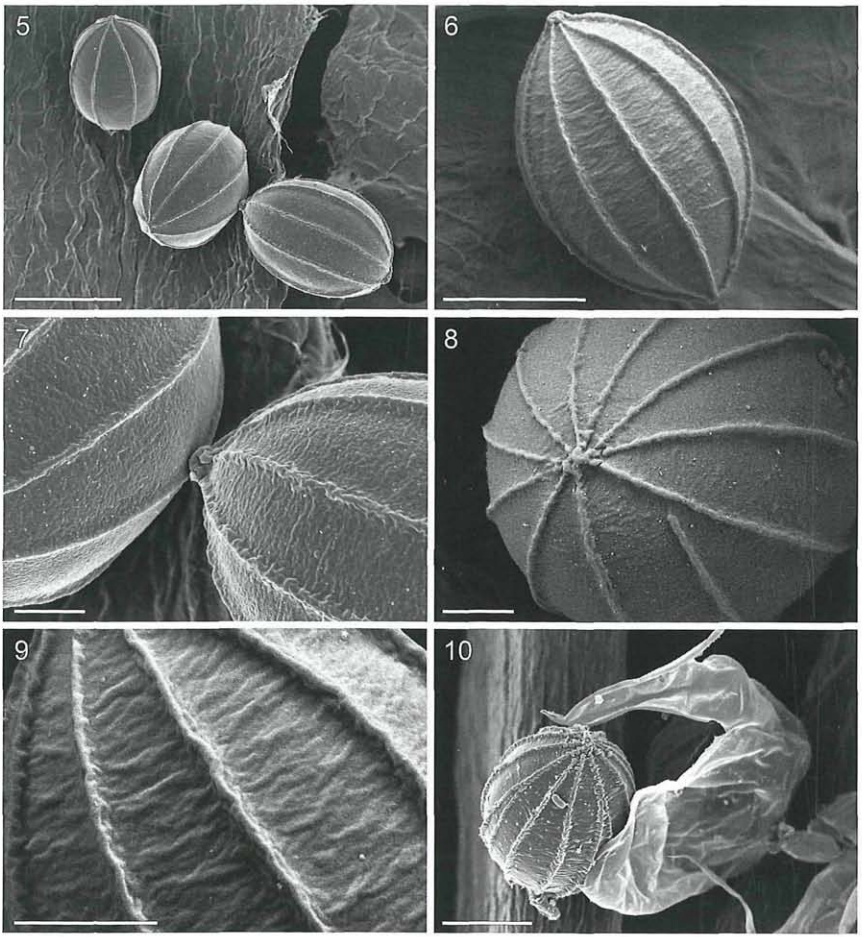

Fig. 5 - 10. *Coelastrella terrestris* isolate SWK 3:53 in SEM. – Fig. 5 – 7. Cell form and cell wall ribs with polar thickenings. Scale bars: Fig. 5 = 10 µm, Fig. 6 = 5 µm, Fig. 7 = 2 µm. - Fig. 8. Cell wall ribs joining below the polar apex (probably caused by culture conditions). Scale bar: 2 µm. – Fig. 9: Transversal fine wrinkles between the longitudinal (meridional) main ribs of the cell wall surface. Scale bar: 2 µm. – Fig. 10. Autosporangium releasing autospores. Scale bar: 5 µm.

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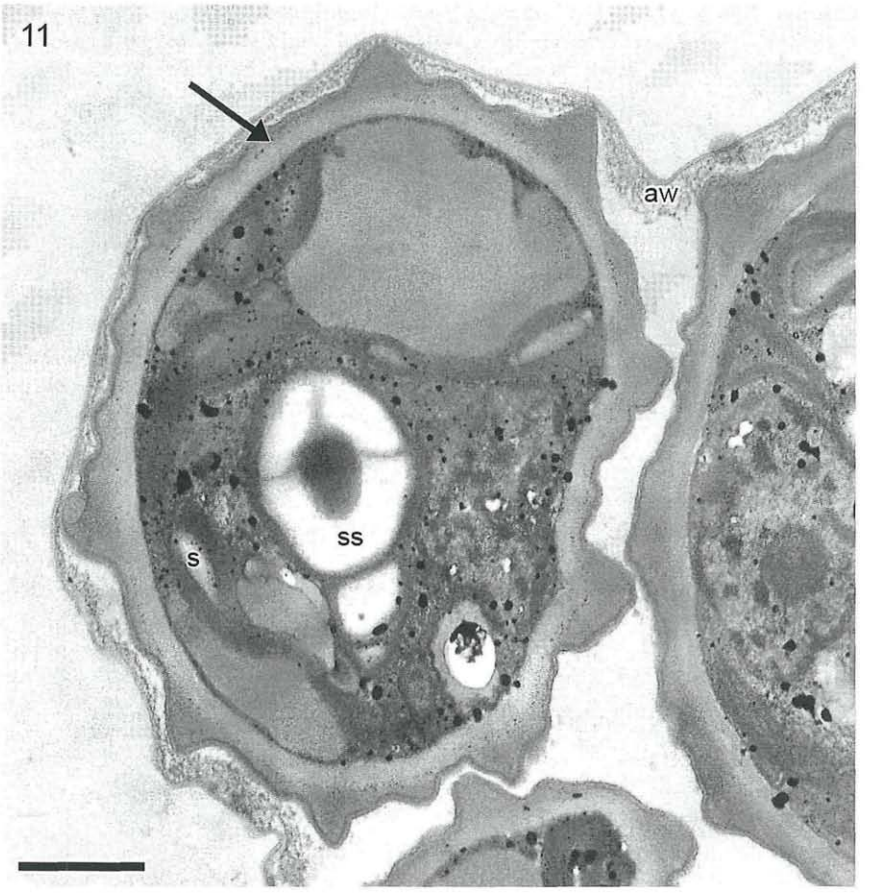

Fig. 11. *Coelastrella terrestris* isolate SWK 3:53 in TEM. Section through autospores within the autosporangium wall (aw). Note the stroma starch grains (s), the starch sheath around the pyrenoid (ss), and the double-layered cell wall of the autospores, with the trilaminar outer layer (arrow). Scale bar: 1 µm.

2. Material and Methods

Soil samples were taken from the surface down to a depth of 2 cm, in October 2004, near the village Obergurgl (Ötztal, Tyrol), at 2350 m above sea level (GPS coordinates: N 46° 50.998', E 11° 00.903'), in alpine grassland dominated by *Carex curvula* ALL., *Sphagnum fuscum* (SCHIMP.) KLINGGR., *Anthelia julacea* (L.) DUM., and *Cladonia arbuscula* (WALLR.) FLOTOW. The samples were transported to the laboratory in Innsbruck in sterile petri dishes. They were stored at 4° C until the isolation procedure started the next day. Samples were crushed and cleaned of stones. A 1 g aliquot was removed and added to 99 ml distilled water for a 10^2 dilution of the original sample. Aliquots of 0.2 ml were spread in triplicate on solidified Bold's basal medium

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(BBM, BISCHOFF & BOLD 1963) in petri dishes for quantitation of algae. Cultures were incubated under standard conditions at 10°–13° C, and illuminated by fluorescent lamps (Philips TLD 58W/840) at 20–30 μmol m^{-2} sec^{-1} on a 12:12 h light:dark cycle.

Single-cell units from a colony were streaked across the whole agar surface with a sterile glass needle under a stereo microscope to obtain unialgal isolates free of fungal and bacterial contaminations. After 6–12 weeks of growth, axenic unialgal colonies were transferred from the petri dishes into glass culture tubes with BBM agar and deposited in the Culture Collection of Algae at the Botanical Institute in Innsbruck, Austria (ASIB, GÄRTNER 1996), for long-term culture under the standard conditions outlined above.

For LM, an Olympus BH-2 light microscope with Olympus PM-10 AK (Automatic Exposure Photomicrographic System) camera or, optionally, with a ProgRes C10 plus Jenoptic digital camera and PICed Cora image analysis software (Jomesa Meßsysteme GmbH) were used. Isolation and purification of algal colonies were done under a Reichert Mak S stereo microscope, 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, for pyrenoids the azocarmine G staining was applied. Additionally neutral red for vacuoles and carmine acetic acid for nuclei were used. Identification was based on cell and colony morphology, using standard references (e.g., KOMÁREK & FOTT 1983, ETTL & GÄRTNER 1995, JOHN & al. 2002). The acetolysis method of ERDTMAN 1969 was applied to confirm the presence of sporopollenin or a similar biopolymer in the cell wall.

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, 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.

For scanning electron microscopy (SEM), algal cells were fixed in 3% glutaraldehyde in 0.1 mol 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.

3. Observations and Discussion

Synonymy: *Coelastrella terrestris* (REISIGL) HEGEWALD & HANAGATA 2000 (Fig. 1 – 11). – *Scotiella terrestris* REISIGL 1964 (basionym). – *Scotiellocystis terrestris* (REISIGL) FOTT 1976. – *Scotiellopsis terrestris* (REISIGL) PUNČOCHÁŘOVÁ & KALINA 1981. – *Scenedesmus terrestris* (REISIGL) HANAGATA 1998.

Material studied: Culture SWK 3:16, 24, 53, isolated by A. TSCHAIKNER (Culture collection A. TSCHAIKNER in the Culture Collection of Algae at the Botanical Institute of the University at Innsbruck, ASIB).

Vegetative morphology. Cells solitary, (5–)14–22 μm long, (3.5–)6–15 μm wide, broadly ellipsoidal to regularly lemon-shaped, rarely asym-

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metrical along the longitudinal cell axis (Fig. 1). Cell wall with 8 – 10 (– 12) meridional ribs, which meet apically in a wart-like thickening (Fig. 5 – 7). These meridional ribs are visible in LM when stained with methylene blue (Fig. 2). Sometimes neighbouring ribs join below the apex (Fig. 8), but this could be depending on culture conditions or age of the culture. In SEM, also transversal fine wrinkles or a rugulose sculpture can be seen between the meridional ribs (Fig. 9, 11). Chloroplast parietal in young cells, later dividing into fragments. Single pyrenoid with dense matrix and a starch sheath consisting of mainly two (sometimes three) starch plates (Fig. 3). Starch also deposited as lenticular grains in the chloroplast as stroma starch (Fig. 11). Many vacuoles are visible within the cell walls, a feature also described in earlier isolated strains (PUNČOCHÁŘOVÁ & KALINA 1981).

Reproduction. Asexual reproduction by 2 – 8 (– 16) more or less lemon-shaped autospores with wall ribs, released by rupture of the mother cell wall (Fig. 10).

Cell wall structure. In ultrathin sections, a double-layered cell wall is visible under TEM, with an inner cellulose component and an outer trilaminar one where acetolysis-resistant material resides. (Fig. 11). This wall structure coincides with the descriptions by ATKINSON & al. 1972, PUNČOCHÁŘOVÁ & KALINA 1981, KALINA & PUNČOCHÁŘOVÁ 1987, and GÄRTNER & INGOLIĆ 1993. It is known that members of *Chlorellaceae* have cell walls with acetolysis-resistant substances (sporopollenin or sporopollenin-like biopolymers), which were also documented earlier for some isolates of *Scotiellopsis* and related taxa by GÄRTNER & INGOLIĆ 1993. Also the cell walls in the present isolates of *Coelastrella terrestris* were tested by acetolysis (ERDTMAN 1969), and intact cell walls could be observed after the treatment, confirming the sporopollenin compound (Fig. 4).

Our material corresponds well in nearly all morphological and ultrastructural features with earlier descriptions published under the name *Scotiellopsis terrestris* (PUNČOCHÁŘOVÁ & KALINA 1981, GÄRTNER & INGOLIĆ 1993). Only minor differences in cell size or shape and in the number of ribs were noted.

Taxonomy. In earlier studies of *Scotiellopsis* and *Coelastrella* taxa, the significance of the number of cell wall ribs and the morphology of polar thickenings appeared to be questionable (GÄRTNER & INGOLIĆ 1993). Now our comparative observations based on strains in long-term culture show clear morphological differences between these genera, also in LM. *Scotiellopsis* taxa have lemon-shaped or broadly ellipsoidal cells with up to 10 (– 12) cell wall ribs and quite distinct polar thickenings. *Coelastrella* species are characterized by globose to broadly ellipsoidal cells with many (16 – 40) fine ribs, without or with very tiny polar thickenings. Ribs and polar thickenings are hardly visible in LM, even after staining. These morphological features are also stable in long-term cultures and

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were included in a determination key presented by KALINA & PUNČOCHÁŘOVÁ 1987.

Based on 18S RNA analyses, HEGEWALD & HANAGATA 2000 transferred two species of the genus *Scotiellopsis* [*S. terrestris* and *S. oocystiformis* (LUND) PUNČOCHÁŘOVÁ & KALINA] to *Coelastrella*. However, as long as molecular analyses of the type species (*S. rubescens*) and other *Scotiellopsis* taxa have not been carried out, we suggest to maintain the *Scotiellopsis* morphotype, with lemon shaped cells and up to 12 meridional wall ribs, as a separate genus also for practical reasons, especially with regard to determination work by LM.

Ecology and distribution. *Coelastrella terrestris* strains have been isolated from a lowland area in Germany (arable land near Braunschweig, isolated by W. OESTERREICHER in 1988, GÄRTNER & INGOLIĆ 1993), from subalpine areas in South Tyrol (soil in a *Pinus sylvestris* forest near Brixen, isolated by H. TRENKWALDER, TRENKWALDER 1975; pasture soil near Bruneck, isolated by C. NIEDERKOFLER in 1989, GÄRTNER & INGOLIĆ 1993), and from alpine areas in Austria (soil on peaks of the Ötztal Alps, 3460 m a.s.l., isolated by H. REISIGL in 1964, REISIGL 1964) and in the Czech Republic (from red and green snow in the Tatra mountains, isolated by F. HINDAK in 1963, and on wet ground in the Krkonoše mountains, isolated by F. HINDAK in 1968, PUNČOCHÁŘOVÁ & KALINA 1981). More than 40 years after its discovery (REISIGL 1964, under *Scotiella terrestris*) it is again recorded from soil in the area of the type collection. Therefore it may be assumed that *Coelastrella terrestris* is confined to terrestrial habitats. Its occasional appearance as a member of the kryoflora (collections by F. HINDAK in 1963, see PUNČOCHÁŘOVÁ & KALINA 1981) seems to be accidental and is perhaps caused by wind dispersal. The general distribution of this aeroterrestrial alga is still unclear.

4. Acknowledgements

The authors are grateful to Mr. Werner KOFLER (Department of Palynology, Institute of Botany, Leopold-Franzens-University Innsbruck) for acetolysis of algal cells. We thank Dr. Christian SCHEUER, Mag. Pramodchandra HARVEY and Prof. Dr. Herwig TEPPNER for helpful comments on this paper and Mag. Dr. Walter OBERMAYER for the layout of the figures (all from Institute for Plant Sciences, Karl-Franzens-University Graz).

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Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 2007

Band/Volume: 46_2

Autor(en)/Author(s): Tschaikner Angelika, Gärtner Georg

Artikel/Article: Observations in a New Isolate of Coelastrella terrestris (REISIGL) HEGEWALD & HANAGATA (Chlorophyta, Scenedesmaceae) from Alpine Soil (Tyrol, Austria). 237-245