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UNIVERSAL PRIMERS AMPLIFY A 23S rDNA PLASTID MARKER IN EUKARYOTIC ALGAE AND CYANOBACTERIA[1]

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The challenge in the development of universal algal primers lies in the genetic diversity contained within the vast array of evolutionary lineages present in this informally named group of organisms. A comparative genomics approach was used previously to identify conserved primers flanking a region of the plastid genome. Our present research illustrates the feasibility of amplifying and sequencing this marker across multiple algal lineages. We present a preliminary framework of 107 novel sequences of this region from 62 red algae, 19 green algae, 14 brown algae, 8 cyanobacteria, 2 diatoms, 1 xanthophyte, and 1 euglenoid, and illustrate levels of divergence of the marker for well-represented groups in a neighbor-joining analysis. This ~410 nt region distinguishes most species included in the analysis. The remarkable universality of these primers suggests potential for their use in assays of environmental samples in which they could be used to simultaneously detect a number of different algal lineages.

Key index words: **biodiversity; cyanobacteria; environmental sampling; plastid 23S rRNA; sequence diversity; universal primer**

Abbreviations: **COI, cytochrome oxidase I; cox, cytochrome oxidase; K2P, Kimura-2-parameter; nt, nucleotide; *rbc*L, large subunit of RUBISCO**

To date, a number of commonly employed sets of primers have been introduced in the phycology literature for PCR-based analyses of algal samples. However, most have limited utility beyond the algal lineage for which they were designed, and their use has been directed primarily at specific phylogenetic or phylogeographic inquiry rather than the general assessment of large numbers of individuals from different algal lineages. For example, Saunders and Kraft (1994) and Harper and Saunders (2001) have published several sets of primers that are widely employed in phylogenetic analyses of the nuclear ribosomal SSU and LSU genes, respectively. Zuccarello et al. (1999) introduced a set of degenerate primers flanking the mitochondrial cox 2-3 spacer region that have been widely used in phylogeographic studies of red algae (Provan et al. 2005), and Saunders (2005) recently published a pair of mitochondrial COI primers.

A number of more broadly amplifying ''universal primers'' have also appeared in the literature. A set of 16S rRNA cyanobacterial primers have been employed for phylogenetic analyses and species identification for almost a decade (Nübel et al. 1997) and have recently been investigated in greater detail for diversity analyses of microbial communities (Boutte et al. 2006). The 18S rRNA gene of green algae has been frequently analyzed through amplification and sequencing using universal eukaryotic primers from Hamby et al. (1988). In 2004, Provan et al. released a suite of universal primers for plastid DNA in either green algae (~5 kb of *rps*11-*rpl*2 gene cluster) or red algae (13–17 kb of *rps*10-*dna*K ribosomal protein gene cluster). However, they approached the design of their primers with a priori separation of the two lineages, stating that ''it is unlikely that truly universal primers could be developed that would amplify in both Rhodophyta and Chlorophyta'' (Provan et al. 2004, p. 47). It has been demonstrated that this is not the case: Stiller and McClanahan (2005) recently reported a primer pair that amplifies both cyanobacterial and plastid 16S rDNA, to the exclusion of bacteria.

Comparative genomics provides a powerful approach for the determination of conserved regions across available genomes, and such comparisons are becoming increasingly valuable now that genomic data are becoming more representative of known lineage diversity. Universal eukaryotic plastid primers were recently designed following analysis of 37 plastid genomes from a variety of algal lineages and land plants (Presting 2006). These primers

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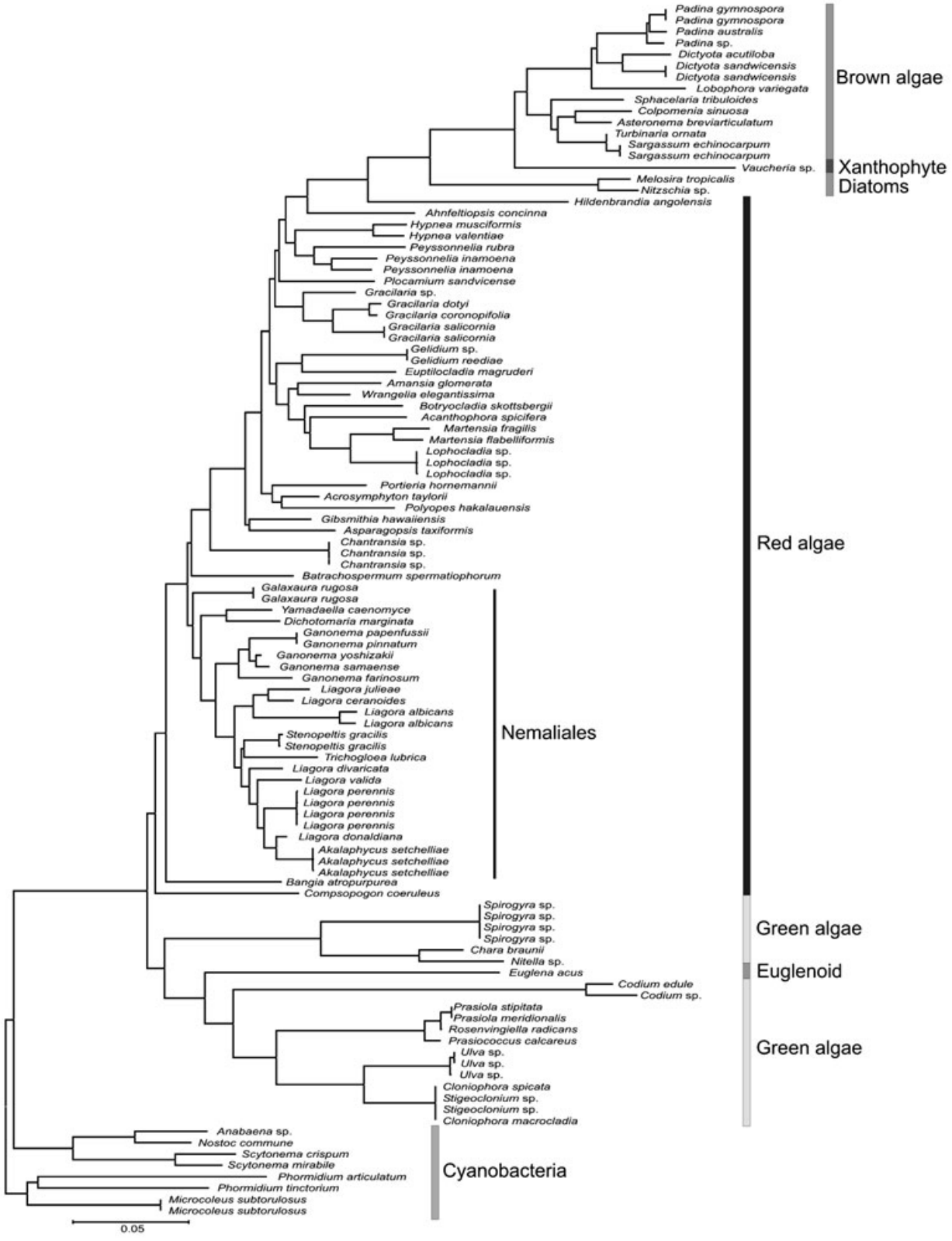

FIG. 1. Neighbor-joining distance tree based on the plastid 23S Domain V plastid rDNA sequence data obtained with a single universal primer pair. The data include 107 algal representatives from the red, green, and brown algae, as well as the diatoms, euglenoids, xanthophytes, and cyanobacteria. GenBank accession numbers and species authorities are provided in Supplementary Table S1.

flank Domain V of the 23S plastid rRNA gene (Harris et al. 1994). Here we demonstrate the ease of using a single pair of universal primers for amplification and sequencing of this plastid marker for multiple eukaryotic algal and cyanobacterial groups, present a preliminary DNA sequence framework based on these data, and illustrate the taxonomic level at which it distinguishes taxa for at least some algal lineages.

The primer pair p23SrV_f1 (5′ GGA CAG AAA GAC CCT ATG AA 3′) and p23SrV_r1 (5′ TCA GCC TGT TAT CCC TAG AG 3′) was designed from these conserved flanking sequences. These exact priming sequences are present only in cyanobacteria and plastids (Presting 2006). A phylogenetically diverse range of samples was obtained for the red (62), green (19), and brown algal lineages (14) and the cyanobacteria (8), with smaller representation from other groups (2 diatoms, 1 euglenoid, and 1 xanthophyte; sample collection information can be obtained from the authors). A culture of the coral symbiont dinoflagellate, *Symbiodinium* sp., was also included in the study, but it failed to amplify and sequence cleanly—a result that is not surprising given the unique 23S rDNA plastid sequence and structure known for members of this genus (Santos et al. 2002).

The DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) or a phenol–chloroform protocol (Saunders 1993). The primer pair was used to generate a ~410 nt amplicon from the plastid genome for the 107 accessions. The PCR amplification was performed in an Eppendorf Mastercycler ep gradient S thermalcycler (Eppendorf, Hamburg, Germany) using the following cycling conditions: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 20 s, 55°C for 30 s, and 72°C for 30 s, with a final extension of 72°C for 10 min. Initial gradient PCR reactions indicated strong amplification with annealing temperatures between 45°C and 62°C for the templates tested (one green and one red alga). The PCR reactions totaled 50 μL in volume and consisted of 5.0 μL of 10× PCR buffer (Promega), 4.0 μL of 2.0 mM $MgCl_2$ (Promega), 3.0 μL of 1.0% BSA solution, 2.0 μL of each primer (0.4 mM), 2.0 μL (20 mM) of each dNTP, 0.2 μL of Promega Taq, 26.0 μL of nanopure water, and 1.0 μL of total genomic DNA. The PCR products were purified (using the Qiagen PCR Purification kit) and sequenced on an ABI 3730XL capillary (Applied Biosystems, Foster City, CA, USA) or ABI 377XL DNA sequencer. Chromatograms were examined, and consensus sequences assembled from forward and reverse reads using ContigExpress of the Vector NTI Suite (InforMax, Frederick, MD, USA) or Sequencher (GeneCodes Corp., Ann Arbor, MI, USA). Sequences were aligned using the Windows version of Clustal X (Thompson et al. 1997). The software package MEGA2 (Kumar et al. 2001) was used to calculate a neighbor-joining distance tree based on K2P comparisons. Sequences have been submitted to GenBank and can be retrieved with accession numbers EF426566–EF426672. The final consensus sequences contained very few insertions and deletions and varied in length from 407 to 414 nt, including primer sequences. Alignment was greatly simplified by the low number of indels, and a final alignment of 377 nt was constructed from the 107 sequences (excluding primers).

The breadth of cyanobacterial and eukaryotic algal diversity analyzed for this study is presented in a neighbor-joining tree of sequences (Fig. 1). With the exception of the single culture of *Symbiodinium* sp., the universal primer pair was successfully used for amplification and sequencing of red, green, and brown algae; diatoms; euglenoids; xanthophytes; and cyanobacteria. With a few exceptions, sequences of closely related species are distinct.

The remarkable universality of the plastid 23S rRNA primers has both advantages and disadvantages. Not surprisingly, contaminant algal sequences are occasionally recovered with the primer pair, and this issue becomes more serious when multispecies collections are used (e.g., turf algal communities) rather than clean macroalgal thalli or cultured isolates. This same universality, however, may be exploited in the development of protocols for quantitatively assaying environmental samples.

Numerous applications exist for algal universal primers, and more are certain to be developed as molecular assessment of algal diversity plays an increasing role in control and detection of invasive species, species identifications for environmental monitoring purposes, testing of commercial algal products, and characterization of herbarium collections. The results presented here demonstrate the amplification in, and provide sequences for, representatives from six eukaryotic algal lineages as well as the cyanobacteria and establish a preliminary framework for the development of such protocols using the 23S rDNA plastid marker.

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Supplementary Material

The following supplemental material is available for this article**:**

Table S1. List of taxa, collection information, and associated voucher information for specimens included in analysis of the 23S rDNA plastid marker.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1529-8817.2007.00341.x (This link will take you to the article abstract).

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