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Newly discovered deep-branching marine plastid lineages are numerically rare but globally distributed

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Ocean surface warming is resulting in an expansion of stratified, low-nutrient environments, a process referred to as ocean desertification [1]. A challenge for assessing the impact of these changes is the lack of robust baseline information on the biological communities that carry out marine photosynthesis. Phytoplankton perform half of global biological CO_2 uptake, fuel marine food chains, and include diverse eukaryotic algae that have photosynthetic organelles (plastids) acquired through multiple evolutionary events [1–3]. While amassing data from ocean ecosystems for the Baselines Initiative (6,177 near full-length 16S rRNA gene sequences and 9.4 million high-quality 16S V1-V2 amplicons) we identified two deep-branching plastid lineages based on 16S rRNA gene data. The two lineages have global distributions, but do not correspond to known phytoplankton. How the newly discovered phytoplankton lineages contribute to food chains and vertical carbon export to the deep sea remains unknown, but their prevalence in expanding, low nutrient surface waters suggests they will have a role in future oceans.

Phylogenetic relationships between the two deep-branching plastid lineages and other phytoplankton were established using an alignment of near full-length 16S rRNA genes that incorporated new plastid sequences from Baselines (see Supplemental Information, published with this article online). These were generated from three North Pacific sites to augment the SILVA database. The general relationships

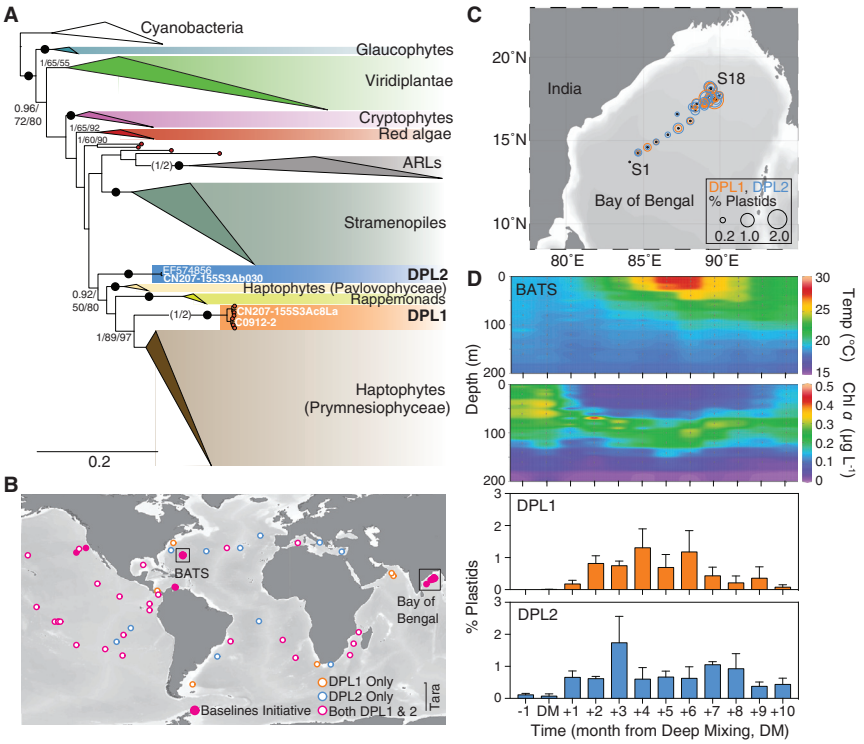

Figure 1. Phylogeny and distribution of the novel deep-branching plastid lineages (DPLs). (A) Reconstruction of plastid 16S rRNA gene sequences illustrating the positions of DPL1 (orange) and DPL2 (blue). Apicomplexan-Related-Lineages (ARLs) and DPL1 branch-lengths are reduced by 50% and the tree is rooted for display purposes; cyanobacteria are the outgroup. Posterior probability/RAxML/PhyML bootstrap percentages are shown when >0.9/50/50 or fully supported in all analyses (black dots). (B) DPL detection in Baselines 16S rRNA V1-V2 amplicon data from non-size fractionated surface samples (closed circles) in the North Pacific (6 samples), Indian Ocean (29), near Curaçao (14) and Sargasso Sea (85 BATS samples), and in Tara Oceans photic-zone 16S miTAGs (<200 m depth; open circles). The 77 Tara 0.2 to <3 µm size-fraction photic-zone samples with DPL1 (n = 32, 22.2 ± 5.5°C) and/or DPL2 (n = 50, 22.1 ± 3.8°C) were warmer than those where not detected (17.6 ± 8.4°C, $p < 0.05$). (C) DPL1 (orange) and DPL2 (blue) percent contributions to Indian Ocean 16S plastid amplicons. (D) Temperature and phytoplankton-derived chlorophyll *a* concentrations (top two plots) show BATS seasonal changes. The X-axis reflects a seasonal composite of 12 years of monthly data [8] adjusted to timing of deep mixing (DM) and months post or prior to DM (numbers). Mean percent DPL1 or DPL2 (± standard error) in plastid-derived 16S amplicons (bottom plots) highlights dynamics in BATS surface samples (0.3 to 7.6 m). Samples from winter (≤21°C; typically –1, DM and +1) had lower relative abundances than during stratification ($p < 0.01$).

illustrated were consistent with prior studies [3–5]. Glaucophytes, Viridiplantae and Red Algae, which arose from the primary endosymbiosis event, emerged in the basal region of the tree while important marine taxa that arose from secondary (or higher level) events, such as haptophytes and stramenopiles [3], formed distinct clades elsewhere (Figure 1A). The novel plastid lineage referred to here as deep-branching plastid lineage 1 (DPL1) was discovered in samples from the edge of the North Pacific Gyre (NPG) and coastal California. Complete DPL1 16S rRNA gene sequences acquired by two PCR approaches had best blastn hits of ≤85% nucleotide identity to unascribed environmental clones in GenBank (Supplemental Information). In phylogenetic reconstructions, DPL1 branched adjacent to haptophytes (Prymnesiophyceae), albeit with long branch-lengths (Figure 1A). Testing of phylogenies using varied taxonomic sampling established that DPL1 sequences do not come from dinoflagellates (at least not those with available molecular data). When fast-evolving dinoflagellate plastid sequences with known haptophyte origins [6] were included, DPL1 branched in unsupported or weakly supported positions adjacent to the Prymnesiophyceae and sister to the

dinoflagellate plastids, or within the Prymnesiophyceae (Figure S1A,B). Diversity of DPL1 (DPL1-clusters C, D, and E) and phylogenetically related novel amplicons (DPL1-related clusters A, B, F–J) was manifested in the 16S V2 variable region (Figure S1C–S1D). DPL1 was present in a Baselines ≥3 μm size-fractionated sample and nearly all non-fractionated samples, but not picoplankton (<3 μm) samples. Thus, we postulate DPL1 sequences originate from a diverse eukaryotic protistan group, with cells ≥3 μm diameter, that bears tertiary plastids of haptophyte origin.

Another plastid lineage unrelated to known phytoplankton, 'DPL2', was recovered from the picoplankton size fraction in oligotrophic NPG waters. They exhibited 99.6% nucleotide identity to a clone from surface waters near Costa Rica (GOS site 25), a Pacific Ocean site with higher temperature (28.3°C) and lower salinity (31.4 ppt) than our NPG site (19.0°C, 33.2 ppt). DPL2 sequences branched in a sister position to haptophytes, rappemonads and DPL1 with moderate to strong bootstrap support, suggesting they are derived from secondary plastids (Figure 1A). Analyses of molecular data alongside fossil records indicate haptophytes arose 1,031–637 million years ago and that the two haptophyte classes diverged 823–328 million years ago [7]. This context indicates an ancient origin of DPL2.

Global distributions of DPL1 and DPL2 were mapped using Baselines 16S amplicon datasets from the North Pacific, Caribbean (near Curaçao), Sargasso Sea (at the Bermuda Atlantic Time-series Study, BATS) and northern Indian Ocean (Bay of Bengal), as well as 16S rRNA gene fragments from Tara Oceans (Table S1). Both novel lineages were broadly distributed but not present in colder Tara samples, and DPL2 was found at more locations (Figure 1B). In Baselines, DPL2 was also in mangrove and saline pond samples from Curaçao, while DPL1 was not. Wherever detected, the newly identified lineages comprised a small percentage of plastid amplicons, 0.93 ± 1.36% (DPL1) and 0.65 ± 0.63% (DPL2), demonstrating these putatively photosynthetic taxa are globally distributed but often numerically rare.

For insight into DPL ecology, we studied warm water environments, where the surface is thermally stratified (BATS) and fresh-water stratified (Bay of Bengal, Indian Ocean). While detected in most Indian Ocean samples, DPL1 (0.39 ± 0.43%, 24 stations) and DPL2 (0.38 ± 0.25%, 28 stations) contributions to plastid-derived amplicons were low (Figure 1C), and trends with environmental parameters unclear. However, at BATS the new lineages exhibited their highest individual sample contributions (10% DPL1; 4% DPL2) to plastid counts and pronounced seasonality. DPL1 and DPL2 contributed higher plastid percentages during stratification than during the winter period (Figure 1D) when deep-mixing (>200 m) brings nutrients from depth into the photic zone and induces phytoplankton blooms [8]. This trend persisted for DPL1 groups when computed against total amplicons (including bacteria), akin to patterns for the oligotrophic cyanobacterium *Prochlorococcus* [9]. When *Prochlorococcus* contributed >10% of total 16S amplicons, temperature (23.92 ± 2.68°C) was not statistically different from samples with highest DPL1/DPL1-related contributions (Figure S1E–S1F). However, DPL1/DPL1-related contributions were lower ($p < 0.001$) when eukaryotic phytoplankton as a whole contributed >10% of total amplicons, during which it was also cooler (20.38 ± 0.89°C, $p < 0.001$) and less stratified. This contrast suggests that among eukaryotes at BATS [8] DPL1/DPL1-related taxa may be relatively effective competitors for macronutrients.

Recent studies highlight previously unrecognized sequence diversity within known marine eukaryote classes. Our discoveries are exceptional in identifying new, deep-branching phytoplankton lineages. Only two other such deep-branching uncultured plastid lineages have been discovered in the last decade: Rappemonads [5] and the coral-associated Apicomplexan-Related-Lineages [4]. The distributions of the lineages we discovered suggest they tolerate a broad range of conditions, but may increase numerically as ocean warming progresses, akin to predictions for *Prochlorococcus* [10]. The discovery of the DPL algal groups in contemporary samples illustrates the importance of time-series sampling for acquiring essential data points for comparison as ocean ecology adjusts to changing climate.

SUPPLEMENTAL INFORMATION

Supplemental Information contains experimental procedures, one figure and one table can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.11.032.

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