

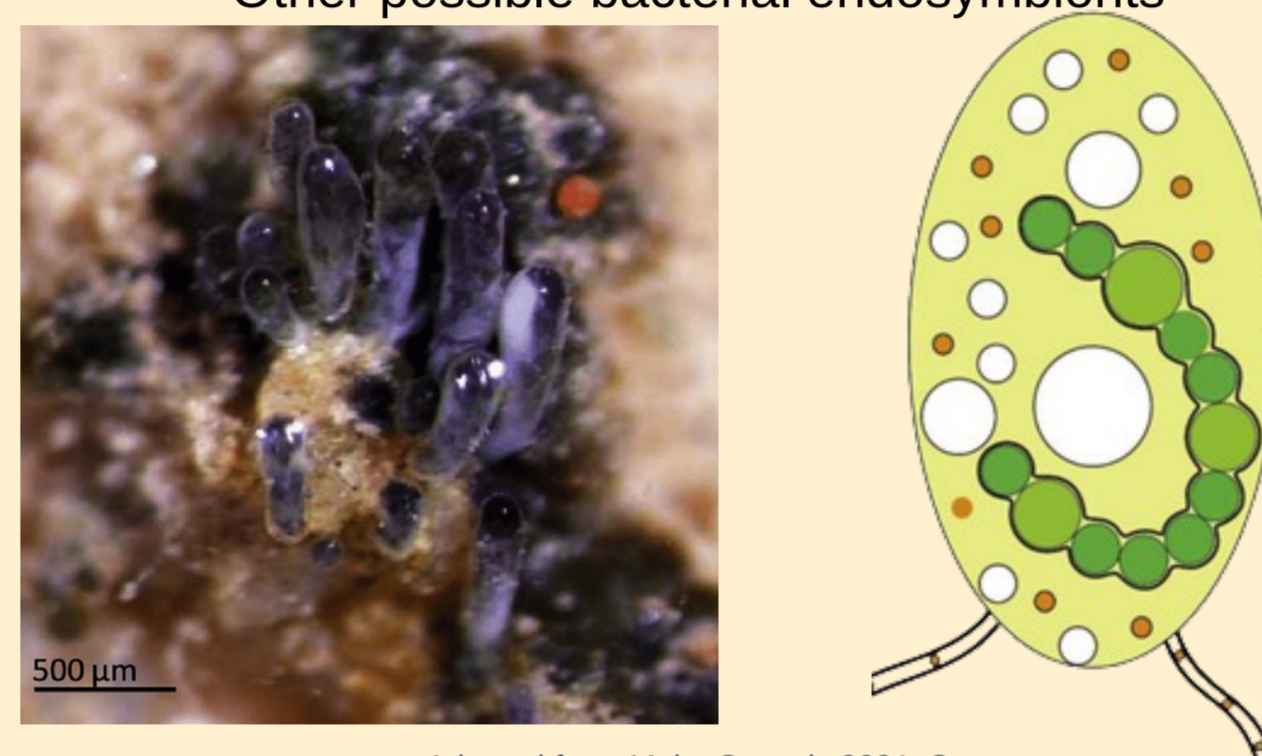
Abstract

Geosiphon pyriformis is the only Arbuscular mycorrhizal fungi (AMF) (subphylum *Glomeromycotina*) that forms a unique symbiosis with cyanobacteria *Nostoc punctiforme*, which survives within a bladder structure that protrudes from the mycelium. Recent studies produced a genome sequence of this fungus and conducted various analyses; however, our knowledge of its metagenome is still lacking.

To address this, we used available Illumina sequence data from *G. pyriformis* to extract sequence information on its endosymbionts. Using a metagenomic approach, which bins reads based on their k-mer frequency, we were able to assemble complete genomes for the *Nostoc* and *Mollicutes/Mycoplasma-related* endobacteria present in its bladder, as evidenced by the BUSCO and gene annotations. This work provides a first glimpse into the genome diversity of endosymbionts in early branching AMF.

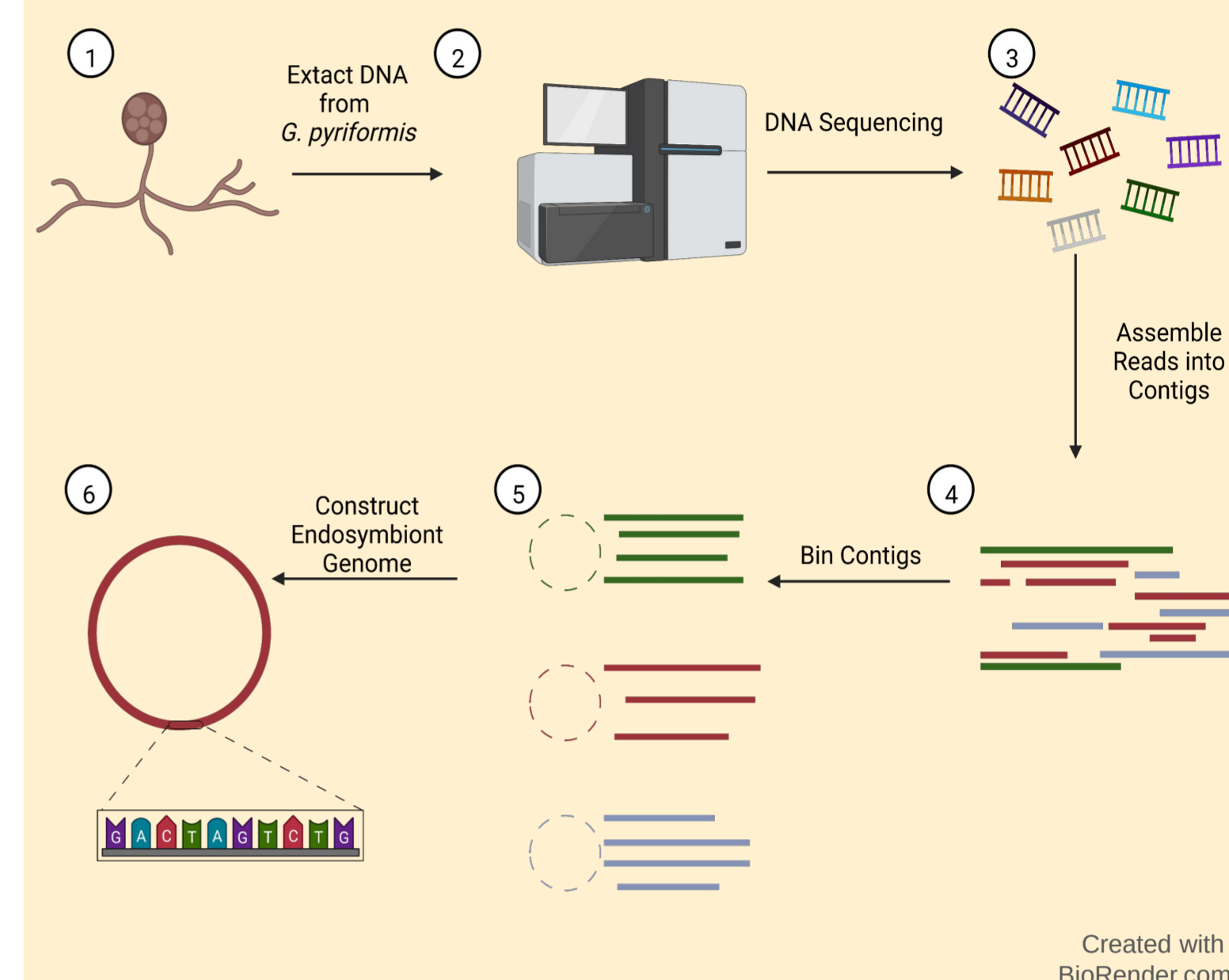
Introduction

- Mutualistic fungal species
- AMF assists in nutrient and water uptake
- **Does not** produce mycorrhiza
- Their Bladders contains:
 - Nuclei from *Geosiphon*
 - Nitrogen-fixing cyanobacteria *Nostoc punctiforme*
 - Other possible bacterial endosymbionts



Adapted from Malar C. et al., 2021, Current Biology

Methods



Results

Table 1: Genome assembly and annotation statistics for the endobacteria present in *Geosiphon pyriformis*.

	NCBI Nostoc Reference	Assembled Nostoc Genome	Mollicutes/Mycoplasma-related endobacteria
Total size	9.1 Mb	11.3 Mb	0.8 Mb
# of Contigs	6	167	19
Genome BUSCO scores	99.40%	98.40%	49.60%
Protein BUSCO scores	99.20%	98.40%	TBD
Number of genes	7215	9706	TBD
GC content	41%	41.59%	34.04%

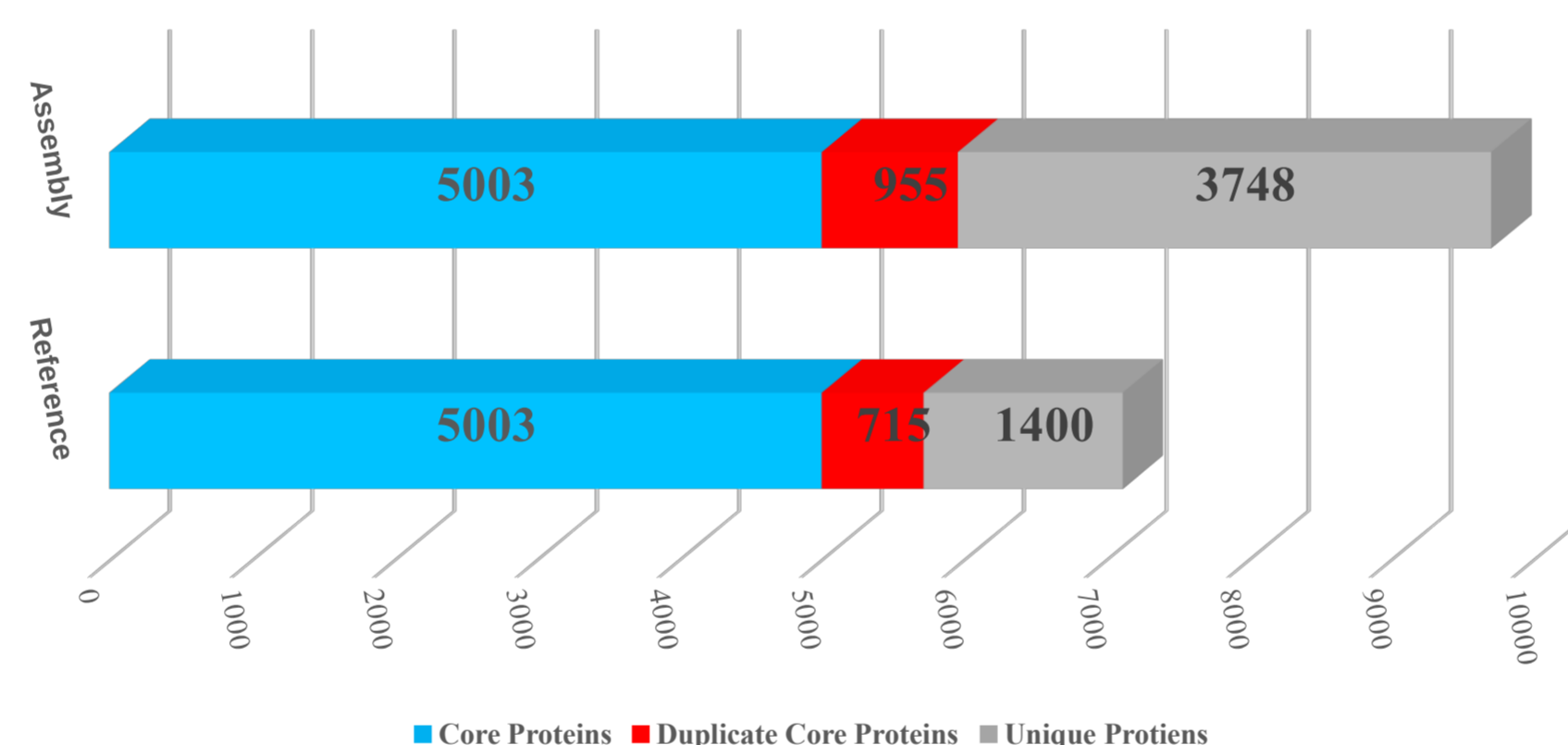


Figure 1: Comparison of annotated genes between assembled *Nostoc punctiforme* genome using a binning approach with reference genome from NCBI (RefSeq genome sequence GCF_000020025.1).

Conclusion

Metagenomic Binning approach identified two endobacteria present in this ancestral AMF species:

Nostoc punctiforme

- 3748 unique proteins were annotated
- Specialized in coding for transposable elements
- Indicative of horizontal gene transfer between endobacteria and host

Mollicutes/Mycoplasma-related endobacteria

- Loss of core mollicutes genes

Future Directions

1. Study gene transfer events
 - Repeat Mask Nostoc contigs to identify family/classes of present transposable elements
2. Genome Annotation of *Mollicutes/Mycoplasma-related* endobacteria
 - Identify which core mollicutes genes are missing
3. Conduct comparative phylogenetic study of the endosymbionts to understand the *Mollicutes/Mycoplasma-related* endobacteria lineages

References

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Figure 2: Cluster of Orthologous genes (COGs) analysis of the unique proteins present in both assembled and reference genome.

