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# Genome Analysis of a Novel Genus of Bacteria

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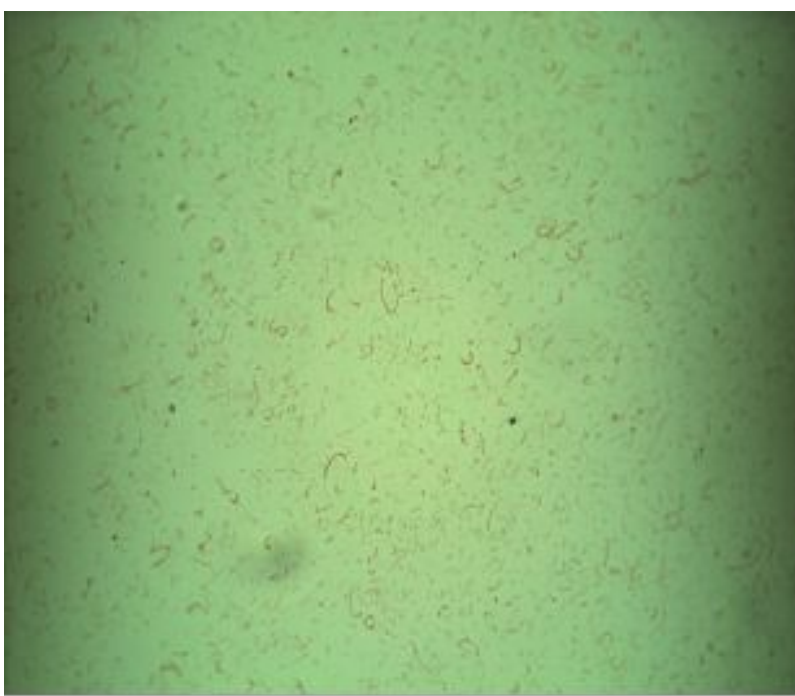
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## Introduction

Gut microbes play an important role in metabolism. *Kyphosus sydneyanus* is an herbivorous fish native to Australasia that consumes brown seaweed including the invasive *Undaria pinnatifida*. Using bacteria isolated from the hindgut of *K. sydneyanus*, we aim to understand the role of novel bacteria in the digestion of seaweed in the fish diet. Seaweed contain carbohydrates, such as carrageenan, beta-galactan, and cellulose, which are broken down by carbohydrate-active enzymes (CAZymes) produced by gut bacteria. Determining which CAZymes the novel isolates encode for will lead to a greater understanding of carbohydrate breakdown and the role of bacteria in the digestion of seaweed in the fish diet. Seaweed contains unique carbohydrates absent in terrestrial plants, so we are interested in identifying which CAZymes are encoded by our isolates compared to isolates from terrestrial organisms.



**Figure 1.** *Kyphosus sydneyanus* (left), Microscopy image of Isolate (right) Images from *Fishes of Australia* (left) and Bikiran Pardesi (right).

## Methods

### Genome Sequencing

Reads were sequenced using Illumina and Nanopore technology

### Genome Assembly

Genome assemblies created using MaSuRCA and UniCycler

### Genome Annotation

Annotations were generated using dbCAN4 and Prokka

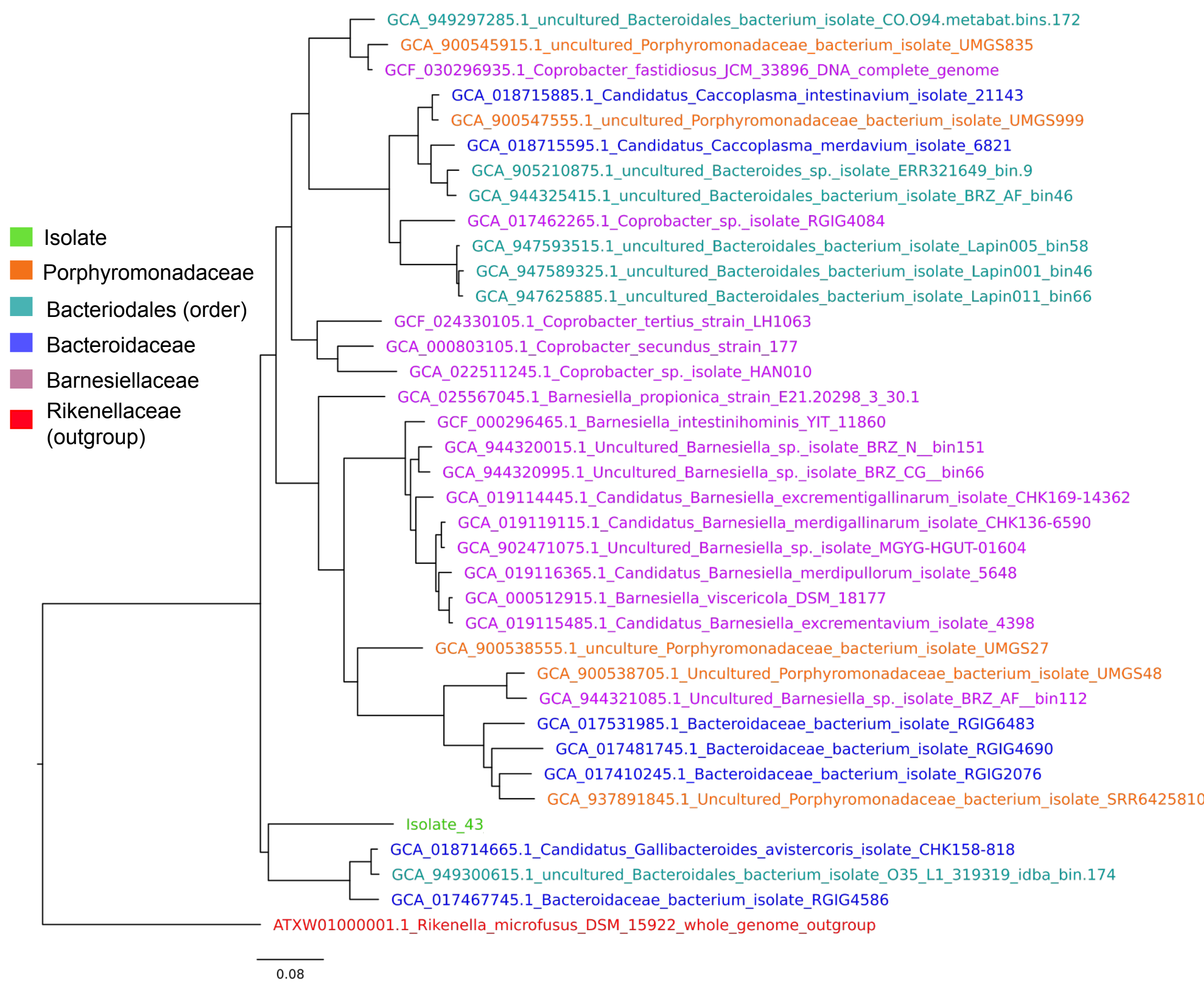
### Analysis

Phylogenetic and genome analysis to identify CAZymes and substrates

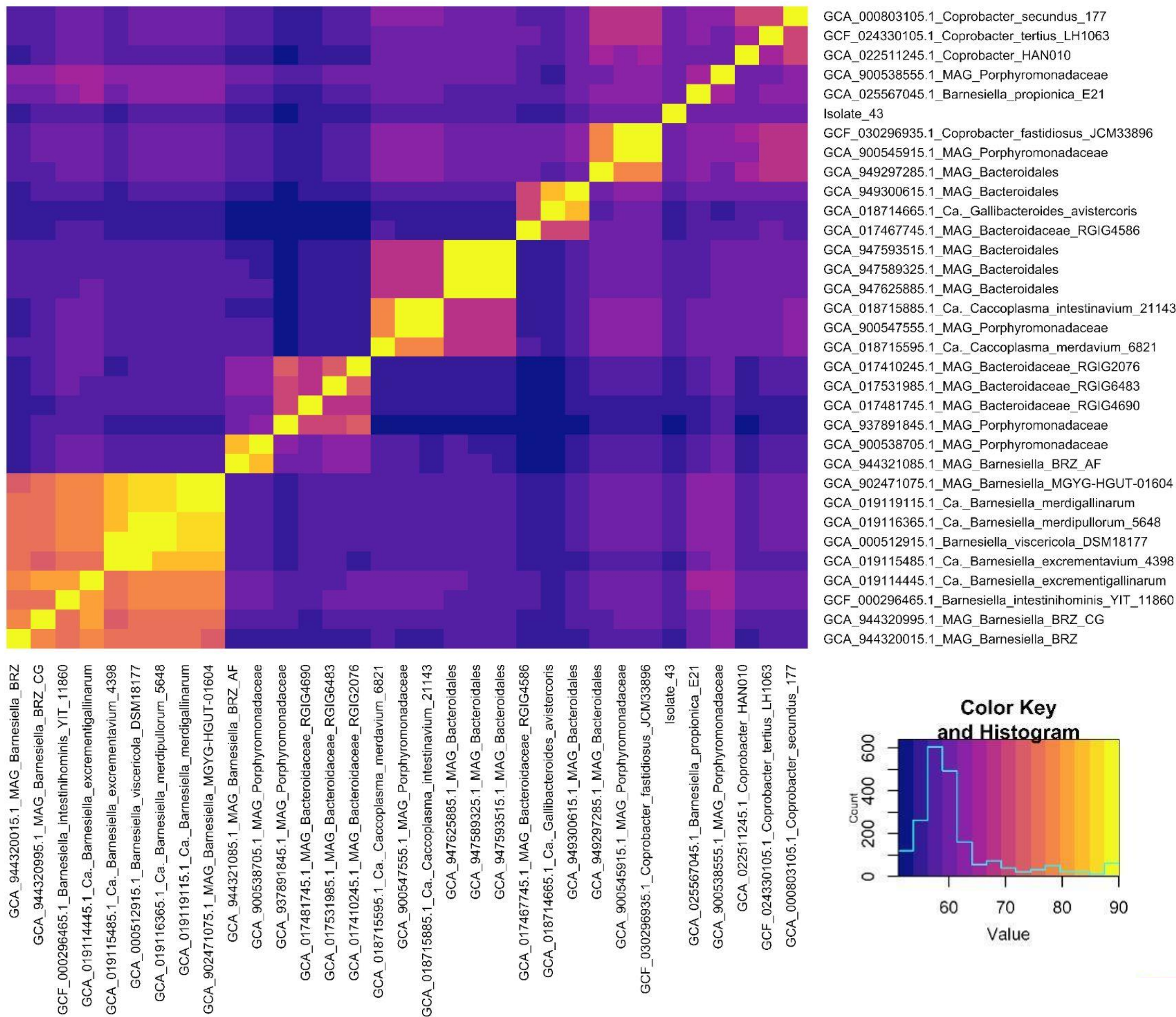
**Table 1.** Genome features from final assemblies of bacterial Isolates 8 and 43

|            | Contigs | Completeness | Contamination | Genes |
|------------|---------|--------------|---------------|-------|
| Isolate 8  | 76      | 98.49%       | 0.5%          | 3,365 |
| Isolate 43 | 13      | 98.68%       | 0.13%         | 3,009 |

## Results



**Figure 3.** Phylogenetic tree displaying the closest related organisms (Bacteroidales order) to the novel bacterial isolates, using NCBI and GTDB databases.



**Figure 4.** Heatmap displaying the AAI values for closest related organisms

## Results

- The isolates are on their own branch in the phylogenetic tree (Figure 3).
- Running BLAST on 16S rRNA sequences indicated the possibility of a novel family of bacteria. The average taxonomic cutoff for individuals to be in the same family is a percent identity of 90% for the 16S rRNA gene. The top BLAST hit for both isolates was *Coprobacter fastidiosus*, with an 89.10% identity.
- Sequencing data for Isolate 8 contained fungal genes, indicating fungal contamination. No further research was done on Isolate 8.
- Average Amino Acid Identity (AAI) values were calculated, confirming that the Isolate 43 belongs to a novel genus. The established threshold for organisms to be in the same genus is an AAI value  $\geq 65\%$ . Values from Isolate 43 compared to other organisms ranged from 52.92-61.39%.
- CAZyme encoding genes were identified for Isolate 43, which are potentially involved in breaking down the seaweed. These CAZymes target substrates such as carrageenan, beta-galactan, and cellulose, which are found in the cell walls of seaweed and other plants.
- Isolate 43 is the only organism in its clade with a class of CAZymes called polysaccharide lyases, which break down sulfated carbohydrates. An example of this is heparin, which is structurally similar to fucoidan, common in brown seaweed. Sulfated carbohydrates, like fucoidan, are found in the cell walls of brown seaweed, but typically not in terrestrial plants.

**Table 2.** Counts of CAZyme-encoding genes in Isolate 43 and related organisms

| Genome          | Glycoside Hydrolases | Glycosyl-Transferases | Polysaccharide Lyases | Carbohydrate Esterases |
|-----------------|----------------------|-----------------------|-----------------------|------------------------|
| Isolate 43      | 61                   | 38                    | 9                     | 6                      |
| GCA_017467745.1 | 82                   | 26                    | 0                     | 7                      |
| GCA_018714665.1 | 99                   | 21                    | 0                     | 6                      |
| GCA_949300615.1 | 104                  | 24                    | 0                     | 7                      |

## Future Directions

Our next steps include comparative genomics and doing further analysis of the novel bacterium in comparison to the most closely related organisms. Understanding these mechanisms can have important industrial applications, allowing researchers as part of a larger collaborative project to create natural fertilizers, products for animal feeds, and vitamin supplements without the need for the host fish.

## Acknowledgements

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