

Supplementary Materials



Fig. S1. An alignment of *cox3* proteins of apicomplexans, dinoflagellates, and of *Chromera* and *Vitrella* *cox3* ORFs. Cox3 sequences of dinoflagellates (*Durinskia*, *Hematodinium*, *Karlodinium*, *Kryptoperidinium*, *Oxyrrhis*), *Chromera*, and *Vitrella*

are all highly derived. The alignment has been constructed in CLC Genomics Workbench v.6.5. and edited manually. Consensus sequence is shown at the bottom, and most conserved motifs are indicated with arrows. Genus abbreviations are the following: P., *Plasmodium*; E., *Eimeria*; B., *Babesia*; T., *Theileria*.

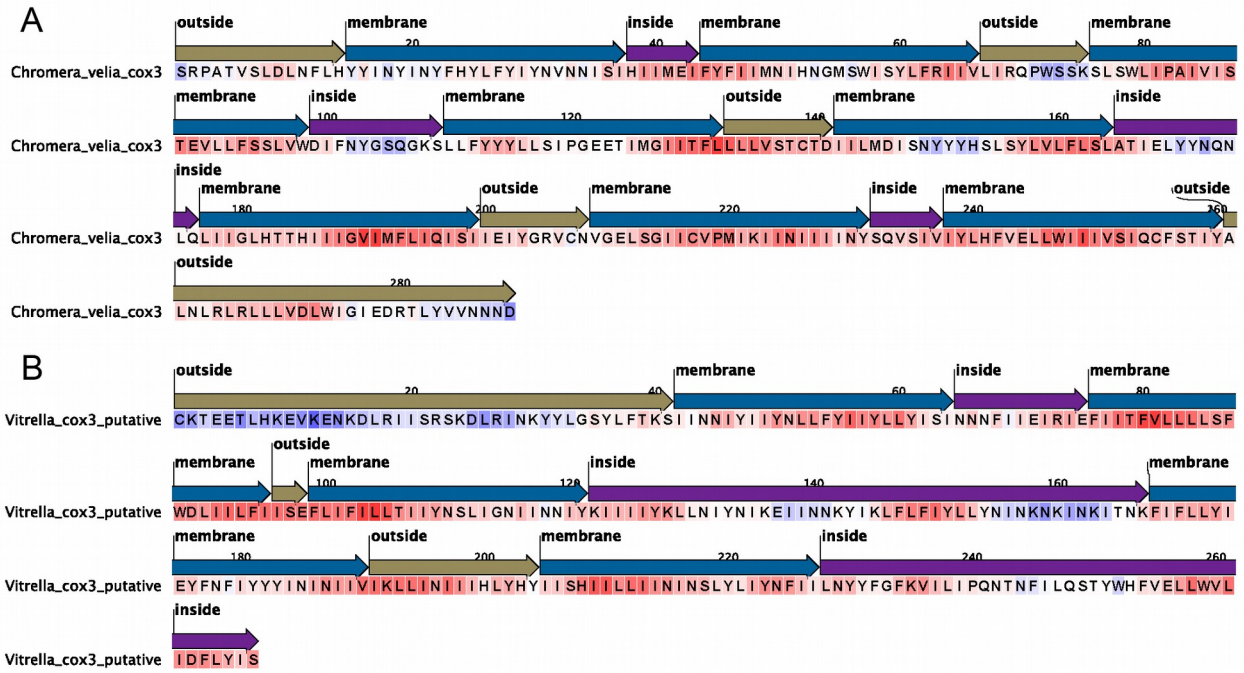


Fig. S2. *Chromera* and *Vitrella* putative *cox3* proteins. Transmembrane domains predicted with CLC Genomics Workbench v.7.0.4 are shown with blue arrows, and Kyte-Doolittle hydrophobicity values are color-coded with red representing the highest values. Due to uncertainty in actual start positions, *Chromera cox1-cox3* ORF sequence downstream of the conserved *cox1* fragment is shown, along with the corresponding portion of the *Vitrella cox3* ORF (see also supplementary fig. S5 showing a full *cox3* ORF of *Vitrella*).

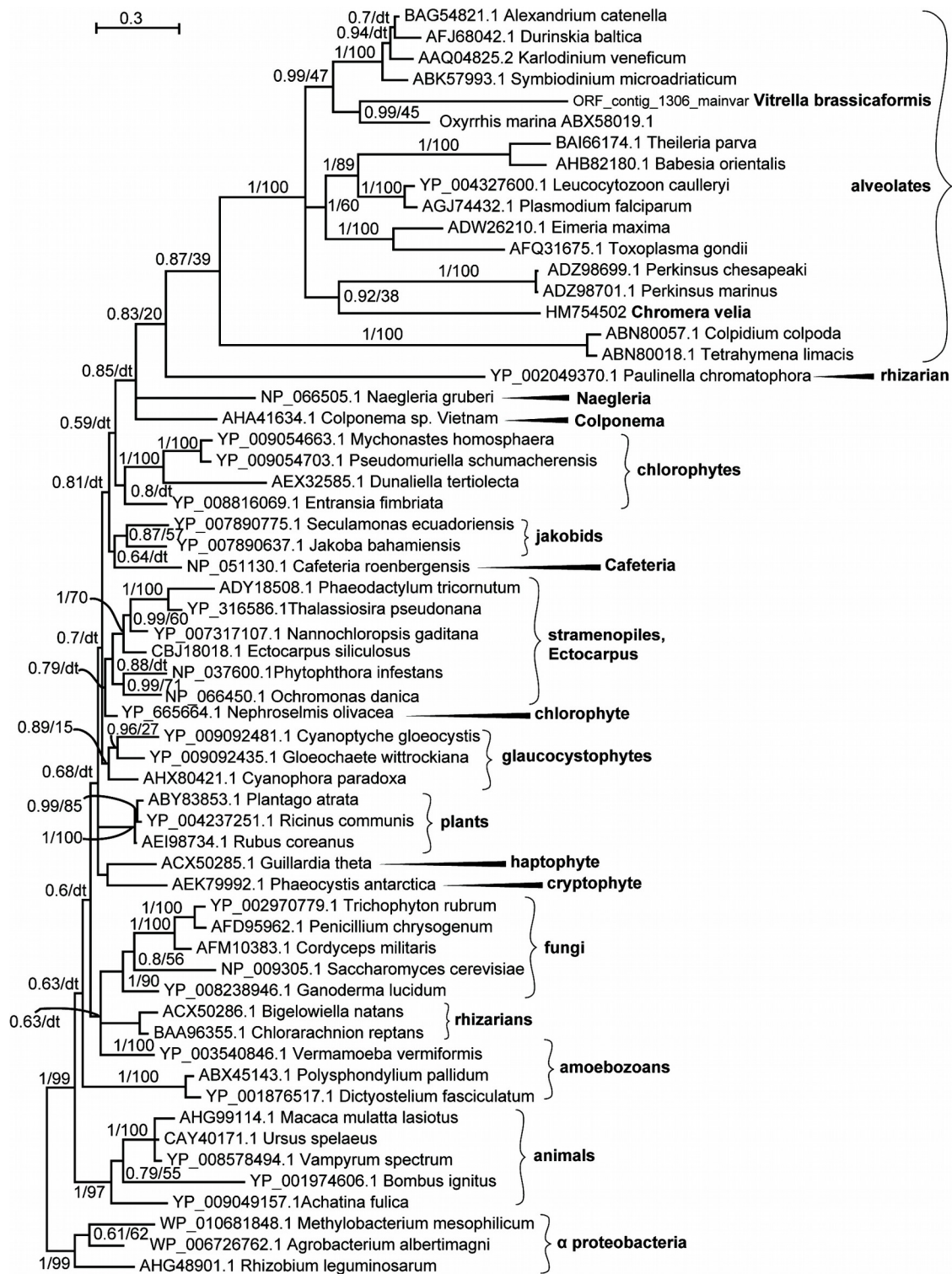


Fig. S3. Bayesian phylogenetic tree as inferred from *cox1* amino acid sequences. Number above branches indicate Bayesian PP/ML bootstrap support.

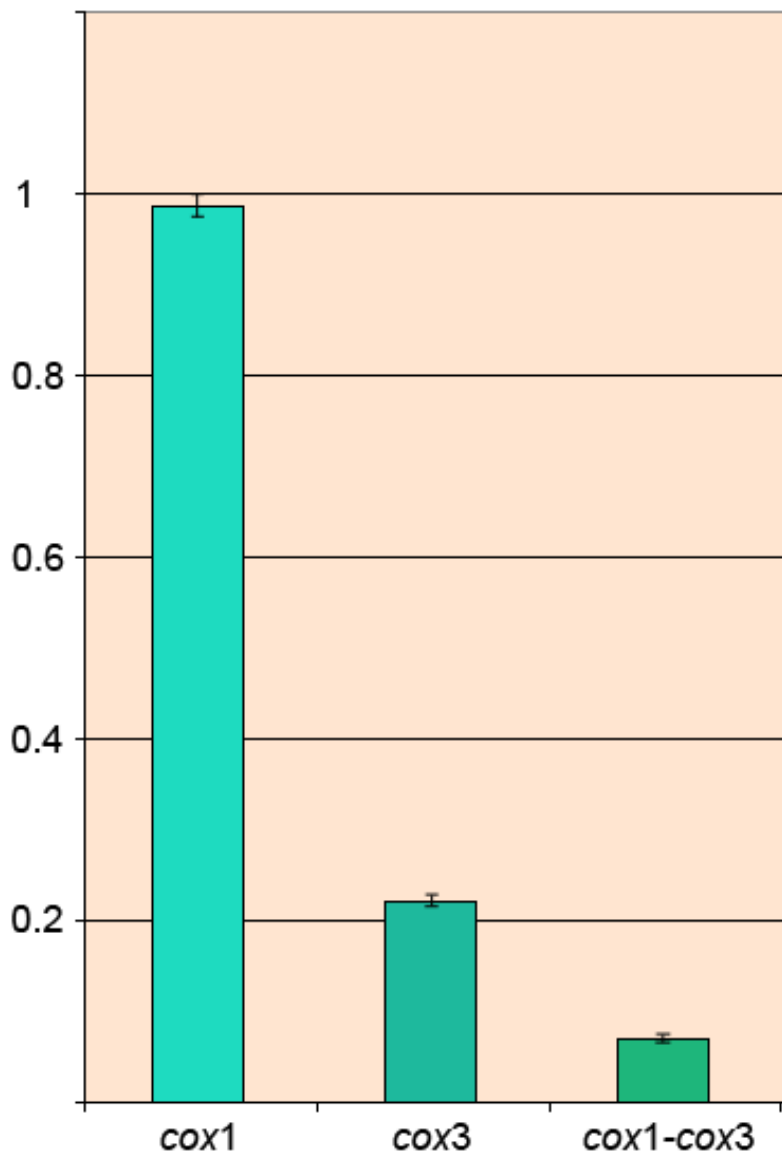


Fig. S4. Relative abundance of *cox1*, *cox3* transcripts, and the junction region (*cox1-cox3*), as determined with quantitative PCR using the respective oligos. Standard deviation was calculated based on two PCR replicates. Relative concentration was determined based on a calibration curve made with a serially diluted sample.

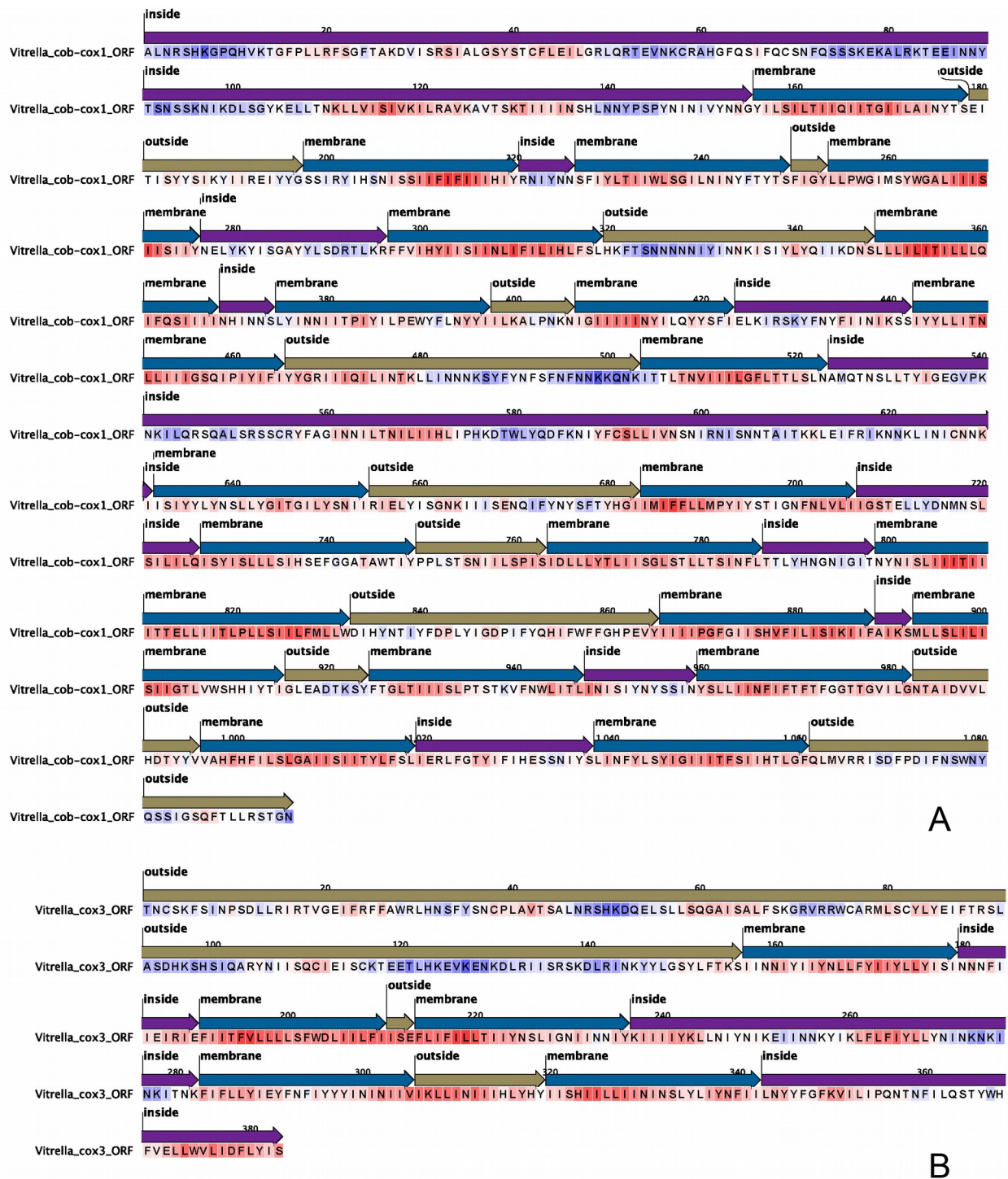


Fig. S5. Coding sequences from two *Vitrella* mitochondrial contigs (assembled from Illumina reads with Velvet): *cob-cox1* fusion ORF, and *cox3* ORF. Transmembrane domains predicted with CLC Genomics Workbench v.7.0.4 are shown with blue arrows, and Kyte-Doolittle hydrophobicity values are color-coded with red representing the highest values.

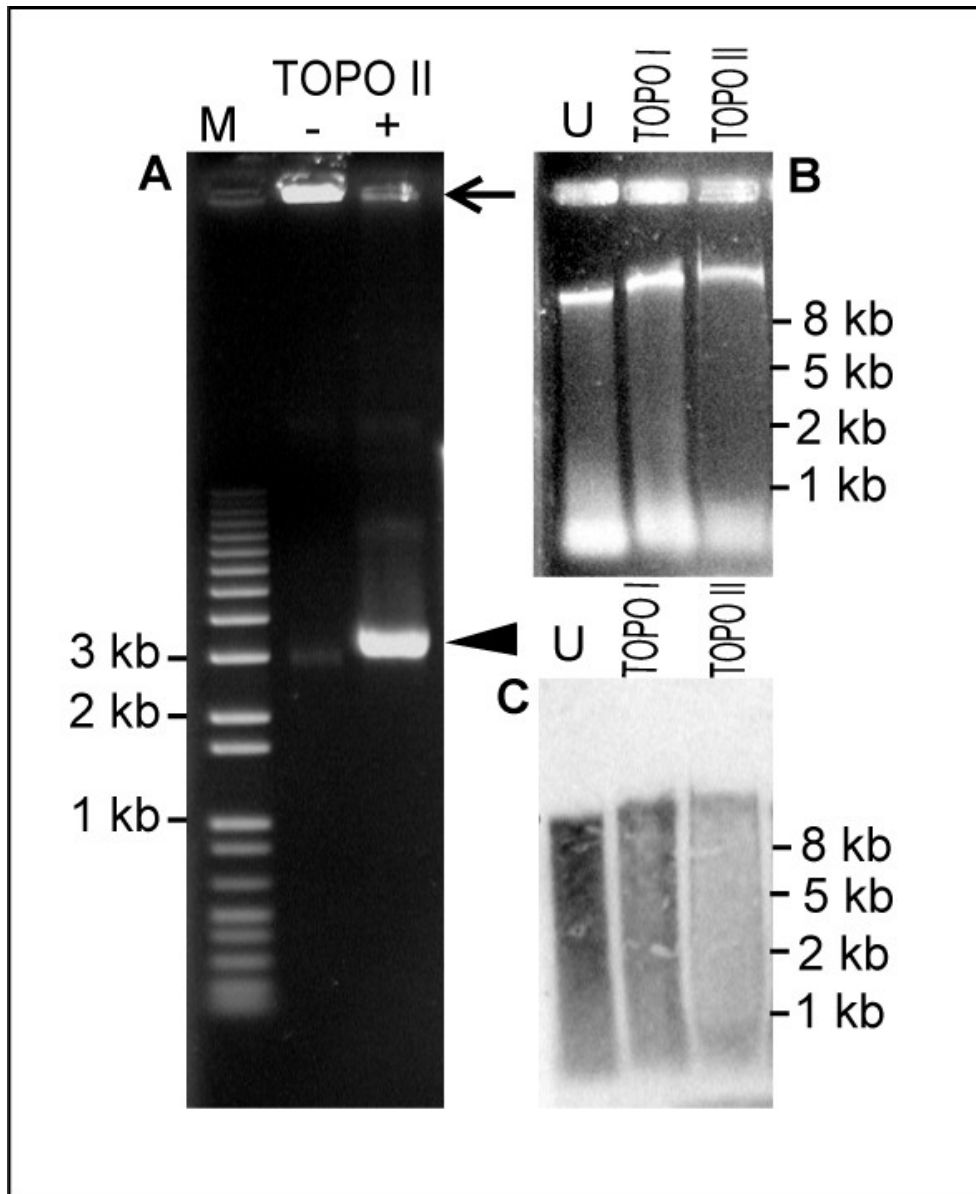


Fig. S6. Linear topology of mitochondrial DNA molecules in *C. velia*, hybridizing with the *cox1* probe. **(A)** The mitochondrial DNA network composed of catenated DNA circles from *Crithidia fasciculata*, isolated as described previously (Jirků et al. 1995), was used as a control. While non-treated mitochondrial DNA network stays in the slot (-), upon topoisomerase II treatment, decatenated circular molecules enter the gel (+). **(B)** Ethidium bromide-stained gel of non-treated total *C. velia* DNA (U), and treated with topoisomerase I (Topo I) and topoisomerase II (Topo II). 1 μ g DNA per lane was resolved in 0.75% agarose gel and stained with ethidium bromide after the run. The DNA was treated either with topoisomerase I or topoisomerase II following protocols described elsewhere (Lukeš et al. 1998). **(C)** Southern blot analysis of the gel in (B) hybridized with the *C. velia cox1* gene as a probe labeled with [α - 32 P]dATP. Hybridization was carried out as described elsewhere (Jirků et al. 1995). Molecular size markers are indicated.

1
Cryptococcus neoformans var. neoformans JEC21
Neosartorya fischeri NRRL 181
Aspergillus flavus NRRL3357
Ajellomyces capsulatus NAm1
Uncinocarpus reesii 1704
Neurospora crassa OR74A
Pyrenophora tritici-repentis Pt-1C-BFP
Talaromyces stipitatus ATCC 10500
Paracoccidioides brasiliensis Pb01
Verticillium albo-atrum VaMs.102
Arthroderma otae CBS 113480
Saccharomyces cerevisiae S288c
Lachanea thermotolerans
Trichophyton rubrum CBS 118892
Candida dubliniensis CD36
Schizophyllum commune H4-8
Komagataella pastoris GS115
Perkinsus marinus ATCC 50983
Karlodinium micrum 1
Karlodinium orcum 2
Chromera velia_gene4425.t1
Chromera velia_gene5763.t1
Vitrella brassicaformis_gene7868.t1
Vitrella brassicaformis_gene19079.t1

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Karlodinium micrum 1
Karlodinium micrum 2
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Saccharomyces cerevisiae_S288c
Lachancea thermotolerans
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Chromera velia gene5763.t1
Vitrella brassicaformis gene7868.t1
Vitrella brassicaformis gene19079.t1

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Fig. S7. Multiple alignment of amino acid sequences of L-LDH (cytochrome) oxidoreductase. Due to occurrence of the enzyme limited to fungi, perkinsids, dinoflagellates, *Chromera* and *Vitrella*, we did not constructed the tree. Alignment was shortened.

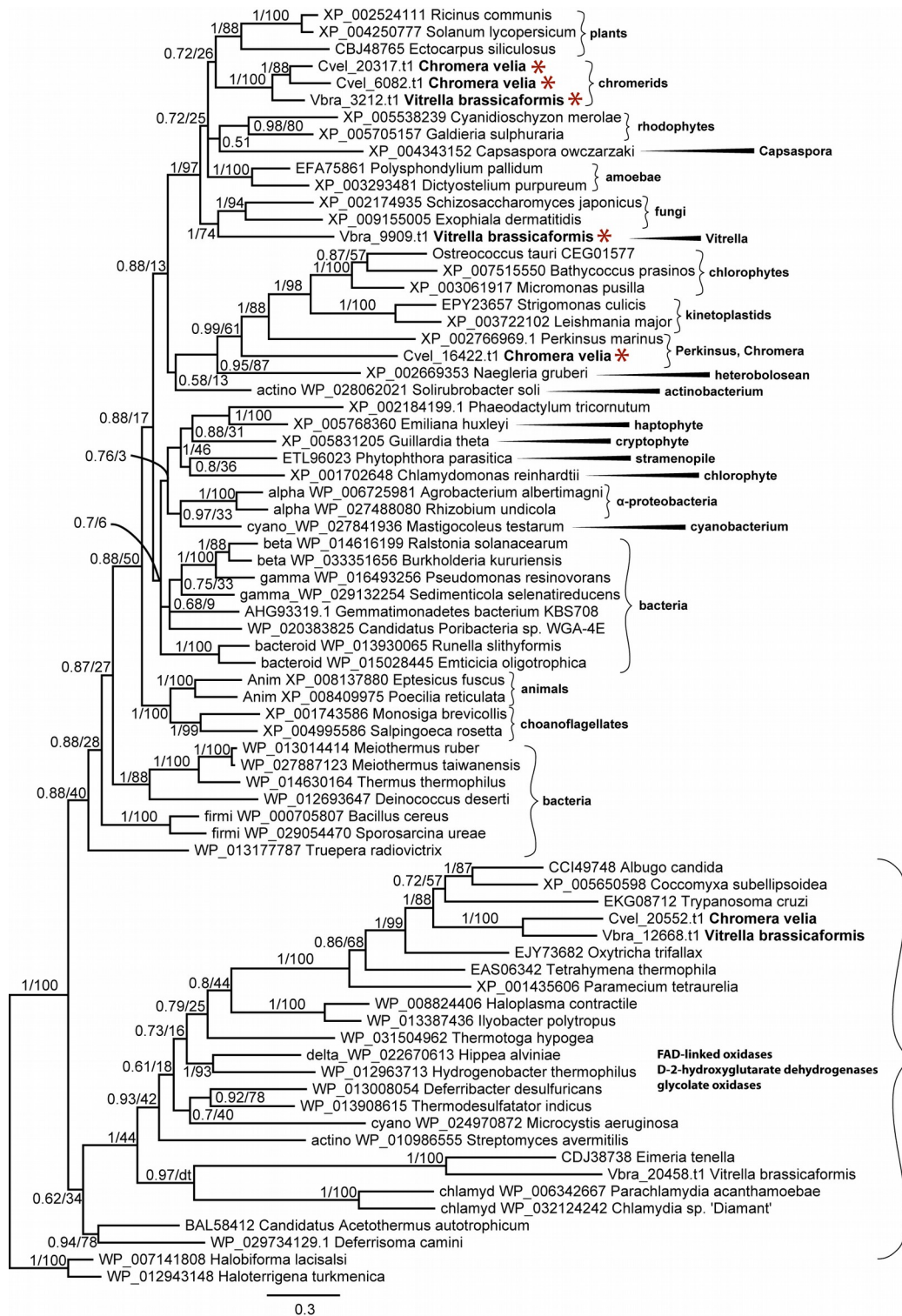


Fig. S8. Bayesian phylogenetic tree as inferred from D-lactate: cytochrome c oxidoreductase amino acid sequences. The supposed mitochondrial-located enzymes are marked by *. Numbers above branches indicate Bayesian PP/ML bootstrap support (1000 replicates).

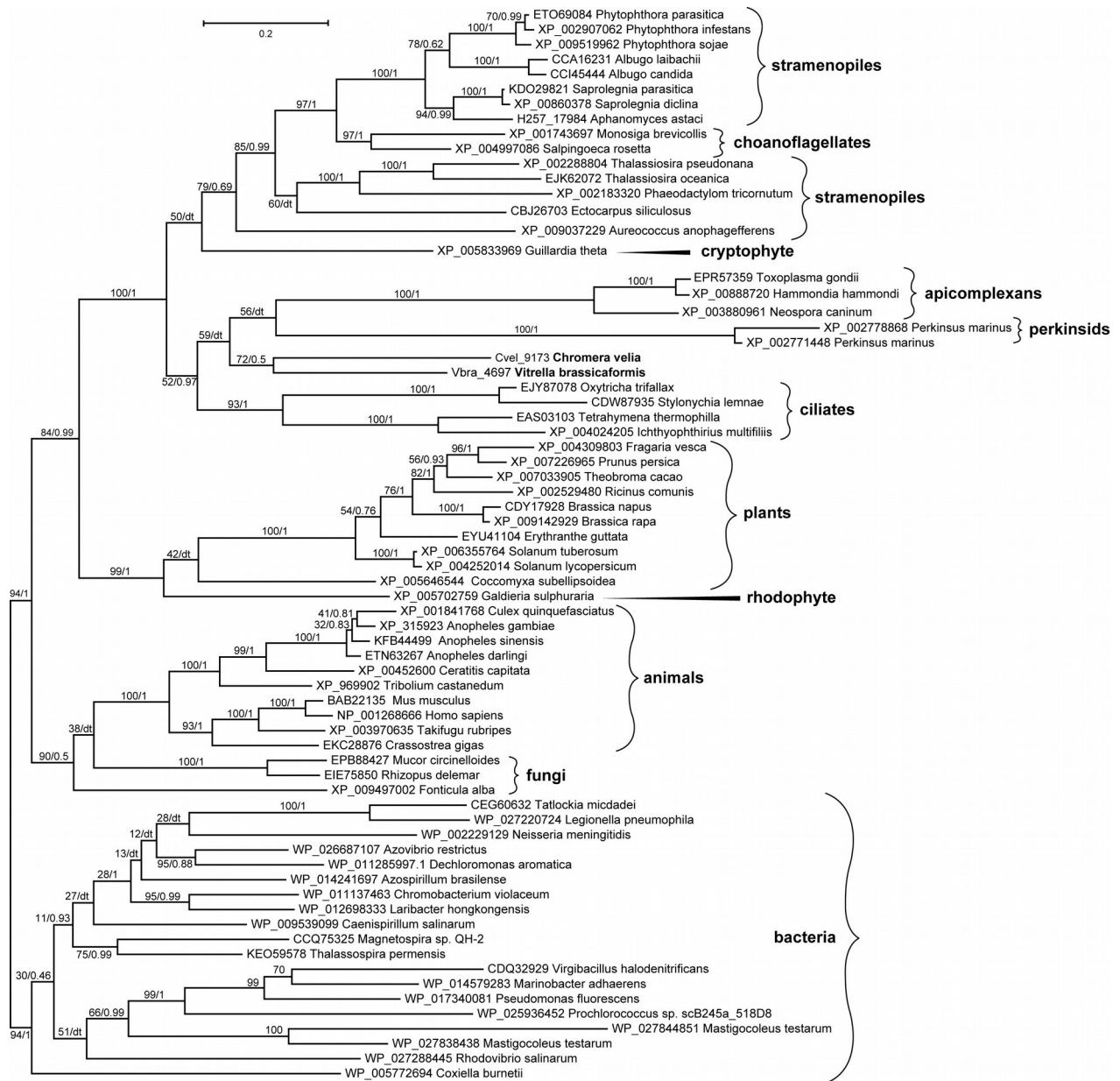


Fig. S9. Maximum likelihood as inferred from electron-transfer flavoprotein: ubiquinone oxidoreductase (ETFQO) amino acid sequences. Numbers above branches indicate ML bootstrap support (1000 replicates) /Bayesian PP.

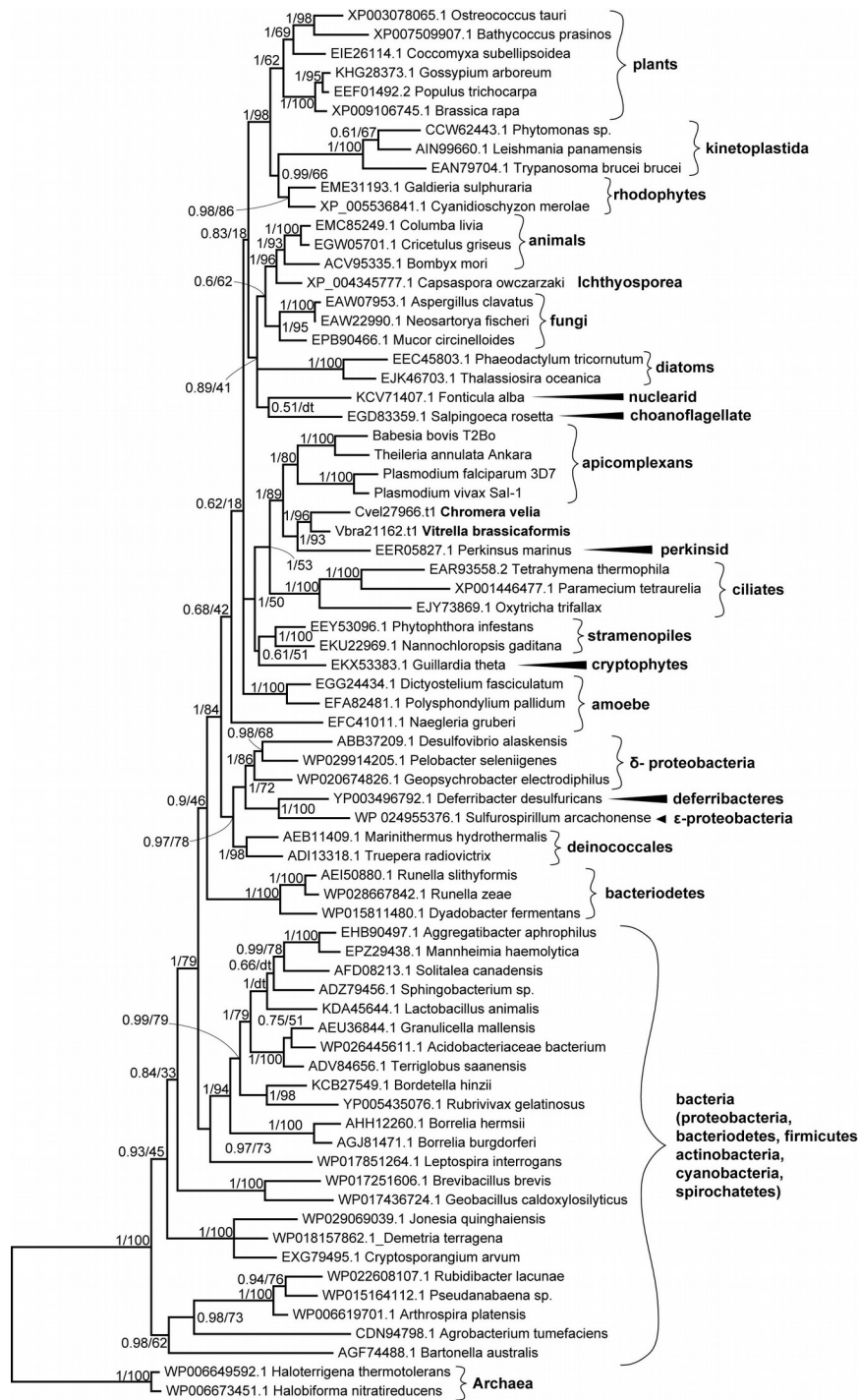


Fig. S10. The extended Bayesian phylogenetic tree as inferred glycerol-3-phosphate: ubiquinone oxidoreductase amino acid sequences. Numbers above branches indicate Bayesian PP/ML bootstrap support (1000 replicates).

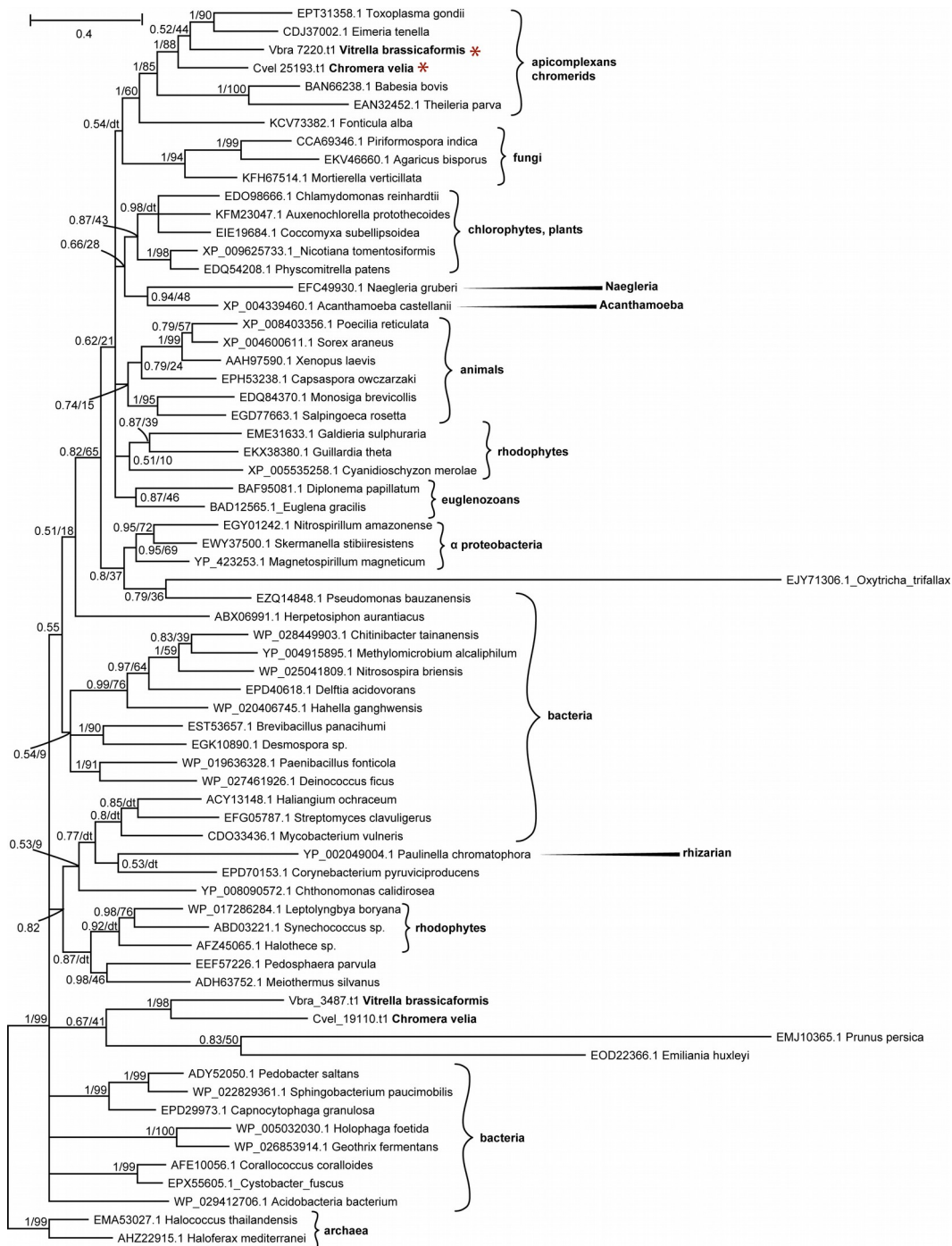


Fig. S11. Bayesian phylogenetic tree as inferred from dihydroorotate: ubiquinone oxidoreductase (DHODH) amino acid sequences. Targeting predictions were inconclusive in the case of this enzyme (see Table 2. For details). We suppose that proteins from *Chromera* and *Vitrella* (marked by *) branching together with apicomplexan homologs are used in the respiratory chain. Numbers above branches indicate Bayesian PP/ML bootstrap support (1000 replicates).

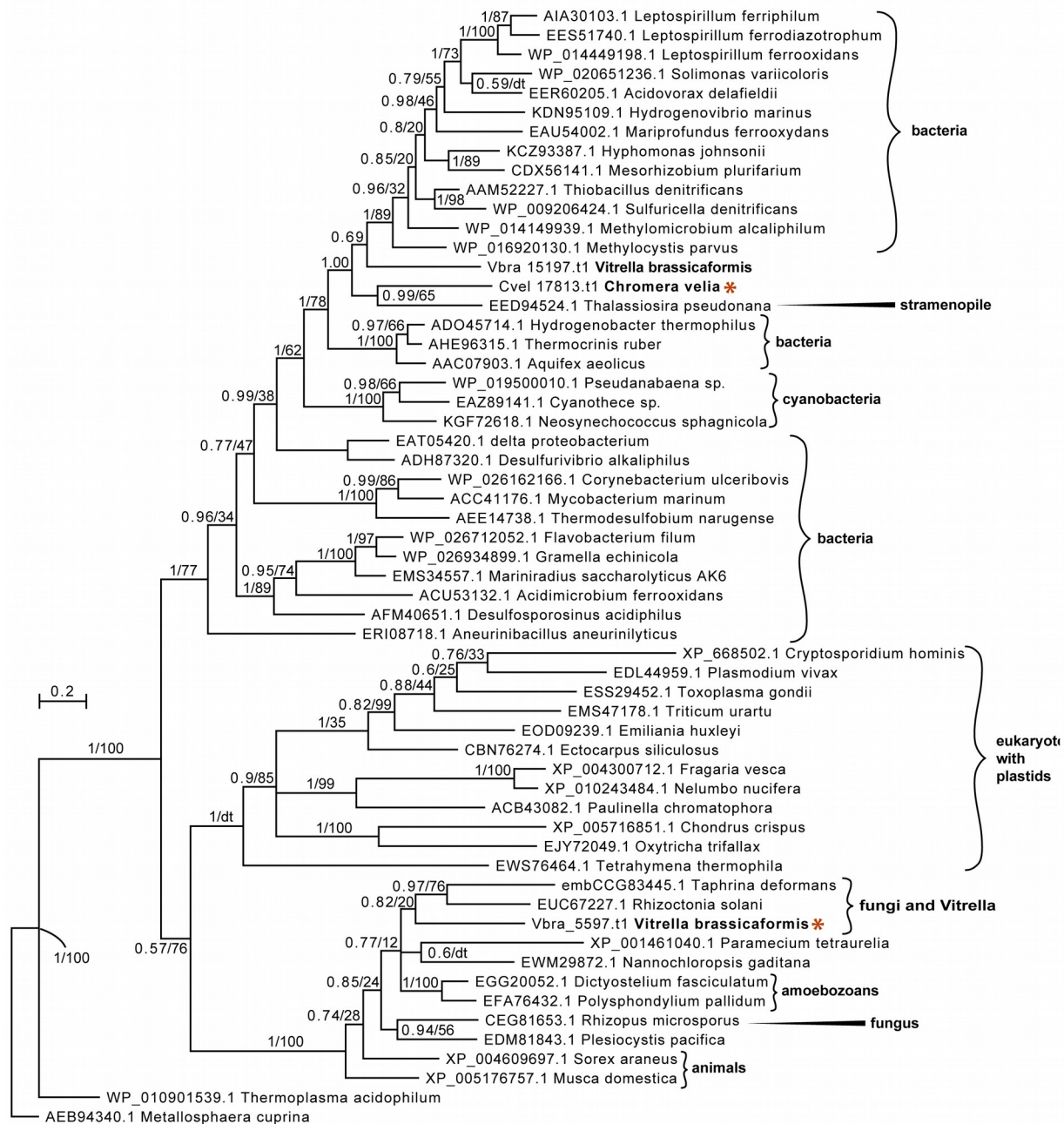


Fig. S12. Bayesian phylogenetic tree as inferred from sulfide: ubiquinone oxidoreductase (SQO) amino acid sequences. The supposed mitochondrial-located enzymes are marked by *. Numbers above branches indicate Bayesian PP/ML bootstrap support (1000 replicates).

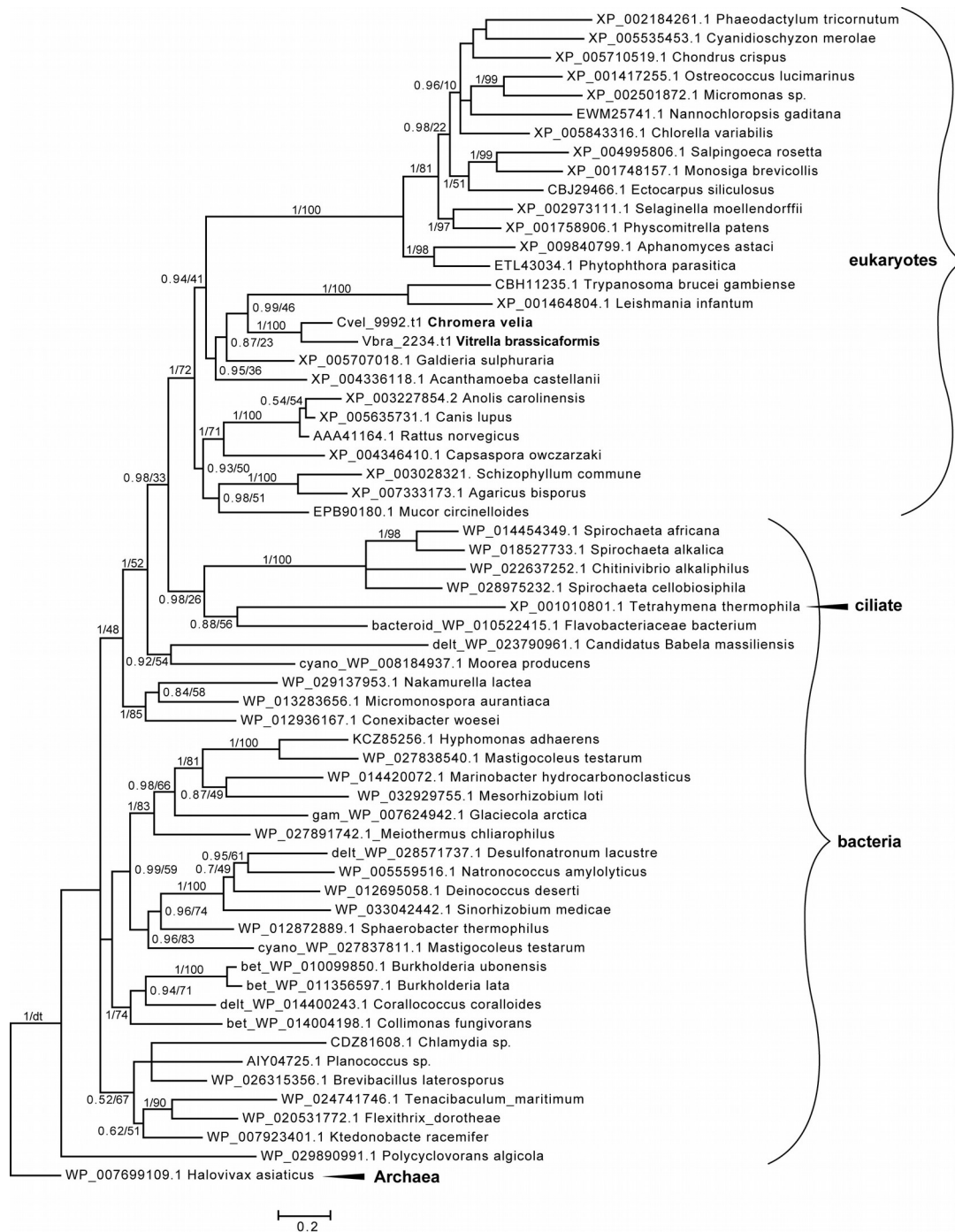


Fig. S13. Bayesian phylogenetic tree as inferred from galacto-1,4-lactone: cytochrome c oxidoreductase amino acid sequences. Numbers above branches indicate Bayesian PP/ML bootstrap support (1000 replicates).

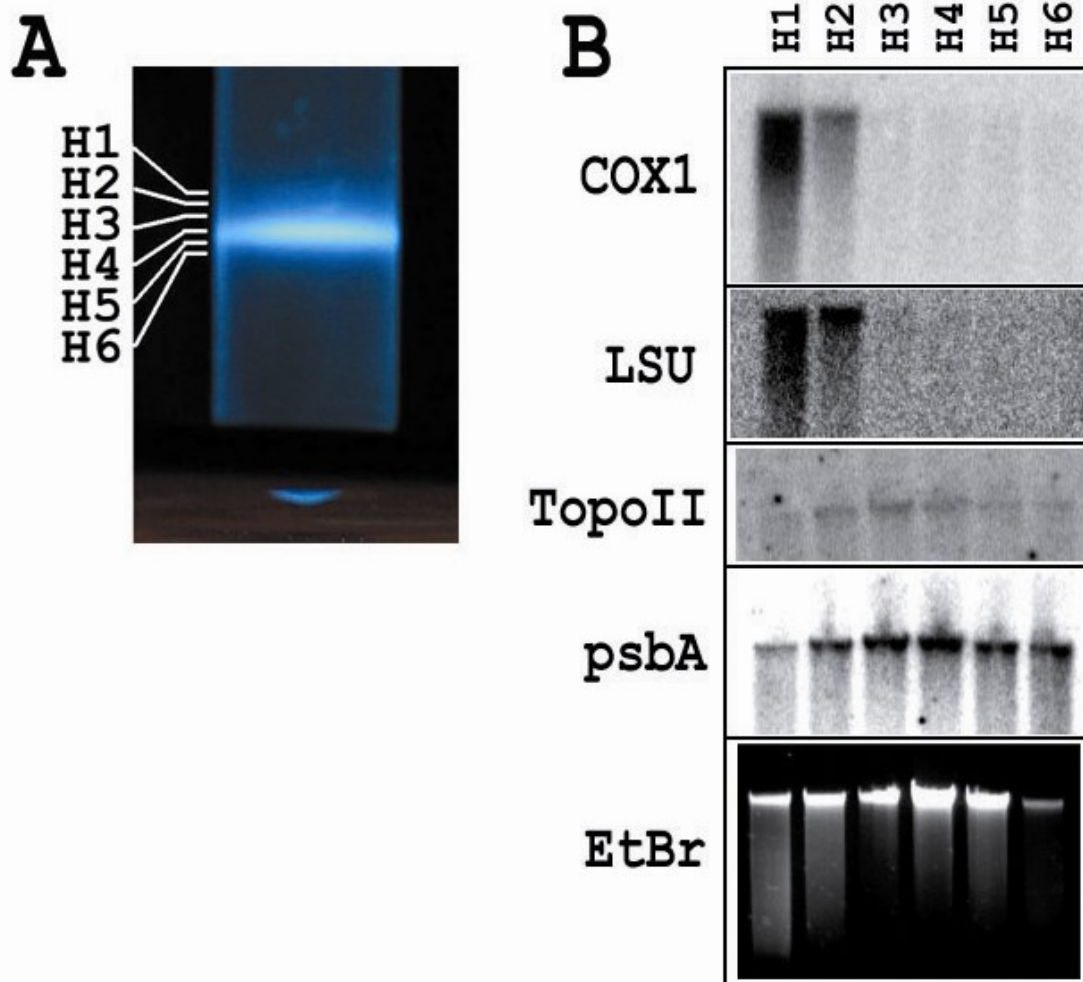


Fig.

S14. Analysis of total DNA from *C. velia*. **(A)** Part of the CsCl-Hoechst 33258 density gradient of total cell DNA. The position of fractions H1 through H6 is indicated. **(B)** Southern hybridization of blotted agarose gel containing undigested DNA isolated from fractions H1 through H6 run in the presence of EtBr (bottom image). The probes used are indicated: *cox1* (cytochrome c oxidase subunit 1), LSU (large subunit of mitoribosomal RNA *rrl8*), *topoII* (nuclear topoisomerase II) and *psbA* (photosystem complex subunit A).

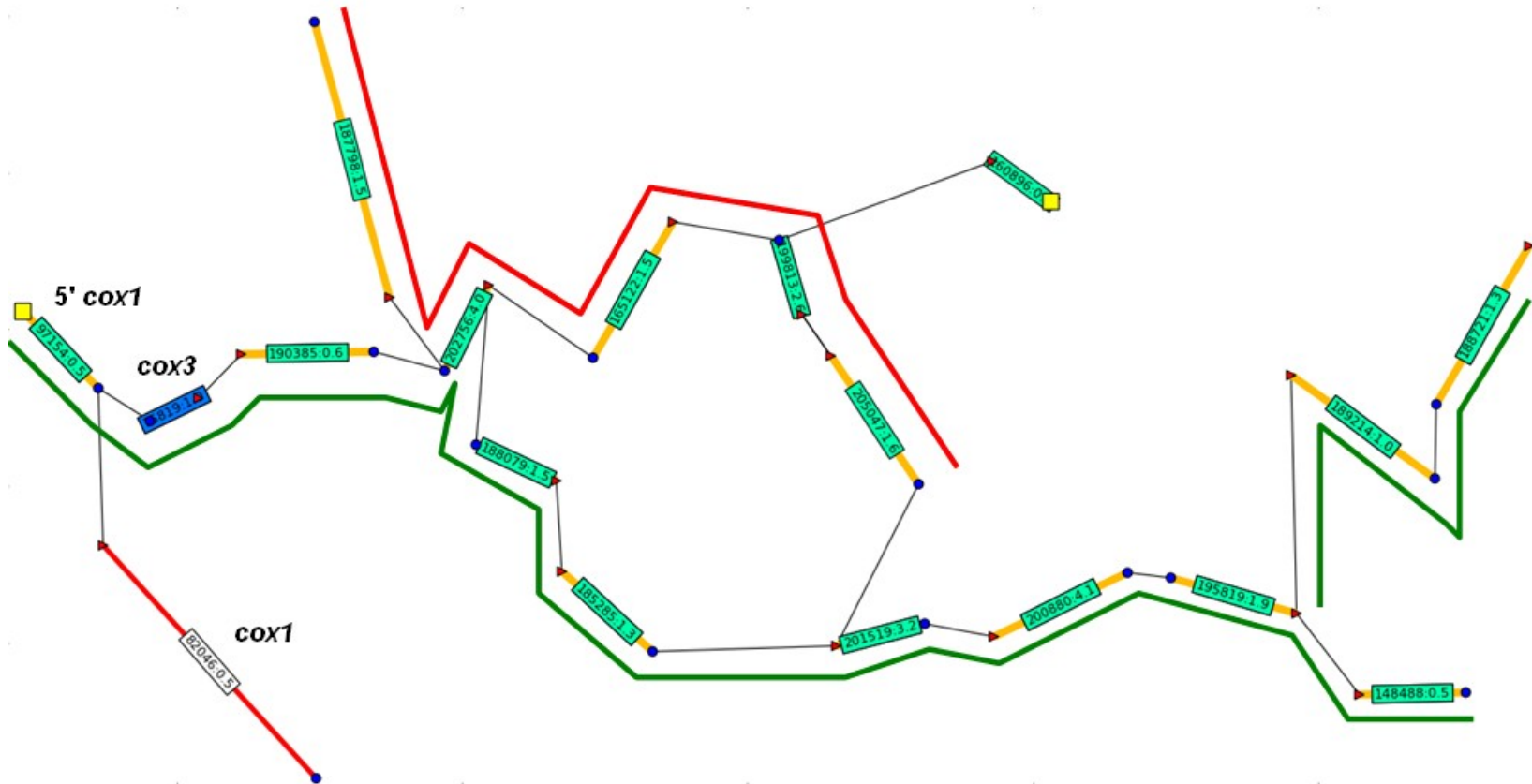


Fig. S15. An example of a contig graph, an output of the Newbler assembly software, visualized by an in-house script. Contigs are represented with bars in the following way: rectangles contain contig numbers and their relative coverage (absolute coverage divided by *cox1* contig coverage in this case) in the format number:coverage. Blue circles and red triangles mark contig 5' and 3' ends, respectively. Direct connections between contigs, i.e. reads flowing from one

contig into another, are shown with thin lines, and yellow rectangles mark contig termini with no direct connections to any other contigs. Contigs containing the *cox1* and *cox3* gene sequences are indicated. This figure shows pathways in the graph starting from one of the highest-coverage contigs, contig 97154, containing a 5'-fragment of *cox1* (amino acids 1-192). From the 5' end this contig reads flow either into a downstream part of the *cox1* gene (contig 82046, marked *cox1*), or into the *cox3* gene (contig 75819, marked *cox3*), and then into contigs 190385 and 202756. From that point there are two possible pathways: either through contig 165122, or through contig 188079. Based on the analysis of 454 read flow and on mapping of paired Illumina reads of the mitochondrial DNA-enriched fraction, it can be concluded that of the two alternative pathways at this point, the pathway indicated with a green line probably represents a genomic sequence, and the 'red' pathway represents no actual sequence. The 'red' pathway contains regions not covered with Illumina reads, and not all contig connections in this path are supported by 454 read flow. All *Chromera* mitochondrial sequences in this paper were constructed and validated in a similar way: i/ contigs with hits to the *cox1* or *cox3* genes, or to rRNA fragments acted as seeds, i.e. starting contigs, for contig graph construction (*cox3* contig 75819 in the example presented); ii/ all possible pathways in the graph in both 5' and 3' directions from seed contigs were considered; iii/ only pathways supported by all of the following were taken as final 'supercontigs': a) by flows of 454 or Illumina MiSeq reads between contigs; b) by complete coverage with paired-end Illumina reads of the mitochondrial DNA-enriched fraction (see an example in supplementary fig. S16); c) by complete coverage with Illumina mate pair inserts 1700-5500 bp in length (data not shown).

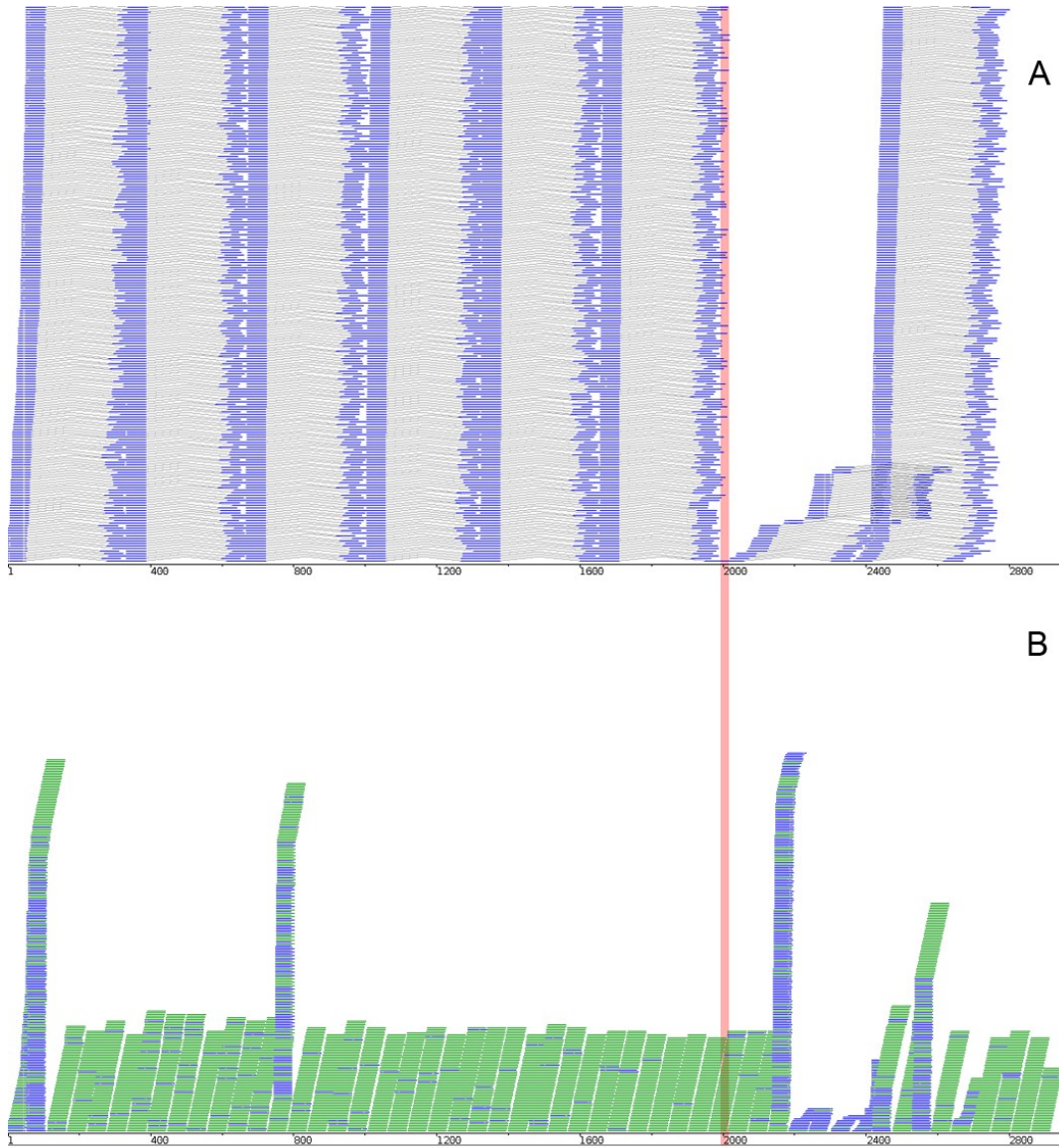


Fig. S16. An example of *Chromera* mitochondrial 'supercontig' with paired Illumina reads of the mitochondrial DNA-enriched fraction (supplementary table S3) mapped on it. Bowtie2 v.2.1.0 was used for mapping with the 'very sensitive' and 'end-to-end' options, and a narrow insert size range of 300-355 bp. Read pairs and individual reads are shown in panels (A) and (B), respectively. As seen in panel B, the full 'supercontig' is covered with reads of the expected insert size, making it a highly supported contig.

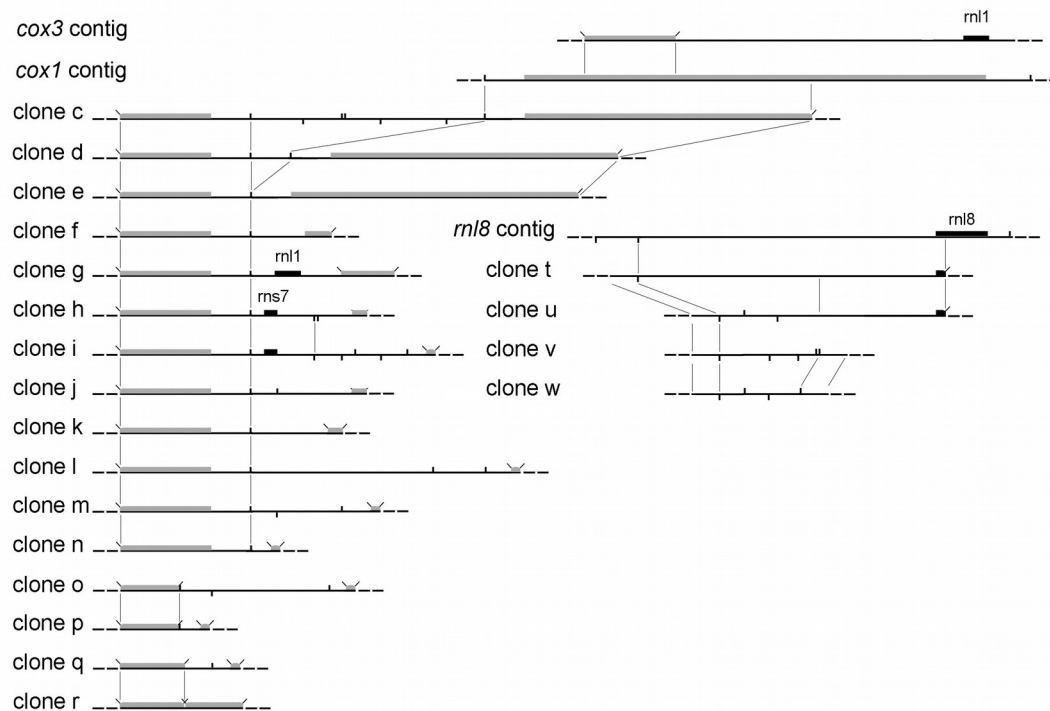


Fig. S17. Diversity of gene arrangements as determined by PCR (clones c-r, t-w). Most clones represent highly variable spacers between *cox1* genes/gene fragments. Few contigs assembled from Illumina and 454 data are shown for comparison (*cox1*, *cox3*, *rnl8* contigs). PCR products were amplified either by 'outward' *cox1*-specific oligonucleotides or by 'outward' and 'inward' rRNA-specific oligonucleotides. Grey rectangles indicate *cox1* gene sequences, black boxes indicate rRNA gene fragments, and short slanted lines at their ends mark truncated gene ends. Small vertical bars indicate short dispersed repeats, probably acting as recombination hot-spots. Sequence regions nearly identical among the clones are aligned between thin vertical or slanted lines.

Table S1. A list of *Chromera* mitochondrial contigs. The contigs shown here were generated with the contig graph analysis approach (supplementary fig. S15) based on initial contigs assembled with Newbler v.2.9 from 454 and Illumina reads (supplementary table S3), compared with PCR-generated clones (supplementary fig. S17), and validated with mapping mate pair reads and paired-end reads of the mitochondrial DNA-enriched fraction (supplementary fig. S16). Length, coverage with reads of the mitochondrial fraction, and GenBank accession numbers are shown.

contig name	length, bp	average coverage, reads of the mitochondrial DNA-enriched fraction	GenBank accession #
11012_rnl8_1	3186	466	
114504_cox1_3_c1_1	1782	2264	
114504_cox1_3_c1_2	11724	368	
114504_cox1_3_c1_4	1623	1881	
114504_cox1_3_c1_5	2046	1975	
114504_cox1_3_c1_6	2116	1870	
114504_cox1_3_c1_7	2130	1897	
114504_cox1_3_c1_9	1620	1881	
114504_cox1_3_c1_10	2043	1934	
114504_cox1_3_c1_11	2113	1909	
114504_cox1_3_c1_12	2127	1204	
114504_cox1_c1_5	3030	2200	
114504_cox1_c1_6	12972	649	
114504_cox1_c1_8	2871	2494	
114504_cox1_c1_9	3294	2473	
114504_cox1_c1_10	3364	2397	
114504_cox1_c1_11	3378	2412	
114504_cox1_c1_13	2868	2494	
114504_cox1_c1_14	3291	2474	
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114504_cox1_c2_14	3564	2528	
114504_cox1_c2_15	3634	2456	
114504_cox1_c2_16	3648	2470	
114504_cox1_c3_5	3251	2249	
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114504_cox1_c4_15	3560	2457	
114504_cox1_c4_16	3574	2471	
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154998_non-coding_3	1042	2803	
154998_non-coding_4	925	2755	
154998_non-coding_5	481	1951	
154998_non-coding_7	919	2668	
154998_non-coding_8	802	2593	
154998_non-coding_9	375	2152	
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274408_non-coding_3	564	3015	
274408_non-coding_4	320	2511	
274408_non-coding_6	584	3131	
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33769_cox1_frag_2	4604	700	
33769_cox1_frag_3	5611	605	
33769_cox1_frag_4	5707	596	
33769_cox1_frag_5	4663	693	
33769_cox1_frag_6	4622	698	
33769_cox1_frag_7	5629	603	
33769_cox1_frag_8	5725	595	
33769_cox1_frag_9	4644	695	
33769_cox1_frag_10	4603	737	
33769_cox1_frag_11	5610	607	
33769_cox1_frag_12	5706	566	
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33769_cox1_frag_14	4621	735	
33769_cox1_frag_15	5628	605	
33769_cox1_frag_16	5724	564	
33769_non-coding_1	1490	154	

33769_non-coding_2	1449	158	
33769_non-coding_3	2456	163	
33769_non-coding_4	2552	161	
33769_non-coding_5	1508	159	
33769_non-coding_6	1467	158	
33769_non-coding_7	2474	163	
33769_non-coding_8	2570	161	
33769_non-coding_9	1489	159	
33769_non-coding_10	1448	277	
33769_non-coding_11	2454	167	
33769_non-coding_12	2551	94	
33769_non-coding_13	1507	154	
33769_non-coding_14	1466	275	
33769_non-coding_15	2473	167	
33769_non-coding_16	2569	92	
34169_cox1_frag_1	2146	197	
36603_cox1_3_1	3706	596	
36603_non-coding_1	4069	161	
36603_non-coding_2	4919	193	
36603_non-coding_3	3863	562	
36603_non-coding_4	3698	166	
36603_non-coding_5	3604	180	
36603_non-coding_6	1579	27	
3725_rRNA_1	12088	196	
37286_rns7_1	2690	170	
41777_rnl12_rns7_1	2021	438	
43250_rns7_1	2736	582	
46689_cox3_frag_c12_1	3879	16	
46689_non-coding_c13_1	1189	2475	
46919_cox3_cox1_1	4478	1566	
46919_cox3_cox1_2	1966	3039	
46919_cox3_cox1_3	2239	3057	
46919_cox3_cox1_4	1965	3041	
46919_cox3_cox1_5	2238	3059	
46919_cox3_cox1_6	2061	3090	
46919_cox3_cox1_7	2185	3029	
46919_cox3_cox1_8	2164	3081	

46919_cox3_cox1_9	2325	3139	
67435_non-coding_1	3670	810	
67435_non-coding_2	835	944	
67435_non-coding_3	6338	185	
67435_non-coding_4	666	1248	
67435_rns7_1	2860	751	
68201_non-coding_2	779	2195	
68201_non-coding_3	658	2108	
68201_non-coding_7	454	1709	
68201_non-coding_10	393	3223	
68201_non-coding_12	702	2191	
68201_non-coding_13	608	1783	
73351_non-coding_8	3169	433	
73351_non-coding_9	3489	470	
73351_non-coding_10	1741	600	
73351_non-coding_11	1413	656	
73351_non-coding_12	2441	573	
73351_non-coding_13	3554	468	
73351_rRNA_3	3427	628	
73351_rRNA_4	2112	646	
75915_rnl8_1	444	1719	
95117_non-coding_1	945	5088	
95117_non-coding_2	2392	2619	
95117_non-coding_6	1391	1349	
95117_non-coding_8	1967	1735	
95117_non-coding_10	1429	1385	
95117_non-coding_11	1509	1341	
95117_non-coding_12	1812	1471	
95117_non-coding_18	1524	1030	
95117_non-coding_19	1541	1270	
95117_non-coding_28	2741	526	
95117_non-coding_29	2413	549	
95117_non-coding_30	3441	522	
95117_non-coding_31	4554	430	
95117_non-coding_32	4489	452	
95117_non-coding_33	3216	436	
95117_non-coding_35	3950	509	
95117_non-coding_36	3379	520	
95117_non-coding_37	4554	430	

95117_non-coding_38	3743	641	
95117_non-coding_39	3415	668	
95117_non-coding_40	4443	620	
95117_non-coding_41	5556	524	
95117_non-coding_42	5491	544	
95117_non-coding_45	4952	599	
95117_non-coding_46	4381	620	
95117_non-coding_47	5556	524	
95117_non-coding_48	2729	566	

Table S2. A non-redundant set of transcripts detected in *Chromera* mitochondrial contigs, excluding full-length *cox1* transcripts or long *cox1* fragments. Strand-specific transcriptomic reads (supplementary table S3) were mapped on mitochondrial contigs (supplementary table S1) with Bowtie2 v.2.1.0, and transcripts were identified using a minimum coverage threshold of 10. For each contig, transcripts are numbered sequentially (see the 'transcript name' column). A substantial number of genome regions, including, e.g., the *cox1* gene, is transcribed from both strands which is indicated in a separate column. Annotated features overlapping each transcript (genes, gene fragments, ORFs, BLAST hits to *Plasmodium* rRNA fragments) are shown in the following way: feature name; feature coordinates within the transcript ('<' and '>' symbols denote features extending beyond the transcript 5' and 3' end, respectively); length of a full ORF (not necessarily contained within the transcript) and its start codon. It should be noted that unidentified transcripts had no reliable BLAST hits to the NCBI nr database at the E-value threshold of 10^{-5} (data not shown). The bottom section of the table lists high-coverage transcripts of rRNA fragments presented in the same fashion. *Rnl1*, *rnl8*, *rnl12*, *rns1*, and *rns7* transcripts were detected, and very short hits to *Plasmodium rns5* were found within a much longer transcript (see contigs 68201_non-coding_2 and 68201_non-coding_3). Due to their high-coverage, a number of short transcripts with no sequence similarity to *Plasmodium* rRNA fragments was annotated as putative rRNAs, until further investigation.

contig	transcript name	size, bp	strands transcribed	feature name	feature coordinates within transcript	full ORF length	start codon
11012_rnl8_1	transcript_1	124					
	transcript_2	86	both	<i>cox3</i> fragment, 449-476 bp ORF1	complement(17..45) complement(<1..>86)		
	transcript_3	144	both	ORF2	<1..59	306	CAT
	transcript_4	139	both				

114504_cox1_3_c1_1	transcri	148	both	ORF2	52..>148	315	CTA
	pt_1						
	transcri	214	both				
	pt_2						
114504_cox1_3_c1_2	transcri	121	both				
	pt_3						
	transcri	168	both				
	pt_4						
114504_cox1_3_c1_2	transcri	132	both	putative rRNA	1..202		
	pt_2						
	transcri	487					
	pt_3						
114504_cox1_3_c1_4	transcri	700		ORF1	5..>700	723	ATG
	pt_4						
	transcri	881	both	ORF2	<1..153	315	CTA
	pt_2			ORF3	complement(496..>881)	387	TAT
114504_cox1_3_c1_4				<i>Plasmodium rn18</i> hit	complement(104..196)		
				<i>rn18</i> transcript	complement(1..228)		
				ORF2	<1..50	315	CTA
				ORF3	complement(393..>744)	675	ATA
114504_cox1_3_c1_5	transcri	744	both	ORF2	<1..50	315	CTA
	pt_2			ORF3	complement(393..>744)	675	ATA
				<i>Plasmodium rn18</i> hit	complement(1..93)		
				<i>rn18</i> transcript	complement(<1..125)		
114504_cox1_3_c1_7	transcri	135	both	ORF2	<1..153	315	CTA
	pt_2	7		ORF3	complement(496..957)	462	ACC
				<i>Plasmodium rn18</i> hit	complement(104..196)		
				<i>rn18</i> transcript	complement(1..228)		
114504_cox1_3_c1_10	transcri	435	both	ORF3	138..>435	675	ATA
	pt_3						

114504_cox1_c3_6	transcript_4	696		ORF2	1..>696	723	ATG
114504_non-coding_c2_2	transcript_1	694	both	ORF1	complement(309..>694)	387	TAT
				<i>Plasmodium rn18</i> hit	complement(<1..9)		
114504_non-coding_c2_5	transcript_1	117	both	ORF1	complement(309..770)	462	ACC
		0		<i>Plasmodium rn18</i> hit	complement(<1..9)		
114504_non-coding_c3_2	transcript_1	594	both				
114504_non-coding_c4_1	transcript_1	711	both	ORF1	138..545	408	ATA
114504_non-coding_c4_3	transcript_1	761	both				
114504_non-coding_c5_4	transcript_1	714	both				
114504_non-coding_c9_9	transcript_1	856	both	ORF1	complement(213..521)	309	AGG
13057_rn12_1	transcript_1	758		ORF1	complement(462..>758)	366	CTT
				ORF2	466..>758	387	GGG
				ORF3	612..>758	537	TGT
				ORF4	complement(703..>758)	480	CTC
	transcript_2	420	both	ORF3	<1..265	537	TGT
				ORF4	complement(<1..299)	480	CTC
				ORF5	13..294	309	TGG
	transcript_3	222					
	transcript_4	93	both	ORF6	complement(<1..>93)	408	AAT
				ORF7	<1..>93	348	GTA
				ORF8	complement(9..>93)	441	TTA
	transcript	92		ORF8	78..>92	441	TTA

	pt_5						
	transcri	116					
	pt_6						
13113_rns7_1	transcri	254		ORF2	<1..>254	876	TTT
	pt_1						
	transcri	175					
	pt_2			ORF3	complement(244..	117	AAA
					1422)	9	
				ORF3a	230..679	450	CGT
				ORF4	complement(630..	639	CTA
					1268)		
	transcri	146					
	pt_4						
	transcri	227					
	pt_5						
	transcri	102					
	pt_6						
13113_rns7_2	transcri	123					
	pt_1						
	transcri	803		ORF1	complement(90..>	744	CCA
	pt_2				803)		
				ORF2	46..801	756	GAC
	transcri	217					
	pt_3						
154998_non-coding_1	transcri	280	both	ORF1	complement(<1..1	318	ATG
	pt_2				04)		
154998_non-coding_3	transcri	751	both				
	pt_1						
154998_non-coding_5	transcri	157	both	ORF1	complement(<1..1	318	ATG
	pt_2				04)		
154998_non-coding_9	transcri	362	both				
	pt_1						
154998_non-coding_10	transcri	328	both				
	pt_1						
19169_cox1frag_1	transcri	273		ORF1	complement(<1..1	106	ATG
	pt_1				06)	2	
	transcri	184		ORF1	complement(<1..>	106	ATG

	pt_2				184)		2	
	transcri	206		ORF2	<1..>184		843	ATG
	pt_3			ORF3	complement(<1..>206)		156	ATG
				ORF4	<1..97		6	
							122	ATG
							7	
	transcri	143		ORF3	complement(<1..>143)		156	ATG
	pt_4			ORF4	<1..>143		6	
							122	ATG
							7	
	transcri	119		ORF10	<1..62		153	ATG
	pt_5			ORF6	<1..>119		9	
							134	ATG
							4	
				ORF8	complement(<1..>119)		909	ATG
				ORF9	complement(<1..>119)		693	ATG
	transcri	298		ORF10	<1..>298		153	ATG
	pt_6						9	
	transcri	107	both					
	pt_9							
203480_non-coding_3	transcri	393	both					
274408_non-coding_4	transcri	319	both					
274408_non-coding_6	transcri	584	both					
33769_cox1frag_1	transcri	118	both					
	pt_1							
	transcri	167	both					
	pt_2							
	transcri	152	both					
	pt_4							
	transcri	229	both					
	pt_5							
	transcri	135	both	ORF2	complement(<1..7		315	ATG

	pt_6				6)			
				<i>Plasmodium rns7</i> hit	complement(134..>135)			
	transcri pt_7	133	both	cox1 fragment, 1- 79 bp	complement(14..92)			
				ORF1	complement(9..>133)			
	transcri pt_8	127	both	ORF3	complement(123..>127)	348	ATG	
33769_non-coding_5	transcri pt_1	150		ORF1	294..1292	999	ATG	
		5		ORF2	complement(282..1274)	993	ATG	
				ORF3	complement(290..700)	411	ATG	
				ORF4	complement(82..594)	513	ATG	
33769_non-coding_6	transcri pt_1	146		ORF1	253..1251	999	ATG	
		4		ORF2	complement(241..1233)	993	ATG	
				ORF3	complement(249..659)	411	ATG	
				ORF4	complement(14..553)	540	ATG	
33769_non-coding_7	transcri pt_1	247		ORF1	1260..2258	999	ATG	
		1		ORF2	complement(1248..2240)	993	ATG	
				ORF3	complement(1256..1666)	411	ATG	
				ORF4	complement(1021..1560)	540	ATG	
				ORF5	748..1251	504	ATG	
				ORF6	300..944	645	ATG	
				ORF7	complement(537..890)	354	ATG	
				ORF8	complement(137..448)	312	ATG	
				ORF9	20..367	348	ATG	
33769_non-	transcri	256		ORF1	1356..2354	999	ATG	

coding_8	pt_1	7	ORF2	complement(1344	993	ATG		
				..2336)				
			ORF3	complement(1352	411	ATG		
				..1762)				
			ORF4	complement(1117	540	ATG		
				..1656)				
			ORF5	844..1347	504	ATG		
			ORF6	396..1040	645	ATG		
			ORF7	complement(633..	354	ATG		
	986)							
	ORF8		complement(233..	312	ATG			
			544)					
	ORF9		116..463	348	ATG			
34169_cox1frag_1	transcri pt_1	990	ORF1	<1..276	375	ATG		
			ORF2	complement(<1..2	378	ATG		
				87)				
			ORF3	complement(172..	486	ATG		
				657)				
			ORF4	269..688	420	ATG		
			ORF5	286..708	423	ATG		
	ORF6		complement(716..	315	ATG			
			>990)					
36603_cox1_3_1	transcri pt_1	572	both	cox1 fragment,	<1..560			
					1193-1766 bp			
		ORF1		49..>572				
		transcri pt_2	132	both				
		transcri pt_3	355					
		transcri pt_4	137		ORF1	complement(473..	408	ATG
			6			880)		
					ORF2	complement(436..	321	ATG
						756)		
					ORF3	39..416	378	ATG
				ORF4	20..379	360	ATG	
36603_non-coding_1	transcri pt_1	166		ORF1	109..486	378	ATG	
				ORF2	complement(227..	369	ATG	
		4			595)			

				ORF3	complement(622..1035)	414	ATG
				ORF4	complement(1364..>1664)	306	ATG
	transcript_2	172	both	ORF5	<1..>172	330	ATG
	transcript_3	417		ORF5	complement(304..>417)	330	ATG
	transcript_4	294		ORF6	<1..13	318	ATG
				ORF6	complement(<1..51)	318	ATG
				ORF7	complement(<1..229)	423	ATG
				ORF8	177..>294	417	ATG
	transcript_5	171		ORF10	<1..>171	441	ATG
				ORF9	complement(<1..>171)	381	ATG
	transcript_6	169		ORF11	complement(<1..>169)	495	ATG
36603_non-coding_2	transcript_1	950	both	ORF1	661..>950	378	ATG
				ORF2	complement(779..>950)	369	ATG
	transcript_2	115		ORF2	complement(<1..96)	369	ATG
		8		ORF3	complement(86..406)	321	ATG
				ORF4	201..617	417	ATG
				ORF5	complement(858..>1158)	306	ATG
36603_non-coding_4	transcript_2	341					
	transcript_3	114		ORF5	complement(<1..470)	537	ATG
		3		ORF6	163..888	726	ATG
				ORF7	complement(265..870)	606	ATG
				ORF8	complement(635..	372	ATG

					1006)			
36603_non-coding_5	transcri pt_1	156 3	ORF1	152..538	387	ATG		
			ORF2	complement(535..942)	408	ATG		
			ORF3	complement(982..1290)	309	ATG		
36603_non-coding_6	transcri pt_1	109 7	ORF1	complement(<1..424)	486	ATG		
			ORF2	117..842	726	ATG		
			ORF3	complement(219..824)	606	ATG		
			ORF4	complement(589..960)	372	ATG		
3725_rRNA_1	transcri pt_1	143						
			transcri pt_2	367	ORF1	<1..119	615	ATG
	transcri pt_3	159	ORF1	complement(<1..>159)	615	ATG		
	transcri pt_4	832	ORF3	1..528	612	ATG		
	transcri pt_5	854	ORF4	<1..161	606	ATG		
			ORF4	complement(<1..426)	606	ATG		
			ORF4a	<1..633				
	transcri pt_6	172	ORF5	complement(<1..>172)	678	ATG		
				ORF6	<1..>172	624	ATG	
				ORF7	<1..>172	606	ATG	
transcri pt_7	98							
37286_rns7_1	transcri pt_1	108						
	transcri pt_2	133						
41777_rnl12_rns7_1	transcri pt_1	114	both					
43250_rns7_1	transcri pt_1	550	ORF1	<1..>550	642	GTG		

	transcri pt_2	99	both				
	transcri pt_3	133	both				
	transcri pt_4	118	both				
46689_cox3frag_ c12_1	transcri pt_1	293 2		ORF1	<1..1073	125 4	ATG
				ORF2	complement(139.. 840)	702	ATG
				ORF3	complement(194.. 1045)	852	ATG
				ORF4	218..922	705	ATG
				ORF5	complement(861.. 1745)	885	ATG
				ORF6	901..1686	786	ATG
				ORF7	932..1579	648	ATG
				ORF8	complement(1310 ..2269)	960	ATG
				ORF9	2125..2745	621	ATG
46689_non- coding_c13_1	transcri pt_1	871		ORF1	complement(527.. >871)	519	ATG
				ORF2	complement(621.. >871)	363	ATG
				ORF3	complement(280.. 738)	459	ATG
	transcri pt_2	130	both				
46919_cox3_cox 1_1	transcri pt_2	134	both	ORF2	32..>134	462	TGT
46919_cox3_cox 1_9	transcri pt_1	232 5		cox1 fragment, 1- 579 bp	complement(1452 ..2030)		
				cox3	complement(582.. 1451)		
				ORF1 cox1-cox3	complement(582.. 2282)	170 1	TCA
				ORF2	1978..2286	309	TCA
				<i>Plasmodium rn1</i> hit	170..247		

				<i>rn/1</i> transcript	137..263		
67435_non-coding_1	transcript_1	145	both				
	transcript_2	176	both				
	transcript_3	127	both	ORF1	<1..>127	327	CAA
	transcript_4	143	both	ORF1	<1..82	327	CAA
				ORF2	complement(69..>143)	318	TCG
	transcript_5	109	both	ORF4	complement(<1..>109)	360	GGA
	transcript_6	196	both	ORF4	<1..61	360	GGA
	transcript_7	361	both				
transcript_8	134	both					
67435_non-coding_2	transcript_1	120	both				
	transcript_2	133	both	ORF1	complement(<1..>133)	555	GAA
	transcript_3	116	both	ORF1	complement(<1..>116)	555	GAA
67435_non-coding_3	transcript_2	158	both				
	transcript_3	311	both				
	transcript_4	148	5	ORF1	<1..546	747	GAA
				ORF2	complement(<1..997)	102	AAC
				ORF3	102..1001	900	TTG
				ORF4	149..853	705	CAG
				ORF5	complement(279..>1485)	128	AAT
transcript_5	532		ORF6	complement(<1..232)	723	AAG	
			ORF7	complement(<1..475)	750	AAG	

					55)		
				ORF8	<1..>532	155	TTA 7
				ORF9	complement(519.. >532)	819	CCA
	transcri pt_6	102		ORF10	<1..>102	693	TCC
				ORF11	complement(<1..> 102)	618	CCC
				ORF8	<1..>102	155	TTA 7
				ORF9	complement(<1..> 102)	819	CCA
67435_non- coding_4	transcri pt_1	236	both	ORF1	<1..159	519	GAA
68201_non- coding_2	transcri pt_1	776	both	ORF1	154..588	435	CTT
				<i>Plasmodium rns5</i> hit	complement(658.. 675)		
68201_non- coding_3	transcri pt_1	607	both	ORF1	<1..419	465	NNN
				<i>Plasmodium rns5</i> hit	complement(489.. 506)		
68201_non- coding_7	transcri pt_1	376	both				
68201_non- coding_12	transcri pt_1	700	both				
68201_non- coding_13	transcri pt_1	606	both				
73351_non- coding_8	transcri pt_4	160	both				
73351_non- coding_10	transcri pt_1	156	both	ORF2	complement(<1..> 156)	447	ATC
73351_non- coding_12	transcri pt_1	150	both				
73351_non- coding_13	transcri pt_1	246	both	ORF1	complement(70..> 246)	384	GAA
	transcri pt_2	105	both	ORF1	complement(<1..> 105)	384	GAA
73351_rRNA_3	transcri	172					

	pt_1							
	transcri	122	both					
	pt_2							
73351_rRNA_4	transcri	153	both	ORF1	complement(<1..>	414	AGA	
	pt_3				153)			
	transcri	122	both					
	pt_4							
95117_non-coding_2	transcri	737	both	ORF2	complement(148..	333	TAT	
	pt_1				480)			
95117_non-coding_6	transcri	601	both					
	pt_1							
95117_non-coding_8	transcri	117	both	ORF1	643..1059	417	TCC	
	pt_1	4		ORF2	complement(207..	306	ATA	
					512)			
				<i>Plasmodium rns5</i>	complement(1056			
				hit	..1073)			
95117_non-coding_10	transcri	639	both					
	pt_1							
95117_non-coding_11	transcri	718	both	ORF1	186..512	327	ACT	
	pt_1							
95117_non-coding_12	transcri	102	both	ORF1	490..816	327	ACT	
	pt_1	2						
95117_non-coding_18	transcri	103	both	ORF1	complement(<1..1	390	AAG	
	pt_1				03)			
				ORF2	complement(<1..7	375	TGT	
					2)			
	transcri	577	both	ORF1	<1..234	390	AAG	
	pt_2			ORF2	<1..250	375	TGT	
				ORF3	complement(<1..3	327	TAT	
					20)			
				ORF4	complement(119..	321	CCC	
					439)			
				putative rRNA	5..123			
95117_non-coding_19	transcri	751	both	ORF1	2..424	423	TAC	
	pt_1							
				ORF2	complement(293..	321	CCC	
					613)			
95117_non-	transcri	132	both					

coding_30	pt_3							
95117_non-coding_35	transcript_1	115	both	ORF1	<1..>115	324	GTT	
				ORF2	complement(<1..13)	432	ATG	
95117_non-coding_38	transcript_3	104	both	ORF3	complement(71..>104)	333	GTT	
	transcript_4	304	both	ORF3	complement(<1..171)	333	GTT	
	transcript_5	138	both	ORF4	36..>138	321	GGC	
95117_non-coding_42	transcript_5	133	both					
rRNA transcripts, sorted by transcript name								
46919_cox3_cox1_1	<i>rn1</i> transcript	127		<i>cox1-cox3</i> transcript 1	complement(<1..>127)			
				<i>Plasmodium rn1</i> hit	34..111			
73351_rRNA_3	<i>rn1</i> transcript	196		<i>Plasmodium rn1</i> hit	80..158			
11012_rn18_1	<i>rn18</i> transcript	214		<i>Plasmodium rn18</i> hit	40..68			
114504_cox1_3_c1_1	<i>rn18</i> transcript	228		ORF2	complement(76..>228)	315	CTA	
				<i>Plasmodium rn18</i> hit	33..125			
114504_cox1_3_c1_10	<i>rn18</i> transcript	228		transcript 2	complement(<1..228)			
				ORF2	complement(76..>228)	315	CTA	
				<i>Plasmodium rn18</i> hit	33..125			
114504_cox1_c3_16	<i>rn18</i> transcript	231		transcript 2	complement(<1..231)			
				ORF2	complement(79..>231)	315	CTA	
				<i>Plasmodium rn18</i> hit	36..128			
114504_non-	<i>rn18</i>	179		<i>Plasmodium rn18</i> hit	<1..84			

coding_c2_10	transcript							
33769_cox1frag_1	<i>rnI8</i> transcript	171		<i>Plasmodium rnI8</i> hit	33..125			
3725_rRNA_1	<i>rnI8</i> transcript	206		<i>Plasmodium rnI8</i> hit	27..100			
75915_rnI8_1	<i>rnI8</i> transcript	444	both	<i>Plasmodium rnI8</i> hit	352..444			
13057_rnI12_1	<i>rnI12</i> transcript	137	both	<i>Plasmodium rnI12</i> hit	complement(75..106)			
41777_rnI12_rns7_1	<i>rnI12</i> transcript	164	both	<i>Plasmodium rnI12</i> hit	44..75			
73351_non-coding_10	<i>rnI12</i> transcript	175	both	ORF1	complement(<1..65)	339	TGG	
				ORF2	142..>175	447	ATC	
				<i>Plasmodium rnI12</i> hit	39..70			
73351_rRNA_3	<i>rnI12</i> transcript	189	both	<i>Plasmodium rnI12</i> hit	106..137			
95117_non-coding_35	<i>rnI12</i> transcript	190	both	ORF2	complement(141..>190)	432	ATG	
				<i>Plasmodium rnI12</i> hit	53..84			
46919_cox3_cox1_1	<i>rns1</i> transcript	261	both	ORF4	complement(<1..23)	318	TAC	
				<i>rns1</i> hit	56..108			
37286_rns7_1	<i>rns7</i> transcript	96		ORF1	<1..87	672	GTG	
				<i>Plasmodium rns7</i> hit	21..60			
41777_rnI12_rns7_1	<i>rns7</i> transcript	101		ORF1	complement(<1..>101)	363	TAC	

	pt			<i>Plasmodium rns7</i> hit	23..62		
43250_rns7_1	<i>rns7</i> transcript	109	both	<i>Plasmodium rns7</i> hit	27..66		
67435_rns7_1	<i>rns7</i> transcript	110	both	<i>Plasmodium rns7</i> hit	28..67		
73351_non-coding_8	<i>rns7</i> transcript	103	both	<i>Plasmodium rns7</i> hit	22..61		
73351_non-coding_10	<i>rns7</i> transcript	106	both	ORF1 <i>Plasmodium rns7</i> hit	complement(<1..> 106) 24..63	339	TGG
73351_non-coding_12	<i>rns7</i> transcript	105	both	<i>Plasmodium rns7</i> hit	23..62		
95117_non-coding_48	<i>rns7</i> transcript	103	both	<i>Plasmodium rns7</i> hit	22..61		
114504_cox1_c3_10	putative rRNA	105		cox1 transcript ORF1	complement(<1..1 05) complement(<1..2 7)	185	TTA 7
19169_cox1frag_1	putative rRNA	141					
46919_cox3_cox1_3	putative rRNA	122		cox1-cox3 transcript transcript 1	complement(<1..1 22) <1..122		
46919_cox3_cox1_8	putative rRNA	104		cox1-cox3 transcript transcript 1 ORF1	complement(<1..1 04) <1..104 complement(<1..5 1)	153	TTC 0
95117_non-	putative	119		transcript 2 ORF1	<1..>119 <1..>119	390	AAG

coding_18	rRNA		ORF2	<1..>119	375	TGT
			ORF4	complement(115..>119)	321	CCC
			ORF3	complement(<1..>119)	327	TAT
114504_cox1_3_c1_2	putative rRNA1	202	transcript 3	1..>202		
114504_cox1_3_c1_2	putative rRNA2	261				

Table S3. Genomic and transcriptomic read datasets generated in frame of this work (marked with an asterisk) and in frame of the *Chromera/Vitrella* genome project (Pain A, Otto, TD, Keeling PJ, Lukeš L, unpublished data), with those used for mitochondrial genome assembly indicated. Adapter removal and the following trimming and filtering settings were applied in CLC Genomics Workbench v. 6.5 to selected Illumina read datasets prior to mitochondrial genome assembly: terminal base runs with Phred quality below 20 were trimmed; longest read fragments with no more than one undetermined base were kept; read length threshold for filtered reads was set to 75 bp. No length threshold was applied to 54 bp reads of the mitochondrial DNA-enriched fraction. 454 reads were trimmed by the Newbler genome assembler itself prior to assembly.

Species	Library type	Sequencing platform	Expected average insert size	Average read length	Read number, million	Used for mitochondrial genome assembly	Estimated insert size range	Average read length after filtering	Read number in million after filtering
Genomic libraries									
<i>C. velia</i>	Illumina PCR-free	Illumina HiSeq	350	101	173				
<i>C. velia</i>	Illumina PCR-free	Illumina HiSeq	350	101	162				
<i>C. velia</i>	Illumina PCR-free	Illumina HiSeq	350	101	219				
<i>C. velia</i>	Illumina mate pair	Illumina HiSeq	8,000	101	152				
<i>C. velia</i>	Illumina	Illumina	3,000	101	133				

	mate pair	a HiSeq							
<i>C. velia</i>	Illumina mate-pair	Illumina HiSeq	3,000	101	47				
<i>C. velia</i>	Illumina Nextera mate pair	Illumina HiSeq	3,000	101	165	yes	1700-5500	95	53
<i>C. velia</i>	Illumina mate pair	Illumina MiSeq	8,000	250	8	yes	230-800	190	3.5
<i>C. velia</i>	Illumina mate pair	Illumina MiSeq	5,000	250	9	yes	230-800	183	3.7
<i>C. velia</i>	Illumina mate pair	Illumina MiSeq	3,000	250	12	yes	230-800	182	4.6
<i>C. velia</i> *	454 single reads	454 GS FLX	N/A	224	0.29	yes	N/A		
<i>C. velia</i> *	454 single reads	454 GS FLX	N/A	225	0.3	yes	N/A		
<i>C. velia</i>	454 single reads	454 GS FLX	N/A	294	0.61	yes	N/A		
<i>C. velia</i>	454 single reads	454 GS FLX	N/A	326	0.63	yes	N/A		
<i>C. velia</i>	454 single reads	454 GS FLX	N/A	292	0.63	yes	N/A		
<i>C. velia</i>	454 single reads	454 GS FLX	N/A	304	0.73	yes	N/A		
<i>C. velia</i> *	Illumina mate pair	Illumina HiSeq	330	54	19	yes	240-	54	13

	TruSeq, mitochondrial DNA-enriched fraction	a GAI				820
<i>V. brassicaeformis</i>	Illumina TruSeq + Kappa Hifi	Illumina HiSeq	350	101	288	
<i>V. brassicaeformis</i>	Illumina TruSeq + Kappa Hifi	Illumina HiSeq	350	101	203	
<i>V. brassicaeformis</i>	Illumina PCR-free	Illumina HiSeq	500	93	155	
Transcriptomic libraries						
<i>C. velia</i>	Illumina TruSeq strand-specific	Illumina HiSeq	300	101	93	
<i>C. velia</i>	Illumina TruSeq strand-specific	Illumina HiSeq	300	101	118	
<i>V. brassicaeformis</i>	Illumina TruSeq strand-specific	Illumina HiSeq	300	101	195	

Table S4. Statistics for the best *Chromera* genome assembly made with Newbler v.2.9, from which mitochondrial contigs were extracted. The assembly was made from read sets indicated in supplementary table S3. Extensive tuning of assembly parameters (data not shown) was employed to achieve as complete assembly of mitochondrial coding sequences as possible.

assembly statistics		insert size estimated by Newbler v.2.9, bp
# single 454 reads	3,188,77	N/A
	4	
# Illumina mate pair reads	26,674,2	1700-5500
	72	
# Illumina paired-end MiSeq reads	11,789,8	230-800
	18	
# Illumina paired-end reads of the mitochondrial DNA-enriched fraction	13,136,9	240-820
	66	
# total reads	54,789,8	
	30	
# reads assembled	45,731,2	
	47	
total assembly size, Mbp	256	
# scaffolds	34,422	
scaffold length threshold, bp	1,000	
largest scaffold, bp	5,019,58	
	7	
scaffold N50, bp	9,382	
large contig length threshold, bp	500	
contig N50, bp	5,465	

Supplementary references:

Jirků M, Kolesnikov AA, Benada O, Lukeš J. 1995. Marine fish and ray trypanosomes have large kinetoplast minicircle DNA. *Mol Biochem Parasitol.* 73:279-283.

Lukeš J, Jirků M, Avliyakov N, Benada O. 1998. Pankinetoplast DNA structure in a primitive bodonid flagellate, *Cryptobia helicis*. *EMBO J.* 17:838-846.