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Class 2 CCA-adding Enzymes in Archaeal Lineages

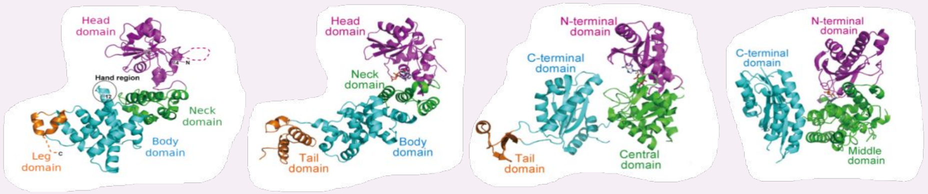
Bled 2025
Ella Cassidy
Computation EvoDevo
Supervisor Sonja Prohaska

What are tRNA-nucleotidyltransferases (CCA-adding enzymes)

CCA-adding enzymes

Superfamily

- class 2 (**CCA2**)
  - PAP
  - CCA
- class 1 (**CCA1**)
  - CCA
  - PAP



Bacteria

Bacteria
Eukaryotes

Archaea

Eukaryotes

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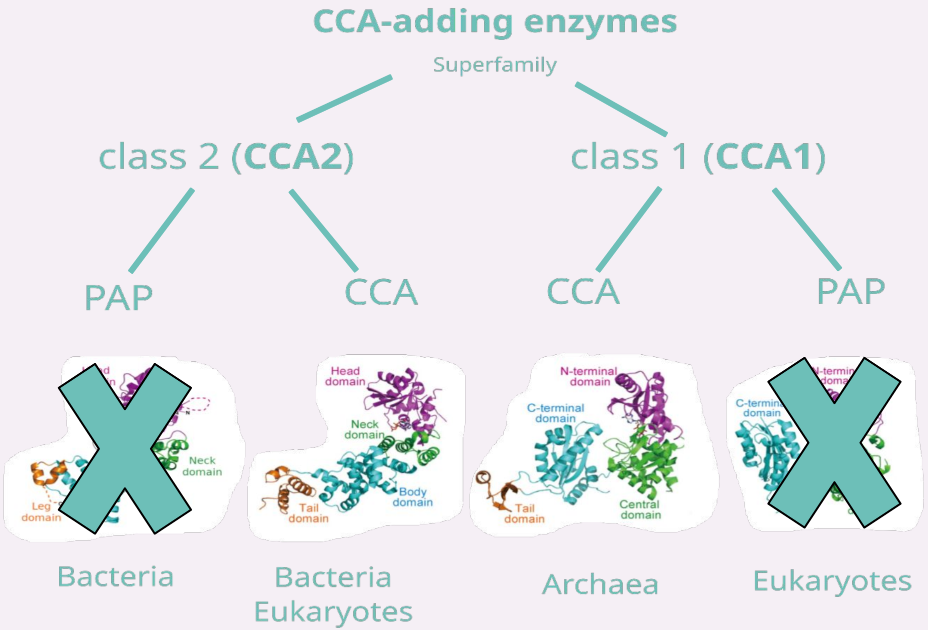

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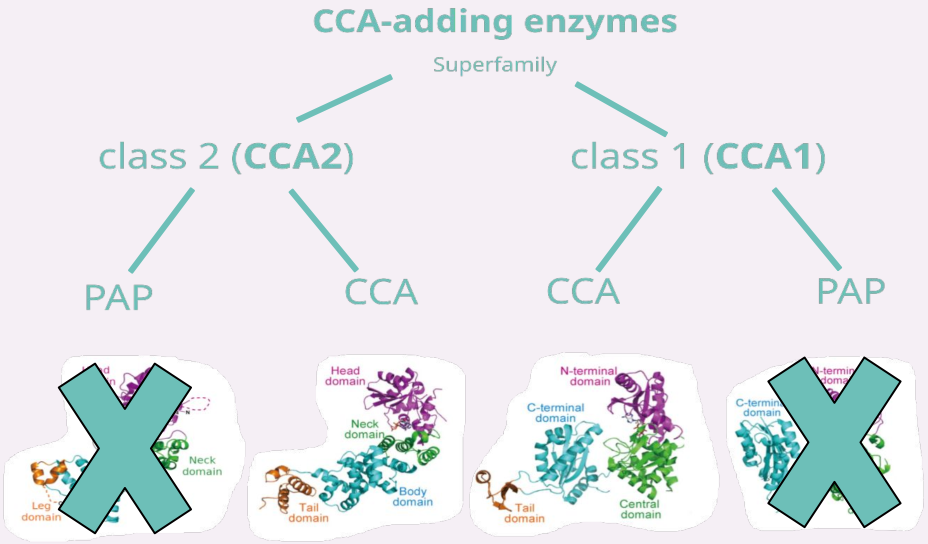

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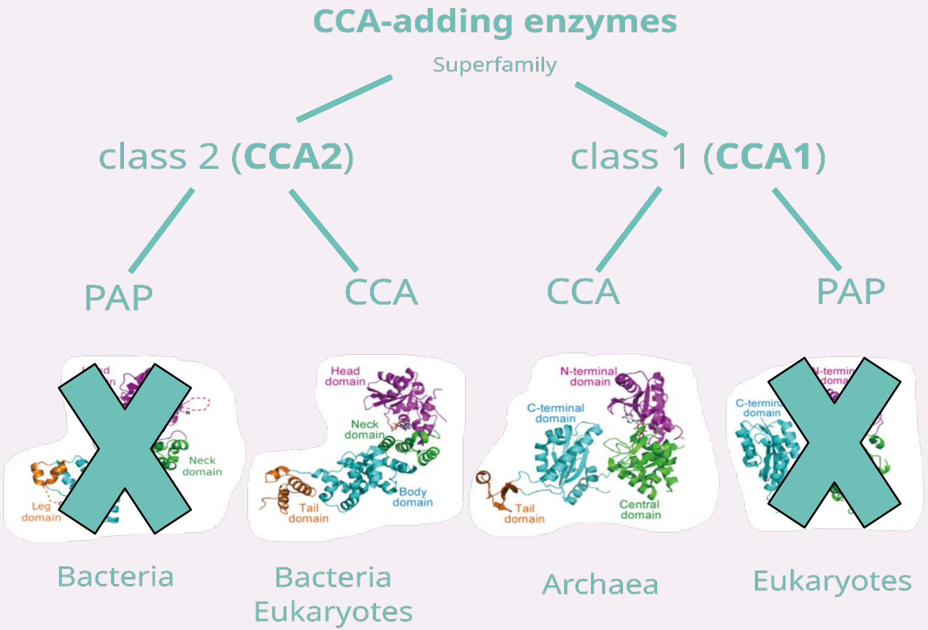

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1. Essential for tRNA maturation

2. Quality control mechanisms

→ Abundant in all living organisms and **essential**

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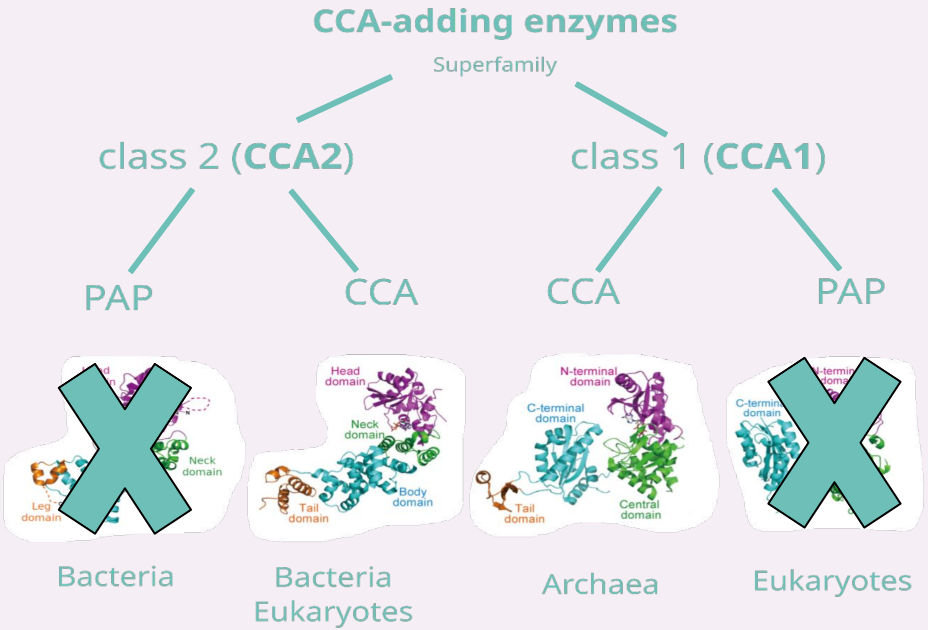

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DO WE FIND CCA2 IN ARCHAEAL LINEAGES?

Why study Archaeal evolution?

→ first discovery only 40 years ago

→ understanding the tree of life

→ not been directly linked to cause human disease **but** they're currently being studied in their possible implications in microbiome studies, existing on skin, tracts, guts (so not just extreme environments) → **regulatory effects?**

→ Important in **hydrosphere**, greenhouse gases (methane) solely metabolic product of methanogenic archaea (some produce, some consume), Ammonia-oxidizing archaea (AOA) nitrification

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Difficulties

- Culturing challenges (difficult to grow, unknown nutrients, slow growth rates)
- Missing phylogeny/not well resolved
- Severely underrepresented in databases

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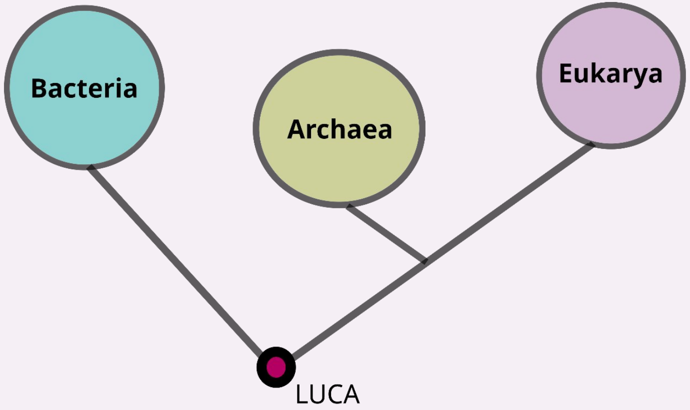
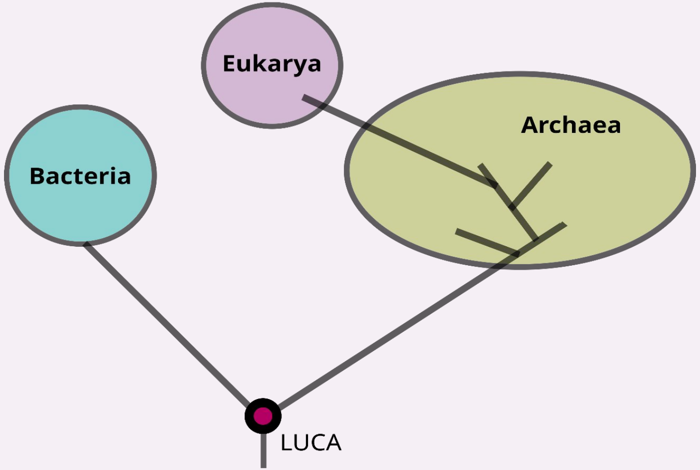


Three-domain tree

Bacteria, Archaea, and Eukarya represent three distinct domains of life. archaea and eukaryotes split from a common ancestor, forming two distinct lineages.

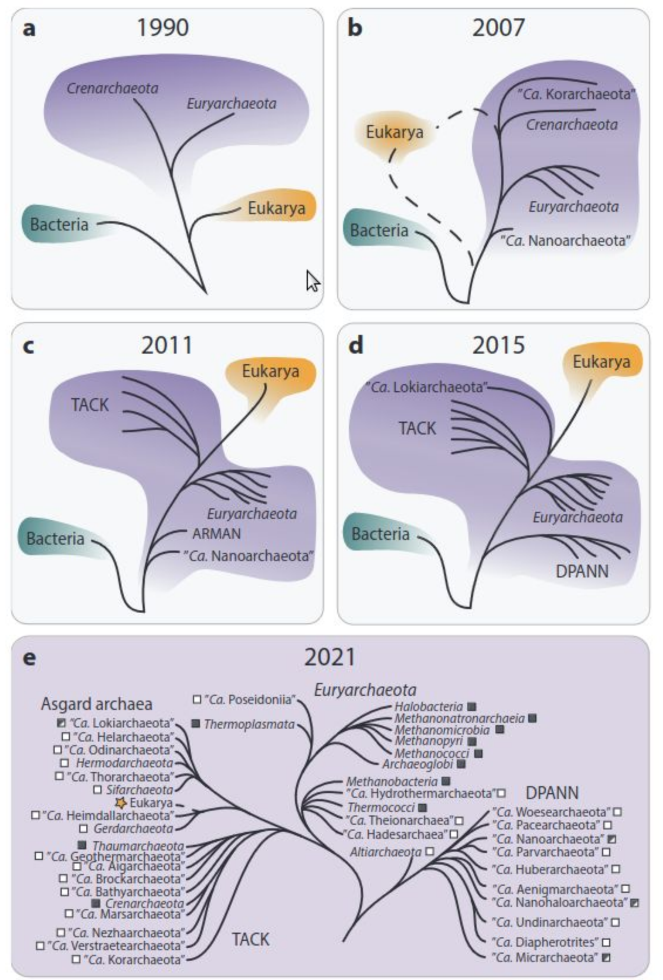


Two-domain tree

Eukaryotes emerge from within Archaea, meaning they are just a special type of Archaeon that later incorporated bacterial features (like mitochondria)

Archaeal current phylogeny

→ expansion of archaeal phylogenies



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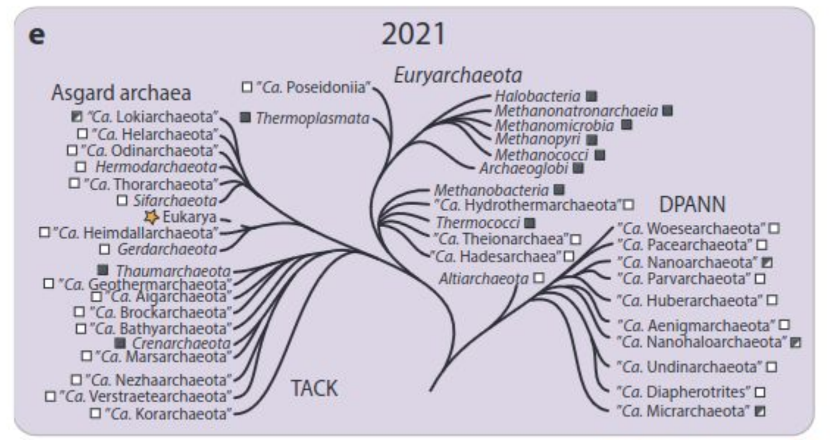
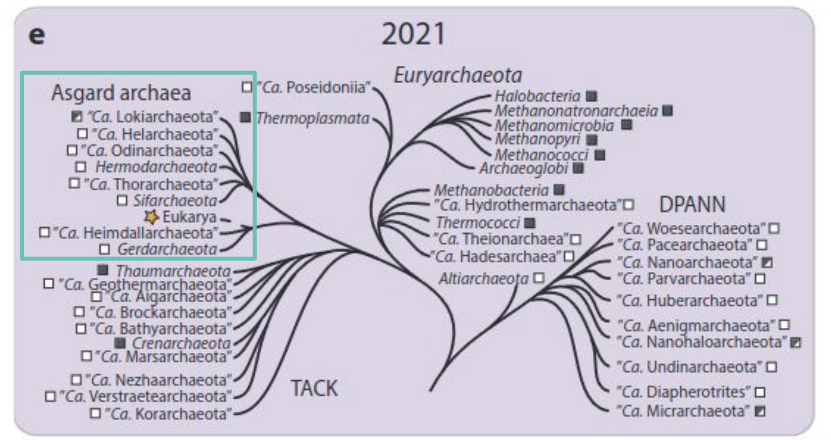


Archaeal current phylogeny

→ expansion of archaeal phylogenies

→ ESPs found in Asgards

- **Endomembrane systems**
- **Small GTPases**
- **Membrane trafficking machineries**
- **Vesicle biogenesis proteins**
- **Post-translational protein modification systems**
- **Cytoskeletal components**



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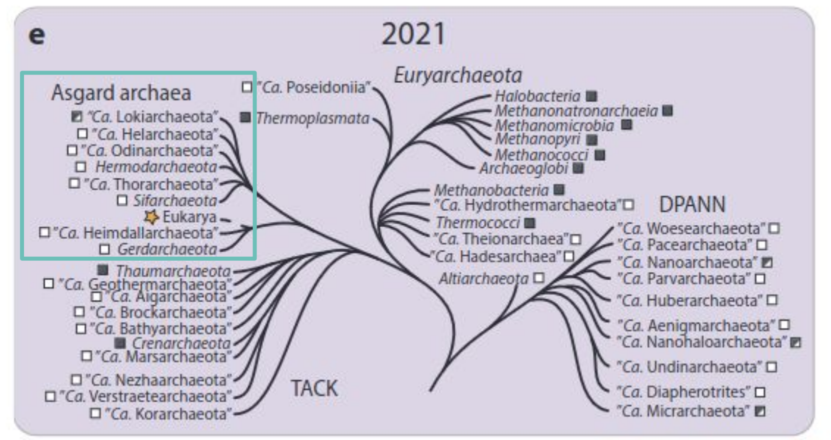
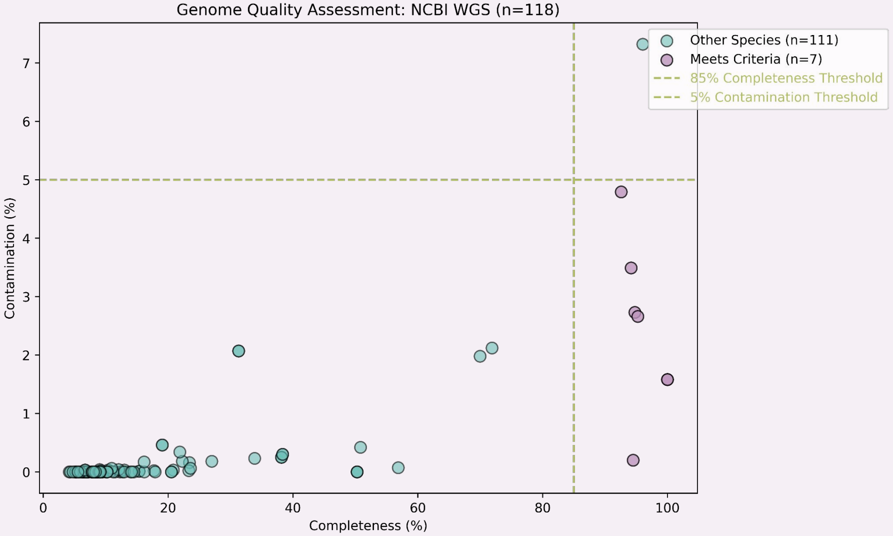
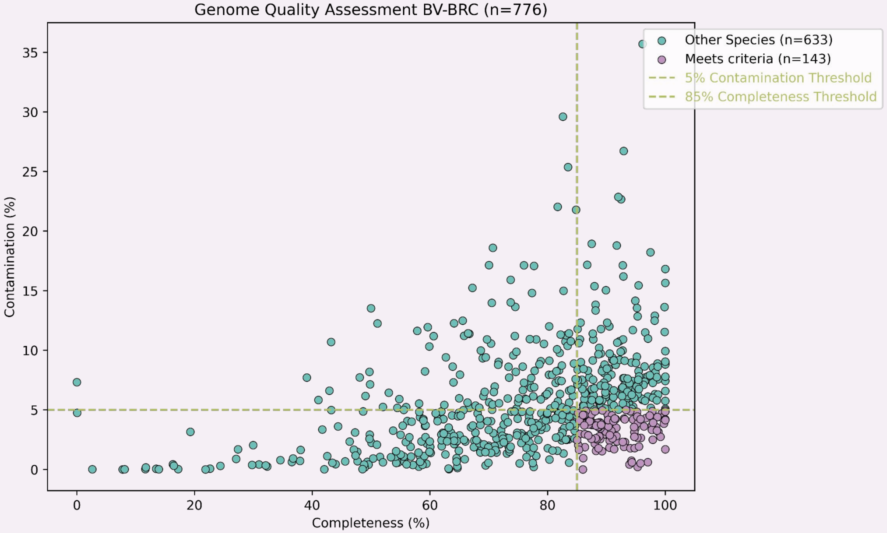
→ Asgard are phyla that eukaryotes originated from in the two-domain theory, specifically the **Heimdallarchaeota** family



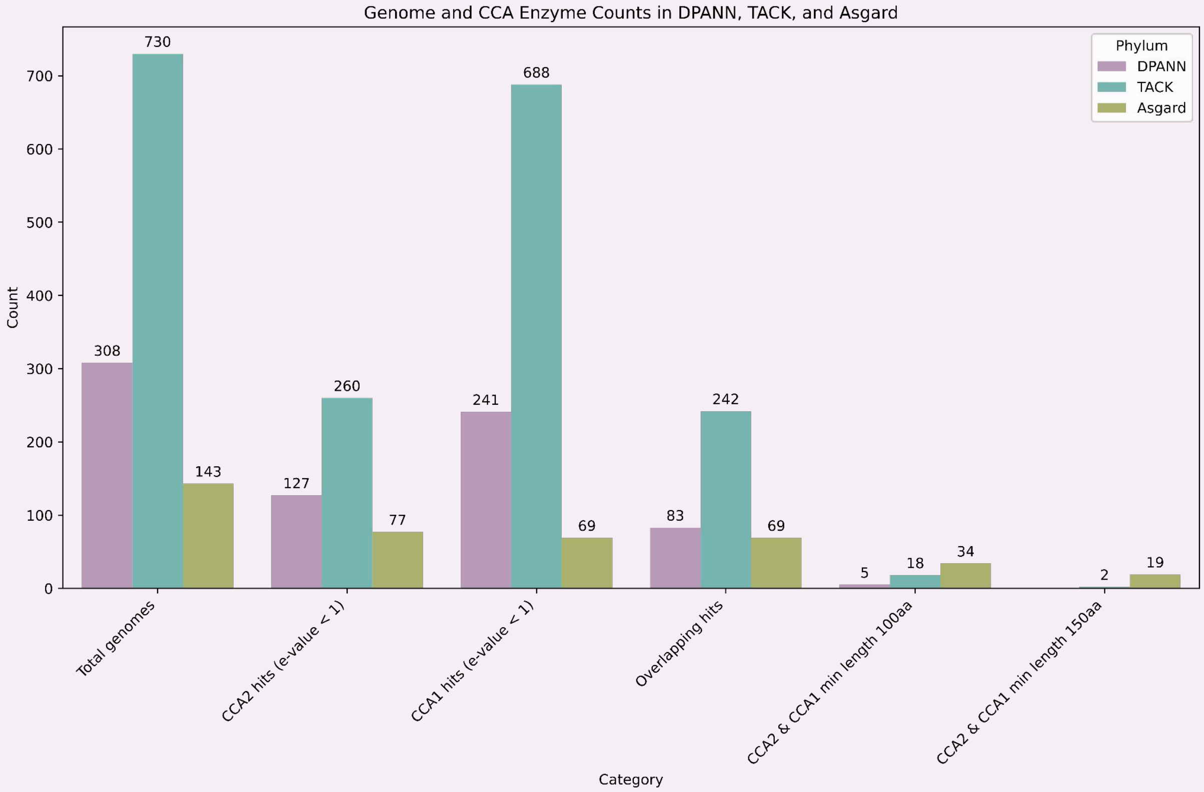
Challenges: NCBI WGS vs. BV-BRC genome quality

- Highlights the low/poor quality of **MAGs** from NCBI database
- NCBI data, only 118 sequences >100kbp for TACK, DPANN and Asgard
- BV-BRC data for Asgards only





Results! Using only BV-BRC data

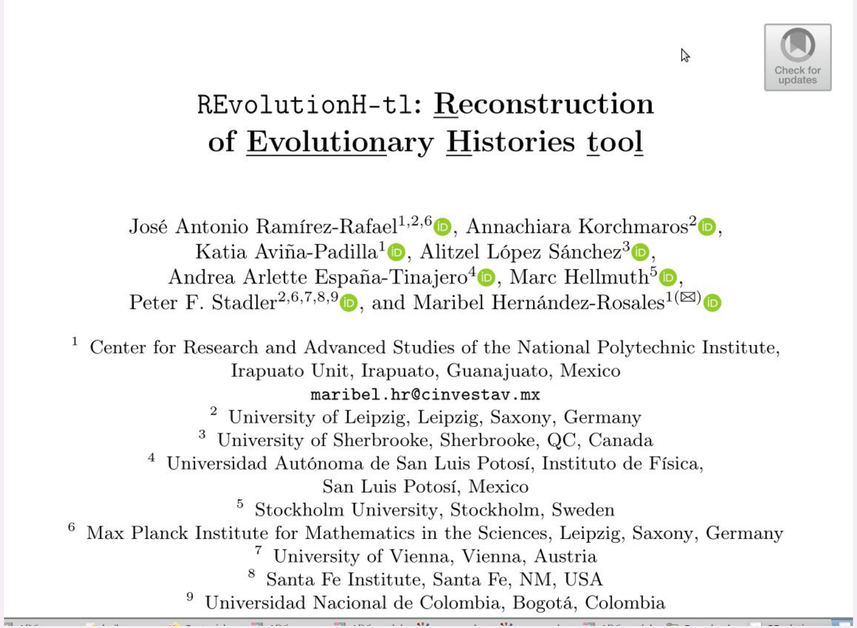


- Blasted CCA1 and CCA2 references against databases created for each phyla
- All sequences containing CCA2 from one family of Asgards
- CCA2 bacterial/eukaryotes ~400aa

→ **Heimdallarchaeota family**

Molecular phylogeny construction

- tool that reconstructs evolutionary histories
- not possible to do this without somewhat complete genomes
- 38 Heimdallarchaeota genomes with >85% complete, and <5% contamination, try to make a molecular phylogeny, without information about CCA1 or CCA2



REvolutionH-tl: Reconstruction of Evolutionary Histories tool

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Katia Aviña-Padilla[1], Alitzel López Sánchez[3],
Andrea Arlette España-Tinajero[4], Marc Hellmuth[5],
Peter F. Stadler[2,6,7,8,9], and Maribel Hernández-Rosales[1](✉)

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[4] Universidad Autónoma de San Luis Potosí, Instituto de Física, San Luis Potosí, Mexico
[5] Stockholm University, Stockholm, Sweden
[6] Max Planck Institute for Mathematics in the Sciences, Leipzig, Saxony, Germany
[7] University of Vienna, Vienna, Austria
[8] Santa Fe Institute, Santa Fe, NM, USA
[9] Universidad Nacional de Colombia, Bogotá, Colombia

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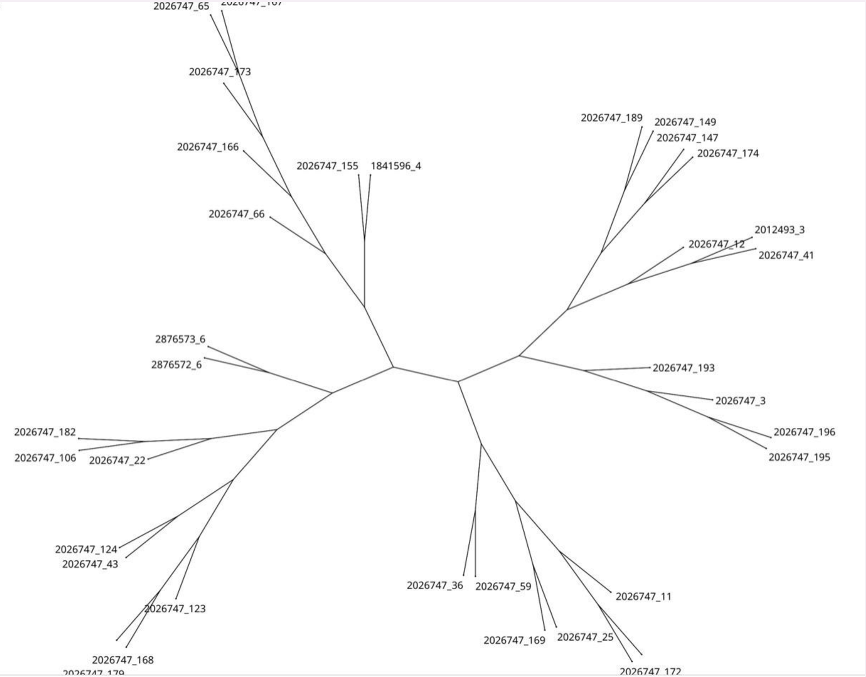

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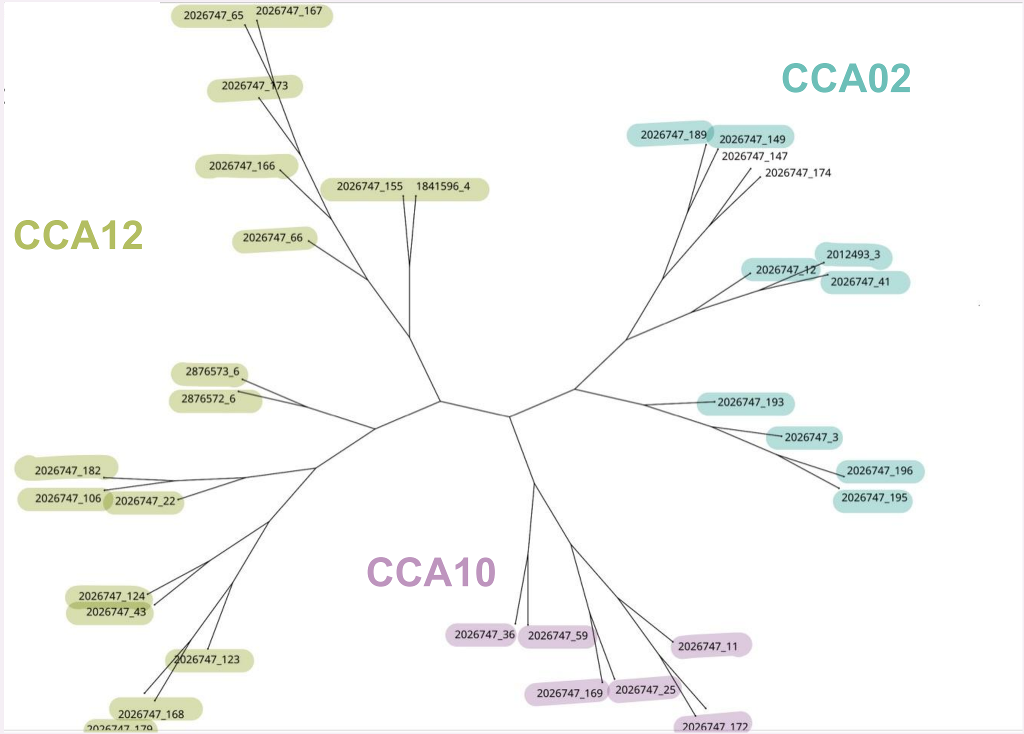
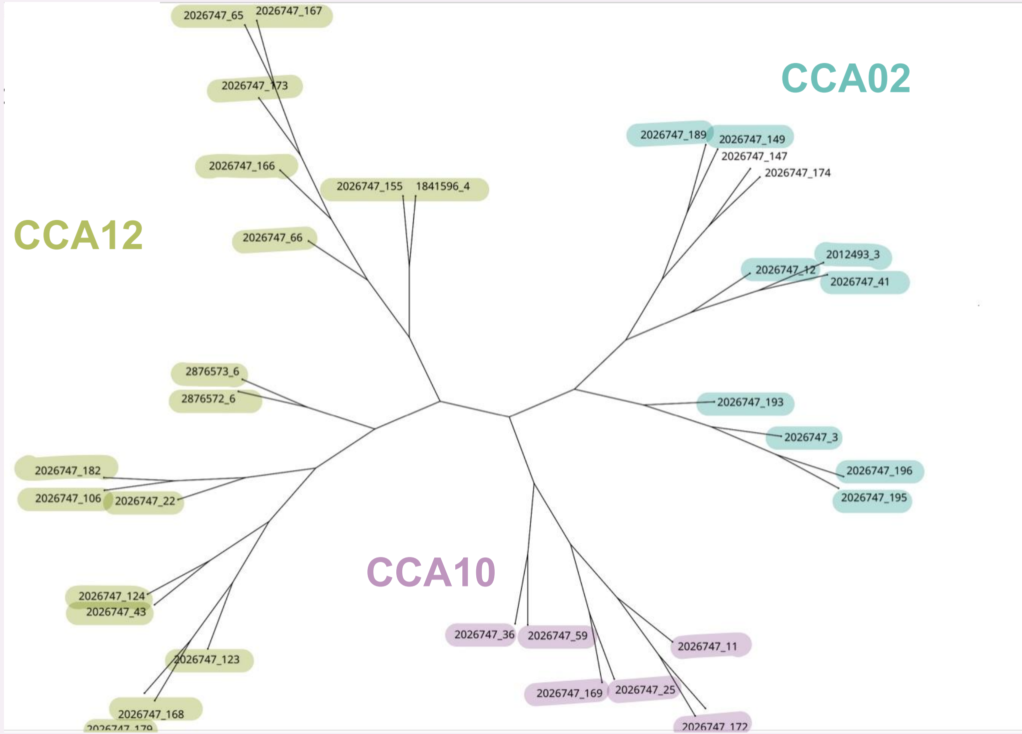


Molecular phylogeny construction

→ **issue:** no meaningful branch lengths to fully understand relationships



Obtaining meaningful branch lengths

better understanding of genetic relatedness, especially when conflicting signals or complex evolutionary histories

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Extract single copy homologs using orthogroup information → **Run clustalo on each orthogroup separately** → **Concatenate all individually aligned protein orthogroups**

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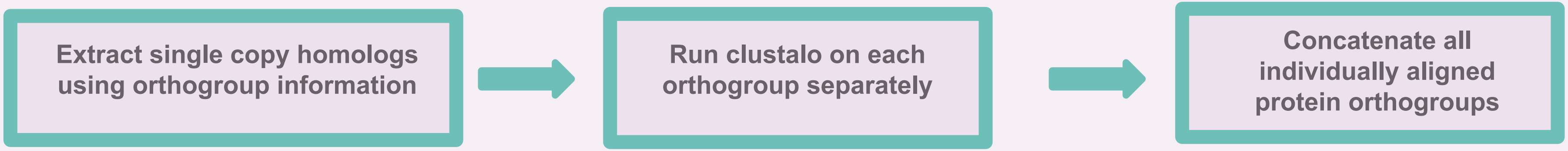

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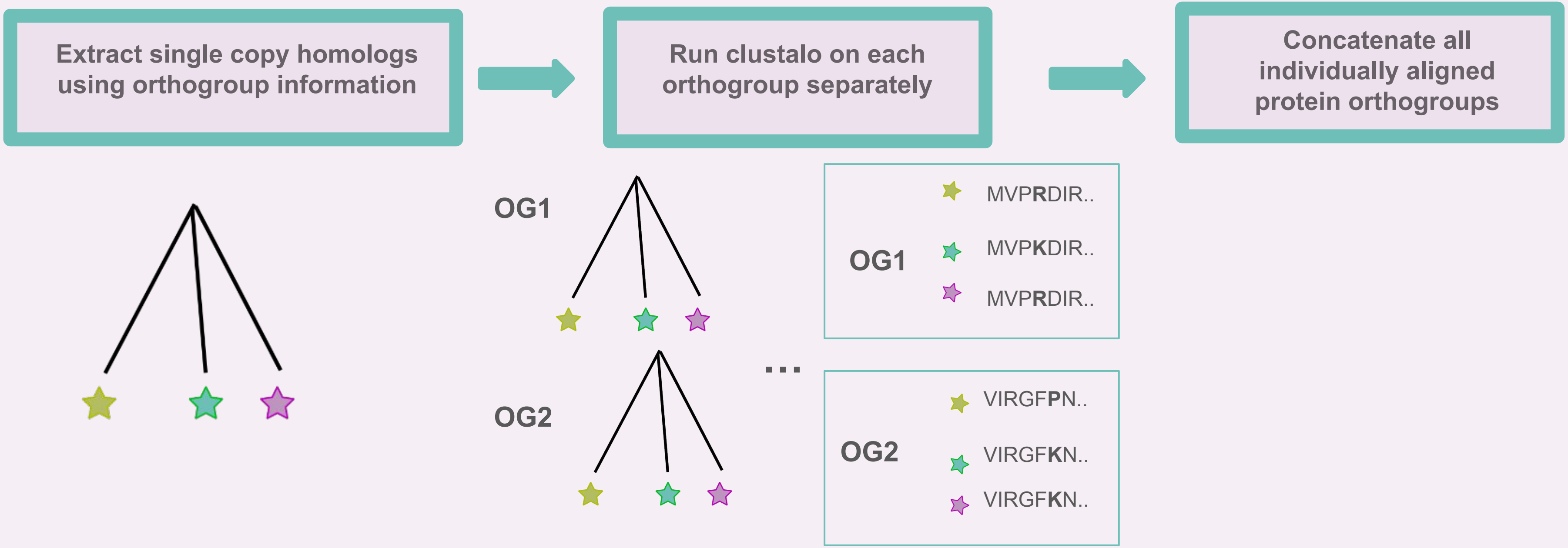

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MVP**R**DIR..
MVP**K**DIR..
MVP**R**DIR..

VIRGF**P**N..
VIRGF**K**N..
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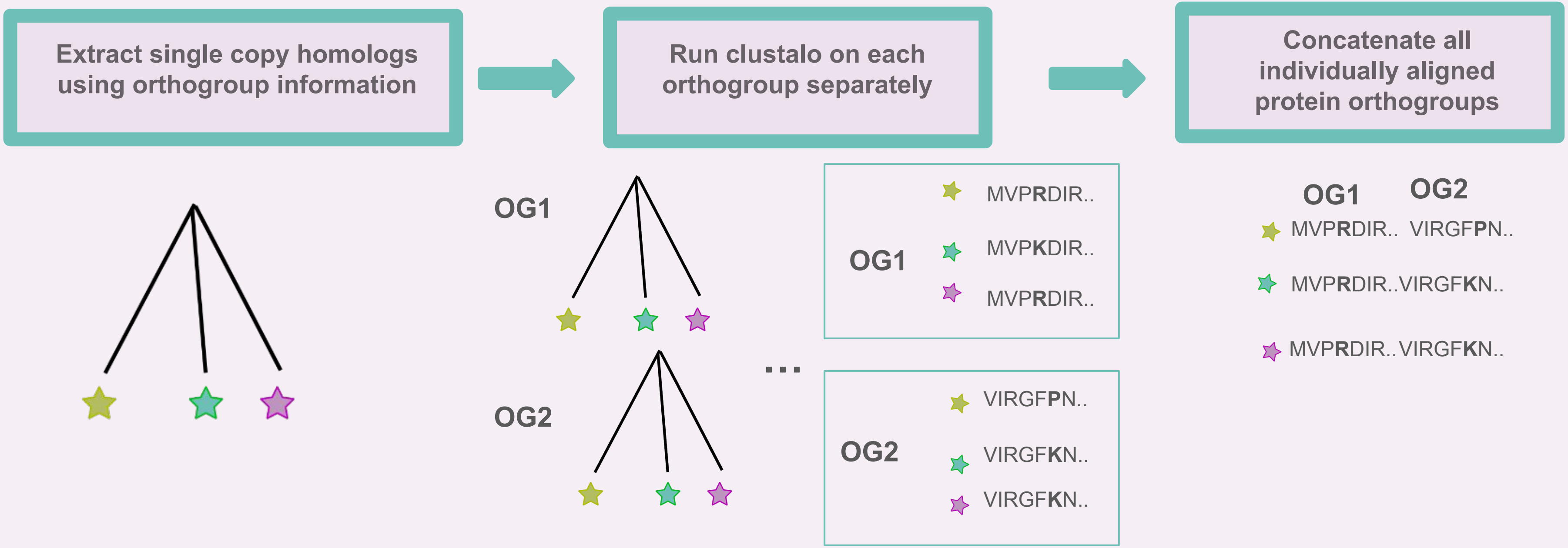

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OG1

★ MVP**R**DIR..
★ MVP**K**DIR..
★ MVP**R**DIR..

OG2

★ VIRGF**P**N..
★ VIRGF**K**N..
★ VIRGF**K**N..

OG1 **OG2**

★ MVP**R**DIR.. VIRGF**P**N..
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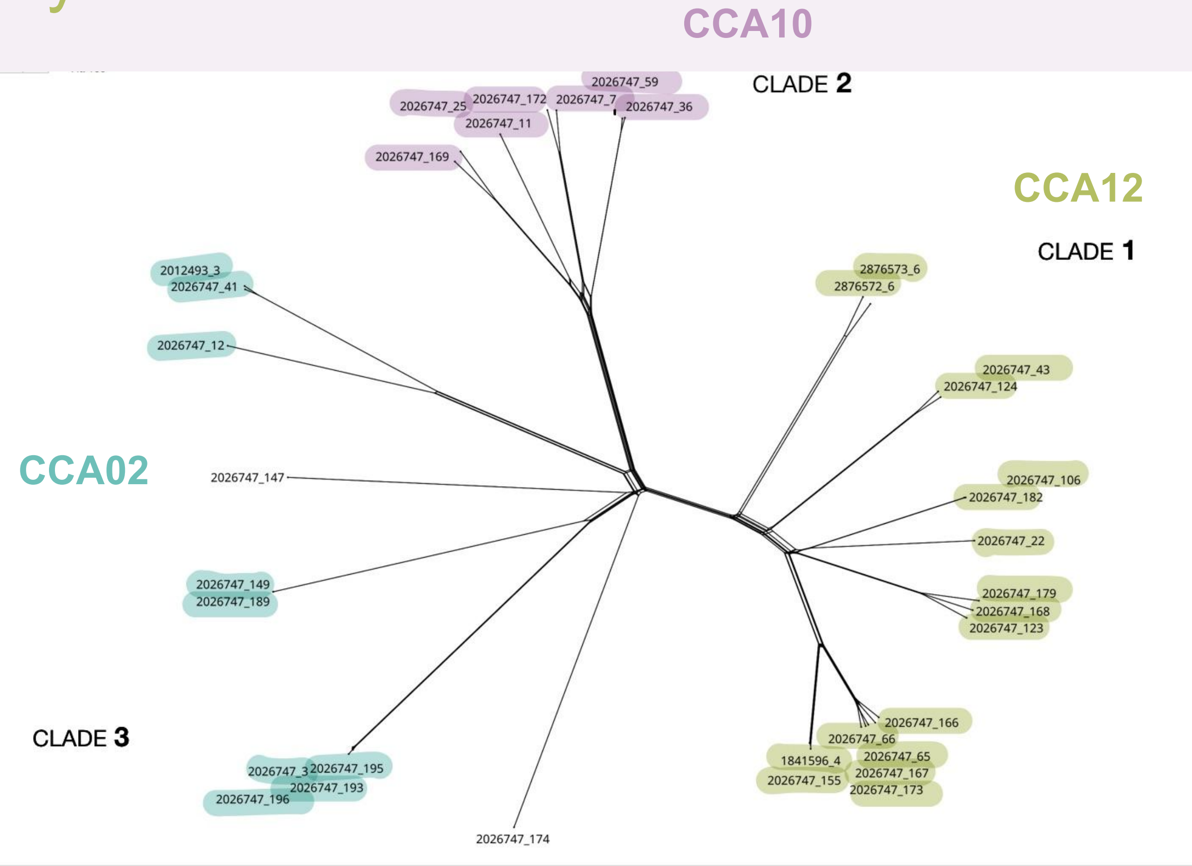
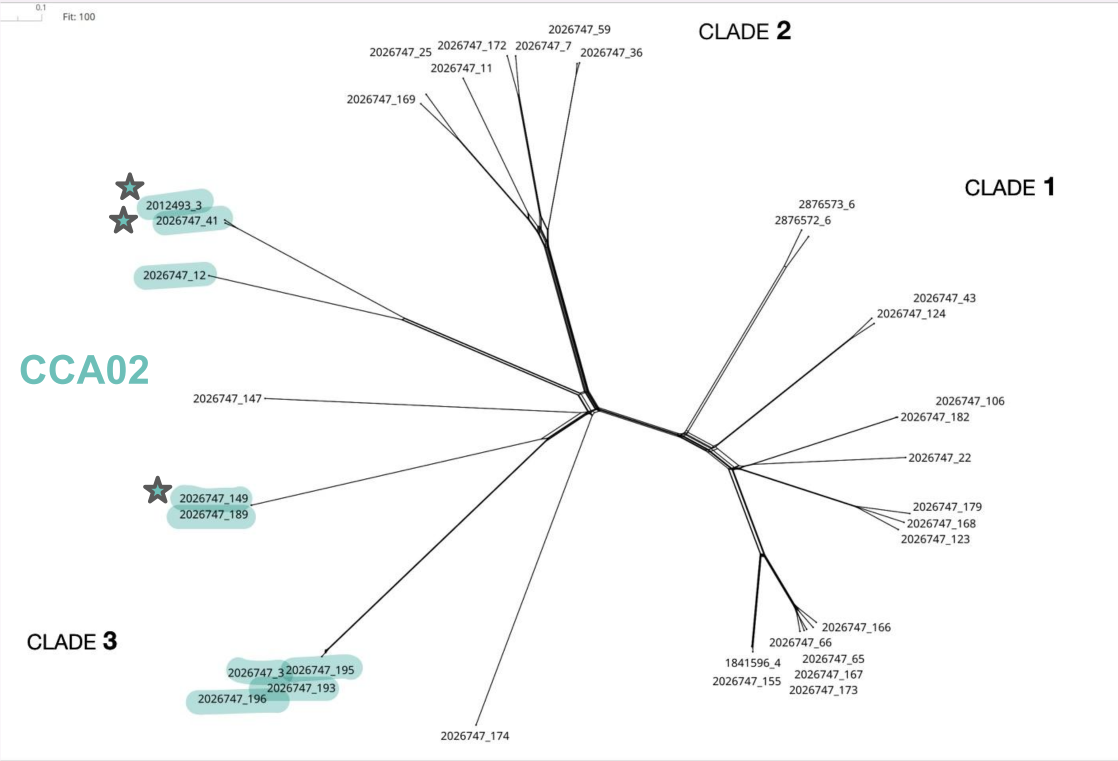
Molecular phylogeny construction

- Constructed phylogeny with meaningful branch lengths
- **But** this is not robust



Finding multiple CCA2 in one clade

- Distinct “CCA2 pairs” found in specific species



Conclusion

Heimdallarchaeota shows a complex evolutionary history of CCA-adding enzymes, where bacterial-like Class II CCA (CCA2) coexists with archaeal Class I CCA (CCA1)

Two explanations:

1. Early Archaeal Acquisition (Supports Two-Domain Hypothesis):

   CCA2 was **present in the archaeal ancestor of eukaryotes** before mitochondria, meaning eukaryotes **inherited** CCA2 rather than acquiring it from bacteria.

2. Later Bacterial HGT (Alternative Hypothesis):

   CCA2 in Heimdallarchaeota came **from bacteria after eukaryotic evolution**, meaning eukaryotes got CCA2 directly from bacteria (possibly during or after symbiosis)

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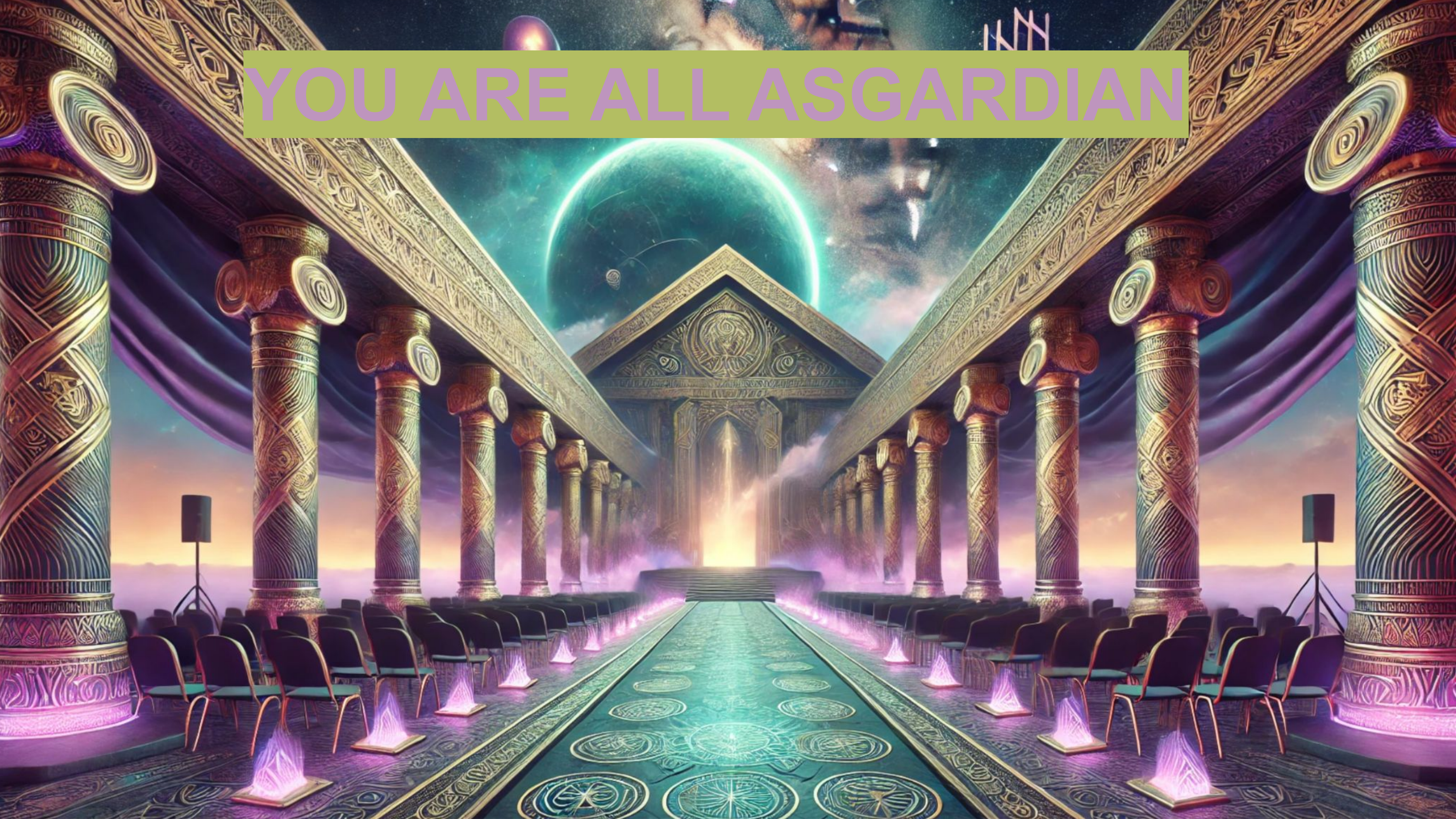
Next steps

- Wet lab verification that these enzymes (CCA2) are, at least partially, active
- Check if CCA2 are they found in clades that also have more eukaryotic-like features?
- Ascertain whether CCA2's found are closely related to bacteria, are they from a recent HGT event

YOU ARE ALL ASGARDIAN



YOU ARE ALL ASGARDIAN

Thanks to

Prof. Dr. Sonja Prohaska

Biochemistry department collaborators
- Heike Betat
- Mario Mörl

Bierinformatics people <3

Are the CCA2 more related to bacteria?

- The clade 3 CCA2 are more related to CPR's and bacteria
- The clade 1 CCA2 have a distinct long branch
- Different origin?