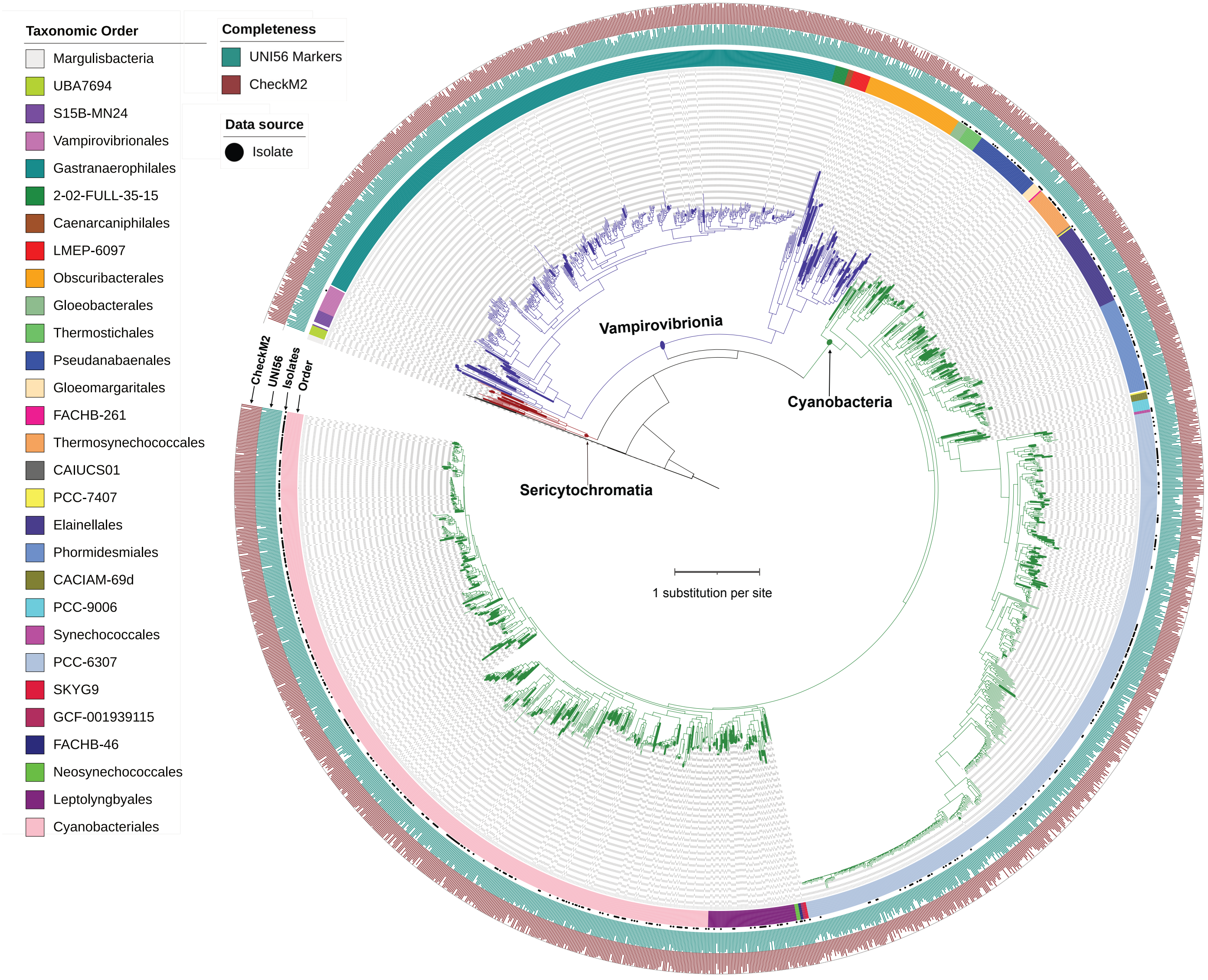

Figure S1. The phylogenetic relationships of 1,973 Cyanobacteriota genomes/MAGs were estimated using IQ-TREE, with Margulisbacteria as outgroup to root the tree. Each major clade is separated by color-coded branches: Sericytochromatia (dark red), Vampirovibrionia (dark blue), and Cyanobacteria (green). MAGs identified in this study (labeled as NeLLi2023 in Supplemental Table S2) are highlighted with thicker branches. Bootstrap support values are not shown. The diagram includes four rings: ring 1 indicates the GTDB taxonomic order for each cyanobacterial lineage; ring 2 uses filled circles to indicate isolates; ring 3 provides a bar plot showing UNI56 markers count; ring 4 provides a bar plot displaying CheckM2 completeness.

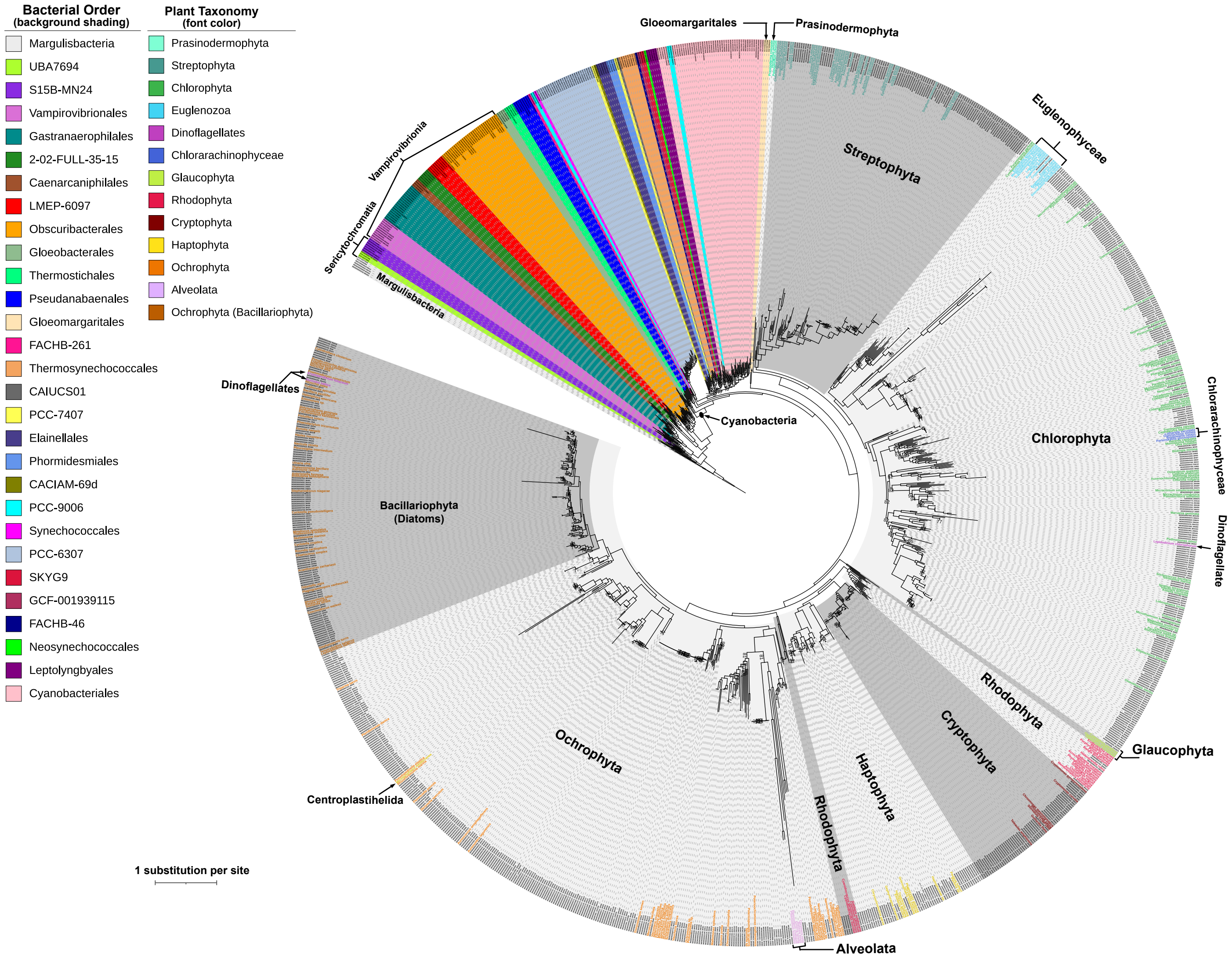

Figure S2. The phylogenetic relationships between cyanobacterial species and plastids from photosynthetic eukaryotes. Cyanobacterial species are represented by dereplicated 259 genomes/MAGs, while plastids are represented by 241 reference plastomes from major photosynthetic eukaryotic groups, and 647 ptMAG contigs containing 16S rRNA genes with at least 10% UNI56 markers. The tree was generated using a concatenated alignment of PLASTID54 markers under the LG+F + I + G4 substitution model with IQ-TREE. Cyanobacterial orders are highlighted with background color, with corresponding color codes for the clade provided in the color key (Bacterial Order). Gloeomargaritales, which is positioned as a sister to all plastids (reference plastomes and ptMAGs), is highlighted with an arrow and labeled. Labels for reference plastomes from diverse photosynthetic eukaryotes are color-coded and indicated in the color key (Plastid Taxonomy). Plastid metagenomes are indicated by labels with black text. Plastids from the major photosynthetic groups are separated with shades of gray and labeled by corresponding species name. The Alveolata clade includes *Chromera* species and four plastomes from Apicomplexa species but excludes dinoflagellates. Dinoflagellate members are separated from Alveolata to indicate their distinct phylogenetic positions.

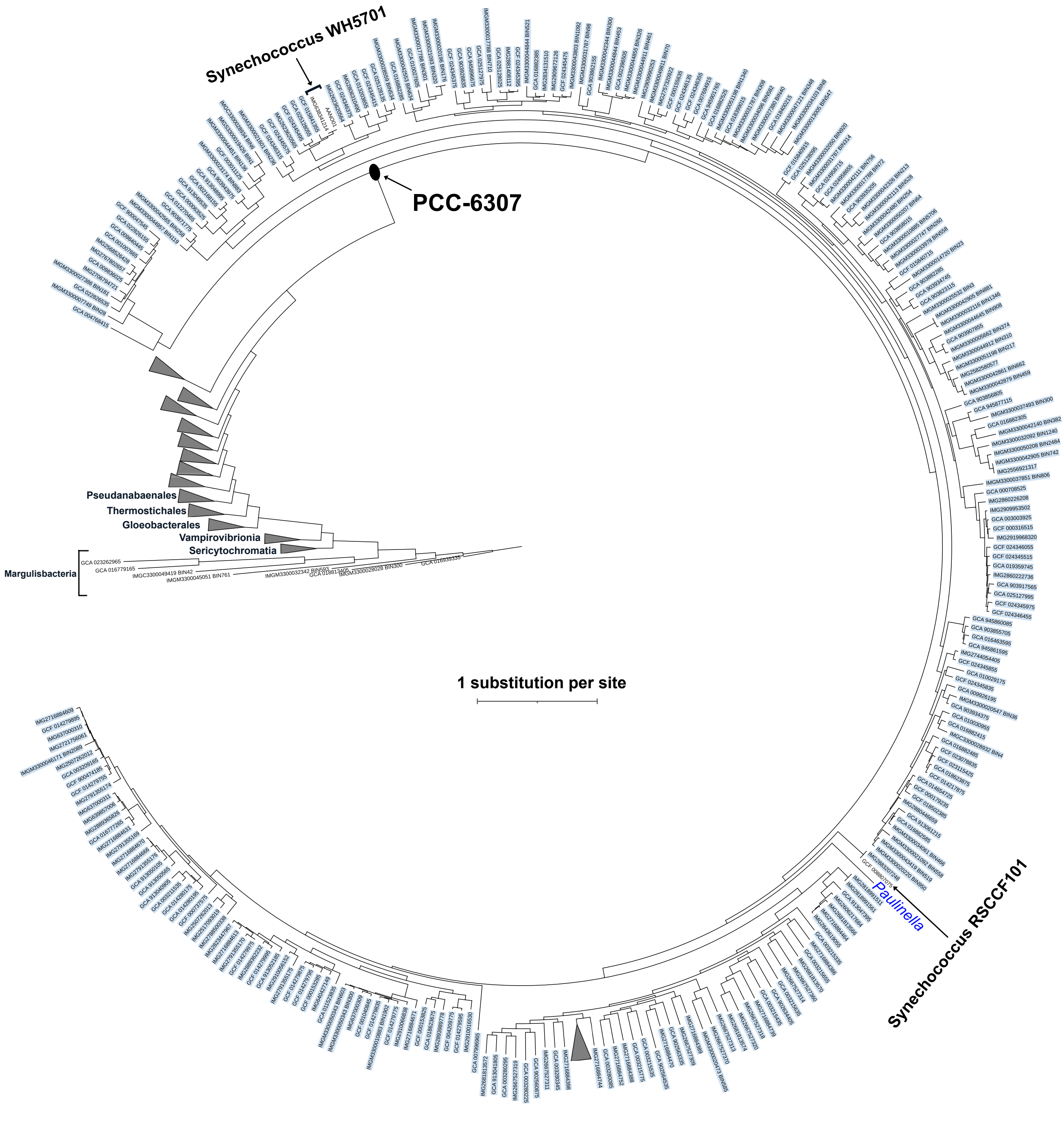

Figure S3. Phylogenetic relationship of the *Paulinella chromatophore* plastid compared to cyanobacterial members of the taxonomic order PCC-6307. The tree highlights the relationships of *Synechococcus* sp. WH5701 and *Synechococcus* sp. RSCCF101 in comparison to the *P. chromatophore* plastid*. Synechococcus* RSCCF101 branches off before the diversification of *P. chromatophore* plastid and is phylogenetically closer to *P. chromatophore*. Only members within the taxonomic order PCC-6307 are shown, while other orders within Cyanobacteriota are collapsed. For a complete overview of the relationship among all orders within Cyanobacteriota, please refer to Fig. 1 and Supplementary Fig. S1.

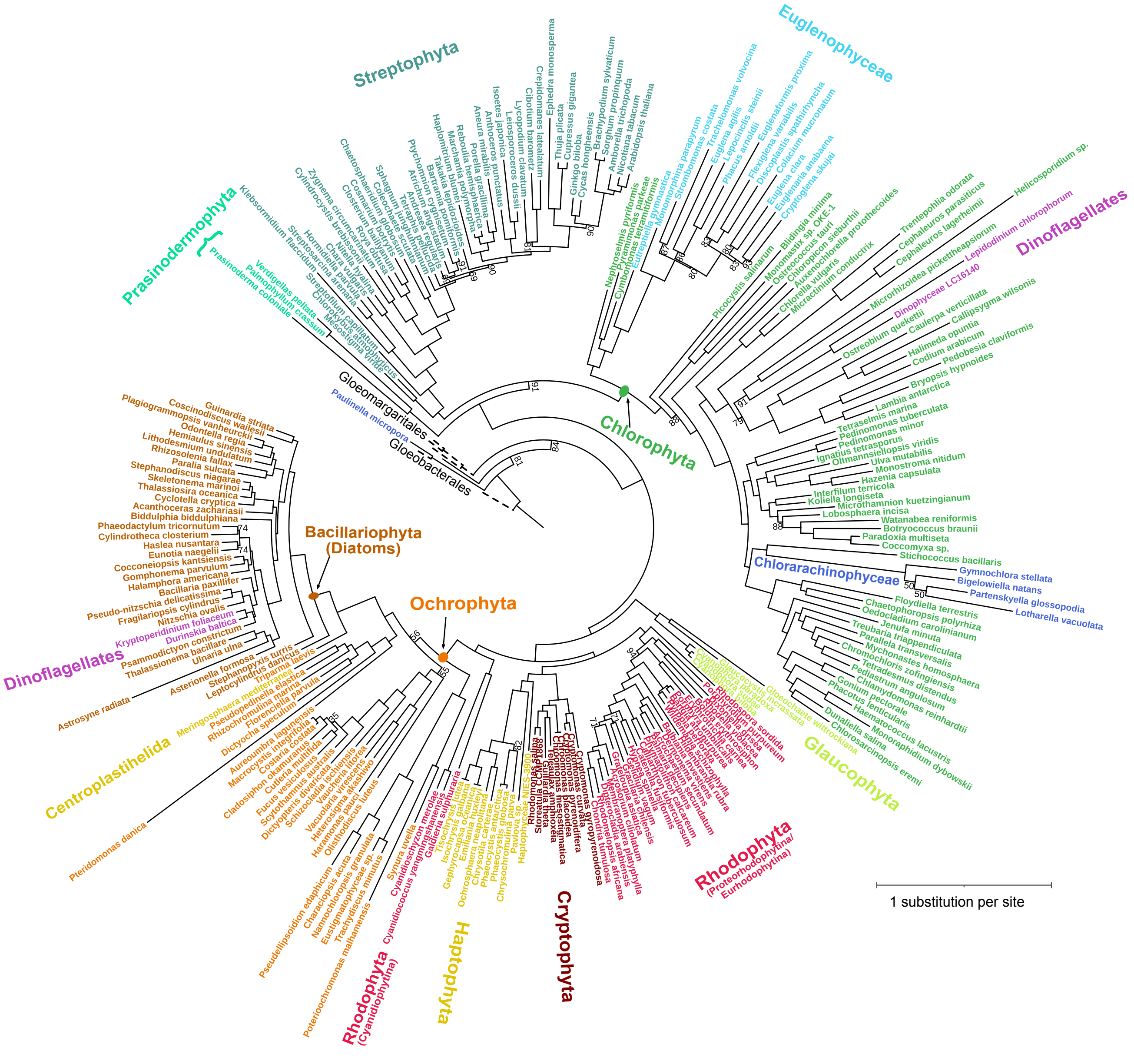

Figure S4. Phylogenetic relationships among different photosynthetic eukaryote plastids estimated with concatenated PLASTID54 genes using IQ-TREE. The dataset includes 236 RefSeq plastomes, *Paulinella chromatophore* plastid, and six cyanobacterial genomes. Ultrafast bootstrap values are displayed only for branches with support below 95%. Leaf labels are colored according to the color key.

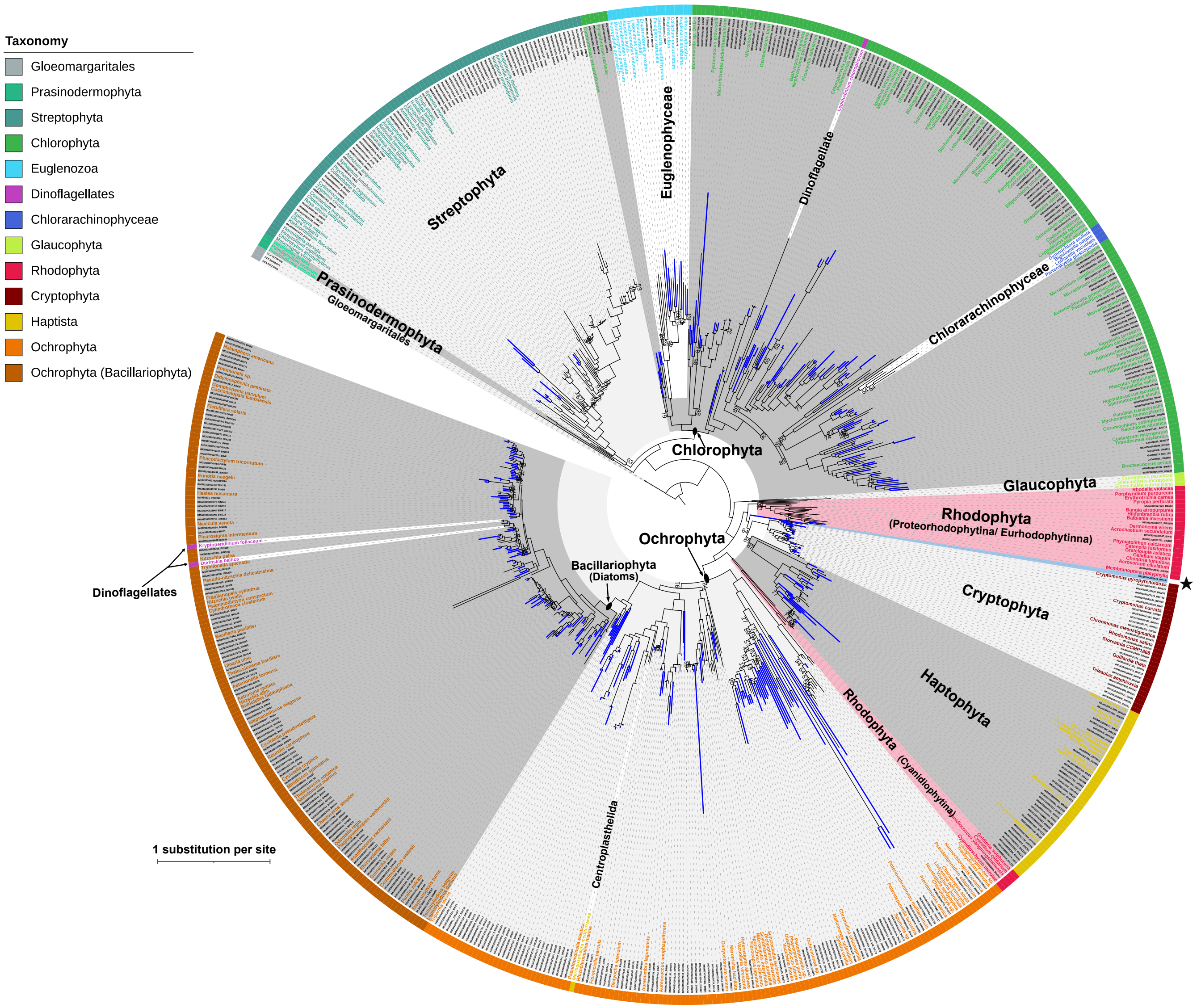

Figure S5. Plastid relationship among different photosynthetic eukaryotes. The maximum-likelihood tree was inferred using a concatenated alignment of PLASTID54 markers under the LG+F+I+G4 substitution model, following the nsgtree pipeline and IQ-TREE. Labels for the 236 reference plastids are color-coded as described in the color key, and labeled clades are delineated from one another with different shades of gray. Complex plastids within Chlorophyta (Euglenophyceae, Chlorarachinophyceae, and *Lepidodinum* spp.), and in dinoflagellates and Centroplasthelida are excluded from the shades. Rhodophyta clades are shaded in red to highlight two independent origins of secondary red algal plastids. A single plastid metagenome that branched off before the diversification of Haptophyta and Cryptophyta is highlighted in a blue shade and indicated by a black star. Novel ptMAGs are highlighted with bright blue branches to distinguish them from redundant ptMAGs derived in this study. Ultrafast bootstrap values below 95% are shown under the branches. Reference plastids from the phylum Bacillariophyta are shown in dark orange to distinguish from the rest of other ochrophytes (orange).

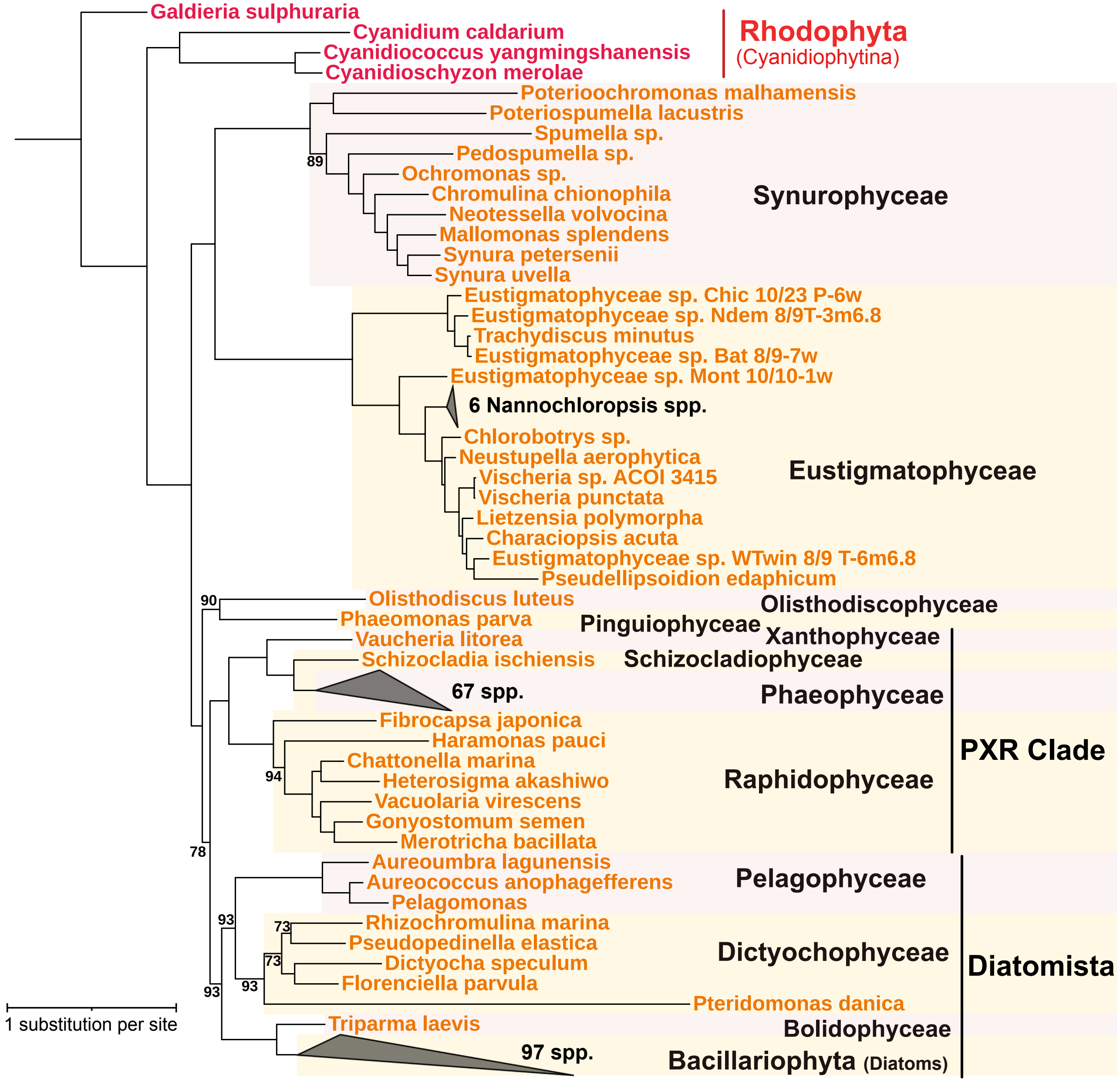

Figure S6. Phylogenetic relationships among reference Ochrophyta plastids. A maximum-likelihood tree was constructed with a concatenated alignment of 54 plastid proteins, applying the LG+F+I+G4 substitution model. The analysis included a total of 213 Ochrophyta reference plastids, with four Rhodophyta plastids serving as outgroup. Only ultrafast bootstrap support values less than 95% are displayed, either above or below the corresponding branches. Clades containing a large number of taxa were collapsed and labeled accordingly. Major groups within Ochrophyta were distinguished with alternating pink or yellow shading and labeled accordingly.

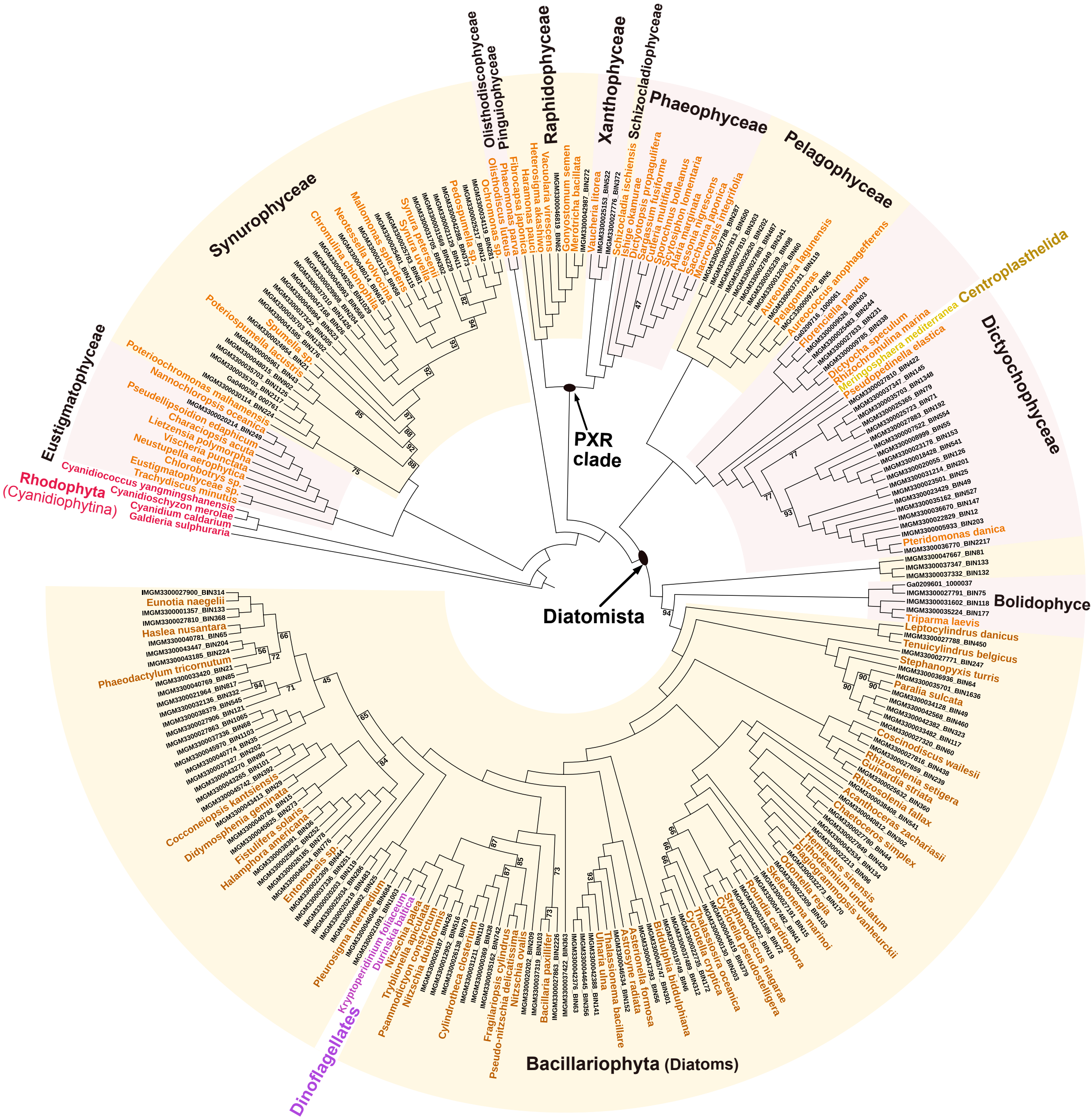

Figure S7. A cladogram depicting the phylogenetic relationships of secondary red algal plastids in Ochrophyta and other photosynthetic eukaryotes. The relationships were estimated using a minimum of 20% PLASTID54 markers using IQ-TREE and the LG+F+I+G4 substitution model. Red algal species (subphylum Cyanidiophytina) was used to root the tree. Only the branches with ultrafast bootstrap support values less than 95% are shown. Complete plastomes available in the NCBI RefSeq/GenBank are labeled with species name, while the ptMAGs are shown in black font. Major classes within Ochrophyta are distinguished with alternating pink or yellow shading and labeled. Plastids from Centroplasthelida and dinoflagellates are excluded from the shaded area. Reference ochrophyte species from the phylum Bacillariophyta are highlighted in darker orange compared to species from other ochrophytes.