Several strains of terrestrial algae isolated from biological soil crusts in Germany and Ukraine were identified by morphological methods as the widely distributed species Dictyosphaerium minutum (=Dictyosphaerium chlorelloides). Investigation of the phylogeny showed their position unexpectedly outside of Chlorellaceae (Trebouxiiophyceae) and distantly from Chlorella chlorelloides, to which this taxon was attributed after revision of the genus Chlorella based on an integrative approach. SSU rRNA phylogeny determined the position of our strains inside a clade recently described as a new genus of the cryptic alga Xerochlorella olmiae isolated from desert biological soil crusts in the United States. Investigation of the morphology of the authentic strain of X. olmiae showed Dictyosphaerium-like morphology, as well as some other characters, common for our strains and morphospecies D. minutum. The latter alga was described as terrestrial and subsequently united with the earlier described aquatic representative D. chlorelloides because of their similar morphology. The revision of Chlorella mentioned above provided only one aquatic strain (D. chlorelloides), which determined its position in the genus. But terrestrial strains of the morphospecies were not investigated phylogenetically. Our study showed that the terrestrial D. minutum is not related to the morphologically similar D. chlorelloides (=Chlorella chlorelloides, Chlorellaceae), and instead represented a separate lineage in the Trebouxiiophyceae, recently described as genus Xerochlorella. Therefore, revision of Xerochlorella is proposed, including nomenclatural combinations, epitypifications, and emendations of two species: X. minuta and X. dichotoma. New characters of the genus based on investigation of morphology and ultrastructure were determined.

Key index words: Chlorophyta; Dictyosphaerium; epitypification; integrative approach; phylogeny; taxonomy; ultrastructure; Xerochlorella

Abbreviations: AIC, Akaike information criterion; KW, Herbarium of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine; ML, maximum likelihood; SAG, Culture Collection of Algae at Göttingen University, Germany; UTEX, Culture Collection of Algae at the University of Texas at Austin

Development of modern taxonomy of microalgae proceeds on the basis of wide usage of molecular-phylogenetic methods. These methods are powerful tools to determine with high accuracy relationships between organisms, their phylogenetic position...
among the numerous groups of algae, and to identify morphologically cryptic taxa (Leliaert et al. 2014). Since many microalgae are organisms with a very limited number of morphological characters for taxonomic purposes (because of simple morphology and life cycle, high adaptive and morphological plasticity, etc.) molecular-phylogenetic methods often are the main or even only tool to delimit taxa. Therefore, algal identification based on molecular markers (including culture independent methods, environmental, and next generation sequencing) represents the current state of the art approach (De Clerck et al. 2013, Leliaert et al. 2015, B. approach (De Clerck et al. 2013, Leliaert et al. 2015, Zimmermann et al. 2015, Büdel et al. 2016).

Despite fast development of new methods and their wide practical application, the main part of accumulated knowledge of algae of different taxonomic groups, such as their diversity, distribution, ecology is still based on the classical morphological approach developed during the middle of 19th century (Friedl and Rybalka 2012, De Clerck et al. 2013, Ettl and Gärtner 2014, Büdel et al. 2016 etc.). Many algal species were originally described based on field material or algal cultures, both of which were later lost in many cases. The type information of these species was usually preserved as line drawings. Further investigation of lost type material by genetic methods is possible only by designating a reference strain as an epitype, with morphological and ecological characters as well as geographical distribution corresponding as close as possible to the original description of the respective taxon.

The so-called integrative approach (combination of classical algal cultivation and microscopy with new molecular-phylogenetic methods) is a feasible instrument to combine morphological and genetic information, particularly for biodiversity studies (Darienko et al. 2015). There exists now many papers devoted to taxonomic revisions of green, streptophycean, xanthophycean, eustigmatophycean algae, cyanobacteria, etc., using such an integrative approach (Neustupa et al. 2011, Rybalka et al. 2013, Bohunická et al. 2015, Skaloud et al. 2016, Darienko et al. 2017, Kryvenda et al. 2018, Mikhailiyuk et al. 2018, Darienko and Pröschold 2019 etc.).

Incompleteness of the algal genetic database sometimes leads to the fact that some newly discovered lineages may be described as new taxa. But in fact these algae were found and described by classical methods many years ago. The reasons for this problem are also the simplicity and plasticity of microalgal morphology. Using an integrative approach including molecular-phylogenetic and follow-up detailed morphological investigations of these taxa may show such disagreements.

An example of such taxonomical disagreement is the recently described desert green algal genus Xerochlorella (Fučíková et al. 2014). This is an authospore-forming alga with small spherical cells (max 10 μm in diameter) and inconspicuous Chlorella-like morphology. More detailed investigation of an authentic strain of this monotypic genus as well as several newly isolated strains led to the conclusion that Xerochlorella is identical to the widely distributed terrestrial species Dictyosphaerium minutum, described more than 80 years ago by classical methods (Petersen 1932, Ettl and Gärtner 2014). Therefore, this paper is devoted to the respective taxonomic revision of the genus Xerochlorella based on an integrative approach.

**METHODS**

**Strains, culture conditions, light microscopy, ultrastructure.** As material for the present study, four unialgal original strains were used. These strains were isolated from terrestrial habitats (biological soil crusts from maritime sand dunes or forest soil) in Europe (Germany and Ukraine). The isolation procedure and culture conditions were described in a previous paper (Schulz et al. 2016). For comparison, an authentic strain of the recently described Xerochlorella alniae, UTEX B 2995 (isolated from desert soil crust [USA]) was used. DNA sequences were compiled from GenBank of other Dictyosphaerium-like strains phylegetically related to Xerochlorella. Short information about all strains and sampling sites are provided in Table 1.

Purified unialgal strains were maintained on solid medium (1.5% agar with 3N BBM and vitamins; Starr and Zeikus 1993) at 20°C with 25 μmol photons·m⁻²·s⁻¹ (Osmalumilux Cool White lamps L36W/840) under a light/dark cycle of 12:12 h light/dark. Morphological examination of these unialgal cultures was performed using Olympus BX51 and Zeiss Axiovert 200 M light microscopes with Nomarski DIC optics. Photomicrographs were taken with digital cameras [Olympus UC30 (Tokyo, Japan) and Zeiss Axiovision (release 4.7, Oberkochen, Germany)] attached to the respective microscopes and processed by software cell Sens Entry and Zeiss Axiovision.

Samples were fixed for transmission electron microscopy (TEM) using a standard chemical fixation protocol (2.5% glutaraldehyde, 1% OsO₄ in 10 mM cacodylate buffer, pH = 6.8) according to Holzinger et al. (2009). Samples were dehydrated in increasing ethanol concentrations, transferred to modified Spurr’s resin and heat polymerized. For TEM, ultrathin sections were prepared, counterstained with uranyl acetate and Reynolds’s lead citrate, and investigated using a Zeiss LIBRA 120 transmission electron microscope at 80 kV. Images were captured with a TRS 2K CCD camera and further processed using Adobe Photoshop software (Adobe Systems Inc., San Jose, CA, USA).

**DNA isolation, PCR and sequencing.** Genomic DNA of all investigated strains was extracted using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions. Nucleotide sequences of the SSU rRNA gene together with ITS-1-5.8S–ITS-2 region were amplified using a set of Taq PCR Mastermix Kit (Qiagen GmbH) and a complex of EAF3 and ITS055R as well as algal-specific primers G800R and G500F. PCR reactions were made in a thermocycler T gradient Thermoblock (Biometra, Analytik Jena, Germany) under conditions described in a previous paper (Mikhailiyuk et al. 2018). PCR products were cleaned using a Qiagen PCR purification kit (Qiagen GmbH) according to the manufacturer’s instructions. Cleaned PCR products were sequenced commercially by Qiagen Company using primers G800R, 536R, 920F, 1400R, 1400F, GF, GR, and ITS2F. Sequences of all primers used in the study with respective references are included in the Table 2. The resulting sequences were assembled and edited using Geneious software (version 8.1.8; Biomatters). They were deposited in GenBank under accession numbers MN267182 – MN267185.

**Phylogenetic analyses.** DNA sequences of our isolates were compared to those from reference strains at NCBI using
For comparison with original strains, we used nucleotide sequences available in GenBank (NCBI) of representatives of the Trebouxiophyceae, with selected Chlorophyceae as the outgroup. Multiple alignment of the nucleotide sequences of the SSU rRNA was made using MAFFT web server (version 7; Katoh and Standley 2013) followed by manually editing in the program BioEdit (version 7.2). Alignment for the phylogeny of the ITS-2 region was performed manually in BioEdit, taking into account the secondary structure of the RNA (see below).

The evolutionary model that is best suited to the database was selected on the basis of the lowest AIC value (Akaike 1974) and calculated in MEGA (version 6; Tamura et al. 2013). Phylogenetic trees were constructed in the program MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003), using an evolutionary model GTR + G + I, with 5,000,000 generations. Gaps were treated as missing character. Two of the four runs of Markov chain Monte Carlo were made simultaneously, with the trees, taken every 500 generations. Split frequencies between runs at the end of calculations were

### Table 1. Summarized information about strains and sequences of Xerochlorella used in the present study (newly obtained sequences are marked with Bold)

| Original species | Strain label/culture number | Collection information | Species designation | SSU rRNA | ITS-1–5.8S rRNA | ITS-2 |
|-----------------|-----------------------------|------------------------|--------------------|----------|----------------|-------|
| Xerochlorella olmiae | UTEX B 2993 | Mojave National Preserve, San Bernardino Co., California, USA, Desert soil crust 35°27.113'N, 115°40.550'W, Louise A. Lewis (2003) | Xerochlorella minuta | MN267184 | – | – |
| Xerochlorella olmiae | BCP-EM3VF21 | Mojave National Preserve, San Bernardino Co., California, USA, Desert soil crust 35°27.113'N, 115°40.550'W, Louise A. Lewis (2003) | Xerochlorella minuta | KF693788 | – | – |
| Dictyosphaerium sp. | UTEX SNO65 | Antarctic (?) | Xerochlorella minuta | GQ502290 | – | – |
| Dictyosphaerium sp. | CCAP 222/3 | Moss epiphyte, Signy Island, South Orkney Islands, Antarctica, Broady (1975) as Dictyosphaerium chloroelloides (formerly listed as Dictyosphaerium minutum) | Xerochlorella minutum | FR865691 | – | – |
| Dictyosphaerium minutum | CCAP 222/3 | Moss epiphyte, Signy Island, South Orkney Islands, Antarctica, Broady (1975) as Dictyosphaerium chloroelloides (formerly listed as Dictyosphaerium minutum) | Xerochlorella minutum | GQ487247 | – | – |
| Dictyosphaerium sp. | CCALA 333 | Slovakia, Vysoké Tatry, peat bog, periphyton | Xerochlorella minutum | MH703761 | – | – |
| Dictyosphaerium chloroelloides | Us-7-12 | Baltic sea coast, sand dunes, soil crust, Zempin, Usedom, Mecklenburg-Vorpommern, Germany, 54°04.172’N; 13°58.035’E, T. Mikhailiyuk, 2013 | Xerochlorella chloroelloides | MN267183 | – | – |
| Dictyosphaerium chloroelloides | SEW-9-1 | Soil crust, beech forest, Germany, 53°02.674’N; 13°48.617’E, K. Glaser and T. Mikhailiyuk, 2014 | Xerochlorella chloroelloides | MN267182 | – | – |
| Dictyosphaerium chloroelloides | Prim-17-2 | Black Sea coast, sand dunes, Danube Delta Biosphere Reserve, Zhebryianska bay, Kiliya District, Odessa Region, 45.48662736 N; 29.633074533 E, Demchenko and T. Mikhailiyuk, 2013 | Xerochlorella chloroelloides | MN267185 | – | – |
| Dictyosphaerium dichotomum | Hg-2-3 | Baltic sea coast, sand dunes, soil crust, Heiligendamm, Mecklenburg-Vorpommern, Germany, 54.146102096 N, 11.86013389 E, T. Mikhailiyuk, 2013 | Xerochlorella dichotoma | MN267186 | – | – |

*Information concerning this strain is from NCBI, but the information from UTEX catalogue is different: Chloromomas rosae, Litchfield Island, Antarctic, red ice, Collection: B. Bidigare (3/26/90), Isolation: R.W. Hoham.

### Table 2. List of primers used in the study for amplification and sequencing.

| Primer name | Sequence [5’–3’] | Reference |
|-------------|------------------|-----------|
| EAF3        | TCGACAATCTGGTTGATCCTGCCAG | Marin et al. (2003) |
| ITS055R     | CTCCCTTGGCTCGTGTTTCAAGACGGG | Marin et al. (1998) |
| G500F       | GAATGAGTACAATCTAAACCCCTTAAC | Darienko et al. (2019) |
| G800R       | CATTACTCCGGTCCTACAGACGAACAGG | Lane (1991) |
| 556R        | GWATTACCAGCGGCCKGCTG | Hoef Emden and Melkonian (2003) |
| 920F        | GAAACTTAAAKGAATTGGH | Hohn and Moon (1993) |
| 1400F       | CTCGCTTCTTGTACACACGCACGCCCGTC | Marin et al. (2003) |
| 1400R       | GGTAGGGCCACGGCGCGGTTGTGTCAC | Marin et al. (2003) |
| GF          | GGATGCGGTTCGCCGTAGGTAACCTGGC | Goff and Moon (1993) |
| GR          | GGATGCGATATGGCTTAAAGTTCAGCGGGTG | White et al. (1990) |

BLASTn queries (http://blast.ncbi.nlm.nih.gov). For comparison with original strains, we used nucleotide sequences available in GenBank (NCBI) of representatives of the Trebouxioiphyceae, with selected Chlorophyceae as the outgroup. Multiple alignment of the nucleotide sequences of the SSU rRNA was made using MAFFT web server (version 7; Katoh and Standley 2013) followed by manually editing in the program BioEdit (version 7.2). Alignment for the phylogeny of the ITS-2 region was performed manually in BioEdit, taking into account the secondary structure of the RNA (see below).
below 0.01. The trees selected before the likelihood rate reached saturation were subsequently rejected. The reliability of tree topology verified by the maximum likelihood analysis (ML; GTR + I + G) was made using the program GARLI 2.0, and bootstrap support was calculated with 1,000 replicates.

Analysis of the ITS-2 secondary structures, genetic similarity. Models of the secondary structure of ITS-2 together with 5.8S-LSU rRNA stem were predicted for all investigated strains of Xerochlorella. Helices were folded with the online software Mfold (Zuker 2003) and visualized in the online tool PseudoViewer (Byun and Han 2009). ITS-2 secondary structure of CCAP 222/3 was predicted based on of two published sequences with different length (GQ502289 and FR865691). A part of 5.8S-LSU stem and neighboring core of UTEX SNO65 are unknown (because of short sequence GQ502290) and therefore could not be analyzed. Complementary base changes (CBCs and hemi-CBCs) as well as mismatches, deletions, single, or unpaired bases were estimated using recommendations published in Demchenko et al. (2012).

Genetic similarity of SSU and ITS rRNA between investigated strains was calculated in the program MEGA using p-distance and uniform rates, and was expressed in percent.

RESULTS

Molecular phylogeny based on SSU rRNA gene and ITS-2. The phylogenetic analysis of SSU rRNA sequences revealed that the newly sequenced original isolates as well as original sequence of UTEX B 2993 clustered together with the authentic strain of Xerochlorella olmiae (BCP-EM3VF21 = UTEX B 2993), as well as with several strains from CCAP, UTEX, and CCALA initially identified as Dictyosphaerium species (Fig. 1). All of these strains formed a highly supported Xerochlorella clade sister to the clades Lobosphaera and Coccolobrys, based on similar, almost identical SSU sequences, with exception of Hg-2-3 which formed a separate, highly supported lineage inside the Xerochlorella clade. This strain differed also morphologically (see below).

To obtain better resolution within the Xerochlorella clade, a phylogenetic analysis of the ITS-2 region of all investigated strains with Lobosphaera as outgroup was performed (Fig. 2). The tree confirmed also two main lineages inside the Xerochlorella clade: strains designated as X. minuta comb. nova (see below) and the morphologically different strain Hg-2-3 identified as X. dichotoma comb. nova.

Comparison of ITS-2 secondary structures, p-distance. To evaluate borders between different species inside the Xerochlorella clade the secondary structure of ITS-2 of all investigated strains was evaluated. Comparison of ITS-2 secondary structure of strains referred to X. minuta (see below) showed generally high similarity (Fig. 3). The most striking differences were localized in helices II and IV, while the 5.8S-LSU rRNA stem was identical in all strains. But many more differences in ITS-2 secondary structure were found between these strains and the strain Hg-2-3 (Fig. 4). All differences were localized in helices I, II, and IV. Results of analysis of secondary structures of all strains are summarized in Table 3. No compensatory base changes (CBCs) between strains referred to X. minuta were found, while differences between these strains varied from 1 to 3 hemi-CBCs, 1–4 mismatches and 2–8 nucleotide differences, all of which were localized in loops or ITS-2 core (Fig. 3, Table 3). Differences between secondary structures of ITS-2 of all X. minuta strains and Hg-2-3 were more prominent: 3–4 CBCs, 4–6 hemi-CBCs, 9 deletions, 2–3 mismatches, and 25–28 nucleotide differences in loops or ITS-2 core (Fig. 4, Table 3).

Pairwise comparison of SSU and ITS rRNA sequences of all strains investigated within the Xerochlorella clade showed a close similarity among all isolates referred to X. minuta (see below; Table 3). The identity of nucleotides of SSU and ITS rRNA of these strains varied from 99.5% to 99.9%. But strain Hg-2-3 considerably differed from those mentioned strains: the identity of nucleotides of the respective region varied from 97.5% to 97.8%.

Morphology and reproduction. The investigated strains represented unicellular green algae. Cells were small, ovoid to wide ellipsoid and almost spherical, (4.3)5.0–6.7(7.5) μm in diameter. Uninucleate (Fig. 5). Cells of the strain Hg-2-3 were slightly larger, (4.5)5.1–8.1(9.6) μm, which formed a separate, highly supported lineage inside the Xerochlorella clade sister to the clades Lobosphaera and Coccobotrys, based on similar, almost identical SSU sequences, with exception of Hg-2-3 which formed a separate, highly supported lineage inside the Xerochlorella clade. This strain differed also morphologically (see below).

To obtain better resolution within the Xerochlorella clade, a phylogenetic analysis of the ITS-2 region of all investigated strains with Lobosphaera as outgroup was performed (Fig. 2). The tree confirmed also two main lineages inside the Xerochlorella clade: strains designated as X. minuta comb. nova (see below) and the morphologically different strain Hg-2-3 identified as X. dichotoma comb. nova.

Comparison of ITS-2 secondary structures, p-distance. To evaluate borders between different species inside the Xerochlorella clade the secondary structure of ITS-2 of all investigated strains was evaluated. Comparison of ITS-2 secondary structure of strains referred to X. minuta (see below) showed generally high similarity (Fig. 3). The most striking differences were localized in helices II and IV, while the 5.8S-LSU rRNA stem was identical in all strains. But many more differences in ITS-2 secondary structure were found between these strains and the strain Hg-2-3 (Fig. 4). All differences were localized in helices I, II, and IV. Results of analysis of secondary structures of all strains are summarized in Table 3. No compensatory base changes (CBCs) between strains referred to X. minuta were found, while differences between these strains varied from 1 to 3 hemi-CBCs, 1–4 mismatches and 2–8 nucleotide differences, all of which were localized in loops or ITS-2 core (Fig. 3, Table 3). Differences between secondary structures of ITS-2 of all X. minuta strains and Hg-2-3 were more prominent: 3–4 CBCs, 4–6 hemi-CBCs, 9 deletions, 2–3 mismatches, and 25–28 nucleotide differences in loops or ITS-2 core (Fig. 4, Table 3).

Pairwise comparison of SSU and ITS rRNA sequences of all strains investigated within the Xerochlorella clade showed a close similarity among all isolates referred to X. minuta (see below; Table 3). The identity of nucleotides of SSU and ITS rRNA of these strains varied from 99.5% to 99.9%. But strain Hg-2-3 considerably differed from those mentioned strains: the identity of nucleotides of the respective region varied from 97.5% to 97.8%.

Morphology and reproduction. The investigated strains represented unicellular green algae. Cells were small, ovoid to wide ellipsoid and almost spherical, (4.3)5.0–6.7(7.5) μm in diameter. Uninucleate (Fig. 5). Cells of the strain Hg-2-3 were slightly larger, (4.5)5.1–8.1(9.6) μm, which formed a separate, highly supported lineage inside the Xerochlorella clade sister to the clades Lobosphaera and Coccobotrys, based on similar, almost identical SSU sequences, with exception of Hg-2-3 which formed a separate, highly supported lineage inside the Xerochlorella clade. This strain differed also morphologically (see below).

To obtain better resolution within the Xerochlorella clade, a phylogenetic analysis of the ITS-2 region of all investigated strains with Lobosphaera as outgroup was performed (Fig. 2). The tree confirmed also two main lineages inside the Xerochlorella clade: strains designated as X. minuta comb. nova (see below) and the morphologically different strain Hg-2-3 identified as X. dichotoma comb. nova.

Comparison of ITS-2 secondary structures, p-distance. To evaluate borders between different species inside the Xerochlorella clade the secondary structure of ITS-2 of all investigated strains was evaluated. Comparison of ITS-2 secondary structure of strains referred to X. minuta (see below) showed generally high similarity (Fig. 3). The most striking differences were localized in helices II and IV, while the 5.8S-LSU rRNA stem was identical in all strains. But many more differences in ITS-2 secondary structure were found between these strains and the strain Hg-2-3 (Fig. 4). All differences were localized in helices I, II, and IV. Results of analysis of secondary structures of all strains are summarized in Table 3. No compensatory base changes (CBCs) between strains referred to X. minuta were found, while differences between these strains varied from 1 to 3 hemi-CBCs, 1–4 mismatches and 2–8 nucleotide differences, all of which were localized in loops or ITS-2 core (Fig. 3, Table 3). Differences between secondary structures of ITS-2 of all X. minuta strains and Hg-2-3 were more prominent: 3–4 CBCs, 4–6 hemi-CBCs, 9 deletions, 2–3 mismatches, and 25–28 nucleotide differences in loops or ITS-2 core (Fig. 4, Table 3).

Pairwise comparison of SSU and ITS rRNA sequences of all strains investigated within the Xerochlorella clade showed a close similarity among all isolates referred to X. minuta (see below; Table 3). The identity of nucleotides of SSU and ITS rRNA of these strains varied from 99.5% to 99.9%. But strain Hg-2-3 considerably differed from those mentioned strains: the identity of nucleotides of the respective region varied from 97.5% to 97.8%.

Morphology and reproduction. The investigated strains represented unicellular green algae. Cells were small, ovoid to wide ellipsoid and almost spherical, (4.3)5.0–6.7(7.5) μm in diameter. Uninucleate (Fig. 5). Cells of the strain Hg-2-3 were slightly larger, (4.5)5.1–8.1(9.6) μm, which formed a separate, highly supported lineage inside the Xerochlorella clade sister to the clades Lobosphaera and Coccobotrys, based on similar, almost identical SSU sequences, with exception of Hg-2-3 which formed a separate, highly supported lineage inside the Xerochlorella clade. This strain differed also morphologically (see below).

To obtain better resolution within the Xerochlorella clade, a phylogenetic analysis of the ITS-2 region of all investigated strains with Lobosphaera as outgroup was performed (Fig. 2). The tree confirmed also two main lineages inside the Xerochlorella clade: strains designated as X. minuta comb. nova (see below) and the morphologically different strain Hg-2-3 identified as X. dichotoma comb. nova.

Comparison of ITS-2 secondary structures, p-distance. To evaluate borders between different species inside the Xerochlorella clade the secondary structure of ITS-2 of all investigated strains was evaluated. Comparison of ITS-2 secondary structure of strains referred to X. minuta (see below) showed generally high similarity (Fig. 3). The most striking differences were localized in helices II and IV, while the 5.8S-LSU rRNA stem was identical in all strains. But many more differences in ITS-2 secondary structure were found between these strains and the strain Hg-2-3 (Fig. 4). All differences were localized in helices
Fig. 1. Molecular phylogeny of Trebouxiophyceae (Chlorophyta) based on the comparison of the nucleotide sequences of the SSU rRNA gene (1776 base pairs). A phylogenetic tree was inferred by the Bayesian method with Bayesian Posterior Probabilities (PP) and Maximum Likelihood bootstrap support (BP); PP values lower than 0.8 and BP lower than 50% not shown. Strains in bold represent newly sequenced algae. Clades were named according to Fučíková et al. (2014) and Bock et al. (2011b). Scale bar: 0.02 substitutions/site.
FIG. 2. Molecular phylogeny of *Xerochlorella* (Trebouxiophyceae, Chlorophyta) based on the comparison of the nucleotide sequences of the ITS-2 region (687 base pairs). A phylogenetic tree was inferred by the Bayesian method with Bayesian Posterior Probabilities (PP) and Maximum Likelihood bootstrap support (BP); PP values lower than 0.8 and BP lower than 50% not shown. Strains in bold represent newly sequenced algae. Scale bar: 0.02 substitutions/site.

FIG. 3. Comparison of ITS-2 secondary structure of *Xerochlorella minuta* strains. The structure of the reference strain (UTEX B 2993) is presented with the marked differences to other strains of the species. Variable bases or basepairs are shown with circles, boxes, and triangles.
Therefore, subsequent division led to large colonies with cells attached to pseudodichotomous branching remnants of mother walls (Fig. 5, h, i, k–o).

Small and large colonies of all strains were usually surrounded by a delicate layer of mucilage. Mucilage was visible after negative staining with Indian ink. The thickness of mucilage varied from 2.1 to 5.0 μm in the different strains (Fig. 6). Individual mucilages envelopes surrounding cells were also observed (Fig. 6, a–d).

Reproduction by autospores was observed with two or four autospores formed in sporangia. Sporangial walls ruptured, but remained in culture forming semilunar or cruciform remnants. Young cells usually attached to the remnants by their narrow ends, but also often detached and laid separately. The culture usually represented a mixture of unicells, remnants of mother cell walls and Dictyosphaerium-like semilunar, cruciform or pseudodichotomous branching colonies depending on the strain (Fig. 5).

Ultrastructure. The ultrastructure was investigated in strains Us-7-12 and Hg-2-3. Generally it was similar (Fig. 7). The chloroplast was always located at one side of the cell covering half or 2/3 of cells’ inner surface and had a smooth or waved edges. The pyrenoid was surrounded by several (5–6) starch grains. The pyrenoid matrix was transversed by two to three thylakoid membranes. The nucleus was located opposite the pyrenoid (Fig. 7, f and j). Several mitochondrial profiles were observed in the cells, they were mostly located close to the

Fig. 4. Comparison of ITS-2 secondary structure of Xerochlorella species. The structure of X. dichotoma (reference strain Hg-2-3) is presented with the differences to X. minuta (reference strain UTEX B 2993). Variable bases or basepairs are shown with circles.

![Image of ITS-2 secondary structure](attachment:image.jpg)
chloroplast (Fig. 7, b, e, f, i and j). Cells were surrounded by a cell wall, and a delicate mucilage envelope was visible on some cells (Fig. 7i). A lot of remnants of cell walls were observed in the samples (Fig. 7, a–e).

**DISCUSSION**

Phylogeny, SSU and ITS rRNA genetic similarity, ITS-2 secondary structure and species delimitation within Xerochlorella. Phylogenetic analysis based on SSU rRNA sequences mostly corresponded with the phylogeny of the Trebouxiophyceae by Fučíková et al. (2014), Darienko et al. (2016), Hodač et al. (2016), Skaloud et al. (2016), and others. All investigated strains formed a highly supported clade assigned to the genus Xerochlorella with the closest genera Cococotrys and Lobosphaera (Fučíková et al. 2014). Therefore, all investigated strains should be identified as Xerochlorella species. ITS-2 phylogeny showed that Xerochlorella represents two main lineages: strains CCAP 222/3, UTEX SNO65, SEW-9-1, Us-7-12, Prim-17-2 together with the authentic strain UTEX B 2993 corresponded to the type species X. olmiae (X. minuta comb. nova, see below), while strain Hg-2-3 represented another species identified initially as Dictyosphaerium dichotomum (Xerochlorella dichotoma comb. nova, see below).

Comparison of ITS-2 secondary structure and pairwise comparison of SSU and ITS rRNA sequences confirmed the presence of two lineages in Xerochlorella. All strains corresponding to X. minuta are characterized by minor differences (1–3 hemi-CBCs and missing CBCs). Comparison of these strains with Hg-2-3 (X. dichotoma) showed conspicuous differences as reflected in 3–4 CBCs and 4–6 hemi-CBCs depending on strain. Therefore, the presence of CBCs in ITS-2, essential differences according SSU and ITS-2 phylogeny as well as in morphology clearly indicate that this genus includes now two species: X. minuta and X. dichotoma.

**Definition of the genus Xerochlorella.** Xerochlorella is a monotypic genus of green algae (Trebouxiophyceae), which was recently described based on SSU rRNA and rbl phylogenies and morphological investigation of two strains (Fučíková et al. 2014). The alga was found during investigation of desert biological soil crusts (Mojave National Preserve, San Bernardino Co., California, USA). It is characterized by simple Chlorella-like morphology, small spherical or ovoid cells and a chloroplast without pyrenoid. The chloroplasts of young cells have smooth margins, later dissects on two lobes and then on several separate chloroplasts. This representative formed a separate lineage in the phylogeny of Trebouxiophyceae. The lineage also included the strain CCAP 222/3, isolated by P.A. Broady from Antarctic soil and deposited in a culture collection under the name Dictyosphaerium minutum. But this strain is non-authentic, since the genus Dictyosphaerium forms another phylogenetic lineage within Trebouxiophyceae (Chlorellales) and Dictyosphaerium-like morphology was not typical for strains isolated from desert soil. Therefore, the new genus Xerochlorella was proposed. Fučíková et al. (2014) mentioned in their paper that X. olmiae was possibly found earlier and perhaps described based on classical methods. But simple morphology does not allow to determine this taxon using light microscopy. Therefore, “... knowing the limitations of the morphological approach, we prefer to establish a new taxon supported by an authentic strain, even though some of our newly proposed taxa could fit some of the uncertain historical morphogenera” (Fučíková et al. 2014).

The investigated strains (Us-7-12, SEW-9-1 and Prim-17-2) were characterized by Dictyosphaerium-like morphology and were preliminary identified as Dictyosphaerium chlorelloides (=Dictyosphaerium minutum) based on the “Syllabus der Boden-, Luft und Flechtenalgen” (Ettl and Gärtner 2014). This taxon was mentioned as Chlorella chlorelloides in earlier manuscripts (Schulz et al. 2016, Borchhardt et al. 2017a, b) taking into consideration the revision of Bock et al. (2011a), in which D. chlorelloides was assigned to the genus Chlorella. But molecular-phylogenetic data obtained later indicated that our strains do not belong to Chlorella and Chlorellales (Mikhailyuk et al. 2019), instead assignment to Xerochlorella was proposed. Prominent Dictyosphaerium-like morphology of the here investigated strains and the presence of several other strains preliminary identified as Dictyosphaerium in the Xerochlorella clade (see Fig. 1) indicated that the generic concept of Xerochlorella should be revised.
Dictyosphaerium morphotype among green algae. The genus *Dictyosphaerium* (type species: *Dictyosphaerium ehrenbergianum*) unites mostly aquatic taxa characterized by a specific morphology: small Chlorella-like cells are attached to variously branched mucilaginous stalks originating from remnants of mother (sporangial) cell walls (Komárek and Perman 1978). As a result colonies of different sizes...
surrounded by mucilage are formed. A molecular-phylogenetic investigation of *Dictyosphaerium* showed that the *Dictyosphaerium*-like morphotype is polyphyletic (Krienitz et al. 2010). Therefore, a position of *Dictyosphaerium* inside *Parachlorella* clade, Chlorellaceae (Bock et al. 2011b) was determined. Many morphospecies of *Dictyosphaerium* and *Pseudodictyosphaerium* were transferred to the genera *Chlorella* and *Xenochlorella*. Fig. 6. Negative staining of *Xenochlorella* species: (a–e) *X. minuta*, mucilage envelopes of different thickness around cells, fragments of sporangial walls and small 2-4-celled colonies: (a, b) UTEX B 2993, (c, d) Us-7-12, (e) Prim-17-2. (f–k) *X. dichotoma*, Hg-2-3, mucilage envelopes around colonies. Scale bars: 10 μm. [Color figure can be viewed at wileyonlinelibrary.com]
(Bock et al. 2011a) and Mychonastes, Chlorophyceae (Krienitz et al. 2011). Numerous new genera inside Chlorellaceae (Mucidosphaerium, Bock et al. 2011b; Heynigia and Hindakia, Bock et al. 2010; Marasphaerium, Compactochlorella, Masaia and Kalenjina, Krienitz et al. 2012) were described based on Dictyosphaerium-like algae. Recent investigation of strains with a Dictyosphaerium-like morphology from water bodies of China showed high phylogenetic and morphological diversity of these representatives inside Parachlorella clade (Chlorellaceae; Song et al. 2018). Perhaps several new genera of Dictyosphaerium-like algae will be described in the future.

Reports of Dictyosphaerium sensu lato in terrestrial habitats are quite limited. The typical aquatic species Dictyosphaerium pulchellum (=Mucidosphaerium pulchellum) was found in soil occasionally (Ettl and Gärtnerr 2014). Dictyosphaerium dichotomum was described from terrestrial habitats of Antarctica (Ling and Seppelt 1998). The widely distributed D. chlorelloides (=D. minutum) is typical for aquatic and terrestrial habitats (Komárek and Perman 1978, Tsarenko 2011, Ettl and Gärtnerr 2014).
Dictyosphaerium terrestr (Frisch and John 1942) described from soil of Great Britain is a doubtful taxon and perhaps does not belong to autospores forming Dictyosphaerium sensu lato (Tsiarenko and John 2011), because reproduction by zoospores was found (Etzl and Gärnter 2014).

It should be noted that initially Dictyosphaerium minutum was described from soil of Denmark (Petersen 1932). It was later transferred to the earlier described species of the aquatic taxon Dictyosphaerium chlorelloides because of a very similar morphology (Komárek and Perman 1978). Later investigation of Dictyosphaerium based on an integrative approach (Bock et al. 2011a) showed that an aquatic strain characterized by a morphology typical for Dictyosphaerium chlorelloides should be assigned to the genus Chlorella, and CB 2008/110 was selected as reference strain of Chlorella chlorelloides. But strains from terrestrial habitats identified as D. chlorelloides/D. minutum were not analyzed in this study (Bock et al. 2011a). The new Xerochlorella clade includes several strains determined as species of Dictyosphaerium found mostly in terrestrial habitats of Europe and Antarctica (see Fig. 1 and Table 1).

The morphological investigation of the authentic strain Xerochlorella olmiae UTEX B 2993 showed characters typical for Dictyosphaerium chlorelloides/D. minutum: the presence of remnants of mother cell walls in culture and formation of typical 2–4-celled Dictyosphaerium-like colonies as well as the presence of pyrenoid in cup-shaped chloroplast. Dissection of the chloroplast on several parts as noted in Fučíková et al. (2014), is due to early stages of autospore formation. The absence of pyrenoid and remnants of the mother cell walls in culture might be explained by the specific state of the respective culture during their investigation which is visible from published micrographs (Fučíková et al. 2014, fig. 1, a–e).

All other strains presented on our phylogenetic trees and attributed to Xerochlorella (CCAP 222/3, CCALA 333, UTEX SNO65) are characterized by Dictyosphaerium-like morphology. CCAP 222/3 was initially identified as Dictyosphaerium minutum and comprehensively described in Broady (1982). CCALA 333 was identified as another Dictyosphaerium species, D. tetrachotomum, but a micrograph of the species in the collection catalogue showed four-celled colony typical for D. minutum (http://ccala.b.utb.cas.cz/en/dictyosphaerium-tetrachotomum-printz). UTEX SNO65 is problematic because it is not available or mislabeled (see Table 1), but according NCBI data this taxon was identified as Dictyosphaerium sp. and isolated from Antarctica (https://www.ncbi.nlm.nih.gov/nuccore/GQ502290).

Based on all of this information, it is clear that the terrestrial species D. minutum is not related to the aquatic taxon D. chlorelloides despite close morphology. Furthermore, the genus Xerochlorella (type species X. olmiae) is in fact synonymous to the widely distributed terrestrial species D. minutum discovered more than 80 years ago (Petersen 1932). This taxon is not a species of Dictyosphaerium or Chlorella (Chlorellaceae), but represents a separate phylogenetic lineage inside the Trebouxiophyceae. Therefore, the validity of the genus Xerochlorella, which unites terrestrial Dictyosphaerium-like algae, should be accepted. Assuming Xerochlorella as a separate genus, we complemented its diagnosis by new data and proposed respective nomenclatural combination, emendation, and epitypification (see below). Chlorella umbelloidea described by Tell (1973) from soil of Argentina is morphologically and ecologically very similar to D. minutum, as already mentioned by Komárek and Perman (1978). Therefore, we additionally propose to consider this taxon as a synonym of Xerochlorella minutata.

The morphologically different strain Hg-2-3 was initially identified as Dictyosphaerium dichotomum (Ling and Seppelt 1998, Etzl and Gärnter 2014). Our strain has all characters typical for this representative: formation of large 8-16-32(64)-celled colonies surrounded by muciilage, pseudodichotomous branching of fragments of sporangial walls, typical morphology of single cells. The reproduction of Hg-2-3 is usually realized by two autospores, but occasionally by four autospores, the same as indicated by Ling and Seppelt (1998). Large colonies originated due to successive cell division from small two-celled (Fig. 5, i, k and l) and occasionally four-celled colonies (Fig. 5, m–o). Phylogenetic analysis showed this strain to belong to the Xerochlorella clade, but with a separate lineage. Therefore, we assume that this strain represents a separate species of Xerochlorella. Thus the respective taxonomic combination and epi-typification of this taxon is proposed (see below).

**Morphological characters of Xerochlorella.** Morphological parallelism is a phenomenon widely distributed among algae. Perhaps morphological similarity of the freshwater Chlorella chlorelloides (Chlorellaceae) and the terrestrial Xerochlorella minutata (separate lineage in Trebouxiophyceae, Trebouxiophyceae incertae sedis; Guiry and Guiry 2019) is an example of such morphological parallelism. But also some morphological characters specific for each taxon were found. A pyrenoid surrounded by 2(4) starch grains is typical for representatives of Chlorellaceae including Chlorella chlorelloides (Bock et al. 2011a, Etzl and Gärnter 2014). TEM micrographs of the investigated Xerochlorella strains showed the presence of a pyrenoid surrounded by several (5–6) starch grains (see Fig. 7). Similar structure of pyrenoid with numerous starch grains was also observed by Broady (1982, figs. 66–67) in Dictyosphaerium minutum (now X. minutata) isolated from Antarctica. A similar pyrenoid structure was found in D. chlorelloides, isolated from granite outcrops of the South of Ukraine (Mikhailiuk and Demchenko 2005).

Another typical morphological character of Xerochlorella is its specific structure of mother (sporangial) cell wall fragments, which form a Dictyosphaerium-like morphology.
Cells of *Dictyosphaerium*-like algae from the Chlorellaceae are connected to thin mucous strands, which originated from mother (sporangial) cell wall fragments (Bock et al. 2011a). Analogical structures of *Xerochlorella* completely maintain the initial semilunar or cruciform shape of ruptured sporangial walls and do not turn to mucous strands. This was already mentioned in the first description of *D. minutum* (Petersen 1932) and during observation of this species by Broady (1982). Ling and Seppelt (1998) also noted as one specific character of *D. dichotomum*: “mother cell wall fragments do not change into mucous strands as is often the case in *Dictyosphaerium*” (p. 59).

The presence of a common mucilage envelope surrounding colonies in *Xerochlorella* is a disputable question. Mucilage envelope and colonies were not observed by Fučíková et al. (2014) in *X. olmiae* strains. Petersen (1932) did also not observe common mucilage envelope in *Dictyosphaerium minutum*, although mentioned slight gelatinization of cell walls. Komárek and Perman (1978), however, noted different development of a mucilage envelope in *D. chlorelloides* from implicit or slight mucilage to prominent strong envelope sometimes with clear individual envelopes surrounding cells. A common mucilage envelope of 3 μm thickness after negative staining with ink was indicated for *D. dichotomum* (Ling and Seppelt 1998, figs. 13, 20). Our observations showed also different phenotypic expression of a common mucilage envelope in strains of *X. minuta* as well as the presence of individual envelopes surrounding cells. A common mucilage envelope of 3 μm thickness after negative staining with ink was indicated for *D. dichotomum* (Ling and Seppelt 1998, figs. 13, 20). Our observations showed also different phenotypic expression of a common mucilage envelope in strains of *X. minuta* as well as the presence of individual envelopes surrounding cells (see Fig. 6). Negative staining of strain Hg-2-3 confirmed the presence of a clear common mucilage envelope with a thickness of 2.1-3.0 μm. Therefore, members of *Xerochlorella* have a common mucilage envelope with different thickness depending on environmental conditions. This mucilage envelope is usually thin, delicate, homogeneous, and start to be visible after negative staining by ink.

Ecology and distribution of *Xerochlorella*. Terrestrial *Dictyosphaerium* (*Xerochlorella minuta*) are widely distributed species. Ettl and Gärtner (2014) noted its wide distribution in Europe (Denmark, Great Britain, Iceland, former USSR) and Asia and some findings in Antarctica. This taxon was mentioned in main check-lists and hand books as widely distributed terrestrial alga (Komárek and Fott 1983, Andreyeva 1998, Tsarenko and John 2011). We found it repeatedly as terrestrial alga from granite outcrops of the South of Ukraine (Mikhailyuk and Demchenko 2005, Mikhailyuk et al. 2011) as well as in biological soil crusts from sand dunes of Germany and Ukraine (Mikhailyuk et al. 2019), forests of Germany (Glaser et al. 2018) and polar regions such as the Antarctic Peninsula and Arctic Svalbard (Borchhardt et al. 2017a,b).

Molecular-phylogenetic data were obtained from *Xerochlorella minuta* strains isolated from terrestrial and amphibian (swamp) habitats of Western and Eastern Europe (Germany, Ukraine, Slovakia), North America (USA), and Antarctica (see Table 1). These data prove that all investigated strains are genetically very close despite distant geographical regions and different ecological conditions (from swamps and moist soils to maritime sand dunes, habitats in hot and cold deserts). All these strains can be considered as representatives of different populations of the same species. Therefore, it is reasonable to assume that *X. minuta*, due to its small cell size and possible high tolerance to temperature and lack of water, can be easily distributed by wind (or other vectors) over long distances which is typical for terrestrial algae (Sharma et al. 2007, Ryšanek et al. 2015). Despite the fact that this species was originally described from Western Europe we propose the American strain (authentic strain of *Xerochlorella olmiae*; see below) as reference strain of *X. minuta*.

The other species of *Xerochlorella*, *X. dichotoma*, was originally described from soils of Antarctica. Cultures were used for species description, but all strains were lost during transportation of the material from Antarctica to Australia (Ling and Seppelt 1998). We do not know other findings of this taxon next to the type locality. Our strain, identified as *X. dichotoma*, was isolated from maritime sand dunes, Germany (Table 1). Despite the fact the type locality of *X. dichotoma* is situated far away from the Baltic Sea, we assume that strains from both regions are representatives of the same species, and hence expect the same cosmopolitan biogeographical distribution as for *X. minuta*. But it is obvious that *X. dichotoma* is a rare species. Based on all this information we propose the here investigated strain as reference of *X. dichotoma*, taking into consideration its complete morphological similarity with the original species description (formation of large colonies by mucilage, pseudodichotomous branching of fragments of sporangial walls, typical morphology of single cells), close ecological characteristic (sand vs. sandy soils) and cosmopolitan distribution of the genus *Xerochlorella*.

Proposed taxonomic changes. *Xerochlorella* Fučíková, P.O. Lewis & L.A. Lewis (2014). *Phycological Research* 62:304, fig. 1, a–e. emend. Mikhailyuk & P.M. Tsarenko

Emended diagnosis: Cells solitary or in colonies surrounded by delicate mucilage envelopes. Cells ovoid to wide ellipsoid and almost spherical, thin-walled, uninucleate, connected to fragments of mother (sporangial) walls with their narrow ends (*Dictyosphaerium*-like morphology). Chloroplasts single, parietal, cup-shaped, with pyrenoid surrounded by several starch grains. Asexual reproduction via autospores. Autospores released by rupture of sporangial wall and further preservation of fragments with connected cells. Sexual reproduction not observed.

Type species: *Xerochlorella minuta* (J.B. Petersen) Mikhailyuk & P.M. Tsarenko comb. nova

*Xerochlorella minuta* (J.B. Petersen) Mikhailyuk & P.M. Tsarenko comb. nova (Figs. 5, a–g; 6, a–e; 7, a–d)
**Basionym:** Dictyosphaerium minutum J.B. Petersen (1932) Bot. Tidsskr. 42:37, fig. 19.

**Synonyms:** Chlorella umbelloidea Tell, Xerochlorella olmiae Fućíková, P.O. Lewis & L.A. Lewis, as “olmiae” (according to Art. 60.8 of the ICN [Turland et al. 2018], the original spelling “olmiae” is corrected to “olmiae”).

**Emended diagnosis:** Cells solitary or in small 2-4-celled (sometimes to 16-celled) colonies surrounded by delicate mucilage envelope (2.1–5.0 μm thickness). Cells small, ovoid to wide ellipsoidal and almost spherical, \((4.3)5.0–(2.5)6.0(7.0)\) μm, connected to semilunar or cruciform fragments of mother (sporangial) walls with their narrow ends. Chloroplasts single, parietal, cup-shaped, located in half or 2/3 of cells inner surface, with pyrenoid surrounded by several \((5–6)\) starch grains. Asexual reproduction mostly via 2, occasionally via 4 autospores, released by rupture of sporangial wall. Zoospore formation and sexual reproduction not observed.

**Type locality:** sandy soil, Stevenson Cove, Casey, Antarctica.

**Lectotype (designated here):** figs. 13-17 in Ling & Seppelt (1998).

**Epitype (designated here):** preserved specimen 160223-7, fixed for TEM embedded material of the strain Hg-2-3 (SAG 2582) (Department of Botany, Innsbruck University). Additionally, KW-A-32502, preserved culture material of reference strain Hg-2-3 (SAG 2582), Algotheca, Herbarium of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (KW).

**Reference strain:** Hg-2-3 was deposited in the Sammlung von Algenkulturen, University of Göttingen, Germany, under number SAG 2582.

**Comments:** The reference strain completely corresponds to the diagnosis of Dictyosphaerium dichotomum (Ling and Seppelt 1998). The species diagnosis was supplemented by the details of pyrenoid structure and refined dimensional ranges for cells. Asexual reproduction mostly via 2, occasionally via 4 autospores, released by rupture of sporangial wall. Zoospore formation and sexual reproduction not observed.

**Proposed nomenclatural changes for Dictyosphaerium chlorelloides.** During this investigation some inaccuracy on the taxon Dictyosphaerium chlorelloides (=Chlorella chlorelloides) was detected. Taxonomic combination *D. chlorelloides* provided *Brachionococcus chlorelloides* as basionym, published by Naumann in 1919 with figures (holotype), belonging to the work from 1921 (Komárk and Perman 1978, p. 252). Naumann’s work was cited in this reference list as published in 1919. The same information was repeated in the paper that provided taxonomic combination of *Ch. chlorelloides*, without any notion of Naumann’s work in the reference list (Bock et al. 2011a). *Brachionococcus chlorelloides* is mentioned among Internet sources as taxon published in Naumann (1919) together with figures (http://ucjeps.berkeley.edu/ina/). However, investigation of the respective literature showed that Naumann’s paper was submitted in 1919, but in fact volume 16(2) of Arkiv för Botanik was published in February 1921. The errors in citation of the basionym do not preclude valid publication of new combinations (Art. 41.6 of the ICN; Turland et al. 2018). Therefore, this inaccuracy was corrected and all previous taxonomic combinations of *Brachionococcus chlorelloides* were kept (see below).
Chlorella chlorelloides (Naumann) C. Bock, Krienitz & Pröschold

Basionym: Brachionococcus chlorelloides Naumann (1921). *Ark. Bot.* 16(2):15, figs. 8–9.

**Synonym:** Dicytosphaerium chlorelloides (Naumann)

**Komárek and Perman (1978)**

This study was supported by a Georgia-Forster fellowship research from the Alexander von Humboldt Foundation (T.M.). The work has been funded by the DFG Priority Program 1374 "Infrastructure-Biodiversity-Exploratories" (subproject Crust-function-KA809, 28, U.K.), by the Priority Program 1991 Taxonomy (GL 909/1-I, K.G.) and by the FWF grant I 1951-B16, A.H. The study was summarized during a stay of T.M. at the University of Innsbruck, Austria funded by a program to revise the culture collection of alpine algae (ASIB) at the Institute of Botany. We thank the managers of the three Biodiversity Exploratories, Martin Giske, and all former managers for their work in maintaining the plot and project infrastructure, Christian Fischer for giving support through the central office, Michael Owonibi for managing the central database, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmüller, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser, and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. Fieldwork permits were issued by the responsible state environmental offices of Brandenburg (according to §72 BbgNatSchG). Our sincere thanks are extended to Sabrina Obwegeser and Beatrix Jungwirth, University of Innsbruck, Austria, for providing help in the TEM investigations. We are grateful to Sergei L. Mosyakin (M.G. Khloody Institute of Botany, NAS of Ukraine) for his valuable advice on nomenclatural issues and Dr. Maike Lorenz, University of Göttingen, for help during strain deposit to SAG.

**Büdel, B., Dulić, T., Darienko, T., Rybalka, N. & Friedl, T. 2016.** Cyanobacteria and algae of biological soil crusts. In Weber, B., Büdel, B. & Belnap, J. (Eds.) *Biological Soil Crusts: Structure, Function, and Management.* Springer, Berlin, pp. 55–80.

**Byun, Y. & Han, K. 2009.** Pseudoviewer3: generating planar drawings of large-scale RNA structures with pseudoknots. *Bioinformatics* 25:1435–7.

**Darienko, T., Gustavs, L., Eggert, A., Wolf, W. & Pröschold, T. 2015.** Evaluating the species boundaries of green microalgae (Coccomyxa, Trebouxiophyceae, Chlorophyta) using integrative taxonomy and DNA barcoding with further implications for the species identification in environmental samples. *PLoS ONE* 10:e0127838.

**Darienko, T., Gustavs, L. & Pröschold, T. 2016.** Species concept and nomenclatural changes within the genera *Elliptochlorella* and *Pseudochlorella* (Trebouxiophyceae) based on an integrative approach. *J. Phycol.* 52:1125–45.

**Darienko, T., Gustavs, L. & Pröschold, T. 2017.** Toward a monograph of non-marine Ulvophyceae using an integrative approach (Molecular phylogeny and systematics of terrestrial Ulvophyceae II). *Phytotaxa* 324:1–41.

**Darienko, T., Kang, W., Orzechowski, A. K. & Pröschold, T. 2019.** *Pleurastracococcus tertiiformae*, a new species of a rare desert trebouxiophycean alga discovered by an integrative approach. *Extremophiles* 23:573–86.

**Darienko, T. & Pröschold, T. 2019.** Reevaluation and discovery of new species of the rare genus *Wontasenia* and establishment of *Massiakochlorella* gen. nov. (Trebouxiophyceae, Chlorophyta) using an integrative approach. *J. Phycol.* 55:493–9.

**De Clerck, O., Guiy, M. D., Leliært, F., Samyn, Y. & Verbruggen, H. 2013.** Algal taxonomy: a road to nowhere? *J. Phycol.* 49:215–25.

**Demchenko, E., Mikhailyuk, T., Coleman, A. W. & Pröschold, T. 2012.** Generic and species concepts in Microglena (previously the *Chlamydomonas monadina* group) revised using an integrative approach. *Eur. J. Phycol.* 47:264–90.

**Ettl, H. & Gärtnér, G. 2014.** *Sylabus der Boden, Luft und Flächental/*gen. 2nd edn. Spektrum Akademischer Verlag, Munich, 773 pp.

**Friedl, T. & Rybalka, N. 2012.** Systematics of the green algae: a brief introduction to the current status. *Prog. Bot.* 73:259–80.

**Fritsch, F. E. & John, R. P. 1942.** An ecological and taxonomic study of the algae of British soils. II. Consideration of the species observed. *Ann. Bot.* 6:371–95.

**Fučíková, K., Lewis, P. O. & Lewis, L. A. 2014.** Widespread desert affiliation of trebouxiophycean algae (Trebouxiophyceae, Chlorophyta) including discovery of three new desert genera. *Phycol. Res.* 62:294–305.

**Glascher, K., Baumann, K., Leinweber, P., Mikhailyuk, T. & Karsten, U. 2018.** Algal richness in BSCs in forests under different management intensity with some implications for P cycling. *Biogesosciences* 15:4181–92.

**Goff, L. J. & Moon, D. A. 1993.** PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores. *J. Phycol.* 29:381–4.

**Guiy, M. D. & Guiy, G. M. 2019.** *AlgaeBase Worldwide Electronic Publication.* National University, Ireland, Galway http://www.a lgaebase.org.

**Hodač, L., Hallmann, C., Spitzer, K., Ekler, J., Füsslauer, F., Brinkmann, N., Lepka, D., Divan, V. & Friedl, T. 2016.** Widespread green algae *Chlorulla* and *Stichococcus* exhibit polar-temperate and tropical-temperate biogeography. *FEMS Microbiol. Ecol.* 92:fiw122.

**Hoeferden, K. & Melkonian, M. 2003.** Revision of the genus *Cryptomonas* (Cryptophyceae): a combination of molecular phylogeny and morphology provides insights into a long-hidden dimorphism. *Protist* 154:371–409.

**Holzinger, A., Ropele, M. Y. & Lütz, C. 2009.** The vegetative arctic green alga *Zygmena* is insensitive to experimental UV exposure. *Micron* 40:831–8.

**Katoh, K. & Standley, D. M. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30:772–80.
Krienitz, L., Bock, C., Kotut, K. & Pröschold, T. 2012. Genotypic diversity of *Dictyochaeterium*-morphospecies (Chlorococcales, Trebouxiophyceae) in African inland waters, including the description of four new genera. *Fotosoa* 21:23–51.

Krienitz, L., Bock, C., Luo, W. & Pröschold, T. 2010. Polyphyletic origin of the *Dictyochaeterium* morphotype within Chlorococcales (Trebouxiophyceae). *J. Phycol.* 46:559–63.

Kryvenda, A., Rybalka, N., Wolf, M. & Friedl, T. 2018. Species distinctions among closely related strains of *Eustigmatophyceae* (Stramenopiles) emphasizing ITS2 sequence-structure data: taxonomy and taxonomic revision of plastid-containing Euglenophyta. *Eur. J. Phycol.* 53:471–91.

Lan, D. J. 1991. 16S/23S rRNA sequencing. In Stackebrandt, E. & Goodfellow, M. [Eds.] *Nucleic Acid Techniques in Bacterial Systematics*. John Wiley and Sons, New York, NY, pp. 115–75.

Leläerti, F., Verbruggen, H., Vanormelingen, P., Steen, F., López-Bautista, J. M., Zuccarello, G. C. & De Clerck, O. 2014. DNA-based species delimitation in algae. *Eur. J. Phycol.* 49:179–96.

Ling, H. U. & Seppelt, R. D. 1998. Non-marine algae and cyanobacteria of the Windmill Islands region, Antarctica, with descriptions of two new species. *Arch. Hydrobiol. Suppl.* 124/Algol Stud. 89:49–62.

Marin, B., Klingberg, M. & Melkonian, M. 1998. Phylogenetic relationships among the Cryptophyta: analyses of nuclear-encoded SSU rRNA sequences support the monophyly of extinct plastid-containing lineages. *Protist* 149:265–76.

Marin, B., Palm, A., Klingberg, M. & Melkonian, M. 2003. Phylogeny and taxonomic revision of plastid-containing Euglenophyta based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. *Protist* 154:99–145.

Mikhailiyuk, T. I. & Demchenko, E. N. 2005. Novye dlja flory i redkie vidi zelyenikh vodorosley s granitnykh obnazyeniy regionalnogo landslachtogo parka “Granimo-Stepnoe Pobuzhie” (Nikolaevskaya obl., Ukraine) [New for the flora and rare species of green algae from granite outcrops of Regional Landscape Park “Granite-Steppe Pobuzhskaya” (Mykolayiv obl., Ukraine)]. *Bot. J.* 90:183–96.

Mikhailiyuk, T., Glaser, K., Tsarenko, P., Demchenko, E. & Karsten, U. 2019. Composition of biological soil crusts from sand dunes of the Baltic Sea coast, in the context of an integrative approach to the taxonomy of microalgae and cyanobacteria. *Eur. J. Phycol.* 54:263–90.

Mikhailiyuk, T. I., Kondratyuk, S. Y., Nyporko, S. O., Darienko, T. M., Demchenko, E. M. & Votysekhovich, A. O. 2011. Lyshainyky, mokhy tjazemni vodorosti granitnykh kanionov Ukrainy [Lichen-forming fungi, bryophytes and terrestrial algae of granitic canyons of Ukraine]. Allerpress, Kyiv, 398 pp. (in Ukrainian).

Mikhailiyuk, T., Lukešova, A., Glaser, K., Holzinger, A., Obwegener, S., Nyporko, S., Friedl, T. & Karsten, U. 2018. New taxa of streptophyte algae (Streptophyta) from terrestrial habitats revealed using an integrative approach. *Protist* 169:406–31.

Naumann, E. 1921. Notizen zur Systematik der Süßwasseralgen. *Arch. Bot.* 16:1–19.

Neustupa, J., Eliáš, M., Škaloud, P., Némcová, Y. & Šejnbohová, L. 2011. *Xylochloris irregularis* gen. et sp. nov. (Trebouxiophyceae, Chlorophyta), a novel subaerial cocoid green alga. *Phycologia* 50:57–66.

Petersen, J. B. 1932. The algal vegetation of Hammer Bakker. *Bot. Tidskr. (Lund)* 42:1–48.

Ronquist, F. & Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1752–4.

Rybalka, N., Wolf, M., Andersen, R. A. & Friedl, T. 2013. Congruence of chloroplast- and nuclear-encoded DNA sequence variations used to assess species boundaries in the soil microalga *Heterococcus* (Stramenopiles, Xanthophyceae). *BMC Evol.* 13:39.

Rysánek, D., Hrčková, K. & Škaloud, P. 2015. Global ubiquity and local endemicity of free living terrestrial protists: phylogeographic assessment of the streptophyte alga *Klebsormidium*. *Environ. Microbiol.* 17:689–98.

Schulz, K., Mikhailiyuk, T., Dreßler, M., Leinweber, P. & Karsten, U. 2016. Biological soil crusts from coastal dunes at the Baltic Sea: cyanobacterial and algal biodiversity and related soil properties. *Microbiol. Ecol.* 71:178–93.

Sharma, N. K., Rai, A. K., Singh, S. & Brown, R. M. 2007. Airborne algae: their present status and relevance. *J. Phycol.* 43:615–27.

Škaloud, P., Friedl, T., Hallmann, C., Beck, A. & Dal Grande, F. 2016. Taxonomic revision and species delimitation of cocoid green algae currently assigned to the genus *Dictyochaeterium* (Trebouxiophyceae, Chlorophyta). *J. Phycol.* 52:599–617.

Song, H., Wang, Q., Liu, X., Hu, Y., Long, J., Liu, G. & Hu, Z. 2018. Phylogenetic diversity and taxonomic problems of the *Dictyochaeterium* morphotype within the *Parachlorella clade* (Chlorococcales, Trebouxiophyceae). *J. Eukaryot. Microbiol.* 65:382–91.

Starr, R. C. & Zeikus, J. A. 1993. UTEX the culture collection of algae at the University of Texas at Austin. *J. Phycol.* 29 (Suppl):1–106.

Tamara, K., Stecher, G., Peterson, D., Filipík, A. & Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725–9.

Tell, G. 1973. Una nueva especie de *Chlorophyceae* pata la Argentina. *Physis (Buenos Aires), See B* 32:555–8.

Tsenenko, P. M. 2011. Order Chlorococcales. In Tsenenko, P. M., Wasser, S. P. & Nevo, E. [Eds.] *Algae of Ukraine: Diversity, Nomenclature, Taxonomy, Ecology and Geography*, Vol. 3. *Chlorophyta*, Gantner Verlag, Ruggell/Liechtenstein, pp. 61–89.

Tsenenko, P. M. & John, D. 2011. Order Chlorococcales. In John, D. M., Whitten, B. A. & Brook, A. J. [Eds.] *The Freshwater Algal Flora of the British Isles*. An identification guide to freshwater and terrestrial algae. Cambridge University Press, Cambridge, pp. 475–99.

Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S. et al. 2018. *International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code)* *Regnum Vegetabile* 159. Koeltz Botanical Books, Glashütten, 254 pp.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis, A., Gelfand, D. H., Sninsky, J. J. & White, T. J. [Eds.] *PCR Protocols: A Guide to Methods and Applications*, Academic Press, London, pp. 315–22.

Zimmermann, J., Glöckner, G., Jahn, R., Enke, N. & Gemeinholer, B. 2015. Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. *Mol. Ecol. Res.* 15:526–42.

Zuker, M. 2003. Mold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31:3406–16.