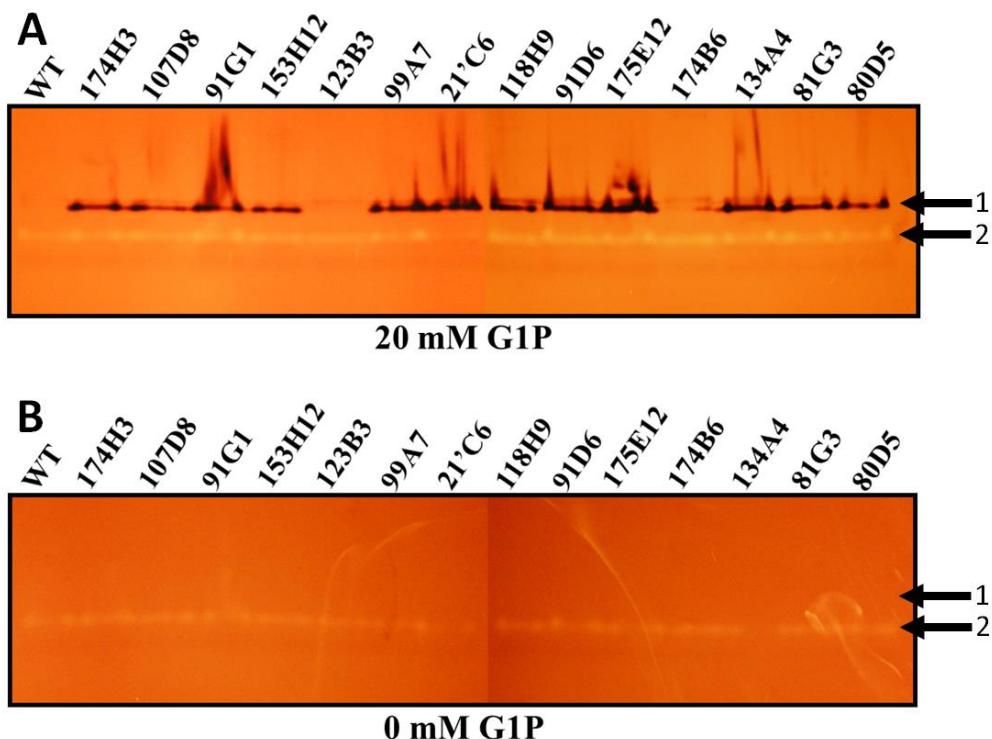


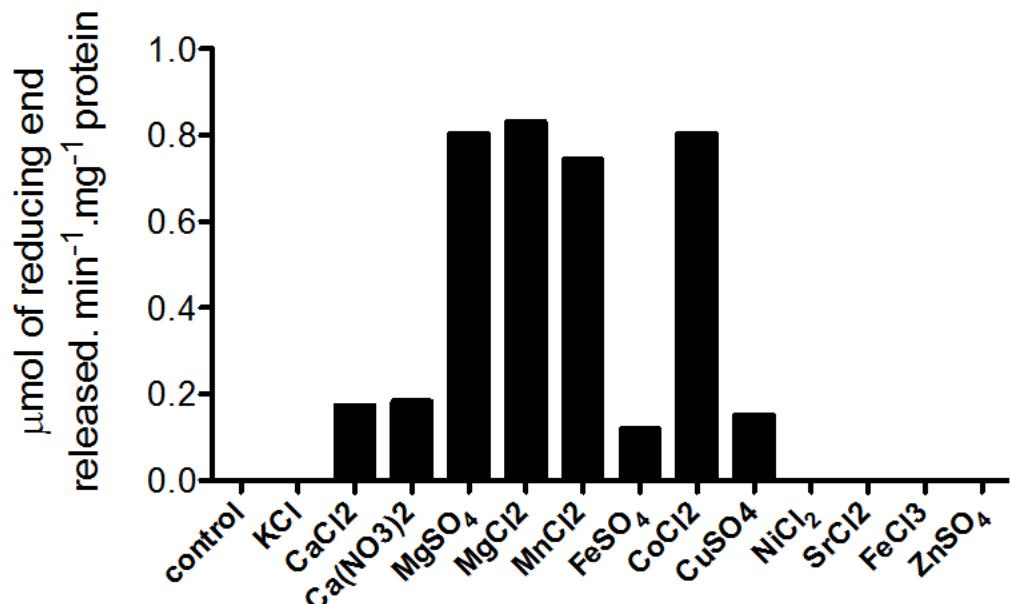
Supplemental figure 1: Cells patches of the five classes of mutant of *Cyanobacterium* sp. CLg1 stained with iodine vapors.

Mutants are grouped according to the percentage of residual water-soluble polysaccharides and starch-like granules measured in the mutant strains in comparison to the wild type strain (WT). Class A mutants (starch-like granule < 100% ; WSP >100 %). The mutant strain 153H12 harbors a yellow phenotype despite the presence of 10 % of starch-like granules (blue square). The intermediate phenotype class B mutants (starch-like granule < 100% ; WSP <100 %); class C (starch-like granule < 100% ; WSP = 100 %); class D mutants (starch-like granule = 100% ; WSP <100 %); Class E mutants (starch-like granule >100% ; WSP <100 %).



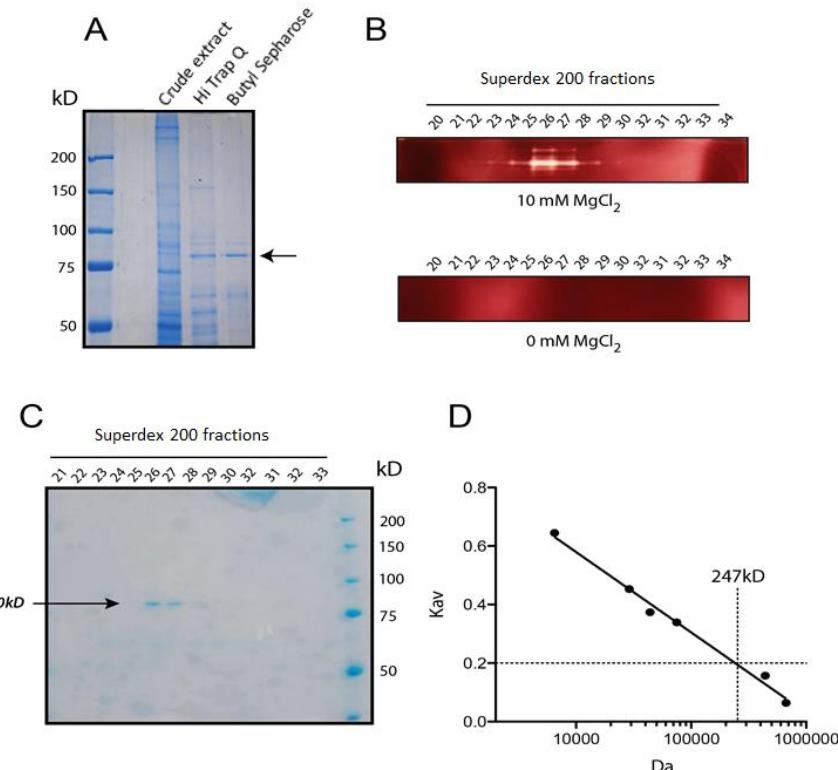
Supplemental figure 2: Zymogram of phosphorylase activity.

Zymogram analysis of phosphorylase activity from wild type and class A mutants. Total protein of WT and class A mutant strains were equilibrated and separated by native PAGE, followed by transfer of proteins to a native PAGE containing 0.6% (w/v) of glycogen. The native gels were then incubated with (A) or without (B) 20 mM Glucose-1-Phosphate (G-1-P). After overnight of incubation, gels are stained with iodine solution. Phosphorylase activity (arrow #1) is induced in all class A mutants, except for 174B6, 123B3 mutants and WT strain. A hydrolytic activity (arrow#2) is present in all crude extracts.



Supplemental figure 3: Effect of cations on the debranching enzyme activity.

Cation free debranching enzyme was incubated in absence (control) or in the presence of 5 mM of K⁺, Ca²⁺, Mg²⁺, Mn²⁺, Fe²⁺, Co²⁺, Cu²⁺, Ni²⁺, Sr²⁺, Fe³⁺, Zn²⁺ and 0,2% of amylopectin at 30°C during 30 minutes. Reactions were stopped by adding a volume of sodium 3,5-dinitrosalicylate (DNS) and boiled 10 minutes at 99°C. After cooling down, the absorbance is measured at 540 nm. The specific activity ($\mu\text{mol of reducing end} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein) was determined in presence of each cation.



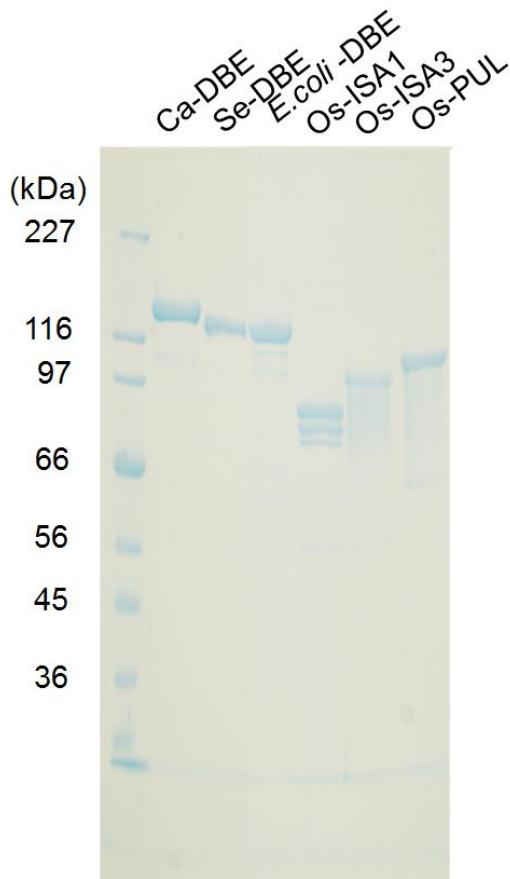
Supplemental figure 4: Purification of cation dependent debranching enzyme activity.

A. Cation dependent activity was purified at homogeneity using an anion exchange chromatography (HitrapQ), hydrophobic column (butyl sepharose) and gel permeation chromatography (superdex 200). The enrichment in GlgX2 protein is followed on SDS-PAGE after each purification step (black arrow). **B.** Debranching enzyme activity was monitored by using zymogram analysis performed in presence or in absence of 10 mM of MgCl₂ during the purification steps. **C.** SDS-PAGE analysis performed on superdex 200 fractions. A polypeptide of weight of 80 kD is visualized in fractions # 26 and #27 after Coomassie blue staining. **D.** Superdex 200 column (GE-Healthcare) pre-equilibrated with 150mM NaCl, tris/acetate buffer pH 7.5, 10 mM DTT was calibrated with standard proteins (669; 440; 75; 29 and 6.5 kD) and dextran blue. The determination of a partition coefficient (Kav) of 0.2 suggests an apparent molecular weight of the cation dependent activity estimated at 247 kD.

MLMGDESMNPQVWPGNPYHLGAKWDGQGTNFALYSENATTVELCFFDRQNQE~~TRIP~~
LTEVQNYVWHAYLPGIMPQQRGYRVDGVYDPEEGHRFNVNKLLIDPYAKALDGEIGF
GEP~~IFGYVWEDED~~E~~DIS~~SELD~~S~~AHLVPKAVVVD~~E~~SDWEGDKPLDIPEHETI~~IYELHV~~
GFTKLHPDIPEDLRGTFAGLAHPSTIVYLKSLGITAIELMPIHHFLSQPGHLVEKDLTNY
WGYDSISYLAPFSGYCATKTPQDVKEFKAMVKALHQEGIEVIMDVVYNHTGEGNHFG
PTLSLRGIDNATYYRVVEEDPRYYMDFTGCGNSLNLKH~~P~~QVMKLIMDSLRYWVLEMH
VDGFRFDLAAALAREELLEVDCLATFFDIIHQDPVLSNIK~~LIAEP~~PWDIGEDGYHVGKFPVL
WSEWNGRYRDTVRNFWRGEKSILAEFAYRV**TGSSDLYQDN**GRTPSASINFITAHDGFT
LNDLVSYNHKHNDANGEENKDGEQFNHSWCNC~~GEEGDSK~~D~~P~~EVLSLRNQQRRNFLVTL
MLSQGVPM~~L~~TAGDEIGRTQKGNNNAFCQDNEISWDWSLEHKNAELLKFVRDLIDLR
HQHPVFRRKWFQGQDIHGSGVSDIGWFNPDGFVTAETQWNLGF~~A~~KAI~~AL~~FLNGQEI
PLKDQQGQRVVDNSFLFFNAHYEAIEFVIPE~~SLG~~KQDWIMVIDTTQSRLLES~~G~~KRYRH
DVS~~I~~KVEARSLVVLKTTN Stop

Supplemental figure 5: Identification of debranching enzyme activity.

Identification of a cation dependent activity by Nano-LC-MS-MS. NCBI blast search identifies seven trypsic peptides common in GlgX proteins (underlined peptides). In addition, three trypsic peptides match specifically with CLg1-GlgX2 (bold underlined peptides) and not with CLg1-GlgX1. These data suggest that the cation dependent activity is probably an homotrimeric or homodimeric complex of GlgX2 (see text).



Supplemental figure 6: SDS-PAGE of purified DBE preparations from rice, cyanobacteria and *E.coli*.

The Ca-DBE (DBE from *Cyanothece* ATCC51142), Se-DBE (DBE from *Synechococcus elongatus* PCC7942) and *E.coli* GlgX were expressed in the form of the trigger factor fusion protein, while OsISA3 (ISA3 from rice) was fused with the thioredoxin tag. The Os-ISA1 (ISA1 from rice) and Os-PUL (PUL from rice) were connected with the His tag. The actual molecular sizes of Os-ISA1, Os-ISA3, Os-PUL, Ca-DBE, Se-DBE and *E.coli* GlgX were calculated to be 84,136, 80,517, 99,166, 80,332, 78,956 and 66,953 Da, respectively. Since all the 3 OsISA1 protein bands were reacted with antibody raised against completely purified Os-ISA1 protein, these bands were considered to be derived from the Os-ISA1 protein, possibly by production from the multiple translation-starting positions. Each purified DBE preparation including about 1.0 µg of protein was loaded onto the SDS-PAGE gel. Molecular mass was indicated in the figure as kDa.

activity	gene	Strains				
		80D5	99A7	118H9	153H12	175E12
Debranching enzymes	<i>glgX2</i>	-	-	-	-	-
	<i>iDBE</i>	-	-	-	-	-
	<i>apu GH13</i>	-	N.D.	-	-	-
	<i>apu GH57</i>	-	-	-	N.D.	-
Branching Enzymes	<i>glgB1</i>	-	N.D.	-	-	-
	<i>glgB2</i>	N.D.	-	-	-	-
	<i>glgB3</i>	-	-	-	-	-
	<i>glgB GH57</i>	N.D.	-	-	-	-
Starch synthases	<i>glgA1</i>	-	-	-	-	-
	<i>glgA2</i>	-	-	-	N.D.	-
	<i>gbss</i>	-	-	-	-	-
Phosphorylase	<i>pho</i>	N.D.	N.D.	-	N.D.	-
a-1,4 glucano transferase	<i>malQ</i>	-	-	-	-	-

Supplemental table 1: Summary table of starch metabolizing genes checked for mutations.

Molecular cloning and sequencing of genes involved in starch metabolism pathway in five class A mutants. Primers used sequenced the others gene coding branching enzyme, of the GH13 family (*glgB1*, and *glgB2*) and of the GH 57 family (*glgB57*), and the others gene coding debranching enzyme: the indirect debranching enzyme (*iDBE*) and the both amylullanase of the GH 13 family (*apu13*) and the GH57 family (*apu57*). This table shows no others mutation in the different gene of the class A mutant.

Gene	Primer	5'-Sequence-3'	Temperature of hybridization
Amylopullulanase	apu13 Cloning For	GCATCCATATTAAAATGATCGCTAAGG	56.6°C
	apu13 Cloning Rev	TTCGTCCCTCGTCCCTCGTCCTT	
	apu13 Sequencing 1	GGTAATCGAGCTTACCGAAAT	
	apu13 Sequencing 2	TCAAGATCTTCTCCTGCTACCGTTA	
	apu13 Sequencing 3	CAGCTAACCCAGTAAATTACTGC	
	apu13 Sequencing 4	GATTGAATTTCGGTAAAGCTCGAT	
	Apu57 Cloning For	GGAGATCTTCTGCCCTCTTCA	
	Apu57 Cloning Rev	AAAATAGCCAATAAACAGAACGCA	
	Apu57 Sequencing 1	ACGAAC TGCGAACCCAGAACCTCC	
	Apu57 Sequencing 2	ACCCAATCCCCTGCCTTCGCAT	
	Apu57 Sequencing 3	GGCATATCAAAGGTACTCGTCATGG	
	Apu57 Sequencing 4	AGGTAAAGACAGGGGCTGTCAA	
Indirect debranching enzyme	idbe Cloning For	TCAATTATAAGTAGGGGTAGGAGA	55.9°C
	idbe Cloning Rev	ATGCTAAAAAAATCGCCAAA	
	idbe Sequencing 1	CACAAC TCTGATAACAGCCTCAA	
	idbe Sequencing 2	GTACACCTGACTCACCACCGTAA	
	idbe Sequencing 3	GATTGGCATATCAAAGGTACTCG	
	idbe Sequencing 4	ACCGCCTAGATAATGAGATTGGAT	
Branching enzyme	glgB3 Cloning Rev	TGACCATCCCATTGGCTCCTA	61°C
	glgB3 Cloning For	AGTGAATAGCCAAAATCAACGAT	
	glgB3 Sequencing 1	GTGGTTTAGGCAACTACGACGGTACA	
	glgB3 Sequencing 2	CGTTACGTATCATGAACTAATT	
	glgB3 Sequencing 3	CCAGGTGCGCCGATGATTT	
	glgB3 Sequencing 4	TCGCCCCCTGAATGATGATAAAAT	
	glgB2 Cloning For	TGCGAAAAGATGTCTGCTATGCT	
	glgB2 Cloning Rev	TCCACTCAGTCAAGTGTGAATCAA	
	glgB2 Sequencing 1	TTTGACGGCTCATGGGTTA	
	glgB2 Sequencing 2	ATTGTGATAGTTCGGGCGCTCAT	60°C
	glgB2 Sequencing 3	AGCGCCCCGAACTATCACAAAT	
	glgB2 Sequencing 4	ACCATAGCGGGAAAGTTGGTG	
	glgB1 Cloning For	TGAAAACAGTCAGACAAGTTTCCGT	
	glgB1 Cloning Rev	TCCCGAACTGAGGTTAAGTATTGA	
	glgB1 Sequencing 1	CACCTCGGTTCTGGCTACA	
	glgB1 Sequencing 2	TGCATCCAGCCCATAATTCCA	
	glgB1 Sequencing 3	TCGCAGAAGAATCAACCGCTTGGT	
	glgB1 Sequencing 4	TGTAGCCAAGAACCGAGGTGT	
Debranching enzyme	glgB57 Cloning For	CGCCATTAGACTCTGCTAGG	59°C
	glgB57 Cloning Rev	TGGAGTTGTGGTTCTCTGTCA	
	glgB57 Sequencing 1	TGCGGTTATTACGGTGGCTT	
	glgB57 Sequencing 2	TAGCTAAACCGCAGCATCG	
	glgB57 Sequencing 3	AACCAAATCCCTCTGGGAGCAA	
	glgB57 Sequencing 4	GAGCTTGCTTGTGGCTAGT	
	glgX2 Cloning For	ATGTTAATGGGAGATGAATCTATGA	
	glgX2 Cloning Rev	TAATTAGTGGTTTTAGTACTACTAACG	

	glgX1 Cloning For glgX1 Cloning Rev glgX1 Sequencing 1 glgX1 Sequencing 2 glgX1 Sequencing 3 glgX1 Sequencing 4	ATGAACCATAAAACGTTACCTG CTATTTGCCATTAATAAAAATGCAAC TCGGATTCCCTTGCTTCAACCGT TCATCGGTGTAGCTTGAGCGAGT CCACGATGGGTGCAAAGAAACT ATGAAGCTAATGGTGAAGAGAACCGA	59.1°C
Starch synthase	GBSS Cloning For GBSS Cloning Rev GBSS Sequencing 1 GBSS Sequencing 2 GBSS Sequencing 3 GBSS Sequencing 4 glgA1 Cloning For glgA1 Cloning Rev glgA1 Sequencing 1 glgA1 Sequencing 2 glgA2 Cloning For glgA2 Cloning Rev glgA2 Sequencing 1 glgA2 Sequencing 2 glgA2 Sequencing 3 glgA2 Sequencing 4	TCCTCATGAATTGGTGACATAGTATGTT CAGATAACAGGTAAAATCGTAACGC AAAACGCCCTGGAAAGCAATA GCAATAGTCAGCACCAAGCCGTGATA TTTATCACGGCTGGTGTGACT ATGTTAACATTTGCTTGCTCTACGGAA AATTGTTAACAAAGTGGGAGAGAAA GATAACGATCGTGATAACTTTAAGGTA GCTCTGCAACATATGACTGGTGTCTGA CCATAATAACGAGCCGAAATTCTGCTA AACTGAATCAACTGAATGAAAACGA CGGGTATCTGTAATCTAGAACTCCTCA ATGATTGGCAAACGGATTGATCCCTGTA ACCACCAACACGAACACTACAGGTACT TTAACCGAATTGATTACAATACTTGGGA GATTGGAATAGACAATACCACCTTCA	59.6°C 60°C 59.8°C
	Phosphorylase Cloning For Phosphorylase Cloning Rev Phosphorylase Sequencing 1 Phosphorylase Sequencing 2 Phosphorylase Sequencing 3 Phosphorylase Sequencing 4 Phosphorylase Sequencing 5 Phosphorylase Sequencing 6	GCTTACTAAGACGAAATTGATTATGAGCGAT TTCACCGTAACGCTATCAGCCGTAAT TTAGGTAACGGTGGTTGGAA CCAAAGATAAAGGTGCGGGGATA CTTCTTCTTAGAAGATATCGTTCATGGTA GAGTTTGTGTGTAATCGACCACGCTT AACTCTGACGGTGCCAATATTGA CTAACACCTGGATCTGGCTCTTCTT	62°C
MalQ	MalQ Cloning For MalQ Cloning Rev MalQ Sequencing 1 MalQ Sequencing 2 MalQ Sequencing 3 MalQ Sequencing 4	CGCAGGTCTGTGATTGTCT CCATCTACCGGTAACGCTGTAA CTTCAGCGACAATCGGCAACT CCACGATCGGTTGGTTGATA TGGTTGGATGACTACGCTCTGT TGCAAATACCCCTTGATGCT	62°C

Supplemental table 2: List of primers used for genes cloning and sequencing of genes involved in the storage polysaccharide metabolism of *Cyanobacterium* sp. CLg1.

Supplemental methods 1: Parameter values for phylogenetic analysis

Determination of Best fit model according to ProtTest 3.2 for the alignment, of GlgX of bacteria and Archaeplastida isoamylase.

This determination was performed with:

- “-all-distribution” parameters including models with rate variation among sites, number of categories and both.
- “-F” parameters including models with empirical frequency estimation.
- “-AIC” parameters displaying model sorted by the Akaike Information Criterion.
- “-S 2” parameters using the optimizing strategy developed for Maximum Likelihood analysis.

Results:

AKAIKE INFORMATION CRITERION

Best model according to AIC: LG+G

Confidence Interval: 100.0

Model	deltaAIC	AIC	AICw	-lnL
LG+G	0.00	170602.28	0.70	85051.14
LG+I+G	1.68	170603.96	0.30	85050.98
LG+G+F	173.72	170776.00	0.00	85119.00
LG+I+G+F	174.89	170777.18	0.00	85118.59
WAG+G	663.83	171266.12	0.00	85383.06
WAG+I+G	665.67	171267.96	0.00	85382.98
RtREV+I+G+F	848.35	171450.63	0.00	85455.32
RtREV+G+F	850.47	171452.76	0.00	85457.38
WAG+G+F	890.11	171492.39	0.00	85477.20
WAG+I+G+F	891.55	171493.84	0.00	85476.92
VT+G	988.07	171590.36	0.00	85545.18
VT+I+G	988.83	171591.11	0.00	85544.56
VT+G+F	1251.84	171854.12	0.00	85658.06
VT+I+G+F	1252.15	171854.43	0.00	85657.22
Blosum62+G	1582.98	172185.27	0.00	85842.63
Blosum62+I+G	1583.76	172186.05	0.00	85842.02
RtREV+I+G	1707.47	172309.75	0.00	85903.87
RtREV+G	1709.75	172312.03	0.00	85906.02
Blosum62+I+G+F	1846.25	172448.53	0.00	85954.27
Blosum62+G+F	1846.52	172448.80	0.00	85955.40
CpREV+G+F	1940.10	172542.38	0.00	86002.19
CpREV+I+G+F	1942.06	172544.34	0.00	86002.17
CpREV+G	2034.40	172636.69	0.00	86068.34
CpREV+I+G	2036.41	172638.70	0.00	86068.35
JTT+G	2129.92	172732.20	0.00	86116.10
JTT+I+G	2131.09	172733.37	0.00	86115.69
JTT+G+F	2571.15	173173.43	0.00	86317.71
JTT+I+G+F	2572.57	173174.85	0.00	86317.43

DCMut+G	3449.82	174052.10	0.00	86776.05
Dayhoff+G	3451.65	174053.93	0.00	86776.97
DCMut+I+G	3451.76	174054.04	0.00	86776.02
Dayhoff+I+G	3453.60	174055.89	0.00	86776.94
DCMut+G+F	3638.15	174240.43	0.00	86851.22
Dayhoff+G+F	3638.78	174241.06	0.00	86851.53
DCMut+I+G+F	3640.09	174242.37	0.00	86851.19
Dayhoff+I+G+F	3640.74	174243.02	0.00	86851.51
MtREV+G+F	4338.60	174940.89	0.00	87201.44
MtREV+I+G+F	4340.54	174942.82	0.00	87201.41
FLU+I+G+F	5672.13	176274.42	0.00	87867.21
FLU+G+F	5672.83	176275.11	0.00	87868.55
MtArt+I+G+F	6601.29	177203.57	0.00	88331.78
MtArt+G+F	6605.84	177208.13	0.00	88335.06
FLU+G	6723.16	177325.45	0.00	88412.72
FLU+I+G	6724.20	177326.48	0.00	88412.24
HIVb+I+G	7423.14	178025.43	0.00	88761.71
HIVb+G	7428.28	178030.56	0.00	88765.28
HIVb+I+G+F	7671.82	178274.10	0.00	88867.05
HIVb+G+F	7675.66	178277.94	0.00	88869.97
MtMam+G+F	9375.19	179977.47	0.00	89719.74
MtMam+I+G+F	9377.24	179979.53	0.00	89719.76
VT+I	9499.48	180101.77	0.00	89800.88
WAG+I	9505.33	180107.61	0.00	89803.80
WAG	9619.70	180221.99	0.00	89861.99
VT	9637.06	180239.35	0.00	89870.67
MtREV+G	9651.66	180253.95	0.00	89876.97
MtREV+I+G	9652.93	180255.22	0.00	89876.61
LG+I	9707.67	180309.95	0.00	89904.97
VT+I+F	9732.05	180334.33	0.00	89898.16
WAG+I+F	9774.08	180376.37	0.00	89919.18
LG	9840.27	180442.55	0.00	89972.28
VT+F	9873.45	180475.73	0.00	89969.87
WAG+F	9892.92	180495.20	0.00	89979.60
LG+I+F	9987.22	180589.51	0.00	90025.75
Blosum62+I	10021.91	180624.20	0.00	90062.10
LG+F	10127.76	180730.04	0.00	90097.02
Blosum62	10152.81	180755.10	0.00	90128.55
Blosum62+I+F	10218.61	180820.90	0.00	90141.45
Blosum62+F	10356.99	180959.28	0.00	90211.64
RtREV+I+F	10470.84	181073.12	0.00	90267.56
RtREV+F	10606.43	181208.71	0.00	90336.36
RtREV+I	11092.73	181695.01	0.00	90597.50
RtREV	11208.58	181810.87	0.00	90656.43
CpREV+I+F	11320.79	181923.08	0.00	90692.54
CpREV+I	11340.27	181942.56	0.00	90721.28
CpREV+F	11426.95	182029.24	0.00	90746.62
CpREV	11437.57	182039.85	0.00	90770.93
JTT+I	11559.32	182161.61	0.00	90830.80
JTT	11706.03	182308.32	0.00	90905.16

JTT+I+F	12008.62	182610.91	0.00	91036.45
MtArt+I+G	12126.46	182728.74	0.00	91113.37
MtArt+G	12129.83	182732.12	0.00	91116.06
JTT+F	12159.34	182761.63	0.00	91112.81
DCMut+I	13201.66	183803.94	0.00	91651.97
Dayhoff+I	13224.06	183826.34	0.00	91663.17
DCMut	13337.48	183939.77	0.00	91720.88
Dayhoff	13359.50	183961.78	0.00	91731.89
DCMut+I+F	13560.91	184163.20	0.00	91812.60
Dayhoff+I+F	13583.06	184185.34	0.00	91823.67
DCMut+F	13700.54	184302.82	0.00	91883.41
Dayhoff+F	13722.27	184324.56	0.00	91894.28
HIVw+I+G+F	13850.90	184453.19	0.00	91956.59
HIVw+G+F	13857.70	184459.99	0.00	91960.99
MtMam+G	14665.21	185267.49	0.00	92383.75
MtMam+I+G	14666.65	185268.93	0.00	92383.46
MtREV+I+F	15727.32	186329.60	0.00	92895.80
MtREV+F	15807.24	186409.52	0.00	92936.76
FLU+I+F	16465.01	187067.30	0.00	93264.65
HIVw+I+G	16496.88	187099.16	0.00	93298.58
HIVw+G	16498.39	187100.67	0.00	93300.34
FLU+F	16619.53	187221.81	0.00	93342.91
FLU+I	17571.87	188174.16	0.00	93837.08
FLU	17712.25	188314.53	0.00	93908.27
HIVb+I	19181.53	189783.81	0.00	94641.91
HIVb	19375.83	189978.12	0.00	94740.06
HIVb+I+F	19810.57	190412.86	0.00	94937.43
HIVb+F	20004.11	190606.39	0.00	95035.20
MtArt+I+F	21099.55	191701.83	0.00	95581.91
MtArt+F	21245.59	191847.87	0.00	95655.94
MtREV+I	22657.05	193259.33	0.00	96379.67
MtREV	22738.64	193340.93	0.00	96421.46
MtMam+I+F	24440.36	195042.65	0.00	97252.32
MtMam+F	24528.71	195131.00	0.00	97297.50
HIVw+I+F	25476.13	196078.41	0.00	97770.21
HIVw+F	25690.42	196292.71	0.00	97878.35
HIVw+I	27881.13	198483.42	0.00	98991.71
HIVw	28055.00	198657.29	0.00	99079.64
MtArt+I	30144.63	200746.91	0.00	100123.46
MtArt	30307.92	200910.21	0.00	100206.10
MtMam+I	33032.04	203634.32	0.00	101567.16
MtMam	33117.07	203719.35	0.00	101610.68

Relative importance of parameters

alpha (+G): 0.698

p-inv (+I): 0.000

alpha+p-inv (+I+G): 0.302

freqs (+F): 0.000

Model-averaged estimate of parameters

alpha (+G): 0.974

p-inv (+I): NaN

alpha (+I+G): 0.980

p-inv (+I+G): 0.001

Determination of the Maximum Likelihood (ML) tree

In order to determine the ML tree, RAxML was performed as follow:

- “-N 1000” parameter indicating 1000 bootstrap repeat
- “-m PROTGAMMALG” : the substitution model performed was LG+G
- “-p 123456” indicating 6 parsimony Random Seed
- “-x 123456” indicating 6 rapid Bootstrap Random Seed
- “-f a” indicating that RAxML was instructed to conduct a rapid Bootstrap analysis and look for the best-scoring ML tree in one single program run.

The tree obtained was rooted on the branch uniting the Archaeplastida sequences. This was done to reflect our current understanding that supports Archaeplastida monophyly.