

Supplementary Information: Dynamic evolution of mitochondrial genomes in
Trebouxiophyceae, including the first completely assembled mtDNA from a lichen-
symbiont microalga (*Trebouxia* sp. TR9)

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Class	Abbreviated name	Organism	Accessions
CHLOROPHYTA			
Prasinophyceae	BPR	<i>Bathycoccus prasinos</i>	NC023273
	MCO	<i>Micromonas</i> sp. RCC299	NC012643
	MON	<i>Monomastix</i> sp. OKE-1	NC022797
	OTA	<i>Ostreococcus tauri</i>	NC008290
Trebouxiophyceae	APR	<i>Auxenochlorella protothecoides</i>	NC026009
	BBR	<i>Botryococcus braunii</i>	NC027722
	CHE	<i>Chlorella heliozoae</i>	KY629615
	CSO	<i>Chlorella sorokiniana</i>	NC024626
	CSP	<i>Chlorella</i> sp. ArM0029B	KF554428
	CSU	<i>Coccomyxa</i> sp. C-169	NC015316
	CVA	<i>Chlorella variabilis</i>	NC025413
	HEL	<i>Helicosporidium</i> sp.	NC017841
	LIN	<i>Lobosphaera incisa</i>	NC027060
	MIC	<i>Micractinium conductrix</i>	KY629619
	PCR*	<i>Prasiola crispa</i>	KR017746-7
	PWI	<i>Prototheca wickerhamii</i>	NC001613
	PZO1	<i>Prototheca zopfii</i> SAG 2021	MF197534
	PZO2	<i>Prototheca zopfii</i> SAG 2063	MF197533
	TAG*	<i>Trebouxia aggregata</i>	EU123944-9
	TR9	<i>Trebouxia</i> sp. TR9	MH917293
	TRE	<i>Trebouxiophyceae</i> sp. MX-AZ01	NC018568
Ulvophyceae	GSA	<i>Gloeotilopsis sarcinoidea</i>	KX306822
	PAK	<i>Pseudendoclonium akinetum</i>	NC005926
	UPE	<i>Ulva pertusa</i>	NC035722
	UPR	<i>Ulva prolifera</i>	NC028538
Chlorophyceae	CMO	<i>Chlamydomonas moewusii</i>	NC001872
	CRE	<i>Chlamydomonas reinhardtii</i>	NC001638
	GPE	<i>Gonium pectorale</i>	NC020437
	PST	<i>Pleodorina starrii</i>	NC021108
	TOB	<i>Tetrademus obliquus</i>	NC002254
STREPTOPHYTA			
	CAT	<i>Chlorokybus atmophyticus</i>	NC009630
	CVU	<i>Chara vulgaris</i>	NC005255
	MVI	<i>Mesostigma viride</i>	NC_008240

Table S1. List of the chlorophytes and streptophytes whose mtDNAs were compared in this study. *, Incomplete sequences.

Organism	RPL10 motif	Annotation	Positions	Length (CDS / tRNA)	Intron length	Direction	Upstream gene	Downstream gene
rpl10 gene								
BPR	-	hypothetical protein CDS	21616 - 22164	549	NA	reverse	nad10	trnH (gtg)
OTA	-	ORF153 CDS	11841 - 12293	462	NA	reverse	nad10	trnH (gtg)
MCO	-	UA	113 - 622	510	NA	forward	nad10	trnH (gtg)
MON	+	orf202b	56108 - 56716	609	NA	reverse	nad10	trnY (gta)
APR	+	hypothetical protein	21089 - 21721	633	NA	forward	rps10	rps3
PWI	+	orf174 = ymf45 CDS	44191 - 44715	525	NA	forward	rps10	rps3
HEL	-	Orf160b CDS	19006 - 19488	483	NA	forward	rps10	rps3
PZO1	+	rpl10	37446 - 37967	522	NA	forward	rps10	rps3
PZO2	+	rpl10	36373 - 36894	522	NA	forward	rps10	rps3
CHE	-	UA	30566 - 31162	597	NA	forward	rps19	rns
CSO	-	orf188	5000 - 5563	564	NA	reverse	rps19	rns
CSP	+	orf180	44706 - 45248	543	NA	reverse	rps19	rns
MIC	+	UA	59296 - 59844	549	NA	forward	rps19	rns
CVA	-	UA	2404 - 3045	642	NA	reverse	rps19	rns
BBR	-	orf203	3996 - 4607	612	2598	forward	rps19	trnR (cgt) / rnl
CSU	+	UA	63474 - 64298	825	NA	forward	rps19	trnR (cgt) / rnl
TRE	-	orf012	73225 - 74091	867	NA	forward	rps19	rnl
TAG*	+	rpl10	15155 - 15685	531	NA	reverse	rps19	rpl5
TR9	+	rpl10	7587 - 8147	561	NA	forward	rps19	rpl5
LIN	+	orf27	60413 - 61084	672	NA	forward	rps19	trnE (ttc)
RCR*	-	UA	300 - 1136	837	NA	forward	?	nad3
tRNA genes								
BBR	NA	trnF (gaa) / UA	30597 - 32959	72	2291	forward	trnQ (ttg)	trnK (ttt)
	NA	trnH (gug) / UA	40824 - 44243	74	3346	forward	trnS (tga)	trnG (tcc)
	NA	trnW (cca) / UA	56454 - 58011	73	1485	forward	trnC (gca)	trnE (ttc)
TRE	NA	trnG (ucc) / UA	67060 - 68774	71	1644	forward	trnH (gug)	trnP (ugg)
TAG*	NA	trnP (ugg) / UA	8283 - 10261	74	1905	reverse	trnG (ucc)	atp6
PCR**	NA	trnI (gau) / UA	22611 - 24434	74	1750	forward	nad6	trnP (ugg)
	NA	trnM (cat) / UA	14989 - 17084	74	2022	forward	trnR (ucu)	nad4
	NA	trnR (ucu) / UA	10742 - 14253	74	3560	forward	rpl6	trnM (cat)

Table S2. Unannotated tRNA genes and ORFs identified as the *rpl10* gene in the studied mitochondrial genomes. RPL10 protein motifs were searched with MotifSearch, which is available at <https://www.genome.jp/tools/motif/>. Algal species are arranged according to their appearance in the phylogram of Figure 2. *GenBank accessions for TAG and PCR (contig 1) are EU123949 and KR017746, respectively. ** GenBank accessions for PCR (contig 2) is KR017747. +: presence of RPL10 motif, and -: absence of RPL10 motif. NA: not applicable, UA: unannotated. ?: no available sequence upstream of the gene.

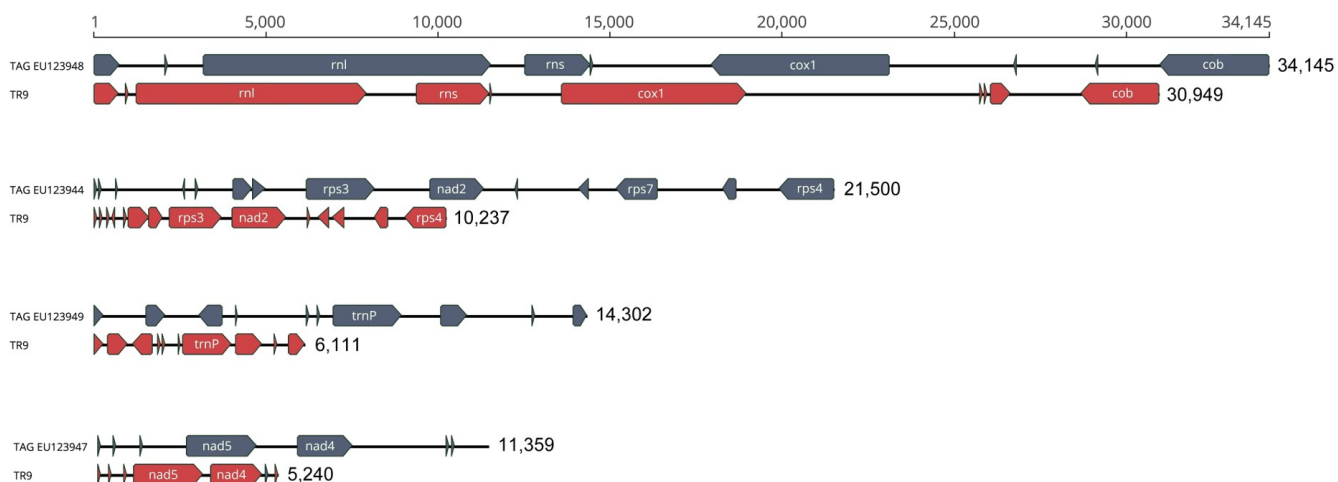


Figure S1. Genetic maps of four regions of the mtDNA of *Trebouxia aggregata* (accessions EU123944, EU123947, EU123948 and EU123949) and their counterparts in *Trebouxia* sp. TR9. Coding regions are indicated as blue and garnet arrows for *T. aggregata* (TAG) and *Trebouxia* sp. TR9 (TR9), respectively. Arrowheads indicate transcription directions. Non-coding regions are indicated by a black line.

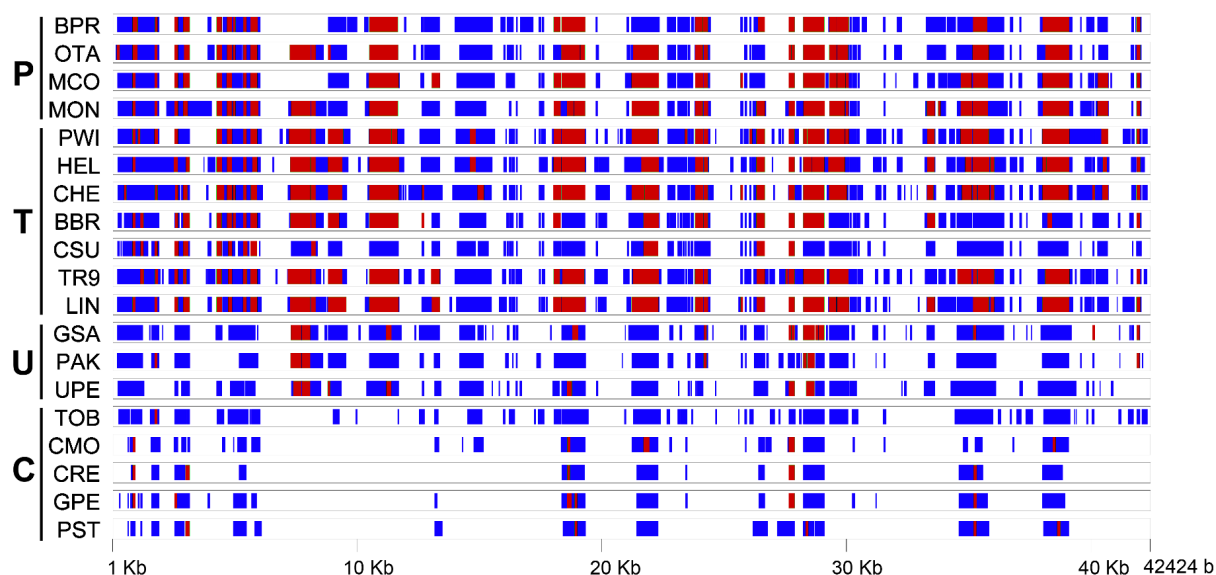


Figure S2. Whole-genome alignment of the *Trebouxia* sp. TR9 mitogenome with other chlorophyte mitochondrial genomes obtained with MultiPipMaker⁶⁸, using *Mesostigma viride* as the reference. Sequence identity is shown by red (75–100%), blue (50–75%), and white (<50%). The systematic classification is indicated on the left (P: Prasinophyceae, T: Trebouxiophyceae, U: Ulvophyceae, C: Chlorophyceae).