



Appendix S2. (A) Infrageneric phylogenetic classification of *Trebouxia* into clades A, C, D, I, and S. Clade C is referred to as clade G in literature prior to Muggia et al. (2020). This color scheme for major clades is used consistently throughout the paper. The ITS-*rbcL* tree was adapted from Medeiros et al. (2021); the tree is rooted using *Asterochloris* and *Myrmecia* and the scale represents nucleotide substitutions per site. **(B)** Single-locus data available on GenBank for molecular systematics of *Trebouxia*. Several genetic loci have been used in studies of lichenized *Trebouxia*, including the nuclear internal transcribed spacer (nrITS), Rubisco large subunit (*rbcL*), cytochrome oxidase subunit 2 (*cox2*), chloroplast 23S rDNA, actin type I (*act1*), photosystem II protein D1 (*psbA*), and the protein-coding gene *RPL10A* (Kroken and Taylor, 2000; Catalá et al., 2016; Garrido-Benavent et al., 2020; Muggia et al., 2020). ITS is by far the most widely sequenced locus for this genus. The protein-coding loci *rbcL* and *cox2* have also been used across numerous studies, but *cox2* is not easily amplified for *Trebouxia* clade C (Muggia et al., 2020).

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