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Dedicated to the late Professor Jadwiga Siemińska

A new species of *Tetradesmus* (*Chlorophyceae, Chlorophyta*) isolated from desert soil crust habitats in southwestern North America

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Abstract. A new species of *Tetradesmus* (*Tetradesmus adustus*) is described from desert soils of southwestern North America. The identification is based on phylogenetic analysis of data from nuclear (ITS2 rDNA) and plastid (*rbc*L, *tuf*A) barcode markers. This newly described species represents the fifth cryptic species of arid-adapted algae in *Scenedesmaceae*. A re-analysis of published sequences attributed to desert *Tetradesmus* in the context of our newly obtained data reiterates the importance of robust phylogenetic analysis in identification of cryptic taxa, such as species of *Tetradesmus*.

Key words: DNA barcoding, *rbc*L, ITS, *tuf*A, desert algae, green algae

Introduction

Microbiotic crusts are complex communities of soil microorganisms and cryptogams that occur in arid and semi-arid habitats. Prokaryotic and eukaryotic members of microbiotic crusts act as important "ecosystem engineers" by powering nutrient cycles, preventing erosion and enhancing water holding capacity in desert soils, as well as influencing the composition of plant communities (Evans & Johansen 1999; Belnap & Lange 2001; Song et al. 2017). Early surveys of algae species composition in desert crusts revealed only a small number of species, owing to the simple morphology of many soil algae and identification methods that only focused on vegetative phases (e.g. Cameron 1960; Cameron 1964; Ocampo-Paus & Friedmann 1966; Lange et al. 1992). More recently, Fletchtner et al. (1998) integrating morphological and life history stages in their identification were able to distinguish almost 40 species collected from a small number of desert crust samples in Baja California (Mexico). Subsequent examinations (e.g., Lewis & Lewis 2005; Büdel et al. 2009) relied on the analysis of DNA sequence data, which has revealed a high level of biodiversity in soils of arid regions, including desert algae. Knowledge of biodiversity of this region was further enhanced by discovery that desert-dwelling green algae belong to multiple clades in *Streptophyta* and *Chlorophyta* (Lewis & Lewis 2005).

Phylogenetics-based taxonomy is key to uncovering novel species, enhancing knowledge of species distributions and revealing overall biodiversity from different habitats. Phylogenetics-based taxonomy is especially relevant for groups of microscopic organisms and those, that do not possess multiple charismatic morphological traits. With the dramatic increase in the number of algal surveys using environmental sampling and DNA barcoding, a growing number of sequences now in public databases are taxonomically unattributed or may have incorrect attributions. We therefore, test species designation of existing sequences in light of our updated taxonomy of *Tetradesmus*.

In *Chlorophyta*, desert algae are members of two classes. *Trebouxiophyceae*, a class known for its many lichenized and free-living terrestrial species, includes members from 12 major lineages (Fučíková et al. 2014). *Chlorophyceae*, on the other hand, is often considered largely aquatic, but also contains multiple lineages of terrestrial algae, including species isolated from deserts (Lewis & Lewis 2005). Thus, in *Chlorophyta*, desert algae are not associated with a single taxon, nor do they tend to be members of predominantly terrestrial lineages; instead desert algae are embedded in clades of aquatic species, as exemplified by the focus genus of this study *Tetradesmus* (*Scenedesmaceae*, *Sphaeropleales*, *Chlorophyta*). *Tetradesmus* includes multiple arid-adapted species, which belong to two separate clades, each containing aquatic as well as terrestrial species (Lewis & Flechtner 2004; Sciuto et al. 2015; Mikhailyuk et al. 2018; Lewis & Flechtner 2019).

Here we present evidence for a new terrestrial species in the mainly freshwater planktonic family

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Scenedesmaceae (*Chlorophyta*), genus *Tetradesmus* G.M. Smith. *Tetradesmus adustsus* is the fourth species in the genus *Tetradesmus* and fifth in the family Scenedesmaceae to be isolated from biological soil crusts or other arid soils.

Materials and methods

Isolation sites

Strain LG2-VF28 was isolated from a sample of loose-packed decomposed granite sandy soil in Baja California, Mexico, strain JT2-VF29 was isolated from the surface of course sand and gravel, near Cadiz, California, U.S.A. (detailed localities are given below).

Culture conditions

Strains JT2-VF29 and LG2-VF28 were grown in KSM liquid medium (Clear & Hom unpubl.) under a 12:12 L:D cycle (photon flux of 200 µmol $m^{-2}s^{-1}$) at a temperature of 25°C. Mixing of the cultures was achieved by orbital shaking at 0.4 radsec. To test variability of cell morphology in different media, the algae were also grown in Bold's Basal medium (BBM, Bold 1949), Storrs medium (Trainor et al. 1991), and a medium that had previously contained a natural grazer of *Scenedesmaceae*, *Daphnia magna* (Zhu et al. 2015), as the presence of chemical cues from predators have been shown to induce distinct morphologies in some members of the *Scenedesmaceae* (Lürling & Van Donk 1996).

DNA extraction, amplification, and sequencing

Culture aliquots of strains LG2-VF28 and JT2-VF29 were concentrated, frozen, and then mechanically disrupted. Genomic DNA was extracted using the Zymo-BIOMICS DNA Miniprep Kit or Qiagen DNEasy Plant Extraction kit (Qiagen, Hilden, Dusseldorf, Germany). The ITS region was amplified with the primer pair ITS1 – ITS4 (White et al. 1990, Hall et al. 2010). For amplification of the *tuf*A gene we used the primer pair tufAF – tufA.870r (Hall et al. 2010; Famá et al. 2012). The *rbc*L gene was amplified using primer pair Scen-RubF1 and Scen-RubR1 (Sciuto et al. 2015). Standard PCR protocols were carried out with GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA) or with Taq-DNA Polymerase (Fisher Molecular Biology, Trevose, PA, USA), according to manufacturer's recommendation. Amplification products were purified with the QIAquick PCR Purification kit (Qiagen, Hilden, Dusseldorf, Germany) or Exosap-IT Express (Life Technologies Corporation, Carlsbad, CA, USA) prior to sequencing. DNA sequencing was performed at University of Connecticut (USA) with the same pairs of primers used for amplification reactions. Consensus sequences for three genes of JT2-VF29 and LG2-VF28 were obtained from forward and reverse reads using Geneious 10.2.2 (https://www.geneious.com) and deposited to NCBI with respective accession numbers MK291427 and MK291430 for the ITS2 region, MK291428 and MK291431 for the *rbc*L gene, and MK291429 and MK291432 for the *tuf*A gene.

Molecular and phylogenetic analysis of focal species

Two types of data sets were prepared: single gene alignments of the *tuf*A, *rbc*L, and ITS2 sequences, and a concatenated data set of all three genes. Each of them included sequences of the two focal strains as well as other available sequences of *Tetradesmus* and selected related genera (Table S1). To create the multiple alignment of the ITS region, sequence data were used together with the secondary structure, which was inferred by homology prediction using the ITS2 Database (Schultz et al. 2006; Koetschan et al. 2012). Substitution models and parameter values for the phylogenetic analyses were selected with Partitionfinder2 (Lanfear et al. 2017) using algorithms greedy (Lanfear et al. 2012) and PhyML (Guindon et al. 2010). The Bayesian Information Criterion (BIC, Schwarz 1978) was used to select the best model.

Maximum likelihood (ML) analyses of data sets for individual genes were performed with following parameters. *tuf*A: model GTR+I+G, nucleotide frequencies A = 0.348646, C = 0.12815, G = 0.207184, T = 0.30602; substitution rates AC = 2.9153, AG = 5.18423, AT = 6.37418, CG = 1.47765, CT = 20.6667, GT = 1.00000; Pinvar = 0.56204; Gamma shape = 0.937424. *Rbc*L: model GTR+I+G, nucleotide frequencies A = 0.289677, C = 0.185267, G = 0.215079, T = 0.309977; substitution rates AC = 0.89969, AG = 0.535725, AT = 1.33955, CG = 0.264598, CT = 2.12243, GT = 1.00000; Pinvar = 0.519836, Gamma shape = 0.400908. ITS2: model K80+I+G, nucleotide frequencies equal; T_i/T_v ratio = 1.7719; Pinvar = 0.519836, Gamma shape = 0.589762. The available ITS2 sequences were highly variable in length, to avoid introducing bias of absent data, the last 65 positions were excluded from the analyses.

The model GTR+I+G was chosen for the ML analysis of the concatenated tree gene data set and implemented with following parameters: nucleotide frequencies A = 0.306869, C = 0.171365, G = 0.223547, T = 0.298219; substitution rates AC = 0.620975, AG = 2.04111, AT = 2.5318, CG = 0.612794, CT = 6.69894, GT = 1.000000; Pinvar = 0.512441; and Gamma shape = 0.66503. ML analyses were performed using PAUP* v. 4.0a (Swofford 2003).

Bayesian interference (BI) was carried out with MrBayes 3.2.6. (Ronquist & Huelsenbeck 2003) available on the CIPRES Science Gateway (Miller et al. 2010). For this analysis the concatenated data set was partitioned by gene and by codon position (for protein-coding genes). The F81+I model was chosen for 1st codon positions of both *tuf*A and *rbc*L, F81 was applied to 2nd codon positions of *tuf*A, JC+I was used for 2nd codon position of *rbc*L, GTR+G was used for the 3rd codon positions of both *tuf*A and *rbc*L, and K80+G was applied to the ITS region. The analysis included two separate MCMC runs, each composed of four chains. Each MCMC chain ran for 200 000 000 generations, sampling trees every 100 generations. Upon completion, the runs were compared using Tracer v.1.7 (Rambout et al. 2018) and the first 25% of generated trees were discarded as burn-in. A 50% majority-rule consensus topology and posterior probabilities were then calculated from the remaining trees.

Re-analysis of published sequences attributed to desert *Tetradesmus*

To analyze previously published sequences of species from *Scenedesmaceae* we prepared an alignment of the *rbc*L gene sequences, including those generated here plus those obtained by Zou et al. (2016) and Kumar et al. (unpublished). ML analysis was implemented using GTR+I+G model with following parameters: nucleotide frequencies A = 0.282483, C = 0.167916, G = 0.219744, T = 0.329857; substitution rate matrix AC = 0.266057, AG = 1.3419, AT = 2.62602, CG = 0.463667, CT = 3.68592, GT = 1.000000; Pinvar = 0.414024; Gamma shape = 0.62218. Given the large number of taxa in the data set with nearly identical *rbc*L sequences, we saved a maximum of 100 best-scoring trees, then prepared a 50% majority rule consensus tree from these saved trees. ML analyses were performed using PAUP* v. 4.0a (Swofford 2003).

BI analysis was carried out as described above with the exception of MCMC chains running for 2×10^6 generations, sampling trees every 1000 generations. The GTR+I+G model was chosen for codon positions 1 and 3, JC+I was used for codon position 2.

Light microscopy

Observation of strains LG2-VF28 and JT2-VF29 kept under the conditions described above were carried out using an Olympus BX60 microscope and DIC optics. The images were taken with Olympus DP25 color camera (2560×1920 pixels).

Results and discussion

Molecular and phylogenetic analysis of focal species

The sequences obtained from strains LG2-VF28 and JT2-VF29 were identical in all three barcode loci studied. Phylogenetic analyses of concatenated and individual gene data sets showed that these two strains compose a highly supported separate clade inside *Tetradesmus*.

Phylogenetic analyses using different methods resulted in comparable but not identical tree topologies. Despite the difference in the order of branching all of them support strains LG2-VF28 and JT2-VF29 as a separate lineage, and for this reason we highlight the BI tree (Fig. 1). The tree resulting from ML analysis of concatenated data and the trees based on the analysis of single genes can be found in supplementary materials (Fig. S1 and Fig. S2 A-C respectively).

Interestingly, strains LG2-VF28 and JT2-VF29 are sister to a clade containing the desert species *T. bajacalifornicus* (plus the recently described *T. arenicola* from Baltic sand dunes by Mikhailyuk et al. in press, which is only included in ITS tree in Fig. S2C) and the aquatic species *T. raciborskii*, separate from the clade containing the other desert–aquatic pair of species (*T. deserticola* and *T. dissociatus*). The mixed association of habitats among this set of *Tetradesmus* species allows us to suggest at minimum two independent origins of these desert species into terrestrial habitats. Given the phylogenetic distinction of these two strains of *Tetradesmus*, we propose a new species.

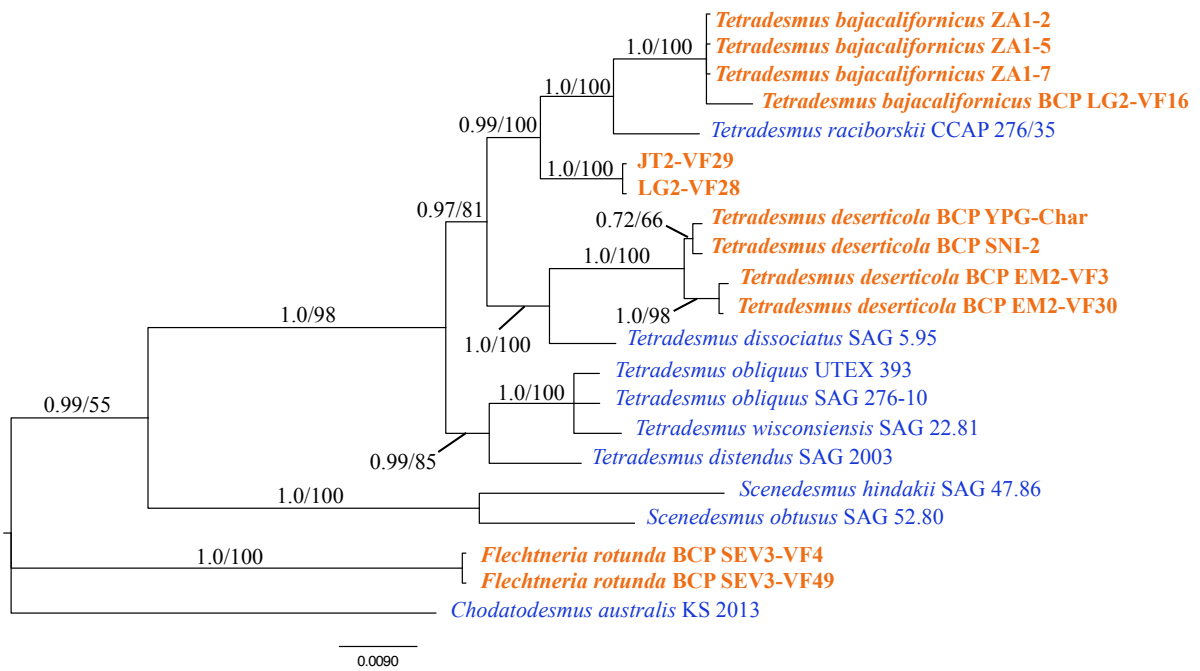

Figure 1. Phylogenetic tree of *Tetradesmus* and selected related genera based on Bayesian inference of the *tuf*A, *rbc*L and ITS2 rDNA. Numbers associated with nodes indicate support values for Bayesian interference (BI) and Maximum likelihood (ML) analyses, respectively. Support values below 0.5 for BI or 50 for ML were omitted. Aquatic strains are labeled in blue, strains isolated from desert soil crusts are labeled in orange boldface font. The scale bar represents expected number of nucleotide substitutions per site.

Tetradesmus adustus Terlova & L. A. Lewis, sp. nov. (Fig. 2A–F)

Diagnosis: Single cells ovoid in shape, with slightly pointed apices in young cells, becoming more spherical with age. Older cells are morphologically similar to *T. bajacalifornicus*, *T. deserticola*, *T. arenicola*. However, young cells are smaller than in these species and never reach more than 8µm in length. Similar to *T. deserticola* with pyrenoid is surrounded by a starch shell, but distinct in that *T. adustus* cells do not form long extensions of the apices. Do not form colonies or crescent-shaped cells as *T. deserticola* or *T. arenicola* and differs in DNA data and is supported as a separate lineage by phylogenetic analysis. Inhabits biological soil crusts. Sequence data of at least one barcode marker is necessary for identification.

Holotype: Fixed cells of strain LG2-VF28 on a permanent slide, deposited in George Safford Torrey Herbarium, University of Connecticut, Storrs, CT, U.S.A., CONN00226475.

Iconotype: Fig. 2A–F.

Type locality: Mexico, Baja California, Sierra San Pedro Mártir, latitude 30.90°N, longitude 115.46°W, elevation 2100 m; collected from loosely packed decomposed granite sandy soil by W.H. Clark, 15 June 1998.

Reference strain number: LG2-VF28.

Type sequences: ITS2 MK291430, *rbc*L MK291431, *tuf*A MK291432.

Description. Young cells are ovoid with slightly pointed apices, 6–8 µm in length and 4–6 µm in width (Fig. 2A–B). Uninucleate. The single chloroplast per cell is cup-shaped and located at the periphery of the cell. It contains a spherical pyrenoid surrounded by an obvious starch hull. In some cases the pyrenoid can occupy a large portion of the chloroplast volume. (Fig. 2B–C).

Older cells and cells preparing for or undergoing division are larger (9–15 µm) and often are spherical in shape (Fig. 2D–E). Four to eight autospores formed

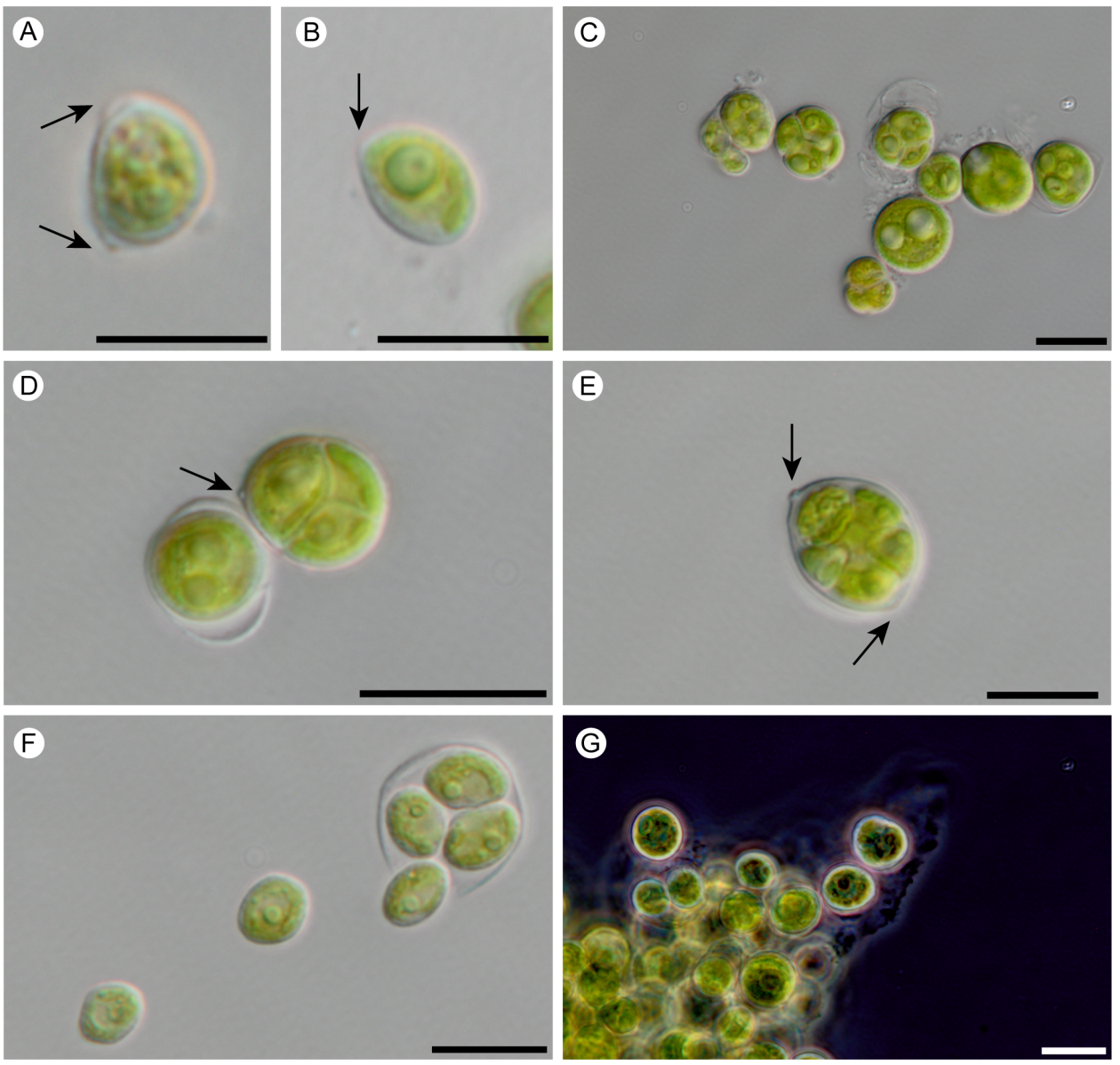

Figure 2. Light photomicrographs of *Tetradesmus adustus*. A, B – cells have slightly pointed apices; C – cells of the JT2-29 strain contain unusually large pyrenoids; D, E – formation of autospores inside mother cells. The number of autospores formed in a single cell varies between four and eight; F – liberation of autospores; G – phase-contrast image of a dense culture, cells are kept together by an extracellular matrix, large pyrenoids surrounded by starch can be seen inside cells. Scales = 10 µm, arrows indicate pointed apices of cells.

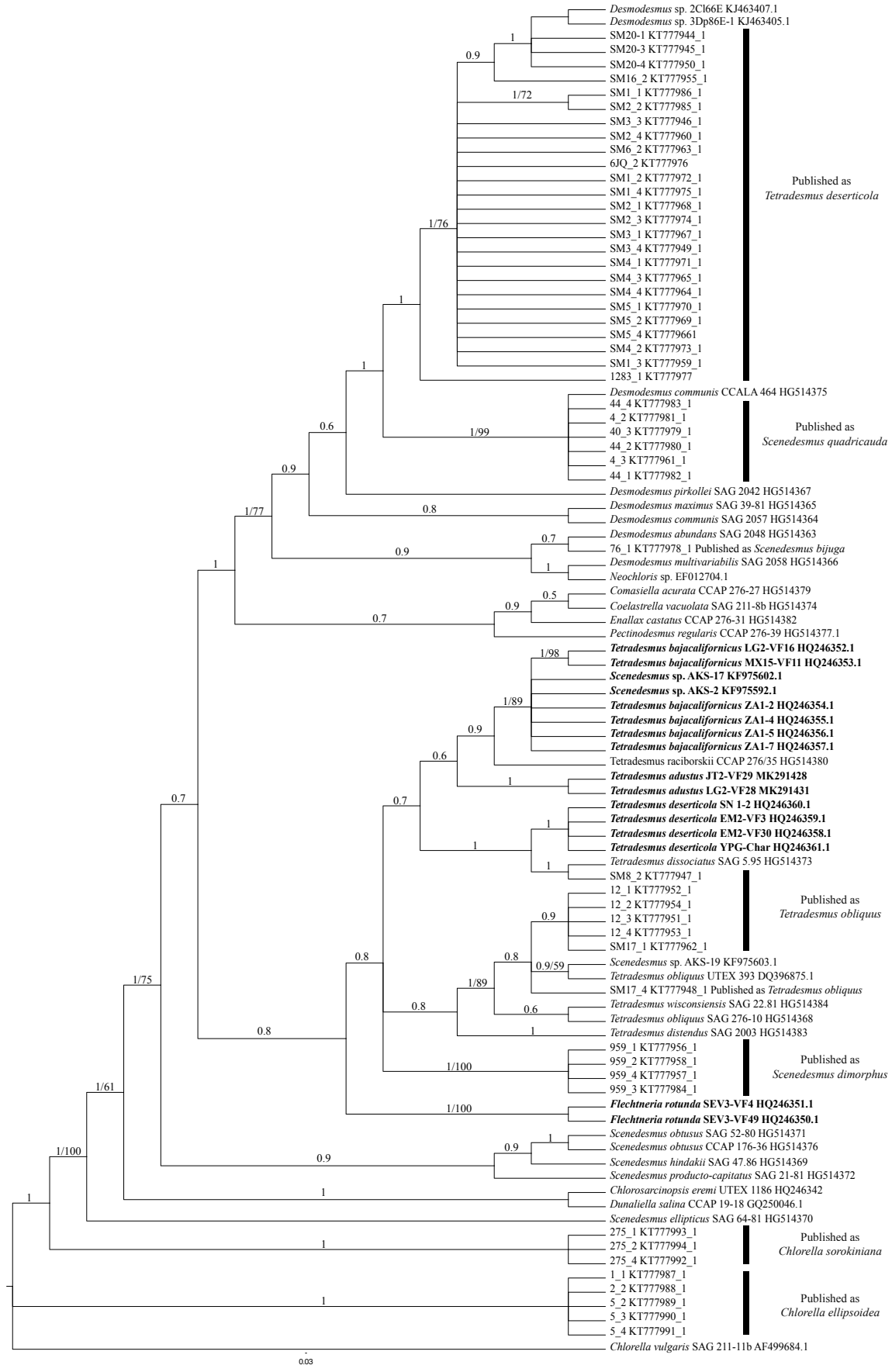

Figure 3. A phylogenetic tree of published sequences in the context of updated *Tetradesmus* taxonomy based on Bayesian inference of the *rbc*L gene. Taxon labels include the strain designation (as appropriate) and the NCBI accession number. Branch labels indicate support value for BI and ML analyses, respectively. Support values below 0.5 for BI or 50 for ML were omitted. Strains isolated from desert soil crusts or other arid environments are labeled in boldface font.

inside a mother cell by longitudinal division (Fig. 2F). In older, denser cultures cells are often aggregated together by an extracellular matrix (Fig. 2G). Under conditions supporting rapid growth in liquid KSM medium cells did not form colonies. Cell morphology remained the same when the strains LG2-VF28 and JT2-VF29 were grown in other media. Formation of coenobia or flagellate cells was not observed in BBM, in the medium that previously contained a culture of *Daphnia magna*, or in nitrogen-deprived conditions (Storrs medium, Trainor et al. 1991). Sexual reproduction was not observed.

Etymology. The specific epithet refers to torrid or parched, which is the state of these algae living in arid habitats.

Additional strain. JT2-VF29

Additional locality. Near Cadiz, San Bernardino County, California, U.S.A.; latitude 34.2644°N, longitude 115.6947°W.

Additional sequences. ITS2 MK291427, *rbc*L MK291428, *tuf*A MK291429

Resolving phylogenetic relationships of published desert *Tetradesmus*

Cryptic species and hidden phylogenetic diversity are common in microscopic green algae, and may even be the rule rather than the exception (e.g., De Clerck et al. 2013; Muggia et al. 2018). DNA-based taxonomy over the last decades has provided a growing understanding of cryptic diversity among algae (e.g., Huss et al. 1999; Zuccarello & West 2003; Trobajo et al. 2010; Sherwood et al. 2018), making molecular phylogenetic analysis a common tool of discovering new species. Resolving and naming cryptic species is crucially important for ecological analyses and evolutionary reconstructions, whereas large non-monophyletic taxa, such as the genus "*Scenedesmus*" (An et al. 1999), hide the true diversity of the group and misrepresent its geographical distribution. Moreover, having a clear understanding of the phylogeny of these species allows us to examine the evolution of traits across species, such as habitat shifts.

In a recent study by Zou et al. (2016) 84 strains of green algae from 11 locations in China were isolated from aquatic and terrestrial habitats including lakes, rivers, and urban soils. Algal strains were identified based on their morphology (the authors were not clear about which traits were used) and sorted into 11 species, including the desert species *T. deserticola*. Four barcode genes (*rbc*L, *tuf*A, ITS, and 16S) were sequenced, and the DNA sequence data were used to test species delimitation with several methodologies. Their results were used to highlight cryptic species in this group. In particular the authors concluded that several of their morphologically identified species were not monophyletic, including the desert species *T. deserticola*.

We performed a phylogenetic analysis, which included *rbc*L sequences generated by Zou et al. (2016), those of various *Tetradesmus* species, and species from other genera in *Scenedesmaceae*. BI and ML analyses resulted in trees of similar topology, and here we show only the BI tree (Fig. 3, with the resulting tree from the ML analysis shown in Fig. S5).

The sampling reported by Zou et al. (2016) shows remarkable diversity of Scenedesmaceae, including a number of well-supported clades that may warrant new species status, but this was not addressed in their publication. None of the sequences obtained by Zou et al. (2016) are clustered with previously described desert species of *Tetradesmus* in our analysis or in their trees (see Fig. 2 in Zou et al. 2016). In contrast, strains AKS-2, AKS-17 and AKS-19 isolated from soils in Chota Nagpur, a dry-deciduous ecoregion in India by Kumar et al. (unpubl.) were highly supported as belonging to *T. bajacalifornicus*. Thus, to our knowledge the distribution of *T. deserticola* is restricted to deserts and other arid habitats, and this species is monophyletic. A number of strains of Zou et al. (2016) are clustered within *Desmodesmus*, others are closely related to *Tetradesmus obliquus* and *Tetradesmus dissociatus*, both of which are common aquatic species. Consequently, the taxonomy associated with the published sequences of Zou et al. (2016) should be updated to reflect their distinction from *T. deserticola*.

The remarkable number of new *Tetradesmus* species that have been recently described illustrates that more research is needed to complete our taxonomic understanding of this genus. Our analyses show once more that the simple morphology of the majority of species from *Scenedesmaceae* makes an analysis of DNA data the most reliable way to identify and differentiate species in this group.

A distinct set of traits that allow these terrestrial species to persist in their harsh environments, coupled with a close evolutionary history with aquatic species, makes *Tetrademus* an excellent system for studying traits associated with the transition to land in chlorophyte green algae, as was recently highlighted by Cardon et al. (2018).

The establishment of a robust phylogenetic and biogeographical data on *Tetradesmus* species is a step, which provides a framework for addressing important evolutionary questions such as mechanisms of adaptation to arid habitats exhibited by multiple species in this genus, or whether all of the *Tetradesmus* species possess traits that may have facilitated the transition from aquatic habitats onto the land. It is possible now to assay differences in response to desiccation and rehydration of desert and aquatic *Tetradesmus* and even investigate genetic background of these differences.

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Supplementary electronic material

Table S1. Focal strains whose sequences were used in this study. Download file

Figure S1. Maximum likelihood tree of *Tetradesmus* and selected related genera based on the analysis of *tuf*A and *rbc*L, and ITS2 data (lnL = –4978.447). Download file

Figure S2. Maximum likelihood trees of *Tetradesmus* based on sequences of individual genes. Download file

Figure S3. Maximum likelihood 50% majority rule consensus tree of 100 best trees of the published sequences in the context of updated *Tetradesmus* taxonomy based on *rbc*L gene (lnL = –9838.873). Download file

References

An, S. S., Friedl, T. & Hegewald, E. 1999. Phylogenetic relationships of *Scenedesmus* and *Scenedesmus*-like coccoid green algae as inferred from ITS-2 rDNA sequence comparisons. *Plant Biology* 1: 418–428.

Belnap, J. & Lange, O. L. 2001. *Biological soil crusts: structure*, *function*, *and management*. Springer, Berlin.

Bold, H. C. 1949. The morphology of *Chlamydomonas chlamydogama*, Sp. Nov. *Bulletin Torrey botanical club* 76: 101–108.

Büdel, B., Darienko, T., Deutschewitz, K., Dojani, S., Friedl, T., Mohr, K. I., Salisch, M., Reisser, W. & Weber, B. 2009. Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. *Soil Microbiology* 57: 229–247.

Cameron, R. E. 1960. Communities of soil algae occurring in the Sonoran desert in Arizona. *Journal of Arizona Academy of Science* 1: 85–88.

Cameron, R. E. 1964. Algae of Southern Arizona. II. Algal Flora (Exclusive of Bluegreen Algae). *Review Algology* 7: 151–177.

Cardon, Z. G., Peredo, E. L., Dohnalkova, A. C., Gershone, H. L. & Bezanilla, M. 2018. A model suite of green algae within the Scenedesmaceae for investigating contrasting desiccation tolerance and morphology. *Journal of Cell Science* 131: jcs212233

De Clerck, O., Guiry, M. D., Leliaert, F., Samyn, Y. & Verbruggen, H. 2013. Algal taxonomy: a road to nowhere? *Journal of Phycology* 49: 215–225.

Evans, R. D. & Johansen, J. R. 1999. Microbiotic crusts and ecosystem pocesses. *Critical Reviews in Plant Sciences* 18: 183–225.

Famá, P., Wysor, B., Kooistra, W. & Zuccarello, G. C. 2012. Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tuf*A gene. *Journal of Phycology* 38: 1040–1050.

Flechtner, V. R., Johansen, J. R. & Clark, W.H. 1998. Algal composition of microbiotic crusts from the Central Desert in Baja California, Mexico. *Great Basin Naturalist* 58: 295–311.

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

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321.

Hall, J., Fučíková, K., Lo, C., Lewis, L. A. & Karol, K. 2010. An assessment of proposed DNA barcodes in freshwater green algae. *Cryptogamie Algologie* 31: 529–555.

Huss, V. A. R., Frank, C., Hartmann, E. C., Hirmer. M., Kloboucek, A., Seidel, B. M., Wenzeler, P. & Kessler, E. 1999. Biochemical taxonomy and molecular phylogeny of the genus *Chlorella* sensu lato (Chlorophyta). *Journal of Phycology* 35: 587–598.

Koetschan, C., Hackl, T., Müller, T., Wolf, M., Förster, F. & Schultz, J. 2012. ITS2 database IV: interactive taxon sampling for Internal Transcribed Spacer 2 based phylogenies. *Molecular Phylogenetics and Evolution* 63: 585–588.

Lanfear, R., Calcott, B., Ho, S. Y. & Guindon, S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T. & Calcott, B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773.

Lange, O. L., Kidron, G. J., Büdel, B., Meyer, A., Kilian, E. & Abeliovich, A. 1992. Taxonomic composition and photosynthetic characteristics of the biological soil crusts covering sand dunes in the Western Negev Desert. *Functional Ecology* 56: 519–527.

Lewis, L. A. & Flechtner, V. R. 2004. Cryptic species of *Scenedesmus* (Chlorophyta) from desert soil communities of Western North America. *Journal of Phycology* 40: 1127–1137.

Lewis, L. A. & Flechtner, V. R. 2019. *Tetradesmus bajacalifornicus* L.A.Lewis & Flechtner, *sp. nov*. and *Tetradesmus deserticola* L.A.Lewis & Flechtner, *sp. nov.* (*Scenedesmaceae*, Chlorophyta). *Notulae algarum* No. 88.

Lewis, L. A. & Lewis, P. O. 2005. Unearthing the molecular phylodiversity of desert soil green algae (Chlorophyta). *Systematic Biology* 54: 936–947.

Lürling, M. & Van Donk, E. 1996. Zooplankton-induced unicell-colony transformation in *Scenedesmus acutus* and its effect on growth of herbivore *Daphnia*. *Oecologia* 108: 432–437.

Mikhailyuk, T., Glaser, K., Tsarenko, P., Demchenko, E. & Karsten, U. 2018. 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. *European Journal of Phycology*. In press.

Miller, M. A., Pfeiffer, W. & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop 14 Nov. 2010*, *New Orleans*, *LA*, pp. 1–8.

Muggia, L., Leavitt, S. & Barreno, E. 2018. The hidden diversity of lichenised *Trebouxiophyceae* (Chlorophyta). *Phycologia* 57: 503–524.

Ocampo-Paus, R. & Friedmann, I. 1966. *Radiosphaera Negevensis* Sp. N., a new chlorococcalean desert alga. *American Journal of Botany* 53: 663–671.

Rambout, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904.

Ronquist, F. & Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Schultz, J., Müller, T., Achtziger, M., Seibel, P. N., Dandekar, T. & Wolf, M. 2006. The Internal Transcribed Spacer 2 Database – a web server for (not only) low level phylogenetic analyses. *Nucleic Acids Research* 34: 704–707.

Schwarz, G. 1978. Estimating the dimension of a model. *The Annals of Statistics* 6: 461–464.

Sciuto, K., Lewis, L. A., Verleyen, E., Moro, I. & La Rocca, N. 2015. *Chodatodesmus australis* Sp. Nov. (Scenedesmaceae, Chlorophyta) from Antarctica, with the emended description of the genus *Chodatodesmus*, and circumscription of *Flechtneria rotunda* Gen. et Sp. Nov. *Journal of Phycology* 51: 1172–1188.

Sherwood, A. R., Neumann, J. M., Dittbern-Wang, M. & Conklin, K. Y. 2018. Diversity of the green algal genus *Spirogyra* (Conjugatophyceae) in the Hawaiian Islands. *Phycologia* 57: 331–344.

Song, G., Li, X. & Hui, R. 2017. Effect of biological soil crusts on seed germination and growth of an exotic and two native plant species in an arid ecosystem. *PLoS ONE* 12: 529–555.

Swofford, D. L. 2003. PAUP*. *Phylogenetic analysis using parsimony (* and Other Methods). Version 4*. Sinauer Associates, Sunderland.

Trainor, F. R., Wallett, S. & Grochowski, J. 1991. A useful algal growth medium. *Journal of Phycology* 27: 460–461.

Trobajo, R., Mann, D. G., Clavero, E., Evans, K. M., Vanormelingen, P. & McGregor, R. C. 2010. The use of partial *cox1*, *rbc*L and LSU rDNA aequences for phylogenetics and species identification within the *Nitzschia palea* species complex (Bacillariophyceae). *European Journal of Phycology* 45: 413–425.

White, J., Bruns, T. T. D., S. Lee, B. & Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* 18: 315–322.

Zhu, X., Wang, J., Lu, Y., Chen, Q. & Yang, Z. 2015. Grazer-induced morphological defense in *Scenedesmus obliquus* is affected by competition against *Microcystis aeruginosa*. *Scientific Reports* 5: 12743.

Zou, S., Fei, C., Gao, Z., Bao, Y., He, M. & Wang, C. 2016. How DNA barcoding can be more effective in microalgae identification: a case of cryptic diversity revelation in *Scenedesmus* (Chlorophyceae). *Scientific Reports* 9: 36822.

Zuccarello, G. C. & West, J. A. 2003. Multiple cryptic species: molecular diversity and reproductive isolation in the *Bostrychia radicans / B. moritziana* complex (Rhodomelaceae, Rhodophyta) with focus on North American isolates. *Journal of Phycology* 39: 948–959.