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## Mansonellosis : A neglected filarial infection

- Mansonellosis is a widespread yet neglected filariasis of humans, caused by infection with any of the three filarial species: *Mansonella perstans*, *M. ozzardi* and *M. streptocerca*.
- M. perstans* infections are endemic in Central and West Africa (Figure 1A), and in a few areas South America (stippled region in Figure 1B). The migration of *M. perstans* from Africa to South America is most likely a consequence of slave trade.
- M. ozzardi* infections are highly prevalent in South America and the Caribbean islands (Figure 1B).
- Transmission to humans is via insect vectors such as the biting midges of *Culicoides* spp. for *M. perstans* and *M. ozzardi*, and additionally via black flies of *Simulium* spp. for *M. ozzardi*.
- The *Wolbachia* endosymbionts of other filaria are placed in supergroups C, D and J in *Wolbachia* phylogeny (Lefoulon et al. 2016). *Mansonella* spp. filariae are unique as their endosymbiont *Wolbachia* is a member of supergroup F, the only clade containing *Wolbachia* from both insect and filarial hosts.
- The goal of the current study was to obtain genome sequence and assemblies of *M. perstans* and *M. ozzardi*, and their *Wolbachia* endosymbionts.

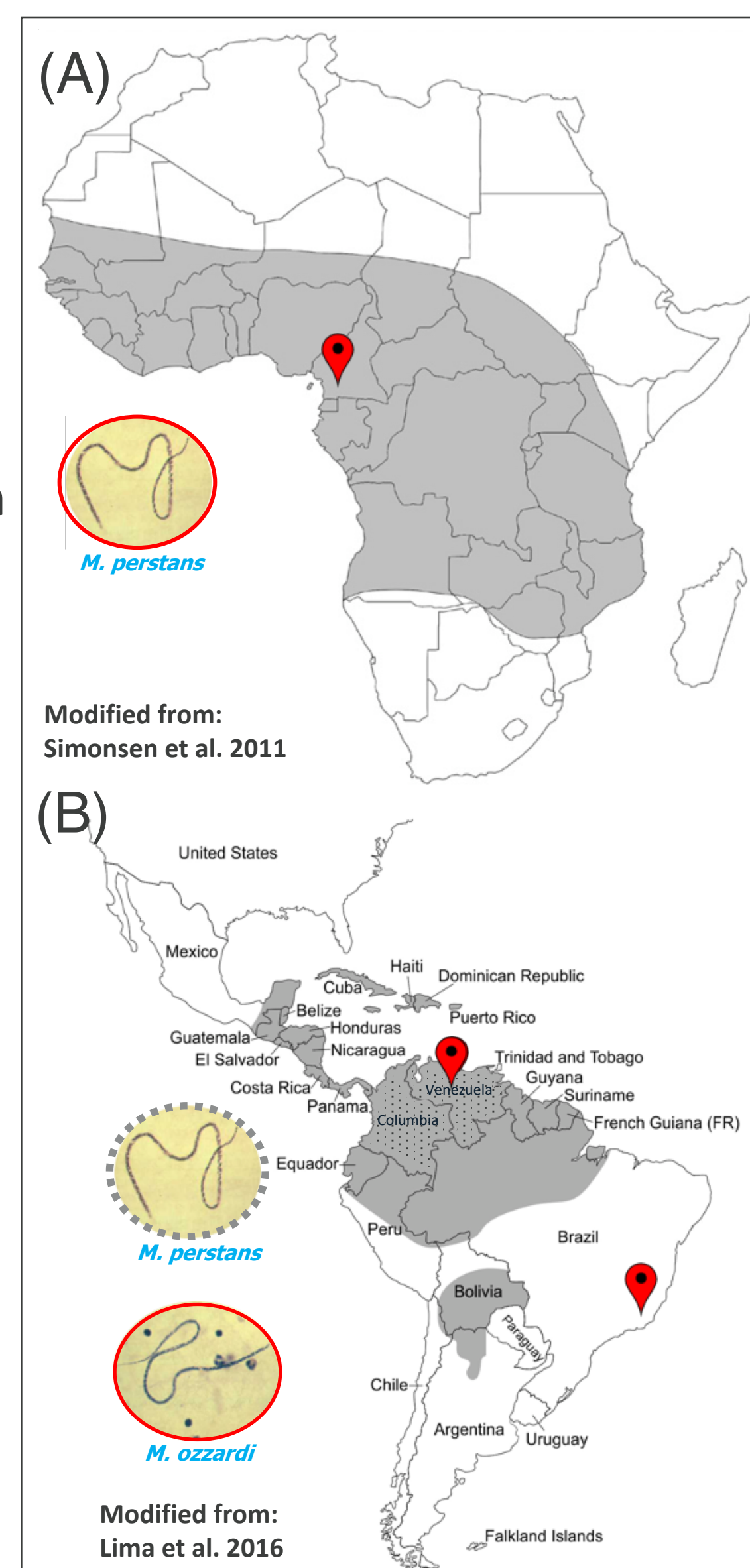
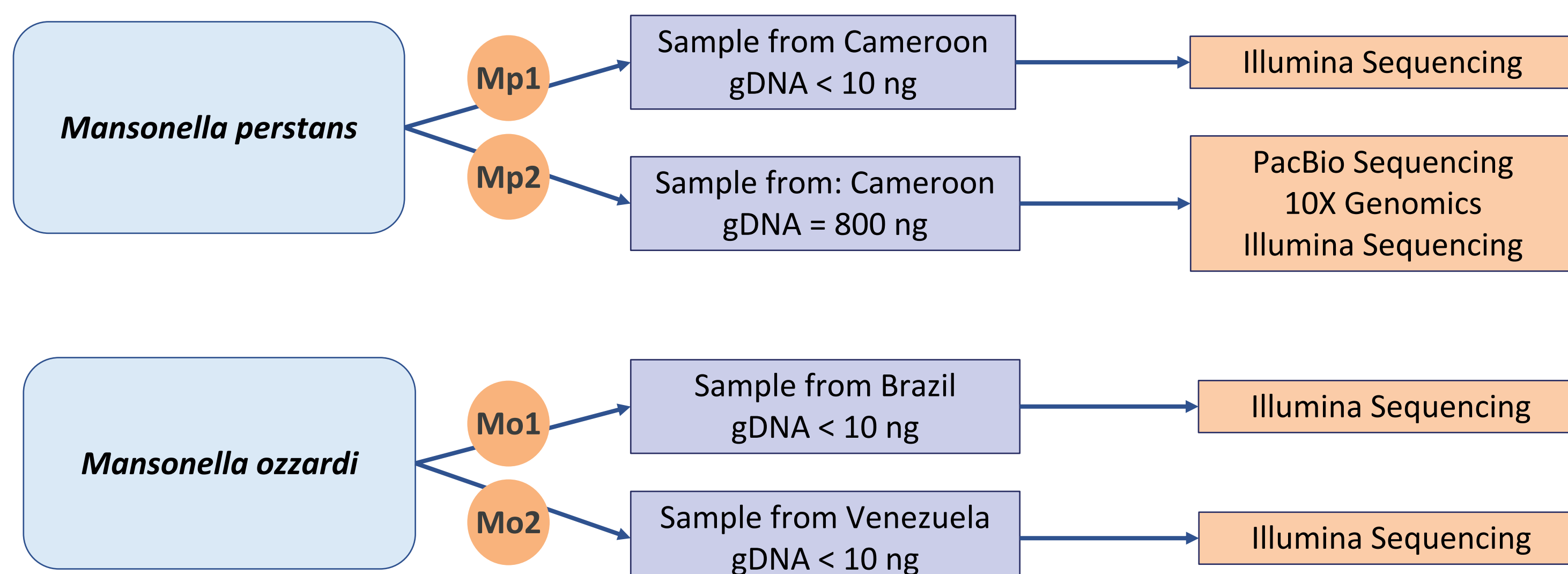


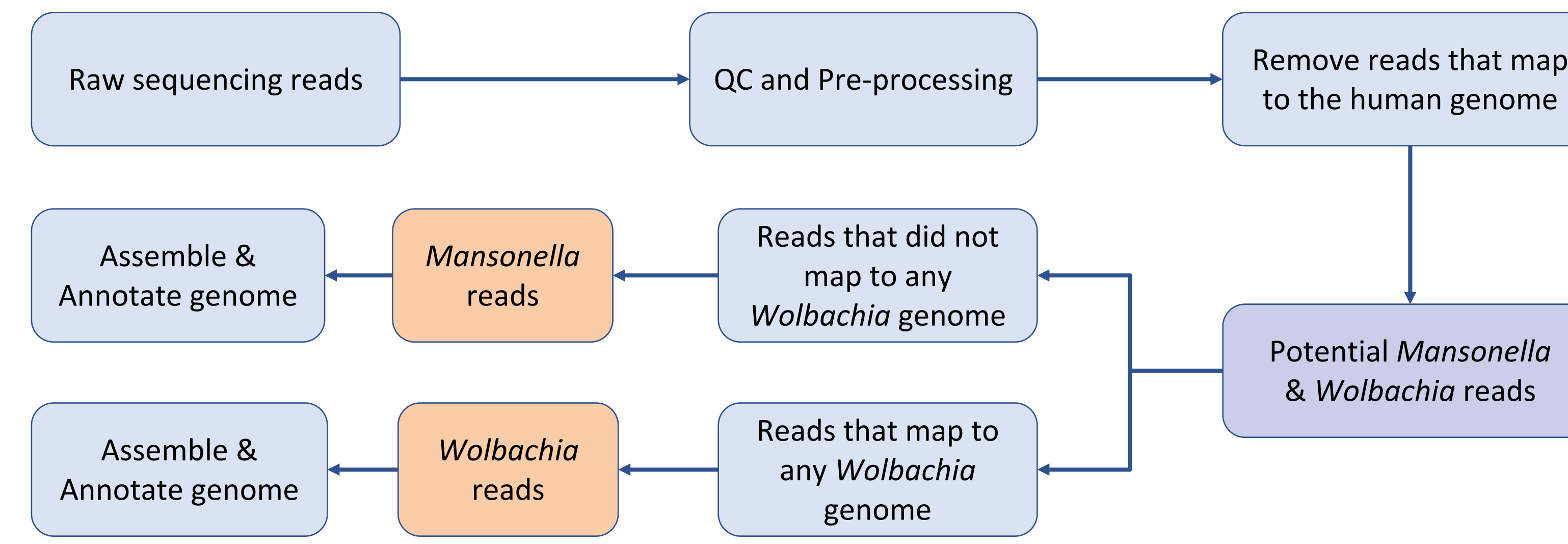
Figure 1: Geographic ranges of (A) *M. perstans* and (B) *M. ozzardi*. The stippled region in (B) represents areas where both *M. ozzardi* and *M. perstans* have been reported to occur. The red pins mark the origin of samples used in this study.

## Mansonella Genomes: Challenges and Solutions

- High levels of human DNA in (up to 95%) as they are derived from patient samples.
  - ✓ Reduce human DNA using NEBNext® Microbiome DNA Enrichment Kit
  - ✓ Sequence as deep as possible
  - ✓ Bioinformatic filtering of reads and contigs originating from human DNA
- Limited amounts of parasite material.
  - ✓ Illumina library preparation using NEBNext® Ultra™ II DNA Library Prep Kit



## Genome Assembly Pipeline



## Mansonella Genome : Draft Assemblies

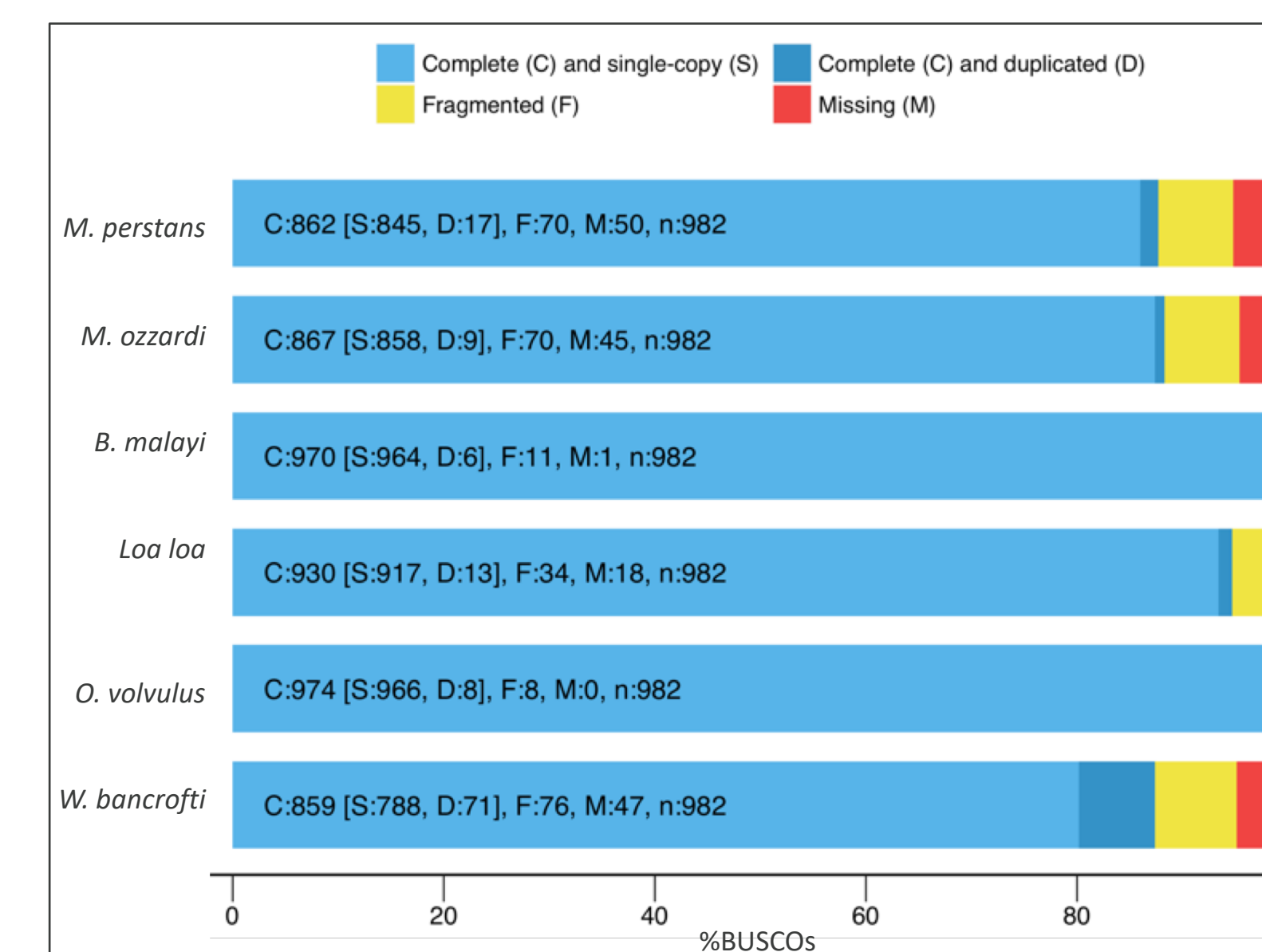
	<i>M. perstans</i>	<i>M. ozzardi</i>	<i>Wolbachia</i> wMpe	<i>Wolbachia</i> wMoz
Sample Name	Mp2	Mo2	Mp1	Mo2
Sequencing Platform	PacBio	Illumina	Illumina	Illumina
Estimated genome size	75 to 85 Mb *	75 to 85 Mb *	0.8 to 1.2 Mb §	0.8 to 1.2 Mb §
Assembled genome size	79 Mb	80 Mb	0.70 Mb	1.5 Mb
Number of scaffolds	1,004	4,912	550	523
Largest scaffold	1.24 Mb	550 kb	5.5 kb	48 kb
Scaffold N50 size	227 kb	73 kb	1.4 kb	10 kb
%GC	30%	30%	36 %	34 %
Predicted genes	10,307	9,119	885	1,871

\* Genome size estimates are based on the k-mer frequency distribution of Illumina reads.

§ Genome size estimates based on other filarial *Wolbachia* and wCLe

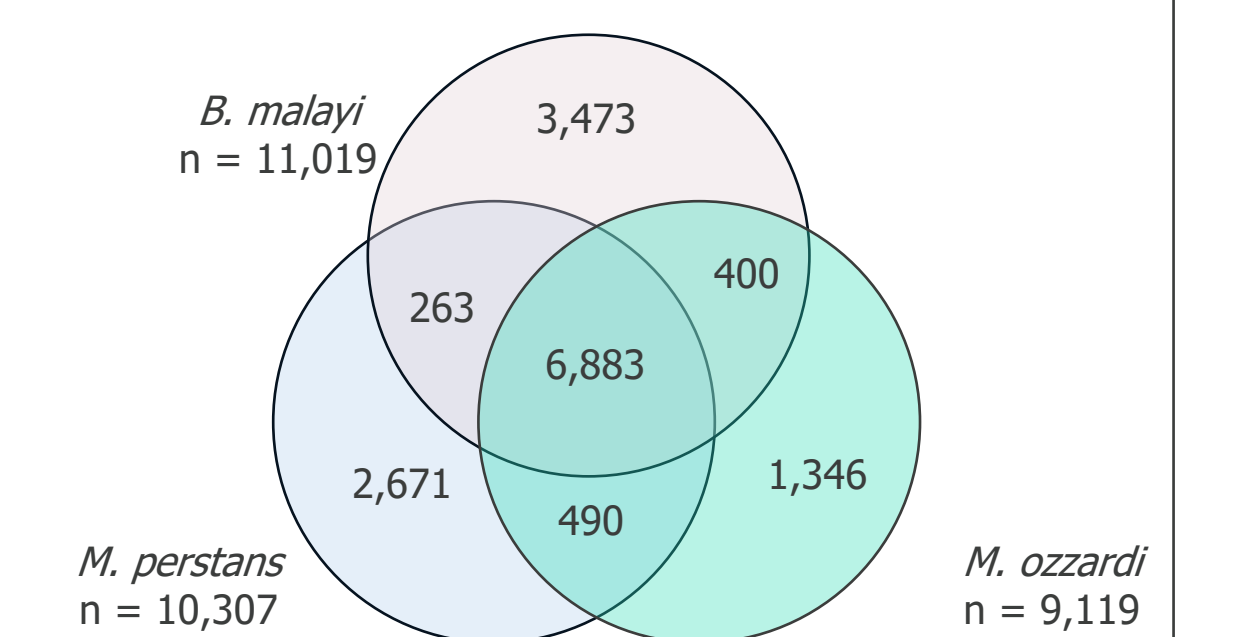
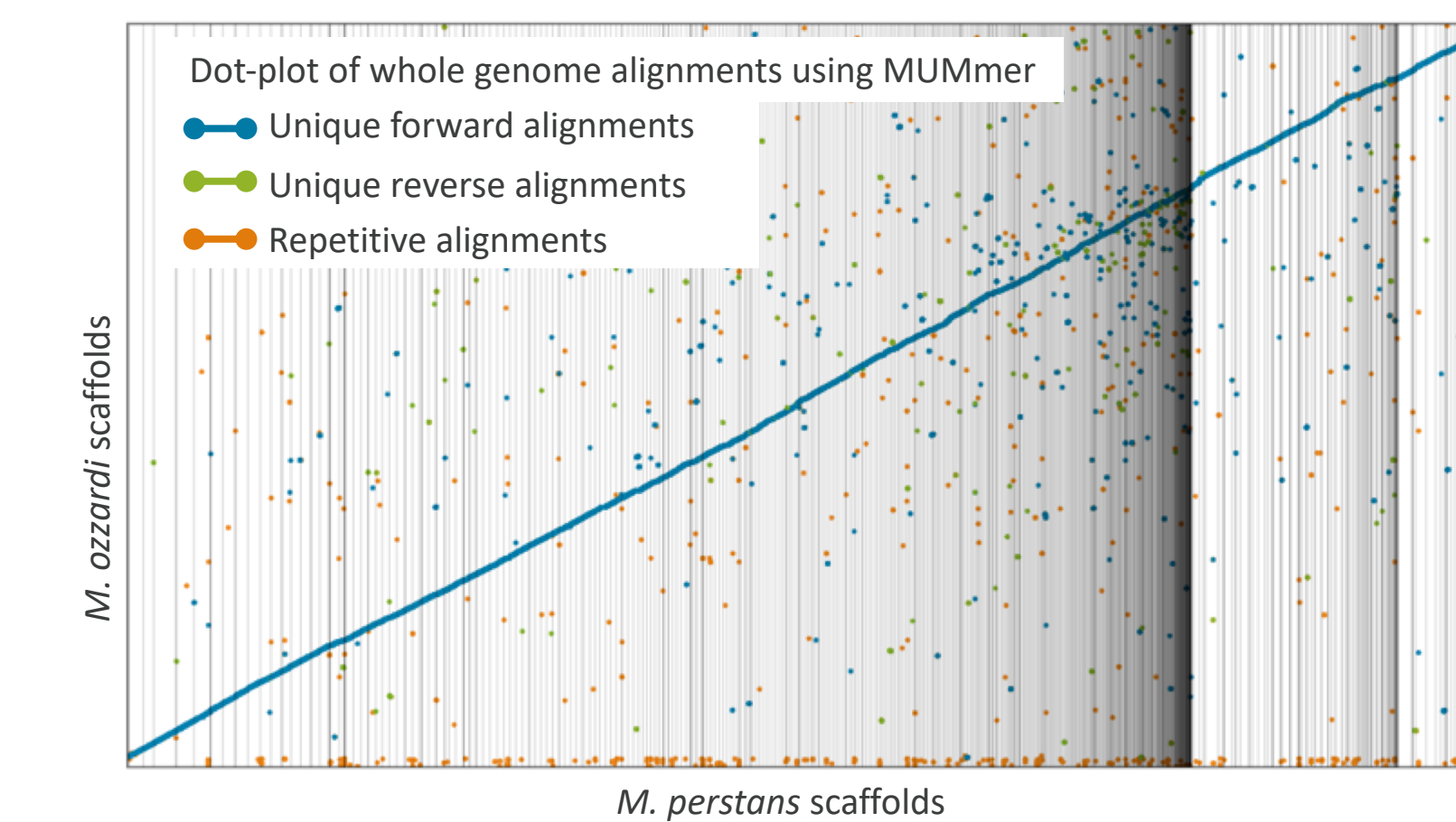
## Completeness of Mansonella Genomes

- The completeness of gene content of assembled draft genomes was evaluated using the BUSCO software (Simão et al. 2015).
- The BUSCO score of a genome is calculated as the percentage of universal Single Copy Orthologs (SCOs) encoded by a genome, as compared to a baseline set of SCOs known to be highly conserved in a particular taxa.
- M. perstans* and *M. ozzardi* encode for more than 85% of the 982 SCOs that are conserved across all nematodes.
- Their BUSCO score is similar to that from other sequenced filarial genomes, suggesting that the *Mansonella* draft assemblies contain most of the protein coding genes they are estimated to encode for.



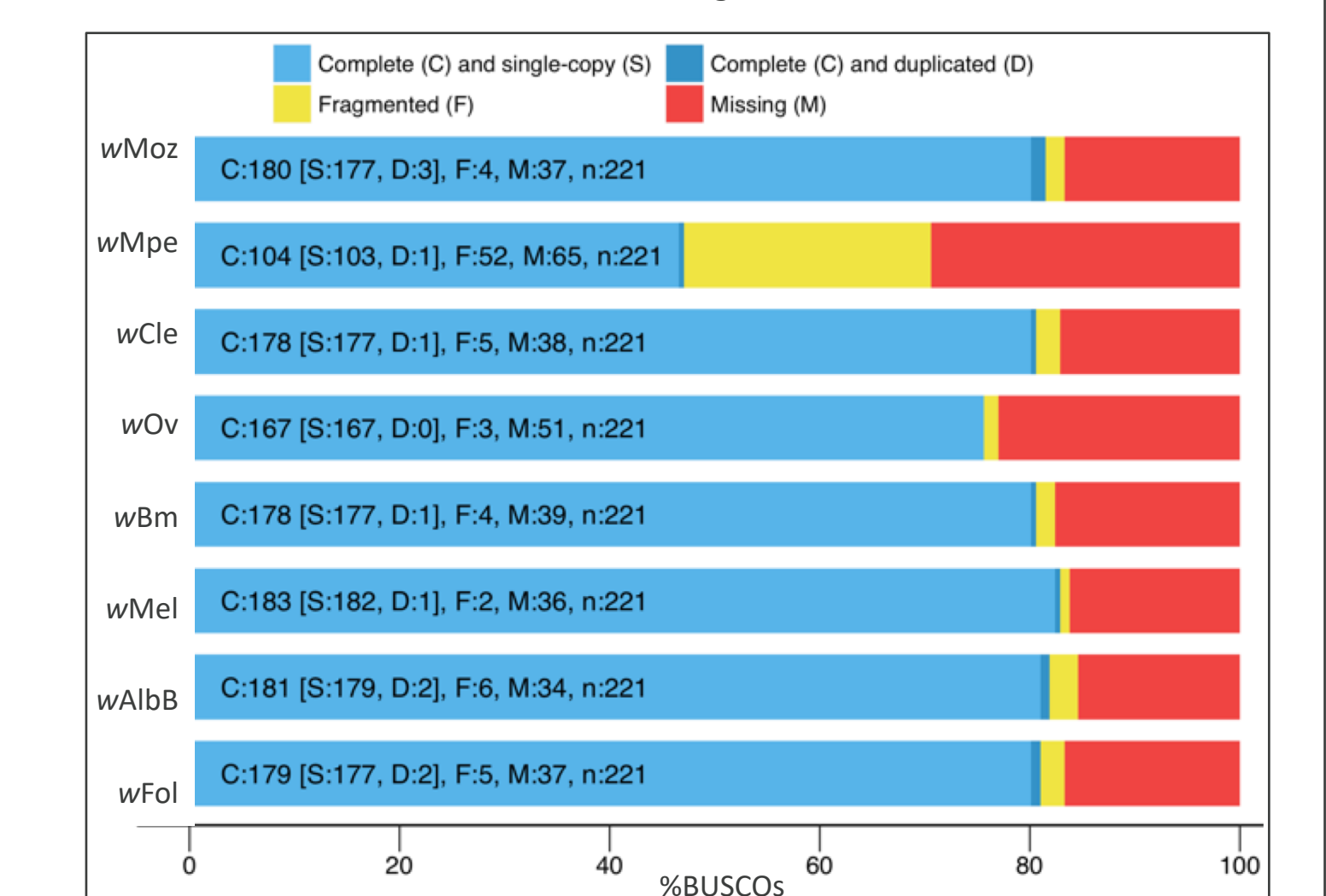
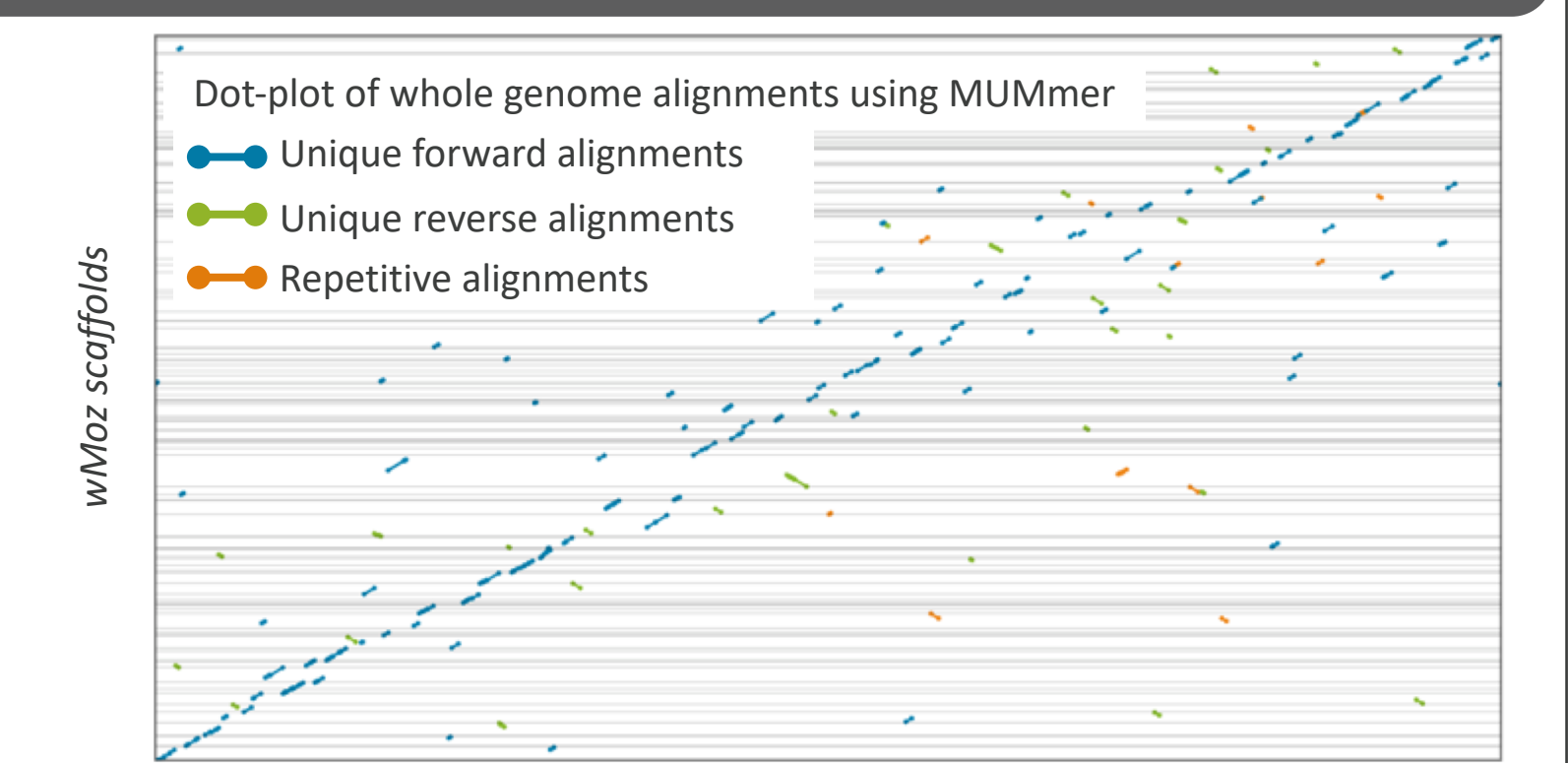
## Genome comparisons

- The two *Mansonella* genomes are largely colinear
- Genome-wide Average Nucleotide Identity, gANI ~ 90%
- Orthology analysis identified the set of shared and unique filarial genes.



## Completeness of Wolbachia Genomes from Mansonella

- Both wMpe and wMoz genomes show high sequence identity (gANI ~ 93%), and collinearity with the bed-bug *Wolbachia* wCLe.
- The *Wolbachia* wMoz genome is nearly complete, with BUSCO score of ~80%, similar to other completely sequenced *Wolbachia* genomes.
- However, the *Wolbachia* wMpe genome shows a large fraction of fragmented and missing SCOs, indicating gaps in the draft assembly.



## Work in Progress

- Refinement and improvement of all the assembled genomes and annotations.
- Intra and inter-species comparisons of genomes.
- Metabolic pathway analysis for understanding the symbiotic relationship of *Mansonella* and their *Wolbachia*.

## Conclusions

- Draft assemblies of *M. perstans* and *M. ozzardi* have been obtained.
- Draft genomes for filarial *Wolbachia* wMpe and wMoz have been obtained. These represent the first ever genomic data from a nematode *Wolbachia* of supergroup F.
- Intra- and inter-species comparisons of genomes will enable investigations and provide insights into the biology and evolution of some of the most highly neglected filarial parasites.

### References:

- Lefoulon, E. et al. (2016) Breakdown of coevolution between symbiotic bacteria *Wolbachia* and their filarial hosts. *PeerJ* 4, e1840.
- Lima, N. F. et al. (2016) *Mansonella ozzardi*: a neglected New World filarial nematode. *Pathogens and Global Health* 110.
- Simão, F. A. et al. (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31.
- Simonsen, P. E. et al. (2011) *Mansonella perstans* filariasis in Africa. *Acta Tropica* 120.