# Supplementary Information

## Title: "Genome-resolved metagenomics suggests a mutualistic relationship between *Mycoplasma* and salmonid hosts"

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# Bacterial biomass monitoring in rainbow trout

**Supplementary Table 1) Real time PCR (qPCR) monitoring of bacterial 16S rRNA gene in intestinal samples of rainbow trout**

| Sample | Dilution | CT value | Group Average | Standard Deviation (SD) |
|---|---|---|---|---|
| Extraction Neg | 1:1 | 40.0 | 38.5 | 2.1 |
| Extraction Neg | 1:1 | 37.1 | 38.5 | 2.1 |
| RT1 | 1:1 | 33.6 | 36.3 | 2.8 |
| RT1 | 1:10 | 34.1 | 36.3 | 2.8 |
| RT2 | 1:1 | 40.0 | 36.3 | 2.8 |
| RT3 | 1:1 | 34.4 | 36.3 | 2.8 |
| RT3 | 1:10 | 34.9 | 36.3 | 2.8 |
| RT5 | 1:1 | 40.0 | 36.3 | 2.8 |
| RT5 | 1:10 | 37.3 | 36.3 | 2.8 |
| PCR.Blk | 1:1 | 39.8 | 39.9 | 0.1 |
| PCR.Blk | 1:1 | 40 | 39.9 | 0.1 |
| Water sample 1 | 1:1 | 26.99 | 27.6 | 2.8 |
| Water sample 2 | 1:1 | 25.12 | 27.6 | 2.8 |
| Water sample 3 | 1:1 | 30.68 | 27.6 | 2.8 |

# Host genomes for host filtering

**Supplementary Table 2) Host genomes for host filtering**

| Species | NCBI Taxonomy ID | Accesion ID |
|---|---|---|
| *Oncorhynchus mykiss* | NCBI:txid8022 | GCF_002163495.1_Omyk_1.0 |
| *Salmo salar* | NCBI:txid8030 | GCF_000233375.1_ICSASG_v2 |
| *Coregonus sp.* | NCBI:txid861768 | GCA_902175075.1_AWG_v1 |

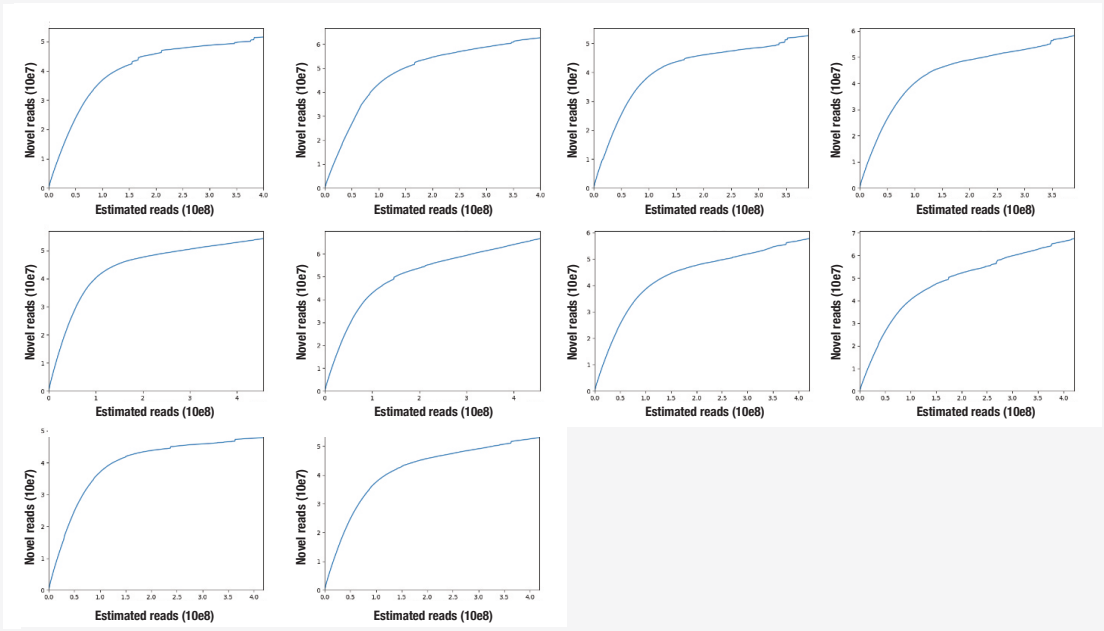
# Data Generation and Quality Control

**Supplementary Table 3) Shotgun sequencing output and quality control output**

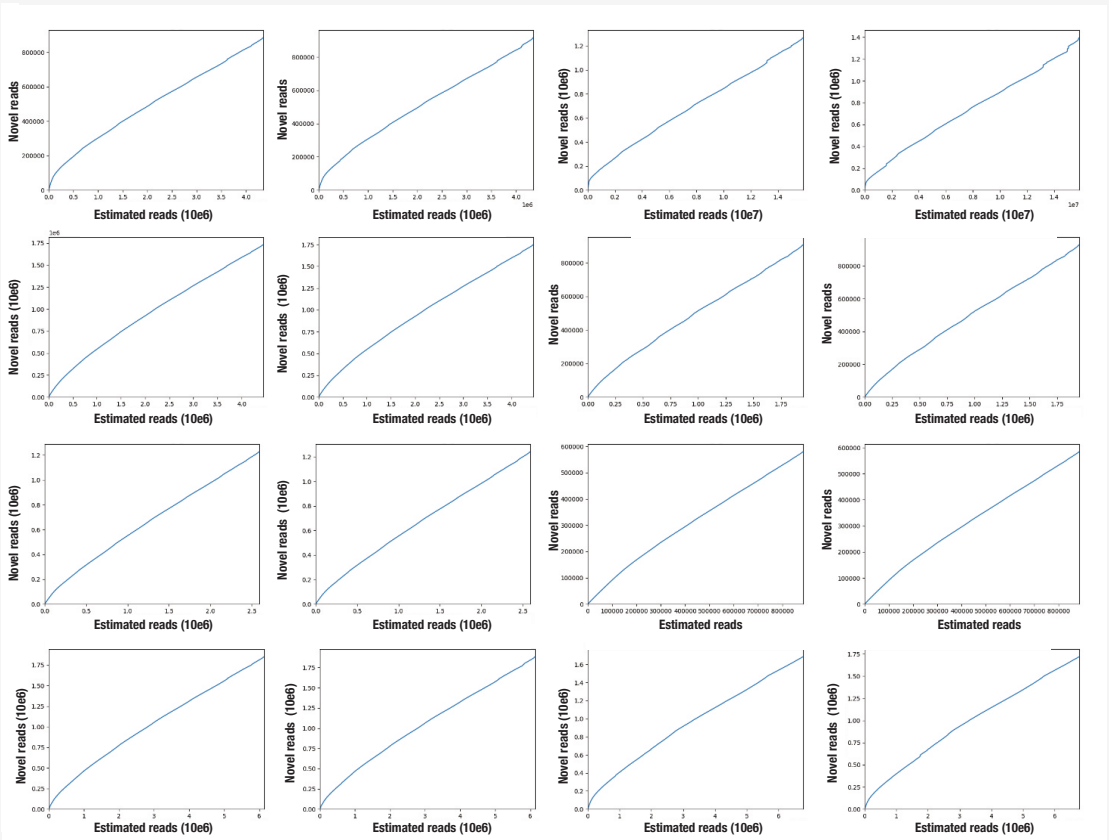
| Sample | Host Species | No. Million Raw Reads | Paired End Length (bp) | No. Million Reads post QC & Filtering |
|---|---|---|---|---|
| RT1_1 | Oncorhynchus mykiss | 216.424 | 150 | 26.061 |
| RT1_2 | Oncorhynchus mykiss | 216.424 | 150 | 31.428 |
| RT2_1 | Oncorhynchus mykiss | 210.547 | 150 | 16.691 |
| RT2_2 | Oncorhynchus mykiss | 210.547 | 150 | 20.406 |
| RT3_1 | Oncorhynchus mykiss | 195.024 | 150 | 10.318 |
| RT3_2 | Oncorhynchus mykiss | 195.024 | 150 | 12.167 |
| RT4_1 | Oncorhynchus mykiss | 227.968 | 150 | 8.168 |
| RT4_2 | Oncorhynchus mykiss | 227.968 | 150 | 10.838 |
| RT5_1 | Oncorhynchus mykiss | 209.142 | 150 | 15.078 |
| RT5_2 | Oncorhynchus mykiss | 209.142 | 150 | 16.206 |
| AS1_1 | Salmo salar | 46.733 | 100 | 0.885 |
| AS1_2 | Salmo salar | 46.733 | 100 | 0.885 |
| AS3_1 | Salmo salar | 24.024 | 100 | 4.349 |
| AS3_2 | Salmo salar | 24.024 | 100 | 4.349 |
| AS4_1 | Salmo salar | 13.521 | 100 | 15.871 |
| AS4_2 | Salmo salar | 13.521 | 100 | 15.871 |

| | | | | |
|---|---|---|---|---|
| AS5_1 | Salmo salar | 53.497 | 100 | 4.448 |
| AS5_2 | Salmo salar | 53.497 | 100 | 4.448 |
| AS6_1 | Salmo salar | 32.376 | 100 | 6.139 |
| AS6_2 | Salmo salar | 32.376 | 100 | 6.139 |
| AS7_1 | Salmo salar | 45.849 | 100 | 1.951 |
| AS7_2 | Salmo salar | 45.849 | 100 | 1.951 |
| AS142_1 | Salmo salar | 53.735 | 100 | 6.778 |
| AS142_2 | Salmo salar | 53.735 | 100 | 6.778 |
| AS391_1 | Salmo salar | 62.770 | 100 | 2.588 |
| AS391_2 | Salmo salar | 62.770 | 100 | 2.588 |
| WF_1 | Coregonus lavaretus | 46.429 | 100 | 21.754 |
| WF_2 | Coregonus lavaretus | 47.398 | 100 | 22.218 |

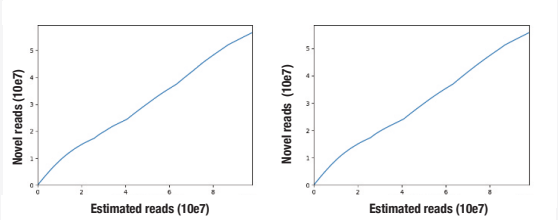
Rainbow Trout




Atlantic Salmon



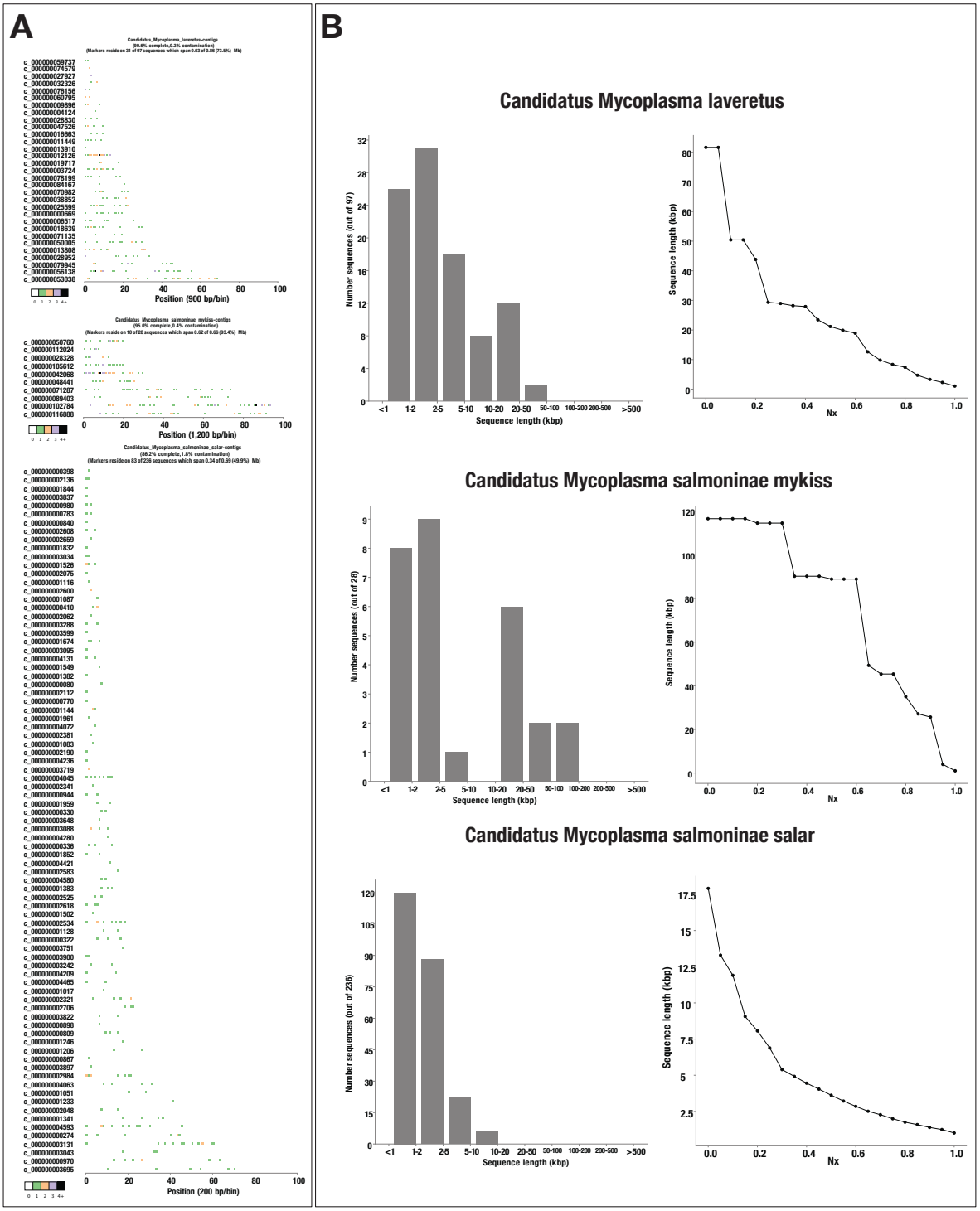

European WhiteFish



**Supplementary Figure 1. Collector's curves of all paired end reads** for rainbow trout, Atlantic salmon, and European whitefish, estimated using khmer. Saturation of curves indicate saturated read depth for 5X of all reads.

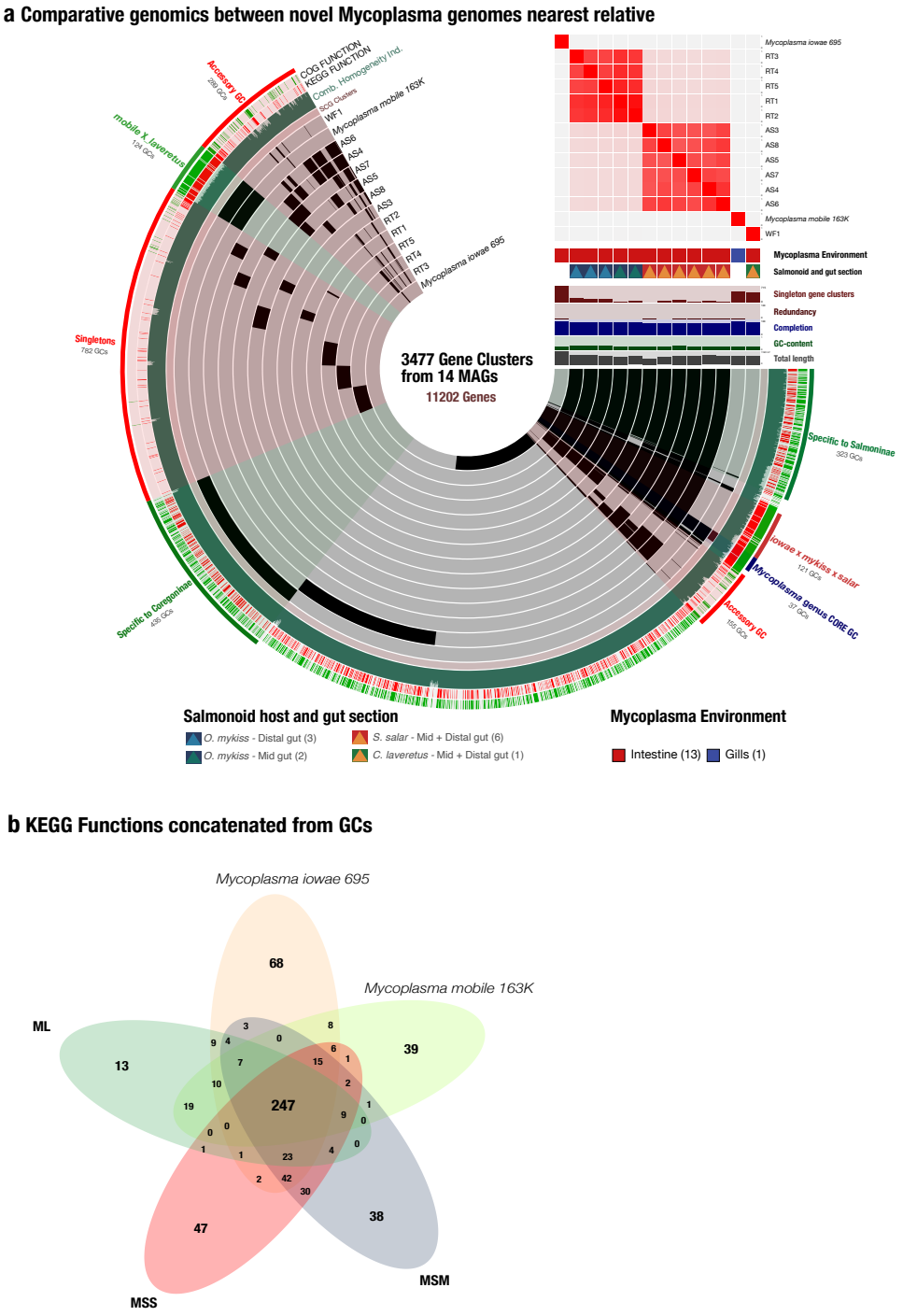
Saturation of read depth for 5X coverage is only fully saturated for rainbow trout. Atlantic salmon and European whitefish would need more data for fully saturated read depth, as seen for a nearly linear progression between accumulated data versus novel reads found. Taken the completion of *Candidatus Mycoplasma lavaretus* (>99% completion) and *Candidatus Mycoplasma salmoninae salar* (>85% completion) compared to *Candidatus salmoninae mykiss* (>95% completion), the lack of read depth is not expected to affect the investigation of dominant species, like *Mycoplasma*, in the metagenomes of Atlantic salmon and European whitefish.

# Quality Control of Assembled *Mycoplasma* MAGs




**Supplementary Figure 2. Quality control of metagenomic assembled genomes (MAGs) from European whitefish (*Candidatus* Mycoplasma laveretus), rainbow trout (*Candidatus* Mycoplasma salmoninae mykiss), and Atlantic salmon (*Candidatus* Mycoplasma salmoninae salar), using CheckM.** A) Hidden Markov Model (HMM) hits across assembled contigs for each *Mycoplasma* related MAG, providing information regarding the extent to which marker genes are collected. The number of marker genes within a fixed size window is indicated by different colours. Sequences without any marker genes are not shown. B) Histogram of contig length and line chart of ratio of MAG described by a given sequencing length for each *Mycoplasma* related MAG.

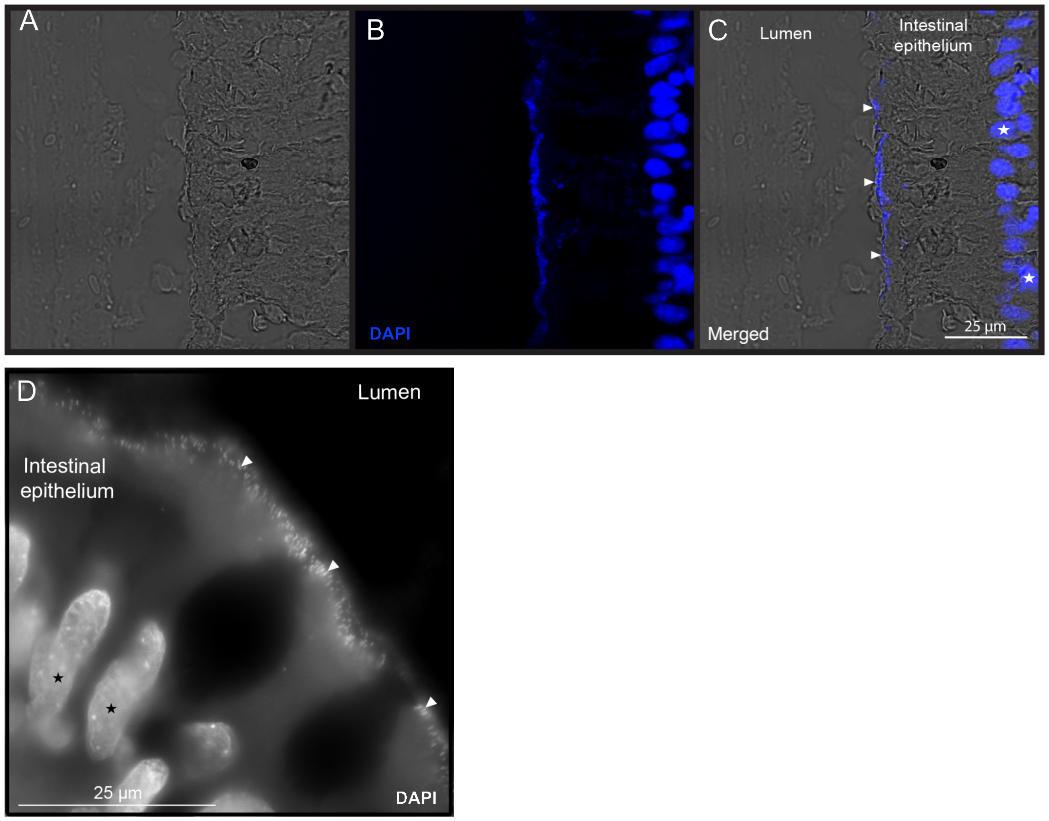
# Comparison of novel MAGs from each host individual from Atlantic Salmon, Rainbow Trout, Common Whitefish




**Supplementary Figure 3. Comparison of novel *Mycoplasma* with nearest relatives across sampled individuals. A) Comparative genomics between the novel *Mycoplasma* single assembled MAGs.** Each one of the 3477 gene clusters (GCs) contains one or more genes contributed by one or more isolated genomes. Bars in the 14 layers

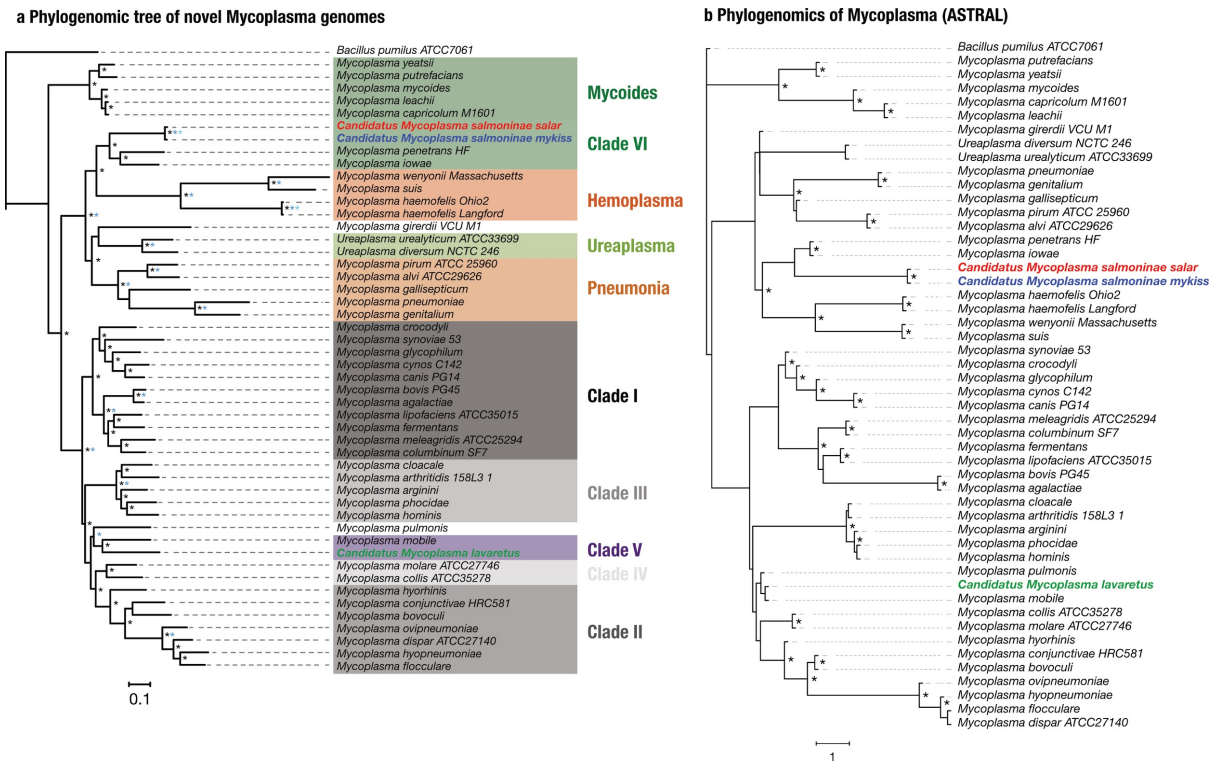
indicate the occurrence of GCs in a given isolated genome, black bars indicate presence of GCs, whereas grey indicates absence. GCs are organised based on their distribution across genomes, and layers of genomes are organised based on their average nucleotide identity (ANI), using Euclidean distance and ward ordination. Heatmap indicates ANI clusters of *Mycoplasma* isolates, where red indicates higher similarity between genomes. The five barplots below the heatmap indicate singleton GCs (dark brown), redundancy (dark brown), completion (blue), GC-content (dark green), and length of genomes (grey). Functional annotation of GCs with KEGG and COG were visualised in outer layers with green and red. The layers are grey if no KEGG or COG annotation were found. Notations in the outer layer of co-clustering of novel genomes are visualised with different colours. Samples from Atlantic Salmon were abbreviated "AS", samples from Rainbow Trout were abbreviated "RT", and samples from Whitefish were abbreviated "WF". **B) Comparison of KEGG classifications of novel *Mycoplasma.*** Venn diagram of KEGG functions concatenated from GCs from a co-assembly of the novel *Mycoplasma* genomes. Venn diagrams also include the nearest relative to the novel *Mycoplasma* genomes, including *Mycoplasma mobile* and *Mycoplasma iowae*.

# Fluorescence in situ hybridization (FISH) of gut epithelia in rainbow trout



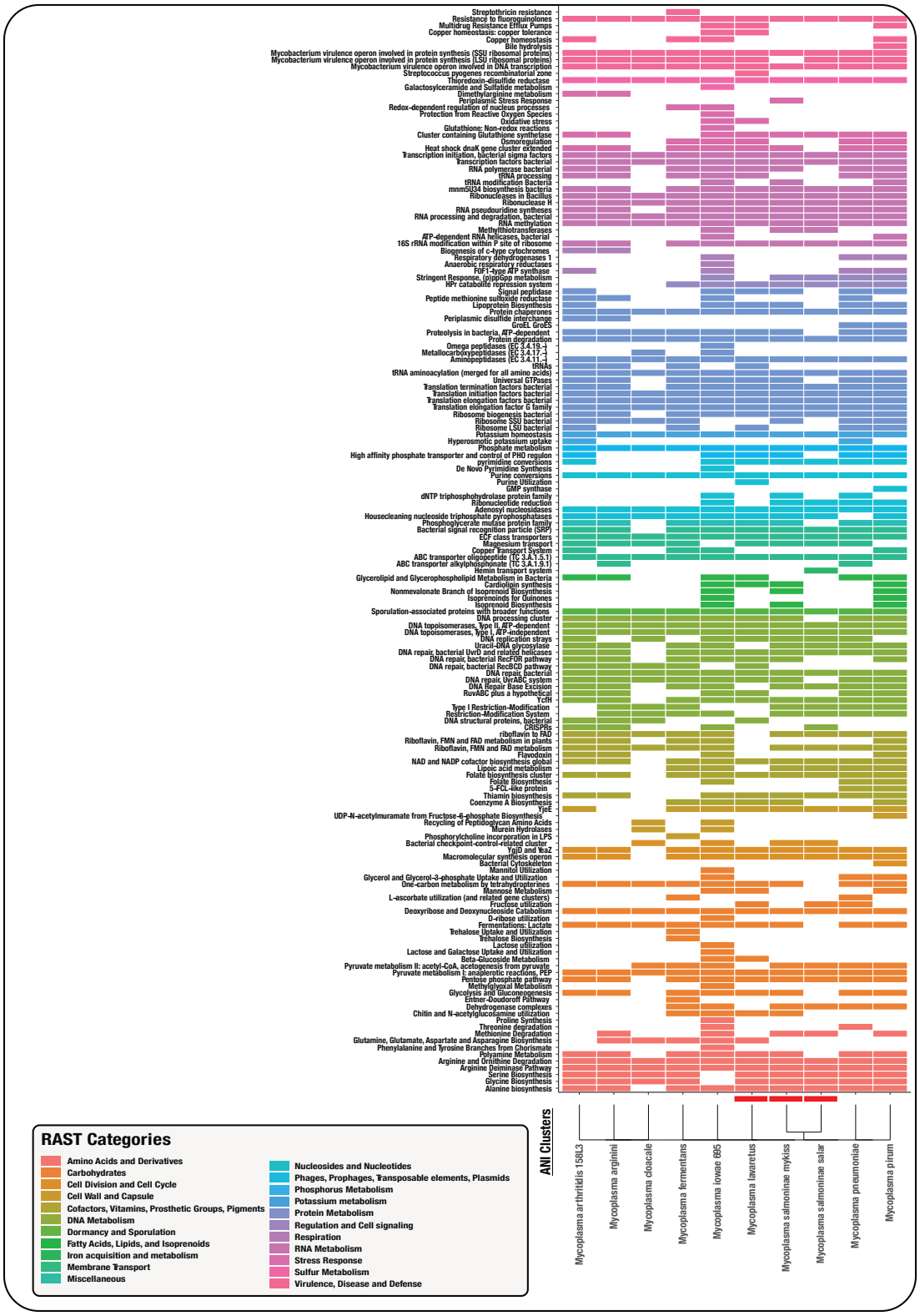

**Supplementary Figure 4. Fluorescence imaging of gut epithelial in rainbow trout.** Four µm rainbow trout intestine sections mounted with Vectashield with fluorescent DNA stain 4',6-diamidino-2-phenylindole (DAPI). Panel A, B and C are confocal images captured on a Leica SPX-5. The tissue was illuminated with A) white light, B) UV light and C) white light and UV light. In panel B and C DNA appears as a blue signal. Panel D was captured with Zeiss Axioplan fluorescence microscope and DNA is visible as white light. Stars represent nuclei of the fish tissue and arrow heads represent bacteria lining the intestinal wall.

# Supporting phylogenomics of unknown *Mycoplasma* MAGs



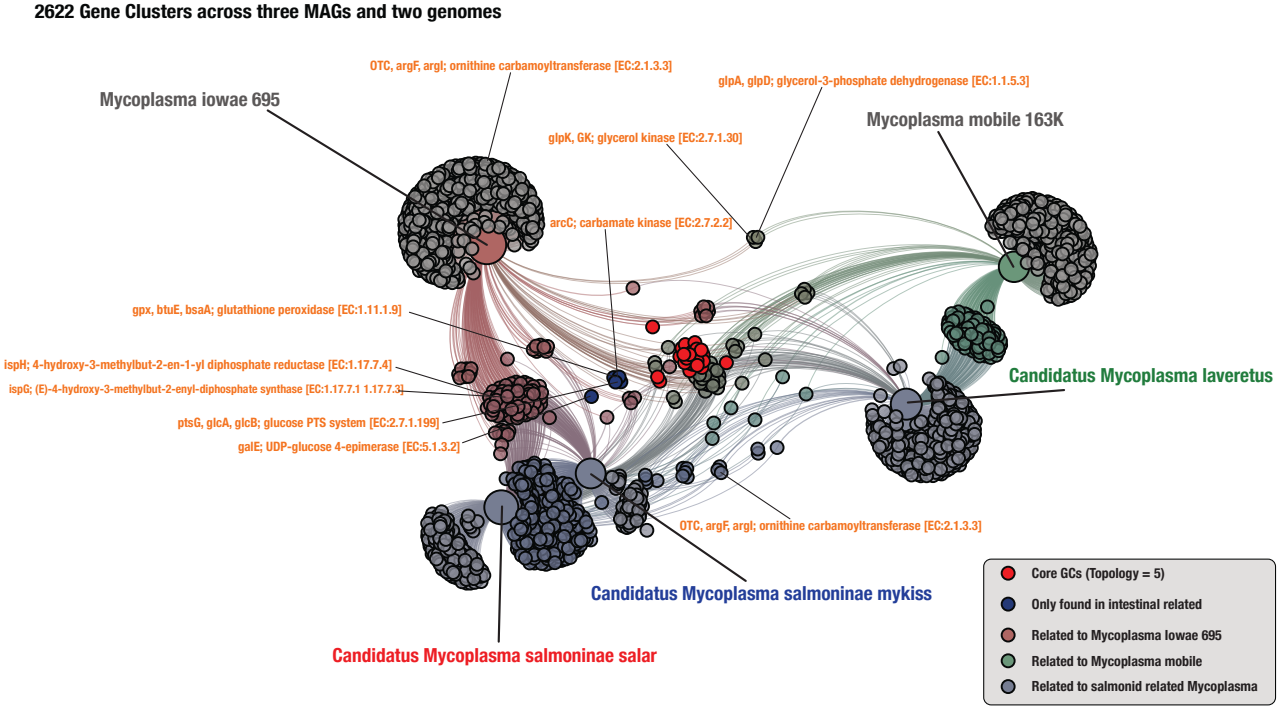

**Supplementary Figure 5. Phylogenomics of unknown *Mycoplasma* MAGs.** A. Phylogenomic tree inference from a concatenated alignment including 50 bacterial genomes (estimated in IQ-TREE). Asterisks indicate high branch supports (black: aLRT supports above 85; dark blue: gene internode certainty above 0.85; light blue: site internode certainty above 0.85). b. Phylogenomic tree estimated from ASTRAL, using the trees for core genes as input. Asterisks indicate branches with local posterior probabilities >0.85.

# Metabolic reconstruction of intestinal related *Mycoplasma* species across multiple hosts

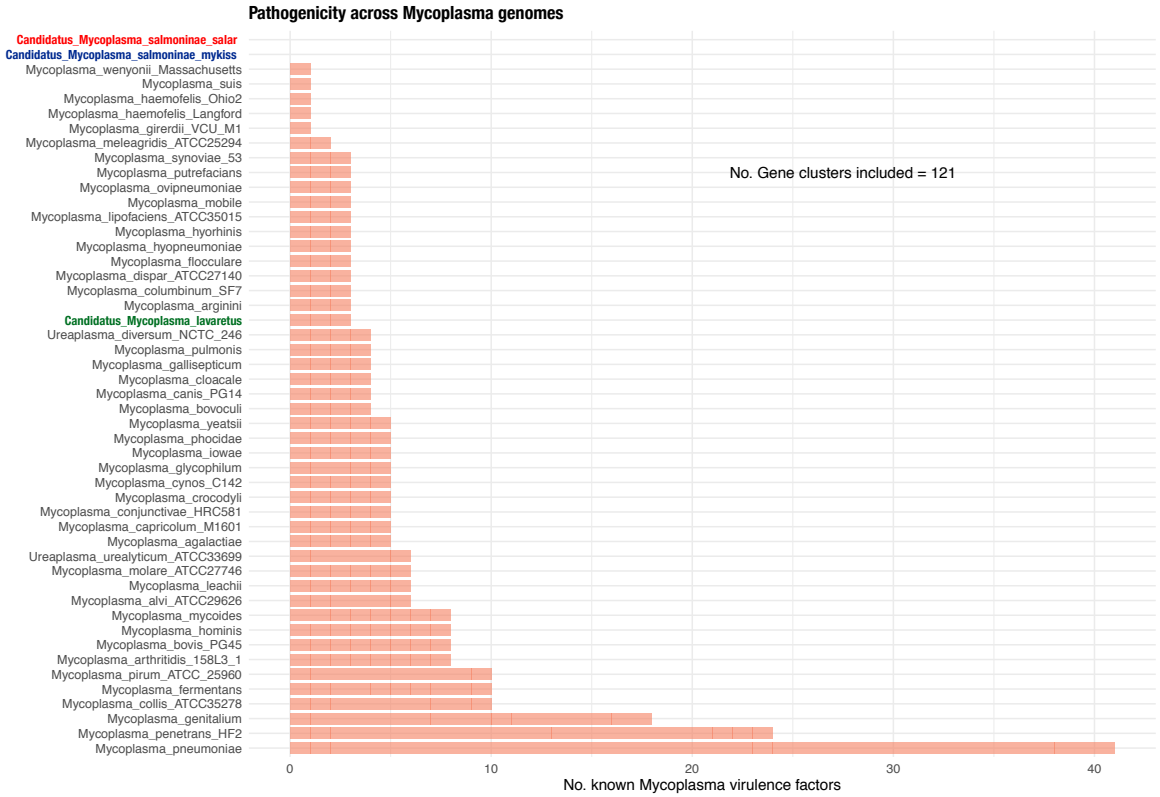



**Supplementary Figure 6. Metabolic reconstruction of intestinal related *Mycoplasma* species across multiple known *Mycoplasma* clusters.**

RAST subsystems are visualised for each *Mycoplasma* genome, including novel genomes. Visualisations are based on presence/absence of completed RAST subsystems. Colours in visualisation are based on RAST categories, which is shown in the legend. Arrangement of genomes are based on average nucleotide identity (ANI) and the dendrogram is based on hierarchical clustering with maximum likelihood of ward distances of ANI. Novel MAGs from salmonids are highlighted with a red bar on the x axis.




**Supplementary Figure 7. Functional network of salmonid related *Mycoplasma* MAGs and their relatives.** Functional network of the three MAGs and two genomes based on a total of 2622 identified gene clusters (GCs). Big nodes represent MAGs and genomes, whereas small nodes indicate GCs. Size and colour of genomic and MAG related nodes represent the number of related GCs and MAG taxonomy, respectively. Colours of functional nodes indicate their occurrence in the different MAGs and genomes. Name and KEGG accession number of functions of interest are highlighted in the figure.




**Supplementary Figure 8. Pathogenicity across *Mycoplasma* genomes based on known virulence factors present in *Mycoplasma*.** Based on search for both functions, including virulence, toxins, anti-toxins, adhesion factors, Large Membrane Proteins (LMPs), and specific genes, including *traG/traE*, *glpF*, *oppA, mgpA/mgpC, katE,* across all gene clusters in the pangenome. Search were based on KEGG, COG, and Pfam.