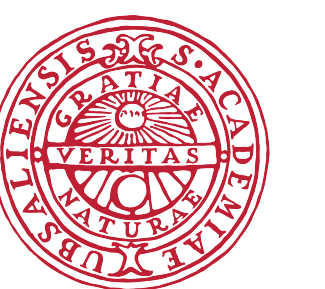


Asgard archaea are the closest archaeal relatives of eukaryotes

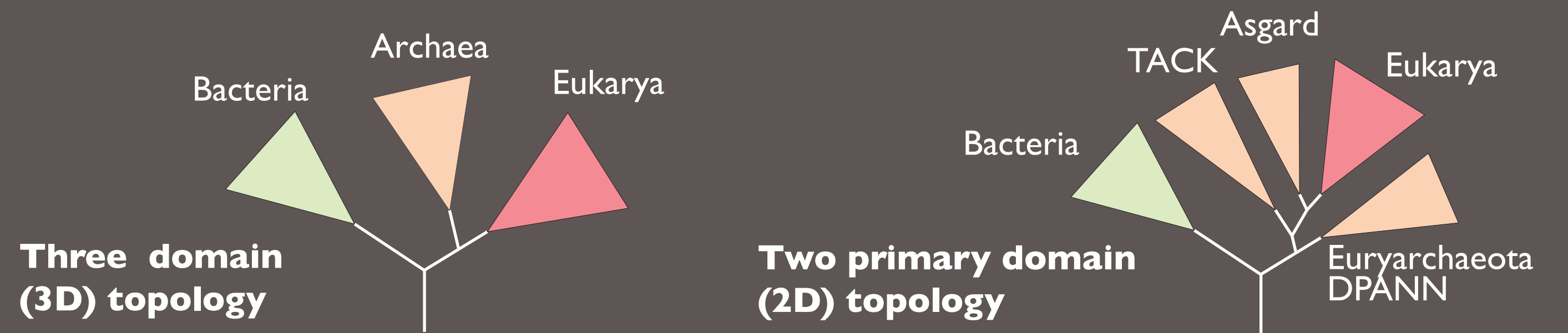
Anja Spang*, Laura Eme*, Jimmy H. Saw, Eva F. Caceres, Katarzyna Zaremba-Niedzwiedzka, Jonathan Lombard, Lionel Guy and Thijs J. G. Ettema



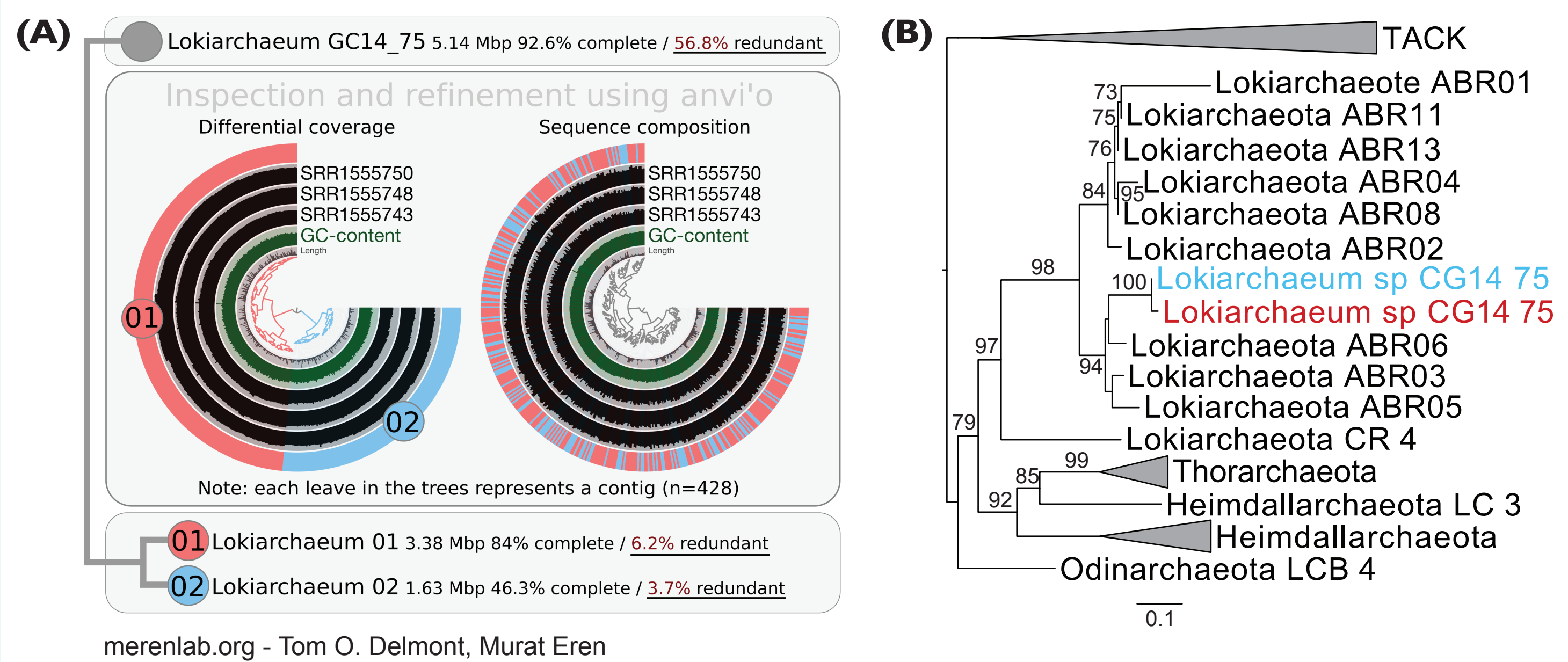
UPPSALA
UNIVERSITET

* contributed equally to this work
Read our full response: <https://goo.gl/Fv86UP>

In their recent contribution, Da Cunha *et al.*¹ claim that Lokiarchaeota are **not** the closest relatives of eukaryotes, and that phylogenetic analyses support a three-domain topology of the tree of life. **We review their main arguments and show that they are untenable.**



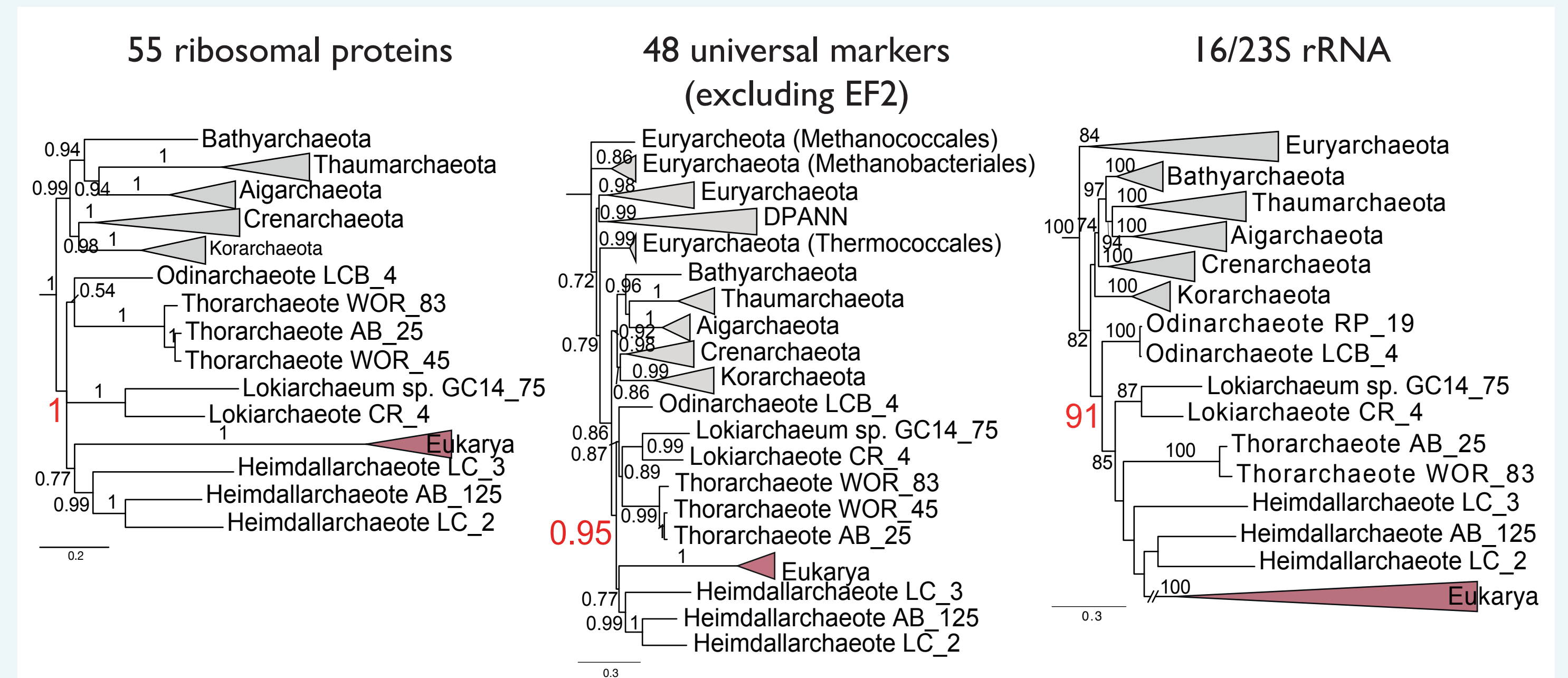
CLAIM: The first published lokiarchaeal genomes² are extensively contaminated.
RESPONSE: The Lokiarchaeal genome represents a composite genome of two closely related strains, as clearly mentioned in^{2,3}. Da Cunha *et al.* incorrectly interpret strain heterogeneity as sign of contamination.



Anvi'o⁴ representation of lokiarchaeota contigs, that comprise two closely related strains (red and blue) (A) as evidenced by single gene trees of redundant marker genes, such as ribosomal protein S12 (B).

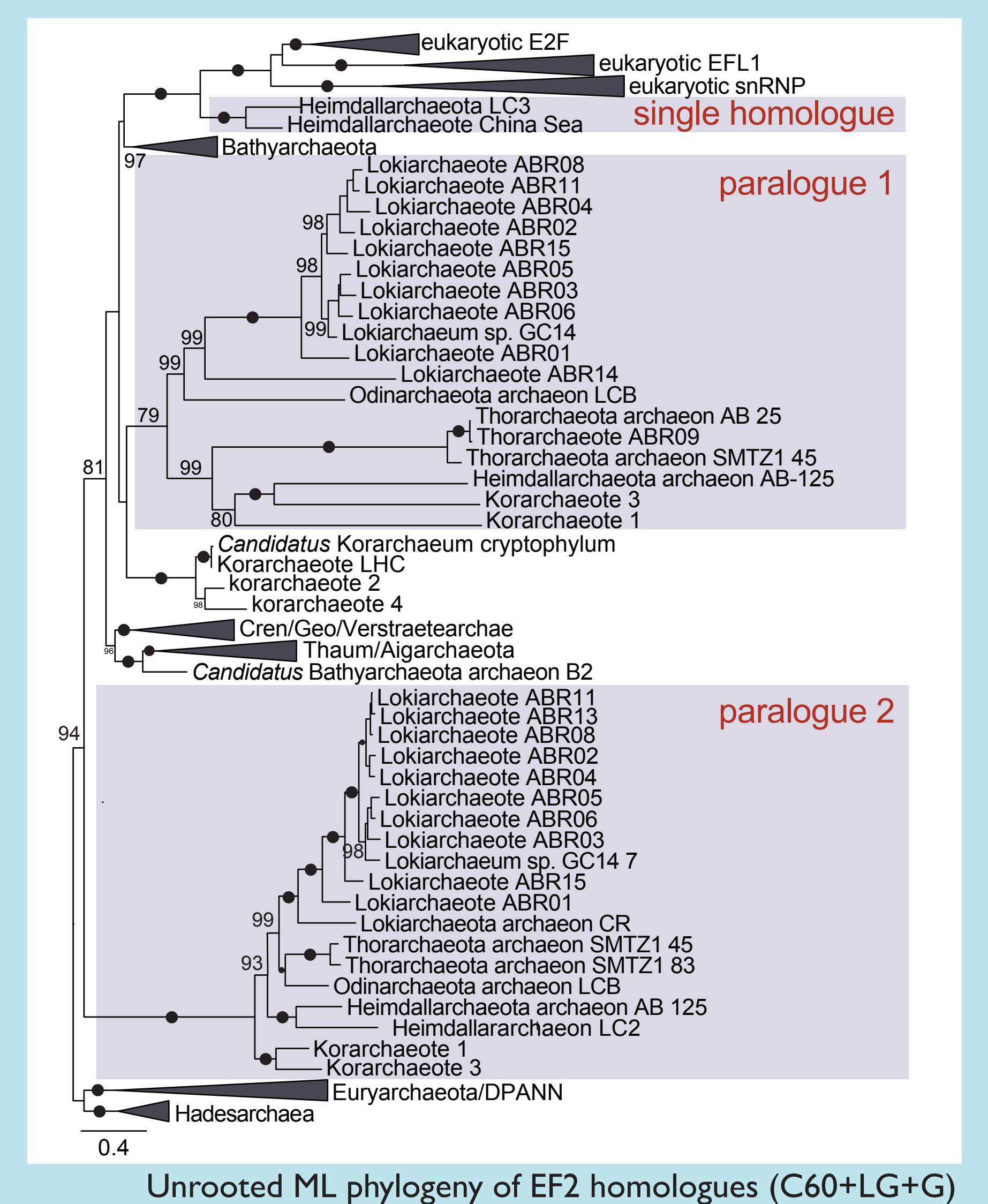
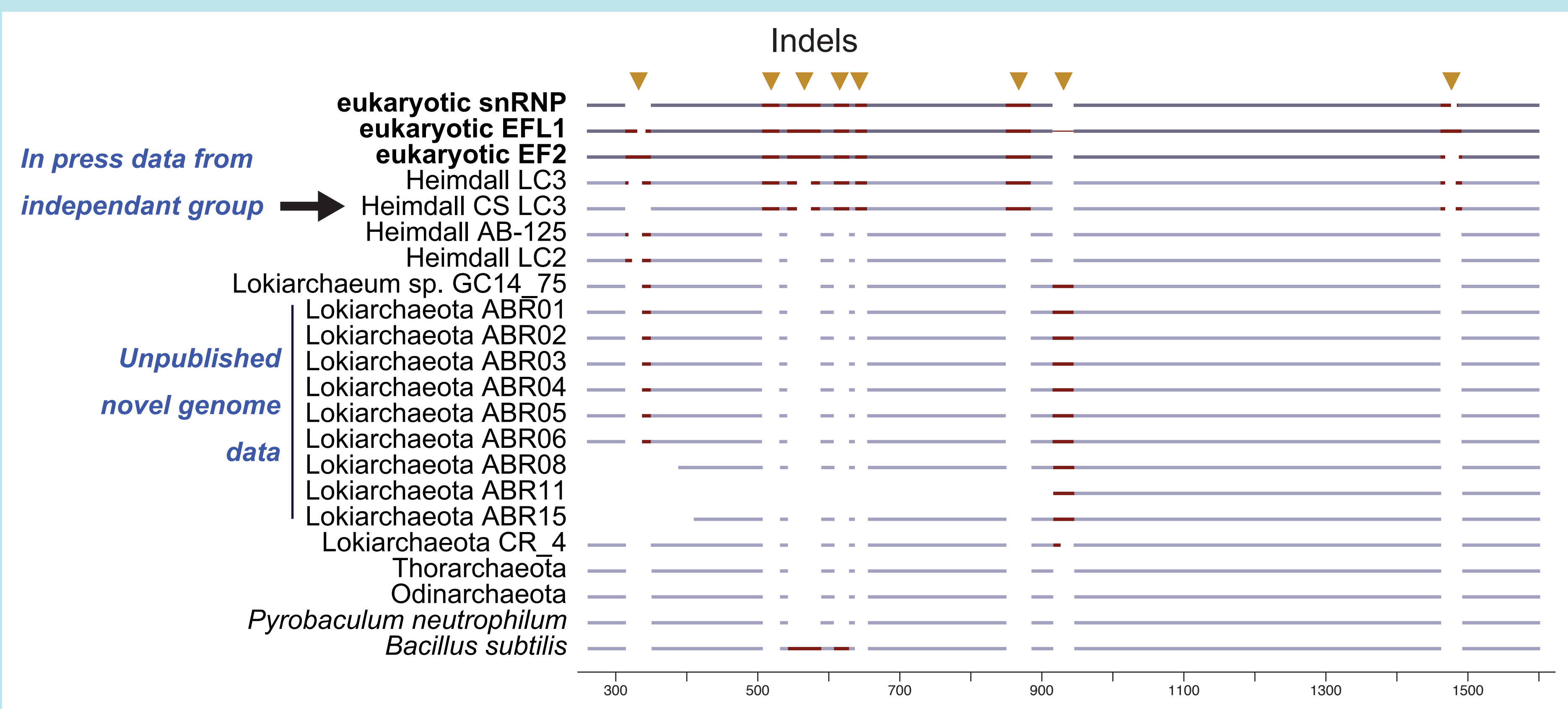
CLAIM: Removing EF2 from the universal gene set breaks the Loki-Eukarya affiliation.

RESPONSE: All our analyses excluding EF2 strongly support the sister-relationship of eukaryotes and Asgard archaea, as explicitly discussed in^{2,3}

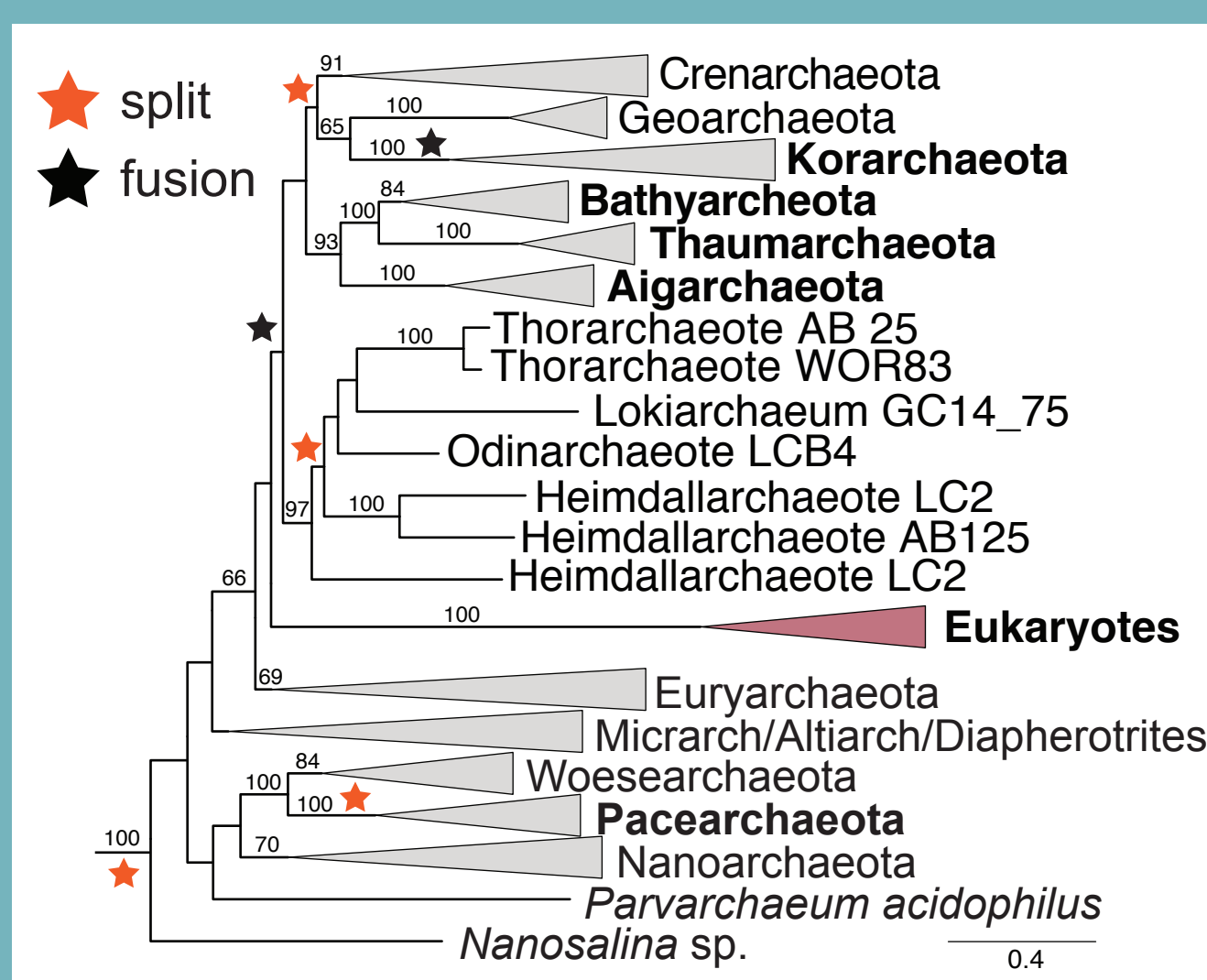


CLAIM: EF2 of Loki- and Heimdallarchaeota contain eukaryotic indels derived from chimeric assemblies.

RESPONSE: All strains of Loki- and Heimdallarchaeota encode the same conserved indels. Indels are not the result of assembly artefacts. Indels unique to the Heimdallarchaeota LC3 group reflect their close relationship with eukaryotic EF2.



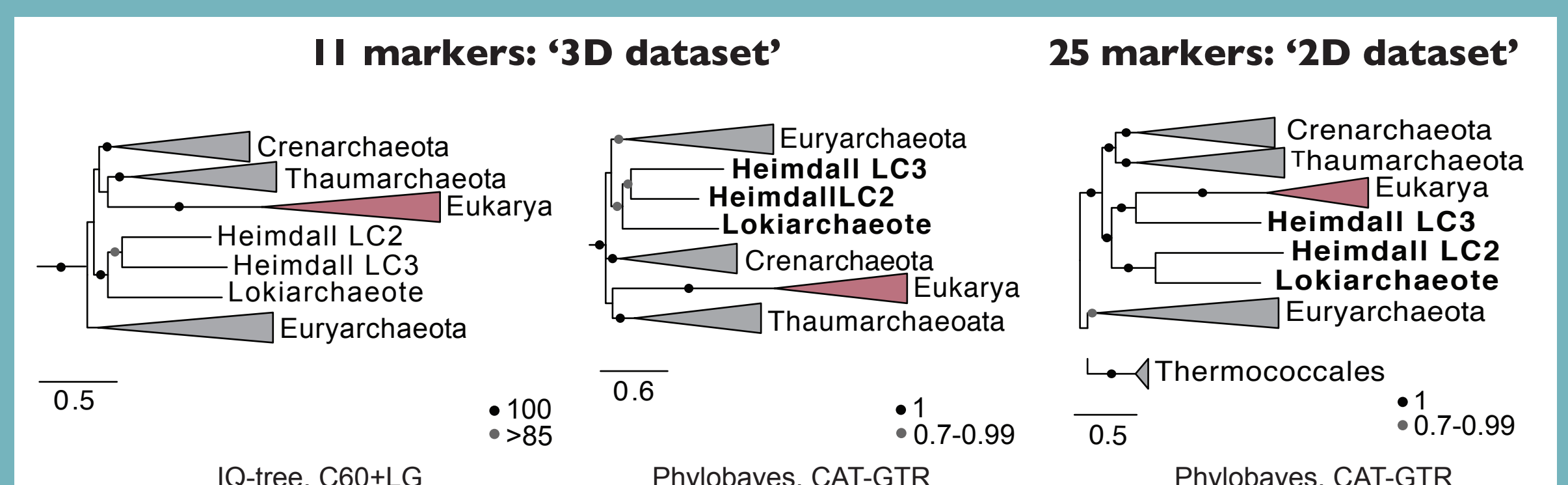
CLAIM: the fused RNA pol A (rpoA) of Heimdallarchaeota LC3 is a contaminant from Thaumarchaeota or Bathyarchaeota.
RESPONSE: rpoA Heimdallarchaeota branches basal to Asgard split homologues². It thus does not represent a contamination from other archaeal clades. rpoA has a complex history and underwent several split events during archaeal evolution.



ML phylogeny of rpoA. Rooted with bacteria (not shown). Taxa in bold face possess a fused rpoA gene. Modified from³

CLAIM: A subset of 11 universal genes, and RNA polymerase A and B subunits recover the 3D topology, whereas the other 25 universal markers recover the 2D topology.

RESPONSE: A re-analysis of these two datasets (taken directly from Da Cunha *et al.*) using sophisticated evolutionary models recovers the 2D topology in both cases:



CONCLUSIONS

The results we have published in recent years are not the final word on the origin of eukaryotes. However, Da Cunha *et al.* use inadequate methodology, misinterpret the data, and make *ad hoc* decisions regarding the taxa and genes included in their analyses. Finally, they ignore the plethora of evidence and discussions presented previously^{2,3}, which addressed many of their points.

The publication by Da Cunha *et al.* provides no evidence that falsify the conclusions drawn by Spang *et al.* and Zaremba-Niedzwiedzka *et al.*

- 1 Da Cunha, *et al.*, Lokiarchaea are close relatives of Euryarchaeota, not bridging the gap between prokaryotes and eukaryotes. PLoS Genetics (2017)
- 2 Spang, *et al.* Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature (2015).
- 3 Zaremba-Niedzwiedzka, *et al.* Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature (2017).
- 4 Eren, *et al.* Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ

