Sequence and secondary structure of chloroplast 16S rRNA from the chromophyte alga *Olisthodiscus luteus*, as inferred from the gene sequence

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The sequence and secondary structure of chloroplast 16S rRNA from the marine chromophyte (chlorophyll a,c containing) *Olisthodiscus luteus** is presented in this report. Few data exist for the *chl a,c* algae, whose evolutionary relationship with respect to the chlorophytic (*chl a,b* containing) plants remains a source of continued debate (1).

DNA Sequencing. The *O. luteus* chloroplast 16S *rrnA* gene was cloned as a 3.5 kilobase-pair *EcoRI* restriction fragment into either M13 or pUC derived DNA sequencing vectors (2). The cloned fragment was digested using Exonuclease III (3) to produce sets of deletion clones for sequence determination. Both the large fragment of *Escherichia coli* DNA polymerase I (Klenow enzyme) and a modified phage T7 polymerase ('Sequenase', U.S. Biochemicals; Cleveland, OH) were used in dideoxynucleotide chain termination sequencing reactions (4). When necessary, *E. coli* single stranded DNA binding protein was included in the primer annealing reactions to reduce secondary structure interference in the template. The *O. luteus* 16S rRNA gene was sequenced from each DNA strand.

Secondary Structure Modeling. Regions of obvious primary structure homology were used to align the *O. luteus* 16S rRNA gene with those from other chloroplast and prokaryotic representatives. Ambiguous regions of similarity between genes were aligned using conserved secondary structure elements as reference domains. Putative helical regions in the 16S rRNA molecule were identified through identifying compensatory base-changes using methods of comparative sequence analysis established by Woese and colleagues (5, 6).

The predicted length of the 16S rRNA product is 1519 nucleotides. A model of *O. luteus* 16S rRNA secondary structure is presented in Figure 1. Although the RNA helices depicted are similar to those found in both chlorophytic chloroplasts and in eubacteria (6), some regions of the *O. luteus* 16S rRNA display extensive primary structure divergence. For example, the helices encompassing residues 814 to 843 and 1219 to 1273 are conserved in secondary structure while showing almost complete sequence divergence from the *E. coli* 16S rRNA (6). The variation observed in some secondary structure elements (pos 144–227) is significantly greater between algal classes than in seed plant taxa (7), suggesting that non-equivalence in taxonomic rank exists among these phylogenetic groups.

*The taxonomic description of this organism is under revision.

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Figure 1. Secondary structure model of *Olisthodiscus luteus* chloroplast 16S rRNA.

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