

Supplementary Material

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Supplementary Table S1. Advantages and disadvantages of each molecular marker tested in this study and other principal markers used in studies with green algae

Marker	Advantage	Disadvantage
<i>tufA</i>	Conserved gene with intermediate evolution rate Good phylogenetic signal All strains tested here were amplified with the same pair of primer used in other studies Easy alignment No introns reported (Fama et al. 2002, Nozaki et al. 2002, O'Kelly et al. 2004, Sáez et al. 2008, Wynne et al. 2009, Garcia-Cuetos et al. 2010, Fučíková et al. 2011, Christa et al. 2013, Lawton et al. 2013)	Few sequences available at the NCBI Transference to the nucleus in some Charophyta (but no reports for Chlorophyta) (Baldauf et al. 1990) - - -
<i>rbcL</i>	Many sequences available at the NCBI Frequently used in phylogenetic studies with Chlorophyceae -	Neither pair of primers tested here amplified all strains Introns reported Specific primers needed for different groups; sequences available may not correspond to the same region of the gene (Nozaki et al. 2002, Mcmanus et al. 2012)
ITS	Very variable Specific discrimination through the secondary structure	Specific primers: available sequences may not correspond to the same region -
UCP4	-	Failed to amplify any strain
COX I	Already in use as DNA barcode for different groups (e.g., animals, brown and red algae, and diatoms) Many sequences available (Ward et al. 2005, Evans et al. 2007, Sherwood et al. 2008, Le Gall and Saunders 2010, McDevit and Saunders 2010) -	Too variable in Green algae: many different primers needed Introns reported Could not be amplified in some Chlorophycean taxa (Turmel et al. 2002, Hall et al. 2010, Fučíková et al. 2011, Saunders and Kucera 2010)
18S	Many sequences available at the NCBI Frequently used in phylogenetic studies with Chlorophyceae -	Introns reported Too conserved to allow specific discrimination Many primers needed; available sequences may not be from the same region of the gene (Luo et al. 2010, Fučíková et al. 2011, Garcia et al. in press)

The markers in bold were tested in this study.