Diagnosing phylogenetic conflict between genes and proteins: Evidence for the origin of plastids

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- What are the relationships between plastids and cyanobacteria?
- Why do the gene tree and the protein tree give a different answer?

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Dataset

- 42 taxa, including 8 outgoup (non-cyano)bacteria, 16 cyanobacteria, and plastids from 1 Glaucophyta, 4 Rhodophyta and 13 Viridiplantae

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- Concatenated dataset (cg75)

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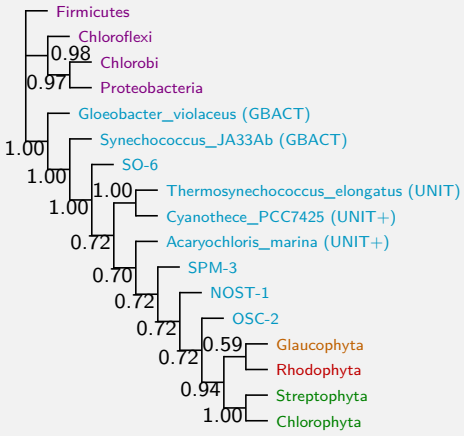
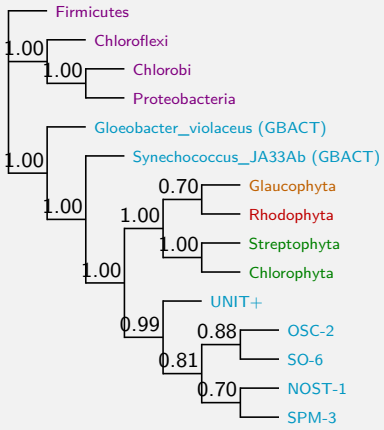
- Various recodings of cg75 using IUPAC ambiguity codes to remove signal associated with some synonymous substitutions. e.g for His: CAC ↔ CAT (synonymous codons) → CAY (degenerate codon)
- Maximum likelihood bootstrap analyses (200 resamplings) using RAxML, with GTR+I+Γ (or LG+I+Γ for cp75, chosen using ProtTest)

Unrecoded ML bootstrap analyses

translation



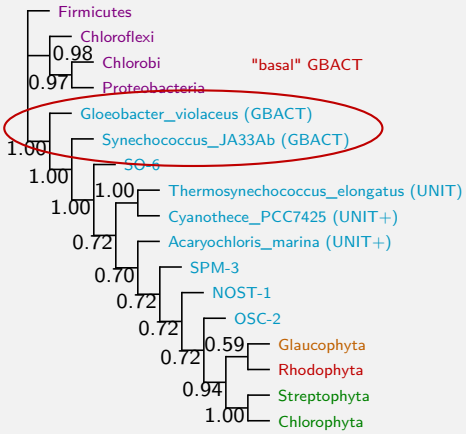
cg75



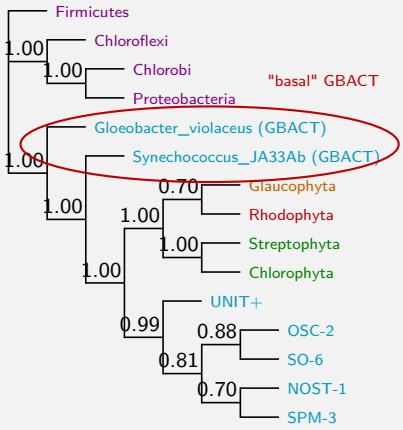
cp75

Unrecoded ML bootstrap analyses

translation

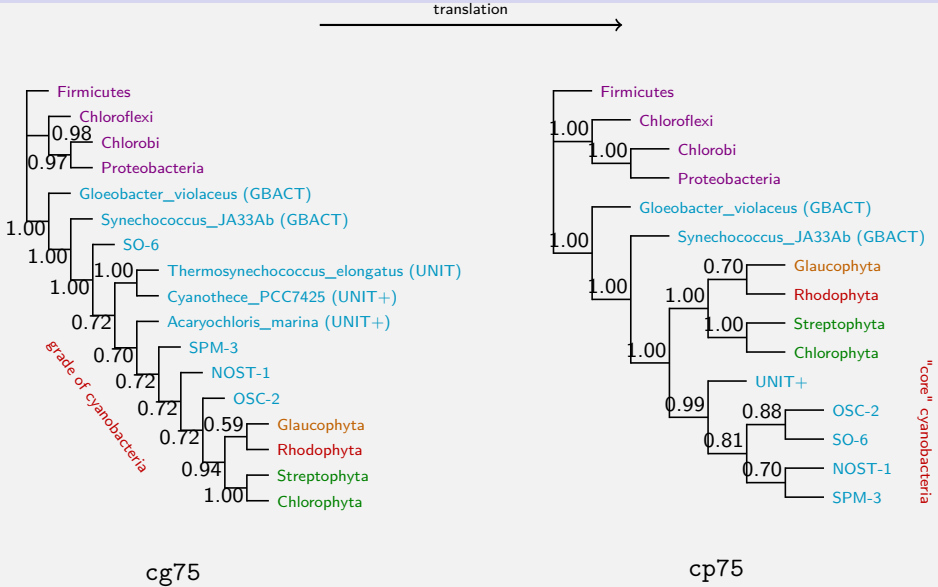


cg75



cp75

Unrecoded ML bootstrap analyses



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  $\rightarrow$ Something is not well modelled.

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- cp75 is a direct translation of cg75
  $\rightarrow$ The trees should be the same.
- But the analyses conflict in the identification of the plastid sister-group.
  $\rightarrow$ Something is not well modelled.
- Data recoding experiments to identify some causes of model mis-specification.

Degenerating synonymous 3rd codon positions

	T		C		A		G	
T	TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
	TTC		TCC		TAC		TGC	
	TTA	Leu	TCA		TAA	Ter	TGA	Ter
	TTG		TCG		TAG		TGG	Trp
C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
	CTC		CCC		CAC		CGC	
	CTA		CCA		CAA	Gln	CGA	
	CTG		CCG		CAG		CGG	
A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
	ATC		ACC		AAC		AGC	
	ATA		ACA		AAA	Lys	AGA	Arg
	ATG	Met	ACG		AAG		AGG	
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
	GTC		GCC		GAC		GGC	
	GTA		GCA		GAA	Glu	GGA	
	GTG		GCG		GAG		GGG	

Example:

ATGAAACAGTTGCTGGAAGCCGGTGTTCACTTC
TTATCAGAACTTTTAGATGCCAGCGCTCACATA
TTAGAAGATATGATTCAAAGTGGAATGCATTTT
CTCGAACAAATGCTAGATGTAGGTGTTCATTTT
TTACAGTCAATGCTTGAAGCTGGTGTTCACTTT
TTAGAAGCACTTTTAGAGGCTGGTGTTCATTTT
TTGGAAGAAATGATGGAAGCGGGTATTCATTTC
TTGGAACAAATGATGGAAGCAGGAGTCCATTTT
ATTCAGCAATTATTGGAAGCAGGAGTCCATCTG
ATTGAAGAATTGTTAGAAGCTGGCGTGCATTTC
TTAGAACAAATGTTAGATGCAGGTGTACATTTT
TTACAAAAAATGATTGAAGCTGGTGTTCATTTT
CTATCAGAAATGATGGAAGCTGGTGCTCATTTC
CTGCCGCAAATGCTGGAAGCCGGTGTCCATTTT
TTAGAAGAAATGATGGAAGCAGGGGTCCATTTT
TTAGCAGAATTACTAGAAGCGGGCGTTCATTTT

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	TTC		TCC		TAC		TGC	
	TTA	Leu	TCA		TAA	Ter	TGA	Ter
	TTG		TCG		TAG		TGG	Trp
C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
	CTC		CCC		CAC		CGC	
	CTA		CCA		CAA	Gln	CGA	
	CTG		CCG		CAG		CGG	
A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
	ATC		ACC		AAC		AGC	
	ATA		ACA		AAA	Lys	AGA	Arg
	ATG	Met	ACG		AAG		AGG	
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
	GTC		GCC		GAC		GGC	
	GTA		GCA		GAA	Glu	GGA	
	GTG		GCG		GAG		GGG	

Example:

```
ATGAAACAGTTGCTGGAAGCCGGTGTTCACTTC
TTATCAGAACTTTTAGATGCCAGCGCTCACATA
TTAGAAGATATGATTCAAAGTGGAATGCATTTT
CTCGAACAAATGCTAGATGTAGGTGTTCATTTT
TTACAGTCAATGCTTGAAGCTGGTGTTCACTTT
TTAGAAGCACTTTTAGAGGCTGGTGTTCATTTT
TTGGAAGAAATGATGGAAGCGGGTATTCATTTC
TTGGAACAAATGATGGAAGCAGGAGTCCATTTT
ATTCAGCAATTATTGGAAGCAGGAGTCCATCTG
ATTGAAGAATTGTTAGAAGCTGGCGTGCATTTC
TTAGAACAAATGTTAGATGCAGGTGTACATTTT
TTACAAAAAATGATTGAAGCTGGTGTTCATTTT
CTATCAGAAATGATGGAAGCTGGTGCTCATTTC
CTGCCGCAAATGCTGGAAGCCGGTGTCCATTTT
TTAGAAGAAATGATGGAAGCAGGGGTCCATTTT
TTAGCAGAATTACTAGAAGCGGGCGTTCATTTT
```

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	T		C		A		G	
T	TTY TTY	Phe	TCN TCN	Ser	TAY TAY	Tyr	TGY TGY	Cys
	TTN TTN	Leu	TCN TCN		TAR TAR	Ter	TGR	Ter
							TGG	Trp
C	CTN CTN CTN CTN	Leu	CCN CCN CCN CCN	Pro	CAY CAY	His	CGN CGN	Arg
					CAR CAR	Gln	CGN CGN	
A	ATH ATH ATH	Ile	ACN ACN ACN ACN	Thr	AAY AAY	Asn	AGN AGN	Ser
	ATG	Met			AAR AAR	Lys	AGN AGN	Arg
G	GTN GTN GTN GTN	Val	GCN GCN GCN GCN	Ala	GAY GAY	Asp	GGN GGN	Gly
					GAR GAR	Glu	GGN GGN	

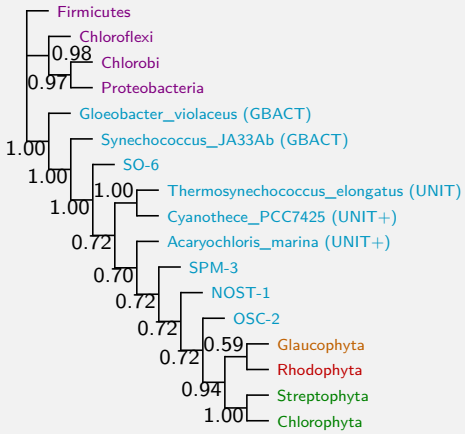
Example:

```
ATGAARCARTTNCTNGARGCNGGNGTNCAYTTY
TTNTCNGARCTNTTNGAYGCNAGNGCNCAYATH
TTNGARGAYATGATHCARAGNGGNATGCAYTTY
CTNGARCARATGCTNGAYGTNGGNGTNCAYTTY
TTNCARTCNATGCTNGARGCNGGNGTNCAYTTY
TTNGARGCNCTNTTNGARGCNGGNGTNCAYTTY
TTNGARGARATGATGGARGCNGGNATHCAYTTY
TTNGARCARATGATGGARGCNGGNGTNCAYTTY
ATHCARCARTTNTTNGARGCNGGNGTNCAYCTN
ATHGARGARTTNTTNGARGCNGGNGTNCAYTTY
TTNGARCARATGTTNGAYGCNGGNGTNCAYTTY
TTNCARAARATGATHGARGCNGGNGTNCAYTTY
CTNTCNGARATGATGGARGCNGGNGCNCAYTTY
CTNCCNCARATGCTNGARGCNGGNGTNCAYTTY
TTNGARGARATGATGGARGCNGGNGTNCAYTTY
TTNGCNGARTTNCTNGARGCNGGNGTNCAYTTY
```

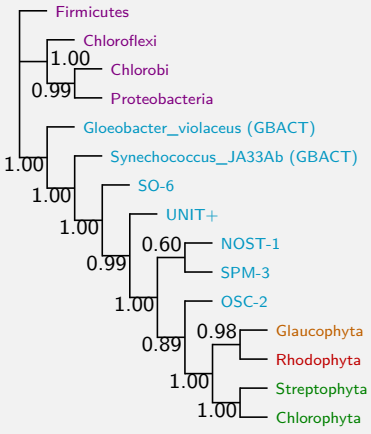
Degenerating synonymous 3rd codon positions

degenerate at 3rd pos.

(27.35% recoded)

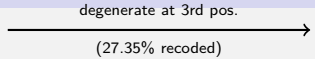


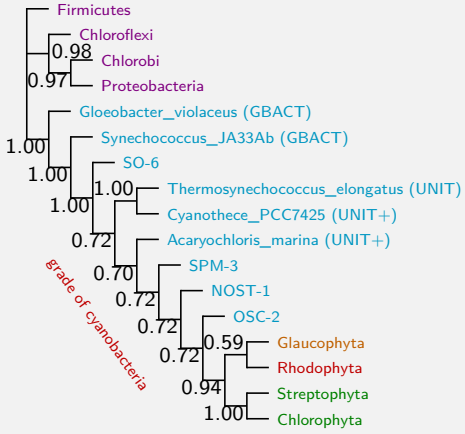
cg75



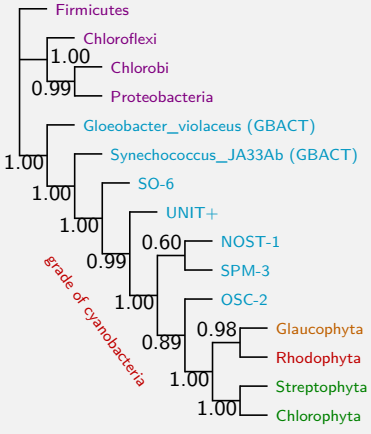
cg75_degenerate3

Degenerating synonymous 3rd codon positions





cg75



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  e.g for Leu: (TTA $\leftrightarrow$ CTA) $\rightarrow$ YTA
  e.g for Arg: (CGA $\leftrightarrow$ AGA) $\rightarrow$ MGA
  e.g for Ser: (TCT $\leftrightarrow$ AGA) $\rightarrow$ WST

Degenerating synonymous 1st and 2nd codon positions

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	CTG		CCG		CAG		CGG	
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	ATC		ACC		AAC		AGC	
	ATA		ACA		AAA	Lys	AGA	Arg
	ATG	Met	ACG		AAG		AGG	
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
	GTC		GCC		GAC		GGC	
	GTA		GCA		GAA	Glu	GGA	
	GTG		GCG		GAG		GGG	

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ATGAAACAGTTGCTGGAAGCCGGTGTTCACTTC
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TTAGAAGATATGATTCAAAGTGGAATGCATTTT
CTCGAACAAATGCTAGATGTAGGTGTTCATTTT
TTACAGTCAATGCTTGAAGCTGGTGTTCACTTT
TTAGAAGCACTTTTAGAGGCTGGTGTTCATTTT
TTGGAAGAAATGATGGAAGCGGGTATTCATTTC
TTGGAACAAATGATGGAAGCAGGAGTCCATTTT
ATTCAGCAATTATTGGAAGCAGGAGTCCATCTG
ATTGAAGAATTGTTAGAAGCTGGCGTGCATTTC
TTAGAACAAATGTTAGATGCAGGTGTACATTTT
TTACAAAAAATGATTGAAGCTGGTGTTCATTTT
CTATCAGAAATGATGGAAGCTGGTGCTCATTTC
CTGCCGCAAATGCTGGAAGCCGGTGTCCATTTT
TTAGAAGAAATGATGGAAGCAGGGGTCCATTTT
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	TTG		TCG		TAG		TGG	Trp
C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
	CTC		CCC		CAC		CGC	
	CTA		CCA		CAA	Gln	CGA	
	CTG		CCG		CAG		CGG	
A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
	ATC		ACC		AAC		AGC	
	ATA		ACA		AAA	Lys	AGA	Arg
	ATG	Met	ACG		AAG		AGG	
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
	GTC		GCC		GAC		GGC	
	GTA		GCA		GAA	Glu	GGA	
	GTG		GCG		GAG		GGG	

Example:

ATGAAACAGTTGCTGGAAGCCGGTGTTCACTTC
TTATCAGAACTTTTAGATGCCAGCGCTCACATA
TTAGAAGATATGATTCAAAGTGGAATGCATTTT
CTCGAACAAATGCTAGATGTAGGTGTTCATTTT
TTACAGTCAATGCTTGAAGCTGGTGTTCACTTT
TTAGAAGCACTTTTAGAGGCTGGTGTTCATTTT
TTGGAAGAAATGATGGAAGCGGGTATTCATTTC
TTGGAACAAATGATGGAAGCAGGAGTCCATTTT
ATTCAGCAATTATTGGAAGCAGGAGTCCATCTG
ATTGAAGAATTGTTAGAAGCTGGCGTGCATTTC
TTAGAACAAATGTTAGATGCAGGTGTACATTTT
TTACAAAAAATGATTGAAGCTGGTGTTCATTTT
CTATCAGAAATGATGGAAGCTGGTGCTCATTTC
CTGCCGCAAATGCTGGAAGCCGGTGTCCATTTT
TTAGAAGAAATGATGGAAGCAGGGGTCCATTTT
TTAGCAGAATTACTAGAAGCGGGCGTTCATTTT

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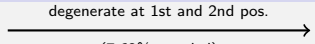
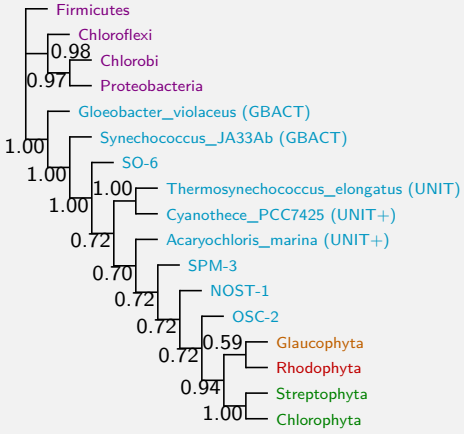
	TTC		WSC		TAC		TGC	
	YTA	Leu	WSA		TAA	Ter	TGA	Ter
	YTG		WSG		TAG		TGG	Trp
C	YTT	Leu	CCT	Pro	CAT	His	MGT	Arg
	YTC		CCC		CAC		MGC	
	YTA		CCA		CAA	Gln	MGA	
	YTG		CCG		CAG		MGG	
A	ATT	Ile	ACT	Thr	AAT	Asn	WST	Ser
	ATC		ACC		AAC		WSC	
	ATA		ACA		AAA	Lys	MGA	Arg
	ATG	Met	ACG		AAG		MGG	
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
	GTC		GCC		GAC		GGC	
	GTA		GCA		GAA	Glu	GGA	
	GTG		GCG		GAG		GGG	

Example:

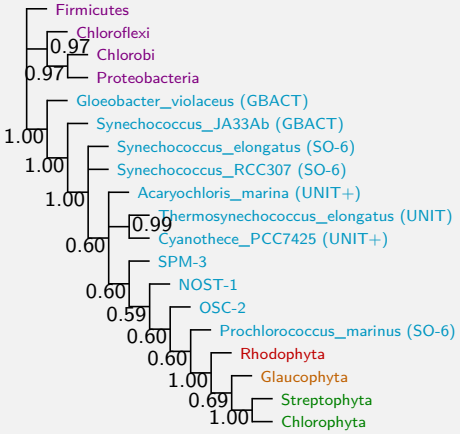
```
ATGAAACAGYTGYTGGAAGCCGGTGTTCACTTC
YTAWSAGAAYTTYTAGATGCCWSCGCTCACATA
YTAGAAGATATGATTCAAWSTGGAATGCATTTT
YTCGAACAAATGYTAGATGTAGGTGTTCATTTT
YTACAGWSAATGYTTGAAGCTGGTGTTCACTTT
YTAGAAGCAYTTYTAGAGGCTGGTGTTCATTTT
YTGGAAGAAATGATGGAAGCGGGTATTCATTTC
YTGGAACAAATGATGGAAGCAGGAGTCCATTTT
ATTCAGCAAYTAYTGGAAGCAGGAGTCCATYTG
ATTGAAGAAYTGYTAGAAGCTGGCGTGCATTTC
YTAGAACAAATGYTAGATGCAGGTGTACATTTT
YTACAAAAAATGATTGAAGCTGGTGTTCATTTT
YTAWSAGAAATGATGGAAGCTGGTGCTCATTTC
YTGCCGCAAATGYTGGAAGCCGGTGTCCATTTT
YTAGAAGAAATGATGGAAGCAGGGGTCCATTTT
YTAGCAGAAYTAYTAGAAGCGGGCGTTCATTTT
```

Degenerating synonymous 1st and 2nd codon positions



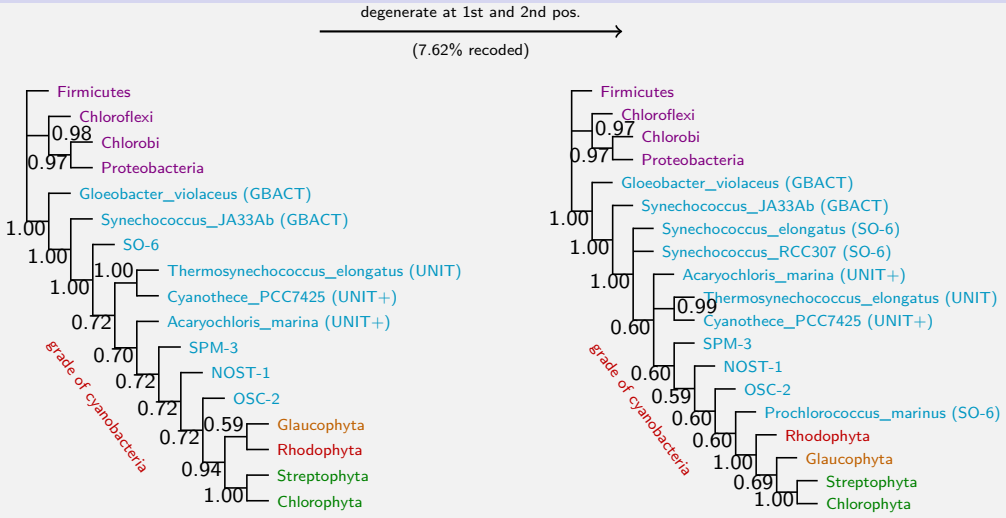


cg75



cg75_degenerate12

Degenerating synonymous 1st and 2nd codon positions



Degenerating synonymous 1st and 2nd codon positions

- Again no "core" cyanobacteria monophyly.

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  $\rightarrow$ Synonymous substitutions at 1st and 2nd codon position are not the only cause of discrepancy between cp75 and cg75.

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- Again no "core" cyanobacteria monophyly.
  $\rightarrow$ Synonymous substitutions at 1st and 2nd codon position are not the only cause of discrepancy between cp75 and cg75.
- Let's try to neutralize all synonymous substitutions...

Degenerating all synonymous codon positions

	T		C		A		G	
T	TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
	TTC		TCC		TAC		TGC	
	TTA	Leu	TCA		TAA	Ter	TGA	Ter
	TTG		TCG		TAG		TGG	Trp
C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
	CTC		CCC		CAC		CGC	
	CTA		CCA		CAA	Gln	CGA	
	CTG		CCG		CAG		CGG	
A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
	ATC		ACC		AAC		AGC	
	ATA		ACA		AAA	Lys	AGA	Arg
	ATG	Met	ACG		AAG		AGG	
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
	GTC		GCC		GAC		GGC	
	GTA		GCA		GAA	Glu	GGA	
	GTG		GCG		GAG		GGG	

Example:

ATGAAACAGTTGCTGGAAGCCGGTGTTCACTTC
TTATCAGAACTTTTAGATGCCAGCGCTCACATA
TTAGAAGATATGATTCAAAGTGGAATGCATTTT
CTCGAACAAATGCTAGATGTAGGTGTTCATTTT
TTACAGTCAATGCTTGAAGCTGGTGTTCACTTT
TTAGAAGCACTTTTAGAGGCTGGTGTTCATTTT
TTGGAAGAAATGATGGAAGCGGGTATTCATTTC
TTGGAACAAATGATGGAAGCAGGAGTCCATTTT
ATTCAGCAATTATTGGAAGCAGGAGTCCATCTG
ATTGAAGAATTGTTAGAAGCTGGCGTGCATTTC
TTAGAACAAATGTTAGATGCAGGTGTACATTTT
TTACAAAAAATGATTGAAGCTGGTGTTCATTTT
CTATCAGAAATGATGGAAGCTGGTGCTCATTTC
CTGCCGCAAATGCTGGAAGCCGGTGTCCATTTT
TTAGAAGAAATGATGGAAGCAGGGGTCCATTTT
TTAGCAGAATTACTAGAAGCGGGCGTTCATTTT

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	T		C		A		G	
T	TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
	TTC		TCC		TAC		TGC	
	TTA	Leu	TCA		TAA	Ter	TGA	Ter
	TTG		TCG		TAG		TGG	Trp
C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
	CTC		CCC		CAC		CGC	
	CTA		CCA		CAA	Gln	CGA	
	CTG		CCG		CAG		CGG	
A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
	ATC		ACC		AAC		AGC	
	ATA		ACA		AAA	Lys	AGA	Arg
	ATG	Met	ACG		AAG		AGG	
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
	GTC		GCC		GAC		GGC	
	GTA		GCA		GAA	Glu	GGA	
	GTG		GCG		GAG		GGG	

Example:

```
ATGAAACAGTTGCTGGAAGCCGGTGTTCACTTC
TTATCAGAACTTTTAGATGCCAGCGCTCACATA
TTAGAAGATATGATTCAAAGTGGAATGCATTTT
CTCGAACAAATGCTAGATGTAGGTGTTCATTTT
TTACAGTCAATGCTTGAAGCTGGTGTTCACTTT
TTAGAAGCACTTTTAGAGGCTGGTGTTCATTTT
TTGGAAGAAATGATGGAAGCGGGTATTCATTTC
TTGGAACAAATGATGGAAGCAGGAGTCCATTTT
ATTCAGCAATTATTGGAAGCAGGAGTCCATCTG
ATTGAAGAATTGTTAGAAGCTGGCGTGCATTTC
TTAGAACAAATGTTAGATGCAGGTGTACATTTT
TTACAAAAAATGATTGAAGCTGGTGTTCATTTT
CTATCAGAAATGATGGAAGCTGGTGCTCATTTC
CTGCCGCAAATGCTGGAAGCCGGTGTCCATTTT
TTAGAAGAAATGATGGAAGCAGGGGTCCATTTT
TTAGCAGAATTACTAGAAGCGGGCGTTCATTTT
```

Degenerating all synonymous codon positions

	T		C		A		G	
T	TTY	Phe	WSN	Ser	TAY	Tyr	TGY	Cys
	TTY		WSN		TAY		TGY	
	YTN	Leu	WSN		TAR	Ter	TGR	Ter
	YTN		WSN		TAR		TGG	Trp
C	YTN	Leu	CCN	Pro	CAY	His	MGN	Arg
	YTN		CCN		CAY		MGN	
	YTN		CCN		CAR	Gln	MGN	
	YTN		CCN		CAR		MGN	
A	ATH	Ile	ACN	Thr	AAY	Asn	WSN	Ser
	ATH		ACN		AAY		WSN	
	ATH		ACN		AAR	Lys	MGN	Arg
	ATG	Met	ACN		AAR		MGN	
G	GTN	Val	GCN	Ala	GAY	Asp	GGN	Gly
	GTN		GCN		GAY		GGN	
	GTN		GCN		GAR	Glu	GGN	
	GTN		GCN		GAR		GGN	

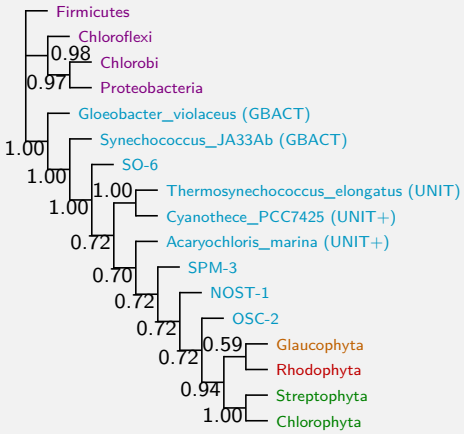
Example:

```
ATGAARCARYTNYTNGARGCNGGNGTNCAYTTY
YTNWSNGARYTNYTNGAYGCNWSNGCNCAYATH
YTNGARGAYATGATHCARWSNGGNATGCAYTTY
YTNGARCARATGYTNGAYGTNGGNGTNCAYTTY
YTNCARWSNATGYTNGARGCNGGNGTNCAYTTY
YTNGARGCNYTNYTNGARGCNGGNGTNCAYTTY
YTNGARGARATGATGGARGCNGGNATHCAYTTY
YTNGARCARATGATGGARGCNGGNGTNCAYTTY
ATHCARCARYTNYTNGARGCNGGNGTNCAYYTN
ATHGARGARYTNYTNGARGCNGGNGTNCAYTTY
YTNGARCARATGYTNGAYGCNGGNGTNCAYTTY
YTNCARAARATGATHGARGCNGGNGTNCAYTTY
YTNWSNGARATGATGGARGCNGGNGCNCAYTTY
YTNCCNCARATGYTNGARGCNGGNGTNCAYTTY
YTNGARGARATGATGGARGCNGGNGTNCAYTTY
YTNGCNGARYTNYTNGARGCNGGNGTNCAYTTY
```

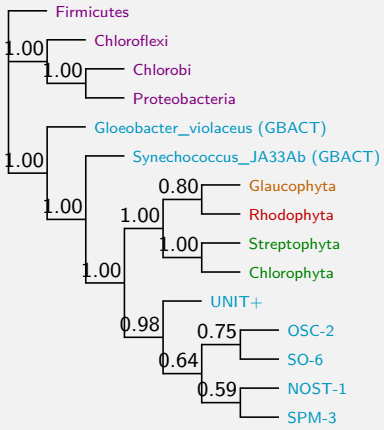
Degenerating all synonymous codon positions

degenerate at 1st, 2nd and 3rd pos.

(34.97% recoded)



cg75

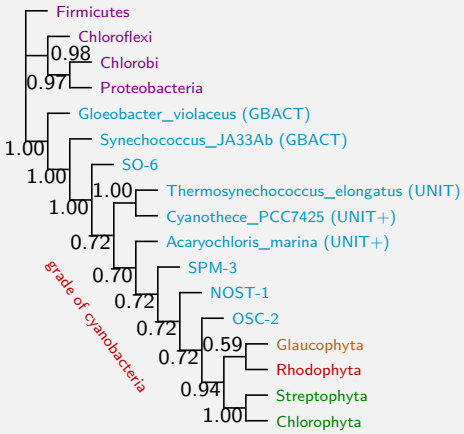


cg75_degenerate123

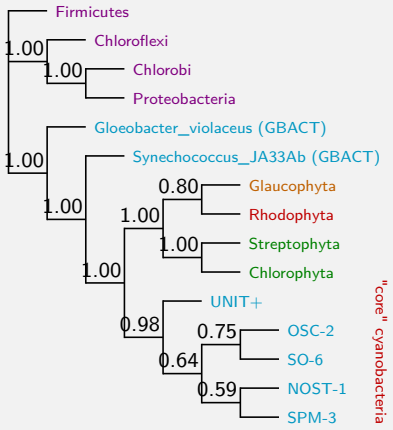
Degenerating all synonymous codon positions

degenerate at 1st, 2nd and 3rd pos.

(34.97% recoded)



cg75



cg75_degenerate123

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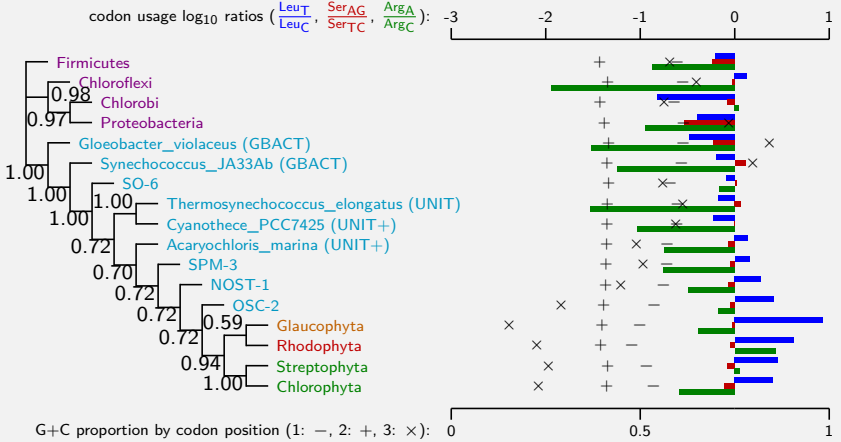
    $\rightarrow$ Convergence between taxa sharing the same biases
  - Let's visualize this...

Effects of composition and codon usage



cg75

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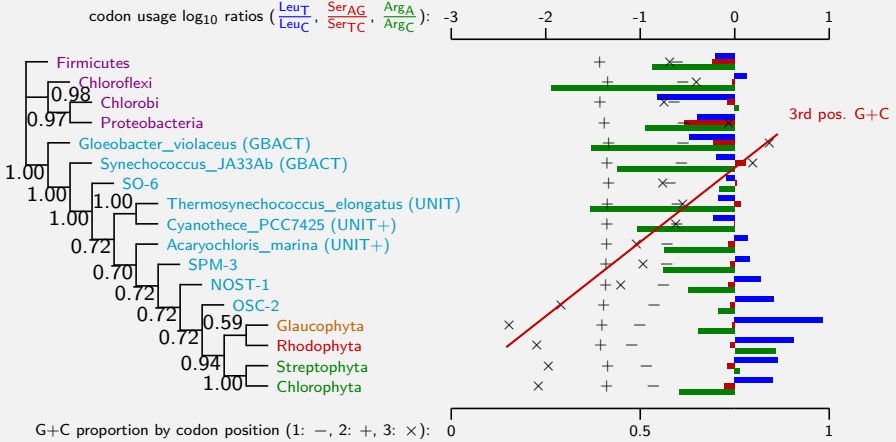

cg75

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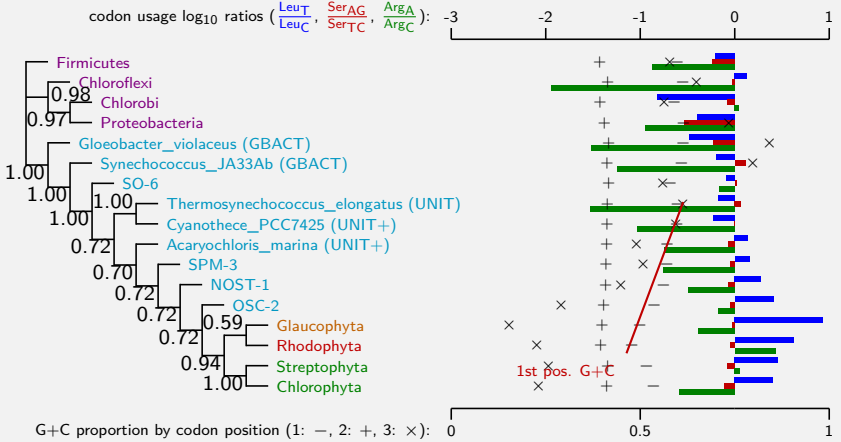

cg75

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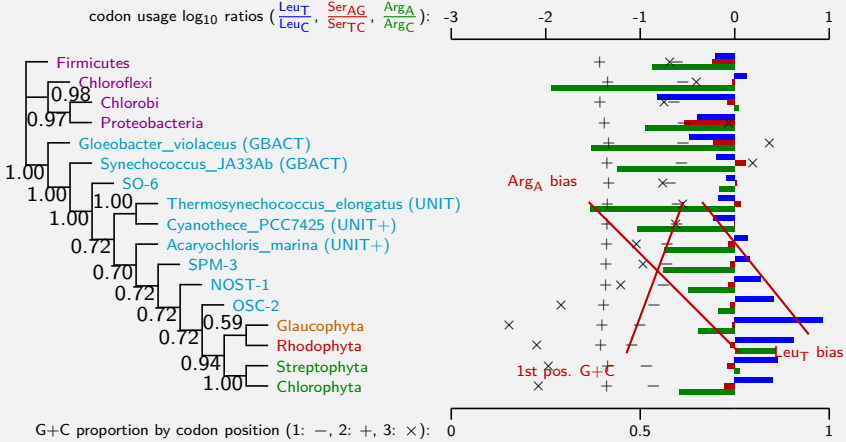

cg75

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- But information potentially useful for small scale resolution is lost in translated / recoded data
- The use of more advanced models could turn misleading signal into useful signal.
  $\rightarrow$ Could we then get both large and small scale resolution using un-recoded nucleotide data?

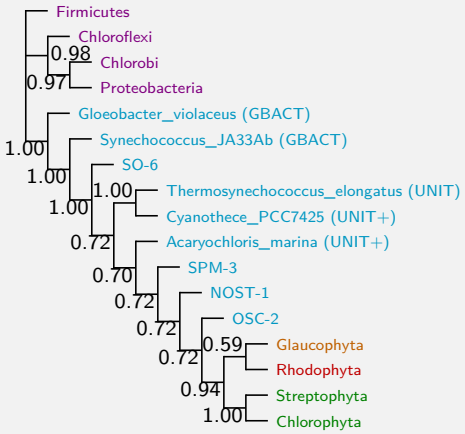
Thanks for your attention

- This work was supported by a Fundação para a Ciência e a Tecnologia (FCT, Portugal) grant to Cymon J. Cox, Centro de Ciencias do Mar (CCMAR) - CIMAR-Lab. Assoc., (PTDC/BIA-BCM/099565/2008).

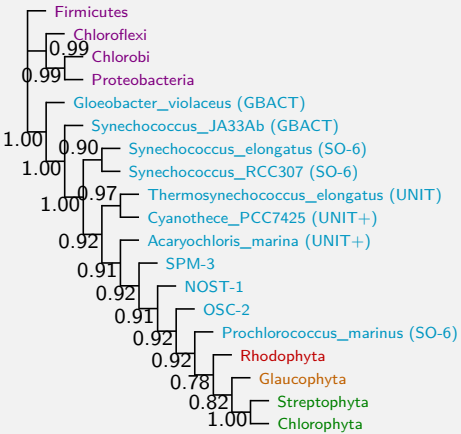
- Contact: `blaise.li@normalesup.org`

Effects of serine synonymous substitutions

degenerate serines (at pos. 1 and 2) →

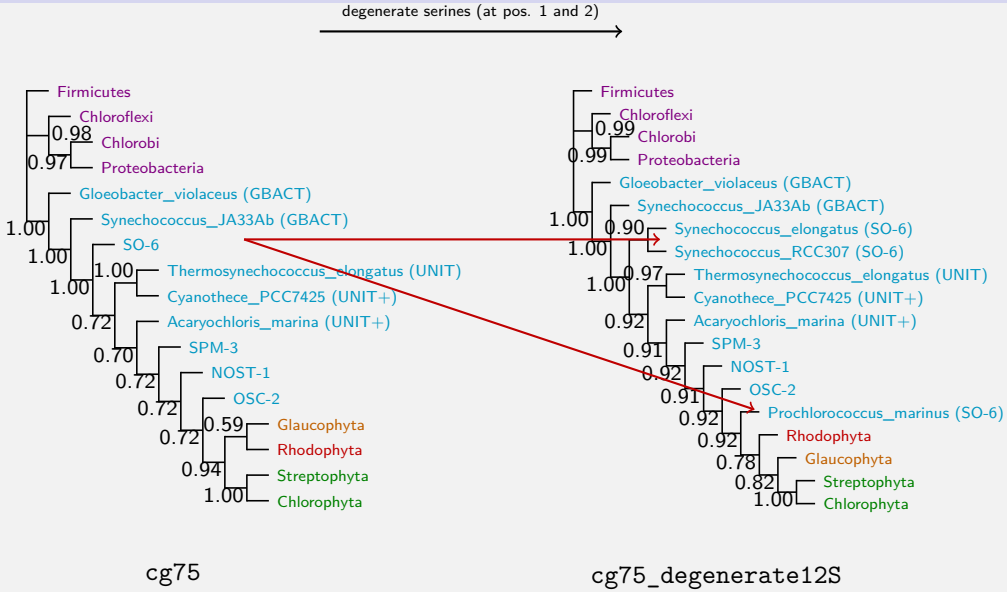


cg75



cg75_degenerate12S

Effects of serine synonymous substitutions



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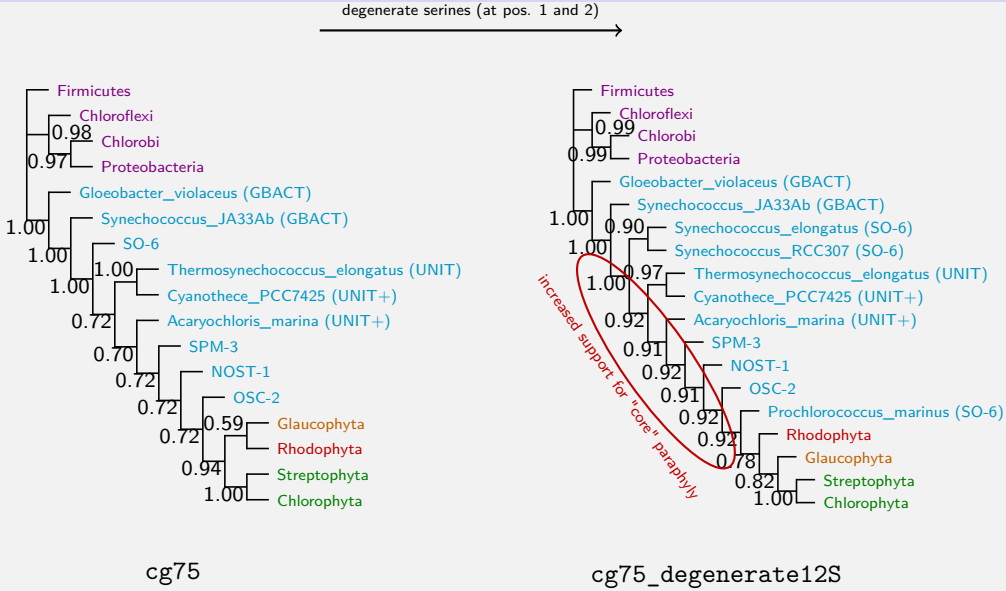

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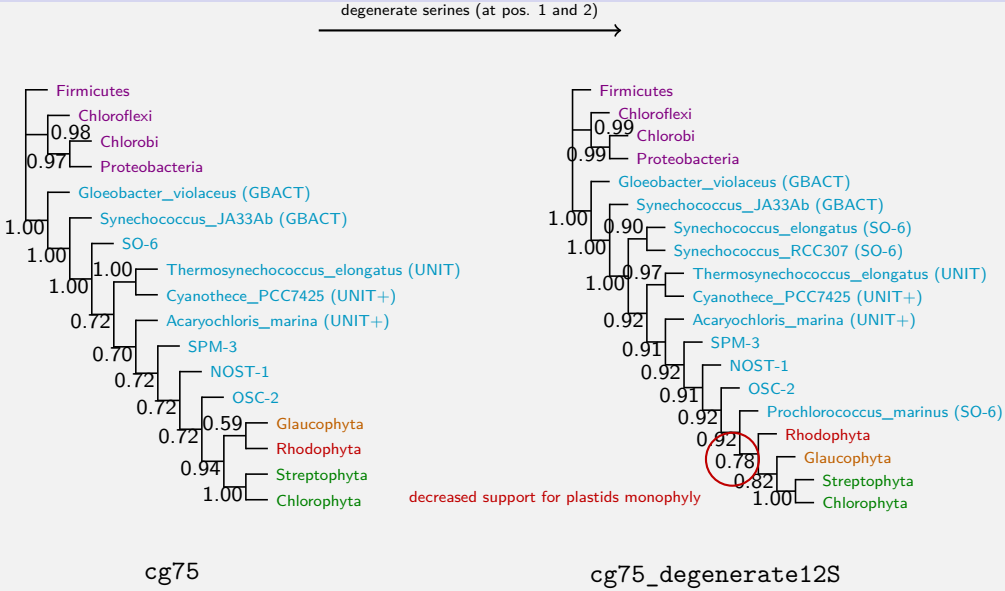

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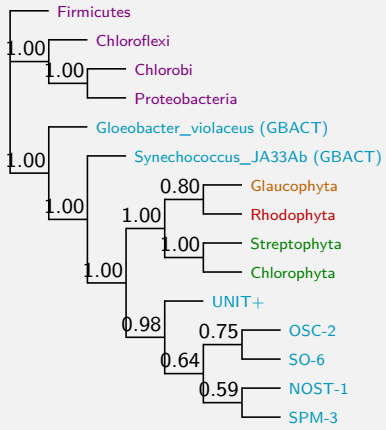
Effects of serine synonymous substitutions

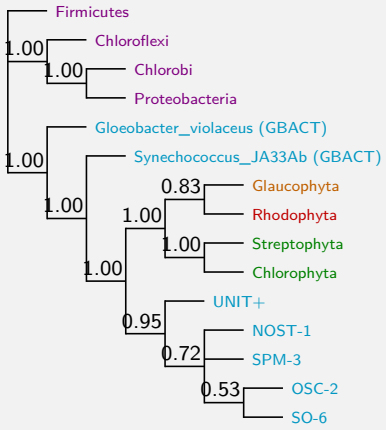
- Serine signal:
  - contributes to SO-6 and plastid monophyly
  - decreases the support of "core" cyanobacteria paraphyly
- Serine signal is not present in translated datasets
- Maybe we should keep this signal in the nucleotide dataset?

Effects of serine synonymous substitutions

restore serine codons



cg75_degenerateLRS3



cg75_degenerateLR3

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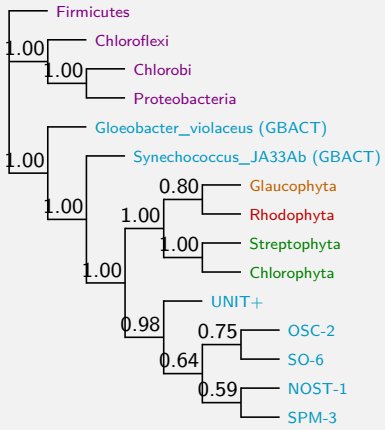
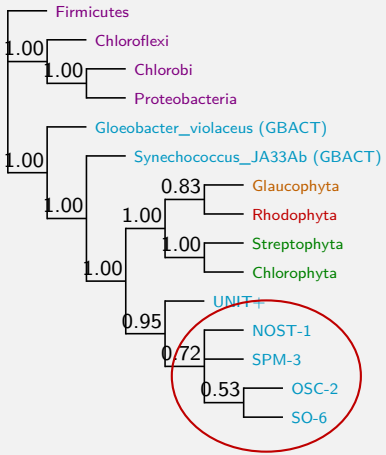
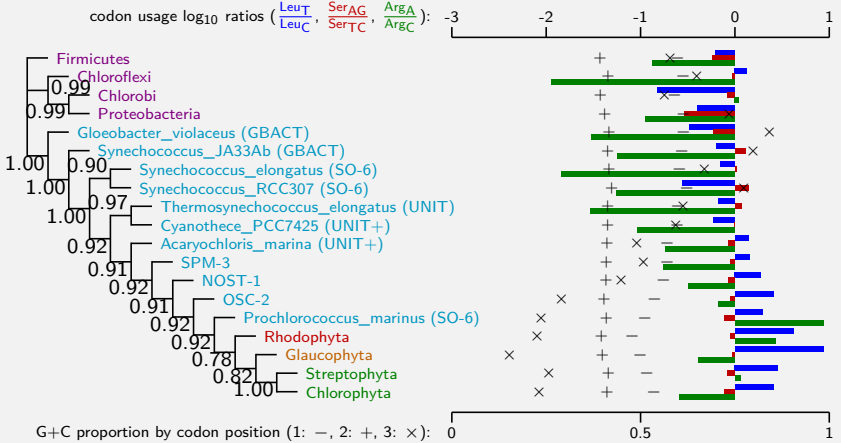


cg75_degenerateLRS3



cg75_degenerateLR3

Effects of composition and codon usage



cg75_degenerate12S

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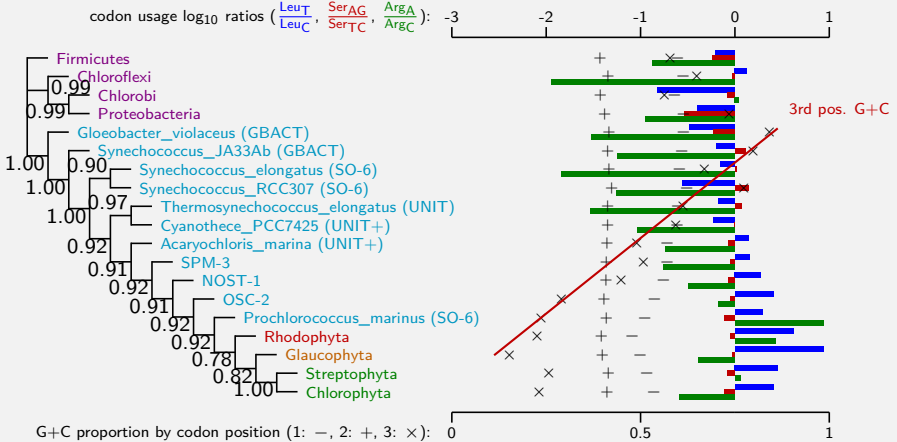

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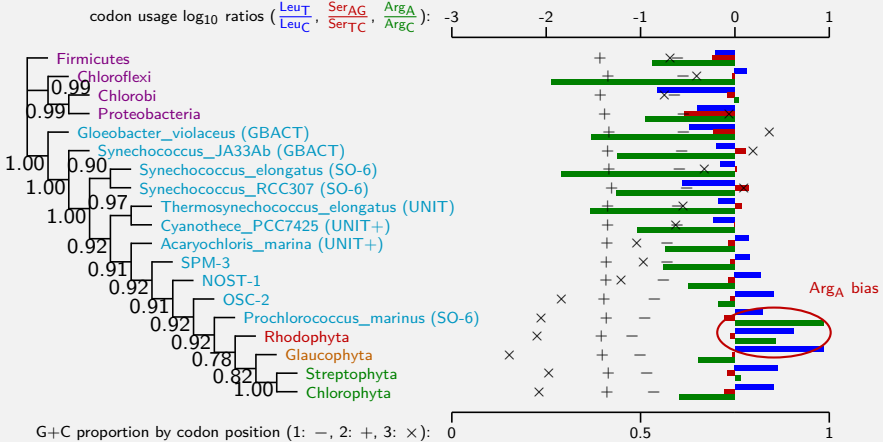

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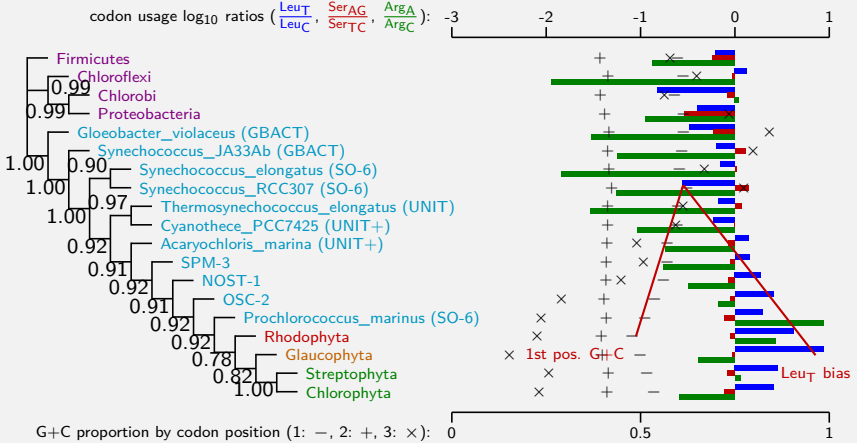

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