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Transfer of the marine red alga *Erythrocystis saccata* (Rhodomelaceae, Rhodophyta) to the tribe Streblocladieae inferred from organellar genome analysis

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The Rhodomelaceae is the largest and most taxonomically complex red algal family, containing 21 tribes, 159 genera and 1,095 species (Guiry & Guiry 2021). The family has recently gone through significant taxonomic revision based on genetic marker and plastid genome sequence analysis (Díaz-Tapia *et al.* 2017, Savoie & Saunders 2019, Bustamante *et al.* 2021). Although extensive, not all taxa in the family have been sequenced to date, this includes the genus *Erythrocystis* J.Agardh (1876: 638) which has yet to be resolved. *Erythrocystis* forms distinctive globular to obpyriform, fluid-filled vesicles and has a large, cylindrical and unicellular rhizoid that penetrates deep into the tissue of various Rhodomelaceae (Kylin 1928, Abbott & Hollenberg 1976). Currently there are two accepted species in the genus, *E. saccata* (J.Agardh) P.C.Silva (1952: 308) distributed in the northeastern Pacific Ocean, and *E. montagnei* (Derbès & Solier) P.C.Silva (1952: 308) from the Atlantic Islands and the Mediterranean Sea (Abbott & Hollenberg 1976, Guiry & Guiry 2021). *Erythrocystis* has been considered parasitic, hemiparasitic, symbiotic, or epiphytic (Kylin 1928, Kugrens & West 1974, Melchionna & De Masi 1977, Preuss *et al.* 2017). Based on observations of the vegetative and reproductive development, Kylin (1928) assigned *Erythrocystis* (as *Ricardia*) to the tribe Laurencieae, stating that it joined the tribe "*sehr gut an*" (very good). This taxonomic conclusion was accepted by Hommersand (1963). Here we performed whole genome sequencing on a specimen of *E. saccata* from Stillwater Cove, Pebble Beach, California (36°33'56.5"N 121°56'39.8"W) to determine its mitochondrial and plastid genome structure and content, as well as infer its phylogenetic relationship at the tribe level.

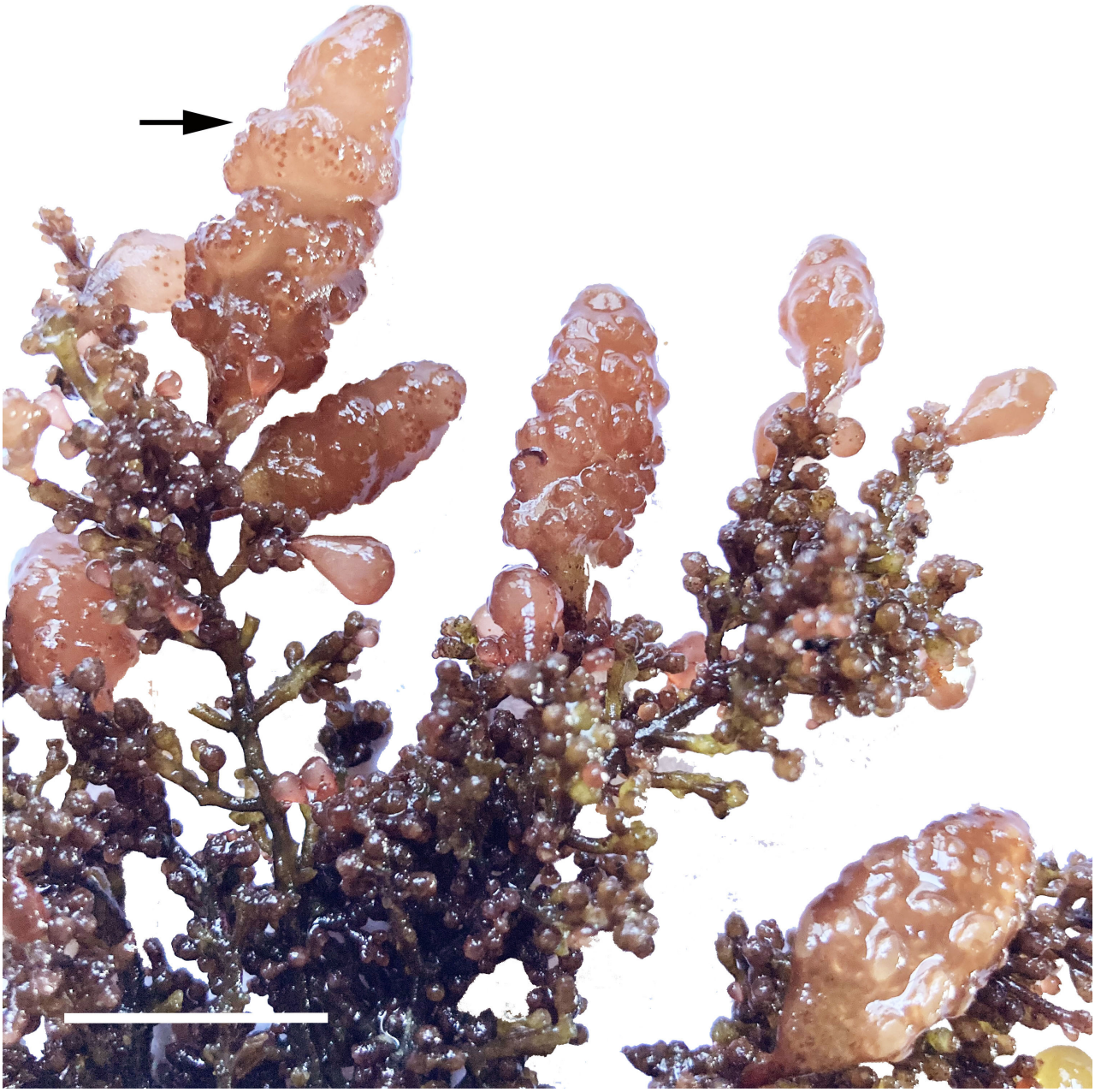

FIGURE 1. *Erythrocystis saccata* growing epiphytically on *Laurencia pacifica* from Stillwater Cove, Pebble Beach, California, USA (UC 2085026). The arrow indicates the specimen analyzed in this study. White bar = 2 cm.

The specimen of *E. saccata* analyzed in this study was deposited in the University Herbarium (UC) under voucher number UC 2085026 (Fig. 1). The DNA was extracted following the modified protocol of Hughey *et al.* (2019). The 150 bp PE Illumina library construction and sequencing was performed by myGenomics, LLC (Alpharetta, Georgia, USA) and yielded 24,502,226 reads (Sequence Read Archive accession SRS8622742). The data were trimmed using the Trim Adapters and Trim Low Quality default settings and saving reads ≥ 75 bp using Geneious Prime version 2019.1.3 (Biomatters Limited, Auckland, New Zealand) with the BBDuk plugin. The reads were assembled *de novo* using the forward read single-end data only (12,251,113 reads) with kmers 71–141 in MEGAHIT (Li *et al.* 2015). The resulting contig file can be accessed via Figshare (https://doi.org/10.6084/m9.figshare.14639412.v1). The plastid and mitogenome contigs, their position and orientation, were identified using the Map to Reference function with default settings (Medium-Low Sensitivity / Fast, iterate up to 5 times) in Geneious Prime with *Osmundea sinicola* serving as the reference sequence (GenBank accessions MH898940, MH898941). Contigs were joined where they overlapped in sequence, and genome accuracy and circularity were confirmed by mapping reads onto the contig/s using the same Map to Reference tool in Geneious Prime. The mitogenome was annotated using MFannot (Beck & Lang 2010) and the plastid genome using Geneious Prime, NCBI ORFfinder, and tRNAscan-SE 1.21 (Schattner *et al.* 2005). The *E. saccata* plastid genome was aligned to other genomes online (https://mafft.cbrc.jp/alignment/server/) using the "auto" strategy with MAFFT (Katoh & Standley 2013, Katoh *et al.* 2019). The RAxML phylogenetic analysis was executed at Trex-online (http://www.trex.uqam.ca/index.php?action=raxml&project=trex) using the GTR + gamma substitution model and 1,000 rapid bootstrap replicates (Boc & Makarenkov 2012). The tree was visualized with TreeDyn 198.3 at Phylogeny.fr (Dereeper *et al.* 2008).

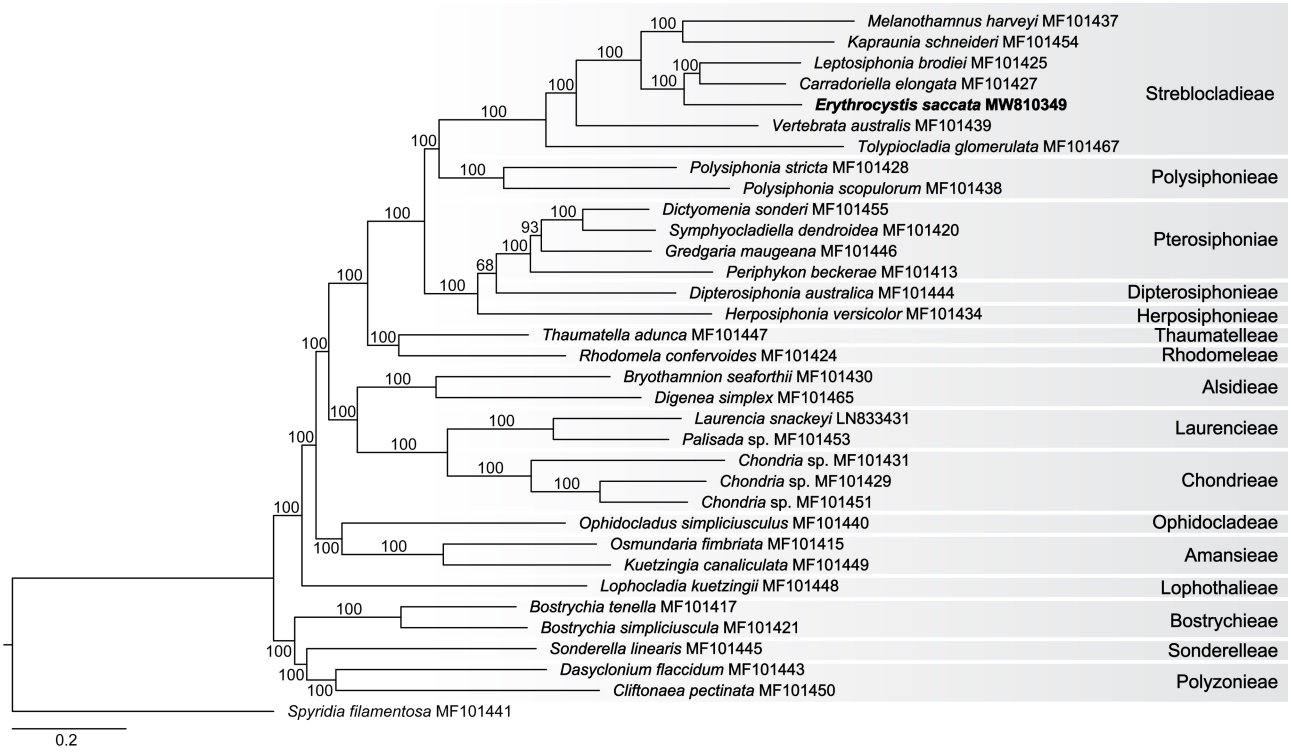

FIGURE 2. RAxML phylogram of the complete plastid genome of *Erythrocystis saccata* (GenBank Accession MW810349) and representative Rhodomelaceae. Numbers along the branches are rapid bootstrap support values based on 1,000 replicates. The legend below represents the scale for nucleotide substitutions.

The mitogenome of *E. saccata* was assembled from 6 overlapping contigs. It is a single circular chromosome, 24,975 bp in length, is A + T rich (76.8%), and contains 45 genes (GenBank accession MW810348) including 20 tRNA, 4 ribosomal proteins, 4 ATP synthase, 2 rRNA, and 15 other genes involved in electron transport and oxidative phosphorylation. The 23 protein coding genes (PCGs) initiate with an ATG codon and all but two genes terminate with TAA (*rps3* and *tatC* with TAG). Twenty-four of the 45 genes are transcribed on the forward strand and the remaining 21 code on the reverse strand. Analysis of the mitogenome shows that the rRNAs and PCGs are similar in chromosomal organization between *E. saccata* and *Vertebrata lanosa* (L.) T.A.Christensen (Salomaki & Lane 2017), however these two mitogenomes differ in the number and type of tRNAs. The plastid genome of *E. saccata* was retrieved from a single contig with overlapping ends. It is a single circular chromosome, 165,292 bp in length, is A + T biased (72.1%), and contains 224 genes (GenBank accession MW810349) including 46 ribosomal proteins, 27 tRNA, 30 photosystem I and II, 23 ycf, 10 phycobiliprotein, 10 cytochrome

b/f complex, 8 ATP synthase, 6 orfs, 5 RNA polymerase, 3 rRNA, and 56 other genes. Nearly all of the PCGs initiate with ATG, with the codon exceptions being *orf88* with GTG and *infC* with TTG. One hundred forty-two of the PCGs terminate with TAA, 36 with TAG, and 16 with TGA. One hundred twenty-one of the 224 genes are transcribed on the forward strand and the remaining 103 encode on the reverse strand. The plastid genome is highly conserved in length, content, and organization to other genera currently assigned to the tribe Streblocladieae (Salomaki *et al.* 2015, Díaz-Tapia *et al.* 2017). The only observed difference is the absence of the gene *pgm*A (phosphoglycerate mutase) in *E. saccata*, which is also absent in some of the plastid genomes of Streblocladieae.

Phylogenomic analysis of the plastid genome data revealed that *E. saccata* was distantly related to the tribe Laurencieae, but was fully resolved with genera in the tribe Streblocladieae (Fig. 2). Within the Streblocladieae, *E. saccata* was sister to *Carradoriella elongata* (Hudson) Savoie & G.W.Saunders and *Leptosiphonia brodiei* (Dillwyn) Savoie & G.W.Saunders. We here propose the transfer of *E. saccata* to the Streblocladieae where it joins twelve other genera currently recognized in the tribe. The placement of *E. saccata* in the Streblocladieae confirms the value of the anatomy of rhizoids as a diagnostic feature (Bustamante *et al.* 2019). Rhizoids in the Streblocladieae are unicellular and cut off from pericentral cells, except in *Eutrichosiphonia* (Bustamante *et al.* 2021). *Erythrocystis saccata* differs from other Streblocladieae in having a combination of characters including extensive cortication, a single unicellular rhizoidal cell, and its obpyriform to ellipsoidal sac-like morphology. Further phylogenomic analyses on the second *Erythrocystis* species, *E. montagnei*, will help to determine how these two species are related as well as better understand their disjunct distribution between the eastern Atlantic and the northeastern Pacific Ocean.

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