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Morphology, ultrastructure and ecology of *Muriella decolor* (Chlorophyta) from subaerial habitats in Poland and the Antarctic

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Abstract: This paper offers a comparison of *Muriella decolor* specimens from different geographical regions and habitats (limestone caves in Poland and ice denuded areas near the Ecology Glacier, King George Island, South Shetland Islands, West Antarctic). Morphological and cytological variability, ecology and life strategies of *M. decolor* were studied in fresh samples, and also in cultures grown on agar plates. The complete life cycle, with detailed ultrastructural (LM and TEM) analysis are presented. The electron microscopic observations prove that materials identified as *M. decolor* collected in Poland and the Antarctic have distinct ultrastructural features. These include the chloroplast lamella arrangement, mitochondrial cristae structure and the cell wall thickness.

Key words: Antarctic moraine, Polish caves, *Muriella decolor* ultrastructure.

Introduction

There is still little known about morphology, ultrastructure and ecology of *Muriella decolor* Vischer, 1936. Subaerial green algae that live on stable exposed surfaces including soil (Nienow 1996; Nestupa 2001; Zancan *et al.* 2006; Nestupa and Štifterová 2013), and natural rocks (Poulíčková and Hašler 2007; Czerwik-

-Marcinkowska and Uher 2011), are perhaps the most obvious and yet often overlooked group of algae, producing extensive green, red, brown or black clusters. Algae growing on rock surface are termed epilithic (Golubic *et al.* 1981), in rocks fissures – chasmoendolithic, and in rock cavities – cryptoendolithic. The hypolithic algae (among them *M. decolor*), inhabiting stone undersurfaces at the stone-soil interface, were found on the Antarctic glacier moraines.

Subaerial coccoid green algae are difficult to classify properly due to their morphological similarity (Fučiková and Lewis 2012). The relationship of main groups of coccoid green algae is shown in the phylogenetic trees of Chlorophyceae and Trebouxiophyceae. These trees show that the morphology of these algae does not always reflect their phylogenetic position. Different phylogenetic species can be hidden under one and the same morphotype (Fučiková *et al.* 2011; Fučiková and Lewis 2012). This similarity pertains to both light and electron microscopy, and according to Hanagata (1998) the molecular studies point to the taxonomic affiliation. Additionally, molecular data can also help to resolve cases of taxonomic inflation caused by morphological variation (Elias *et al.* 2013). Cases of morphological crypticism and convergence are documented across classes Chlorophyceae and Trebouxiophyceae in the phylum Chlorophyta (Hanagata 1998; Friedl and Rybalka 2012; Fučiková and Lewis 2012; Leliaert *et al.* 2012; Škaloud *et al.* 2014), and are especially common in subaerial/aerial coccoid algae.

From the historical point of view the genus *Muriella* was placed in Trebouxiophyceae (Friedl and Rybalka 2012), but according to Hanagata (1998), Fučiková*et al.* (2011) and Fučiková and Lewis (2012) it belongs to the Chlorophycean genus *Pseudomuriella*. Class Chlorophyceae is represented by species occupying a variety of terrestrial habitats, including soils with free-living soil algae, rocks in caves, permanent snow and ice denuded areas (Hoffmann 1989; Van den Hoek *et al.* 1995; Rindi *et al.* 2009; John *et al.* 2011). According to Ettl and Gärtner (1995) *Muriella decolor* was found on soil of pine forest in Brixen and Dolomites, South Tyrol (Italy). Soil habitats are the most frequent, non-aqueous ecosystems for green algae (Zenova *et al.* 1995). Coccoid soil algae are a common component of all terrestrial ecosystems, in which they play a significant role as primary producers; some species are nitrogen fixers, having the ability to improve soil structure. It is generally known that green algae act as a reservoir for plant nutrients, incorporate organic carbon and nitrogen into the soil system through photosynthesis and nitrogen fixation, influence soil structure and control the activity of other edaphic organisms (Metting 1981; Johansen 1993). This is a useful adaptation mechanism in coping with the relative environmental and climatic extremes that can be found at the soil surface. According to several authors, *M. decolor* is considered as one of pioneer colonizers of stone, cave rocks, wet rocks along trail, soil and ice denuded areas (Ortega-Calvo *et al.* 1991; Tiano *et al.* 1995; Flechtner *et al.* 1998; Cecchi *et al.* 2000; Lamenti *et al.* 2000; Massalski *et al.* 2001; Johansen *et al.* 2002; Crispim and Gaylarde 2005; Czerwik-Marcinkowska and

Mrozińska 2011; Czerwik-Marcinkowska 2013). All caves belong to habitats of extreme conditions characterized by low nutrients content, limited light and water availability (Chang and Chang-Schneider 1991; Pedersen 2000; Mulec *et al.* 2008), and similarly such harsh conditions occur also in the Antarctic regions (Pankow *et al.* 1991; Broady 1996; Olech 2002). However, many groups of organisms (liverworts, mosses, some ferns, flowering plants, algae and cyanobacteria) prefer such conditions for the colonization and growth (Dobat 1970; Broady 1989; Kuehn *et al.* 1992; Sanchez *et al.* 2002; Mulec *et al.* 2008; Mulec and Kosi 2009).

This study reports the comparison of ultrastructural similarities and differences of *Muriella decolor* growing in Polish caves and in Antarctic deglaciated areas. Although there are no irrefutable proofs that the materials from caves and moraines represent the same species – the taxonomic plasticity of *M. decolor* allows to assume that there is only one species and not just *Muriella*-like morphology. Studying the same species occurring in two extreme microhabitats a number of similarities in the phenotypic features were found. Thus, it can be assumed that both studied populations belong to the same species although as distinct ecotypes.

Our preliminary 18S sequence results indicate that the *Muriella* specimens belong to the class Chlorophyceae (unpublished data). A detailed molecular sequence analysis is the subject of a forthcoming study.

Material and methods

Samples were collected between 2010 and 2012 from the walls and ceiling of 11 caves in the Polish Jura (Table 1), and soil samples were taken from the contemporary moraines of Ecology Glacier (62°09'S, 58°28'W) on King George Island, South Shetland Islands, Antarctic, in the years 1991–1993 and 1995–1996. Samples from Antarctic moraines were taken from hypolithic habitats. The caves in Polish Jura were created by underground waters dissolving Jurassic marine limestones (Michalik and Partyka 1992). Environmental variables (temperature, pH and humidity) were measured using digital thermohygrometer Testo 608 H2, photoactinometer and pH-meter. In the caves on the surface of wet rocky walls, temperature was 0,8°C; pH – 6.6–7.2 and humidity – 80%; in the Antarctic, on ice-denuded areas, the temperature was 1.8°C, pH – 7.7 and humidity – 82%.

Collected cells (both the algal crust from caves and the soil samples from moraines) were cultured on Bold's Basal Medium (Bischoff 1963), at 20°C in a 12-h light/12-h dark cycle at 3000 $\mu Em^{-2}\ s^{-1}$lx provided by 40W cool fluorescent tubes. For transmission electron microscopy (TEM) the cells were collected from the cultures by gentle centrifugation. The pellet of material was dispersed in fixative. The initial fixative was 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2, applied at room temperature for 2 h. Three washes of 10 min each were done in the same buffer, and then the material was post-osmicated in 1% OsO_4 in 0.1 M phosphate

Table 1

Location and basic morphometric characteristics of the eleven studied caves after Szelerewicz and Górny (1986), Bisek *et al.* (1992), and Gradziński *et al.* (1995, 1998).

Cave	Location	Altitude (m)	Length (m)	Depth (m)	Orientation of cave entrance	Lithology (Jurassic)
Biała	Jamki Gorge	*ca.* 398, 407	84	9	NW	limestones
Ciemna	Prądnik Valley	372	209	10	NW, S	limestones
Dzika	Kluczwody Valley	390	61		SW, NW	limestones
Głęboka	Zborów Mt.	380	160	22.4	NW, SW, N, E	limestones
Jasna	Strzegowa Village	430	81		NW, SW	limestones
Krakowska	Jamki Gorge	410	90	11.5	NW	limestones
Na Łopiankach	Półrzeczki Gorge	260	70		W	limestones
Nietoperzowa	Będkowska Valley	447	326		W	limestones
Twardowskiego	City of Kraków	220	430	-12.7	SW	karst
Za Kratą	Zelka Mt.	215	70	17	NE	limestones
Zbójecka	Jamki Gorge	*ca.* 370, 372, 376	189	15	NW, W, vertical	limestones

buffer, in the refrigerator (at 4°C) for 2 h. Following a short wash in the same buffer, and dehydration in ethanol series (10, 20, 30, 50, 70, 90%), and three changes in an absolute ethanol followed by one change of 50/50 an absolute ethanol-propylene oxide, and two changes of propylene oxide, the material was infiltrated overnight with a 1:1 mixture of propylene oxide and Spurr resin (Spurr 1969). The material was embedded in Spurr's hard mixture and polymerized at 70°C for 24 h. Silver (50 nm) and gold (70 nm) sections were cut with glass knives on a Reichert-Jung Supernova ultramicrotome, and collected on unsupported 600-mesh acetone-treated copper grids. The grids were contrasted for 5 min with 5% uranyl acetate in 50% ethanol, followed for 2 min with lead citrate solution (Reynolds 1963). Observations and photographs were made with a TESLA BS 500 transmission electron microscope.

Muriella decolor was identified according to: Ettl and Gärtner (1988, 1995), Hoffmann (1989), Massalski *et al.* (2001) and John *et al.* (2011). The amount of cells grown from Antarctic soils samples were insufficient for the molecular studies, and the identification was based on the morphological characteristics (both light and electron microscopy). The samples from the caves and from the ice denuded areas demonstrated morphological similarities, likewise the habitats. In this study the scheme of Graham *et al.* (2009) was followed, supported by a number of recent phylogenetic studies (Friedl and Zeltner 1994; Huss *et al.* 1999).

Results

Muriella decolor was found in 11 caves in the Polish Jura (Kraków–Częstochowa Upland, Southern Poland) and also in the soil from the contemporary mo-

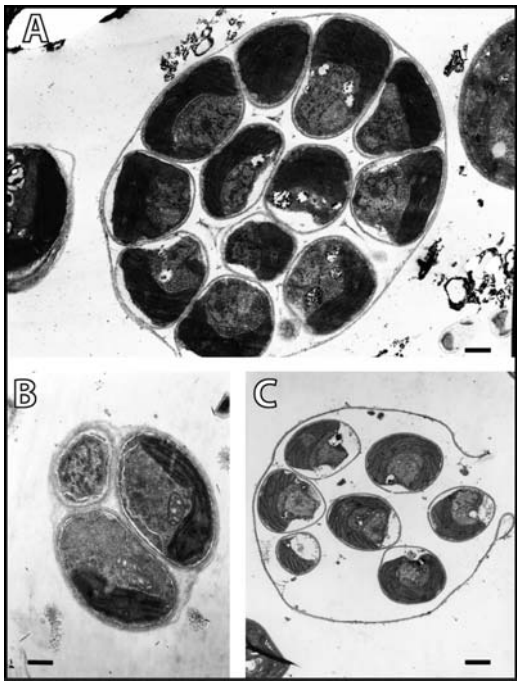

Fig. 1. *Muriella decolor* – TEM images of autosporangium. **A**. Autosporangium with 12 autospores (Antarctic). Scale bar 1 μm. **B**. Autosporangium with three autospores (caves). Scale bar 2 μm. **C**. Autosporangium with an opened cell wall with seven autospores (Antarctic) on the cross section. Scale bar 1 μm.

raines of the Ecology Glacier (Antarctic). This species from both localities consists of single cells, almost sphaerical or broadly ellipsoid, thin-walled but thickening with age. In mature cells the asexual reproduction is by autospores. The species observed in the caves and in the Antarctic has different number of autospores (Fig. 1, Table 2). Cells divide by autosporulation forming 2, 4, 8 or 16 autospores per autosporangium. *M. decolor* found in caves, basing on the molecular phylogenetic data (unpublished) revealed that the species belongs to the phylum Chlorophyta,

Table 2

Comparison of the cell structure of *Muriella decolor* from Antarctic moraine (Massalski *et al.* 2001) and from Polish caves (Czerwik-Marcinkowska and Mrozińska 2011).

Cell structure	Antarctic moraine	Polish caves
Chloroplasts (distribution) arrangement	parietal	parietal
Lamella arrangement	parallel, diagonal, radial	parallel
Cell wall	thin, stratified wall	thick, not stratified wall
Mitochondrion	elongated cristae running across entire length of mitochondrion	cristae not running across mitochondrion
Autospores (number)	8, 16, 32	4, 8

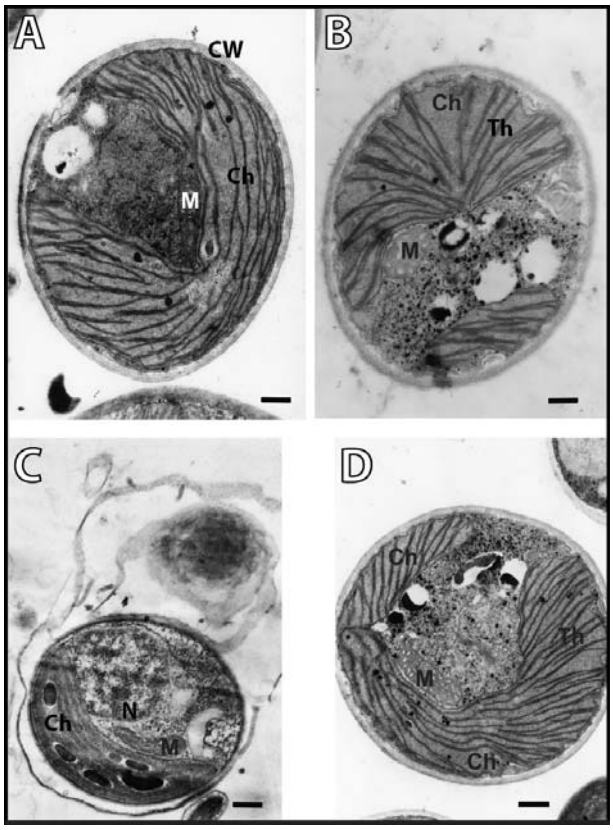

Fig. 2. *Muriella decolor*. **A**. Vegetative cell with parietal chloroplast, mitochondrion and stratified cell wall (Antarctic). **B**. Vegetative cell showing radially arranged thylakoids in chloroplast and mitochondrion (Antarctic). **C**. Autospore still within mother cell wall with one parietal chloroplast, mitochondrion and nucleus (caves). **D**. Vegetative cell with one large and one small chloroplasts and with mitochondrion (Antarctic). Scale bars 1 μm.

class Chlorophyceae, which includes the coccoidal as well as filamentous forms (Van den Hoek *et al.* 1995; Rindi *et al.* 2009; John *et al.* 2011; Leliaert *et al.* 2012).

Light microscopy. — Cells of *Muriella decolor* collected from the caves walls of the Polish Jura, in general appearance were similar to those grown from the Antarctic soil samples (Fig. 2). The algae were single-celled, spherical, measuring from 5.0 μm to 7.0 μm in diameter, adult cells up to 6 μm in diameter, with either one or two parietal chloroplasts, single in young cells, becoming multiple with age. The chloroplast is U-shaped and/or cup-shaped, without the pyrenoid. Asexual reproduction occurred *via* autospores, released by splitting of mother cell wall. Autosporangia contained different number of spores ranging from 4 to 32 spores. In the species found in the caves the number of spores does not exceed 16, whereas in the Antarctic *Muriella* the number of spores often reaches even 32. The cell walls of young cells comparing with mature cells were thinner.

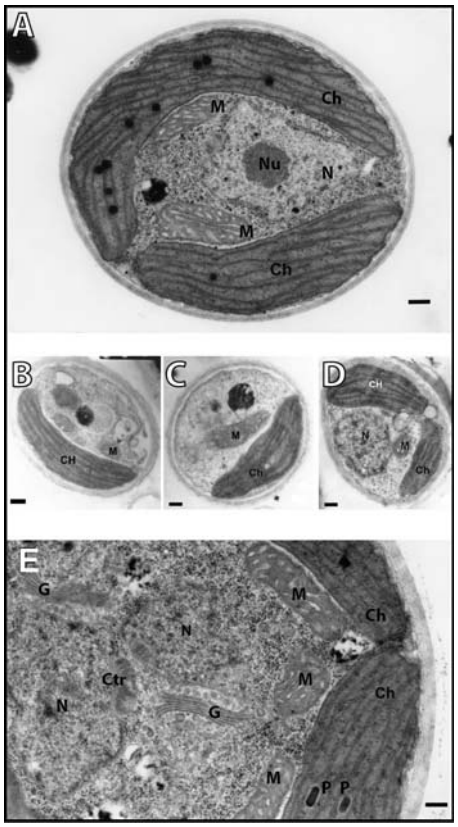

Fig. 3. *Muriella decolor*. **A**. Two parietal chloroplasts with lamella arranged either parallel or diagonal, two elongated mitochondria (note two parallel crista running across entire length of mitochondria) (Antarctic). **B**–**D**. Single cells showing one (B–C) or two (D) parietal chloroplasts, two mitochondria and large vacuole, note thick cell wall (caves). **E**. Part of young autosporangium showing two nuclei, two Golgi bodies, numerous mitochondria, two obliquely sectioned centrioles, and plastoglobules in chloroplasts (Antarctic). Scale bars 1 μm. Symbols explanations: M – mitochondrion, N – nucleus, Nu – nucleoli, Cw – cell wall, Ch – chloroplasts, Th –thylakoid, P – plastoglobules, G – Golgi body, Ctr – centriole.

Ultrastructure observation (TEM). — Numerous plastoglobules are either sphaerical or cylindrical with rounded ends (Fig. 3). There are no starch grains in the chloroplast, and the chloroplast lamella contains from two to three thylakoids. The lamella arrangement at least in some cells appears to be somewhat unusual. The lamella in Chlorophyta are more or less parallel to each other, whereas in *Muriella* from the Antarctic they were often arranged in diagonal and/or radial manner. Some chloroplasts are branched (Figs 2–3). The nucleus has a very large nucleolus (Fig. 2). Each cell contains a single Golgi body (Figs 2–3), and two, sometimes three large, elongated mitochondria with very long cristae (Fig. 2). In mature cells beginning to divide the centriols (obliquely sectioned) close to the nucleus were observed. Osmophilic plastoglobules, are spherical in young cell (Fig.

3) which is typical for many subaerial algae. In the dividing cells the droplets are cylindrical with rounded ends (Figs 2–3). Asexual reproduction is made by autospores (Fig. 1). In the life cycle the mature cells, after reaching the appropriate size, begin the autosporangium building process. Large, mature vegetative cells became multinucleate, with more and larger lipid droplets in the chloroplast. In autosporangia the young autospores were closely packed together (Fig. 1A). At a later developmental stage the autospores became more spherical, and arranged more loosely. Finally, they were released through the ruptured parent cell wall of the autosporangium (Fig. 1C).

Discussion

Muriella decolor was observed in the ice denuded areas of the Antarctic (Massalski *et al.* 2001) and from the caves in Poland (Czerwik-Marcinkowska and Mrozińska 2011). According to Skowroński *et al.* (2002), Mulec *et al.* (2008) and Lam (2010) algae living in the subaerial environment should have certain morphological and physiological adaptations to succeed in such extreme conditions (light, temperature, high relative humidity and/or seeping water, and substratum characteristics).

Van Den Hoek *et al.* (1995) and Leliaert *et al.* (2012) among the principal characteristics of the Chlorophyta give starch occurring in grains as the most important reserve polysaccharide, and a new mode of cell division that is mediated by a system of microtubules that develops parallel to the plane of nuclear division. The lamellae arrangement in *M. decolor* from the Antarctic is, to our knowledge, at least unusual for the chlorophycean alga. No such lamellae arrangement diversity, probably due to different environment, was observed in *M. decolor* found in the caves. Comparing the cell ultrastructure of *M. decolor* from caves and ice denuded areas there are differences between the mitochondrial cristae (Table 2). In the cells growing in caves the cristae are shorter than those in the cells from the Antarctic. Different size of cristae could be the result of the cells being in a different life cycle stage. *M. decolor* living in such specific subaerial habitats (limited light and humidity, low nutrient input) can be plastic in general morphology and/or ultrastructure.

The molecular studies (Rindi *et al.* 2007; Vinogradova and Darienko 2008) and our 18S results (unpublished data) have shown that the *Muriella* specimens are to be affiliated with Chlorophyceae class. However, many key questions regarding the relationships of the green algae lineage remain largely unresolved. Fučiková *et al.* (2014) recently described ten new families and three new genera of coccoid green algae, considering analyses of a multigene data. Hanagata (1998) and Fučiková *et al.* (2011) and point out that *Pseudomuriella* and *Bracteococcus* belong to Chlorophyceae, and also emphasize close relationship of *Muriella* (*Pseudomuriella*) *aurantiaca* and *Bracteacoccus*.

The ultrastructure of *M. decolor* was up to now not studied in details, except of Massalski *et al.* (2001), and there is still little known about its morphology, ultrastructure and ecology. *M. decolor* material now studied came from two subaerial habitats (from ice denuded area and caves). The morphological (LM and TEM) analysis showed similarities and differences regarding the size of both young and mature cells, chloroplast (lamella arrangement) and mitochondrial cristae structure. The further study on the genus *Muriella,* including molecular data of both field material and holotypes species from the Culture Collections will be continued.

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