① Identify speciation and duplication nodes in the gene tree
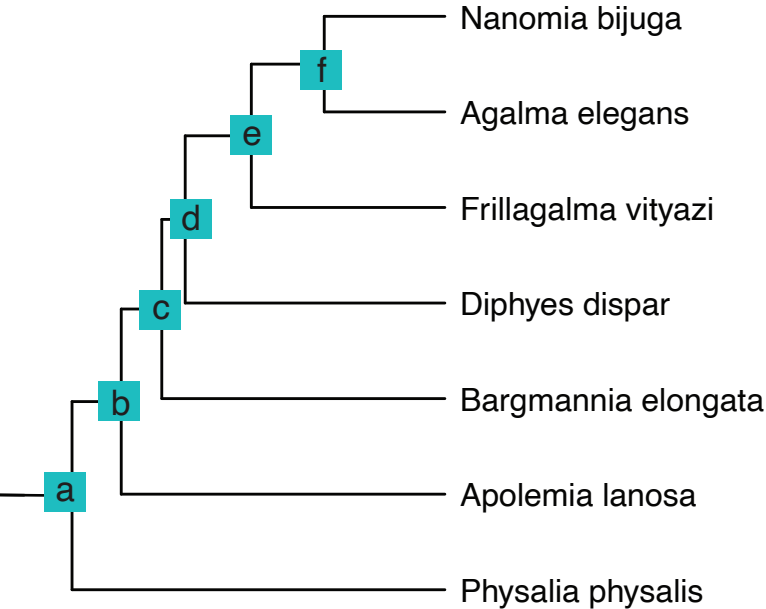
Siphonophore phylogeny
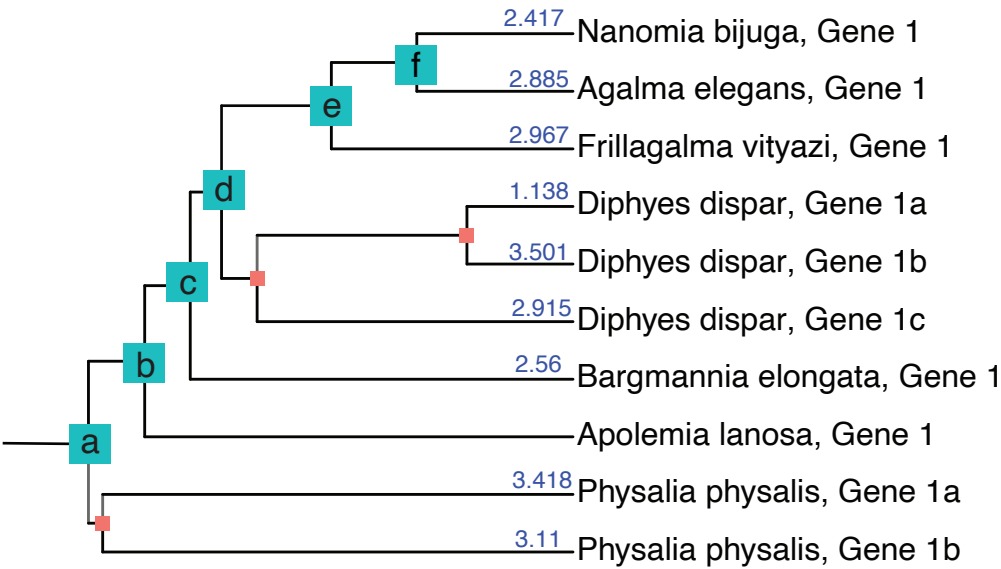
- Nanomia bijuga
- Agalma elegans
- Frillagalma vityazi
- Diphyes dispar
- Bargmannia elongata
- Apolemia lanosa
- Physalia physalis

Nodes: a, b, c, d, e, f

An exemplar gene tree

- Nanomia bijuga, Gene 1
- Agalma elegans, Gene 1
- Frillagalma vityazi, Gene 1
- Diphyes dispar, Gene 1a
- Diphyes dispar, Gene 1b
- Diphyes dispar, Gene 1c
- Bargmannia elongata, Gene 1
- Apolemia lanosa, Gene 1
- Physalia physalis, Gene 1a
- Physalia physalis, Gene 1b
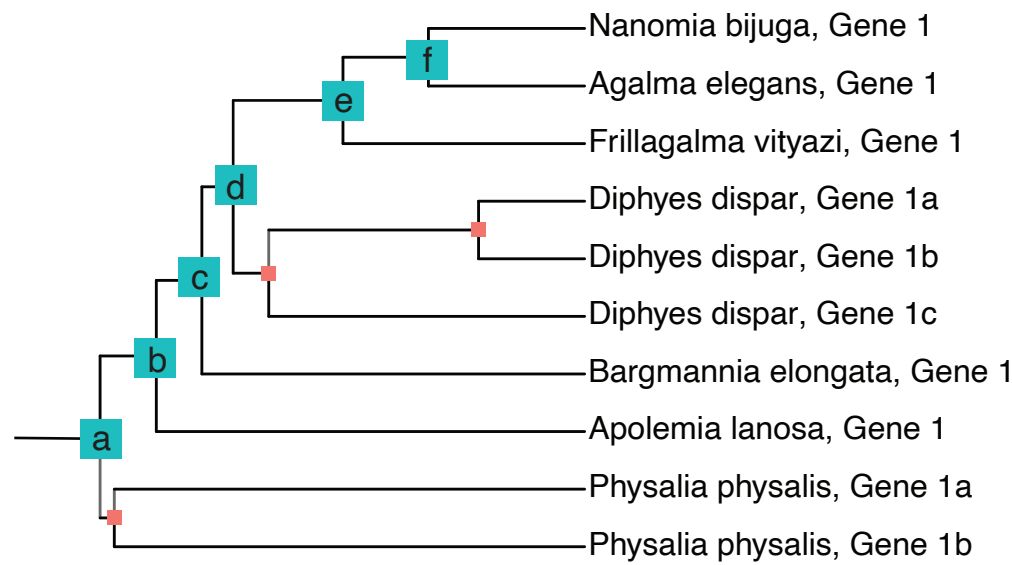
■ Speciation event   ■ Duplication event

② Map expression values to tips of gene tree

- 2.417 Nanomia bijuga, Gene 1
- 2.885 Agalma elegans, Gene 1
- 2.967 Frillagalma vityazi, Gene 1
- 1.138 Diphyes dispar, Gene 1a
- 3.501 Diphyes dispar, Gene 1b
- 2.915 Diphyes dispar, Gene 1c
- 2.56 Bargmannia elongata, Gene 1
- Apolemia lanosa, Gene 1
- 3.418 Physalia physalis, Gene 1a
- 3.11 Physalia physalis, Gene 1b

③ Reconstruct ancenstral expression values at nodes

- 2.7 / 2.417 Nanomia bijuga, Gene 1
- 2.753 (f) / 2.885 Agalma elegans, Gene 1
- 2.724 (e) / 2.967 Frillagalma vityazi, Gene 1
- 2.739 (d) / 2.396 / 1.138 Diphyes dispar, Gene 1a
- 2.836 (c) / 3.501 Diphyes dispar, Gene 1b
- 2.697 / 2.915 Diphyes dispar, Gene 1c
- (b) / 2.56 Bargmannia elongata, Gene 1
- 2.836 (a) Apolemia lanosa, Gene 1
- 3.418 Physalia physalis, Gene 1a
- 2.86 / 3.11 Physalia physalis, Gene 1b
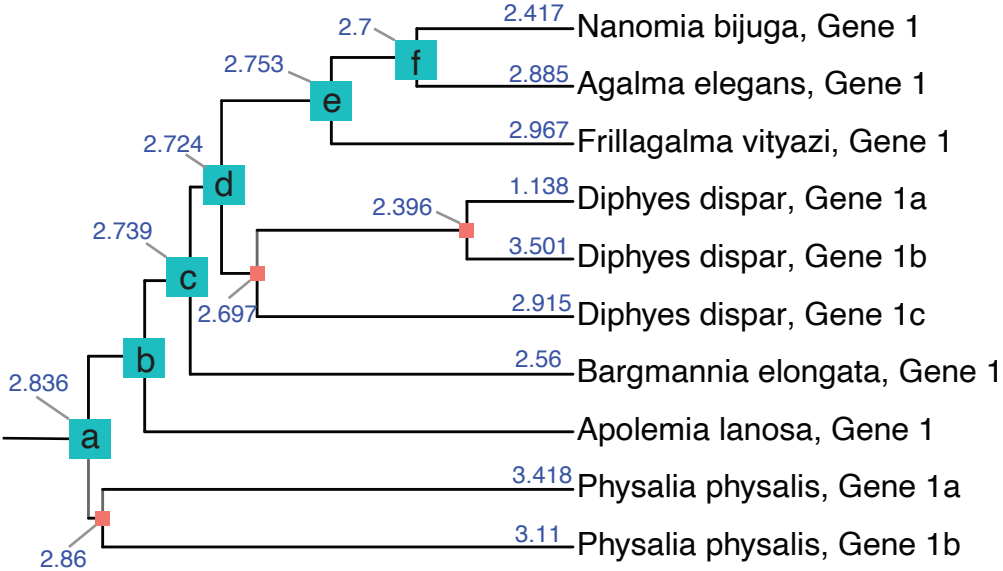
④ Calculate scaled change across branches

- −1.48 / −4.29 Nanomia bijuga, Gene 1
- 0.63 / 2.81 Agalma elegans, Gene 1
- 2.1 Frillagalma vityazi, Gene 1
- −3.41 / −28.12 Diphyes dispar, Gene 1a
- 24.71 Diphyes dispar, Gene 1b
- −1.77 / 1.64 Diphyes dispar, Gene 1c
- −1.11 Bargmannia elongata, Gene 1
- Apolemia lanosa, Gene 1
- 1.82 / 2.81 Physalia physalis, Gene 1a
- 4.07 / 1.26 Physalia physalis, Gene 1b
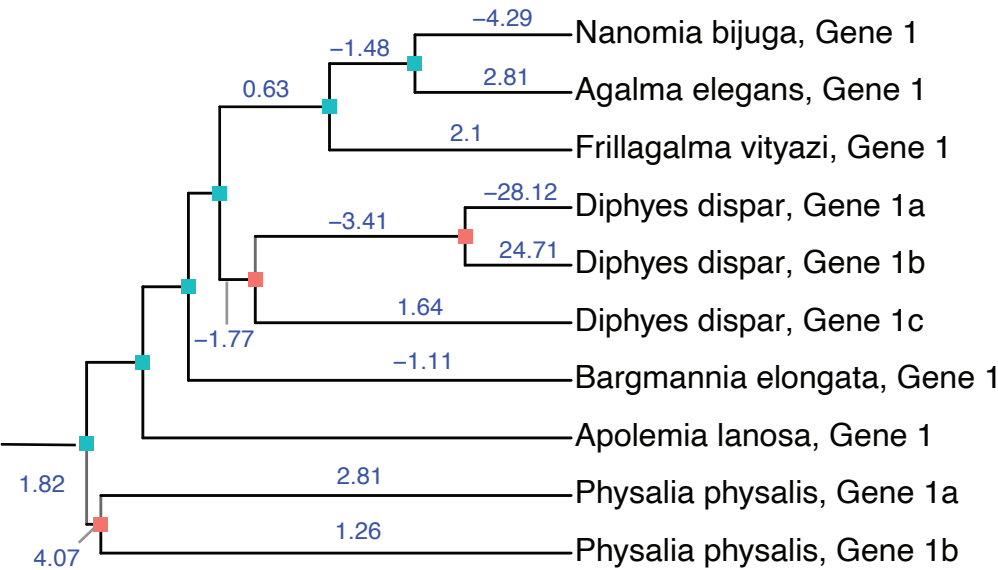
$$\text{Scaled change} = \frac{\text{Child node} - \text{parent node}}{\text{Calibrated branch length}}$$

⑤ Isolate corresponding branches across all gene trees

Species phylogeny

- L Nanomia bijuga
- K Agalma elegans
- J Frillagalma vityazi
- I Diphyes dispar
- H Bargmannia elongata
- G Apolemia lanosa
- F Physalia physalis

Nodes: A, B, C, D, E

— Focal branch

Gene tree 1

- L Nanomia bijuga, G1
- E / K Agalma elegans, G1
- D / J Frillagalma vityazi, G1
- C Diphyes dispar, G1a
- Diphyes dispar, G1b
- B Diphyes dispar, G1c
- A / H Bargmannia elongata, G1
- G Apolemia lanosa, G1
- Physalia physalis, G1a
- Physalia physalis, G1b

Gene tree 2

- L Nanomia bijuga, G2a
- E / K Agalma elegans, G2a
- D / J Frillagalma vityazi, G2
- I Diphyes dispar, G2
- K Agalma elegans, G2b
- L Nanomia bijuga, G2b
- H Bargmannia elongata, G2
- A Apolemia lanosa, G2a
- Apolemia lanosa, G2b
- F Physalia physalis, G2

...n gene trees