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Research Article

# *Chloropyrula uraliensis* gen. et sp. nov. (Trebouxiophyceae, Chlorophyta), a new green coccoid alga with a unique ultrastructure, isolated from soil in South Urals

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**Abstract** Soil hosts diverse communities of photosynthetic eukaryotes (algae) that have not yet been fully explored. Here we describe an interesting coccoid green alga isolated from a soil sample from a forest-steppe in South Urals (Bashkortostan, Russia) that, based on a phylogenetic analysis of 18S rRNA gene sequence, appears to represent a new phylogenetic lineage related to the genus *Leptosira* within the class Trebouxiophyceae. This new alga is characterized by uninucleate cells with a shape ranging from spherical to ellipsoid or egg-like, occurring solitary or more often grouped in irregular masses or colonies. Remarkably, cells with a characteristic pyriform shape are encountered in cultures grown on a solid medium. The cells harbour a single pyrenoid-lacking parietal chloroplast with the margin undulated or forming finger-like projections; in mature cells the chloroplast becomes divided by deep incisions into more or less separate lobes. Transmission electron microscopy of vegetative cells revealed an unprecedented structure in the form of a cluster of microfibrils located in the cytoplasm near the plasma membrane, often appressed to the chloroplast. Reproduction takes place via autospores or biflagellated zoospores. The unique suite of characters of our isolate distinguishes it from previously described coccoid green algae and suggests that it should be classified as a new species in a new genus; we propose it be named *Chloropyrula uraliensis*.
**Key words** *Chloropyrula uraliensis*, green algae, new species, Trebouxiophyceae, ultrastructure, Ural Mountains.

Despite more than two centuries of effort to catalog the diversity of microscopic organisms, there is still no prospect of reaching the ultimate goal of having described all the species inhabiting our planet (De Clerk et al., 2013). This is not only due to our inability to cultivate many, if not most, microorganisms in laboratory conditions, or due to difficulties with properly recognizing the actual species diversity behind morphologically uniform cohorts of organisms, but probably also because many species have simply not yet been encountered (at least by a specialist competent to realize their novelty). This is naturally pertinent especially for species living in rare or inaccessible habitats, but surprising discoveries can be easily made in common and seemingly well-studied habitats. The purpose of this study is to describe such a novel interesting organism, specifically a unicellular green alga, isolated from a soil sample from a forest-steppe in the temperate zone near the European-Asian boundary.

Green algae are one of the most diverse and most successful major groups of photosynthetic eukaryotes (Friedl & Rybalka, 2012; Leliaert et al., 2012). Compared to most other algal groups, they have proven to be particularly talented for colonizing terrestrial habitats, which is attested not only by the spectacular success of one of the green algal lineages having transformed into the hugely diverse clade of land plants (embryophytes), but also by numerous independent origins of other, less conspicuous, green algal groups inhabiting soil, biofilms on various subaerial surfaces (tree bark, rock walls, artificial substrates), or constituting the photobiont partner in lichen symbioses (Lopez-Bautista et al., 2007). Terrestrial algae are concentrated especially in the green algal class Trebouxiophyceae, which embraces a diverse array of mostly coccoid algae with an autosporic or zoosporic mode of reproduction (asexual reproduction *via* formation of immotile autospores or motile zoospores, respectively), but also species with simple filamentous and even

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parenchymatous thalli (Leliaert et al., 2012). The known diversity of trebouxiophytes is ever growing. An important source of the expansion are numerous new cryptic or semi-cryptic species and genera being established on the basis of molecular characters by splitting traditional broadly defined taxa, e.g., the genera *Chlorella* Beijerinck or *Dictyosphaerium* Nägeli (Bock et al., 2011a, 2011b; Krienitz et al., 2012). However, taxa of a more pronounced morphological and/or phylogenetic novelty have been also described over the past few years, particularly from aeroterrestrial habitats (Zhang et al., 2008; Neustupa et al., 2009, 2011, 2013; Somogyi et al., 2011; Novis & Visnovsky, 2012). The pace of describing the new taxa indicates that the extent of the unknown trebouxiophyte diversity may still be vast.

During a study of terrestrial algae in the territory of the Republic of Bashkortostan (South Urals, Russia) we isolated a strain of a morphologically novel coccoid green alga that proved to occupy an isolated phylogenetic position within the class Trebouxiophyceae upon sequencing its 18S rDNA region. This prompted us to conduct a more detailed characterization of this alga, which revealed additional interesting features and led us to describe it as a new species in a new genus, *Chloropyrula uraliensis*.

## 1 Material and methods

### 1.1 Isolation and cultivation

The strain of *Chloropyrula uraliensis* characterized in this study was isolated from a soil sample taken in a ravine in a zone of a broadleaf forest near the village Krasnousolsky (53°55′42.24″N, 56°31′22.58″E) (Republic of Bashkortostan, Russia). The strain was cultivated in liquid and on agarized BBM media (Andersen, 2005). The cultures were maintained at 20–23 °C with illumination of 40 μmol/m$^2$ per second provided by 18W cool fluorescent tubes (Philips TLD 18W/33, Philips Lighting Poland S.A., Pila, Poland).

### 1.2 Microscopy

The morphology of vegetative and reproductive cells was examined using an Axio Imager A2 light microscope. Microphotographs were taken with an AxioCam MRc camera at 400× magnification using a differential interference contrast (DIC) optics. A technique of Fritz & Triemer (1985) was employed to visualize microfibrilar structures. To increase the permeability of the plasma membrane, sodium dodecylsulphate (2% SDS in water) was added to the cell suspension that was then gently shaken for 10 min (Zachleder & Cepák, 1987). The cells were then washed twice by distilled water, air-dried on slides, stained with 1% Calcofluor white (Sigma-Aldrich, St. Louis, MO, USA), and observed in an Olympus BX51 microscope equipped with a mercury burner U-RFL-T as a light source.

For observations in a transmission electron microscope (TEM), samples were fixed for 2 hours at 5 °C in a 2% solution of glutaraldehyde in 0.05 mol/L phosphate buffer, post-fixed for 2 hours at 5°C in 1% osmium tetroxide in 0.05 mol/L phosphate buffer and overnight at 5 °C in 1% uranyl acetate in methanol. After dehydration through an ethanol series, cells were embedded in Spurr's medium (Spurr, 1969) via isobutanol. Ultrathin sections, cut with a diamond knife on an Ultracut E (Reichert-Jung, Wien, Austria), were post-stained with lead citrate and examined using a JEOL 1011 TEM (JEOL Ltd., Tokyo, Japan).

For a more detail study of chloroplast, cells were examined with a Leica TSP SP2 laser scanning confocal microscope, equipped with an Ar-Kr laser using a 488 excitation line and an AOBS filter-free system collecting emitted light between 498 and 700 nm. A Leica 63×/1,4 N.A. oil immersion objective fitted on a Leica DM IRE2 inverted microscope was used for the observations. 3D reconstructions of the chloroplast morphology were produced using the program ImageJ 1.34p (Abramoff et al., 2004) and the "Volume viewer" plugin.

### 1.3 Acquisition and analyses of rDNA sequence data

The sequence of the genomic region comprising an almost full 18S rRNA gene, ITS1 region, 5.8S rRNA gene, and ITS2 region was obtained from two overlapping PCR segments following the procedure described in Němcová et al. (2011), with additional employment of the ITS1 and ITS4 primers (White et al., 1990) for sequencing the ITS region. The newly obtained sequence, excluding the primer regions, was deposited at GenBank with the accession number JX070625.

The 18S rRNA gene sequence of *C. uraliensis* was added to an updated comprehensive alignment of trebouxiophyte sequences and a representative set of sequences from other chlorophyte classes obtained from GenBank (as described in Neustupa et al., 2011). After masking unreliably aligned positions, a maximum likelihood (ML) tree was inferred on the comprehensive alignment using RAxML 7.8.4 run at the CIPRES (Cyberinfrastructure for Phylogenetic Research) Portal (http://www.phylo.org/sub_sections/portal/) and employing the strategy of rapid bootstrapping followed

by a thorough ML search on the original dataset with the GTR + Γ substitution model (Stamatakis et al., 2008). The resulting tree showed the *C. uraliensis* sequence branching with strong support (100) in a position sister to three sequences of *Leptosira* spp. (not shown). To simplify additional phylogenetic analyses and presentation of their results, the alignment was reduced to a selection of trebouxiophyte sequences representing all major lineages. The final alignment (51 sequences, 1650 positions including 539 variable positions and 339 parsimony-informative positions; available at http://www1.osu.cz/~elias/data/Chloropyrula_paper.html) was subjected to a ML analysis using the same strategy as applied on the comprehensive alignment. In addition, a Bayesian inference was applied using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) run at the CIPRES Portal, employing the GTR + Γ + I substitution model; two parallel MCMC runs were carried out for $3 \times 10^6$ generations, each with one cold and three heated chains. Samples were taken every 100 generations. Initial 7500 samples of each run were discarded as "burn-in" and a consensus tree was calculated from the remaining 45 002 trees.

## 2 Results

### 2.1 Taxonomic treatment

**Chloropyrula** Gaysina, Eliáš, Němcová & Škaloud, gen. nov.

Diagnosis: Cells solitary or grouped in pseudofilamentous, regular or irregular masses; uninucleate; egg-like, pyriform, spherical, elliptical or rarely irregular in shape. The chloroplast parietal with several more or less deeply separated lobes, lacking a pyrenoid. The chloroplast margin undulated or divided into finger-like projections. A cluster of microfibrils located near the plasma membrane. Asexual reproduction by autospores and biflagellate zoospores, sexual reproduction not observed. The genus differs from other genera by the 18S rRNA sequence.

Type species: *Chloropyrula uraliensis* Gaysina, Eliáš, Němcová & Škaloud.

Etymology: From *chloros*—"green" in Greek, and *pyrus*—"pear" in Latin, in reference to the pyriform shape of cells often seen in culture.

**Chloropyrula uraliensis** Gaysina, Eliáš, Němcová & Škaloud, sp. nov.

Diagnosis: An alga with the general morphological characteristics of the genus. The cell shape ranging from spherical to pyriform or ovoid, with the latter found especially at the periphery of cell clusters. When grown on an agar-solidified medium, the species makes a visible growth, sometimes in the form of a very solid dark green mass. Spherical cells (3.4−)5.8–15.0 (−28.2) μm in diameter; egg-like, pyriform, elliptical cells (7.7−)10.8–22.6(−34.5) μm in length and (5.3−)8.0–16.4(−25.3) μm in width. Autorporangia 16.1–24.4 μm in diameter, producing 4–16 autospores 3.4–13.6 μm in diameter. Zoosporangia spherical, 11.7–16.3 μm in diameter, releasing biflagellate zoospores with a parietal chloroplast, 3.3–6.8 μm in width, 6.9–11.7 μm in length, with a stigma 1.6–2.0 μm in diameter, and with granules in the cytoplasm. Freshly released zoospores round, changing to elongate when moving. Upon settling the zoospores become egg-like, pyriform, and finally round before shedding the flagella. Cells in old cultures become yellowish and very granulated.

Holotype: Resin-embedded cells deposited in the Culture Collection of Algae of the Charles University in Prague (CAUP) as the item TYPE-H 8402. Living cultures of the alga are maintained in CAUP as the strain CAUP H 8402 and in the Bashkortostan Collection of Algae and Cyanobacteria (BCAC, Ufa, Russia) as the strain BCAC 229. [Correction added on 22 May 2013, after first online publication: The type strain number 'H 8401' should be listed as 'H 8402'.]

Type locality: Soil in a ravine in a zone of a broadleaf forest near the village Krasnousolsky (53°55′42.24″N, 56°31′22.58″E), Republic of Bashkortostan, Russia.

Etymology: In reference to the geographic location of the type locality (*uraliensis*—coming from the Ural Mountains).

### 2.2 Morphology of *C. uraliensis*

Morphological features of *C. uraliensis* as observed in the light microscope are summarized in Fig. 1: A–E (interpretative line drawings) and Fig. 2: A–H (representative photos of various life stages). Cultures were examined in different conditions and life stages: mature cultures (7–10 days old) and zoospore liberation both on agar-solidified and in liquid media, and young cultures (1–3 days after zoospore liberation) in a liquid medium. Morphological features of *C. uraliensis* were found to depend on culture conditions. Integration of cells into more or less irregular clusters or pseudofilamentous cell masses was observed more frequently on the agar-solidified medium. In the liquid medium the formation of colonies with egg-like or ellipsoid cells on the periphery was observed only in young cultures (Fig. 2: A). The dimensions of young cells in the liquid medium were (3.7−)5.9–9.4(−14.7) μm in diameter for spherical cells, and (8.6−)9.8–13.5(−15.8) μm in length and (5.5−)7.3–10.5(−12.6) μm in width for

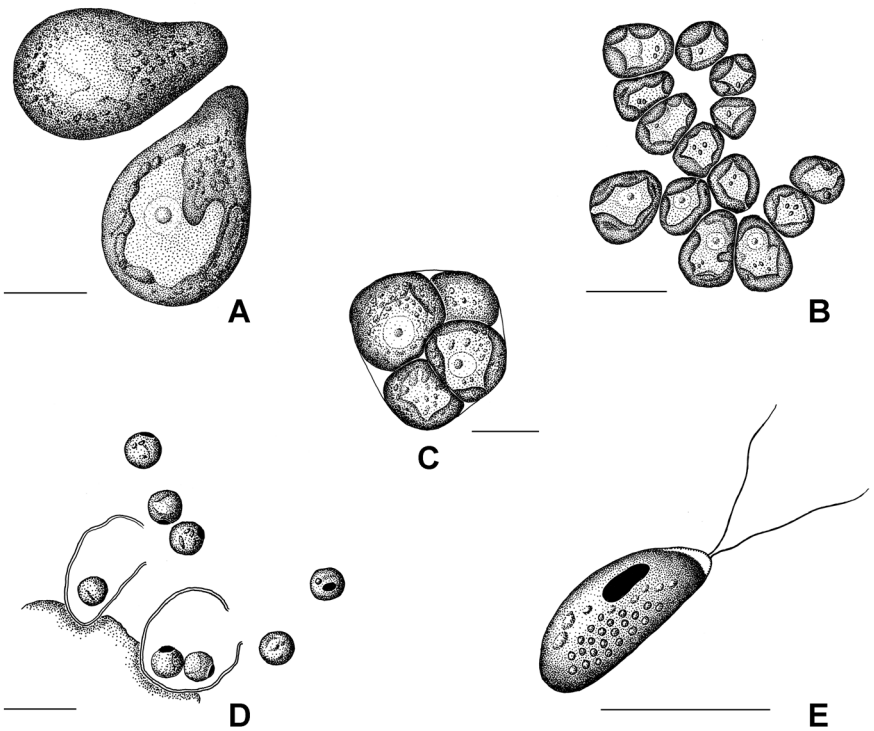


**Fig. 1.** *Chloropyrula uraliensis* gen. et sp. nov., an interpretative line drawing of various cell morphologies and life stages. **A**, Pyriform cells. **B**, Growth of a young colony on a solid medium. **C**, Autospores in an autosporangium. **D**, Zoospores leaving a zoosporangium. **E**, A zoospore. Scale bar = 10 μm.

egg-like or ellipsoid cells. Cells in mature cultures in liquid media were generally spherical, (3.4−)7.7–18.1 (−28.2) μm in diameter (Fig. 2: B). On solid media spherical cells were (3.4−)7.4–15.4(−21.7) μm in diameter; egg-like, pyriform and elliptical cells were (7.7−)14.1–25.0(−34.5) μm in length and (5.3−)10.0–18.1(−25.3) μm in width (Fig. 1: A, B and Fig. 2: C, D). Small colonies of irregular cells (5.3−)5.5–8.2 (−11.7) μm in length, (2.6−)3.2–4.6(−5.3) μm in width, were occasionally observed in old cultures (over 1 month) on the solid medium. Pyriform cells characteristic for the species were observed only on solid media (Fig. 1: A and Fig. 2: D).

Autosporangia were spherical, 16.1–24.4 μm in diameter. Autospores were spherical, 3.4–13.6 μm in diameter (Fig. 1: C and Fig. 2: G). The number of autospores per autosporangium varied widely, from 4 to 16 (rarely to 32). Zoosporangia were spherical, 11.7–16.3 μm in diameter, developing from any vegetative cell (Fig. 1: D and Fig. 2: E). Zoospores were biflagellate with flagella 7.7–10 μm in length. The zoospore body was 3.3–6.8 μm in width and 6.9–11.7 μm in length, with apparent granules in the cytoplasm and a stigma (1.6–2 μm in diameter) situated mostly in the front or the central part of the cell (Fig. 1: E and Fig. 2: F). Zoospores were round after release, elongated while moving; after settling they underwent a transformation series by changing the shape from egg-like to pyriform to round and by loosing the flagella at the end. The release of zoospores was observed in 7–10 days old liquid cultures having stored one day in darkness and then illuminated in a microscope. Another way to get the zoospores was keeping young agar cultures one day in darkness after adding liquid BBM medium; zoospores were then observed in the morning of the next day after illumination. An occasional zoospore release in a mature culture on agar was also seen. Cells in old cultures (2–6 months old) became yellowish and very granulated (Fig. 2: H).

The chloroplast of young cells was parietal with several lobes, in mature cells becoming divided by deep broad incisions and sometimes exhibiting holes, lacking a pyrenoid. The chloroplast margin was undulated or divided into finger-like projections (Fig. 2: I, J).

### 2.3 Ultrastructural features of *C. uraliensis* vegetative cells

Young autospores were surrounded by the mother cell wall (Fig. 3: A). A newly formed cell wall was composed of three layers, an inner electron-dense layer, a middle lighter layer, and an outer layer formed by densely packed microfibrils, centrifugally loosing their packed arrangement (Fig. 3: D, G). The nucleus was located centrally, surrounded by the cytoplasm and a

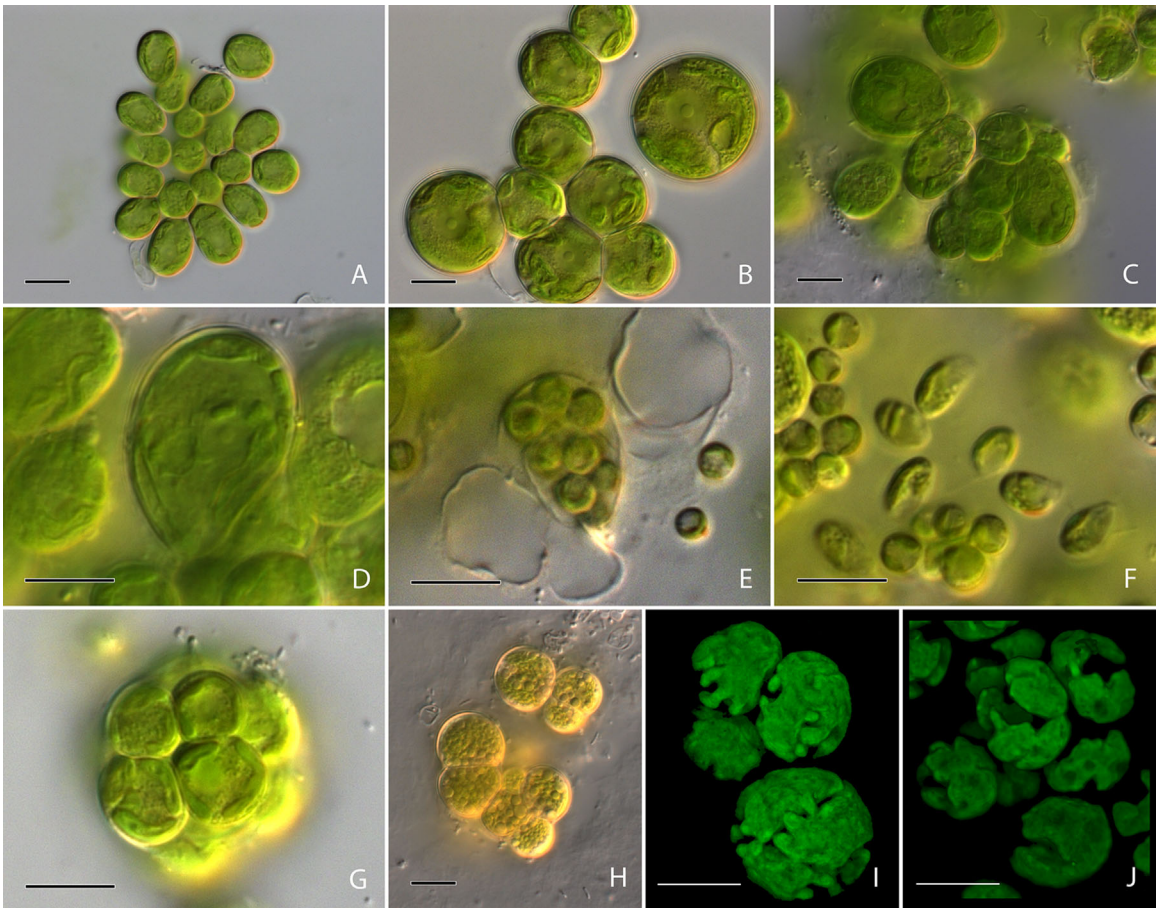


**Fig. 2.** *Chloropyrula uraliensis* gen. et sp. nov., morphology in a light or a confocal microscope. **A**, Growth of a young colony in a liquid medium. **B**, Growth of mature cells in a liquid medium. **C**, Growth on a solid medium. **D**, A pyriform cell on a solid medium. **E**, A zoosporangium with zoospores before release. **F**, Zoospores. **G**, An autosporangium with autospores. **H**, Old cells. **I**, A three-dimensional reconstruction of chloroplasts in young cells. **J**, A three-dimensional reconstruction of chloroplasts in mature cells. Scale bar = 10 μm.

large lobed parietal chloroplast. The cytoplasm contained vacuoles filled with an electron-transparent content. The abundance of vacuoles was higher in mature cells compared to young autospores. A reserve polysaccharide was stored inside the chloroplast in the form of starch grains (Fig. 3: B). No pyrenoid was observed on cross-sections of the chloroplast. The most interesting ultrastructural feature was a large cluster of microfibrils (MF), located in the vicinity of the cell wall and often appressed to the chloroplast. Upon careful observation the MF cluster was visible also on light microphotographs (Fig. 2: B) or as a rounded depression on confocal images of chloroplasts (Fig. 2: J). The MF clusters were about 1.5–2.5 μm in diameter, the matrix of the cluster was darker in the middle, while the fringe had a lighter appearance (Fig. 3: C). The cluster was composed of microfibrils 2–4 nm in diameter. Moreover, we documented uncoiling of the MF cluster next to the plasma membrane (Fig. 3: E–G). Bunches of 3–5 MFs were formed. We presume that the MFs are extruded to the cell surface through the plasma membrane. To check the nature of the MF clusters we applied Calcofluor white that reacts non-specifically with various polysaccharides (Herth & Schnepf, 1980). Both the cell wall and MF clusters were stained (Fig. 3: H–K).

### 2.4 Phylogenetic position of *C. uraliensis*

Sequencing the 18S rDNA gene of *C. uraliensis* revealed no introns. BLASTN searches against the non-redundant nucleotide database at the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/) revealed that the sequence is consistently most similar to sequences from trebouxiophyte algae, with the sequences from *Leptosira obovata* Vischer, *Leptosira terrestris* (F. E. Fritsch & R. P. John) Printz, and *Parietochloris alveolaris* (H. C. Bold) S. Watanabe & G. L. Floyd being the best three hits, all exhibiting

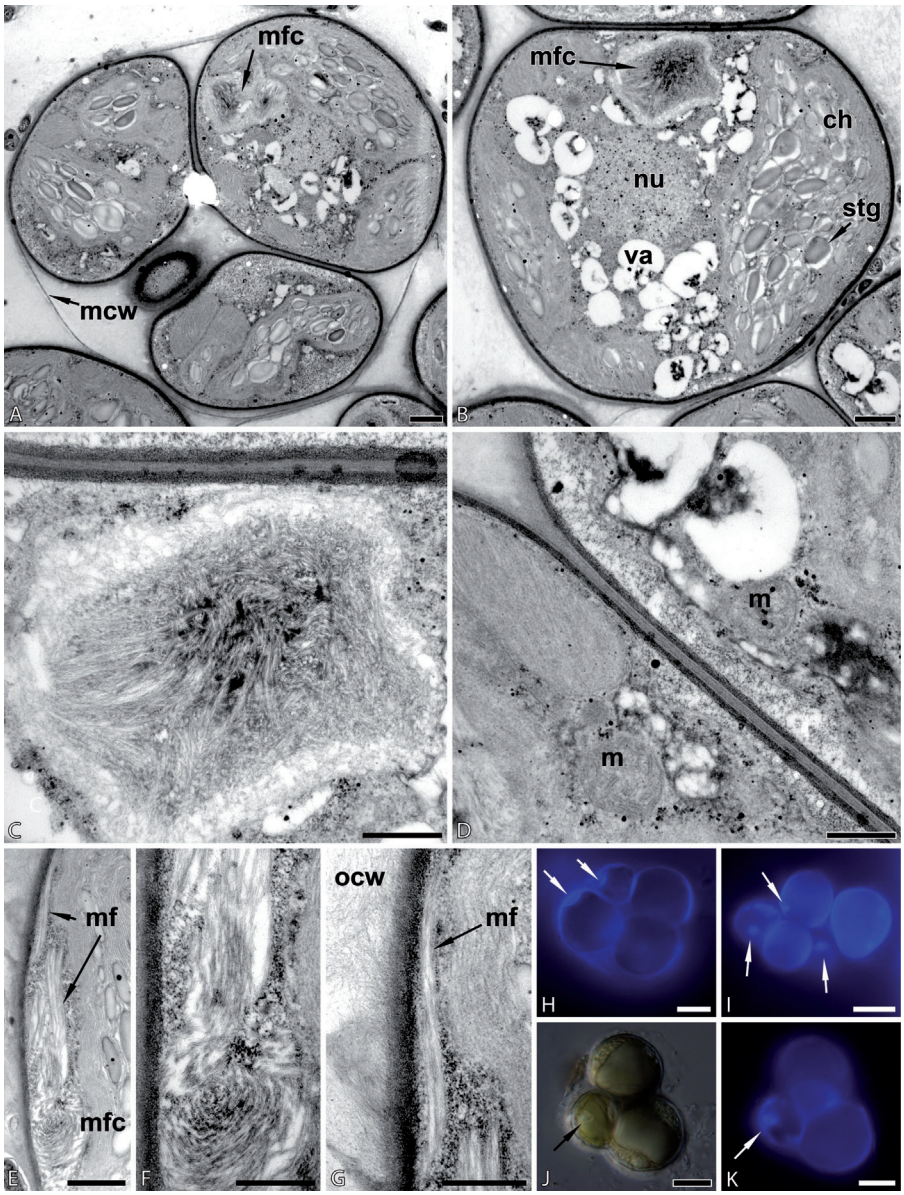


**Fig. 3.** *Chloropyrula uraliensis* gen. et sp. nov., ultrastructure and cell wall characteristics. **A**, Young autospores surrounded by the mother cell wall (mcw), note the microfibrilar cluster (mfc) in the right upper cell. **B**, A mature cell; chloroplast (ch), microfibrilar cluster (mfc), nucleus (nu), starch grain (stg), vacuole filled with an electron-transparent content (va). **C**, A detail of the microfibrilar cluster located in the vicinity of the cell wall. **D**, A detail of the three-layered cell wall with an inner electron-dense layer, a middle lighter layer, and an outer layer formed by densely packed microfibrils that become looser towards the fringe; mitochondrion (m). **E**–**G**, Uncoiling of the microfibrilar cluster. E, Bunches of microfibrils (mf), microfibrilar cluster (mfc). F, A detail of the microfibrilar cluster. G, A detail of uncoiled bunches of microfibrils (mf) positioned next to the plasma membrane; the outer cell wall (ocw) formed by microfibrils. **H**, **I**, Epifluorescence microscopy on dried cells stained with 1% Calcofluor white. **J**, Permeabilized stained cells in a bright field. **K**, The same detail seen through a fluorescence microscope. The arrows in H–K indicate microfibrilar clusters. Scale bars: A, B, E = 1 μm; C, D, F, G = 0.5 μm; H–K = 10 μm.

96% identity to *C. uraliensis*. The trebouxiophyte affiliation of *C. uraliensis* was confirmed by a phylogenetic analysis including a wide selection of trebouxiophyte 18S rDNA sequences and representative sequences from three other classes of "core" chlorophytes (Chlorophyceae, Ulvophyceae, Chlorodendrophyceae). In the resulting phylogenetic tree (Fig. 4), the *C. uraliensis* sequence was placed in a strongly supported clade (ML bootstrap support of 93%, Bayesian posterior probability of 1.00) together with sequences from the genus *Leptosira* Borzì, but the divergence between *Leptosira* spp. and *C. uraliensis* was very deep and consistent with treating the new alga as a new genus.

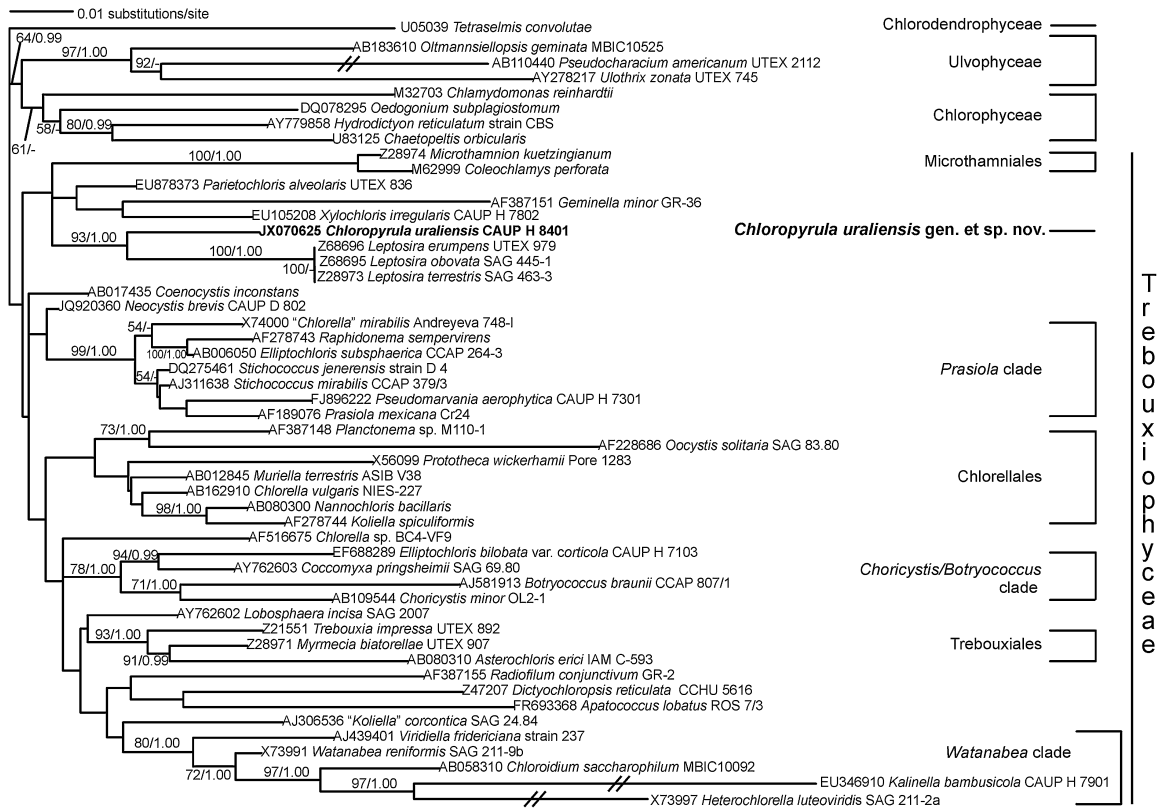


**Fig. 4.** The phylogenetic position of *Chloropyrula uraliensis* gen. et sp. nov. based on the 18S rRNA gene sequence. The phylogeny portrayed is a ML tree inferred using RAxML (employing the GTR + Γ + I substitution model). Numbers at branches represent bootstrap support values from the RAxML rapid bootstrapping search/Bayesian posterior probabilities (only values >50/0.95 are shown). Labels at terminal leaves comprise a DDBJ/EMBL/GenBank accession number of the sequence, source organism, and a strain number (if known).

## 3 Discussion

The alga characterized in this study exhibits a suite of features that is new to science. Among the known algal species it might resemble most closely the genus *Botryokoryne* Reisigl with its single species *Botryokoryne simplex* described by Reisigl (1964) from soil in Ötztal Alps (Austria) as an alga forming cell clusters, with young cells oval, becoming elongate, pyriform to irregularly bottle-shaped. However, *B. simplex* was noted to have a pyrenoid with a starch envelope, whereas *Chloropyrula uraliensis* is apparently devoid of it, and the mature *B. simplex* cells are elongate (20–30 μm in length, 9–10 μm in width), whereas those of *C. uraliensis* are more heterogeneous in shape, being spherical as well as egg-like elliptical, or pyriform (14–25 μm in length, 10–18 μm in width). *Botryokoryne simplex* has been poorly studied since its description, no culture appears to be maintained in public culture collections, and no data is available to investigate its phylogenetic position by molecular phylogenetic methods. While it is possible that our new alga may be related to *B. simplex*, we suggest that the differences outlined above are substantial enough to treat the two species as different taxa at the generic level.

Molecular data have proven essential for establishing the actual phylogenetic relationships between individual green algal taxa at various taxonomic levels, and the 18S rRNA gene is the marker of choice when an obviously novel organism needs to be classified, because it has by far the best sampling across the green algal phylogenetic diversity and generally provides excellent phylogenetic resolution. Indeed, the 18S rRNA gene sequence from *C. uraliensis* does not match closely any of the species currently represented by the 18S rRNA gene sequence in the GenBank database (the most similar sequences differ in at least 75 positions), indicating that it represents a taxon of a significant phylogenetic novelty. Phylogenetic analyses conducted on the basis of 18S rRNA gene sequences shows *C. uraliensis* being most closely related to species of the genus *Leptosira* with good statistical support (Fig. 4). *Leptosira* is classified the class Trebouxiophyceae (Friedl, 1996). Its actual phylogenetic relationship to other trebouxiophytes and the monophyly of the whole class of Trebouxiophyceae were questioned on the basis

of phylogenomic analyses of chloroplast genomes (Turmel et al., 2009), but a more recent analysis using a larger sampling of chloroplast genome sequences and an alternative phylogenomic method does recover *Leptosira* nested within maximally supported monophyletic trebouxiophytes (Lang & Nedelcu, 2012). The robust relationship of *C. uraliensis* to *Leptosira* spp. in the 18S rRNA gene phylogeny therefore seems sufficient to conclude that *C. uraliensis* is a trebouxiophyte alga, in spite of the absence of statistical support for trebouxiophyte monophyly in the 18S rDNA tree.

However, despite the specific relationship between *C. uraliensis* and *Leptosira* spp., their divergence in the 18S rDNA tree is very deep and suggests that the members of these two sister lineages are substantially different. Indeed, few morphological attributes are shared by the genus *Leptosira* and *C. uraliensis*. First, cells of *Leptosira* spp. are typically arranged in short branched filaments, whereas cells of *C. uraliensis* grow in more or less irregular groups without obvious filaments. Second, the chloroplast of *Leptosira* spp. is often band-like and with a smoother margin than that of *C. uraliensis*. Third, most *Leptosira* species, including those represented by the 18S rDNA sequence in our phylogenetic analysis (Fig. 4), possess a pyrenoid (Lukešová, 1991; Ettl & Gärtner, 1995), whereas no pyrenoid could be observed in *C. uraliensis* by light or electron microscopy. It should be noted that the type of species of the genus *Leptosira*, *L. mediciana* Borzì, may lack a pyrenoid (Borz, 1883; Lukešová, 1991), but it is very different from *C. uraliensis* in both the vegetative morphology (forming true irregularly branched filaments) and the much smaller size of zoospores (2.5 μm in length and 0.5–2 μm in width). Some species formerly classified in the genus *Leptosira* are now known to be unrelated, as they belong to classes Chlorophyceae or Ulvophyceae based on molecular phylogenetic evidence (Friedl, 1996).

The large clumps (almost as big as the nucleus) of microfibrils within the cytoplasm revealed by TEM (Fig. 3) are one of the most salient features of *C. uraliensis*. To our knowledge, such a structure has never been described from a green alga. A question obviously emerges whether this structure is somehow related to the synthesis of the cell wall. The structure and biosynthesis of fibrillar cell wall polysaccharides has been studied in only a few trebouxiophyte algae. In *Oocystis* Nägeli ex A. Braun and *Eremosphaera* De Bary the cellulose microfibrils are synthetized by membrane-localized terminal complexes (Quader & Robinson, 1981; Brown, 1985; Saxena & Brown, 2005). Another trebouxiophyte, *Chlorella variabilis* Shihira & R. W. Krauss (strain NC64A), synthesizes chitin instead of cellulose microfibrils, and chitin synthase was suggested to be integrated to the membrane and the cell wall (Kawasaki et al., 2002). There is still a great deal of uncertainty about the subcellular site of the microfibril synthesis. Brown et al. (1970) hypothesized, based on morphological evidence, that cellulose microfibrils in *Pleurochrysis* Pringsheim (Haptophyta) are synthesized in the dictyosome during scale formation. However, modern molecular and immunochemical tools have to be used to verify this statement. The clumps of microfibrils within the cytoplasm of *C. uraliensis* do not seem to be connected with a dictyosome. Microfibrils are extruded to the cell surface probably through the fusion with the plasma membrane of vesicles containing uncoiled microfibrils. More effort should be invested into analyzing the chemical structure of microfibrils and the site of their synthesis.

In conclusion, our study has brought to light a new trebouxiophyte alga that deserves to be recognized as a new species in a newly established genus. By this discovery we have expanded further the known diversity of soil algae, which nevertheless still remain poorly explored and there is no doubt that additional sampling will uncover additional currently unknown taxa, possibly including additional species of the *Chloropyrula* lineage.

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