

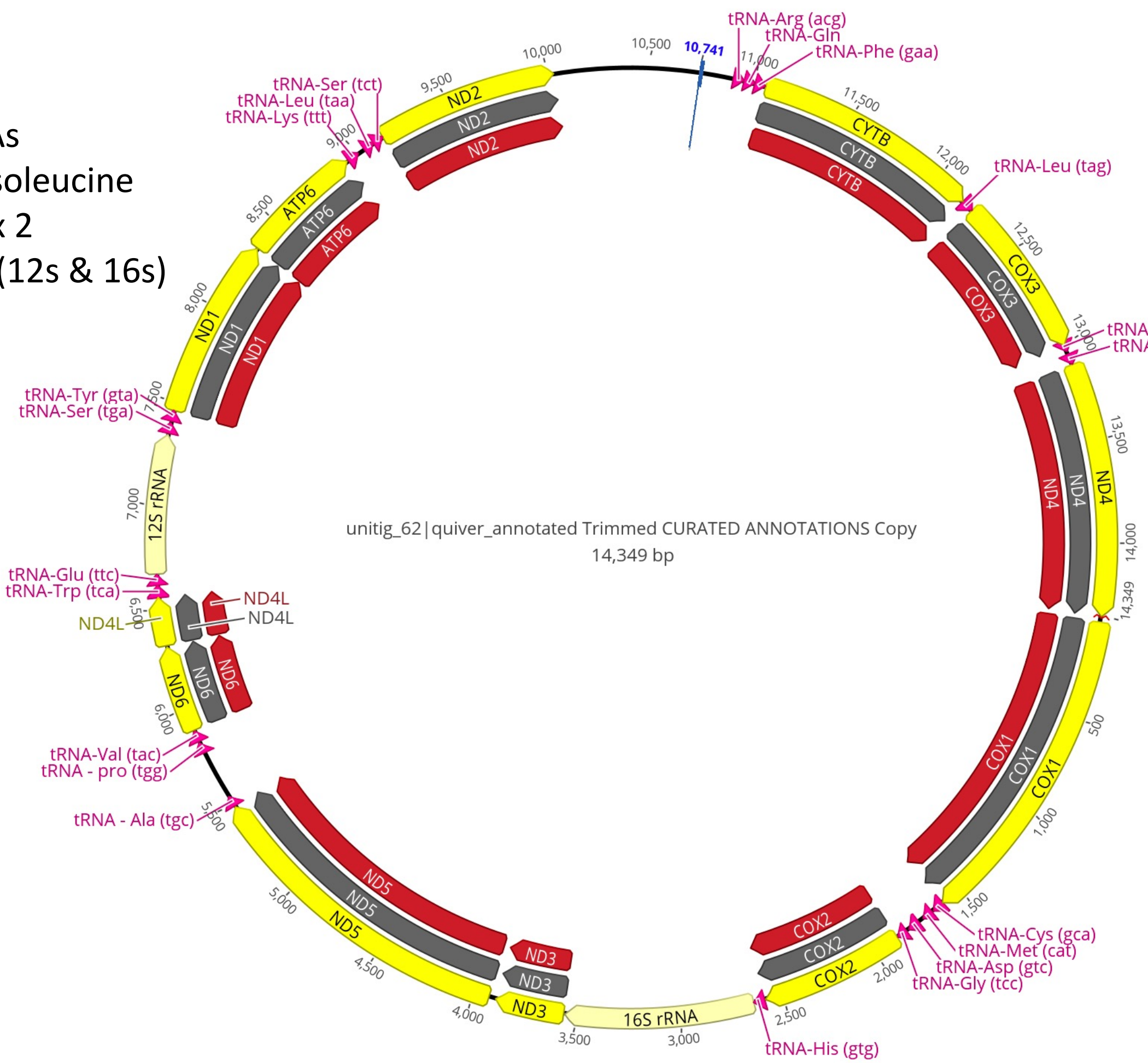
Abstract

Halicephalobus mephisto, or the Devil Worm, is a subterranean nematode found nearly 1.3 kilometers below Earth’s surface. When underground, the worm was residing in thermal waters and exposed to hypoxic and methane-rich conditions. The ability to thrive in a stressful environment indicates that there must have been an evolutionary event that distinguished this particular species as an extremophile compared to other nematodes. While nuclear genome sequencing revealed stress-related protein family expansions, it is reasonable to assume that the mitochondrial genome will also exhibit molecular adaptations, as the organelle is vital to the survival and energy production of an organism. The mitochondrial genome of *Halicephalobus mephisto* is composed of 35 total genes encoding for the 12s and 16s ribosomal subunits, 21 tRNAs, and 12 protein-coding genes involved in oxidative phosphorylation. Deviating from typical mtDNA structure, this particular species lacks the ATP synthase subunit 8 (*ATP8*) gene and the tRNA responsible for the delivery of isoleucine during translation, as well as the duplication of the leucine and serine tRNAs. We identified signatures of positive selection by dN/dS analysis in the *COX1* and *COX2* gene. Surprisingly, however, positive selection was not detected in the most recent *H. mephisto* branch, but rather, the ancestral clade IV lineage, indicating that that organism and its descendants may all have a stress-adapted version of this gene. Heat-induced gene expression data has indicated significant upregulation of this protein product in the presence of a biological stressor, only serving to highlight the gene’s role in environmental adaptation. Therefore, non-synonymous mutations in the *COX1* gene of clade IV nematodes are hypothesized to provide a molecular adaptation that allows *Halicephalobus mephisto* to colonize unconventional environments, and may contribute toward its ability to withstand hypoxia.

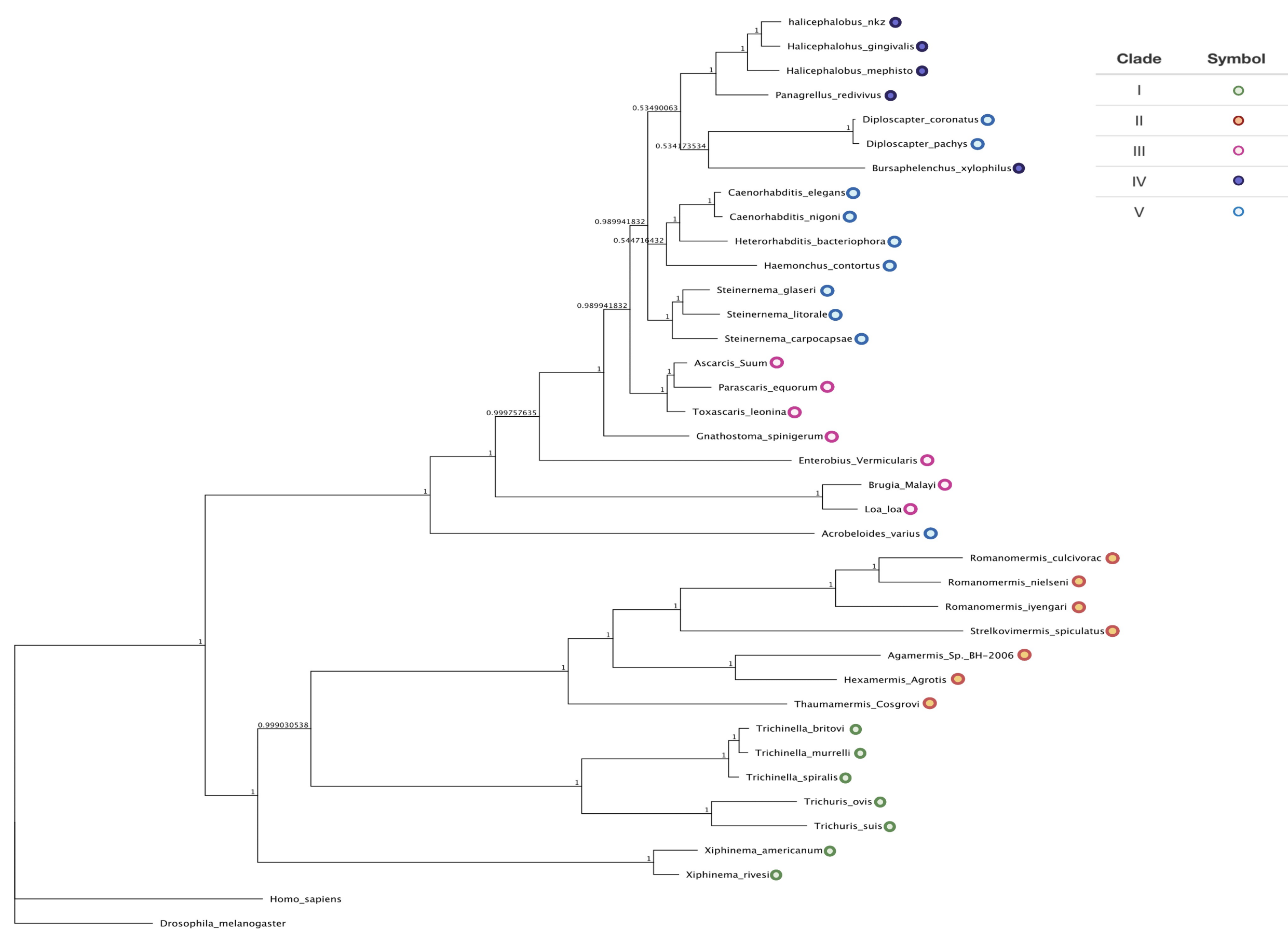
Annotated mtDNA of *Halicephalobus mephisto*

Includes:

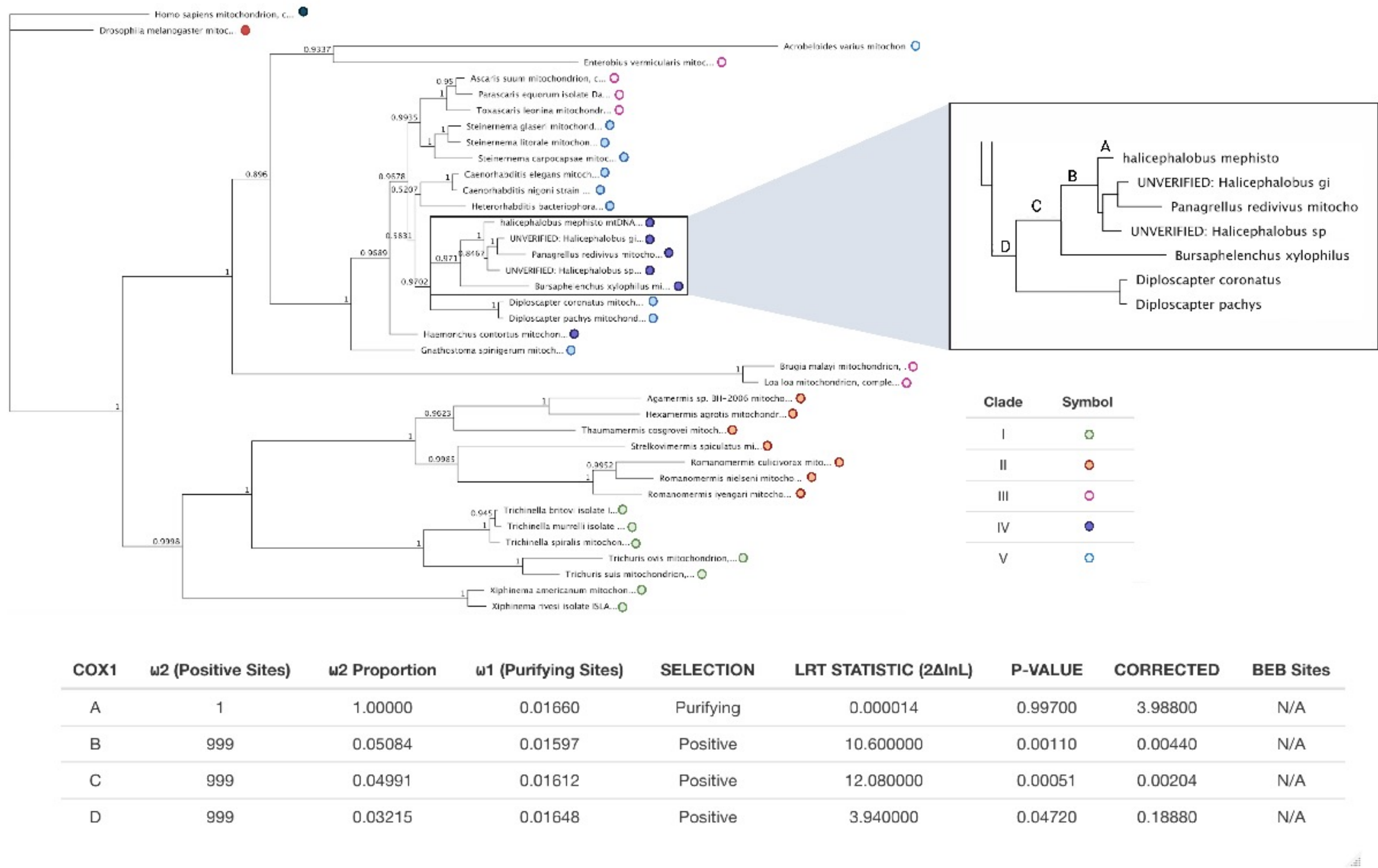
- 12 CDS
- 21 tRNAs
 - no isoleucine
 - Ser x 2
- 2 rRNA (12s & 16s)



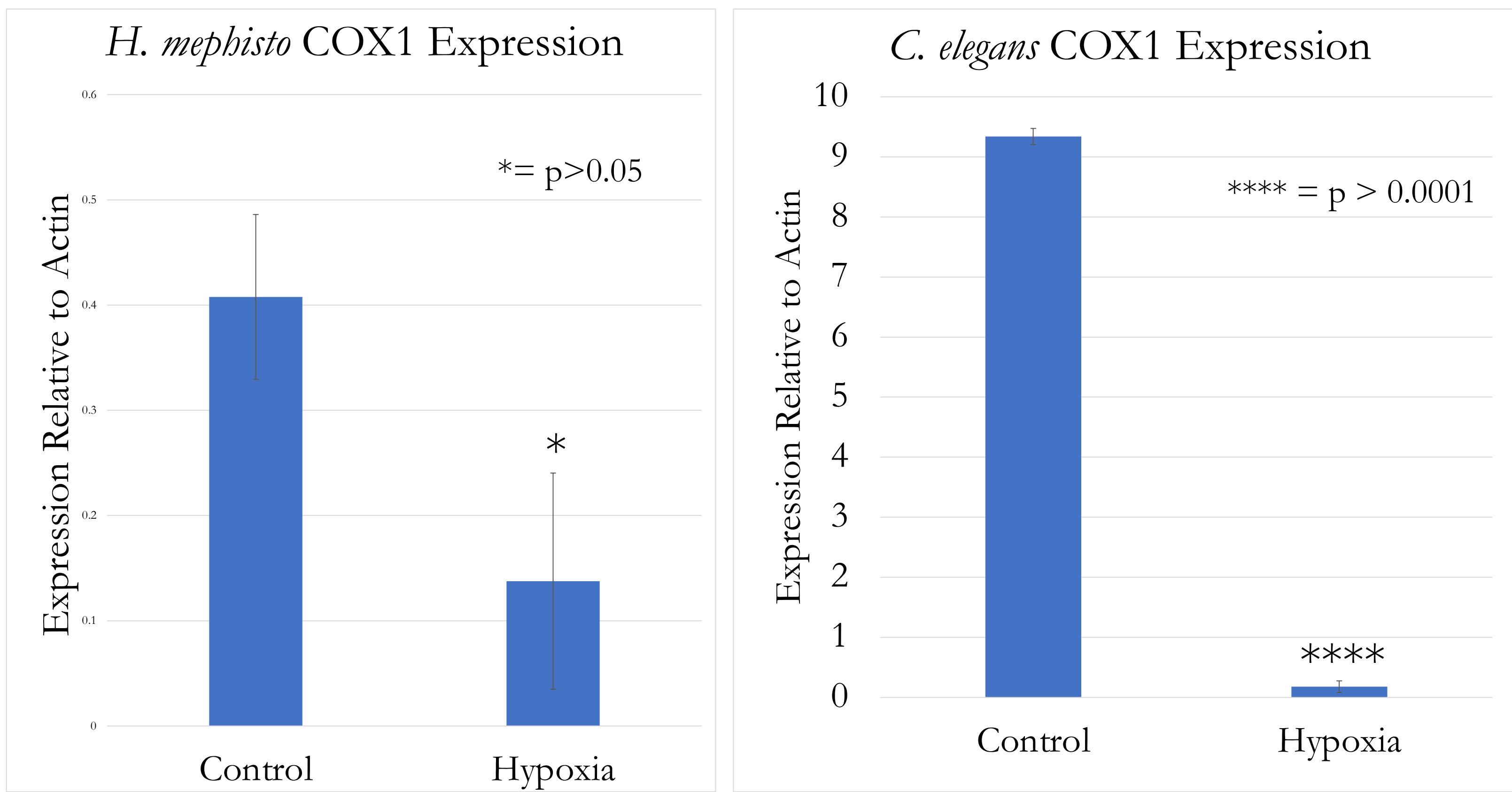
Catenated Protein Tree



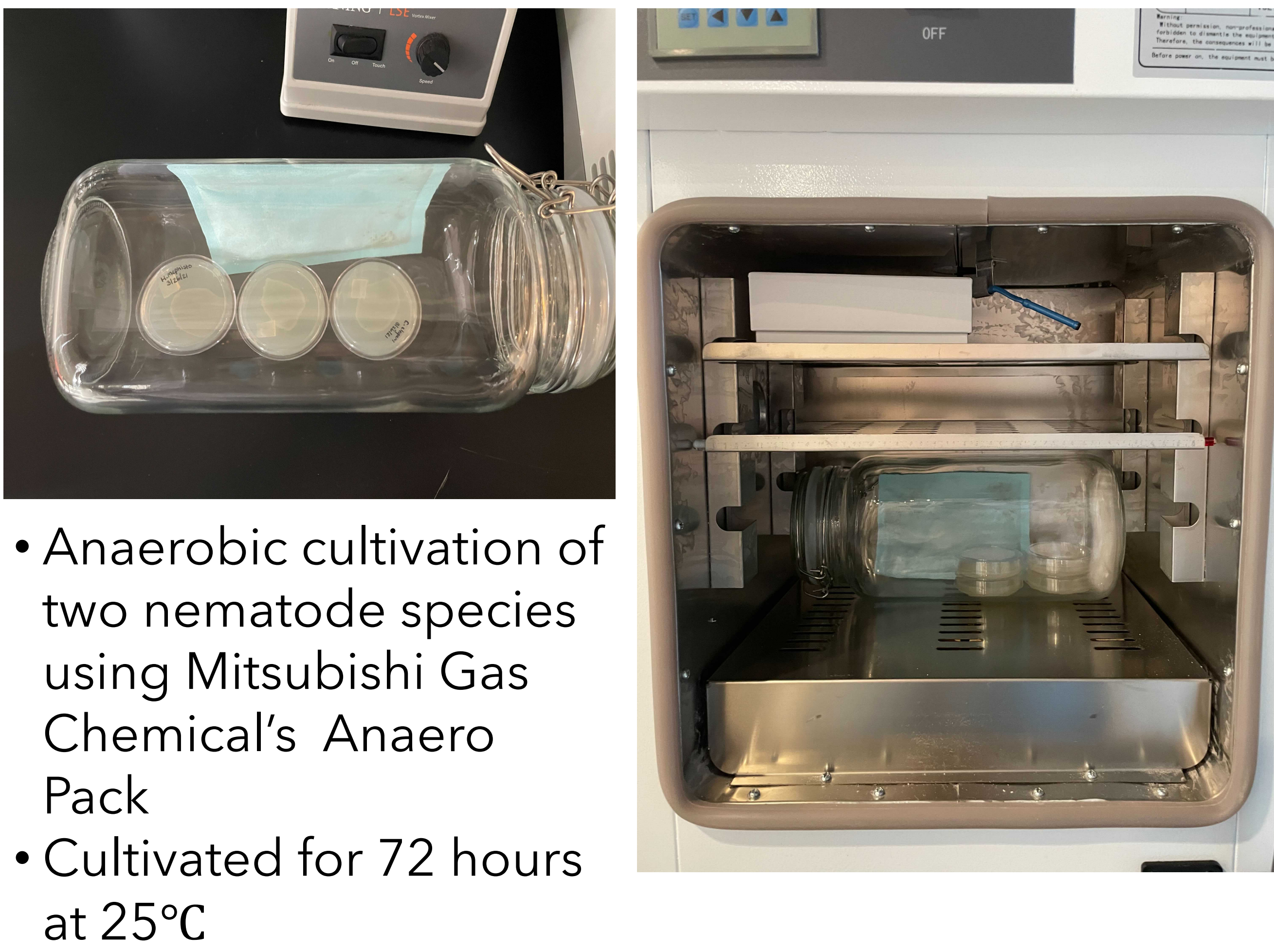
COX1: Phylogenetic Analysis & dN/dS



Gene Expression Analysis



Methods / Hypoxia Set-Up



- Anaerobic cultivation of two nematode species using Mitsubishi Gas Chemical’s Anaero Pack
- Cultivated for 72 hours at 25°C

Main Findings

- Positive selection indicates pre-adaptation of clade IV lineage nematodes to hypoxia
- Possible regrouping of *Diploscapter pachys* as a clade IV nematode based on mtDNA
- Positive selection places evolutionary emphasis on COX subunits 1 and 3 of complex IV in OXPHOS
- Highlights *H. mephisto* as a model for stress resilience

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