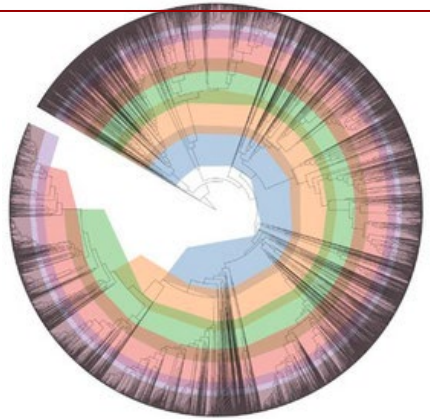
ENVIRONMENTAL METAGENOMICS

Physalia course, online, 11-15 November 2024

Long read assembly

Nikolay Oskolkov, Lund University, NBIS SciLifeLab
Samuel Aroney, Queensland University of Technology



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NB: original course material courtesy:
Dr. Antti Karkman, University of Helsinki
Dr. Igor Pessi, Finnish Environment Institute (SYKE)
As. Prof. Luis Pedro Coelho

A bit about me

- Bachelor at University of Queenslar
- DPhil at Oxford University, UK
  - Internship with Zooniverse – Software development
- Post-doc at Queensland University of Technology, Australia
  - In the Woodcroft group → →
  - sandpiper.qut.edu.au

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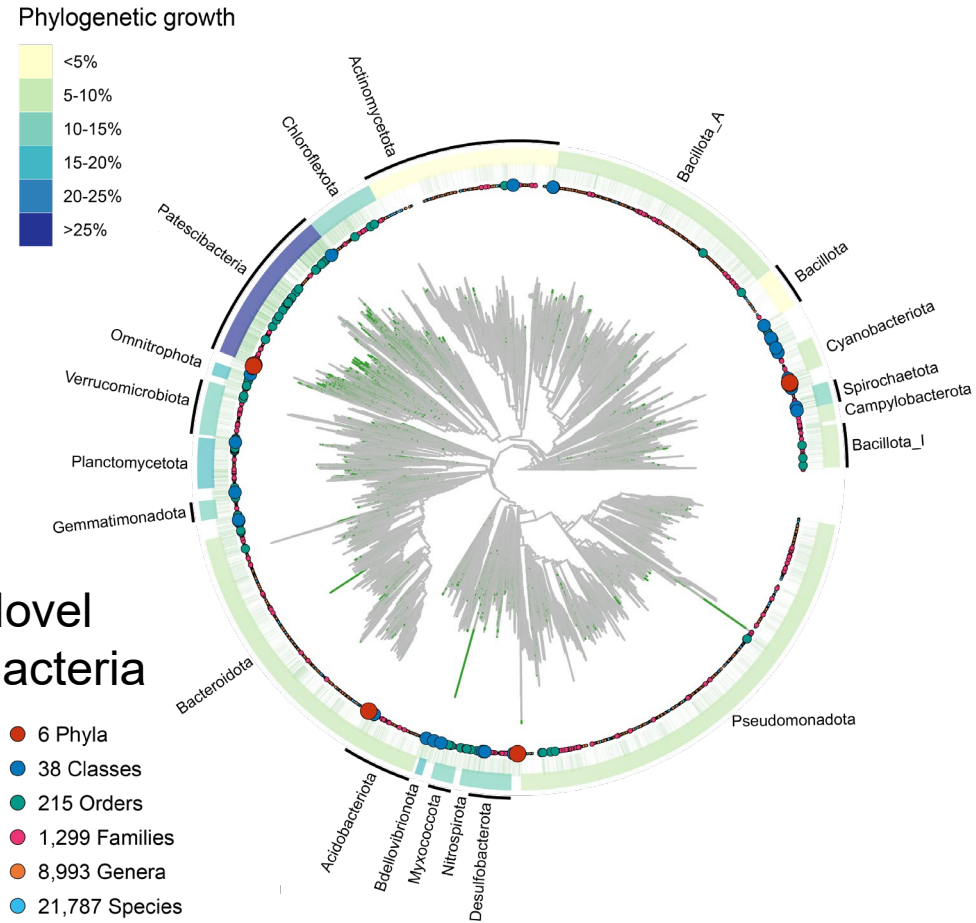
Sandpiper

A bit about my work

- Method development
- Global-scale genome recovery
- Permafrost thaw

CoverM

Aviary



Novel Bacteria

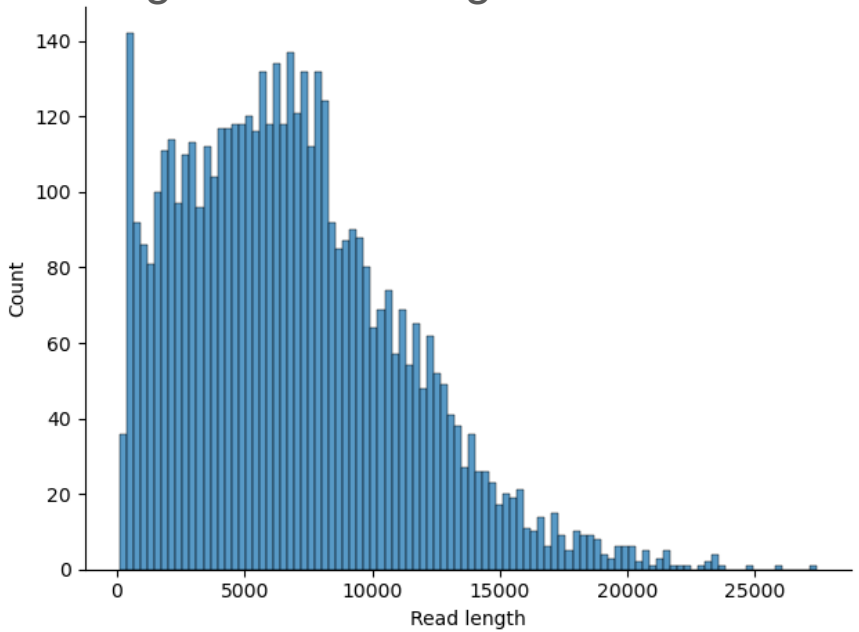
- 6 Phyla
- 38 Classes
- 215 Orders
- 1,299 Families
- 8,993 Genera
- 21,787 Species

Long reads are longer than short reads

When short reads first appeared, they were 35bps!

Nowadays, short reads are 150-300bp and long reads are longer

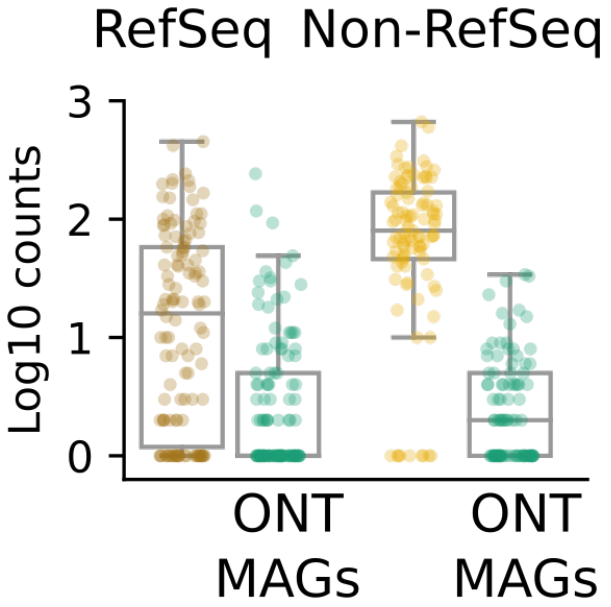
PacBio



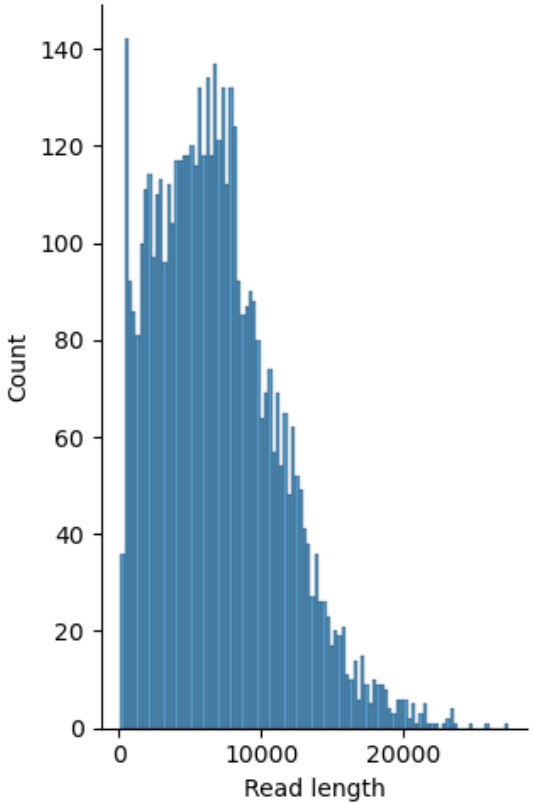
We can get better assemblies with long reads!

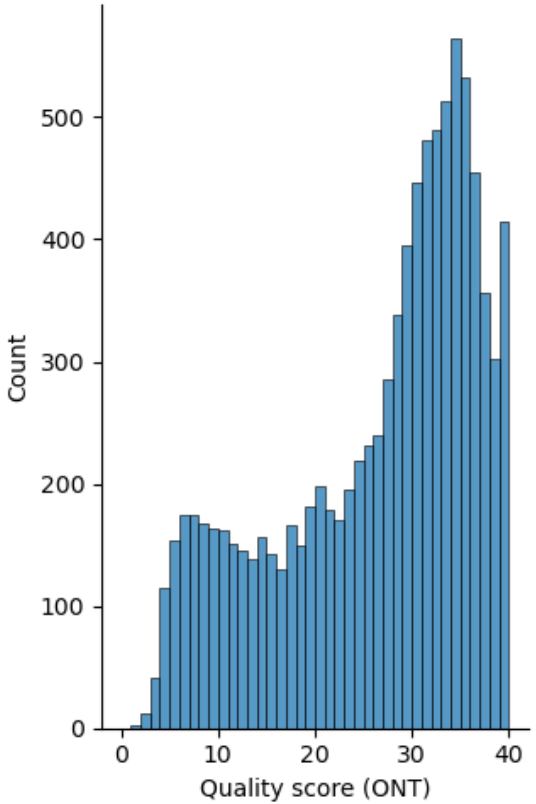
Some individual reads are longer than contigs from short-read assembly

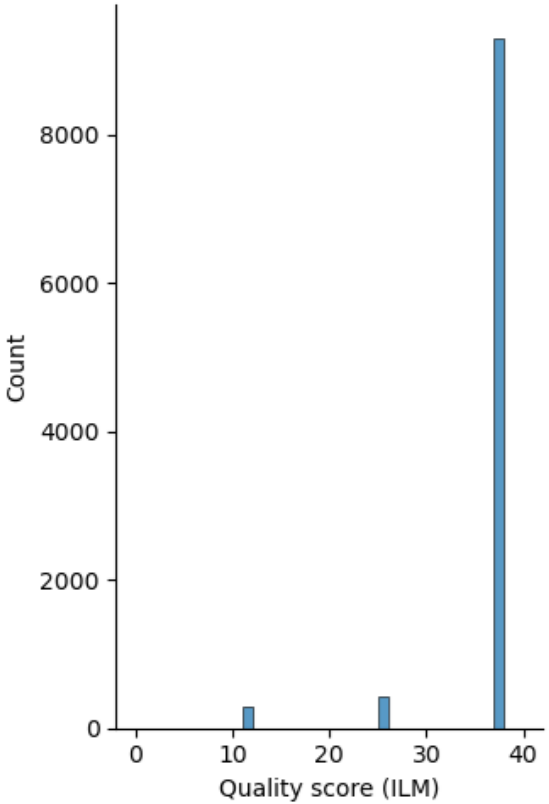
It can cover repeats and other hard to assemble regions



Why do we even use short reads then?





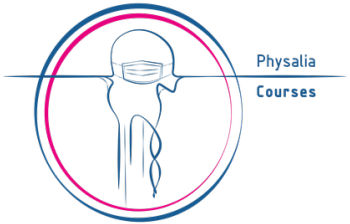


Best of both worlds: do both!

In fact, the data I showed before was from hybrid assembly!

How do you handle hybrid assembly?

1. Polish the long-reads with Illumina
2. Use a hybrid assembler (that takes ONT + ILM)
3. Polish the output of a long-read assembler with ILM
4. Use a short read assembler, then scaffold with the long reads
5. …?



ONT data is in FASTQ format (like Illumina)

```
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```
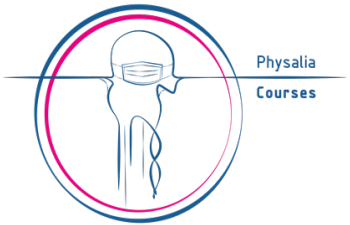
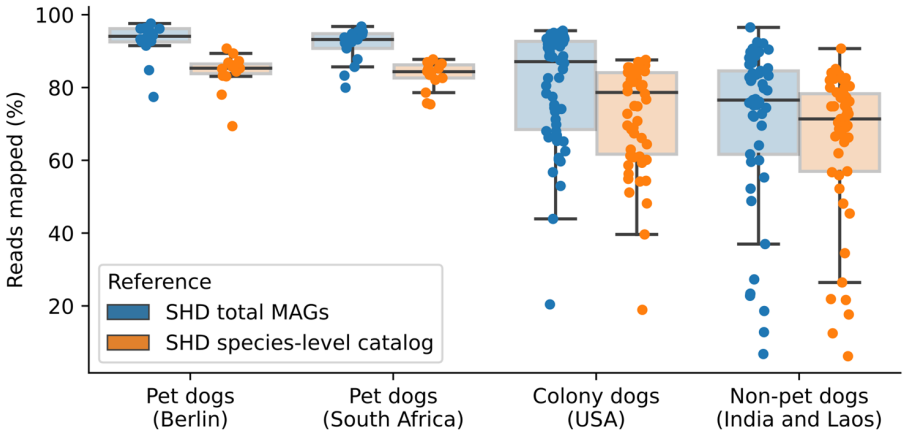

How do results look like with long-reads?

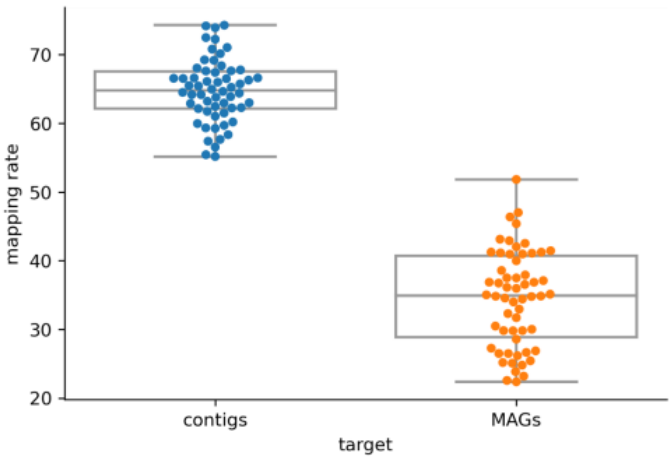
		#contig	Average length	N50
Sample A	mNGS	277,884	1,865	8,778
	PacBio-HiFi	2,575	99,842	269,406
	Nanopore	3,414	65,685	199,639
Sample B	mNGS	146,857	2,402	28,310
	PacBio-HiFi	1,447	157,228	1,081,788
	Nanopore	1,795	123,512	658,841
Sample C	mNGS	170,813	1,956	18,485
	PacBio-HiFi	822	171,215	1,270,126
	Nanopore	1,370	126,533	891,411



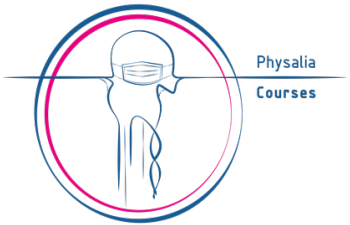
Results depend on the habitat!



Dog gut
(20 Gbp + 20 Gbp) x 50 samples



Soil
(40 Gbp + 40 Gbp) x 52 samples

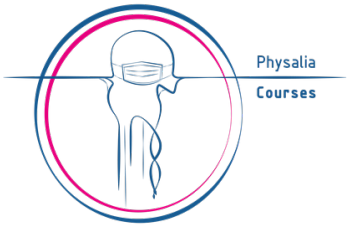


In dog guts, 20 Gbp was enough to get excellent representation of the community, but in soil even double that was not enough! — could also be Euks!

From Anna Cuscó (dog) & Yiqian Duan (soil)

A few other notes on long-reads

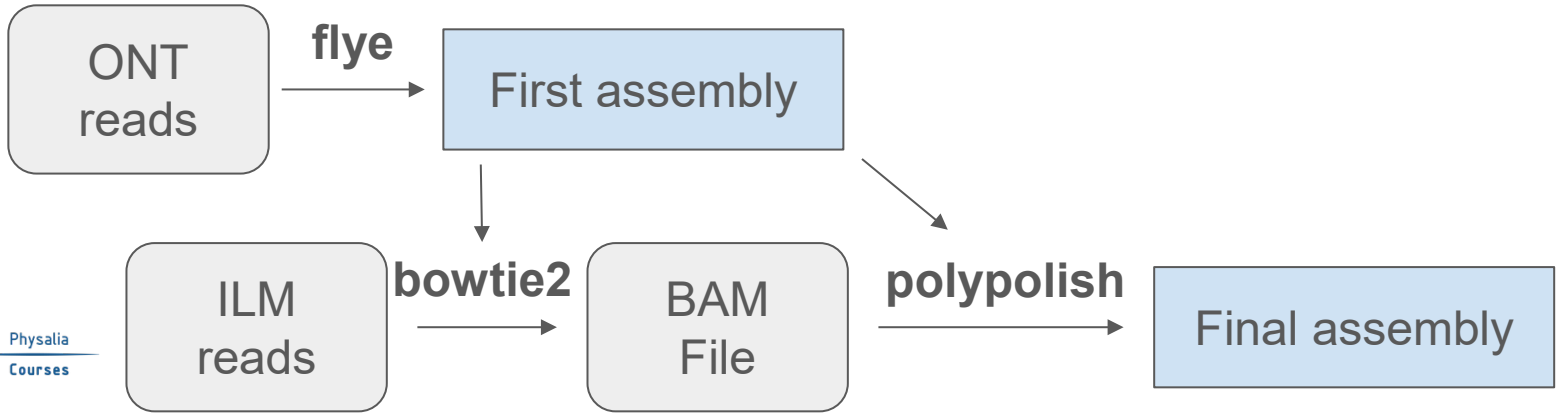
- Still a very rapidly evolving field (both in the wetlab & in the drylab)
- Differences between ONT versions matter a lot
- We might be able to get even more information in the future
  - Methylation
  - Non-canonical nucleotides
- **Long-reads are the future**



Exercises overview

1. Assemble with **flye** (long-read only)
2. Polish with **polypolish** (using the short reads)

In order to polish, we need to align the short reads to the contigs from the **flye** assembly



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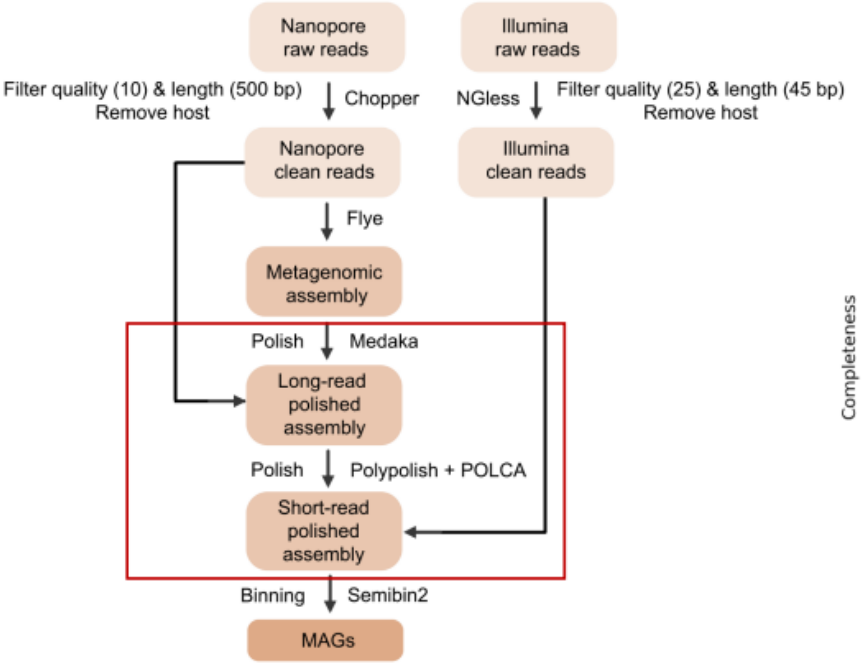
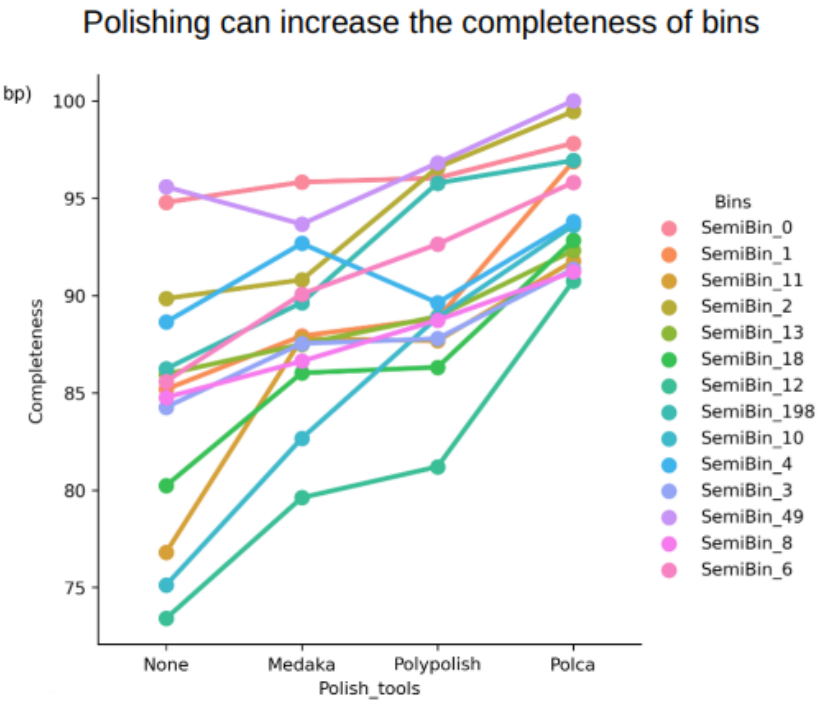
Real workflow





From Yiqian Duan