

Genomes of the *Wolbachia* endosymbionts from the human filarial parasites *Mansonella perstans* and *Mansonella ozzardi* reveal multiple origins of nematode-*Wolbachia* symbiosis



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Wolbachia in Mansonella is different from other filarial Wolbachia

- *Wolbachia* are Gram-negative, obligate intracellular bacteria in some filarial nematodes and about 60% of arthropods
- *Wolbachia* are obligate mutualists in filarial parasites
 - Antibiotics targeting *Wolbachia* have been used to treat filariasis
- The filarial parasites *Mansonella perstans* and *Mansonella ozzardi* are known to harbor *Wolbachia*
 - Clearance of *M. perstans* mf following doxycycline treatment has been reported
- *Wolbachia* have been consistently detected in *M. ozzardi*, but in *M. perstans*, the presence of *Wolbachia* may be isolate-dependent

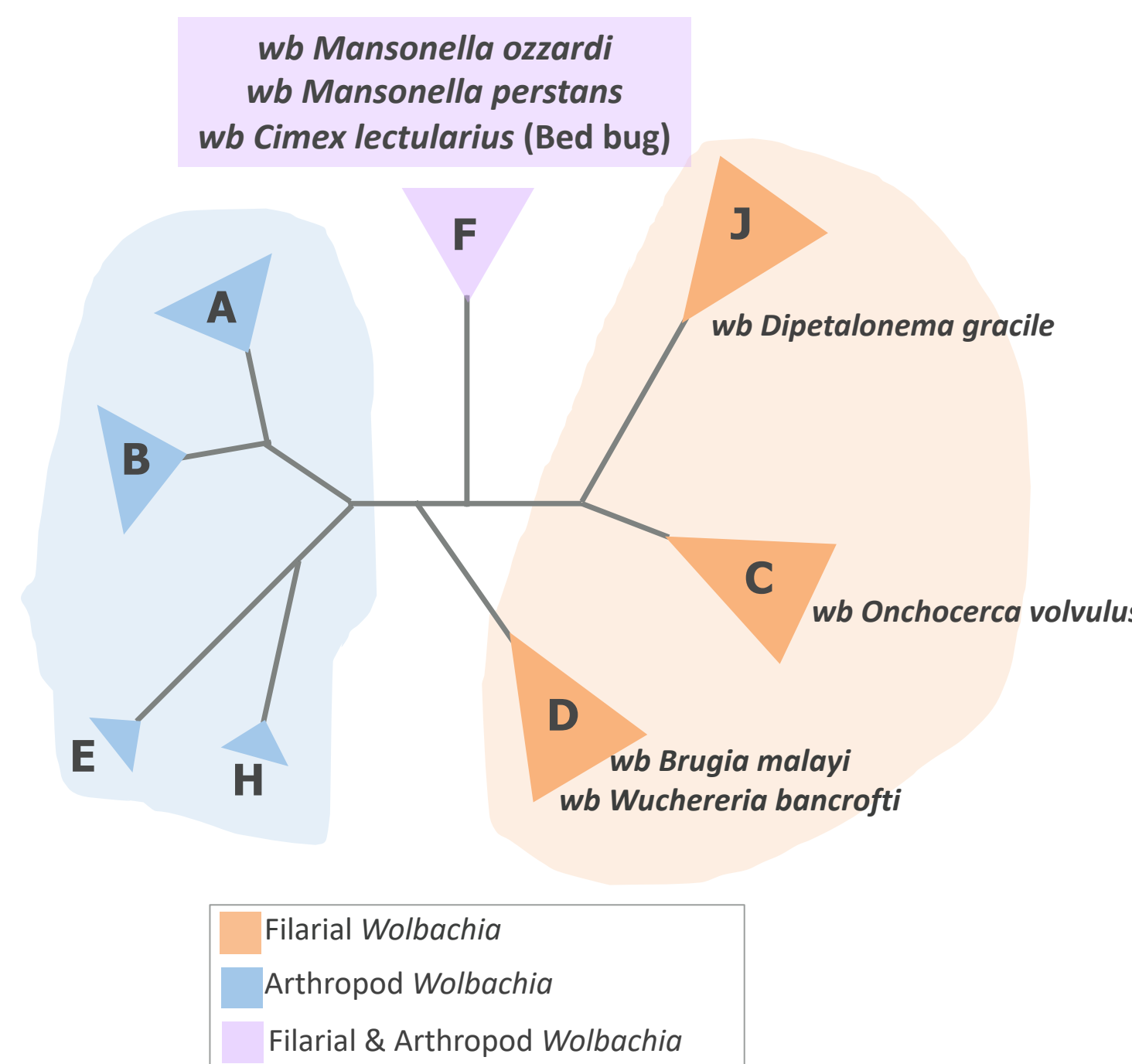
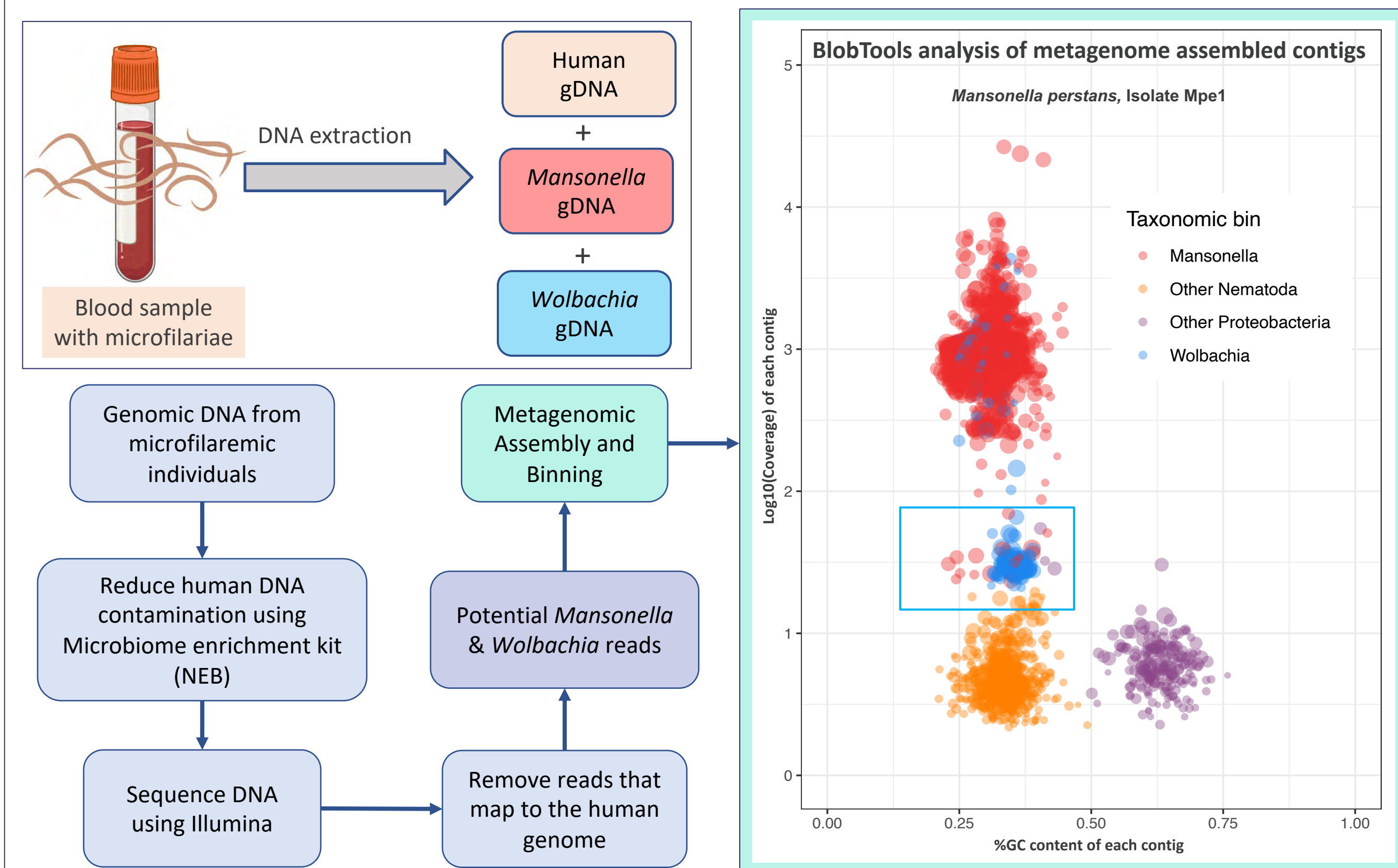


Figure 1: Unique phylogenetic position of *Wolbachia* from *Mansonella* in supergroup F

- Phylogenetically, the majority of *Wolbachia* from filarial parasites cluster into supergroups C, D and J, while arthropod *Wolbachia* are clustered in supergroups A, B, E, H and S.
- *Wolbachia* from *Mansonella* (wMpe, wMoz) are different from other filarial *Wolbachia* as they are placed in supergroup F, with *Wolbachia* from insects such as the bed bug
- Only available complete supergroup F genome is *Wolbachia* in the bed-bug *Cimex lectularius* (wCle), where *Wolbachia* is essential for normal growth and reproduction of the insect host via provisioning of B vitamins
- Genome sequences of wMpe and wMoz are needed for a better understanding of *Mansonella-Wolbachia* symbiosis
- We present here the genomes of wMpe and wMoz, representing the first genomes from filarial *Wolbachia* of supergroup F
- We also present two new genomes of arthropod *Wolbachia* from supergroup F

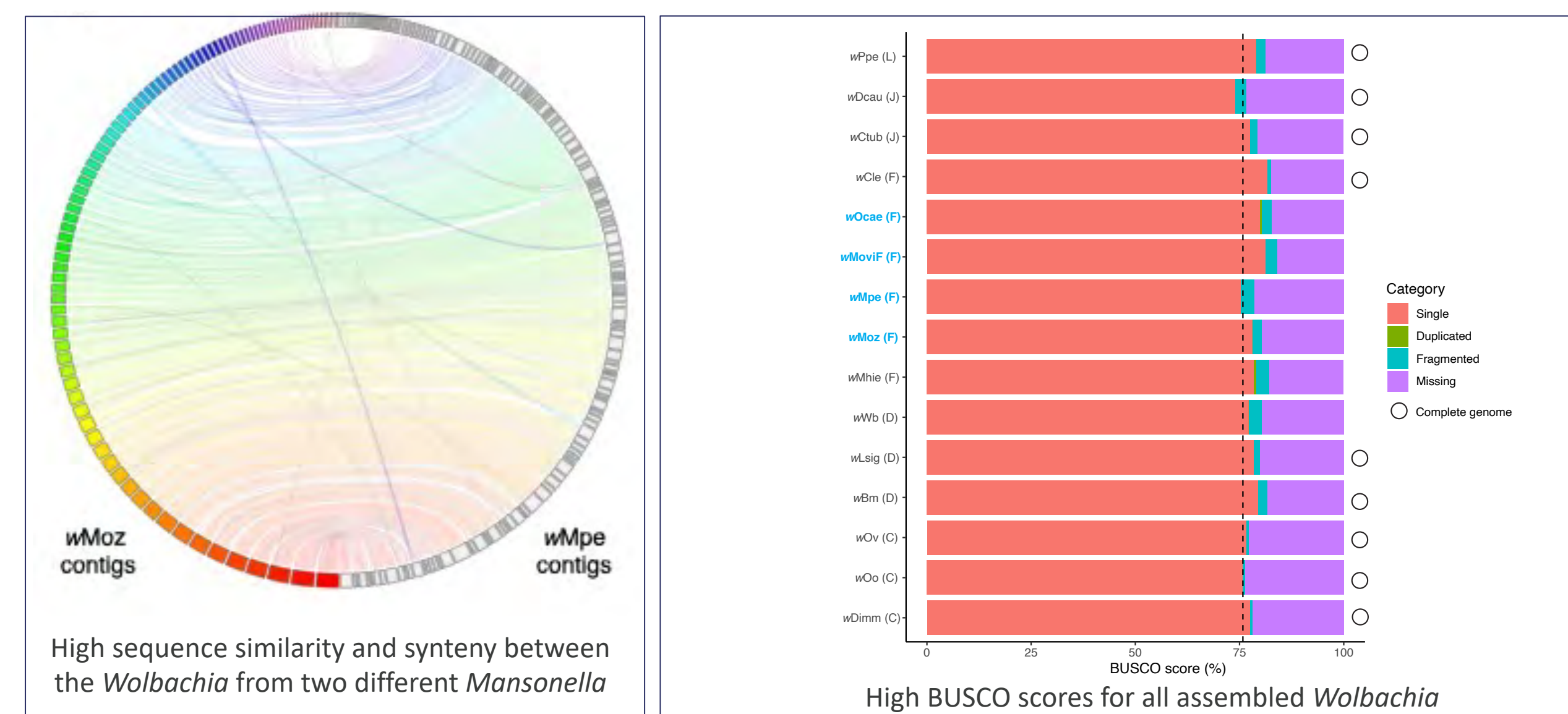
Metagenomic assembly of Mansonella clinical samples



Four new genomes of Wolbachia from supergroup F

	wMoz	wMpe	wOcae	wMovIF
Host organism	<i>Mansonella perstans</i>	<i>Mansonella ozzardi</i>	<i>Osmia caerulea</i>	<i>Melophagus ovinus</i>
Host taxa	Filaria	Filaria	Arthropoda (Mason bee)	Arthropoda (Sheep ked)
Assembled genome size	1,073,310 bp	1,058,123 bp	1,201,397 bp	1,008,858 bp
Number of scaffolds	93	170	186	196
Largest scaffold	37.481 kb	28.485 kb	21.794 kb	20.403 kb
Scaffold N50 size	17.225 kb	10.041 kb	8.755 kb	7.443 kb
Predicted genes	1,079	1,058	1,173	1,009
Source of Illumina reads	This study	This study	Gerth et al. 2014	Nováková et al. 2015

High quality draft genomes of Wolbachia from Mansonella



Variable levels of Wolbachia in different Mansonella isolates

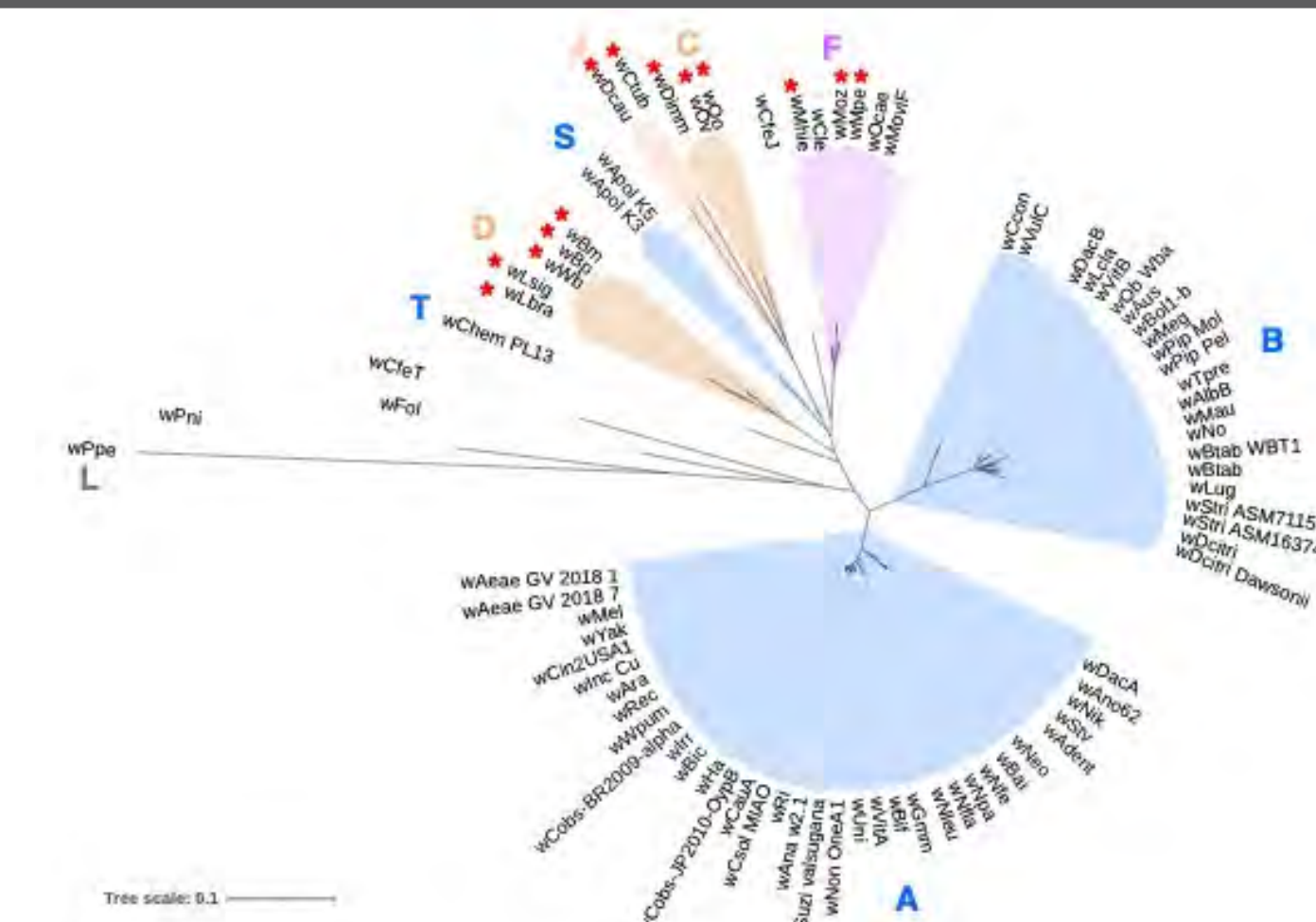
- Relative coverage of assembled contigs used to estimate *Wolbachia* levels per microfilariae

	Sampling site	<i>Mansonella</i> coverage M	<i>Wolbachia</i> coverage W	Ratio M : W	<i>Wolbachia</i> per microfilaria = 1000 x W/M
Mpe1	Cameroon	1,032	30	34 : 1	30
Mpe2	Cameroon	633	*	*	*
Moz1	São Paulo	57	3*	19 : 1*	*
Moz2	Venezuela	360	13	28 : 1	37

* Not reliable due to low coverage or size of *Wolbachia* contigs

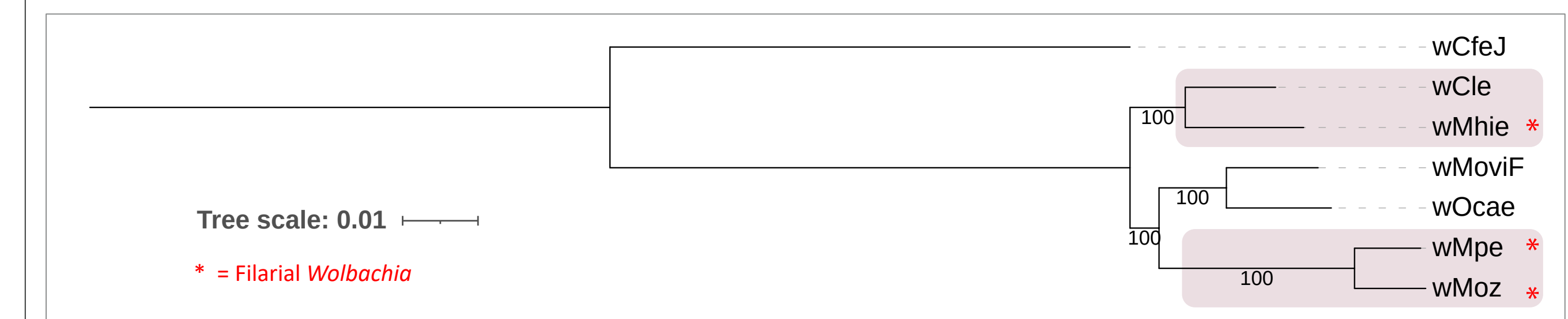
Phylogenomic analysis of all known Wolbachia genomes

- Phylogenomic analysis of 81 *Wolbachia* genomes
- 91 conserved orthologs
- Tree based on a supermatrix of total 62,160 nucleotides
- The placement of wMoz and wMpe in supergroup F is robustly supported by genomic data
- Filarial *Wolbachia* are marked by red asterisks (*)



Two origins of Wolbachia – filaria symbiosis within supergroup F

- Total 6 genome sequences are now available for *Wolbachia* in supergroup F
 - *Wolbachia* wMoz* and wMpe*, wOcae and wMovIF assembled in this study
 - Arthropod *Wolbachia* wCle, from the bed-bug *Cimex lectularius* (Nikoh et al. 2014)
 - Filarial *Wolbachia* wMhie*, from gecko parasite *Madathamugadia hiepei* (Lefoulon et al 2020)
- Phylogenomic analysis of 525 conserved single copy orthologs, total 469,845 nucleotide supermatrix
 - The genome of the cat flea *Wolbachia* wCfeJ was used as an outgroup



- The filarial *Wolbachia* are placed in two separate clades (shaded boxes) within supergroup F
- Two independent origins of symbiosis between a filaria and *Wolbachia*
 - One symbiotic association event in the lineage leading to wMoz and wMpe
 - Another event in the lineage leading to wMhie
- In both cases, the most recent common ancestor is an arthropod *Wolbachia*
 - Filarial *Wolbachia* are derived from ancestral arthropod *Wolbachia*

Biotin pathway absent in all supergroup F Wolbachia except wCle

- The bed-bug *Wolbachia* wCle provides the host with biotin (vitamin B7) and has an operon containing the biosynthetic enzyme genes.
- The biotin operon is absent in wMpe and wMoz, as well as in other *Wolbachia* in supergroup F
- Other *Wolbachia* e.g. wBm from *Brugia malayi* also do not have biotin pathway

	bioA	bioD	bioC	bioH	bioF	bioB
wCle	✓	✓	✓	✓	✓	✓
wMpe	X	X	X	X	X	X
wMoz	X	X	X	X	X	X
wMhie	X	X	X	X	X	X
wOcae	X	X	X	X	X	X
wMovIF	X	X	X	X	X	X
wBm	X	X	X	X	X	X

Conclusions and Future Directions

- High quality draft genomes of wMpe and wMoz have been assembled
- Metagenomic assembly and binning were critical to disentangle genomes from complex clinical samples
- *Mansonella* isolates from the same sampling site can have widely varying levels of *Wolbachia*
- Phylogenomic analysis reveals two independent origins of filaria-*Wolbachia* symbiosis
- Nutritional supplement of biotin is a feature unique only to wCle, and is not found in other members of supergroup F
- These genomes will enable further investigations into the biology, evolution, and use of anti-*Wolbachia* strategy for treatment of Mansonelliasis

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