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THE EXTREMOPHILE *ENDOLITHELLA MCMURDOENSIS* GEN. ET SP. NOV. (TREBOUXIOPHYCEAE, CHLORELLACEAE), A NEW *CHLORELLA*-LIKE ENDOLITHIC ALGA FROM ANTARCTICA[1]

Teresa P. Martins[4], *Vitor Ramos*[2,4], *Guilherme S. Hentschke, Raquel Castelo-Branco*[4], *Adriana Rego*

Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal

Maria Monteiro

Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal
School of Science, University of Waikato, Hamilton, New Zealand

Ângela Brito

Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal
Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal

Paula Tamagnini

Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal
Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal
Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

S. Craig Cary

School of Science, University of Waikato, Hamilton, New Zealand
International Centre for Terrestrial Antarctic Research, University of Waikato, Hamilton, New Zealand

Vitor Vasconcelos

Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal
Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

Lothar Krienitz

Department of Experimental Limnology, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, D-16775 Stechlin, Germany

Catarina Magalhães

Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal
Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

and Pedro N. Leão[3]

Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal

The McMurdo Dry Valleys constitute the largest ice-free region of Antarctica and one of the most extreme deserts on Earth. Despite the low temperatures, dry and poor soils and katabatic winds, some microbes are able to take advantage of endolithic microenvironments, inhabiting the pore

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[2]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança Portugal.
[3]Author for correspondence: e-mail pleao@ciimar.up.pt.
[4]These authors contributed equally to this work.
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spaces of soil and constituting photosynthesis-based communities. We isolated a green microalga, *Endolithella mcmurdoensis* gen. et sp. nov, from an endolithic sandstone sample collected in the McMurdo Dry Valleys (Victoria Land, East Antarctica) during the K020 expedition, in January 2013. The single non-axenic isolate (*E. mcmurdoensis* LEGE Z-009) exhibits cup-shaped chloroplasts, electron-dense bodies, and polyphosphate granules but our analysis did not reveal any diagnostic morphological characters. On the basis of phylogenetic analysis of the 18S rRNA (SSU) gene, the isolate was found to represent a new genus within the family Chlorellaceae.

Key index words: **18S rRNA; extremophiles; genus; LEGEcc; microalgae; sandstone**

***Abbreviations*: BI, Bayesian inference; DAPI, 4′,6-diamidino-2-phenylindole; LEGEcc, Blue Biotechnology and Ecotoxicology culture collection; OTU, operational taxonomic unit; QIIME, Quantitative insights into microbial ecology**

The remarkable diversity of green algae reflects a great adaptive capacity, allowing for survival in the most extreme environments on Earth such as marine deep waters (Zechman et al. 2010, Hodač et al. 2016, Spalding et al. 2016), deserts (Friedmann and Ocampo 1976, Lewis and Lewis 2005), hypersaline habitats (Vinogradova and Darienko 2008), or habitats with temperatures below freezing point (Morgan-Kiss et al. 2008). Even in these extreme settings, green microalgae play an important ecological role (Lyon and Mock 2014), but their diversity is far from being understood, especially in unexplored polar environments (Hodač et al. 2016). The life-limiting environmental conditions that occur in Antarctica are triggers for speciation and development of endemic species, making this a particularly promising location for the exploration of microbial diversity (Vincent 2000).

In Antarctica, trebouxiophycean microalgae have been known since the description of *Chlorella antarctica* (Wille 1928), and many studies have highlighted the considerable diversity of this group in cold environments (Friedmann and Ocampo 1976, Friedmann 1982, Carvalho et al. 2017, Nedbalová et al. 2017). The McMurdo Dry Valleys constitute the largest ice-free region of Antarctica (Horowitz et al. 1972) and one of the world's most extreme deserts. The average annual temperature is about −22°C, winter temperatures typically of −60°C, precipitation levels are lower than 100 mm water equivalent, the land is battered by katabatic winds and intense UV radiation, and the soils are poor, with low levels of carbon, nitrogen, and small amounts of clay (Wynnwilliams 1990, Ugolini and Bockheim 2008, Cary et al. 2010). Despite all these extreme conditions, life exists. Soil and rocks act as refuges, protecting life from intense solar radiation and desiccation providing milder microclimate conditions that ease the growth of microbial life (Pointing et al. 2009) with significant diversity (Wood et al. 2008). In fact, some microbes are able to inhabit porous rocks such as sandstones (Friedmann 1982, Hughes and Lawley 2003), constituting photosynthesis-based endolithic communities (Friedmann and Ocampo 1976) that rely heavily on cyanobacteria and microalgae. In Antarctic land environments, green algae, especially *Chlorella*-like forms, are particularly abundant and diverse (Hodač et al. 2016) and have an important role in nutrient cycling and as primary producers, providing organic matter in the oligotrophic soils (Tscherko et al. 2003).

Herein, we describe the new trebouxiophycean microalga *Endolithella mcmurdoensis* gen. et sp. nov, isolated from an endolithic habitat in the McMurdo Dry Valleys, Antarctica, that was found by pyrosequencing analysis to be dominated by cyanobacteria.

MATERIALS AND METHODS

Sampling, isolation, and culturing. Endolithic samples (Fig. 1, A and B) were collected during the K020 Mission, that took place in January 2013, on Victoria Valley, located in McMurdo Dry Valleys region, in South Victoria Land, Antarctica (77°20.502 S, 161°39.306 E). Collected samples were preserved at −80°C in LifeGuard Solution (Mo Bio Laboratories, Carlsbad, CA, USA). Isolate LEGE Z-009 was obtained from an isolation process originally intended for isolating cyanobacteria (Rego et al. 2019). An aliquot of the endolithic sample was washed and centrifuged three times (4500*g*, 3 min) with nitrogen-free $BG11_0$ medium (Rippka 1988). It was then inoculated into a 100 mL Erlenmeyer flask with liquid Z8 medium (Kotai 1972) as an initial enrichment culture and placed at 19°C, under a 12:12 h light (25 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}): dark cycle. When visible growth was detected, an aliquot was transferred and streaked onto a solid $BG11_0$ medium plate (1.5% agar supplemented with 25 μL of cycloheximide), and placed at 12°C. Single colonies were then microscopically inspected, selected, and re-streaked aseptically in the same medium and/or in Z8, until isolation was achieved. The isolated strain was deposited in the LEGE Culture Collection in CIIMAR, and is maintained cryopreserved and at 12°C and 19°C, in liquid Z8 medium, under the identification LEGE Z-009.

Pyrosequencing (16S rRNA-based) analysis of the endolithic community. Environmental DNA (eDNA) was extracted from the same Victoria Valley sample used for isolation of the microalga strain, using a modification of the CTAB extraction protocol (Barrett et al. 2006). The 16S small subunit (SSU) rRNA gene was initially amplified by PCR using the universal primer pair 27F/1492R (Weisburg et al. 1991) and then sequenced by pyrosequencing technology. Briefly, the 16S rRNA gene was amplified for the V3/V4 hypervariable region with barcoded fusion primers containing the Roche-454 A and B Titanium sequencing adapters, an eight-base barcode sequence, the forward (5′-ACTCCTACGGGAGGCAG-3′) and reverse (5′-TACNVRRGTHTCTAATYC-3′) primers (Wang and Qian 2009). Amplicons were quantified by fluorimetry with PicoGreen (Invitrogen), pooled at equimolar concentrations and sequenced in the A direction with GS 454 FLX Titanium chemistry, according to the manufacturer's instructions (454 Life Sciences, Roche, Basel, Switzerland) at Biocant (Cantanhede, Portugal). The 454-machine-generated FASTA (.fna) and quality score (.qual) files were processed using the

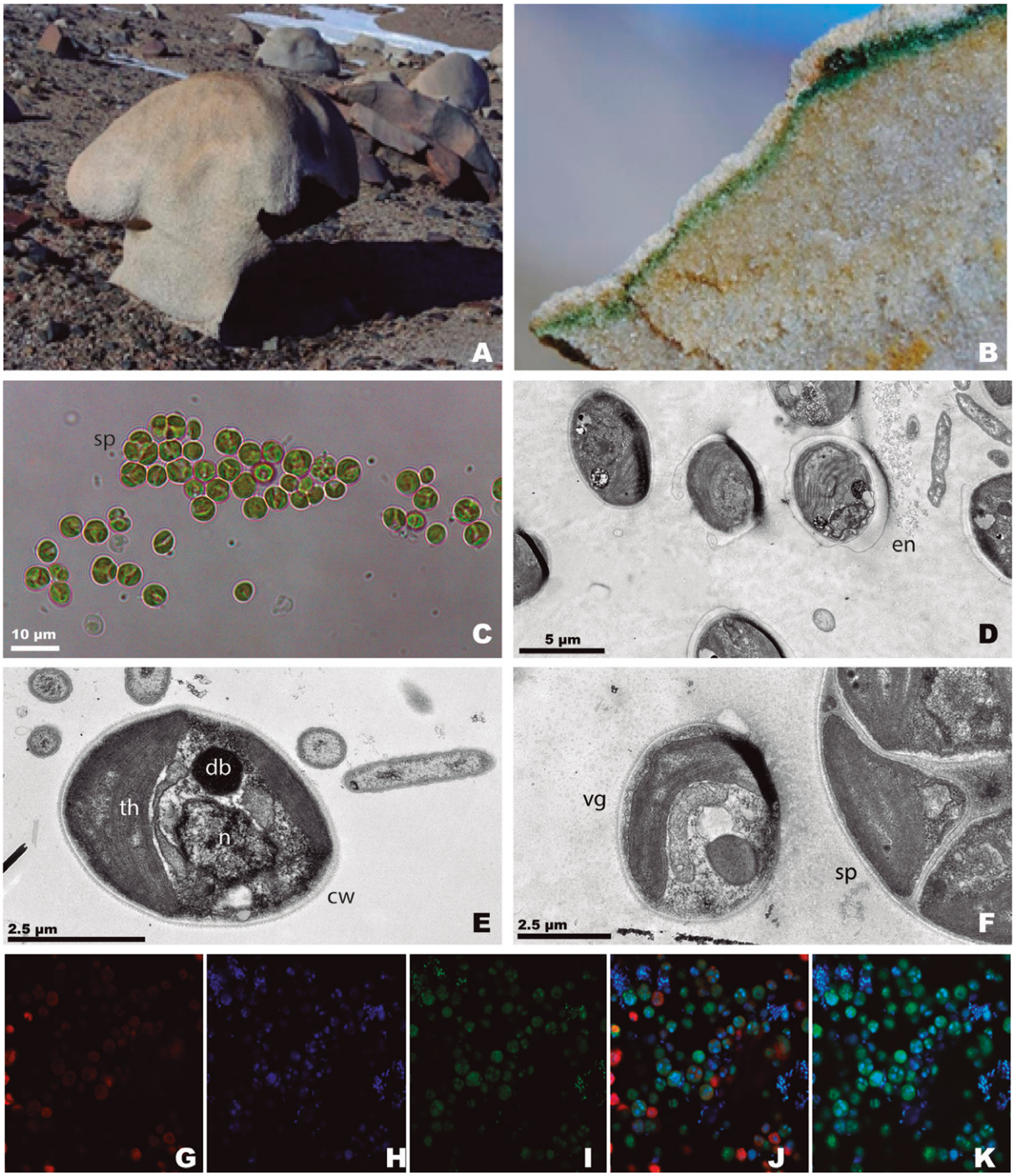

FIG. 1. (A,B) Colonization of the cryptoendolith-bearing sandstone in McMurdo Dry Valleys. (C) Light photomicrographs of the chlorophyte microalga LEGE Z-009 (1000× magnification). (D–F) TEM photomicrographs of the chlorophyte microalga LEGE Z-009 culture: th, thylakoids; db, electron-dense body; cw, cell wall; en, cell envelope; n, nucleus, vg, vegetative cell, sp, sporangium. (G–K) Fluorescence photomicrographs of Endolithella mcmurdoensis LEGE Z-009 with DAPI staining highlighting the co-localization of polyphosphate granules and microalgal cells; chloroplast epifluorescence (G), DAPI blue-fluorescence emission upon DNA binding (H), DAPI green-fluorescence emission (1000× magnification) upon polyphosphate granules binding (I), merged G, H and I (J) and merged H and I (K). [Color figure can be viewed at wileyonlinelibrary.com]

QIIME (Quantitative insights into microbial ecology) pipeline (Caporaso et al. 2010). Initially, raw reads were demultiplexed and subjected to a quality filtering—sequences with a quality score below 25 were removed. The next step, Pick OTUs (Operational Taxonomic Units; Sneath and Sokal 1973) was performed using the pick_open_reference_otus.py workflow (open-reference method; i.e., unmatched sequences are clustered de novo and included in the downstream analysis). No action was undertaken to remove eventual plastid-derived sequences from the data set. The OTU table

obtained from the open-reference method was selected to the downstream analyses. Essentially, all sequences (average length 428 bp) were clustered into OTUs at 97% sequence similarity using UCLUST (Edgar 2010) and the reads aligned to the Greengenes v13_8 (GG; DeSantis et al. 2006) database using PyNAST. For the taxonomic assignment, the RDP Classifier 2.2 (Wang et al. 2007) was used with the UCLUST method. Sequencing reads from this study were deposited in the European Nucleotide Archive (ENA) under the accession number PRJEB34393.

Microscopy. The isolated strain was observed and photographed under a Leica DMLB microscope. Measurements of 40 cells from a growing-phase culture were performed using a dedicated software (Leica LAS EZ; Leica Microsystems). For TEM studies, BG11- and N-depleted $BG11_0$-grown microalgal cultures were centrifuged and the cells were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for at least 2 h, followed by a buffer wash (5 min) and overnight incubation with osmium tetroxide (2% w/v). A new buffer wash was performed, and samples were incubated overnight with 1% uranyl acetate (w/v). Dehydration was performed using an ethanol series (25–100%; v/v), and twice propylene oxide (10 min each). Subsequently, samples were embedded in mixtures of propylene oxide and Epon resin, followed by Epon for at least 24 h, before being placed in embedding molds with Epon, and allowed to polymerize at 65°C for 48 h. Ultrathin sections were cut with an ultramicrotome Leica Reichert SuperNova, placed on electron microscopy grids, and visualized using an electron microscope (JEOL JEM-1400Plus).

Polyphosphate granules and DNA were visualized with DAPI staining according to previous standardized protocol (Mukherjee and Ray 2015). Preparations were visualized under Laser Scanning Confocal Microscope Leica SP2 AOBS SE (Leica Microsystems) using the following wavelength channels: 430–460 nm for DNA staining, 520–530 nm for polyphosphate granules, and 640–680 nm for chlorophyll epifluorescence. Images were merged using Adobe Photoshop software.

DNA extraction, PCR amplification, and cloning. Genomic DNA extraction was performed with the DNeasy® Plant Mini Kit (QIAGEN) following the manufacturer's instructions for plant DNA extraction. Plastid-encoded 16S small subunit (SSU) rDNA was amplified by PCR using the primers CYA106F, CYA359F, CYA781R (Nubel et al. 1997), and 1492R (Weisburg et al. 1991). Nuclear-encoded 18S SSU rDNA amplification was performed using the internal primers 18S402F, 18S895F, 18S919R, 18S1339R (Katana et al. 2001), and the external 18SF and 18SR primers (Moon-van der Staay et al. 2000). The nuclear internal transcribed spacer region (ITS1-5.8S-ITS2) was amplified using primers 18S895F and ITS055R (Marin et al. 2003). All PCRs were performed according with the conditions originally reported for the used oligonucleotides. PCR products were separated in a 1.5% (w/v) agarose gel stained with GelRed™ (Biotium, Freemont, CA, USA) and DNA fragments with the expected size were excised from the gel and purified using the NucleoSpin™ Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany). The purified 16S rDNA PCR product was sequenced (GATC Biotech, Konstanz, Germany) with CYA106F/1492R primers while purified 18S rDNA and ITS PCR products were TA cloned using a pGEM®-T Easy Vector System Kit (Promega, Madison, WI, USA), and transformed into *Escherichia coli* ONE SHOT® TOP10 chemically competent cells (Invitrogen). Colonies were selected by blue-white screening, and the presence of the appropriate insert was evaluated by colony PCR. Colonies with an insert of the expected size were grown overnight at 37°C in liquid LB medium supplemented with 100 $\mu g \cdot mL^{-1}$ of ampicillin, with shaking. Plasmid DNA was isolated using the NZY Miniprep kit (NYZTech, Lisbon, Portugal) and sequenced (GATC Biotech) using M13 primers. All nucleotide sequences were manually inspected for quality, trimmed, and assembled using the Geneious 8.1.9. software package (Biomatters, Auckland, New Zealand). Sequences obtained in this study were deposited in the NCBI GenBank database under accession numbers MN394916 (16S rRNA gene), MK682757 (18S rRNA gene), and MK682753 (ITS).

Phylogenetic analysis. The 18S rRNA gene Maximum Likelihood (ML) and Bayesian inference (BI) trees presented here were constructed in two rounds. In the first round, to place our species within the Trebouxiophyceae tree, we performed a ML phylogeny with our sequence and with sequences of reference strains representing Chlorellales, Prasiolales, and Trebouxiales. Then, we included sequences retrieved in GenBank by BLAST. In the second round, to obtain trees with higher phylogenetic support, we selected the phylogenetically most closely related taxa to *Endolithella mcmurdoensis* and performed ML and BI analysis. In the ITS2 phylogenies (ML and BI), we included the OTUs with available ITS2 sequences from our second round 18S rDNA phylogenies and sequences retrieved from GenBank by BLAST.

For all generated trees, sequences were aligned by ClustalW, and the GTR+G+I evolutionary model was selected by MEGA 7.0 (Kumar et al. 2016). The robustness of ML trees was estimated by bootstrap percentages, using 1000 replications in MEGA 7.0 (Kumar et al. 2016). Bayesian trees were constructed in two independent runs, with four chains each, for 5×10^5 generations using MrBayes (Ronquist and Huelsenbeck 2003) in Cipres Gateway (Miller et al. 2010). The outgroup used was *Oocystis marssonii* (AF228688).

Internal transcriber spacer 2 (ITS2) structures. The ITS2 region of *Endolithella mcmurdoensis* MK682757 (280 bp) and the phylogenetically closest related sequences: *Auxenochlorella protothecoides* FN298931 (354 bp) and *A. protothecoides* LN610704 (354 bp) were annotated using ITS2 database (Koetschan et al. 2010) and folded with Mfold (Zuker 2003). Images were built using 4SALE (Seibel et al. 2006).

RESULTS

Diversity in the endolithic community and isolation of Endolithella mcmurdoensis *LEGE Z-009.* To obtain an overview of the microbial composition of the endolithic sample, 16S rRNA-based pyrosequencing analysis was performed and revealed that the endolithic community collected from the McMurdo Dry Valleys sample (Fig. 1, A and B) was dominated by Cyanobacteria (44%) and Actinobacteria (30%), followed by lower abundances of Chloroflexi (10%) and Proteobacteria (6%). The photosynthetic microbial community was clearly dominated by an *Acaryochloris*-like unicellular cyanobacterium representing 99% of the cyanobacterial/chloroplast OTUs present in the sample (Fig. S1 in the Supporting Information), which is in line with our own microscopic examination that revealed a clear dominance by coccoid cyanobacteria (Fig. S2 in the Supporting Information). These results are in accordance with other endolithic communities previously characterized (Walker and Pace 2007), where oxygenic phototrophs, especially cyanobacteria, represented the major fraction of community, followed by Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Proteobacteria, and Crenarchaeota. In addition, the

dominance of *Acaryochloris* suggested a high level of adaptation to this type of environment, which may result from its high chlorophyll d content (Partensky et al. 2018).

Although, from our pyrosequencing analysis, we found no evidence for chloroplast-derived 16S rRNA gene OTUs in the original endolithic sample, the strain *Endolithella mcmurdoensis* LEGE Z-009 was isolated from the sample, following repeated streaking in agar plates under laboratory conditions.

Morphology. Endolithella mcmurdoensis was not detected by microscopic observation of the endolithic community sample; therefore, morphological analysis and taxon description were performed based only on the cultured isolate strain *E. mcmurdoensis* LEGE Z-009.

Description. Endolithella T. Martins, V. Ramos, Hentschke & Krienitz gen. nov.

Cells living in endolithic layers, solitary, ovoid, and uninucleate. Cell wall two-layered. Asexual reproduction is by autospores. The genus differs from other members of Chlorellaceae by its extreme habitat in the polar region, endolithic life style, 18S rRNA gene, and ITS2 secondary structure. TYPE SPECIES: *Endolithella mcmurdoensis* T. Martins, V. Ramos, Hentschke & Krienitz.

ETYMOLOGY: The generic name reflects the endolithic habitat.

Endolithella mcmurdoensis T. Martins, V. Ramos, Hentschke & Krienitz sp. nov. (Fig. 1, C–K)

Individuals solitary, sometimes forming small chains with two to four cells. Mature vegetative cells spherical or sub-spherical, 3.2–5.2 μm in diameter (average: 4.42 μm), with double-layered cell wall covered by mucilage (Fig. 1C). Chloroplast cup-shaped, without pyrenoids (Fig. 1, D–F). From TEM analysis (Fig. 1, D–F), electron-dense bodies and polyphosphate granules present, the latter confirmed by fluorescence microscopy (Fig. 1, G–K). Reproduction by two (rarely) or four ovoid autospores with 2.0–3.8 μm in diameter (average: 3.1 μm). Sporangia spherical or sub-spherical, 4.4–6.6 μm in diameter (average: 5.3 μm).

Comments: There is no diagnostic morphological character in *Endolithella*. The taxonomic identification is only possible considering the typical extreme habitat (endolithic in Antarctica) and the molecular data presented below.

HOLOTYPE: authentic strain permanently cryopreserved, *Endolithella mcmurdoensis* LEGE Z-009, deposited in the Blue Biotechnology and Ecotoxicology Culture Collection (LEGEcc) at CIIMAR, Porto, Portugal (http://lege.ciimar.up.pt), collected 01/23/2013, leg. Catarina Magalhães. The strain has been deposited in the same collection as an active culture.

ISOTYPE: an air-dried herbarium specimen of the authentic strain LEGE Z-009 deposited at the Botanical Museum Berlin-Dahlem under the designation B 40 0043196.

TYPE LOCALITY: Antarctica, South Victoria Land, Victoria Valley, located in McMurdo Dry Valleys region.

HABITAT: Endolithic.

ETYMOLOGY: The specific epithet refers to the geographic origin of the type strain.

Phylogenetic position. The nuclear-encoded 18S rRNA gene (1,796 bp) and the ITS2 were used for the molecular characterization of *Endolithella mcmurdoensis* LEGE Z-009. Blast searches against the GenBank database indicated best identity scores for *Pseudochlorella pringsheimii* KY364701 (95.89%) and *Nannochloris* AS 2-10 AY195968 (95.84%). Both sequences were included in the first round trees, but proved to be not phylogenetically closely related to *E. mcmurdoensis*, reinforcing supporting the description of the new genus.

An initial 18S rRNA gene phylogenetic tree (Fig. S3 in the Supporting Information), with a total of 87 OTUs and 1487 nucleotide analyzed positions and containing genera of Trebouxiales, Chlorellales, and Prasiolales, was constructed. In this tree, *Endolithella mcmurdoensis* LEGE Z-009 is positioned within the family of Chlorellaceae, weakly supported at the base of a large clade containing (sequentially) the *Nannochloris* (bootstrap value 62), *Parachlorella* (bootstrap value 99), and *Chlorella* (bootstrap value 49) clades. Basal to this group (*E. mcmurdoensis* included) are the *Auxenochlorella* clade, as well as genera of Prasiolales and Trebouxiales. *Endolithella mcmurdoensis* LEGE Z-009 is positioned outside any of the already known clades of Chlorellaceae.

In subsequently constructed trees (Fig. 2, Fig. S4 in the Supporting Information), only the most phylogenetically closely related taxa to *Endolithella mcmurdoensis* were included in the analysis. Again, the new taxon proved not to be phylogenetically closely related to any currently known clades of Trebouxiophyceae. In the ML tree, the new genus is positioned basal to *Nannochloris* (boostrap value 99), *Parachlorella* (bootstrap value 100), and *Chlorella* (bootstrap value 100) clades, as also observed for the first round tree, but now with stronger phylogenetic support (Fig. S4). In the BI tree (Fig. 2), *E. mcmurdoensis* LEGE Z-009 is basal to the *Auxenochlorella*-clade and again, not phylogenetically related to the other clades of Trebouxiophyceae. The average standard deviation of split frequencies for this analysis was 0.009191 and the average potential scale reduction factor (PSRF) for parameter values was 0.998. In all first and second round trees, all genera present strong phylogenetic support. The ITS2 ML and BI phylogenies corroborate with these results showing *Endolithella* as a weak supported sister clade of *Auxenochlorella* (Fig. S5 in the Supporting Information).

ITS2 secondary structure. The ITS2 secondary structures (Figs. 3, S6 and S7 in the Supporting Information) are consistent with the usual structures for eukaryotes with four helices, a pyrimidine–pyrimidine mismatch in helix II, an A-rich region between

helices II and III and a TGGT motif 5′ in helix III, the longest one (Schultz et al. 2005). Comparisons between *Endolithella mcmurdoensis* LEGE Z-009 ITS2 secondary structure (280 bp, from MK682757) with the closest phylogenetic sequences showed that *Endolithella* presents a unique ITS2, differing in length and structure from *Auxenochlorella protothecoides* CCAP 211/7A (354 bp, from FN298931) and *A. protothecoides* CCAP 211/17 (354 bp, from LN610704). This analysis corroborates our 18S rDNA and ITS2 phylogenies in supporting *Endolithella* as a new genus.

DISCUSSION

We described the new chlorellacean genus and species *Endolithella mcmurdoensis* based on 18S rRNA gene and ITS2 data from the strain LEGE Z-009. The isolate was obtained after repeated streaking in solid media, despite chloroplast sequences being absent from the pyrosequencing data set, underpinning the importance of culture-dependent approaches to retrieve non-abundant biodiversity (Rego et al. 2019). From the morphological description presented here, *E. mcmurdoensis* LEGE Z-009 can be related to *Auxenochlorella*, *Pumilliosphaera*, and *Meyerella* by the absence of a pyrenoid. This character distinguished *E. mcmurdoensis* from *Chlorella*-like pyrenoid-bearing genera (Gaysina et al. 2013, Neustupa et al. 2013). *Endolithella mcmurdoensis* LEGE Z-009 presents electron-dense bodies and polyphosphate granules that play important roles as environmental stress adaptation structures. Electron-dense bodies in green algae, previously documented in *Mychonastes homosphaera* from lake Kinneret (Hanagata et al. 1999), are related to metabolite deposition, working usually as poly-P accumulation structures (Ota and Kawano 2018). For instance, under early sulfur-depletion conditions, poly-P is accumulated in electron-dense bodies (Ota et al. 2016b), being hydrolized to Pi in a later stage, and released to the cytosol, acting as a protein-protective chaperone (Gray et al. 2014, Gray and Jakob 2015). The accumulation of polyphosphate and other nutrients such as lipids is known to be considerably high in members of Chlorellaceae (Ota et al.

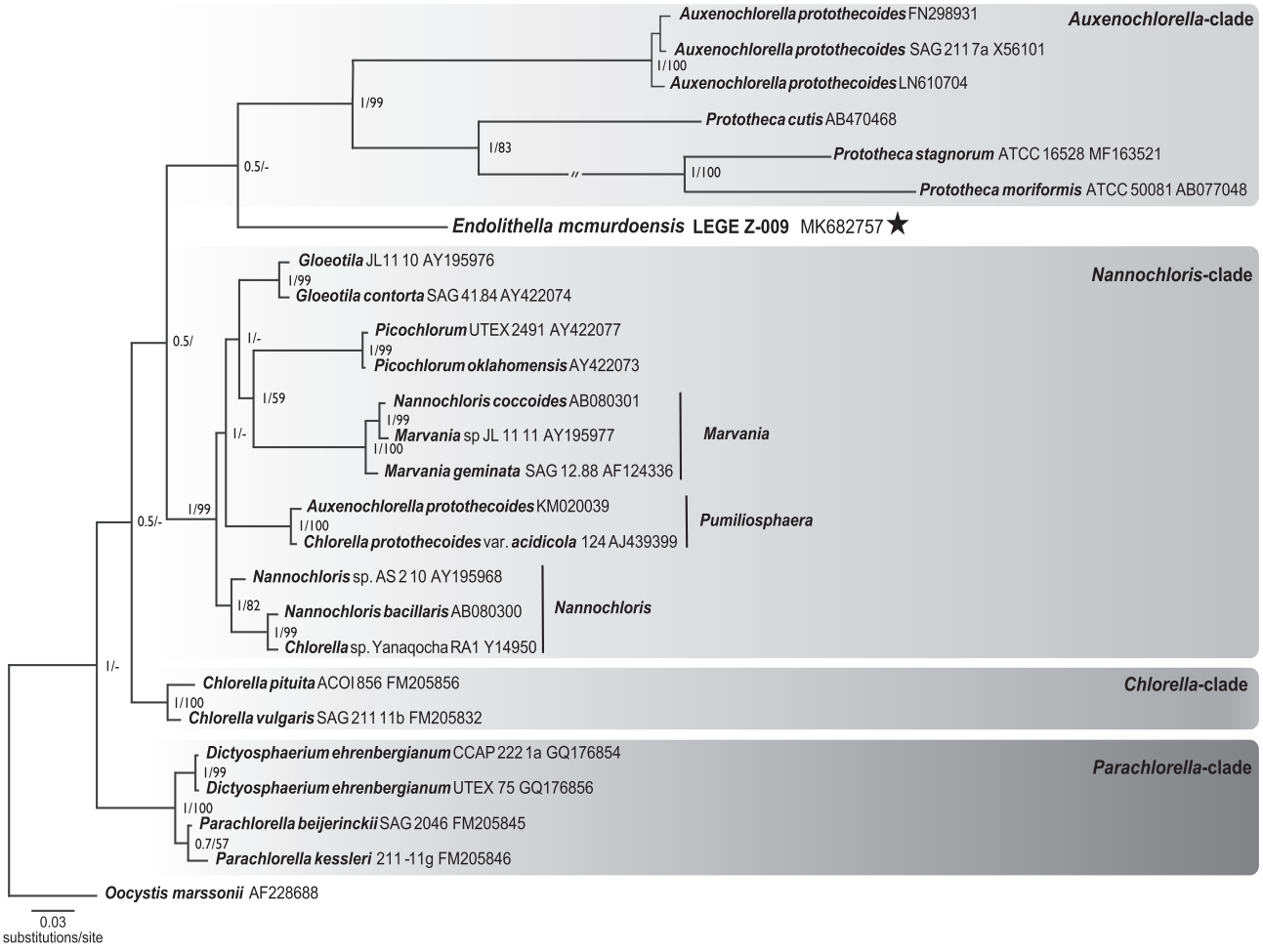

FIG. 2. 18S rRNA gene Bayesian phylogenetic analysis. Posterior probabilities and Bootstrap values (BI/ML, respectively) are indicated at nodes. "–" denotes no support.

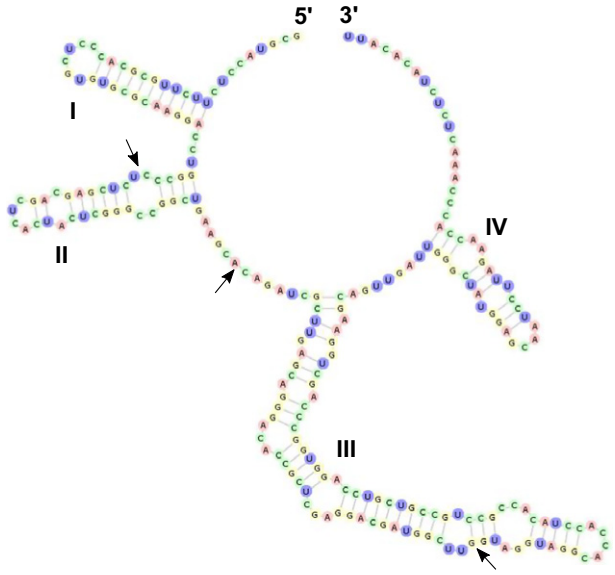

FIG. 3. ITS2 secondary structure of Endolithella mcmurdoensis LEGE Z-009 (280 bp, from MK682757) visualized with 4SALE. ITS2 helices are numbered I–IV and typical motifs are highlighted (arrows): the pyrimidine–pyrimidine mismatch in helix II; the A-rich region between helices II and III, as well as the UGGU motif 5′ to the apex of helix III. [Color figure can be viewed at wileyonlinelibrary.com]

2016a), working as survival strategy in harsh, adverse conditions, such as strong UV radiation and temperature fluctuations (Achbergerová and Nahalka 2011) but also under N deprivation (Chu et al. 2013), which is precisely the condition under which this alga was isolated.

Despite the lack of a diagnostic morphological character, our phylogenies and ITS2 secondary structure data confirm that *Endolithella mcmurdoensis* LEGE Z-009 is a new taxon, with an uncertain position among the already known clades of Chlorellaceae. Comparing the topologies of the BI and ML trees (Fig. 2 and Fig. S4, respectively), *E. mcmurdoensis* LEGE Z-009 is basal to *Chlorella*, *Parachlorella*, and *Nannochloris* clades in the ML trees, but in the BI tree the new taxon is more closely related to the extremophile *Auxenochlorella* clade. In both trees, the phylogenetic relationships between *E. mcmurdoensis* LEGE Z-009 and the other clades are only weakly supported but it was possible to infer that *Endolithella* is a distinct genus. We hypothesize that *E. mcmurdoensis* LEGE Z-009 could be a new lineage in the Chlorellaceae family, a proposition that has to be confirmed by further studies.

Herein, we are presenting only the BI tree (which includes ML bootstrap values) because this tree shows the close relationship between *Endolithella mcmurdoensis* LEGE Z-009 and the extremophile clade of *Auxenochlorella* and *Prototheca*. The last two genera were described from sap of trees samples and were characterized as being dependent of exogenous organic compounds (Ueno et al. 2005, Darienko and Pröschold 2015). *Endolithella mcmurdoensis* LEGE Z-009 is also an extremophile taxon, living in Antarctica cold desert sandstones.

The potential of polar environments as collection sites for new microalgae taxa discoveries begins with the description of *Chlorella antarctica* in 1924 (Wille 1928). However, strains isolated from these environments were considered to be "non-indigenous," growing from spores that reached polar regions by wind dispersal. This view lasted until Friedmann and Ocampo (1976) reported endolithic microalgae growing in Antarctica. This was followed by the discovery of many microalgae species (including Cyanobacteria) in polar regions (Nedbalová et al. 2017, Davey et al. 2019). Our endolithic sample, presenting a conspicous thick layer of different photosynthetic microorganisms inside a porous rock, is in line with an "indigenous" origin. Still, recent studies (Hodač et al. 2016) show that, within the same microalgae species, specimens from polar regions can show no Compensatory Base Changes in ITS2 secondary structures when compared to specimens from temperate zones. In light of these findings, and despite the endolithic character of the LEGE Z-009 isolate, we cannot fully attest to the Antarctic endemic character of *Endolithella mcmurdoensis*.

The identification of this new genus represents an important taxonomic novelty and it is in line with recent studies that point toward a much more extensive and interesting biodiversity in Antarctica than previously thought (Chown et al. 2015).

Finally, we highlight the importance of studies on polar microalgae communities due to their potential in biotechnological aplications like protection from UV radiation (Miao et al. 2002), energy production (Chen et al. 2012), or nanoparticle biosynthesis (Gallardo et al. 2014), considering their survival strategies and ability to grow and produce organic compounds in these extreme habitats with light, temperature, and nutrient limitations.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. (A) Taxonomy summary bar chart of relative frequency of 10 most abundant phyla in the initial sample, based on 16S rRNA gene sequences (B) Summary bar chart of the most abundant taxonomic frequency distributions at Genus level for the oxygenic photosynthetic community (both taxa are Cyanobacteria).

Figure S2. Environmental sample photomicrographs. Bright field image (A) of the same optical field as in the fluorescence image (B).

Figure S3. 18S rRNA gene Maximum Likelihood phylogenetic analysis of trebouxyophycean strains. Bootstrap values are indicated at nodes. The analysis involved 87 nucleotide sequences and there were a total of 1487 positions in the final dataset.

Figure S4. 18S rRNA gene Maximum Likelihood phylogenetic analysis of a selection of chlorellacean strains. Bootstrap values are indicated at nodes. The analysis involved 26 nucleotide sequences and there were a total of 1664 positions in the final dataset.

Figure S5. ITS2 secondary structure of *Auxenochlorella protothecoides* FN298931, visualized with 4SALE.

Figure S6. ITS2 secondary structure of *Auxenochlorella protothecoides* LN610704, visualized with 4SALE.

Figure S7. ITS2 secondary structure of *Auxenochlorella protothecoides* LN610704, visualized with 4SALE.