Supporting information

Figure S1. **A)** *G. muris* cells propagate as trophozoites in the mice small intestine. The cell trophozoites undergo encystation into cysts and are shed in the feces. The cysts are prompted to excyst as they encounter the conditions of the digestive tract. The excyzoites transition into the vegetative trophozoites. Cell material for genomic DNA was harvested from *G. muris* cysts from infected mice. **B)** Sub-telomeric regions in *G. muris*. Sequence motifs in the 10 unitigs terminating in $(TAGGG)^n$ telomeric sequences (purple). Ribosomal DNA sequences (black). Satellite-like repeated sequences (green).

Figure S2. Chromosome-scale synteny between *G. muris* and *G. intestinalis* WB. *G. muris* chromosomes are color coded. The links between the two genomes representing sequence similarity identified by MUMmer promer[96], are colored after the *G. muris* chromosomal color. Circular plot was drawn with circlize[94].

Figure S3. Sequence logo around start codon (A) and stop codon (B) in *G. muris*. C) *G. muris* gene expression from *in vivo* trophozoites. Genes showing top-ranked FPKM ratios (log2 (Trophs/Cysts)) between *in vivo* derived trophozoites and cysts (cut-off value ≥4). The value is indicated in the teal circle. An orange dot indicates that the ortholog of this gene is upregulated (>2 fold) in *G. intestinalis* at 7 hrs, 12 hrs or 22 hrs into encystation [40].

Figure S4. *Trans*- and *cis*-introns in *G. muris*. A) Aligned *trans*- and *cis*-splice junctions in *G. muris.* **B)** Splice-site associated motifs in *trans*-spliced exons. Base-pairing stems are underlined. Bases in red denote the predicted cleavage motif. **C)** Consensus alignment of the cleavage motifs in *G. muris*. **D-E)** Predicted secondary structures of **D)** *cis*-spliced and **E)** *trans*-spliced genes. The secondary structure was predicted using the mfold webserver using default settings and manually curated. GU-rich stretches and cleavage motifs are boxed.

Figure S5. A) Conservation of the VSP C-terminal in *G. muris* and *G. intestinalis.* Sequence logos of conserved motifs in the C-terminal of VSPs in *G. muris* (n=25) (upper panel) and *G. intestinalis* WB (n=183) (lower panel) were generated using MEME v5.0.5. Transmembrane and cytosolic pentapeptides were predicted by the Phobius webserver. **B) VSP and ψVSP evolutionary dynamics.** The ψVSP arrays (defined as >3 ψVSP genes arrayed) in the *G. muris* genome arranged by chromosome, termini arranged to the right. Arrays of ψVSPs without chromosomal context are shown below as orphan arrays. **C)** VSP cluster mostly together in a phylogeny whereas most ψVSP gene arrays show relaxed clustering. Intact VSP genes are colored red, while ψVSP genes are colored according to their resident ψVSP array in panel A. Genes with black label are not found in arrays. **D) Expression measurements of VSP and ψVSP in *G. muris.*** VSP and ψVSP RNA-seq read counts (FPKM) from trophozoites, cysts and excyzoites. Size of the circle corresponds to expression level.

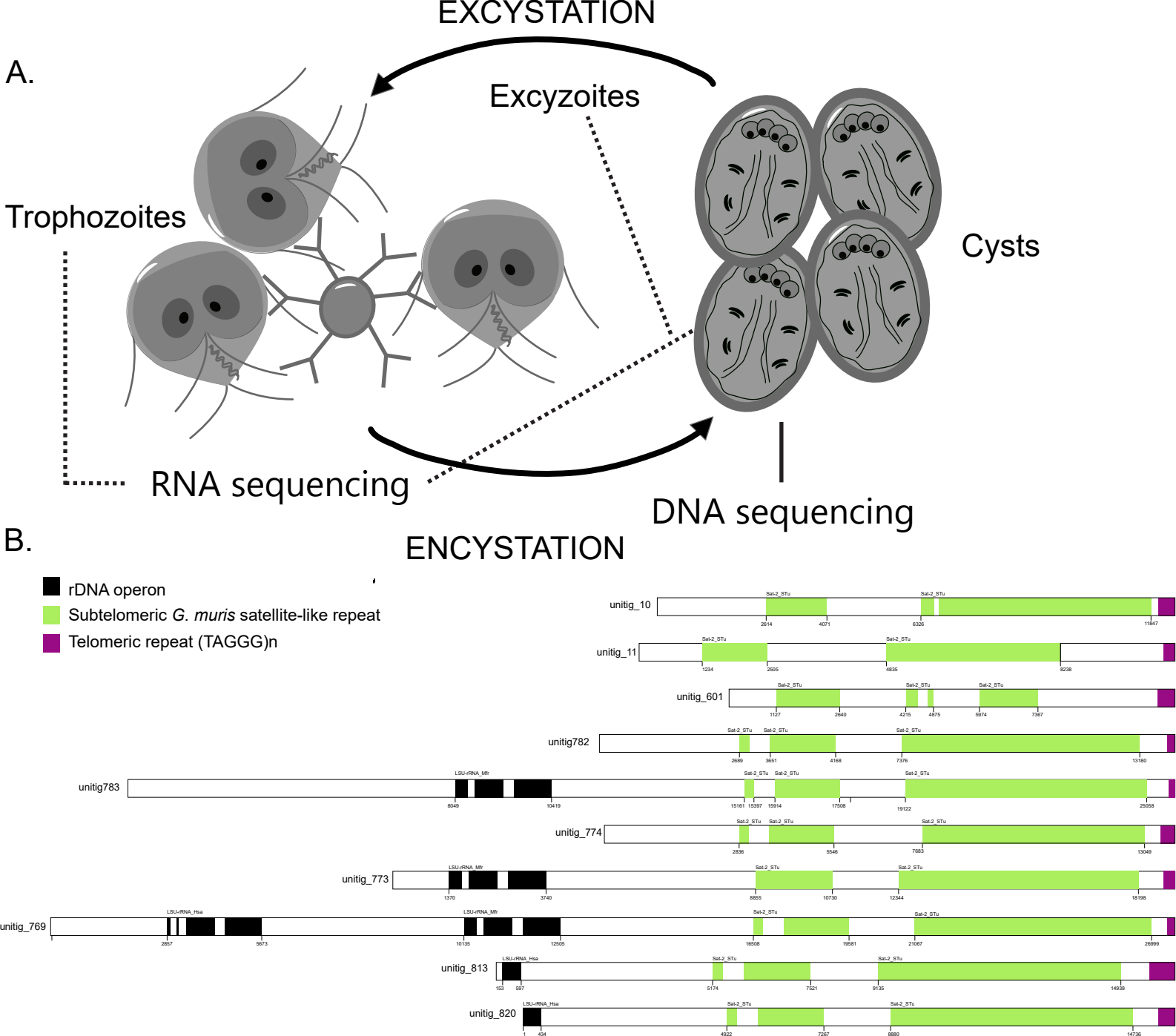
Figure S6. A) Phylogenetic tree of *G. muris* and *G. intestinalis* NEKs. B) Phylogenetic tree of *G. muris* and *G. intestinalis* ARPs. C) Network analysis of *G. muris* and *G. intestinalis* NEKs and ARPs. *G. muris* genes are represented in circle whereas *G. intestinalis* genes are shown in triangle. Blue indicates NEK and pink as ARP. Edges are weighted and scaled by reciprocal BLAST scores with evalue 1e-05 as the cutoff.

Figure S7. Phylogenetic tree of C1 family cysteine proteases in diplomonads. Black: *G. muris*, Red: *S. salmonicida*, Cyan: *G. intestinalis* GS/M, Green: *G. intestinalis* P15, Purple: *G. intestinalis* WB.

Figure S8. Phylogenetic analyses of HGT genes. A) Fructokinase. **B)** Mannose-6-phosphate isomerase. **C)** Transcriptional regulator/sugar kinase. **D)** Arginase. **E)** 2,5-diketo-D-gluconic acid reductase 1. **F)** 2,5-diketo-D-gluconic acid reductase 2. **G)** Carboxymuconolactone decarboxylase. **H)** Ketosteroid isomerase. **I)** Tryptophanase. **J)** Tae4. **K)** Quorum-quenching N-acyl-homoserine lactonase.

Table S1. A) One-to-one ortholog list between *G. muris* and *G. intestinalis* WB. Orthologs inferred by OrthoMCL. **B)** RNA-Seq expression values in excel file. Sorted according to FPKM values. **C)** VSP and ψVSP gene metadata. '-' in the pentapeptide motif represents lack of the 3' end sequence. **D)** List of ventral disc proteins in *G. muris*. **E)** Proteases detected in *G. muris* and *G. intestinalis*. **F)** Detailed information about hits of unique genes in *G. muris.*

Methods S1. Additional methods section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Trophozoites
Cysts
RNA sequencing
DNA sequencing
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Telomeric repeat (TAGGG)n
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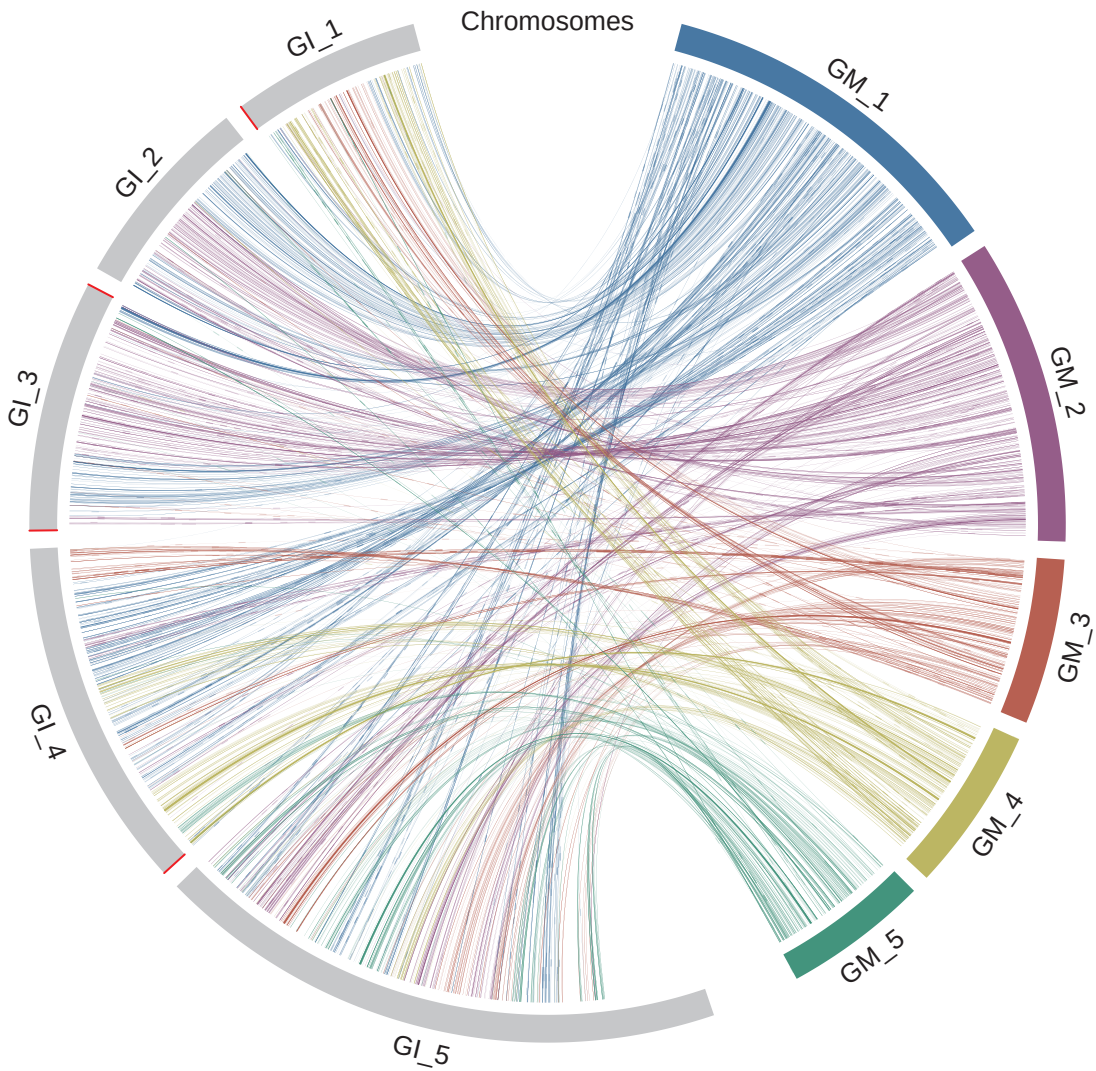
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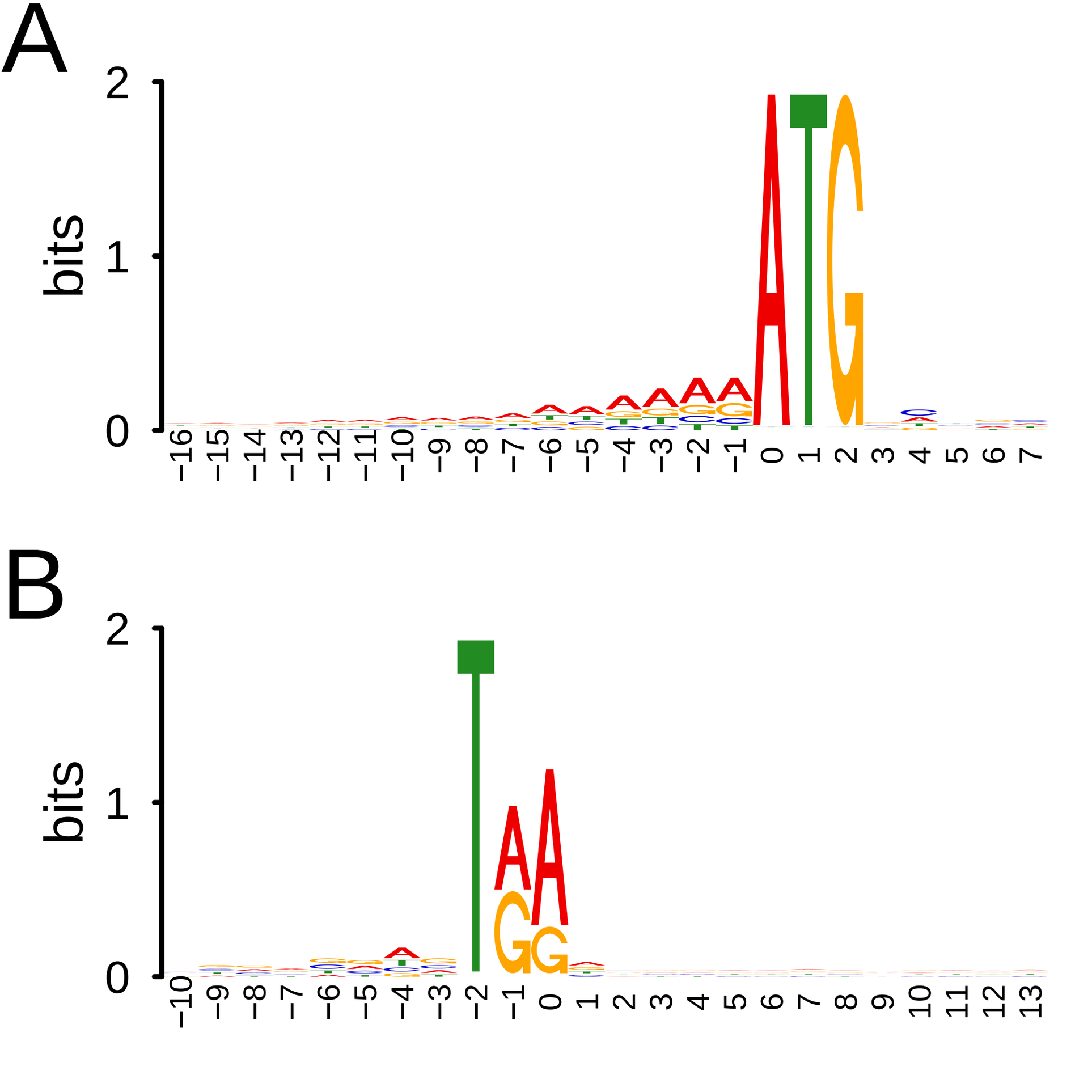
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1
0
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C

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7				GMRT_15329
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6				GMRT_12476
6				GMRT_15303
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LOG2 Trophs/Cysts	Encyst up 90 min	Encyst up 7h	Encyst up 22h	Gene id
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Cis-spliced introns

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C.

Cleavage motif

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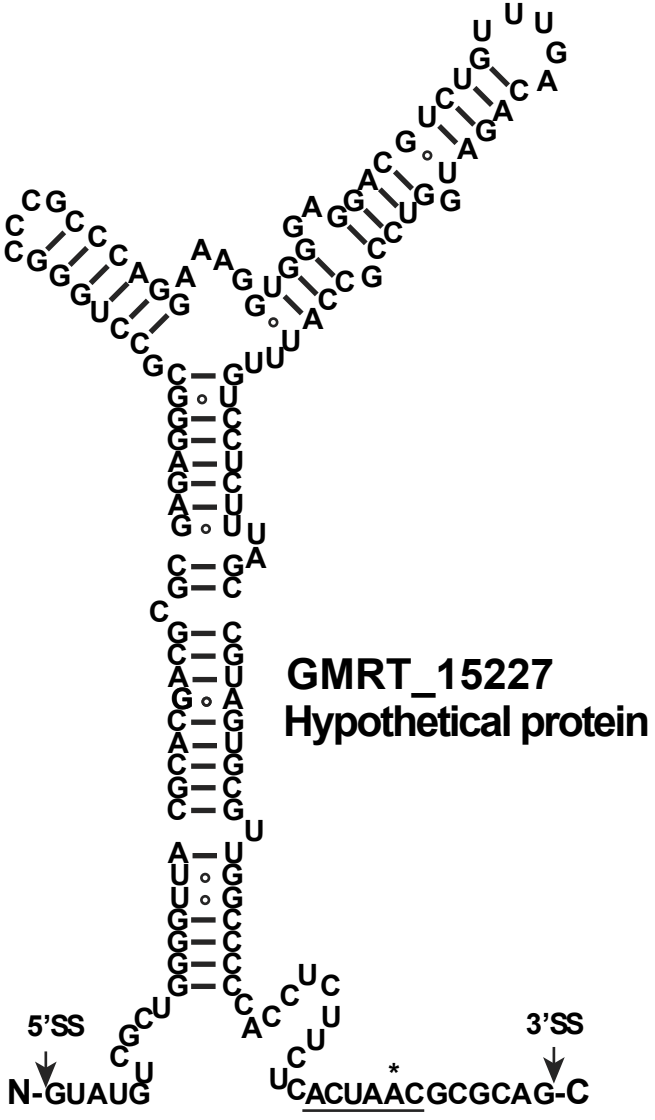

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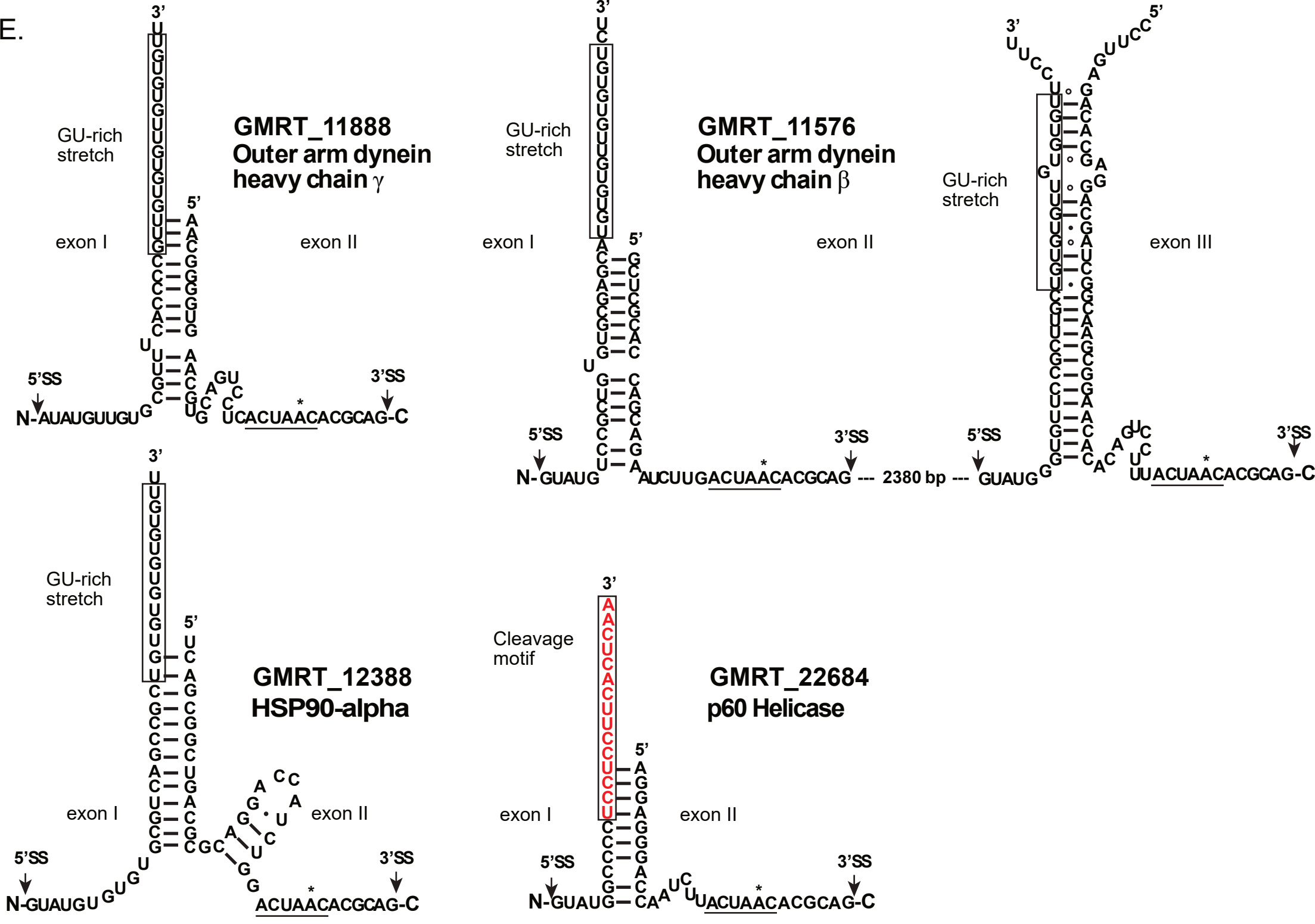
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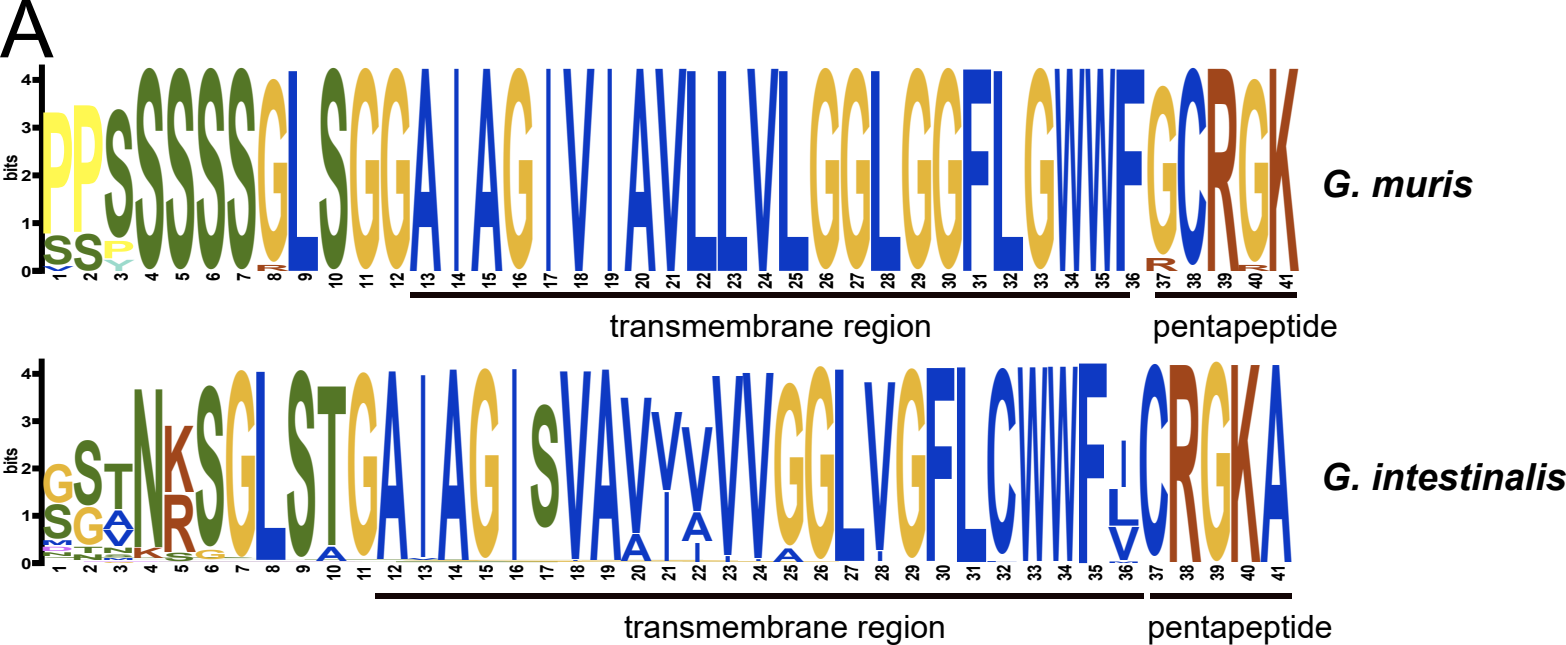
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B

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Chromosome 2 array 2

Chromosome 3 array 1

Chromosome 3 array 2

Chromosome 4 array 1

Chromosome 4 array 2

Chromosome 5 array 1

Chromosome 5 array 2

Chromosome 5 array 3 (internal)

Orphan array 1

Orphan array 2

Orphan array 3

Orphan array 4

Orphan array 5

Orphan array 6

Orphan array 7

C

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Internal pVSP array

VSP cluster 3

VSP cluster 1

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VSP directly adjacent to a pVSP

Part of a twinned pVSP array

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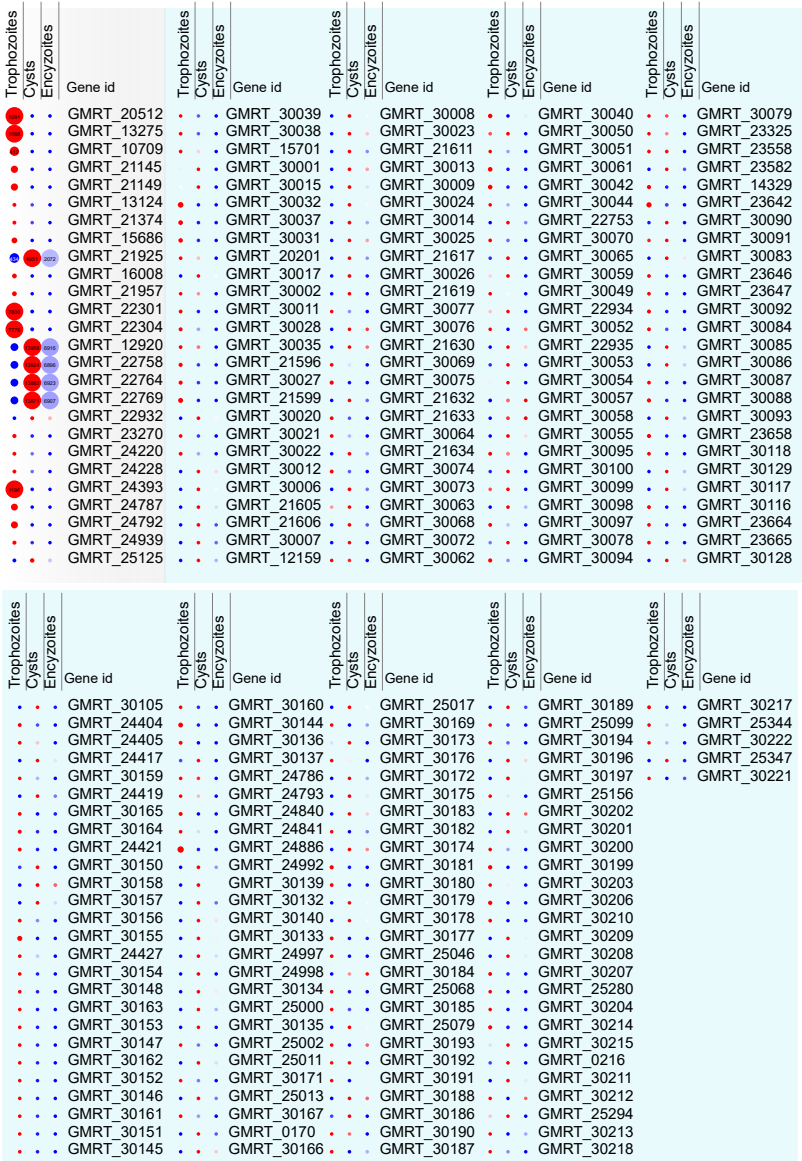
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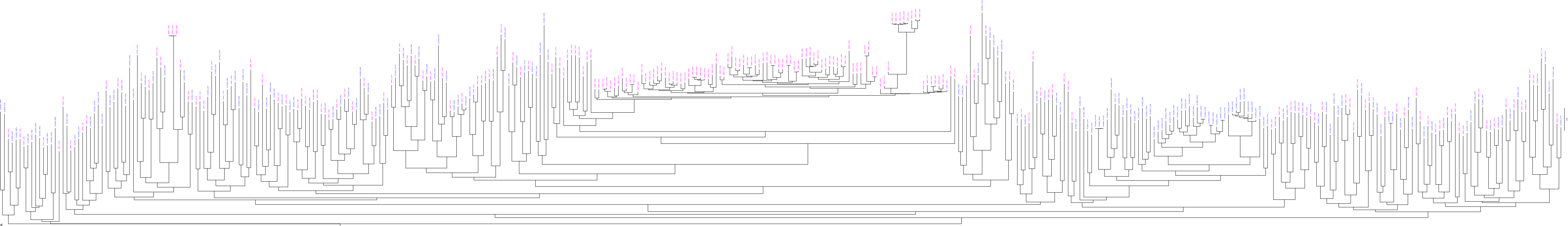

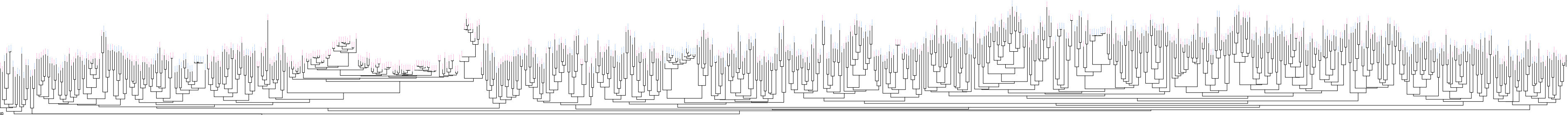

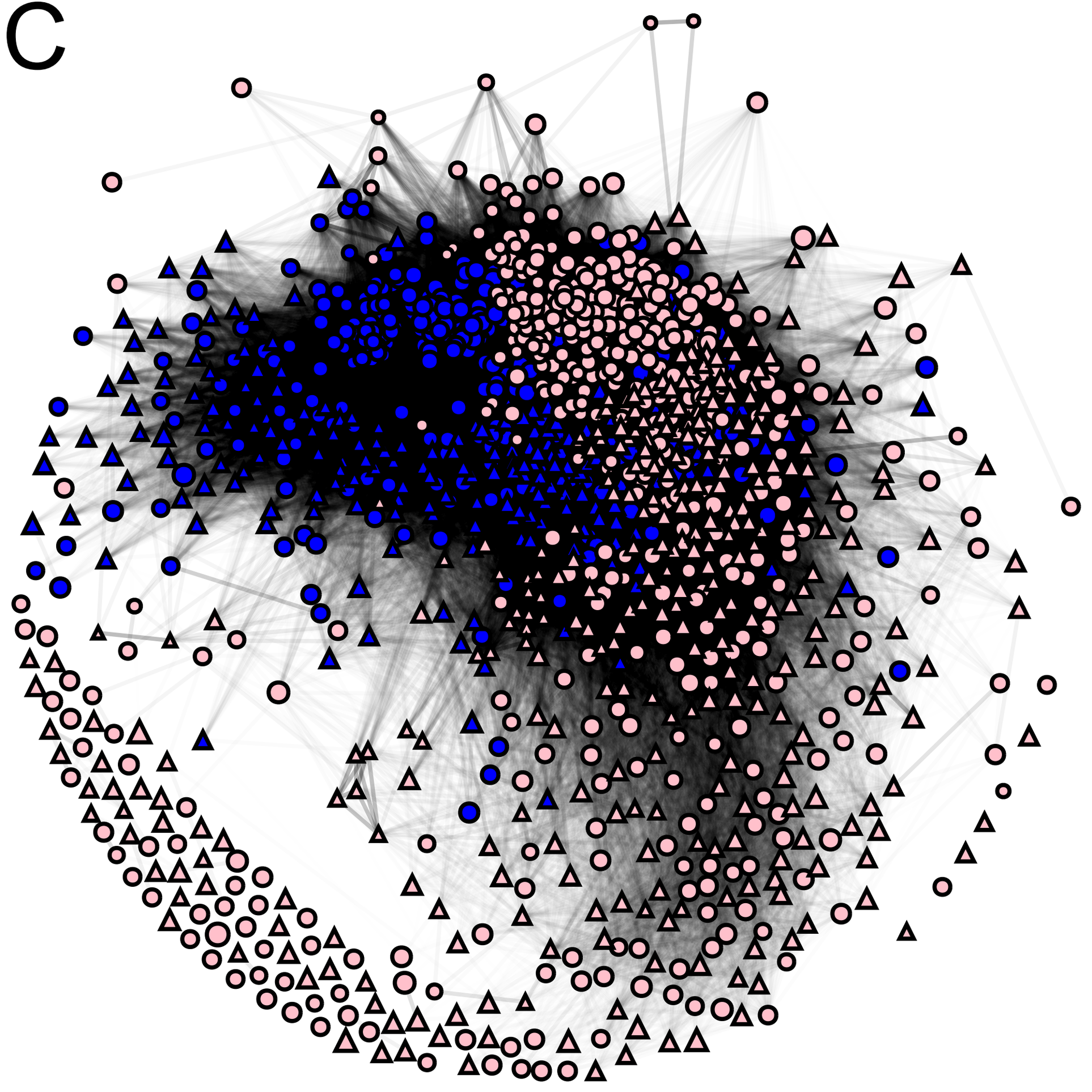
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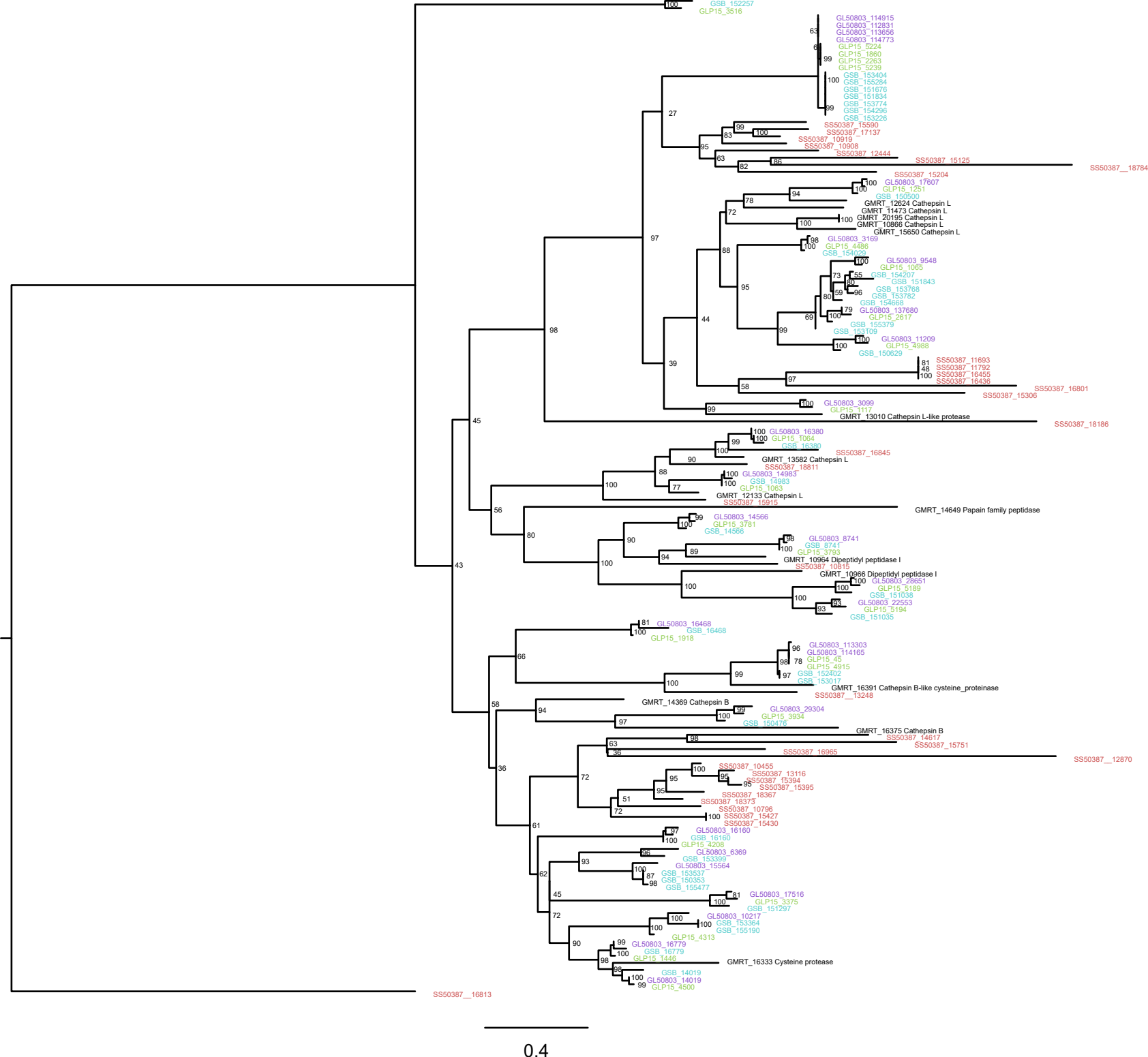
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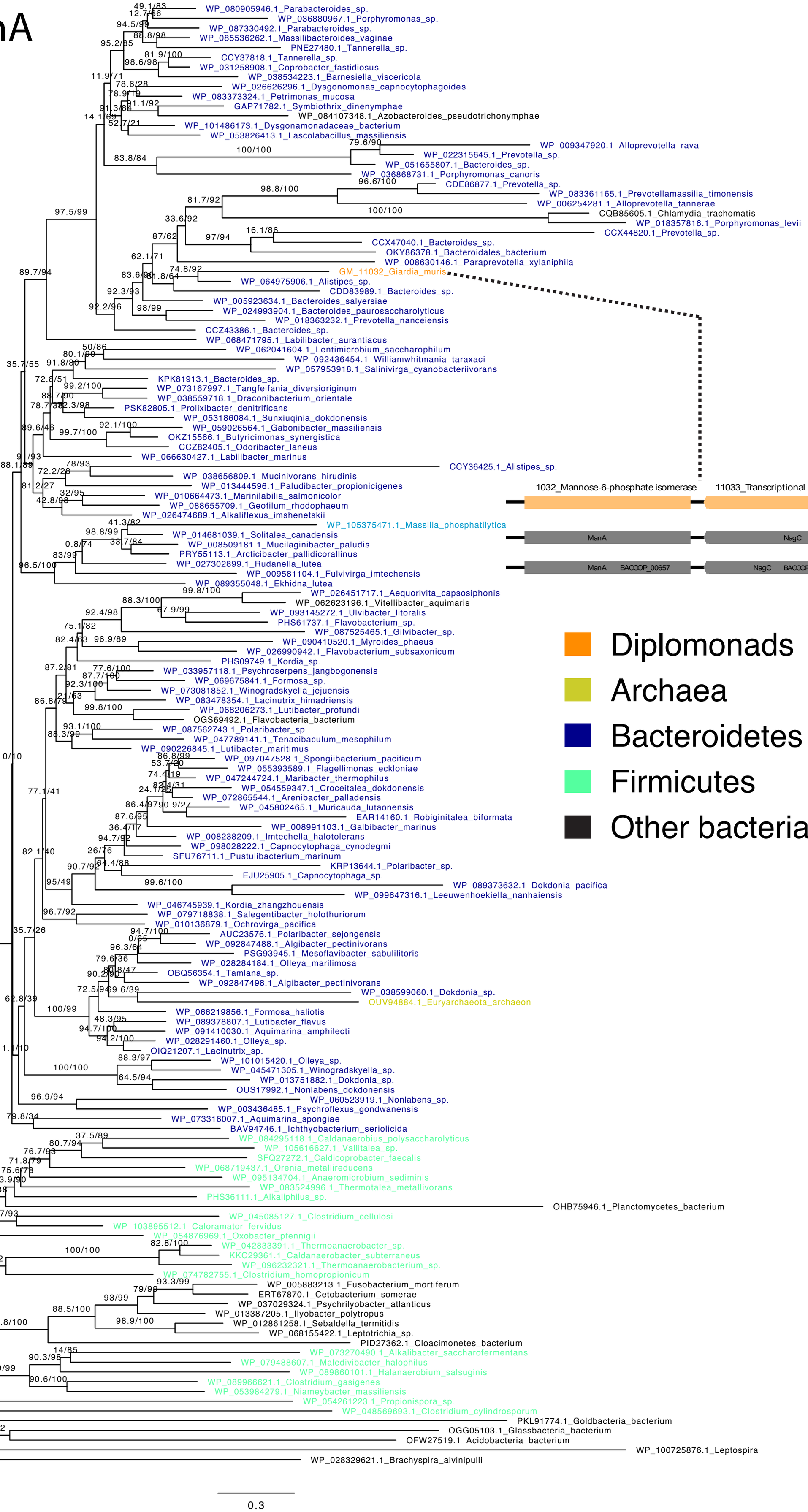
A. Fructokinase

B. ManA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ManA
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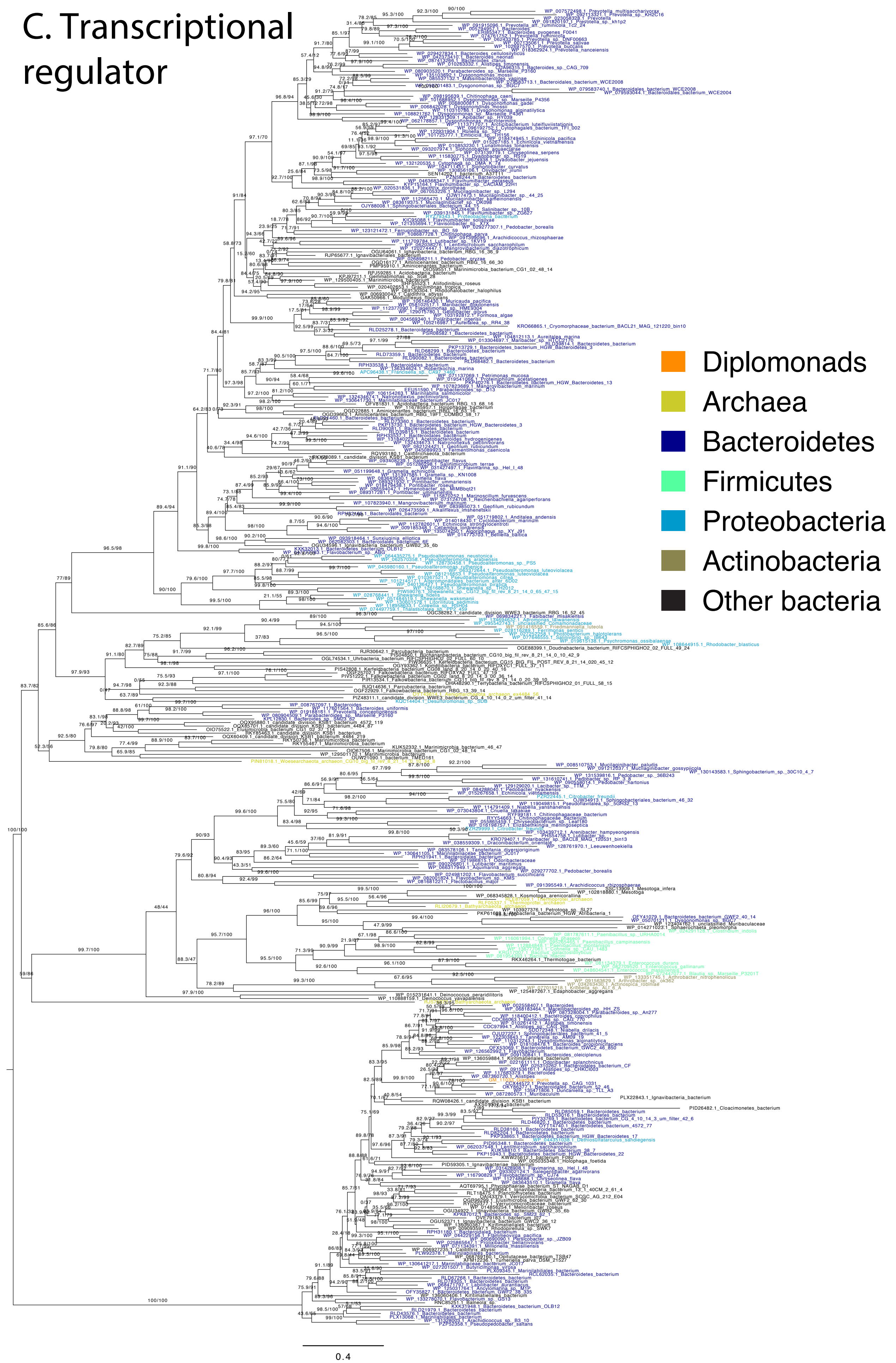
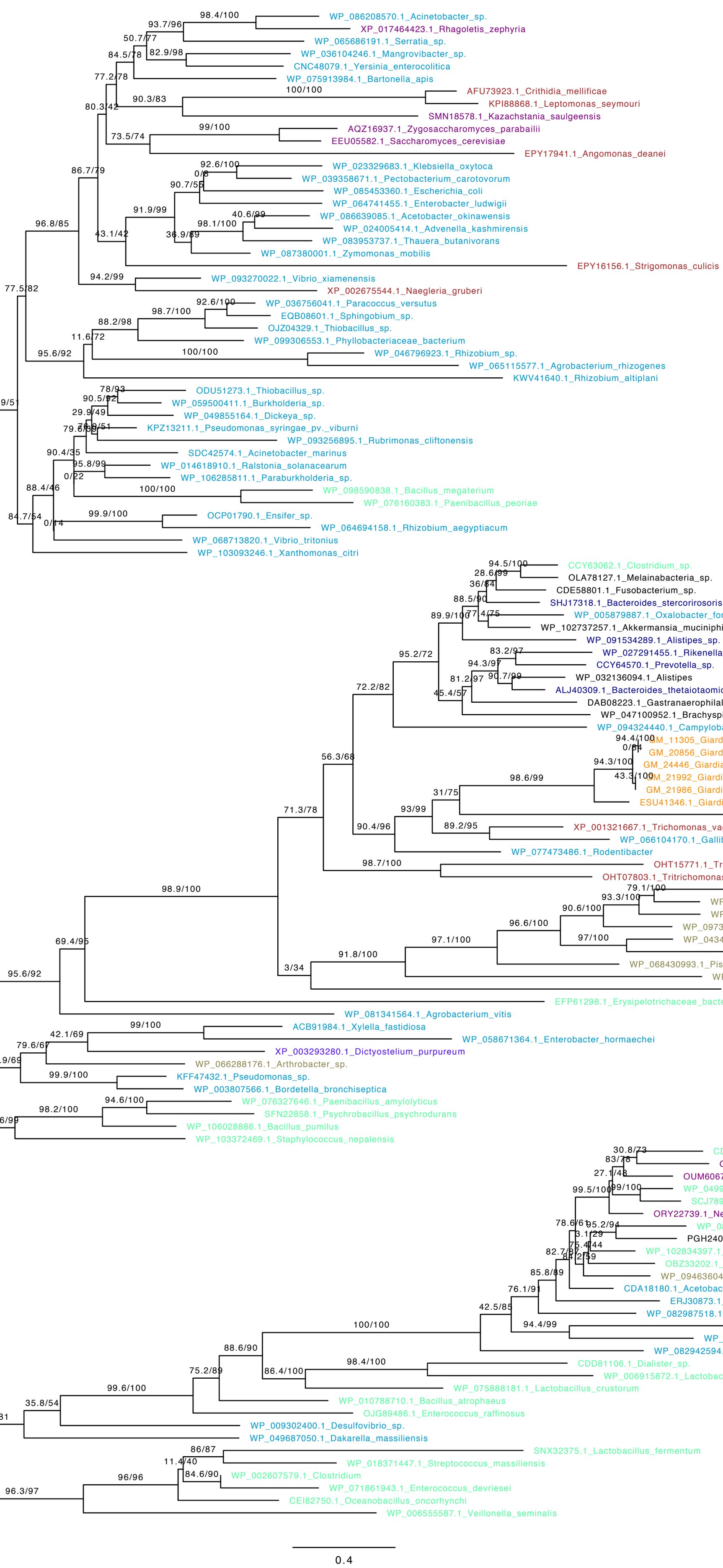
Bacteroides coprocola
Diplomonads
Archaea
Bacteroidetes
Firmicutes
Other bacteria
0.3

C. Transcriptional regulator

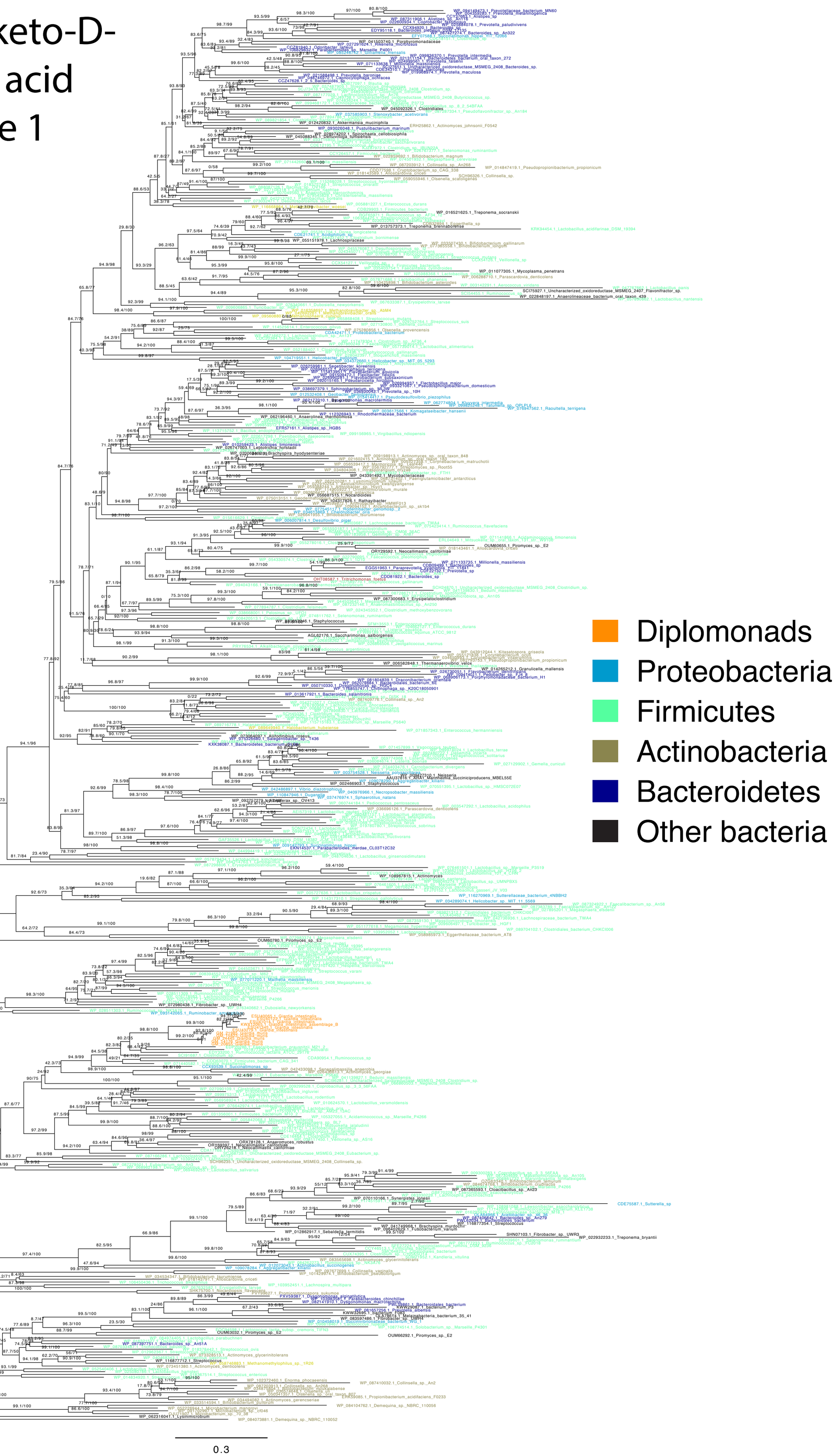


D. Arginase



- Diplomonads
- Excavata
- Amoebozoa
- Opisthokonta
- Proteobacteria
- Firmicutes
- Actinobacteria
- Bacteroidetes
- Other bacteria

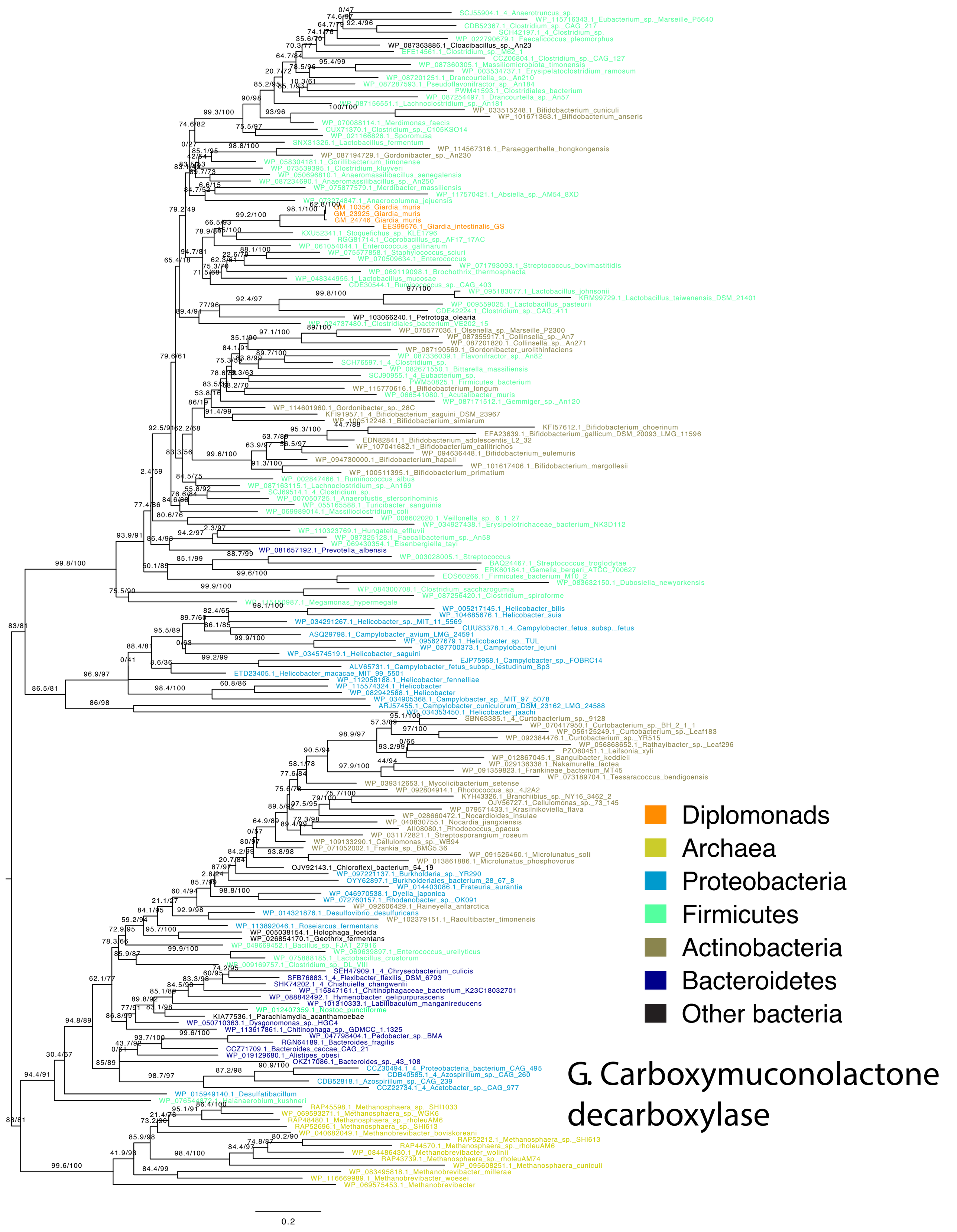
E. 2,5-diketo-D-gluconic acid reductase 1



F. 2,5-diketo-D-gluconic acid reductase 2

- Diplomonads
- Excavata
- SAR
- Archaea
- Proteobacteria
- Firmicutes
- Actinobacteria
- Bacteroidetes
- Other bacteria

0.3


Diplomonads
Archaea
Proteobacteria
Firmicutes
Actinobacteria
Bacteroidetes
Other bacteria
G. Carboxymuconolactone decarboxylase
0.2

Diplomonads

Proteobacteria

Actinobacteria

Bacteroidetes

Cyanobacteria

Other bacteria

H. Ketosteroid isomerase

0.4

I. Tryptophanase

- Diplomonads
- Amoebozoa
- Opisthokonta
- SAR
- Archaea
- Proteobacteria
- Firmicutes
- Actinobacteria
- Bacteroidetes
- Other bacteria

J. Tae4

- Diplomonads
- Opisthokonta
- Proteobacteria
- Bacteroidetes
- Other bacteria

K. Quorum-quenching N-acyl-homoserine lactonase

Methods S1 – Compact genome of *Giardia muris* reveals important steps in the evolution of intestinal protozoan parasites" by Xu et al. (2020)

DNA preparation from *G. muris* for long read sequencing

G. muris excyzoites (~9×10^7 cells) were centrifuged at 500 ×g for five minutes to remove the excystation media and the pellet was dissolved in Lysis Buffer (50 mM EDTA, 1% SDS and 10 mg/ml Proteinase K) by vortexing. The lysed cells were incubated at 56 °C with occasional vortexing for 2 hours followed by addition of 1 µl RNase A (100 mg/µl) and incubation at room temperature for 10-15 minutes. The genomic DNA was extracted by addition of 0.5 volumes each of phenol and CHISAM (chloroform: isoamyl alcohol 24:1). The sample was mixed carefully by vortexing and centrifuged at 13,000 rpm for 10 minutes, the water phase was collected and an equal volume of CHISAM was added to the sample and mixed by vortexing followed by a second centrifugation with same settings. The water phase was collected, and the DNA was precipitated with an equal volume of isopropanol. The precipitate was recovered by immediate centrifugation at 13,000 rpm, 4 °C, 30 min. The supernatant was removed, and the pellet was washed with 70% ice cold ethanol and then centrifuged at 13,000 rpm, 4 °C, 10 minutes. The supernatant was removed, air-dried briefly and resuspended in TE-buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). The genomic DNA was further purified using Genomic-tip 20/G (Qiagen) according to the manufacturers instructions.

Finally, the genomic DNA was purified using phenol-chloroform extraction according to the "Extracting DNA Using Phenol-Chloroform" protocol (https://www.pacb.com/wp-content/uploads/2015/09/Experimental-Protocol-Extracting-DNA-using-Phenol-Chloroform.pdf). The DNA solution was mixed with an equal volume of

the phenol/chloroform/isoamyl alcohol solution (25:24:1). The tube was mixed vigorously for 1 min and spun at high speed for 5 min. The aqueous phase was removed, and the tube was back extracted using EB buffer. The original and back-extracted material was combined and extracted as above using chloroform/isoamyl alcohol (24:1). The aqueous phase was precipitated using 2.5× volume 100% ethanol and NH_4OAc (0.75 M final concentration) while adding glycogen (20 ug /extraction). The precipitate was recovered by high speed centrifugation and washed two times by double volumes of 80% ethanol. The pellet was air-dried and brought up in a small volume of EB buffer. The concentration and quality of the extracted DNA was determined by NanoDrop and agarose gel electrophoresis.

RNA purification

Total RNA from trophozoites were extracted using TriZol according to the method in Franzén et al.[1]. The total RNA was DNase treated using the Turbo DNA-free kit (Applied Biosystems, AM1907) as described in Franzén et al.[1]. RNA from cysts and excyzoites were extracted using TriZol as described above and the resulting RNA was DNase treated (Turbo DNase, 2 U/µl) in a reaction volume of 100 µl for 30 minutes at 37°C. The RNA was subsequently cleaned using the cleanup protocol from the RNeasy Mini Kit (Qiagen, 74104) and eluted in 30 µl of DEPC treated water. The quality and quantity were evaluated using Qubit and BioAnalyzer.

Sequencing

Total genomic DNA was sequenced using both Illumina MiSeq and PacBio RS II sequencers at the SNP&SEQ platform and Uppsala Genome Center respectively at the Science for Life Laboratory (Uppsala University). The trophozoite total RNA was divided in two parts and stranded transcriptome mRNA library was prepared using the appropriate TruSeq kit at GATC Biotech (Konstanz, Germany). The finished libraries were sequenced using the Illumina HiSeq 2000 system at GATC Biotech (Konstanz, Germany). RNA sequencing

libraries from cysts and excyzoites were prepared using the TruSeq stranded mRNA sample preparation kit and sequenced by HiSeq 2500 at the SNP&SEQ platform.

Raw DNA and RNA sequence reads are archived at NCBI Sequence Read Archive (SRA) under accession numbers SRR8858297-SRR8858305.

Biosamples and accession numbers

Biosample accession	**SAMN11231833**		**SAMN11231832**		**SAMN11231834**
Sample description	Cysts		Trophozoites		Excysted trophozoites
DNA/RNA	DNA	RNA	DNA	RNA	RNA
Sequencing instrument	PacBio RS II	Illumina HiSeq 2500	Illumina MiSeq	Illumina HiSeq 2000	Illumina HiSeq 2500
Usage	Genome assembly	RNA-Seq comparison	Assembly base correction	RNA-Seq comparison	RNA-Seq comparison
SRA accession	SRR8858302-SRR8858305	SRR8858298	SRR8858300	SRR8858301	SRR8858299
# Reads	200,601	31,397,561	38,048,422	121,271,107	30,381,809
Read len (bp)	-	125	251*2	51	125
# Total Bbp	2.5	3.9	19.1	6.2	3.8

Repeat detection

RepeatMasker v3.3.0 (http://www.repeatmasker.org/) was run to detect genome repeats. Sequence comparison in RepeatMasker was performed by cross_match v1.080812. RM database v20110920 with RepBase Update 20110920 was used as RepeatMasker library.

Heterozygosity estimation

Samtools mpileup with B flag was used to generate pileup file from MiSeq reads mapped bam file. Sites with at least 20X coverage and 10% of alternative bases were considered as allelic heterozygous sites.

Genome annotation

Two gene prediction programs were used, Prodigal v2.60 and GlimmerHMM v3.0.1. Training set for GlimmerHMM consists of genes predicted from Prodigal which have hits against *G. intestinalis* with evalue <1e-50 and similar length to the hit gene. The union of the two sets of predicted genes was taken for functional annotation. Genes were manually annotated using information from BLASTP results against NR database as well as HMMER (v3.0) search results of domain information against Pfam (v27.0).

tRNA and rRNA

18S, 28S and 5.8S ribosomal RNAs were annotated after BLASTN similarity to the deposited *G. muris* ribosomal RNAs in NCBI (X65063), and the start and stop positions were adjusted to be inline with the record. 5S rRNAs were predicted by searching against Rfam 11.0 using infernal v1.0.2. There are 78 pieces of 28S rRNAs, 73 18S rRNAs, 68 5.8S rRNAs and 10 5S rRNA annotated.

tRNAs were predicted by tRNAScan-SE v1.23 with the most sensitive co-variance model. BLASTN with -task blastn (which is more sensitive for distant matches) of the 40 *G. intestinalis* ncRNAs against *G. muris* genome revealed 10 ncRNAs with evalue <0.1 and not overlapping genes. Alignment of the 10 ncRNAs using MUSCLE v3.8.31 showed a clear motif on the 3' end, CCTTYNHTNAA, which is similar to what was found on *G. intestinalis* (CCTTYNHTHAA). This motif was used to search for other potential ncRNAs in *G. muris* using scan_for_matches (http://blog.theseed.org/servers/2010/07/scan-for-matches.html). Additionally, results were obtained from BLASTN search against miRBase, similarity search against Rfam and prediction of small RNAs based on small RNA-Seq data using Shortstack v1.2.4. All the search and prediction results were combined following the rule that ncRNAs do not overlap (or overlap little) with the annotated genes and with themselves. Manual efforts were also applied. In the end, they are 219 ncRNAs annotated, with 16 from similarity

search against *G. intestinalis* ncRNAs, 8 from Rfam searches, 17 from Shortstack prediction, and 178 from motif search.

Protein kinases

Protein kinases were assigned into group, family and subfamily. Alignment files for different group, family and subfamily were downloaded from the kinase database (http://kinase.com). HMMER 3.0 (http://hmmer.janelia.org) was used to build HMM profiles from the alignment and the protein sequences were searched against the HMM profiles. The search results were then combined with Pfam domain search results where genes contain significant Pfam Pkinase domain (PF00069) (score >25). *G. intestinalis* protein kinase annotations were taken from reference.

Ankyrin repeat proteins

Ankyrin repeat proteins (ARPs) were assigned to genes that contain ankyrin repeat domains (score >=20) but not Pkinase domain. According to if an ARP contains zink finger domains or other domains, they were further divided into 3 subgroups.

Cysteine-rich proteins

Alignments of the proteins with more than 10% cysteines using MAFFT v7.215 revealed that a subset of them contains a conserved pentapeptide motif (GR)CR(GRKE)K at the C-terminus. Cysteine-rich proteins were divided into three subgroups depending on the presence and absence of the transmembrane domains and the pentapeptide motif.

Synteny

MUMmer v3.23 was used to align the draft genomes of *G. muris* and *G. intestinalis*. Promer was used in order to align the genome at protein level. Mummerplot was used to generate the alignment dot plot. Show-coords was used to view a summary of all the alignments produced by promer. Alignment coordinates were used to calculate the percentage of similarity between the two genomes as well as to draw the similarity in Circos plot.

OrthoMCL v2.0.2 was used to cluster genes using match cutoff of 40% and e-value cutoff of 1e-10. The clustering was done within *G. muris*, between *G. muris* and *G. intestinalis*, and among *G. muris*, *G. intestinalis* and *S. salmonicida*.

Phylogenetic trees for NEKs and ARPs

All the NEKs from *G. muris* and *G. intestinalis* were aligned with MAFFT v7.407 (auto setting), and FastTree v2.1.10 No SSE3 was used to construct approximately-maximum-likelihood tree using default WAG+CAT model. ARPs tree was constructed the same way as NEKs tree.

Phylogenetic analysis of lateral transfers

One sequence from *G. muris* was used as a query for each enzyme to retrieve at least 5000 hits with e-value <0.001, using BLASTP against the nr database and the organism-specific proteomes. The resulting datasets were filtered automatically by finding all sequences with greater than 90% sequence identity to another sequence using the software skipredundant (EMBOSS v6.5.7.0 [2]) with default settings. Datasets were aligned using MAFFT v6.603b[3], and regions of ambiguous alignment were automatically trimmed using BMGE v1.12 (blosum30 and block size of 2) [4]. Sequences with a length less than 50% of the multiple alignment were removed, and the datasets were aligned and trimmed again as described above. An initial phylogenetic analyses were performed using FastTree v2.1.8 SSE3, OpenMP [5]. Sequences with a phylogenetic distance < 0.3 were removed in an iterative process to create the final datasets. This final datasets were aligned in forward and reverse orientation using MAFFT and PROBCONS v1.12 [6]. The four resulting alignments were combined with T-COFFEE v11.00.8cbe486 [7] into a maximal consensus alignment, which was trimmed using BMGE as in previous steps. Maximum Likelihood (ML) trees were computed using IQtree v1.6.5 [8] under LG4X substitution model [9]. Branch supports were

assessed using ultrafast bootstrap approximation (UFboot) with 1,000 ultrafast bootstraps [10] and 1,000 replicates for SH-like approximate likelihood ratio test (SH-aLRT) [11].

Promoter motif search

MEME suite v4.10.2 was used for promoter motif analysis. A maximum of 120 bp upstream of annotated genes were used in search of potential promoter. The longest possible sequence was used if the intergenic region is shorter than 120 bp, and the sequence was discarded if the intergenic region is shorter than 8 bp. 4139 sequences in total were used in motif search, and MEME was set to search for 10 most likely motifs with sizes from 6 to 30 bp.

VSP and ψVSP phylogenetic analysis

Nucleotide sequences of *G. muris* VSPs and ψVSP genes were aligned using MAFFT in MAFFT v7.416[3], and regions of ambiguity were trimmed using BMGE v1.12 (DNAPAM100:2, [4]). Maximum Likelihood (ML) trees were computed using IQtree v.1.6.5 [8] under the best model (TIM2+F+R6) with 1,000 ultrafast bootstraps [10] and 1,000 bootstrap replicates for SH-like approximate likelihood ratio test (SH-aLRT)[11].

References

1. Franzén O, Jerlström-Hultqvist J, Einarsson E, Ankarklev J, Ferella M, Andersson B, et al. Transcriptome Profiling of Giardia intestinalis Using Strand-specific RNA-Seq. PLoS Computational Biology. 2013; 9(3):e1003000. doi:10.1371/journal.pcbi.1003000.

2. Rice P, Longden I, Bleasby A. EMBOSS: The European Molecular Biology Open Software Suite. Trends in Genetics. 2000; 16(1):276–277. doi:10.1016/j.cocis.2008.07.002.

3. Katoh K, Misawa K, Kuma K, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic acids research. 2002; 30(14):3059–3066. doi:10.1093/nar/gkf436.

4. Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC evolutionary biology. 2010; 10(1):210. doi:10.1186/1471-2148-10-210.

5. Price MN, Dehal PS, Arkin AP. FastTree 2--approximately maximum-likelihood trees for large alignments. PLoS ONE. 2010; 5(3):e9490. doi:10.1371/journal.pone.0009490.

6. Do CB, Mahabhashyam MSP, Brudno M, Batzoglou S. ProbCons: Probabilistic consistency-based multiple sequence alignment. Genome research. 2005; 15(2):330–40. doi:10.1101/gr.2821705.

7. Notredame C, Higgins DG, Heringa J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. J. Mol. Biol. 2000; 302(1):205–217. doi:10.1006/jmbi.2000.4042.

8. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 2015; 32(1):268–274. doi:10.1093/molbev/msu300.

9. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods. 2017; 14(6):587–589. doi:10.1038/nmeth.4285.

10. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 2018; 35(2):518–522. doi:10.1093/molbev/msx281.

11. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 2010; 59(3):307–321. doi:10.1093/sysbio/syq010.