

Supplementary Material

Cultures

T. subtilis was grown with *Imantonia rotunda* as food source in Erdschreiber medium (Foyt 1934) at 16°C for 14 days under light/dark cycles of 14h/10h, respectively, and finally grown in dark for periods of 5-10 days to maximize the *Telonema* cells from the culture. About 2 L of *T. subtilis* culture were harvested by centrifugation at 4000 rpm for 10 min and flash frozen in liquid nitrogen. *R. contractilis* was monoxenically grown with the green alga *Chlorogonium elongatum* as food source as described earlier (Sakaguchi and Suzuki 1999). The cells were cultured at 20°C for 14 days under light/dark periods of 14h/10h, respectively. After confirming that most of the *C. elongatum* cells were consumed by *R. contractilis*, ~0.9 liters of culture was collected by centrifugation at 500g for 3 min and the cells were transferred into RNAlater solution (Ambion, Austin, USA). *Plagioselmis nannoplanctica* culturing will be described elsewhere as part of an independent project involving *Colloidietyon*.

cDNA library construction and 454 pyrosequencing

Normalized cDNA libraries were constructed by Vertis Biotechnology AG (Germany) according to their Random-Primed (RPD) cDNA protocol. Frozen cells were ground under liquid nitrogen and total RNA isolated from the cell powder using the mirVana RNA isolation kit (Ambion). Poly(A)⁺ RNA was prepared from total RNA. First-strand cDNA synthesis was primed with a N6 randomized primer and second-strand cDNA was synthesized according to the classical Gubler-Hoffman protocol (Gubler and Hoffman 1983). Double stranded DNA (dsDNA) was blunted and 454 adapters A and B ligated to the 5' and 3' ends. dsDNA carrying both adapter A and adapter B attached to its ends was selected and amplified with PCR using a proof reading enzyme (24 cycles). To ensure a reduction in highly expressed genes, an equalization of the gene representation was performed with a method developed by Vertis Biotechnology. For 454 sequencing the cDNA in the size range of 250 – 600 bp was eluted from a preparative agarose gel. Half a plate of a GSFLX instrument (Standard chemistry) was sequenced for *T. subtilis* by the Norwegian ultra-high throughput sequencing service unit at the University of Oslo, yielding about 210,000 reads. For *R. contractilis*, half a plate was sequenced by Macrogen Inc (South-Korea) generating about 360,000 reads.

Bootstrapping of genes analyses

Bootstrapping analyses in which the genes instead of the sites are randomly sampled from the total number of genes were performed. We constructed 200 replicates, each containing 127 concatenated genes, which resulted in supermatrices of variable length (ranging from 25,045 aa to 33,017aa). This procedure was repeated on 2 sets of species, that is with *T. subtilis* and *R. contractilis* included (**Supp. Figure 1**) and excluded (**Supp. Figure 2**). Overall, the relationships we obtained were very similar to those based on the original alignment. In particular, this approach confirmed the evolutionary affinities of telonemids, centrohelids et haptophytes. In **Supp. Figure 1**, cryptomonads weakly branched together with some excavates (Discoba, Hampl et al. 2009). After removing *T. subtilis* and *R. contractilis*, cryptomonads were placed again as sister to haptophytes (**Supp. Figure 2**). Importantly, we could not detect any clear correlation between the inferred evolutionary relationships and the length of the alignments or the combination of genes that were sampled in each replicate. Hence, the bootstrapping of genes approach did not reveal any obvious conflict in the phylogenetic signal in the data.

Phylogenetic analyses after removing both, or one of *T. subtilis* or *R. contractilis*

In order to see the impact on the topology and supports of *T. subtilis* and *R. contractilis*, analyses were performed using concatenated datasets that did not contain these species (**Supp. Figure 3**), as well as with one or the other removed (**Supp. Figure 4 and 5**). In all cases the major groups of eukaryotes were recovered as in Figure 1 and the relationships among them were very consistent. Interestingly, we observed more robust support for the association between cryptomonads and haptophytes (corresponding to node 1 in Figure 1, see the main text) and the sister position of this grouping to SAR (node 2 in Figure 1) when both species were not included (**Supp. Figure 3**). This is consistent with our interpretation of the ancient origin of telonemids and centrohelids. Indeed, if relatively few sequence synapomorphies accumulated during a brief period of shared common ancestry with cryptomonads and haptophytes, and even fewer now remain following hundreds of millions of years of divergence, one expects that *T. subtilis* and *R. contractilis* will randomly branch elsewhere in the tree, thus lowering the statistical support for the whole CCTH/CCTH-SAR groups.

A “separate” analysis was also conducted on the dataset that lacked *T. subtilis* and *R. contractilis*. Here we specifically examined the relationships among 5 major groups — (1) cryptomonads plus haptophytes, (2) SAR group, (3) Plantae, (4) excavates, and (5) unikonts (opisthokonts + Amoebozoa). 123 genes (amounting to a total of 28’166 aa), which contained at least one representative taxon for each group of interest, were selected from the 127 genes used in the concatenation. The best tree was identical to the Bayesian and ML analyses of the supermatrix and REL values were consistently higher than those on Figure 1 (**Supp. Figure 3**).

When *R. contractilis* was removed from the alignment in isolation (**Supp. Figure 4**), *T. subtilis* branched within a clade also including cryptomonads and haptophytes (CTH group), and this group was sister to SAR. The Bayesian and ML approaches gave two different unsupported topologies for the position of *T. subtilis* within the CTH group, a poor resolution that was also observed in Figure 1. This means that adding *R. contractilis*, another enigmatic lineage that likely diverged soon after the origin of the CCTH-SAR grouping, did not help in recovering a good support for placing the telonemids. When *T. subtilis* was removed to see how *R. contractilis* alone influenced the results (**Supp. Figure 5**), we again recovered the same major eukaryotic groups and relationships, notably an assemblage enclosing cryptomonads, haptophytes, and centrohelids (CCH group) and its sister position to SAR. Consistent with our previous observations, the CCH group and the CCH-SAR relationship received in this analysis the lowest ML support of all analyses (60% BP), indicating once more that only weak phylogenetic signal remains in centrohelids sequences (probably less than in telonemids). It is worth noting that this lack of sequence synapomorphies resulting in poor phylogenetic signal is likely the main reason for the many unsuccessful attempts to place centrohelids in the tree of eukaryotes until this study (Cavalier-Smith and Chao 2003; Sakaguchi et al. 2005; Cavalier-Smith and von der Heyden 2007; Sakaguchi et al. 2007).

Topology comparisons based on the supermatrices

To better assess the phylogenetic position of *T. subtilis* and *R. contractilis*, we conducted topology comparisons using the approximately unbiased (AU) test. For each tested tree, per-site log likelihoods for the supermatrices were calculated using RAxML (Stamatakis 2006) and the AU tests were performed using CONSEL (Shimodaira and Hasegawa 2001) with default scaling and replicate values. The test trees were constructed by using the Baye-

sian and ML topologies shown in Figure 1, Supp. Figure 2 and Supp. Figure 3 and placing *T. subtilis* and *R. contractilis* on different branches (we did not test positions within monophyletic groups that received maximal supports) (**Supp. Figure 6, A-E**). These analyses generally confirmed the trends observed in the tree reconstructions, that is: (1) alternative positions for *T. subtilis* cannot be rejected, but only if placed within or sister to the CCTH or CTH groups; an exception was a non-rejected position on the branch leading to the red algae when *R. contractilis* was absent from the alignment (**Supp. Figure 6D**), but this branching was discarded when *R. contractilis* was present, underlying the importance of taxon-sampling (**Supp. Figure 6A and B**). (2) Alternative positions for *R. contractilis* even outside that of the CCTH-SAR groupings were kept in the set of plausible trees, precisely as sister to or within the excavates (**Supp. Figure 6B and E**); a sister relationship to the red algae was also accepted when *T. subtilis* was absent (**Supp. Figure 6E**), but it was similarly rejected when both species were analyzed together (**Supp. Figure 6 A and B**). Altogether these analyses suggest once more the early origin of telonemids and centrohelids; because several deep branchings could not be rejected for centrohelids, it is possible that this group diverged even earlier.

The AU tests retained in the pool of candidate trees a relationship between *T. subtilis* (or *R. contractilis*) and red algae when only one of these two species was considered. Although no obvious relationship with red algae was found in our single-gene tree reconstructions, this signal could be explained by genes of red origin that were transferred from a red algal endosymbiont to the nucleus in the ancestor of CCTH-SAR if the chromalveolate hypothesis is correct (Lane and Archibald 2008). In this context, it is interesting to note that a relationship between *R. contractilis* and red algae was previously observed on 18S rRNA (Cavalier-Smith and Chao 2003) and α - and β -tubulin phylogenies (Sakaguchi et al. 2005) and was not rejected in an analysis of six housekeeping genes (Sakaguchi et al. 2007). Yet neither of these genes has been shown to have a red algal ancestry in chromalveolate species.

References

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Table S1. OTU (Operational Taxonomic Unit) names and chimera constructions. Percentage of missing data per species and per genes

Taxon	Species in chimera	14-3-3
<i>Aureococcus anophagefferens</i>		0
<i>Acanthamoeba castellanii</i>		0
<i>Alexandrium</i>	Alexandrium fundyense (2%), Alexandrium tamarense (4	0
<i>Arabidopsis thaliana</i>		0
<i>Batrachochytrium dendrobatidis</i>		0
<i>Bigelowiella natans</i>		19
<i>Chlamydomonas</i>	Chlamydomonas incerta (34%), Chlamydomonas reinhardi	0
<i>Cercomonas longicauda</i>		0
<i>Cryptococcus neoformans</i>		0
<i>Cyanophora paradoxa</i>		0
<i>Cryptosporidium</i>	Cryptosporidium hominis (14%), Cryptosporidium parvum	0
<i>Dictyostelium discoideum</i>		0
<i>Danio rerio</i>		0
<i>Drosophila</i>	Drosophila melanogaster (94%), Drosophila pseudoobscura	0
<i>Euglena gracilis</i>		0
<i>Emiliana huxleyi</i>		0
<i>Eimeria tenella</i>		100
<i>Gracilaria changii</i>		14
<i>Gymnophrys cometa</i>		100
<i>Gallus gallus</i>		0
<i>Glaucocystis nostochinearum</i>		39
<i>Galdieria sulphuraria</i>		0
<i>Guillardia theta</i>		0
<i>Histiona aroides</i>		1
<i>Homo sapiens</i>		0
<i>Hartmannella vermiformis</i>		0
<i>Isochrysis galbana</i>		0
<i>Imantonia rotunda</i>		100
<i>Jakoba</i>	Jakoba bahamensis (40%), Jakoba libera (36%)	0
<i>Karlodinium micrum</i>		0
<i>Laminaria sp</i>		100
<i>Malawimonas</i>	Malawimonas jakobiformis (52%), Malawimonas californica	0
<i>Mastigamoeba balamuthi</i>		0
<i>Monosiga brevicollis</i>		0
<i>Mus musculus</i>		0
<i>Mesostigma viride</i>		0
<i>Naegleria</i>	Naegleria gruberi (89%), Naegleria fowleri (2%)	0
<i>Neurospora crassa</i>		0
<i>Nematostella vectensis</i>		0
<i>Ostreococcus lucimarinus</i>		0
<i>Oxyrrhis marina</i>		0
<i>Oryza sativa</i>		0
<i>Paramecium</i>	Paramecium caudatum (2%), Paramecium tetraurelia (77	0

<i>Phycomyces blakesleeenanus</i>		0
<i>Phytophthora</i>	Phytophthora infestans (50%), Phytophthora palmivora (1	1
<i>Plasmodium</i>	Plasmodium berghei (4%), Plasmodium yoelii (71%), Plas	0
<i>Pavlova lutheri</i>		0
<i>Perkinsus marinus</i>		15
<i>Plagioselmis nannoplanctica</i>		100
<i>Pythium oligandrum</i>		14
<i>Porphyra</i>	Porphyra purpurea (1%), Porphyra yezoensis (63%)	100
<i>Prymnesium parvum</i>		0
<i>Physcomitrella patens</i>		0
<i>Physarum polycephalum</i>		24
<i>Pinus taeda</i>		0
<i>Populus trichocarpa</i>		0
<i>Phaeodactylum tricorutum</i>		0
<i>Reclinomonas americana</i>		100
<i>Raphidiophrys contractilis</i>		0
<i>Rattus norvegicus</i>		0
<i>Schizochytrium sp</i>		69
<i>Stachyamoeba lipophora</i>		29
<i>Schizosaccharomyces pombe</i>		0
<i>Solanum tuberosum</i>		0
<i>Tetrahymena</i>	Tetrahymena pyriformis (7%), Tetrahymena thermophila (0
<i>Toxoplasma gondii</i>		0
<i>Theileria</i>	Theileria annulata (64%), Theileria parva (57%)	0
<i>Thalassiosira pseudonana</i>		1
<i>Trimastix pyriformis</i>		0
<i>Telonema subtilis</i>		0
<i>Ustilago maydis</i>		0
<i>Volvox carteri</i>		0
% missing positions		13
% missing OTUs		10
sequence length		168

Species in red represent the species with new data generated for this study
Genes in blue are the genes that were omitted for the separate analyses

	actin	arf3	arpc1	atp6	calm3	calr	capz	cct-A	cct-B	cct-D	cct-E	cct-N	cct-T	cct6A	crfg	ctsl1	ef2-EF2
	0	0	0	0	0	0	0	0	0	38	0	0	100	0	0	3	3
	0	42	100	0	100	36	8	43	66	100	43	100	100	100	100	0	100
50	0	0	100	3	0	100	100	100	100	100	100	100	100	62	100	100	73
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
1	0	51	0	0	1	0	0	52	100	66	39	100	100	100	100	0	71
0	100	100	0	0	1	0	100	15	0	64	0	0	63	100	65	100	0
2	0	100	0	0	21	100	21	100	55	100	23	60	100	100	100	100	43
0	0	0	0	0	0	100	100	0	0	0	0	0	0	0	0	100	0
1	0	100	0	0	0	100	100	57	47	79	63	100	100	100	100	100	78
0	0	0	0	0	0	100	100	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	42	0	0	0	0	0	0	0	0
100	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	6	0	0	0	0	100	100	100	100	100	100	100	100	100	100	0
0	0	0	0	0	0	0	34	10	1	58	2	0	53	0	0	0	0
15	100	100	100	100	100	100	100	100	100	100	100	100	64	100	100	100	56
24	1	100	0	10	100	100	100	70	100	100	58	100	100	100	100	100	76
47	0	100	100	100	100	100	100	76	100	100	100	100	100	61	100	42	100
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	100	0	0	66	100	100	100	100	100	100	100	100	100	100	100	53
23	0	100	100	100	100	100	100	100	100	100	100	51	100	50	100	100	74
0	0	100	0	0	100	100	100	52	51	100	55	53	100	27	30	0	33
100	0	31	0	0	100	100	100	64	100	100	100	100	69	100	100	0	100
0	0	0	0	0	0	0	5	0	0	18	0	0	0	0	0	0	0
0	0	56	12	20	23	25	100	52	100	100	100	100	100	100	24	100	47
0	5	58	0	1	20	100	100	100	100	100	100	78	100	41	100	100	46
50	18	75	53	54	100	100	100	100	100	100	58	100	100	100	100	100	100
0	0	46	0	0	100	100	100	74	100	53	100	100	100	100	100	0	0
0	0	100	100	0	0	100	100	100	100	100	100	100	100	100	100	100	72
38	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
0	0	100	0	0	66	2	14	100	100	100	81	100	100	100	100	100	63
0	0	100	0	46	0	26	100	100	100	39	45	100	27	100	100	100	44
0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	100	0
0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0
32	0	40	0	20	100	100	100	60	100	100	100	100	100	100	100	100	86
0	0	0	0	0	0	0	7	0	0	0	2	0	2	0	0	0	0
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0	0	0	0	0	0	0	9	0	7	0	0	0	0	0	0	0	1
0	0	100	0	0	100	100	0	0	0	0	0	0	0	0	0	100	0
39	0	100	3	0	30	100	57	64	100	100	100	100	100	100	26	10	81
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0	11	0	0	0	100	100	0	0	0	0	0	0	8	0	0	100	44

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0	0	57	0	3	51	35	0	0	2	0	0	0	55	0	0	0
0	0	0	0	0	100	2	0	0	0	0	0	0	0	0	100	0
0	0	52	0	1	60	100	62	100	100	52	100	100	72	100	100	2
0	25	100	17	37	100	100	78	86	100	100	76	100	60	51	100	33
81	22	100	100	100	100	100	100	100	100	100	100	100	100	100	33	100
24	0	100	0	3	100	100	55	61	100	100	100	100	66	100	50	61
0	100	100	0	1	100	100	71	100	27	76	100	63	100	9	100	0
25	0	100	0	19	20	100	67	49	48	100	100	100	100	100	100	100
0	22	100	0	0	39	100	42	60	31	35	13	40	71	100	14	34
0	1	55	100	100	0	11	50	100	33	100	0	100	69	0	33	0
0	0	46	0	1	0	100	37	11	15	16	0	9	59	5	11	33
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	100	0	1	0	0	2	2	0	3	0	4
0	0	21	0	0	45	100	100	74	100	77	100	56	100	100	100	100
2	0	62	0	28	0	0	64	57	46	100	19	100	59	35	0	0
0	0	0	100	0	0	0	0	0	0	0	0	0	48	100	0	0
47	0	64	1	0	100	100	100	100	100	100	100	100	100	100	100	82
1	100	49	100	100	100	100	100	100	100	100	100	100	100	100	24	33
0	0	0	0	1	100	100	0	0	0	0	0	0	0	0	100	0
20	0	32	0	0	8	0	45	46	48	48	28	100	57	18	100	57
0	1	0	0	0	100	100	0	1	0	0	0	0	0	0	0	0
0	0	0	100	1	100	0	12	5	10	0	8	36	100	19	0	0
3	0	100	0	0	100	100	69	17	59	100	0	14	0	0	100	0
3	0	0	0	14	0	100	0	2	2	0	2	0	0	0	100	0
0	0	100	15	19	0	100	100	70	100	86	100	63	100	100	0	0
61	3	36	0	44	0	65	22	50	67	100	36	100	69	66	0	16
0	0	100	0	0	100	100	0	0	0	0	0	0	0	0	100	0
0	0	0	0	1	0	100	0	0	0	0	0	0	0	3	100	0
11	9	48	15	16	48	60	44	44	50	45	44	53	49	47	54	31
3	7	36	14	11	42	57	28	28	40	32	38	46	36	42	51	11
363	158	326	130	147	187	155	469	435	403	422	425	298	418	295	129	654

fh	fibri	gdi2	gln	gnb2l	gnb	gnbpa	grc5	h3	h4	hla-B	hmt1	hsp90	if2b	if2g	if6	ino1
0	100	9	100	0	0	0	0	0	0	0	100	0	0	0	0	100
34	0	3	26	29	100	26	0	2	0	2	48	100	20	94	0	49
100	37	100	100	100	100	100	14	100	100	40	100	2	100	51	100	100
0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
5	0	0	35	0	0	0	0	0	0	0	0	21	0	0	0	0
100	34	100	100	39	53	100	1	0	0	100	100	29	100	100	0	100
100	0	100	63	0	100	100	0	100	0	100	1	63	0	7	39	0
58	26	100	100	56	7	29	100	100	100	0	100	100	100	100	100	3
0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0
100	100	44	100	0	100	100	0	0	13	46	100	4	100	100	100	100
100	0	11	0	0	100	100	0	0	0	0	0	0	0	0	0	100
0	0	0	24	0	0	0	0	0	6	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
100	0	100	100	0	100	100	0	0	100	0	100	4	100	100	0	100
11	0	0	0	0	0	1	0	0	0	0	100	0	0	13	0	100
100	100	100	100	100	100	100	60	100	0	100	100	100	100	27	14	100
100	49	100	100	23	100	100	0	0	0	32	20	63	100	100	12	100
100	66	37	100	100	100	100	15	100	100	40	32	100	100	100	100	100
0	100	0	0	0	0	0	4	0	0	0	0	0	0	0	0	100
100	100	5	100	100	100	100	0	0	0	39	57	100	100	64	14	100
100	13	100	100	100	100	100	0	1	0	38	100	100	0	100	100	41
100	100	100	100	100	100	100	0	0	23	100	27	3	100	48	0	38
100	100	42	100	35	100	100	0	100	17	100	100	72	100	100	18	100
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	100	2	100	2	100	33	0	17	100	100	100	100	100	100	100	100
100	100	0	100	100	100	100	0	20	100	36	100	3	100	1	6	100
100	19	100	100	55	100	65	53	48	100	100	100	100	100	100	100	100
37	24	30	100	0	100	27	15	100	100	9	100	62	100	100	100	0
100	18	47	100	100	100	100	0	100	100	36	100	0	100	46	17	100
100	100	100	100	32	100	100	0	100	100	100	100	75	10	100	100	100
100	11	49	100	0	100	100	0	100	0	100	100	56	0	100	9	100
100	6	31	0	0	100	12	0	0	0	0	4	100	100	100	15	0
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0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
100	100	100	34	0	100	100	10	0	100	100	100	100	100	72	100	100
0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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100	42	52	100	16	100	100	100	100	100	100	100	0	0	100	17	100
0	0	0	0	0	0	9	0	2	0	8	0	0	0	2	0	0
0	0	0	0	100	100	100	0	0	7	100	0	39	100	0	0	0

0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	26	100	34	0	0	0	0	0	39	28	5	0	0	0	0
100	0	0	0	0	100	100	0	0	0	0	0	0	0	0	0	100
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100	0	100	100	1	100	100	0	0	0	18	100	10	100	63	5	100
100	100	100	100	100	100	100	59	100	100	100	100	100	100	100	100	100
100	9	52	100	22	100	100	0	26	100	28	100	49	0	61	0	57
100	100	100	100	100	100	100	4	0	0	100	100	72	100	39	17	13
100	100	100	100	29	100	100	12	0	100	27	100	46	19	100	63	54
60	0	26	100	41	0	100	0	1	17	33	39	46	0	0	0	1
73	16	28	100	11	0	0	0	5	100	0	0	0	100	17	100	17
41	0	45	100	2	20	45	0	0	0	27	12	100	0	0	0	0
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73	12	17	100	42	31	0	0	0	0	44	58	0	7	70	39	43
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
55	100	100	100	46	100	100	41	0	0	48	100	100	100	100	100	79
100	100	100	100	10	66	43	8	100	100	100	51	79	100	100	100	100
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64	0	19	100	0	40	100	0	0	0	50	23	39	0	50	0	28
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100	0	100	54	0	100	100	0	0	0	0	100	100	0	2	0	100
0	0	0	0	0	0	0	0	5	0	0	0	0	7	3	0	0
100	0	26	100	0	100	17	0	100	100	0	0	100	100	100	0	50
46	29	43	100	0	100	64	41	0	22	0	100	12	100	73	77	86
0	0	0	12	0	0	0	0	0	0	0	0	100	0	0	0	0
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58	29	37	56	23	60	55	9	23	31	37	49	34	41	43	27	49
50	24	28	53	15	57	50	4	21	29	28	43	22	40	31	21	42
427	211	254	175	223	235	206	164	133	101	309	244	479	107	375	211	352

l12e-A	l12e-D	mcm-A	metap2	ndf1	nop	nsf1-G	nsf1-I	nsf1-J	nsf1-K	nsf1-L	nsf1-M	nsf2-A	nsf2-F	oplah	orf2	osgep
0	0	0	0	0	0	0	0	0	0	0	100	0	19	100	0	0
0	0	100	70	88	36	0	100	33	0	29	20	69	100	91	0	100
0	59	100	100	100	100	100	100	45	100	100	35	100	100	100	100	100
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100	0	100	30	100	100	100	100	44	100	100	100	67	100	100	100	100
0	0	7	100	0	100	0	0	0	41	0	77	0	17	100	100	100
2	15	100	100	81	100	100	100	100	0	100	100	100	100	100	100	100
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0	0	0	0	100	0	0	0	0	0	0	0	0	0	100	0	0
0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	2	0
0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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0	0	100	0	0	0	0	16	0	7	27	0	0	100	0	0	100
0	31	100	100	100	100	100	100	30	90	100	100	55	100	100	100	100
100	0	100	42	100	54	100	29	100	100	100	12	45	100	100	100	100
0	100	72	100	100	100	35	100	100	100	100	100	100	100	100	100	100
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0	0	100	29	42	100	14	0	0	100	0	100	14	100	100	8	100
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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0	0	100	0	0	100	100	100	0	100	100	100	100	100	100	25	100
39	71	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
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100	63	100	33	100	47	100	0	0	100	100	20	42	100	100	100	100
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100	0	0	0	0	0	0	17	0	0	0	0	0	0	0	5	0
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0	0	0	0	0	0	0	0	0	0	0	100	0	0	7	0	0
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100	100	100	100	100	100	100	100	100	27	100	100	100	100	100	100	100
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0	0	0	0	100	100	0	0	0	0	0	0	0	0	100	0	100
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100	100	100	100	100	89	63	50	48	8	71	23	71	100	36	100	100
100	100	100	100	100	100	100	100	100	100	100	100	68	100	100	100	100
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0	0	80	17	100	29	0	0	0	48	0	0	0	25	77	0	25
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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2	0	100	77	53	72	36	47	34	100	0	100	25	36	100	46	100
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23	12	57	44	55	49	44	42	35	43	42	43	40	50	66	43	61
22	8	53	36	44	42	39	39	28	36	38	39	28	42	60	42	57
94	148	320	273	402	229	264	205	233	212	225	257	493	321	573	155	271

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0	100	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0
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100	18	24	41	100	100	100	100	100	100	48	0	100	100	6	52	0
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100	40	6	100	0	100	0	0	0	100	100	28	100	100	20	65	0
100	42	7	0	38	100	12	0	0	100	100	100	100	100	16	25	15
100	14	100	16	13	100	0	100	0	100	100	33	100	100	0	100	100
8	100	0	0	0	0	0	0	0	0	28	60	0	0	0	0	0
21	17	100	100	100	100	100	100	100	100	100	100	100	41	32	42	0
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0	0	0	0	1	0	2	0	0	0	0	0	100	0	0	0	0
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100	0	0	0	0	0	100	0	0	0	100	0	100	0	0	0	0
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100	100	100	100	100	100	100	100	100	100	69	100	44	100	100	100	0
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0	14	0	0	5	0	100	0	0	0	0	28	0	100	0	31	0
0	0	0	0	0	28	0	6	0	0	0	100	0	0	0	100	0
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54	3	0	27	43	20	0	7	100	100	0	0	100	47	0	45	0
14	0	9	39	0	0	0	0	0	69	0	0	100	3	0	0	0
100	100	6	0	100	100	100	100	100	0	100	100	100	100	0	35	0
65	3	5	62	15	8	27	0	32	0	0	100	100	80	0	14	2
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0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0	1
51	43	26	32	37	31	35	36	32	40	47	44	50	49	22	44	15
47	38	22	26	33	28	33	32	26	38	39	35	47	38	19	31	14
154	295	175	180	162	149	162	169	157	172	257	273	156	276	173	352	163

	rpl12b	rpl15a	rpl16b	rpl17	rpl18	rpl19a	rpl1	rpl20	rpl21	rpl26	rpl27	rpl2	rpl30	rpl32	rpl3	rpl4B	rpl5
	0	0	0	0	0	0	100	0	0	50	100	0	0	0	0	0	0
100	20	0	17	28	0	100	0	0	50	0	23	0	0	17	0	0	0
9	33	0	0	20	1	100	0	37	50	0	100	0	35	100	100	100	0
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0	100	0	0	0	0	0	0	0	0	100	0	0	0	0	36	100	0
2	100	0	100	0	0	0	0	100	100	100	100	100	0	0	100	8	4
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0	6	0	0	0	0	100	1	100	100	0	11	0	0	3	39	0	0
100	100	66	7	100	0	72	54	100	100	100	100	29	100	84	100	100	0
0	8	0	0	3	0	100	0	0	100	0	100	0	0	0	0	0	0
100	100	21	100	33	100	100	0	100	100	0	100	0	100	41	100	100	0
15	7	0	0	38	0	14	18	1	50	100	33	100	17	56	42	32	0
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100	0	0	0	100	100	0	1	4	50	17	8	0	100	0	15	0	0
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0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0
100	0	7	0	8	0	100	0	100	100	0	28	0	15	46	38	38
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47	100	52	100	100	33	100	27	100	100	100	63	0	100	64	100	100
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100	20	0	0	61	0	11	0	0	50	0	12	0	0	0	0	0
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34	0	0	100	0	0	100	0	0	50	0	100	0	100	0	0	0
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100	100	0	0	0	1	0	100	0	50	0	100	0	0	100	0	0
0	10	100	100	0	0	50	0	0	100	100	36	100	0	50	100	100
100	20	34	100	100	0	0	100	100	100	100	3	0	100	35	54	9
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0	0	0	0	22	0	0	22	0	50	0	0	0	0	0	0	14
0	0	0	0	0	0	0	0	0	50	1	0	0	0	0	0	0
0	2	0	0	0	0	0	0	3	50	0	3	100	4	0	2	0
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0	1	0	0	5	0	20	100	100	100	0	0	0	100	14	0	100
0	0	0	23	22	0	0	0	0	50	13	0	0	0	0	0	34
0	0	0	0	0	0	0	0	8	100	0	0	0	0	10	0	0
0	0	0	12	0	0	0	0	0	50	0	0	0	0	0	0	0
14	13	15	18	18	9	25	16	24	63	20	17	12	25	17	20	17
12	10	12	15	14	8	22	14	22	26	19	14	11	24	6	14	14
130	184	152	121	130	141	169	113	98	230	113	242	91	100	352	211	157

	rpl7-A	rpl9	rpp0	rps10	rps11	rps13a	rps14	rps15	rps16	rps18	rps1	rps22a	rps23	rps2	rps3	rps4	rps5
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	100	100	100	0	0	0	2	0	0	1	69
12	0	100	100	12	1	0	0	3	100	4	88	0	0	49	67	29	10
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16	100	14	100	100	0	100	1	100	0	0	0	0	19	11	5	21	100
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	100	100	100	0	0	100	44	0	2	100	27	0	100	100	8	3	
0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
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7	0	5	100	0	0	0	0	0	0	40	100	0	0	13	0	0	0
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0	0	0	0	0	26	100	0	0	0	0	0	0	0	0	8	0	0
37	100	5	13	35	7	0	0	2	0	0	9	1	0	38	0	50	0
0	0	100	0	100	100	0	100	30	49	100	100	100	37	100	100	100	100
0	0	0	0	0	0	0	0	0	100	100	0	0	14	0	0	100	
0	0	100	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0
100	100	100	0	0	0	0	100	100	0	10	100	0	33	0	100	0	
1	0	0	0	0	0	0	0	0	100	0	1	0	0	0	0	0	0
20	100	100	100	100	100	100	100	100	100	100	100	100	100	34	0	100	100
0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
5	3	39	0	11	0	46	100	0	0	1	0	0	3	10	0	0	0
34	0	1	100	3	0	0	100	0	0	100	1	9	2	50	12	18	
100	100	51	17	100	100	50	27	100	50	100	42	100	44	100	41	100	
0	100	8	100	0	0	0	0	100	100	0	100	0	0	0	8	3	
100	100	100	100	0	0	100	100	100	100	47	0	29	4	5	25	100	
100	26	29	0	1	0	8	100	100	21	24	0	100	57	5	13	8	
0	0	100	0	0	0	0	0	0	0	100	0	0	13	100	0	0	
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13	2	23	0	0	100	0	6	0	100	100	32	3	0	0	8	0	
0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	
0	0	0	100	0	0	100	5	100	100	100	0	17	0	0	5	0	

3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	100	100	0	100	100	0	0	100	100	5	100	0	0	100	9	29
100	100	0	100	6	0	1	44	100	26	25	0	12	20	0	41	45
100	100	100	100	38	68	43	44	18	100	27	100	100	100	62	68	64
6	0	7	0	6	18	0	0	0	0	7	0	0	13	0	1	0
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100	0	33	18	100	0	0	0	100	100	0	100	52	0	2	47	0
0	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0	0
100	0	0	0	2	0	0	0	0	0	6	0	0	0	17	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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0	0	1	0	0	0	0	1	3	4	0	0	1	0	0	0	0
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0	18	1	0	0	56	0	0	9	0	0	0	0	0	20	8	0
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12	100	53	0	6	100	100	0	100	100	20	24	0	13	100	40	0
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0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
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28	0	0	100	1	26	0	7	0	0	73	0	2	0	0	0	0
0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	17	0	0	0	60	0	0	0	0	0	0	0
0	1	0	0	0	0	0	1	0	4	0	0	0	0	0	0	0
0	0	0	100	100	0	0	0	0	0	0	0	0	0	0	65	100
0	0	1	0	22	60	2	0	0	1	14	0	0	0	0	0	13
0	100	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	22	19	26	12	15	20	15	22	20	20	13	12	10	13	11	15
15	21	15	25	10	11	18	12	21	17	14	11	10	3	8	4	11
169	121	199	78	116	145	128	124	127	121	214	130	129	198	181	237	175

0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
0	0	0	0	0	0	12	0	2	0	0	0	0	0	0	0	11
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	8	100	100	100	100	100	100	100	100	0	6	43	0	35	100	20
33	0	5	100	71	62	37	7	100	100	0	0	100	0	13	12	100
100	100	52	100	100	100	100	100	100	100	100	76	100	100	80	100	100
0	0	40	100	71	18	100	100	100	100	49	45	100	0	80	100	100
0	8	0	100	67	49	100	100	54	100	15	0	100	0	42	10	100
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0	0	0	33	65	35	0	45	69	69	0	0	0	0	49	18	34
100	0	5	100	45	100	35	100	100	52	52	36	100	100	0	53	100
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0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
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0	0	0	0	1	22	100	100	100	52	0	0	100	0	62	100	0
0	0	32	62	57	100	41	51	43	20	5	0	60	4	48	22	44
0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	100
25	0	100	100	100	100	44	100	100	100	59	49	100	0	100	68	100
100	100	100	100	100	100	100	100	100	53	57	100	100	100	60	100	23
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
0	0	0	13	69	52	12	11	63	30	26	28	100	0	59	35	100
0	11	32	1	0	0	0	0	100	100	0	0	0	0	2	0	0
0	100	0	68	100	38	0	20	86	0	0	0	26	100	40	10	100
0	0	44	16	11	56	4	19	8	76	10	0	83	0	14	13	100
1	0	0	5	26	0	15	5	0	0	0	6	0	0	0	0	0
100	0	0	100	100	100	100	100	100	38	0	55	100	0	21	67	100
28	0	0	36	80	76	0	1	100	85	0	11	71	100	21	6	100
0	0	0	0	0	6	0	0	100	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	100	11	0	0	0	0	0	0	100
18	16	22	46	47	48	38	46	58	45	15	17	50	21	38	35	56
15	12	17	40	35	40	32	43	49	35	6	4	43	18	24	21	51
161	165	176	171	332	251	250	151	224	266	413	413	353	142	457	434	128

xpb	% miss.poss	% miss.genes	seq length
0	11	8	25908
100	47	25	15845
100	75	57	7107
0	2	2	28748
0	3	2	28423
100	61	46	11602
100	29	20	20597
100	71	58	8713
0	7	7	27192
100	55	39	13422
0	12	10	25623
0	1	0	29063
0	3	2	28526
0	3	3	28462
100	55	46	12909
0	12	8	26183
100	73	59	7831
100	66	48	9809
100	88	77	3549
0	11	10	26303
100	61	42	11509
100	71	56	8252
100	38	26	17263
100	79	69	6313
0	1	0	29154
100	62	44	11478
100	57	42	12509
100	90	74	2926
100	53	42	13745
100	75	67	7815
100	79	64	5950
100	55	38	13366
100	48	36	14977
100	5	5	27921
100	3	2	28585
100	69	52	8802
0	7	9	27329
0	4	4	27818
6	3	2	28370
0	16	17	24817
100	65	49	10016
0	2	1	28550
0	19	21	23860

0	4	4	28227
0	10	2	26181
0	13	11	25432
100	64	53	10278
100	54	38	13333
100	90	74	2692
100	54	32	13992
24	51	36	14506
100	64	48	10754
100	28	7	21158
100	45	36	16546
66	18	3	24204
0	1	1	28918
0	2	2	28527
100	49	33	15182
100	39	17	17820
0	14	16	25007
100	76	57	7255
100	76	64	7477
0	7	7	27269
100	33	12	20029
0	11	9	25932
48	31	21	19907
64	29	16	20788
0	4	3	27806
100	57	45	12323
100	39	14	18256
100	12	10	25910
12	11	9	26160
<hr/>			
60			
57			
307			

Supp. Figure 1 and Figure 2

Cladograms representing the majority rule consensus trees resulting from the bootstrapping of genes analyses. In Supp. Figure 1 *T. subtilis* and *R. contractilis* were included; these 2 species were excluded from the analysis resulting in Supp. Figure 2. Black dots are on nodes defining groups that were recovered in all 200 replicates (100%); when not maximal the percentages of trees recovering the groups are indicated. The white thick bars are the groups that were originally included in the chromalveolates. Assemblages indicated by capitalized names correspond to the hypothetical supergroups of eukaryotes.

Supp. Figure 3

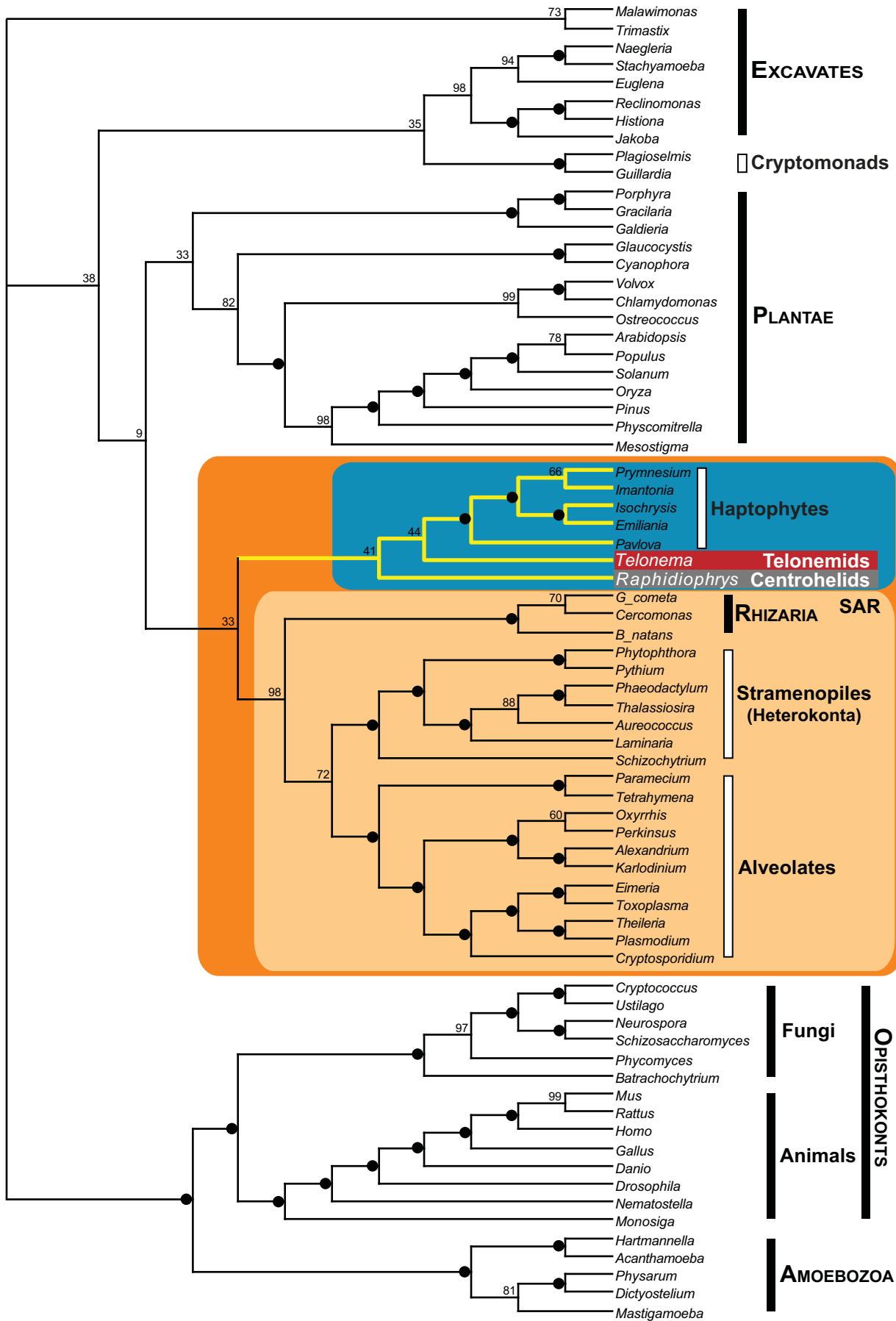
Phylogeny summarizing the relationships among the major groups of eukaryotes when *T. subtilis* and *R. contractilis* are not included in the analysis. This tree was obtained with phylobayes ran under the CAT model (consensus between two independent Markov chains), and subsequently schematized in FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>) with the “Cartoon” option. Black dots correspond to 1.0 posterior probability (PP) and 100% ML bootstrap (BP), otherwise values at node represent PP (above) and BP (below) when not maximal. Black squares show the constrained bifurcations used in the separate analysis and RELL bootstraps (RBP) are indicated.

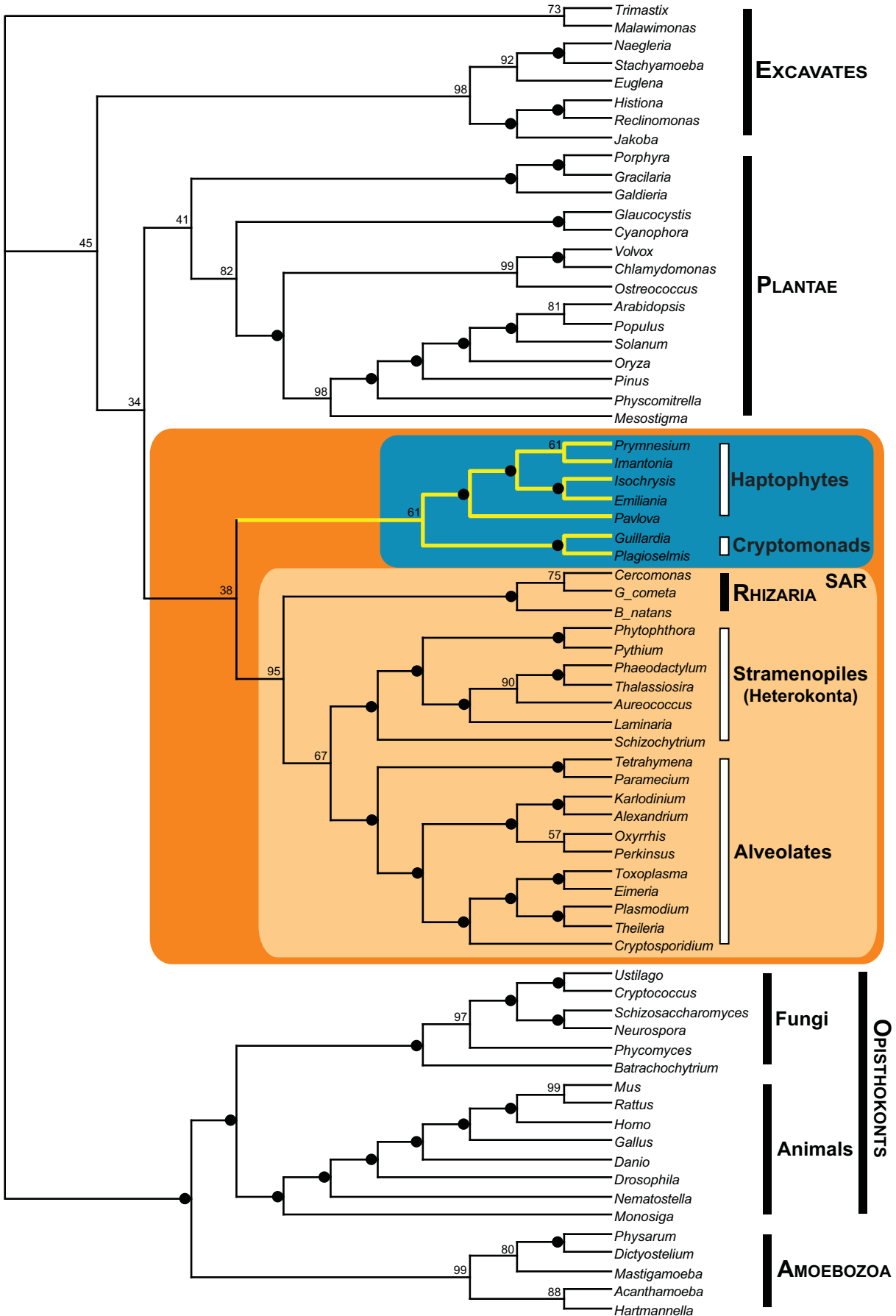
Supp. Figure 4 and Figure 5

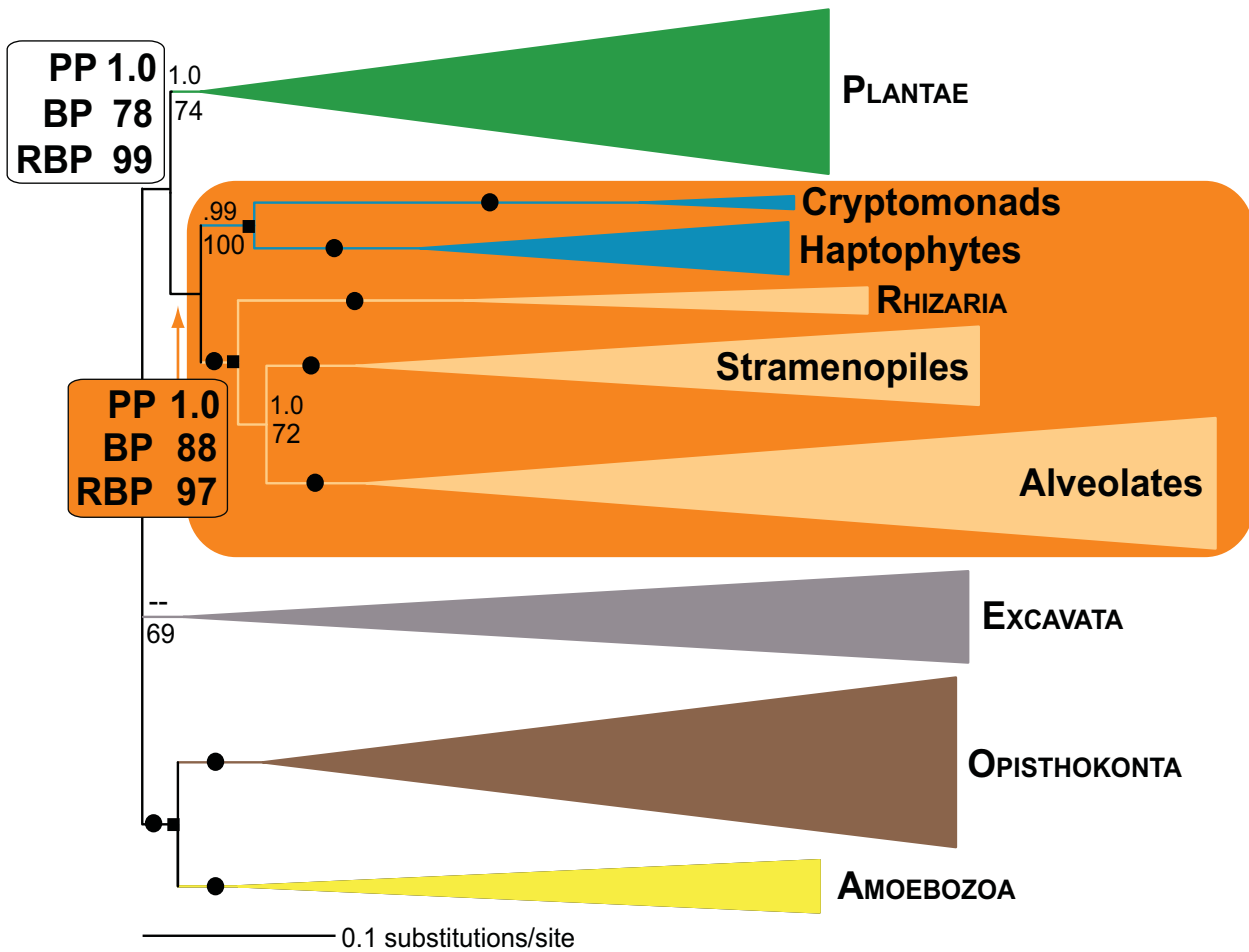
These trees represent a Bayesian phylogeny of eukaryotes, obtained from the consensus between two independent Markov chains, run under the CAT model implemented in phylobayes. The curved dashed lines indicate the alternative branchings recovered in the ML analysis of the same dataset. Black dots correspond to 1.0 posterior probability (PP) and 100% ML bootstrap (BP), otherwise values at node represent PP (above) and BP (below) when not maximal. The white thick bars are the groups that were originally included in the chromalveolates. Assemblages indicated by capitalized names correspond to the hypothetical supergroups of eukaryotes. The scale bar represents the estimated number of amino acid substitutions per site.

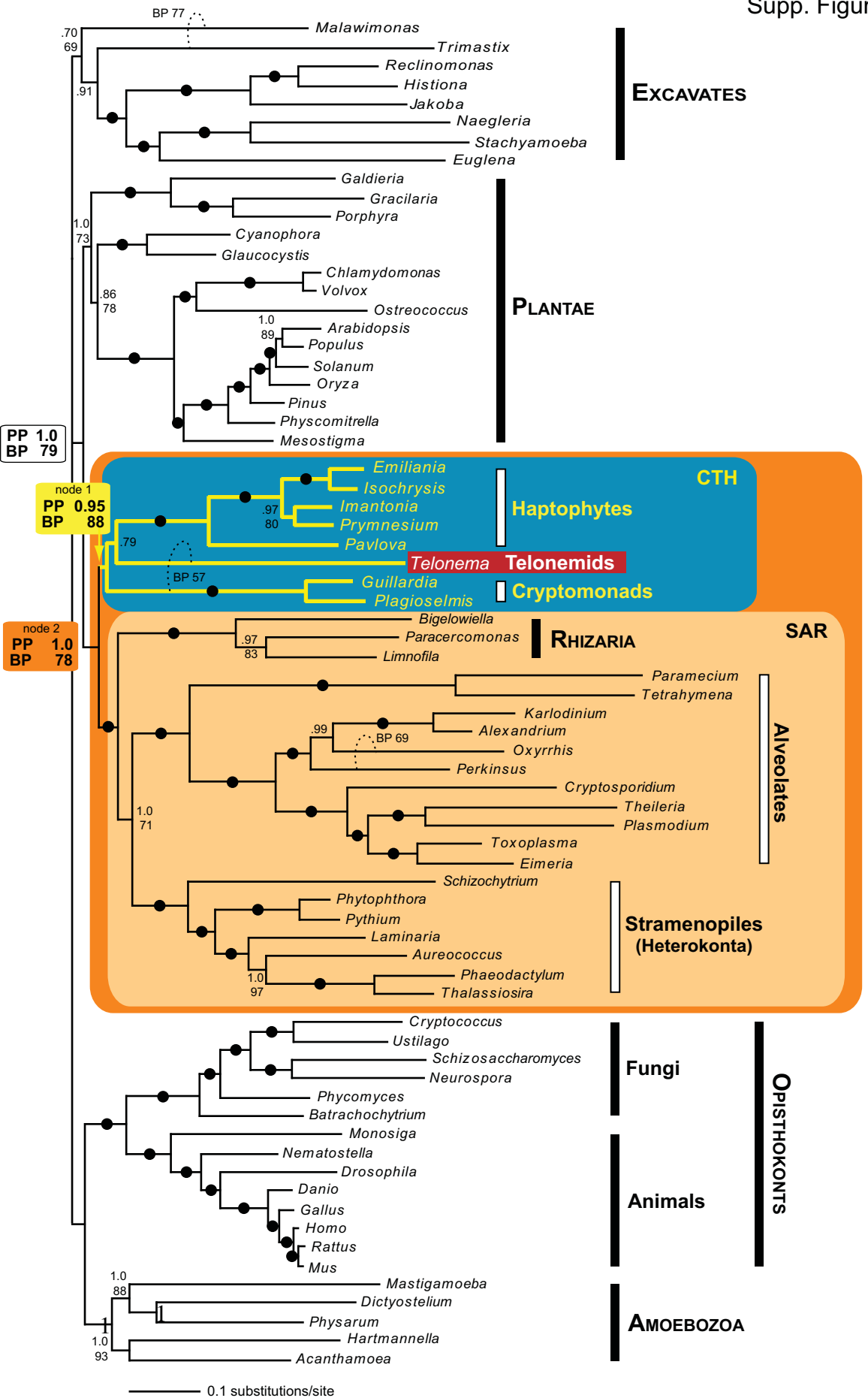
Supp. Figure 6

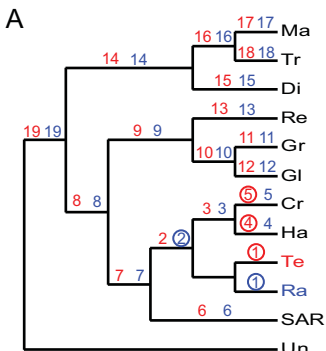
Summary of the AU tests based on the concatenated alignments, showing the alternative branching points that were tested (numbers on branches) and the *P*-values higher than 0.05. The values in circles correspond to the positions that were not rejected by the AU tests. When both *T. subtilis* and *R. contractilis* were present, only one species was moved at a time leaving the other in its inferred position. (A) Bayesian tree as in Figure 1, *T. subtilis* or *R. contractilis* were successively placed on alternative branches; (B) ML tree as in Figure 1, *T. subtilis* or *R. contractilis* were successively placed on alternative branches; (C) Bayesian tree as in Figure 1, both *T. subtilis* and *R. contractilis* were successively placed on alternative branches; (D) Bayesian tree as in Supp. Figure 2, *T. subtilis* was successively placed on alternative branches; (E) Bayesian tree as in Supp. Figure 2, *T. subtilis* was successively placed on alternative branches. Ma: *Malawimonas*; Tr: *Trimastix*; Di: *Discoba*; Re: *Red algae*; Gr: *Green algae*; Gl: *Glaucophytes*; Cr: *Cryptomonads*; Ha: *Haptophytes*; Te: *T. subtilis*; Ra: *R. contractilis*; Un: *Unikonts*.



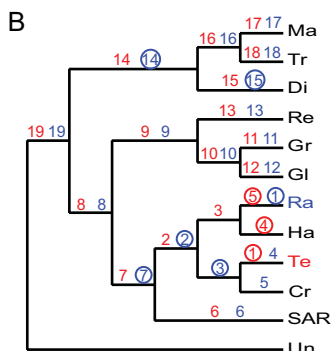




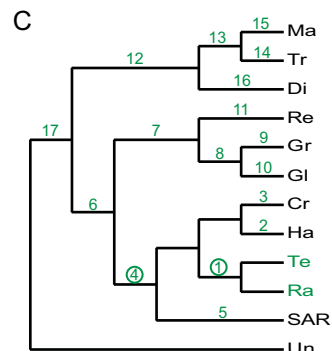




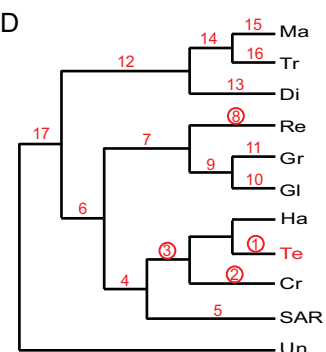
ID	$\Delta\ln L$	<i>P</i> value
1	-9.4	0.718
5	9.4	0.461
4	29.6	0.138
1	-35.1	0.987
2	35.1	0.076



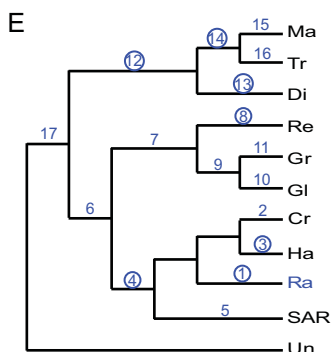
ID	$\Delta\ln L$	<i>P</i> value
1	-38.5	0.923
4	38.5	0.157
5	41.5	0.125
1	-10.1	0.826
2	10.1	0.523
14	83.2	0.087
7	46.9	0.083
15	92.8	0.062
3	32.4	0.054



ID	$\Delta\ln L$	<i>P</i> value
1	-38.4	0.718
4	38.4	0.104



ID	$\Delta\ln L$	<i>P</i> value
1	-10.2	0.781
2	10.2	0.437
3	22.2	0.244
8	70.5	0.091



ID	$\Delta\ln L$	<i>P</i> value
1	-14.7	0.916
3	14.7	0.336
12	65.1	0.149
8	57.6	0.127
4	32.7	0.125
13	72.6	0.115
14	83.8	0.074