

8 | Evolution | Announcement

Complete chloroplast genome of the marine red alga *Rhodochorton tenue* (Rhodochortonaceae, Rhodophyta) from San Juan Island, Washington

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ABSTRACT We present the complete chloroplast genome sequence of *Rhodochorton tenue* from San Juan Island, Washington. The chloroplast genome of *R. tenue* is 192,037 bp in length, contains 244 genes, and is similar in content to *Acrochaetium secundatum*. *Rhodochorton tenue* is genetically distinct from *Rhodochorton purpureum* from the North Atlantic Ocean.

KEYWORDS acrochaetiales, chloroplast genome, plastid, *rbcL*, *Rhodochorton purpureum*

R hodochorton tenue Kylin was originally described as a short plant 3–5 mm high with more or less branched filaments and a creeping basal layer (1). The type locality of R. tenue was cited as San Juan Island, Friday Harbor Marine Laboratories Preserve, where it was said to densely cover rocks and stones at the high-water mark (1). Later workers accepted R. tenue (2, 3); however, based on similarities in morphology and life histories in culture, West (4) proposed that R. tenue be placed into synonymy under the generitype of *Rhodochorton* Nägeli, *Rhodochorton purpureum* (Lightfoot) Rosenvinge. The only two DNA marker sequences of R. tenue deposited in GenBank are from slowly evolving (SSU and LSU) genes, and the authors did not comment on the status of the name (5). To determine the systematic relationship between R. tenue and R. purpureum, the complete chloroplast genome of R. tenue was assembled and analyzed.

The specimen of *R. tenue* analyzed here was collected on rocks in the supralittoral from the Friday Harbor Marine Laboratories Preserve, San Juan Island, Washington, voucher number UC 2100479. The DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following a previously published protocol (6). The 150-bp paired-end library was constructed with the KAPA HyperPlus Kit (Roche) and sequenced on an Illumina NovaSeq 6000. The analysis generated 21,656,624 reads that were filtered using the default BBDuk settings in Geneious Prime 2019.1.3 (Biomatters Limited). The chloroplast genome was assembled *de novo* using the filtered reads with a kmer ≥ 69 in MEGAHIT 1.2.9 (7). The assembly yielded 63,951 contigs with an N_{50} of 518 and GC content of 54.6%. Two *R. tenue* chloroplast contigs, 156,855 and 26,268 bp, with 267× and 275× coverage, respectively, were identified by a two-way Nucleotide BLAST search using the default settings. Nuclear and mitochondrial coverages were $659\times$ and $216\times$, respectively. The final complete chloroplast genome was circularized by iteration over

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TABLE 1 Plastid genome content of Rhodochorton tenue

Gene groups	Genes		
ATP synthase	atpA, atpB, atpD, atpE, atpF, atpG, atpH, atpl		
Cytochrome complex	ccs1, ccsA, petA, petB, petD, petF, petG, petJ, petL, petM, petN		
Hypothetical chloroplast orfs	ycf19, ycf20, ycf21, ycf22, ycf23, ycf33, ycf34, ycf35, ycf36, ycf37,		
	ycf39, ycf41, ycf45, ycf46, ycf52, ycf53, ycf54, ycf55, ycf60, ycf65		
Maintenance	dnaB, rne, rnz		
Metabolism	accA, accB, accD, acpP, argB, carA, cbbX, chIB, chII, chIL, chIN, dfr,		
	fabH, gltB, ilvB, ilvH, moeB, odpA, odpB, pgmA, preA, rbcL, rbcS,		
	syfB, syh, thiG, thiS, trpA, trpG, upp		
Open reading frames	rf55, orf118, orf166, orf257, orf396, orf456, orf493, orf770		
Photosystem I	psaA, psaB, psaC, psaD, psaE, psaF, psaI, psaJ, psaK, psaL, psaM,		
	ycf3, ycf4		
Photosystem II	psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL,		
	psbN, psbT, psbV, psbW, psbX, psbY, psbZ, psb30		
Phycobiliproteins	apcA, apcB, apcD, apcE, apcF, cpcA, cpcB, cpcG, cpcS, cpeA, cpeB,		
	nblA		
Protein quality control	dnaK, clpC, ftsH, groEL		
Redox system	acsF, bas1, dsbD, ftrB, grx, pbsA, trxA		
Ribonuclease	rnpB		
Ribosomal proteins (large subunit)	rpl1, rpl2, rpl3, rpl4, rpl5, rpl6, rpl9, rpl11, rpl12, rpl13, rpl14, rpl16,		
	rpl18, rpl19, rpl20, rpl21, rpl22, rpl23, rpl24, rpl27, rpl28, rpl29,		
	rpl31, rpl32, rpl33, rpl34, rpl35, rpl36		
Ribosomal proteins (small subunit)	rps1, rps2, rps3, rps4, rps5, rps6, rps7, rps8, rps9, rps10, rps11,		
	rps12, rps13, rps14, rps16, rps17, rps18, rps19, rps20		
Ribosomal RNAs	rnl(x2), rrn5(x2), rns(x2)		
RNA polymerase	rpoA, rpoB, rpoC1, rpoC2, rpoZ		
Transcription factors	lysR, ntcA, ompR, ycf29		
Transfer RNAs	trnA-TGC(x2), trnC-GCA, trnD-GTC, trnE-TTC, trnF-GAA, trnG-GCC,		
	trnG-TCC, trnH-GTG, trnl-GAT(x2), trnK-TTT, trnL-CAA, trnL-TAA,		
	trnL-TAG, trnM-CAT(x2), trnN-GTT, trnP-TGG, trnQ-TTG, trnR-ACG,		
	trnR-CCG, trnR-TCT, trnS-GCT, trnS-GGA, trnS-TGA, trnT-GGT,		
	trnT-TGT, trnV-GAC, trnV-TAC, trnW-CCA, trnY-GTA		
Translation	infB, infC, tsf, tufA		
Transport	cemA, secA, secG, secY, sufB, sufC, tatC, ycf38, ycf63		
tRNA processing	tilS		

the two gaps using the filtered reads and Map to Reference function with the Low Sensitivity/Fastest setting in Geneious Prime. The genome start position was adjusted to correspond with the chloroplast genome of *Acrochaetium secundatum* (GenBank accession number MH026107). The annotation was performed using the default settings in GeSeq 2.03 (8), followed by manual corrections of gene start and stop positions according to NCBI ORFfinder and Sequin 15.5 (9). Nucleotide identities were calculated by BLAST search using the default settings.

The complete circular chloroplast genome of *R. tenue* is 192,037 bp in length and has a GC content of 33.5%. The genome contains 244 genes including 206 protein-coding, 32 tRNA, and 6 rRNA genes (Table 1). Genome content and structure are highly similar to those of *A. secundatum* (10). The plastid-encoded *rbcL* gene sequence of *R. tenue* differed in nucleotide identity from *R. purpureum* from the Isle of Man, Irish Sea, and Newfoundland and Labrador, Canada, by 1.98%–2.5%. These identities are similar to numbers reported for other florideophyte congeners and support the treatment of *R. tenue* as distinct from *R. purpureum*.

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DATA AVAILABILITY

The complete chloroplast genome sequence of *Rhodochorton tenue* is available in GenBank under accession number OR608737. The associated BioProject, SRA, and BioSample numbers are PRJNA1034540, SRS19378060, and SAMN38060163, respectively. The chloroplast genome referenced in the text was *Acrochaetium secundatum* GenBank accession number MH026107.

REFERENCES

- Kylin H. 1925. The marine red algae in the vicinity of the biological station at Friday harbor, wash. Lunds Universitets Årsskrift, Ny Följd, Andra Afdelningen 21:1–87.
- Drew KM. 1928. A revision of the genera Chantransia, Rhodochorton and Acrochaetium with descriptions of the marine species of Rhodochorton (Naeg.) gen. emend. on the Pacific coast of North America. Univ Calif Publ Bot 14:139–224.
- 3. Papenfuss GF. 1945. Review of the *Acrochaetium-rhodochorton* complex of the red algae. Univ Calif Publ Bot 18:229–334.
- West JA. 1969. The life histories of *Rhodochorton purpureum* and *R. tenue* in culture. J Phycol 5:12–21. https://doi.org/10.1111/j.1529-8817.1969. tb02569.x
- Harper JT, Saunders GW. 2002. A re-classification of the acrochaetiales based on molecular and morphological data, and establishment of the Colaconematales ord. nov. (Florideophyceae, Rhodophyta). Euro J Phycol 37:463–476. https://doi.org/10.1017/S0967026202003840
- Garcia AN, Ramos JH, Mendoza AG, Muhrram A, Vidauri JM, Hughey JR, Hartnell College Genomics Group. 2022. Complete chloroplast genome

of topotype material of the coast live oak *Quercus agrifolia* Née var. *agrifolia* (Fagaceae) from California. Microbiol Resour Announc 11:e0000422. https://doi.org/10.1128/mra.00004-22

- Li D, Liu CM, Luo R, Sadakane K, Lam TW. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31:1674–1676. https://doi.org/ 10.1093/bioinformatics/btv033
- Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq – versatile and accurate annotation of organelle genomes. Nucleic Acids Res 45:W6–W11. https://doi.org/10.1093/nar/ gkx391
- Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD, Sayers EW. 2018. GenBank. Nucleic Acids Res 46:D41–D47. https://doi. org/10.1093/nar/gkx1094
- Costa JF, Lin S-M, Macaya EC, Fernández-García C, Verbruggen H. 2016. Chloroplast genomes as a tool to resolve red algal phylogenies: a case study in the Nemaliales. BMC Evol Biol 16:205. https://doi.org/10.1186/ s12862-016-0772-3