Internode certainty and related measures

@JLSteenwyk https://jlsteenwyk.com/

Outline

- PhyKIT, what is it and why?
- A refresher on incongruence
- Technical comments for practical
- Quiz at 4:40pm

PhyKIT

a toolkit for examining multiple sequence alignments and trees

Motivation

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- “Code available upon request….”

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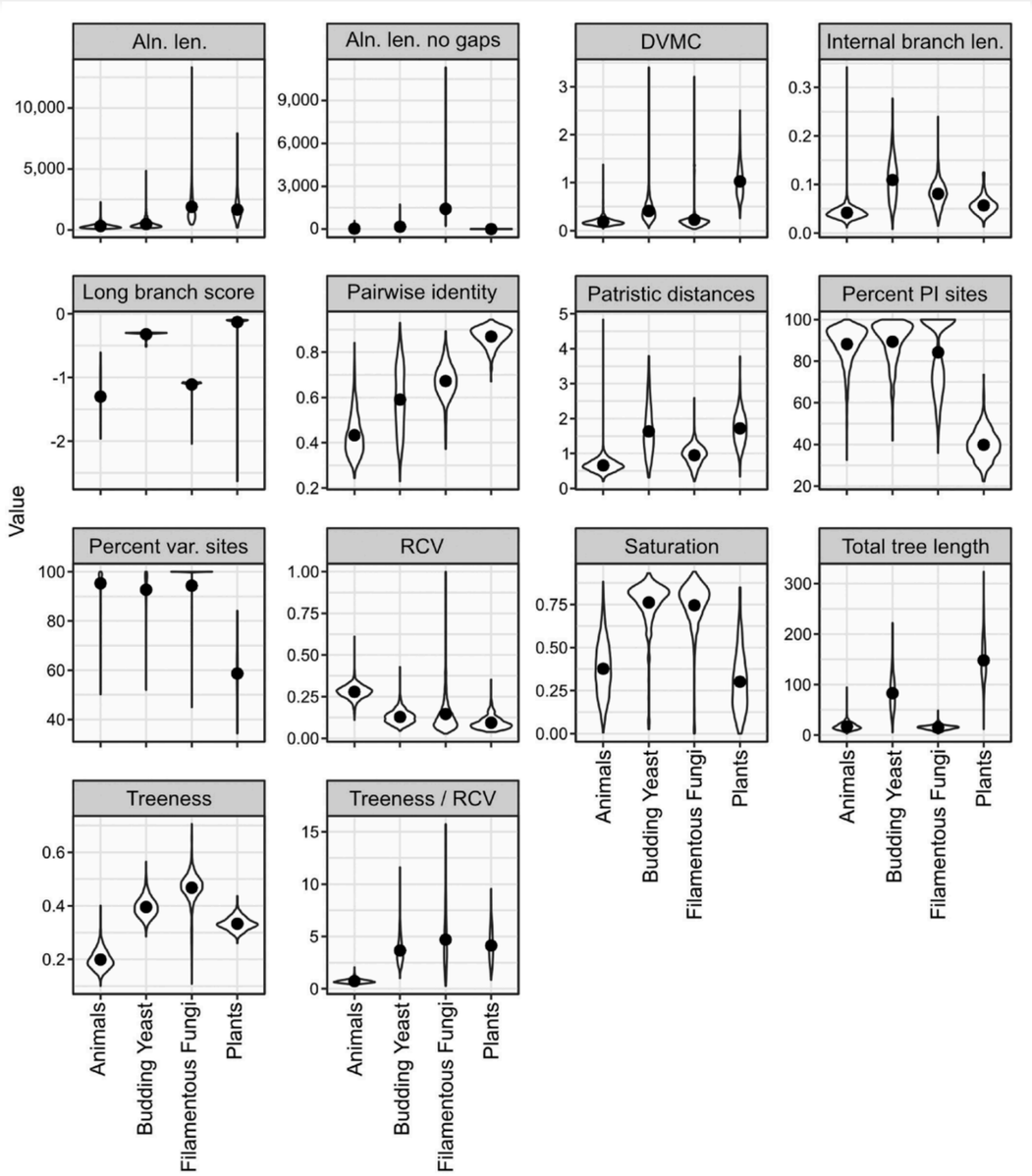
Motivation

- “Code available upon request….”
- “Can you send me your script?”
- I can’t read their code
- Documentation is horrendous or nonexistent

PhyKIT, a Swiss-army knife toolkit

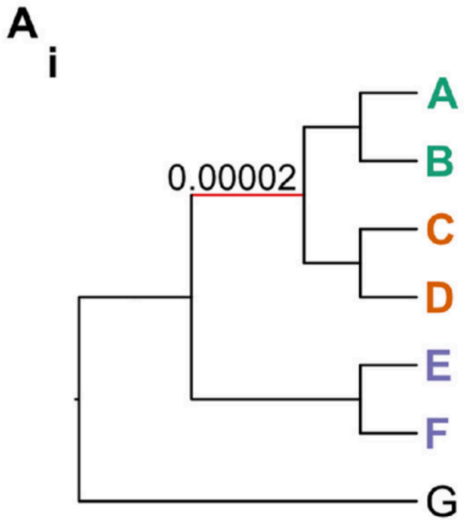
- Helps with processing and analyzing MSAs and trees
- Three exemplary use cases
  - Summarize information content
  - Identify radiations / polytomies
  - Quantify gene-gene coevolution

Information theory-based summary of data



- Phylogenomic subsampling
- Identifying potential sources of error
- Quantifying evolutionary rate, etc.

The polytomy test



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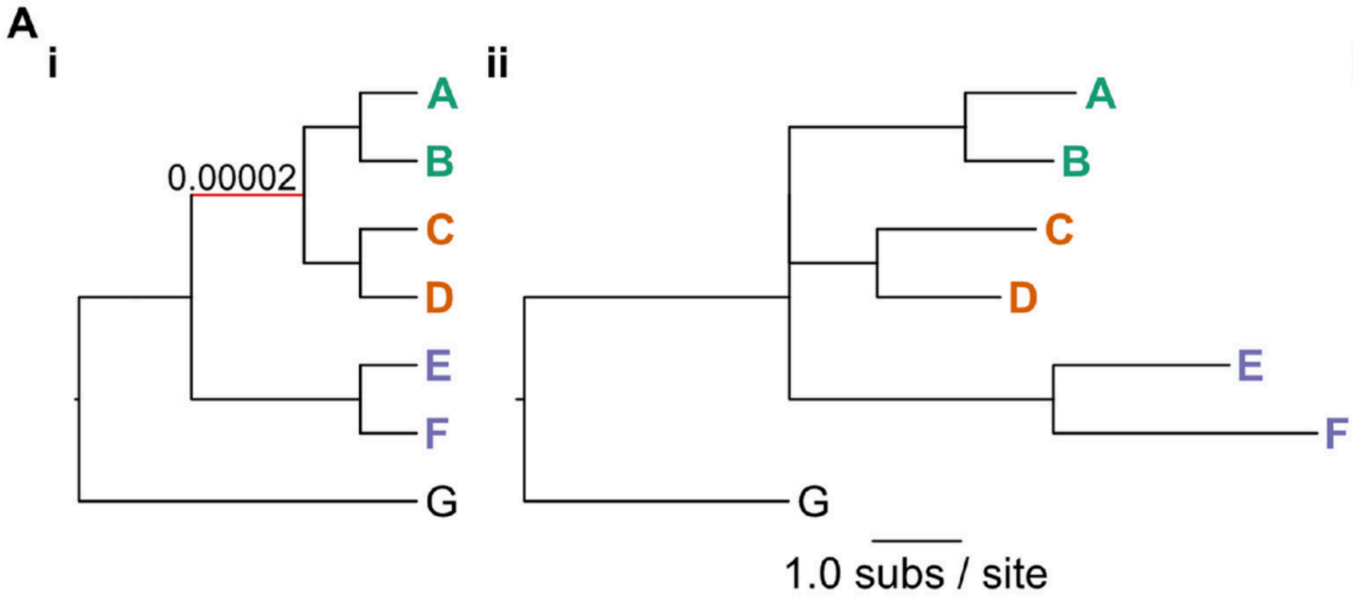
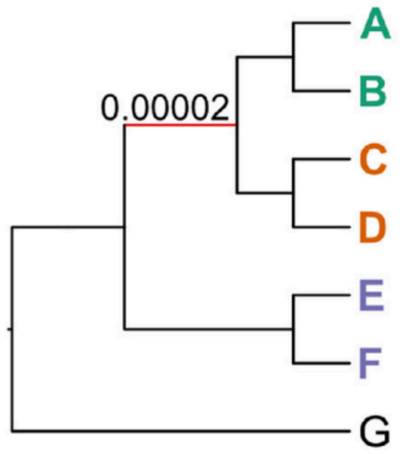


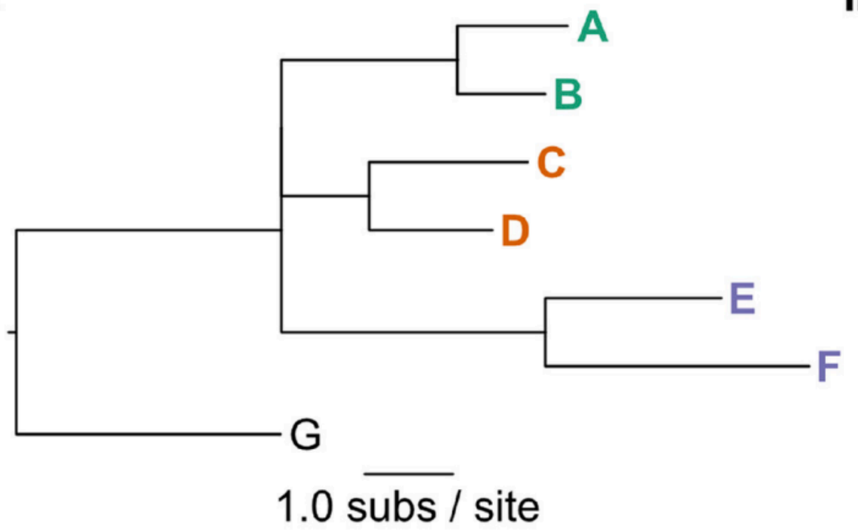
The polytomy test

A

i



ii



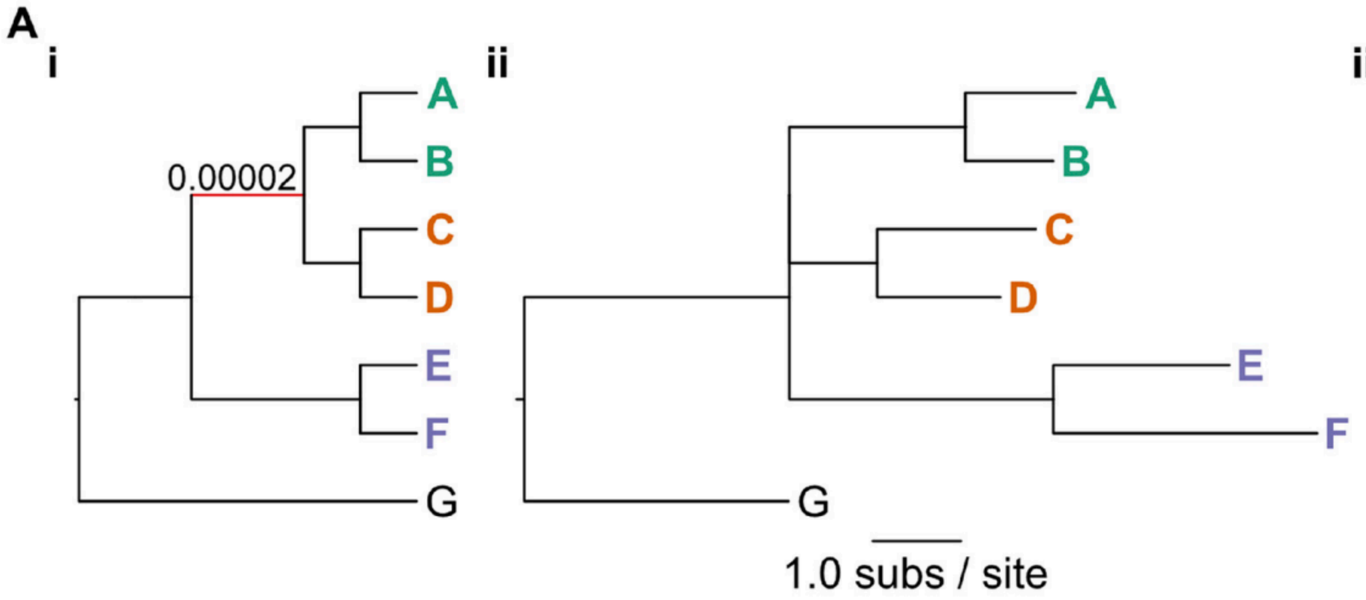
iii

Gene support frequency-based

Chi-squared	5.97
P-value	0.05
(((A,B),(C,D)),others);	301
(((A,B),(E,F)),others);	364
(((C,D),(E,F)),others);	335

The polytomy test

A



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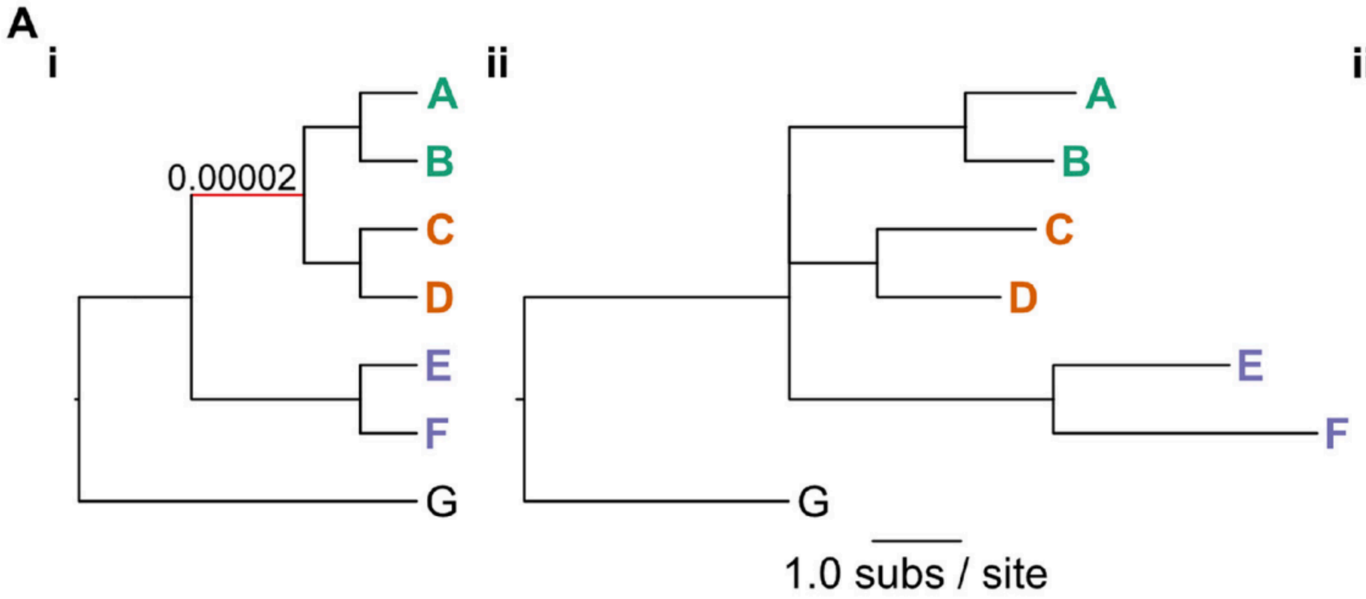

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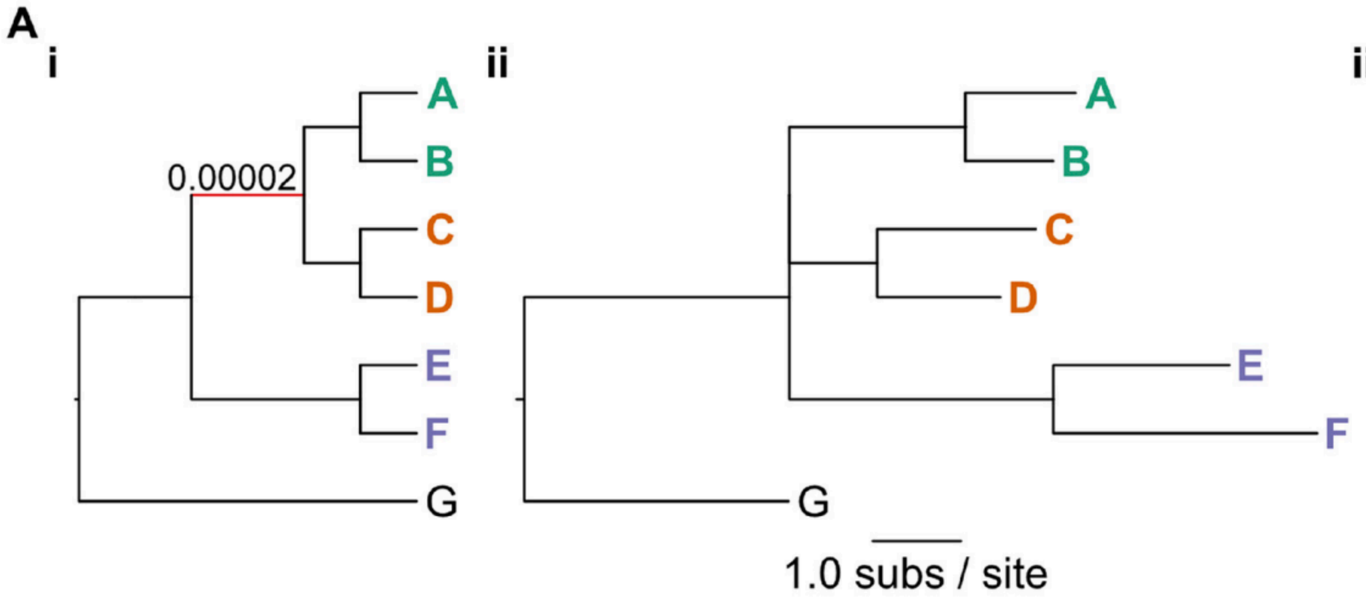

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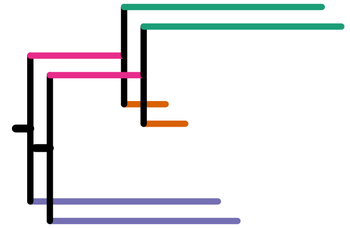
Gene-gene coevolution predicts shared function

- gene coevolution refers to:
  - two genes that covary in parallel across speciation events
  -often observed among genes that share function, are coexpressed, or are part of the same multi-meric complexes



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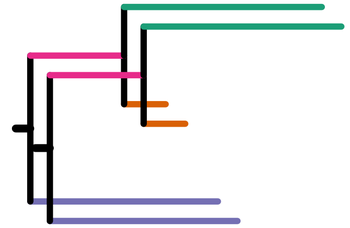

PhyKIT

a toolkit for examining multiple sequence alignments and trees

PhyKIT: a broadly applicable UNIX shell toolkit for processing and analyzing phylogenomic data

Jacob L Steenwyk ✉, Thomas J Buida, III, Abigail L Labella, Yuanning Li, Xing-Xing Shen, Antonis Rokas ✉

Bioinformatics, btab096, https://doi.org/10.1093/bioinformatics/btab096
Published: 09 February 2021 **Article history** ▾

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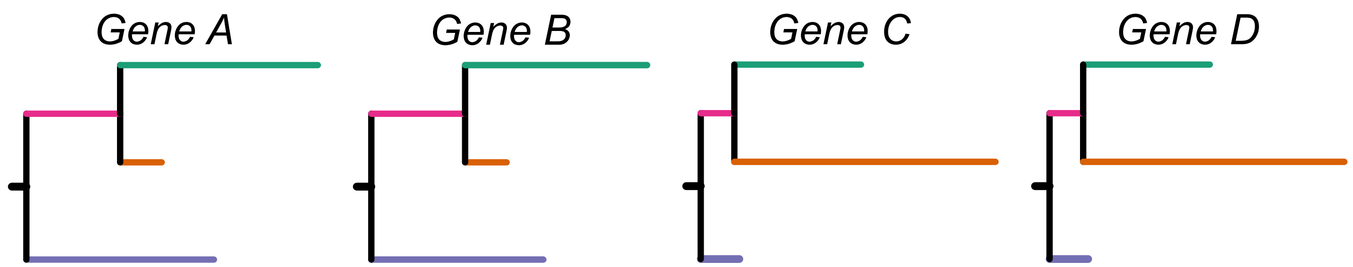
Steenwyk et al. (2021), Bioinformatics

The mirror principle to detect gene coevolution

Gene trees



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*gene trees are constrained to the species tree topology; mathematical corrections and transformations not shown

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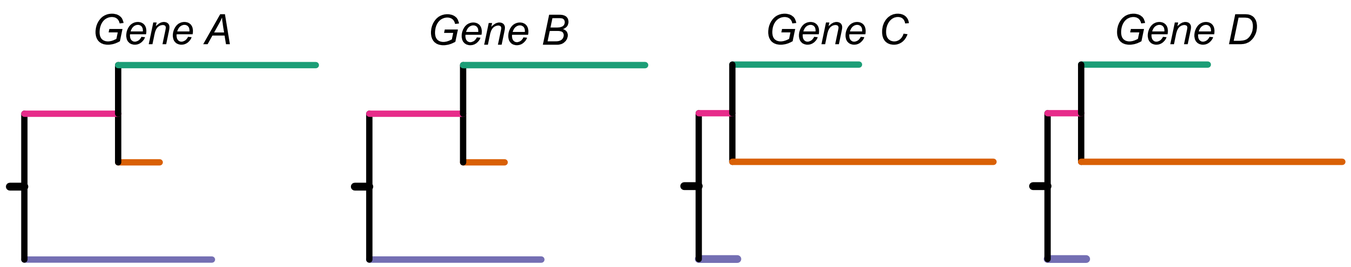
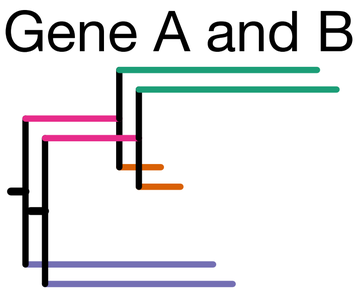


The ‘mirror’ principle



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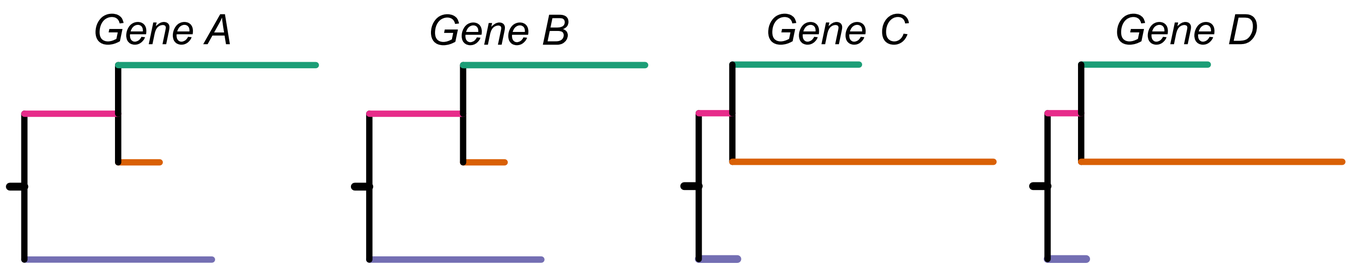

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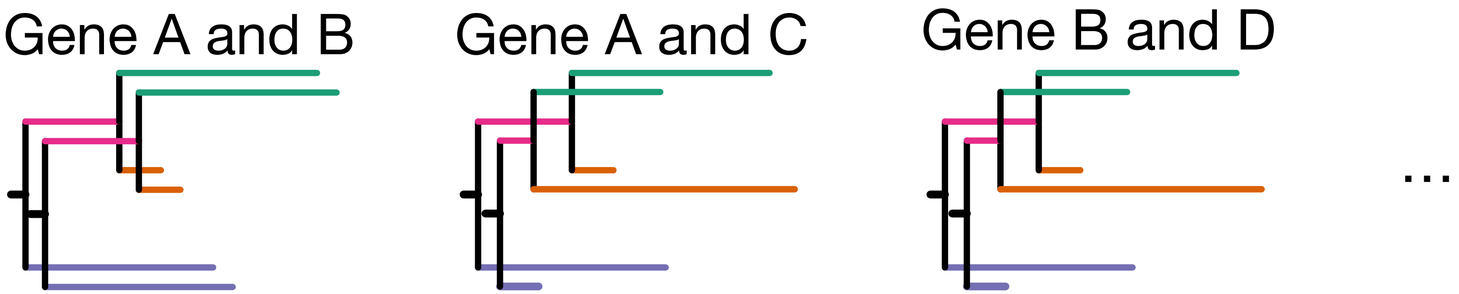

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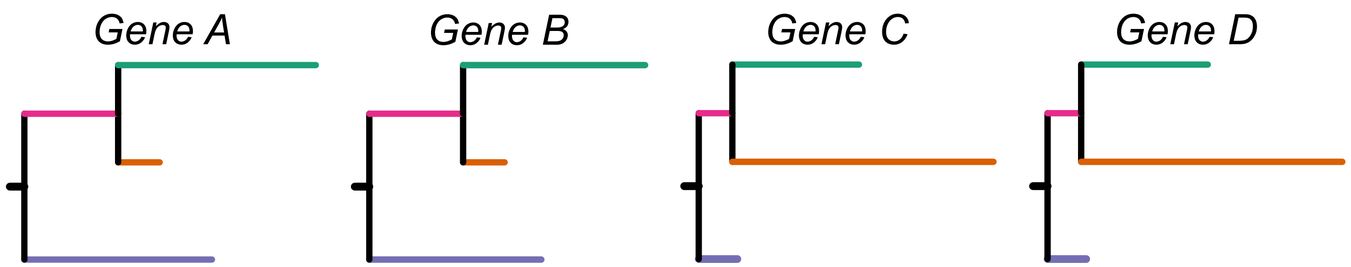

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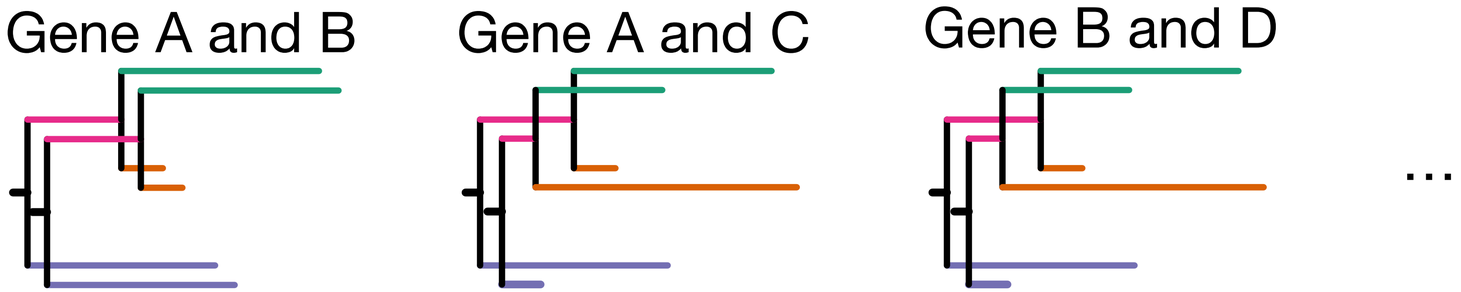
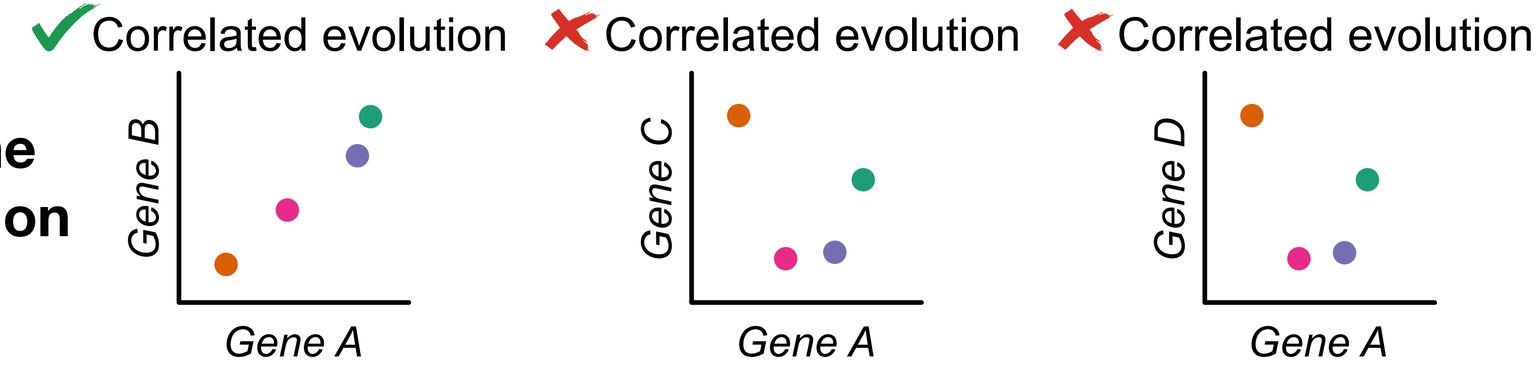


Examine covariation



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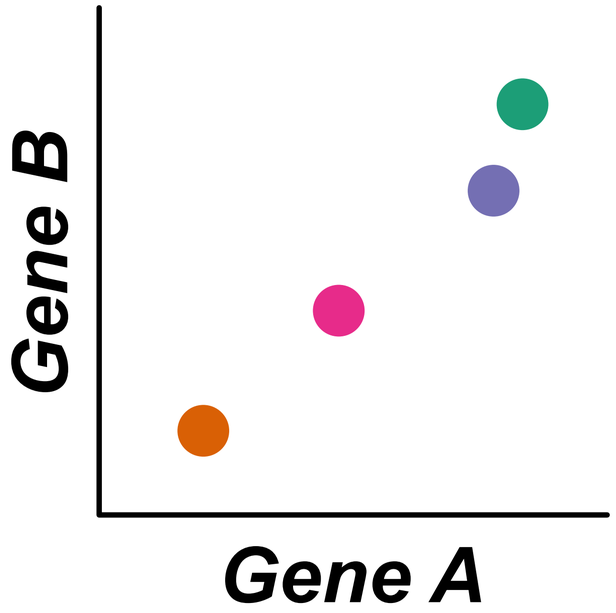
Genes of a feather evolve together

✔ Correlated evolution



- Coevolving genes tend to share function, be coexpressed, or are part of the same multimeric complexes

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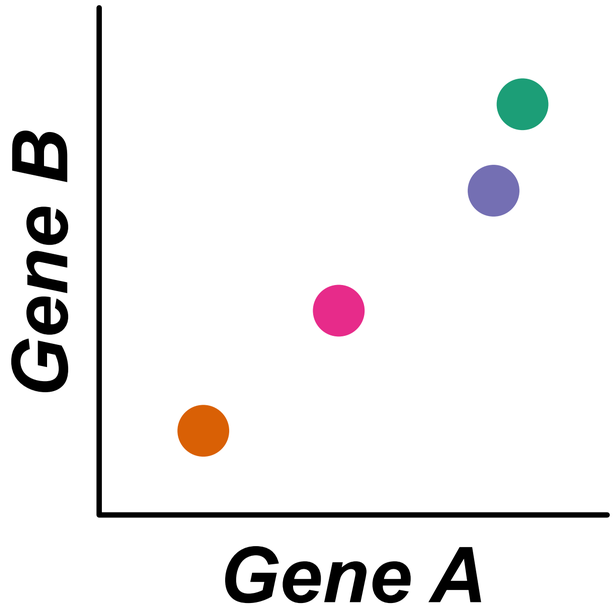

- Coevolving genes tend to share function, be coexpressed, or are part of the same multimeric complexes
- **But can we build a genetic network?**

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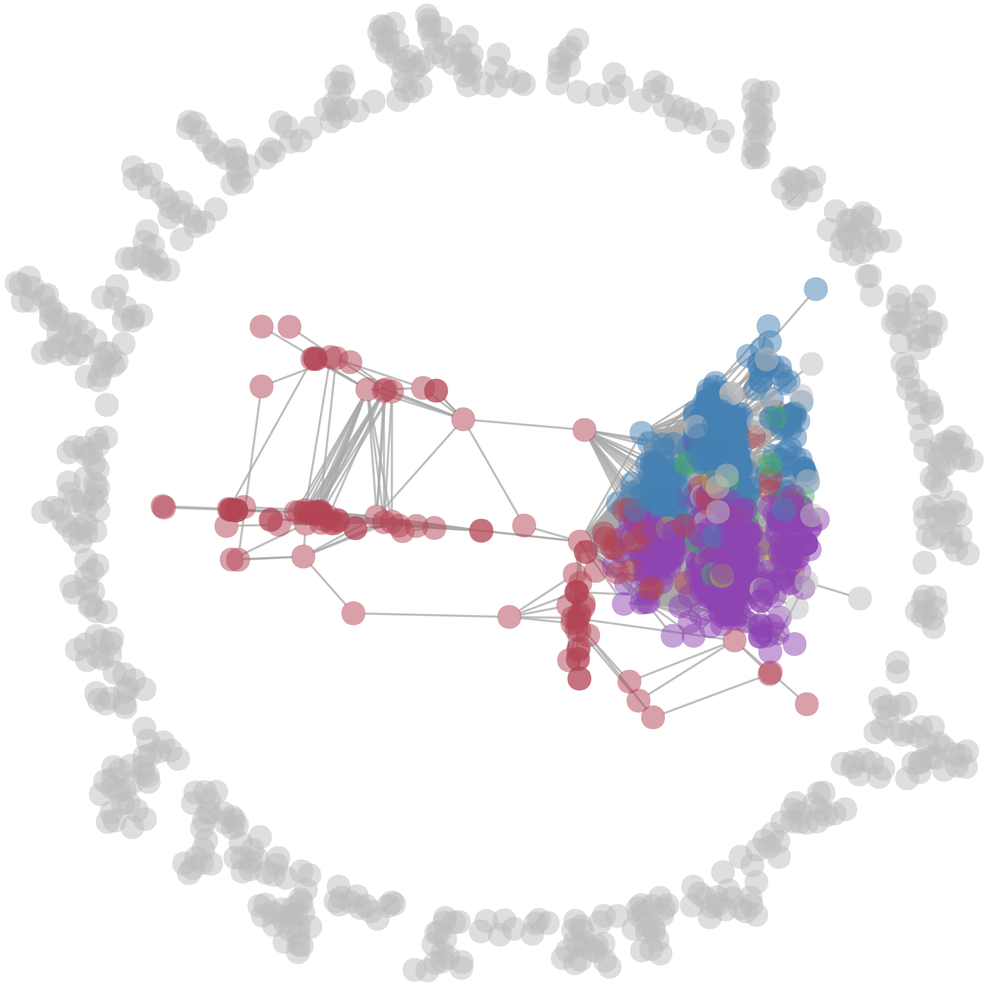
Steenwyk et al. (2021), Bioinformatics

*gene trees are constrained to the species tree topology; mathematical corrections and transformations not shown

A gene coevolutionary network for budding yeast



- Nodes are genes
- Genes that are predicted to be cofunctional are connected

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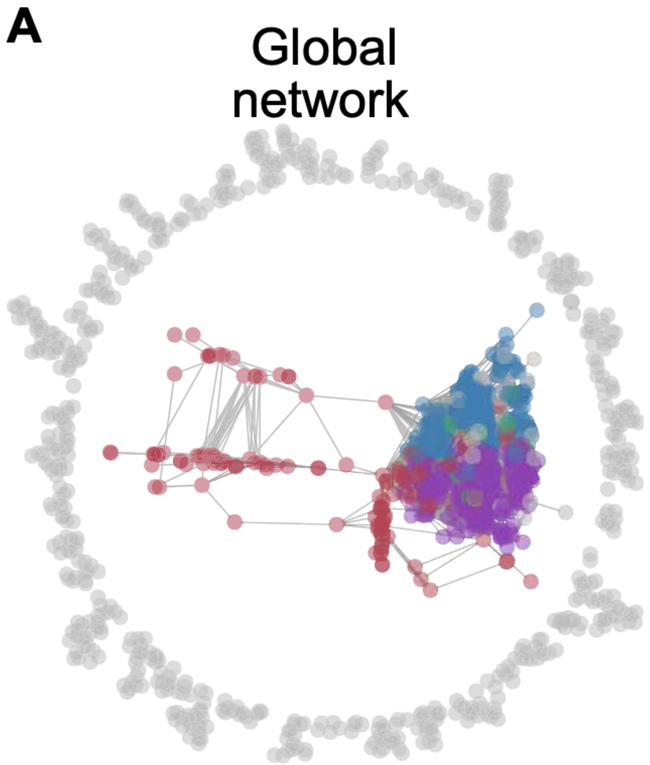
Steenwyk et al. (2022), Science Advances

Gene coevolution networks capture bio-information



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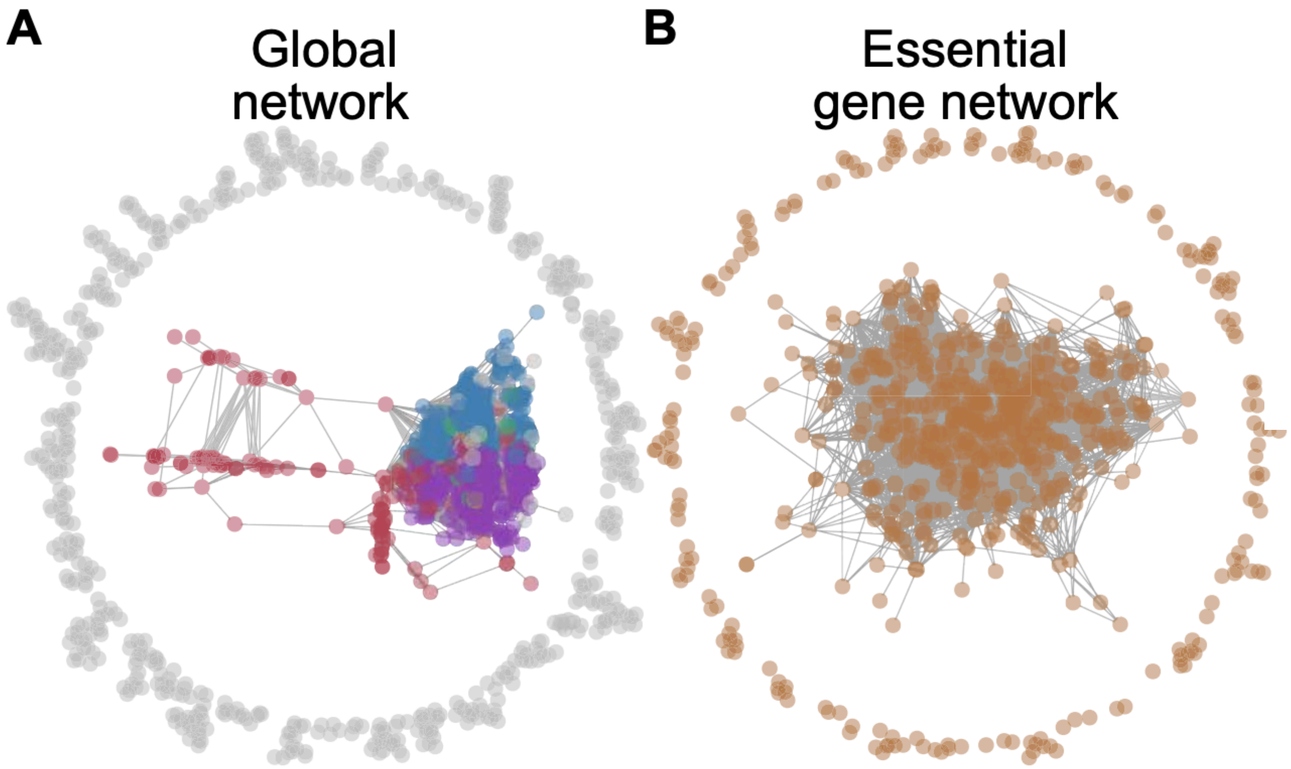

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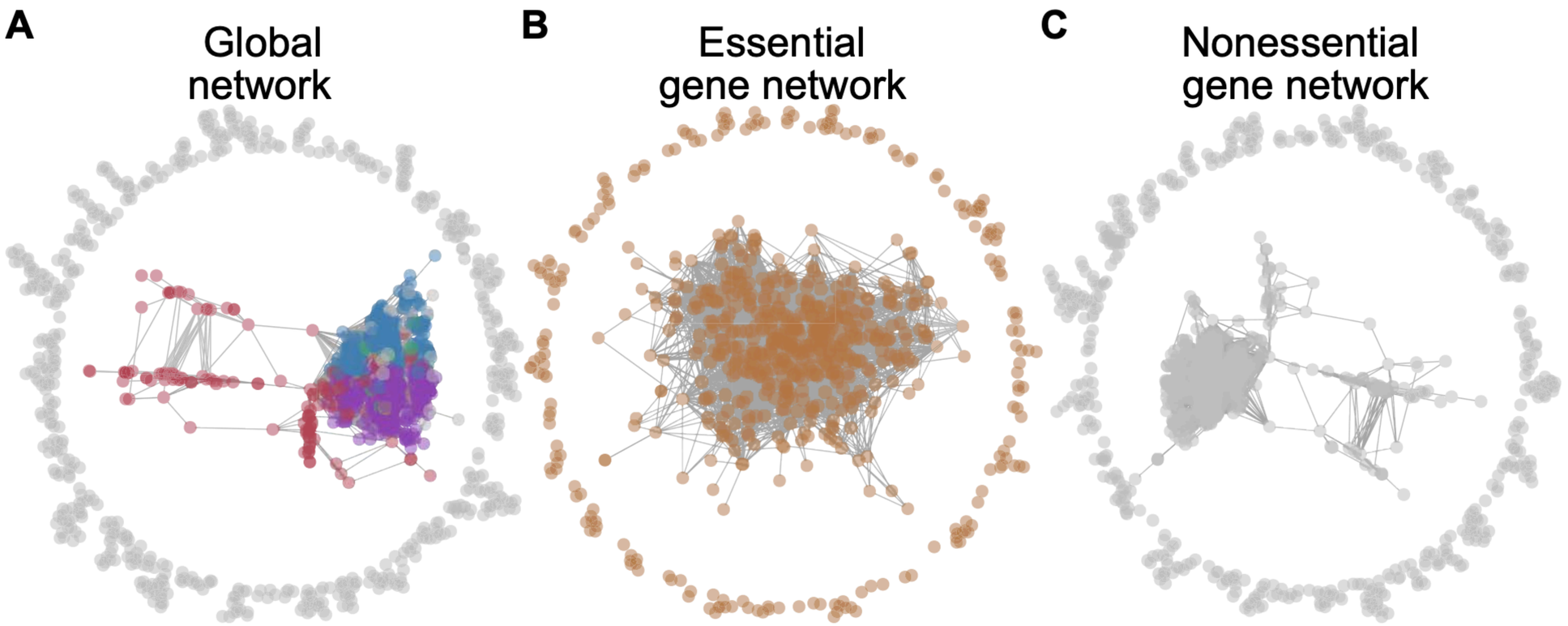

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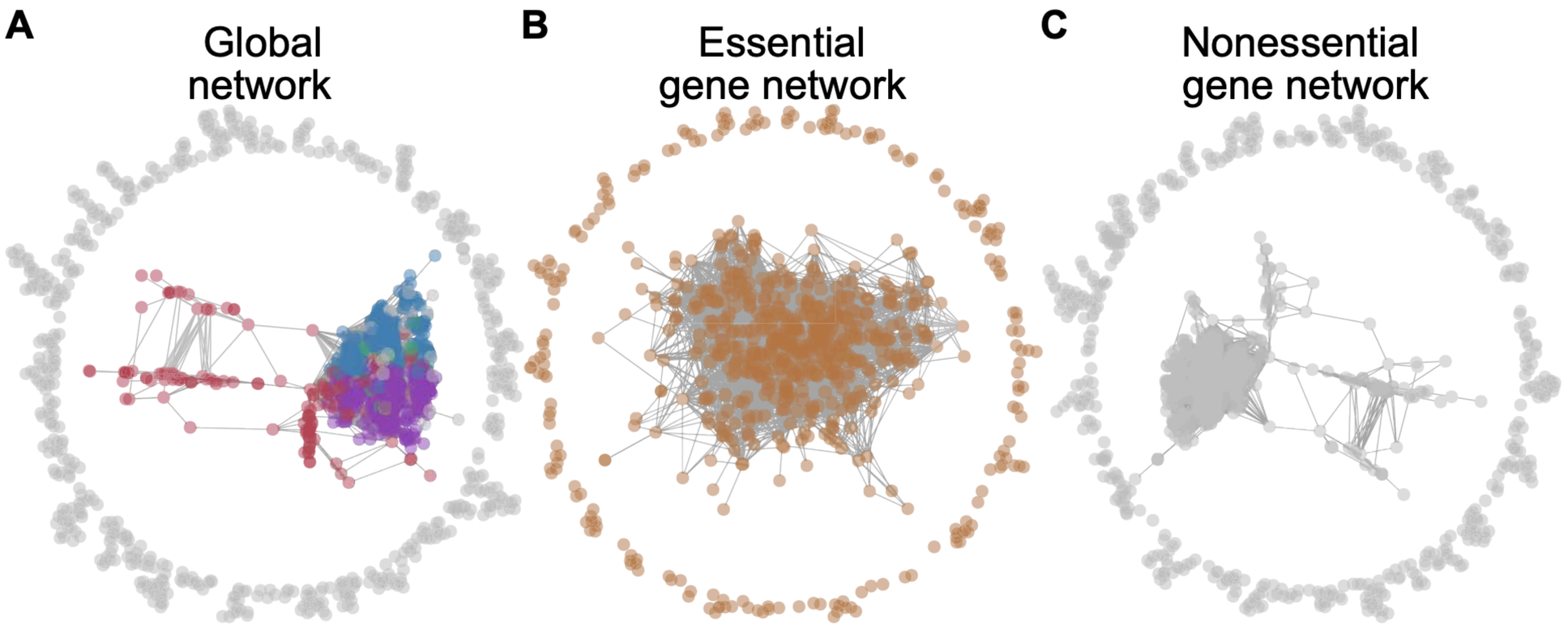

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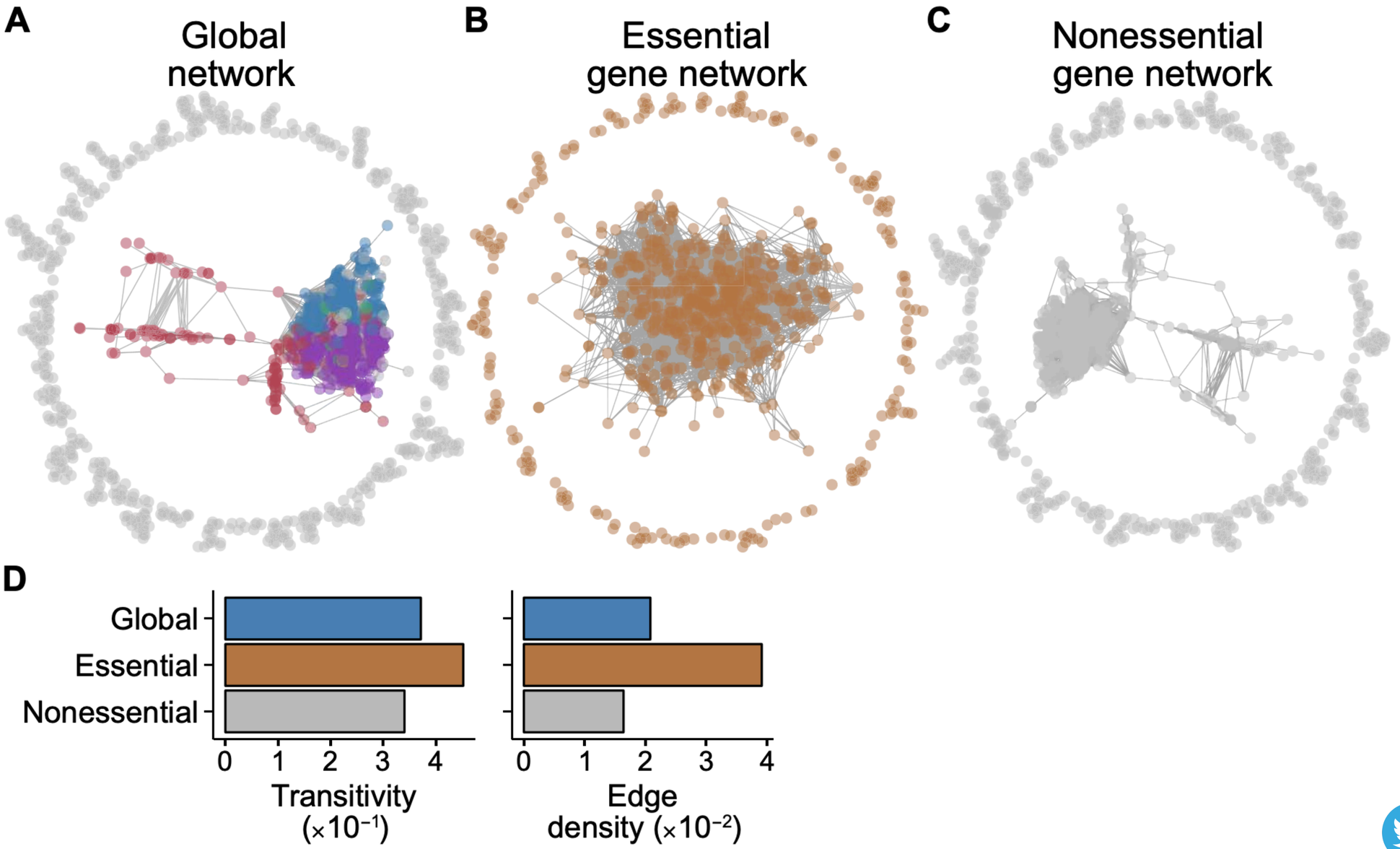

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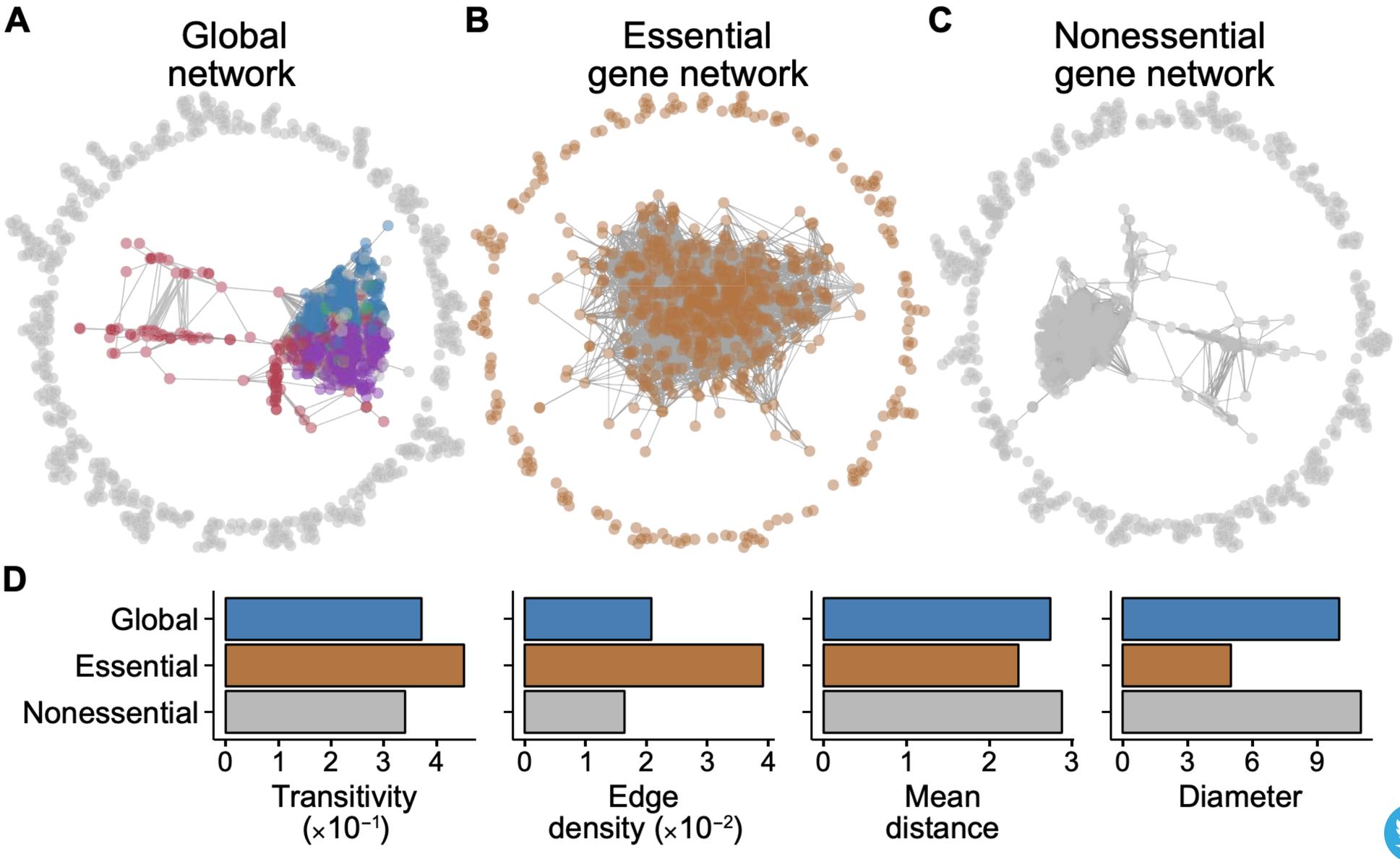

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PhyKIT is not your only option

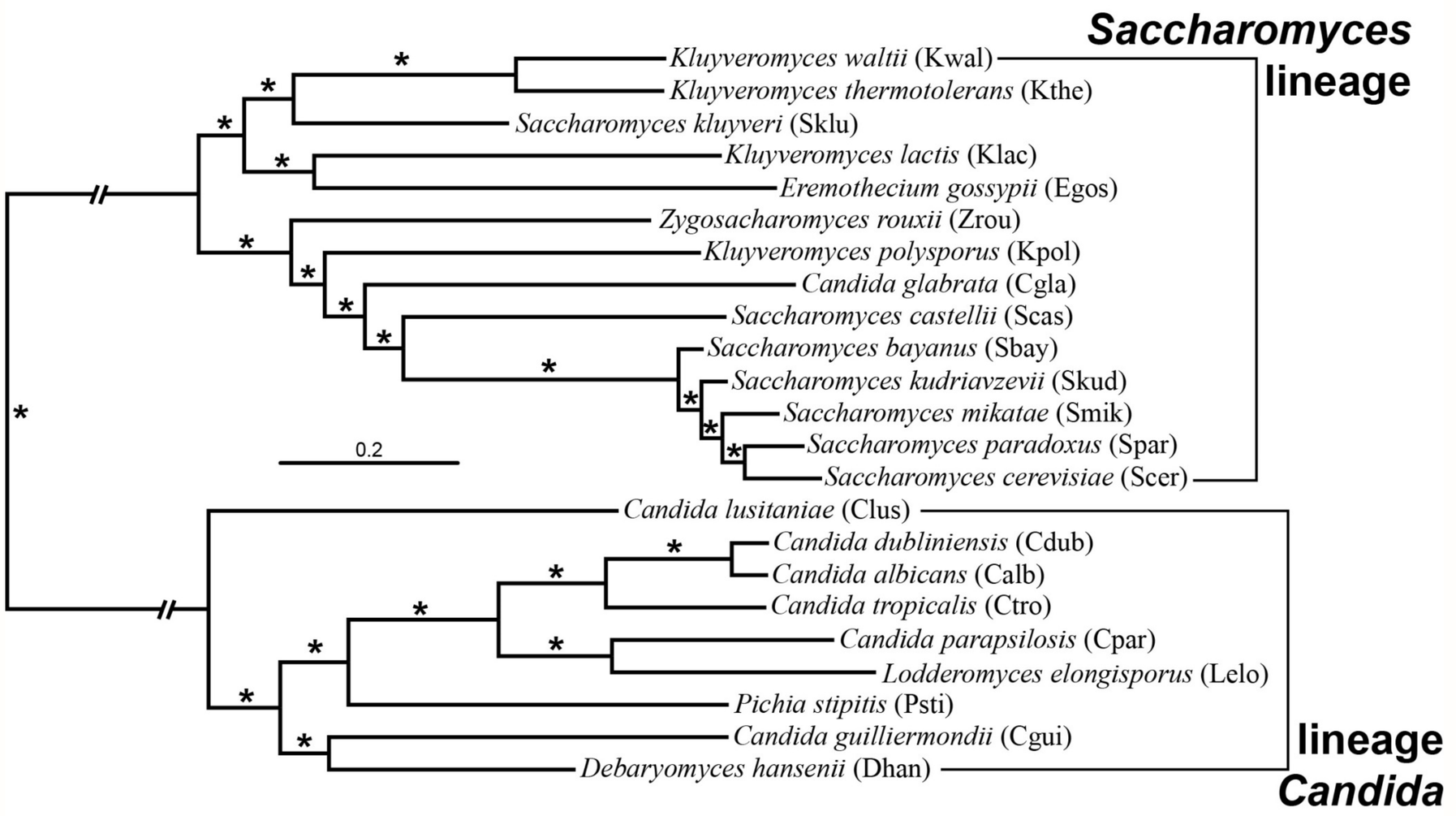
- Phyx
- Newick utilities
- Phylommand
- Gotree

A refresher…

A refresher…

The next few slides are from Antonis Rokas

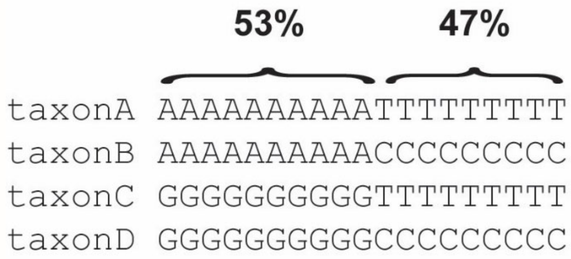
Concatenation Yields an Absolutely Supported Phylogeny



Slide from Antonis Rokas

Salichos & Rokas (2013) Nature

Bootstrap Support is Misleading When Used in Large Datasets



Slide from Antonis Rokas

Rokas & Carroll (2006) PLoS Biol.

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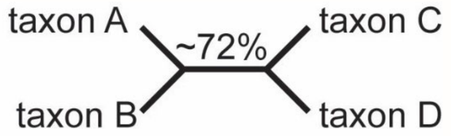
100 characters



53% **47%**

```
taxonA AAAAAAAAAATTTTTTTTT
taxonB AAAAAAAAAACCCCCCCCC
taxonC GGGGGGGGGGTTTTTTTTT
taxonD GGGGGGGGGGCCCCCCCCC
```

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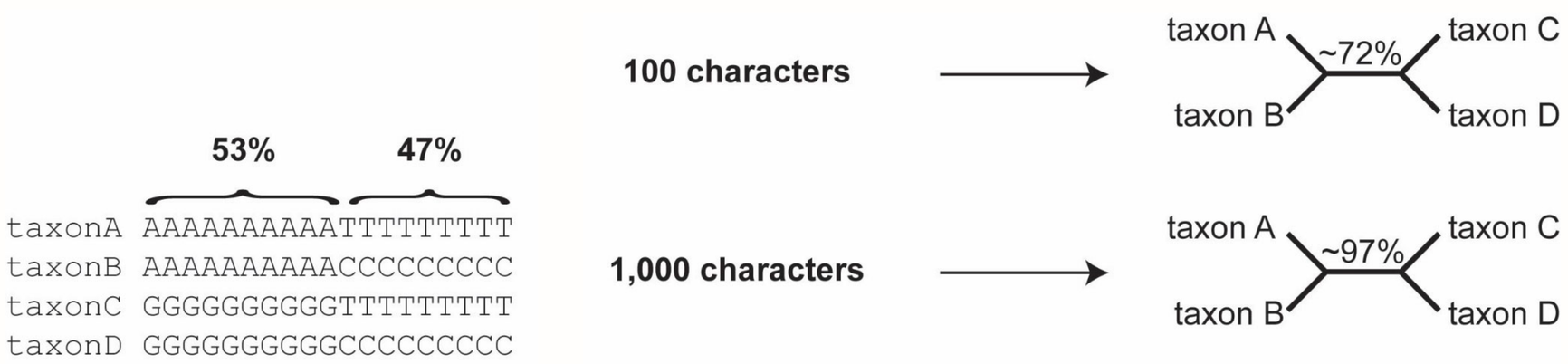

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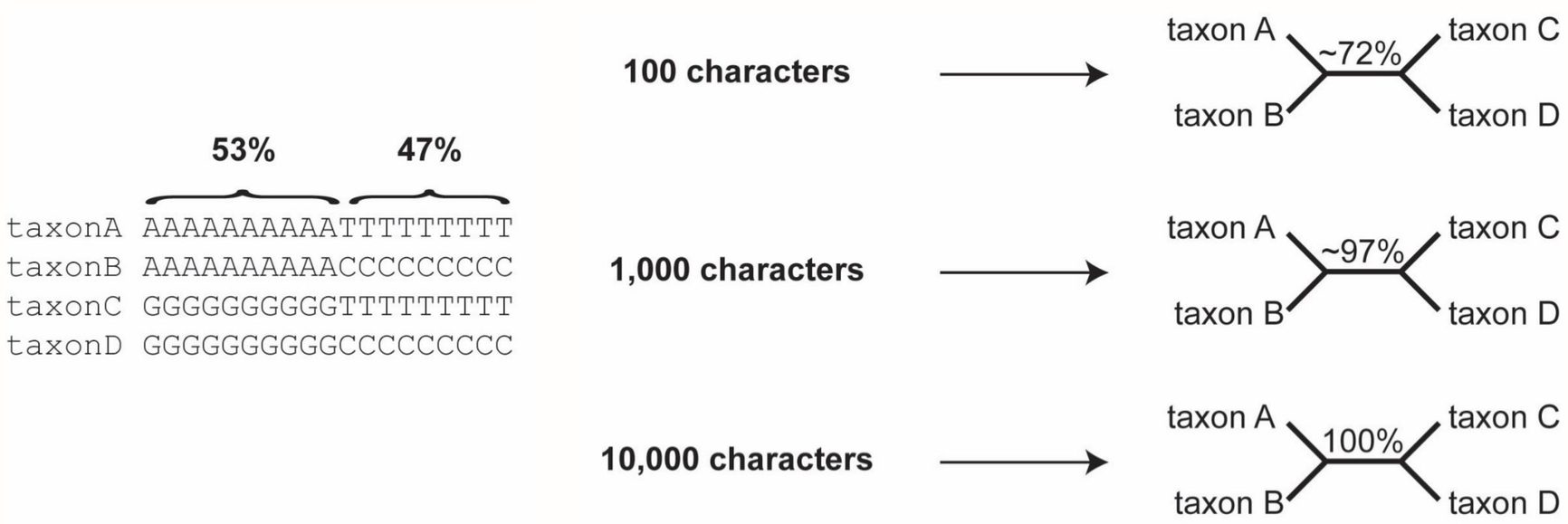

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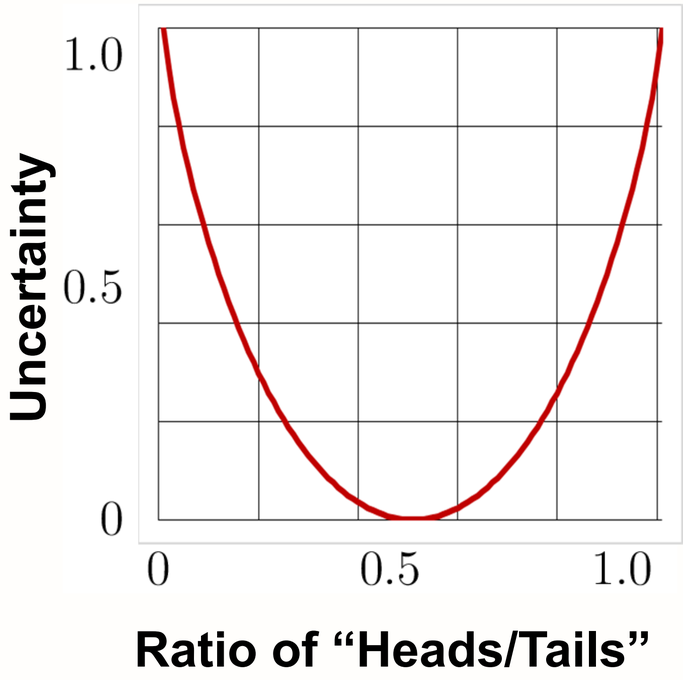
Rokas & Carroll (2006) PLoS Biol.

Quantifying Incongruence

Internode Certainty (IC): a measure of the support for a given internode by considering its frequency in a given set of trees jointly with that of the most prevalent conflicting internode in the same set of trees

Tree Certainty (TC): the sum of IC across all internodes

IC and TC are implemented in the latest versions of RAxML



Salichos et al. (2014) Mol. Biol. Evol.; Kobert et al. (2016) Mol. Biol. Evol.; Zhou et al. (2018) bioRxiv

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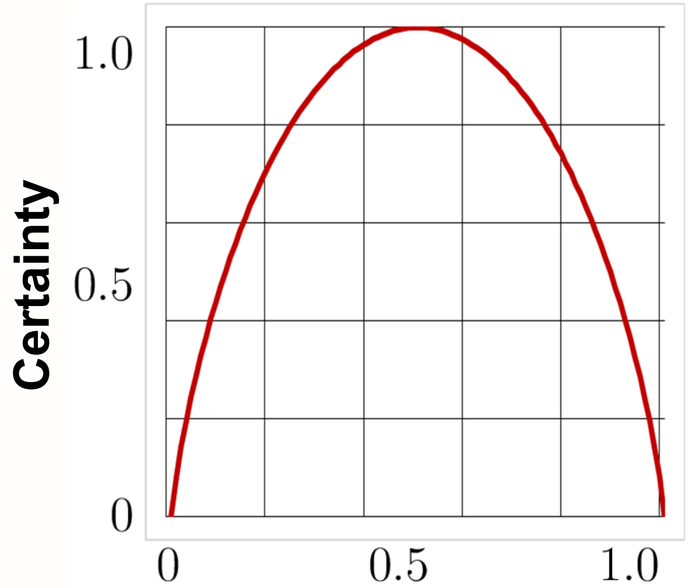


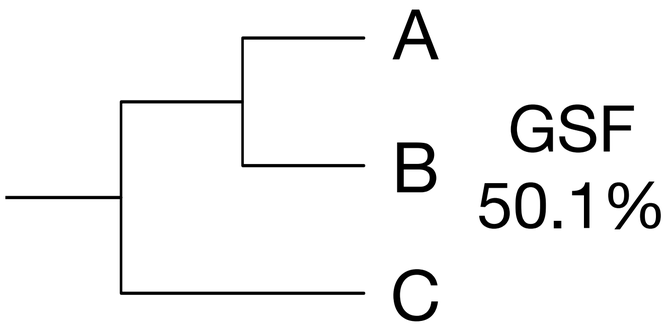
Ratio of Support for Two Conflicting Internodes

Salichos et al. (2014) Mol. Biol. Evol.; Kobert et al. (2016) Mol. Biol. Evol.; Zhou et al. (2018) bioRxiv

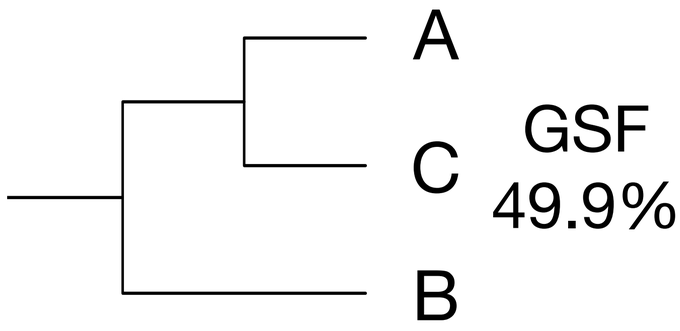
Slide from Antonis Rokas

Internode certainty (in other words…)

Case 1: High conflict



GSF
50.1%



GSF
49.9%

IC ~ 0

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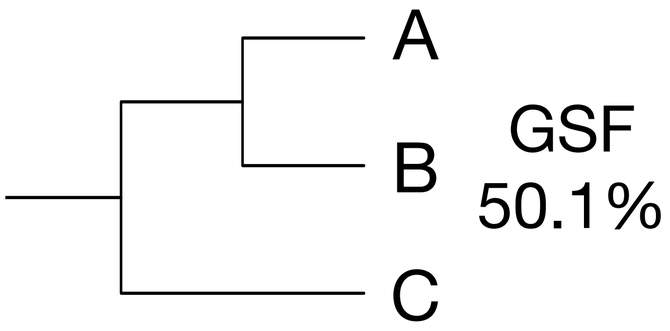

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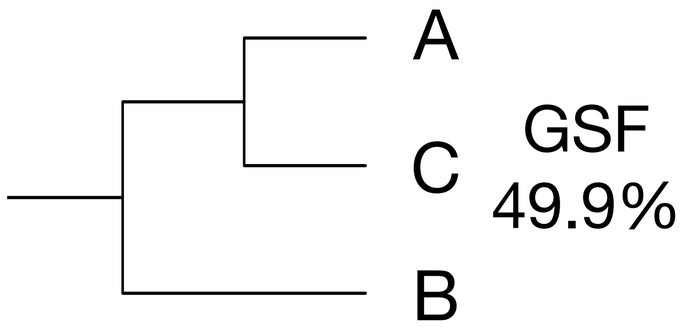

GSF
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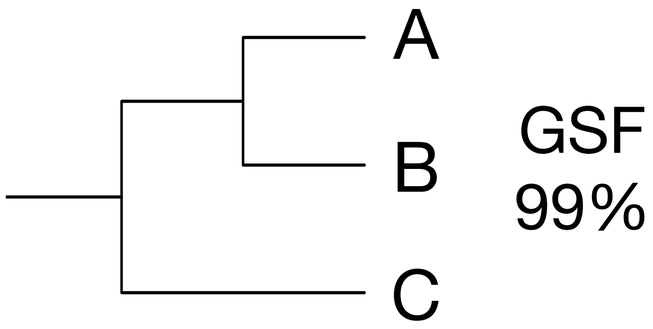
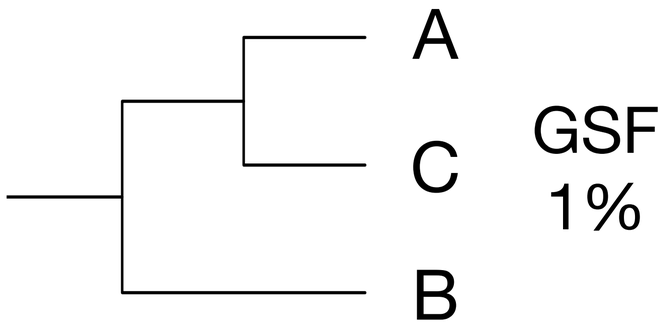
Case 2: Low conflict



GSF
99%



GSF
1%

IC ~ 1

Developments of internode certainty

- The original implementation was originally developed for phylogenies with complete taxon representation
    - **Salichos and Rokas (2013) Nature**
    - **Salichos *et al*. (2014) Mol. Biol. Evol.**

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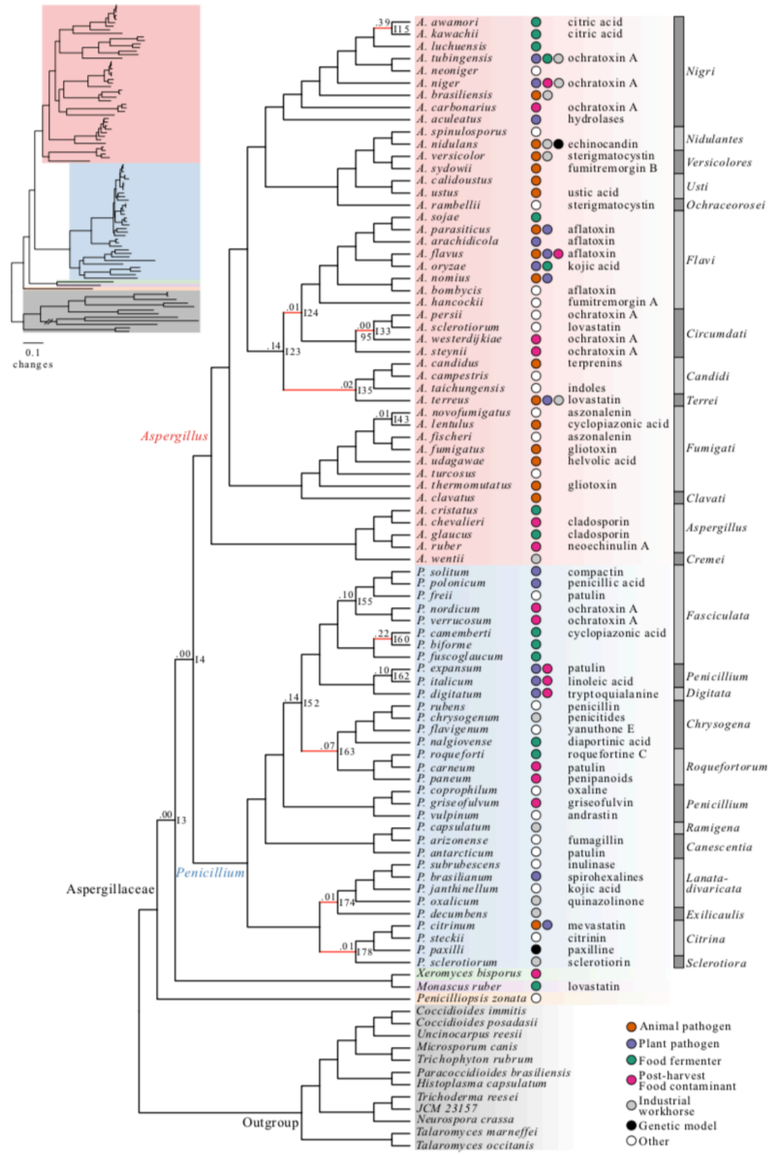
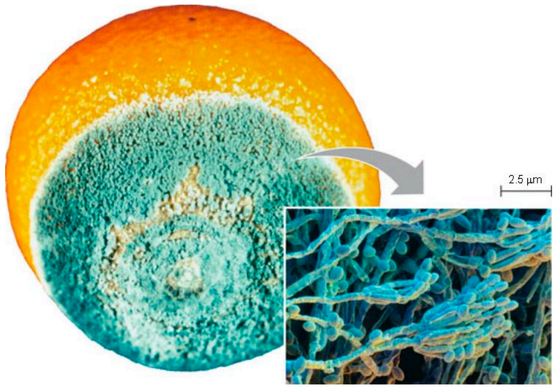
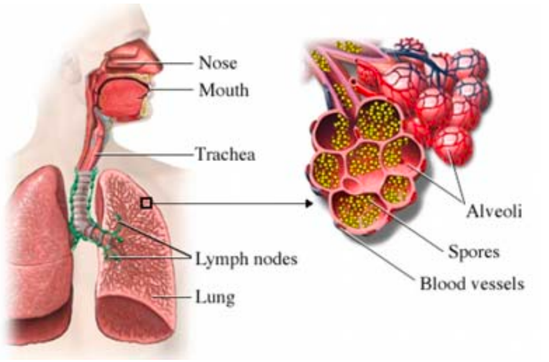
- Quartet based IC measures, QuartetScores, are more accurate with partial gene trees
    - **Zhou *et al.* (2020) Systematic Biology**

81 genomes from mainly Aspergillus and Penicillium







Steenwyk *et al.* (2019), *mBio*

Notes on implementation

• Verbose usage of RAxML's calculations of IC provides detailed information about conflicting bipartitions *RAxML_verboseSplits.suffix*

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```
1. Uncinocarpus_reesii
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3. Penicilliopsis_zonata
4. Xeromyces_bisporus
5. Monascus_ruber
6. Penicillium_camemberti
7. Penicillium_digitatum
8. Penicillium_roqueforti
9. Aspergillus_fumigatus
10. Aspergillus_niger
11. Aspergillus_oryzae

partition:
---** ----- -   1189/92.385392/0.850774
--*-* ***** *   26/2.020202/0.850774
```

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Taxa names

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Taxa names

Partition information
xx/yy/zz
xx = Trees supporting ref.
yy = gene support freq.
zz = Internode certainty

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partition:
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--*-* ***** *   26/2.020202/0.850774
```

Verbose can only be used with trees that have full taxon representation

Taxa names

Partition information
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xx = Trees supporting ref.
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Notes on implementation

• Exact bipartition topology for a given bipartition can be examined among files with the following syntax *RAxML_verboseIC.suffix.0 … RAxML_verboseIC.suffix.N-1*

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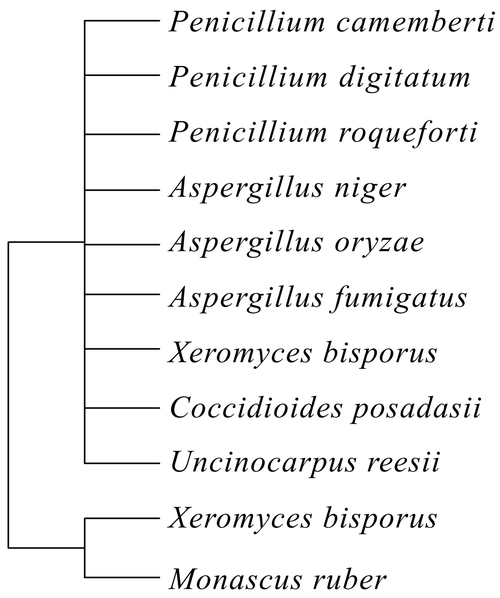
Topology 1




```
---** ----- -
1189/92.385392/0.850774
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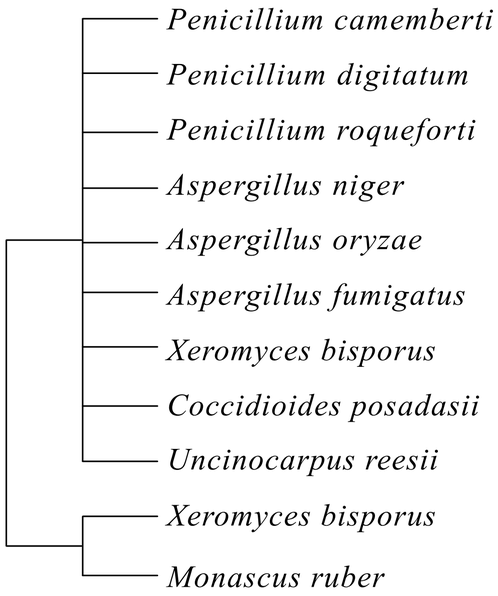
Topology 1




```
---** ----- -
1189/92.385392/0.850774
```

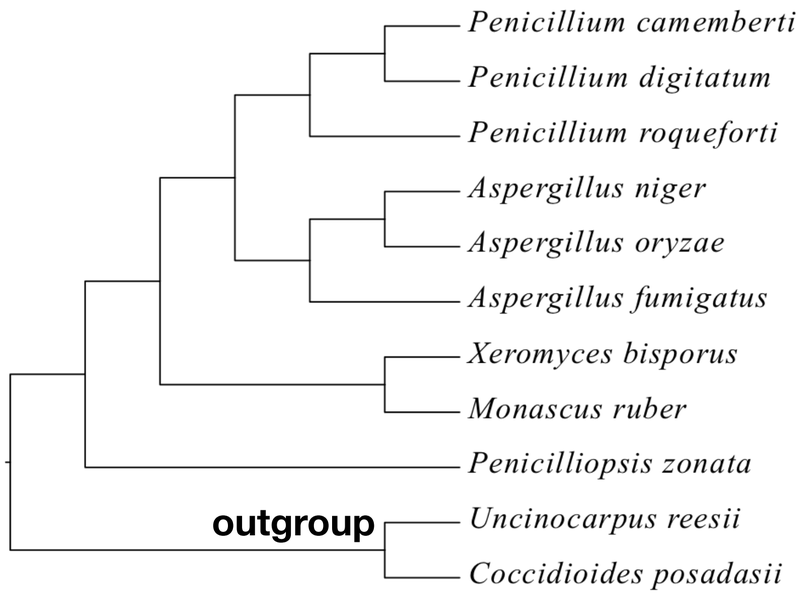
Topology 2


```
--*-* ***** *
26/2.020202/0.850774
```

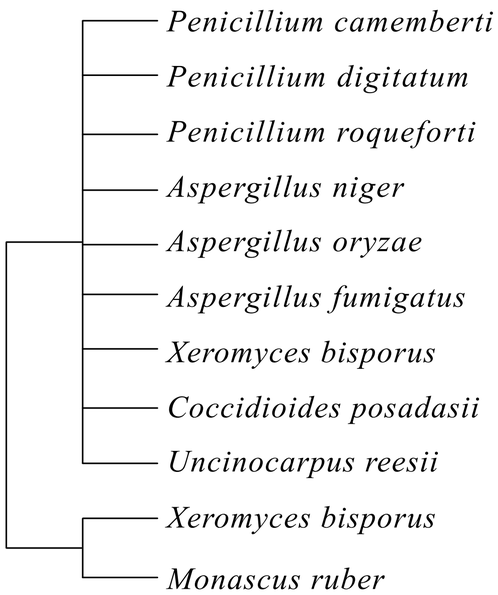
Notes on implementation

- Exact bipartition topology for a given bipartition can be examined among files with the following syntax *RAxML_verboseIC.suffix.0 … RAxML_verboseIC.suffix.N-1*

Concat topology

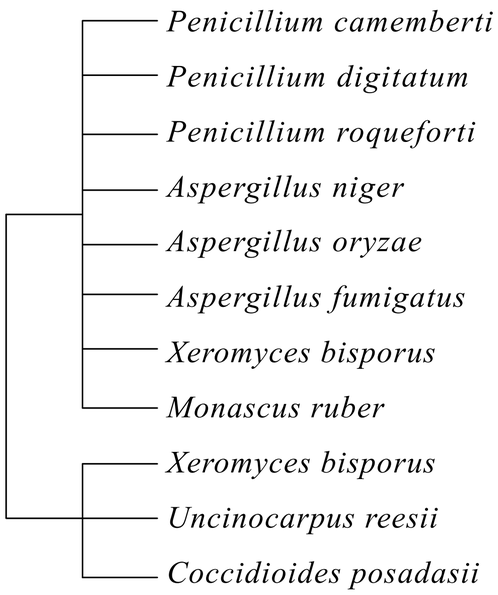


Topology 1



---** ----- -
1189/92.385392/0.850774

Topology 2



--*-* ***** *
26/2.020202/0.850774

Measures related to internode certainty

IC-All

- computed by taking into account all conflicting bipartitions with that have ≥5% support and not only the most supported conflict

TC-All

- The sum of IC-All values

Relative tree certainty

- A value from 0 (no certainty) to 1 (high certainty)

Concordance factors

Concordance factors

- proportion of the genome for which a given clade is true

Baum (2007) Taxon

Gene or site concordance factors

- proportion of genes or sites for which a given clade is true
- more precisely, percentage of decisive gene trees (or sites) with a given branch

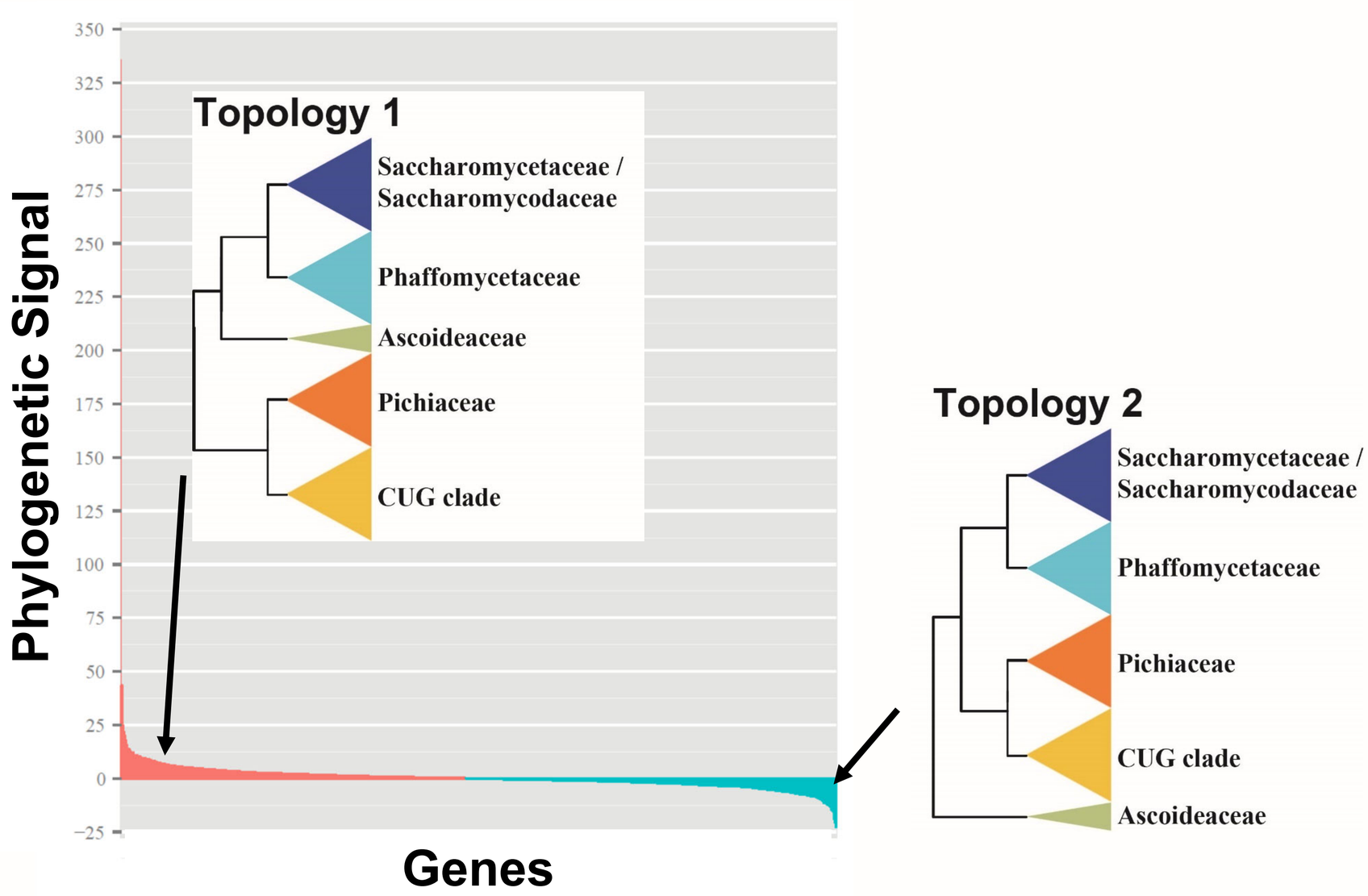
Minh (2020) Molecular Biology and Evolution

Great additional analysis when bootstrapping becomes unreliable!!

A refresher…

The next few slides are from Antonis Rokas

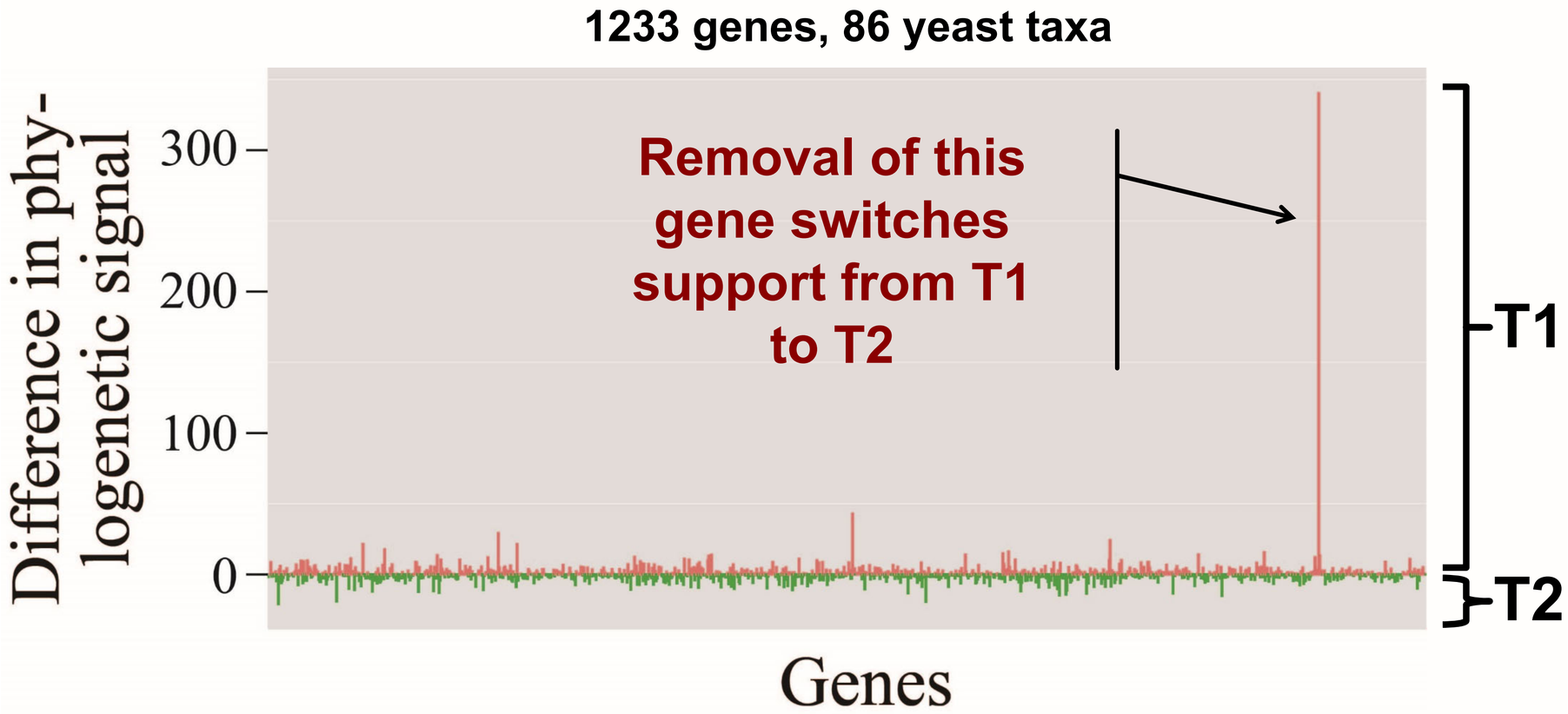
...But in Others It Stems from One or Two Genes



Slide from Antonis Rokas

Shen et al. (2017) Nature Ecol. Evol.

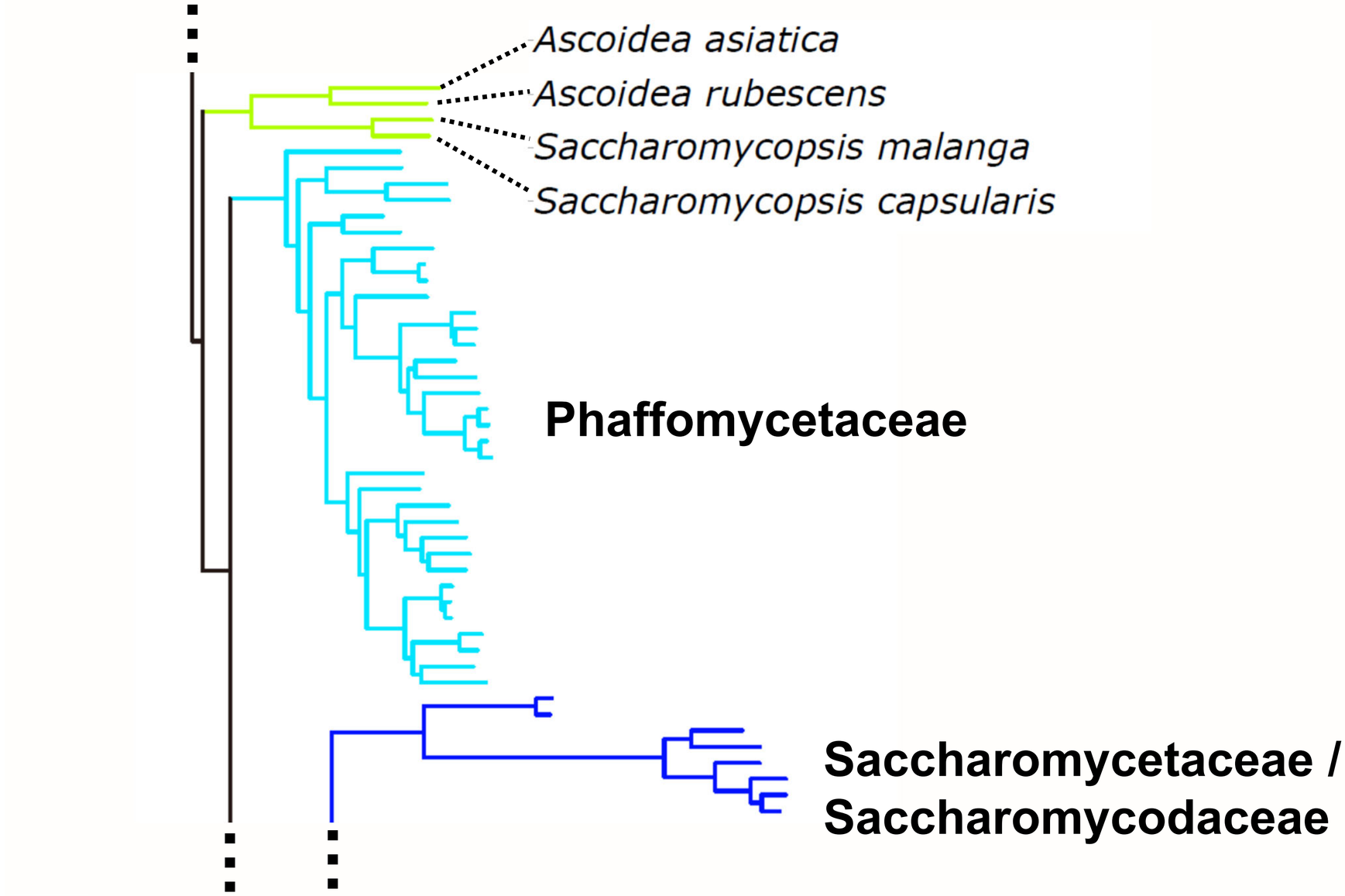
Phylogenetic Signal per Gene for the Two Hypotheses



Slide from Antonis Rokas

Shen et al. (2017) Nature Ecol. Evol.

Sampling of 3 Additional Taxa "Breaks" the Long Branch

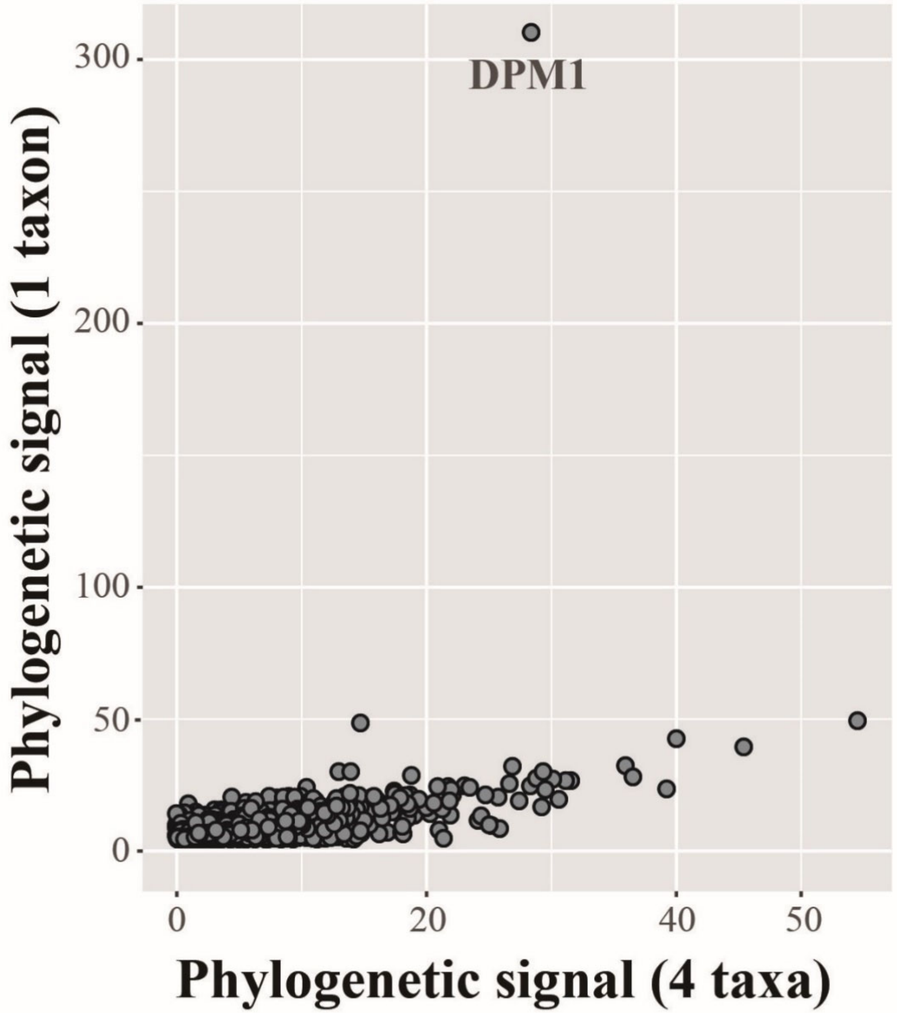


Slide from Antonis Rokas

n, Opulente, Kominek, Zhou et al. (2018) Cell

Sampling of 3 Additional Taxa Decreases Gene's Signal

2,408 genes, 329 – 332 yeast taxa



n, Opulente, Kominek, Zhou et al. (2018) Cell

Slide from Antonis Rokas

Internode certainty and related measures

@JLSteenwyk https://jlsteenwyk.com/

Quiz time :D

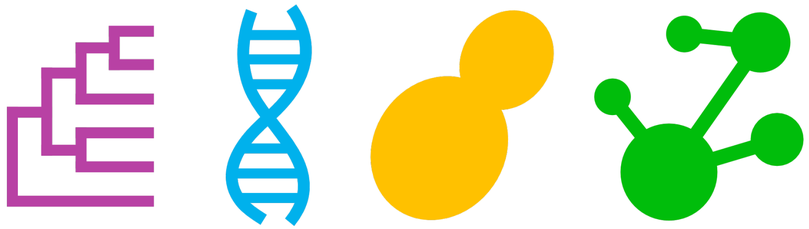
Internode certainty and related measures

• When viewing the trees, root on the clade with *U. reesii* and *C. posadasii*

4iii)
• Don’t forget to put the path to IQ-Tree

5i)
• “Do not execute the following commands” only pertains to 5i

5xi)
• This command takes some time…try subsetting the data before reading it into *R*



Jacob L. Steenwyk

@JLSteenwyk https://jlsteenwyk.com/

2iii

- Tree certainty:

4.190492

- Relative tree certainty:

0.523811

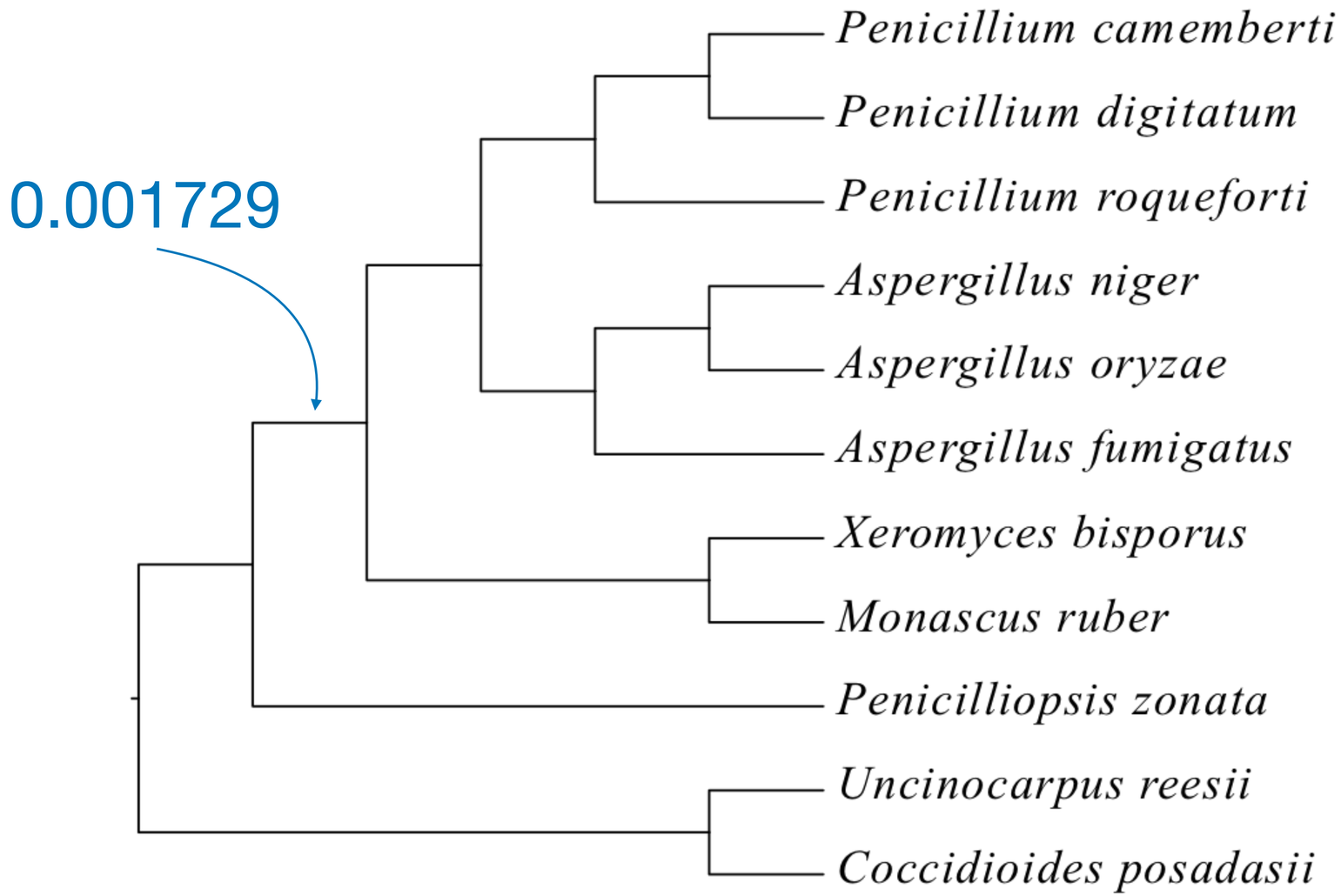
- Tree certainty all including all conflicting bipartitions:

4.128041

- Relative tree certainty all including all conflicting bipartitions:

0.516005

3v


0.001729
Penicillium camemberti
Penicillium digitatum
Penicillium roqueforti
Aspergillus niger
Aspergillus oryzae
Aspergillus fumigatus
Xeromyces bisporus
Monascus ruber
Penicilliopsis zonata
Uncinocarpus reesii
Coccidioides posadasii

4ii

What is the number of genes and gene support frequency that support the two topologies?

First topology:
375/29.137529

Second topology:
340/26.418026

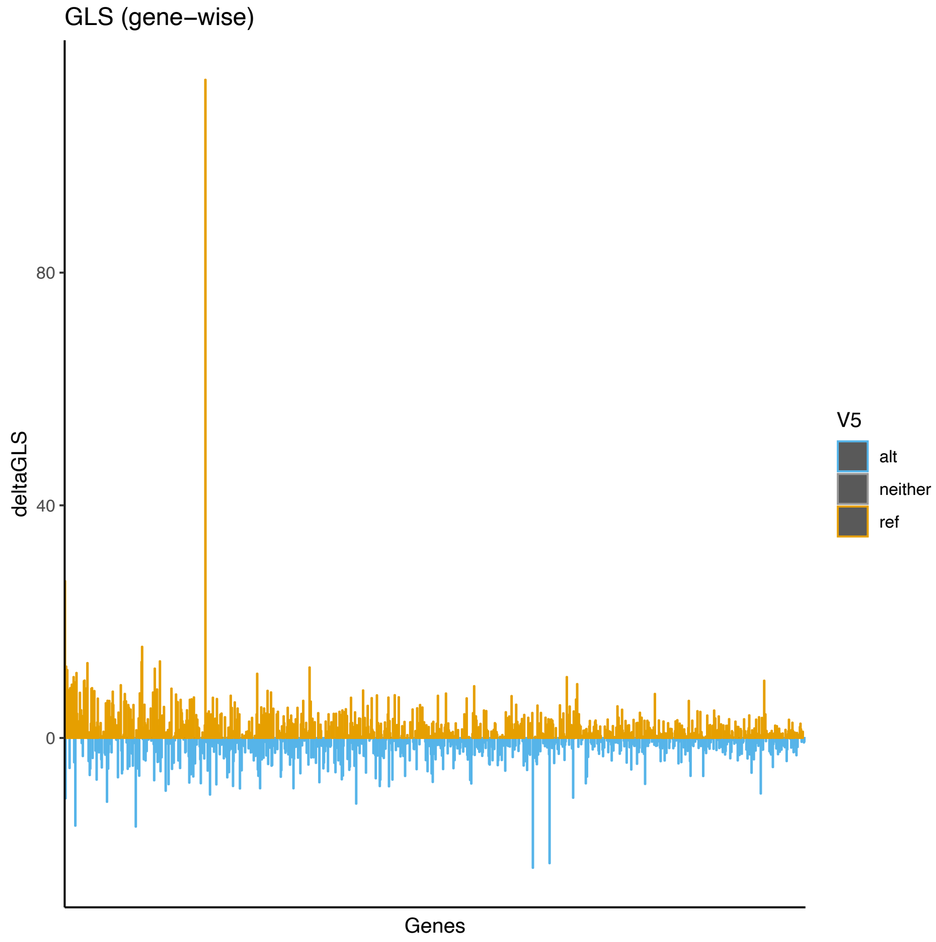
4iv

What is gene and site concordance factor values for the reference topology and alternative topology?

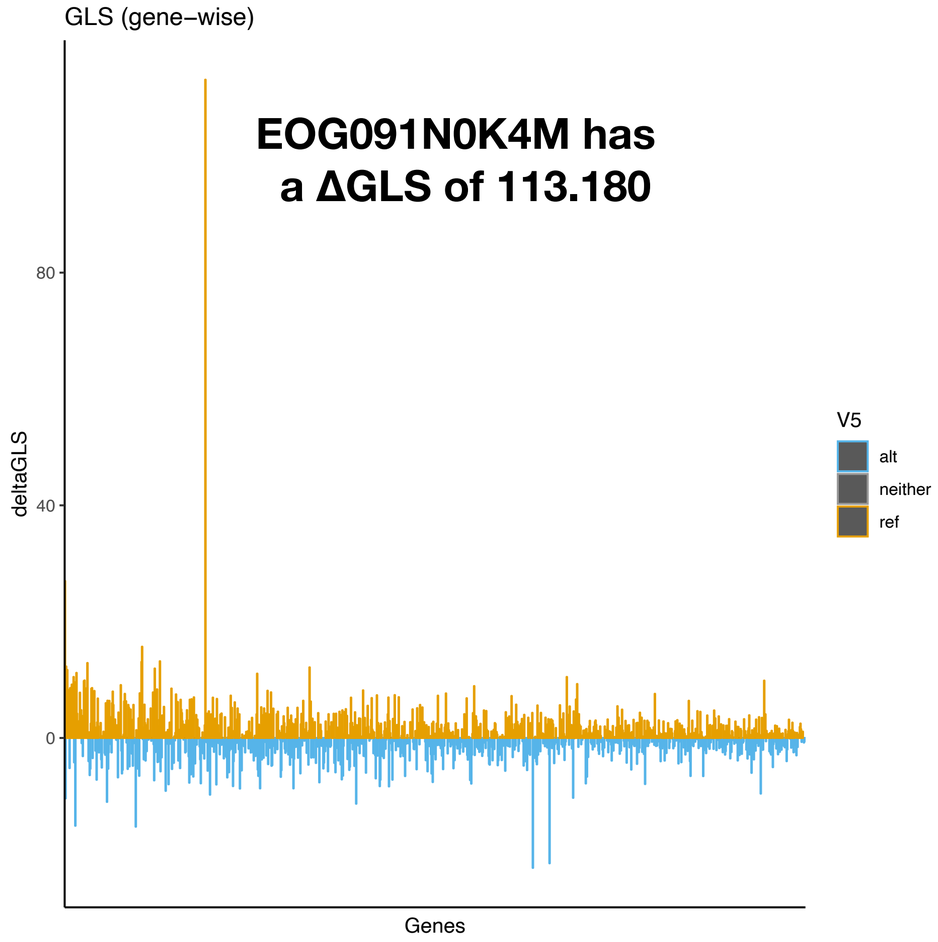
Reference topology:
0.155/32.1

Second topology:
0/36.2

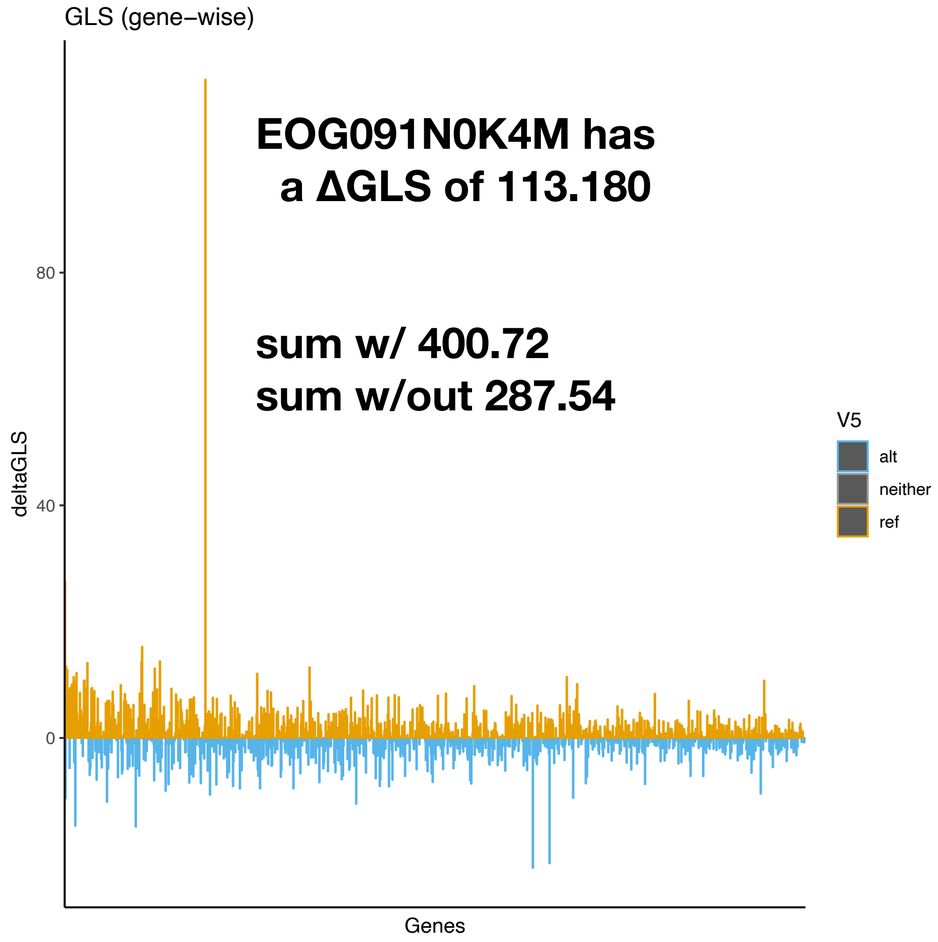
5



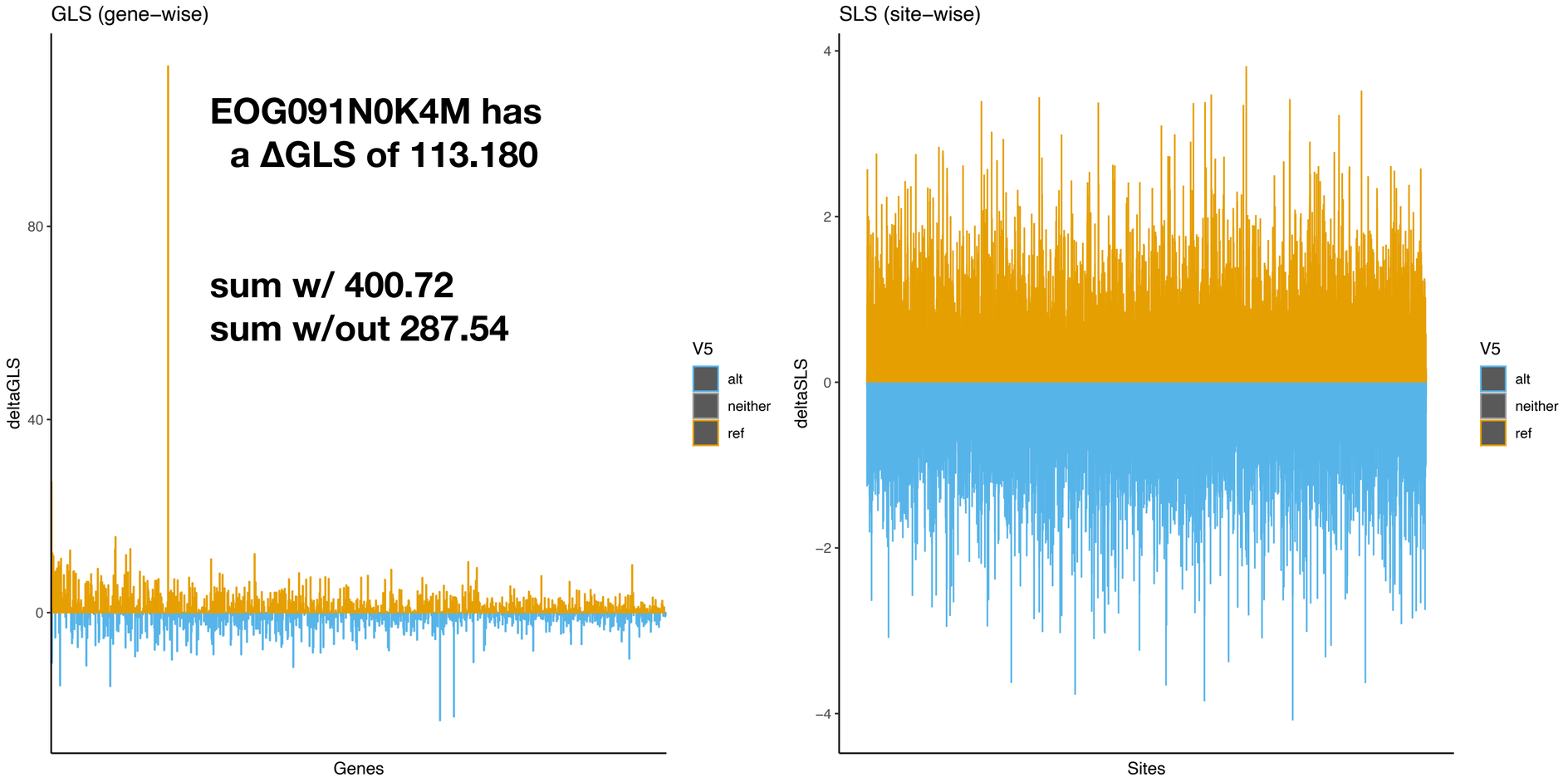
5


GLS (gene−wise)
EOG091N0K4M has
a ΔGLS of 113.180
80
40
0
deltaGLS
Genes
V5
alt
neither
ref

5


GLS (gene−wise)
EOG091N0K4M has
a ΔGLS of 113.180
sum w/ 400.72
sum w/out 287.54
80
40
0
deltaGLS
Genes
V5
alt
neither
ref

5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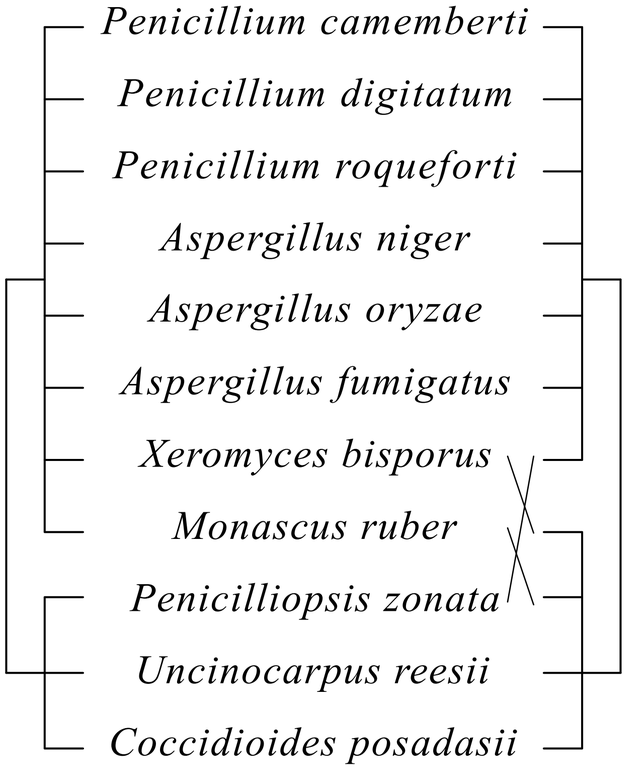
deltaGLS
80
40
0
Genes
V5
alt
neither
ref
SLS (site−wise)
deltaSLS
4
2
0
−2
−4
Sites
V5
alt
neither
ref

Why do gCF and GSF differ?

Consider the topologies being examined

RAxML



IQ-Tree