

Supplementary Information for: Widespread *Trichuris incognita* reveals hidden diversity and reshapes understanding of human whipworm infections

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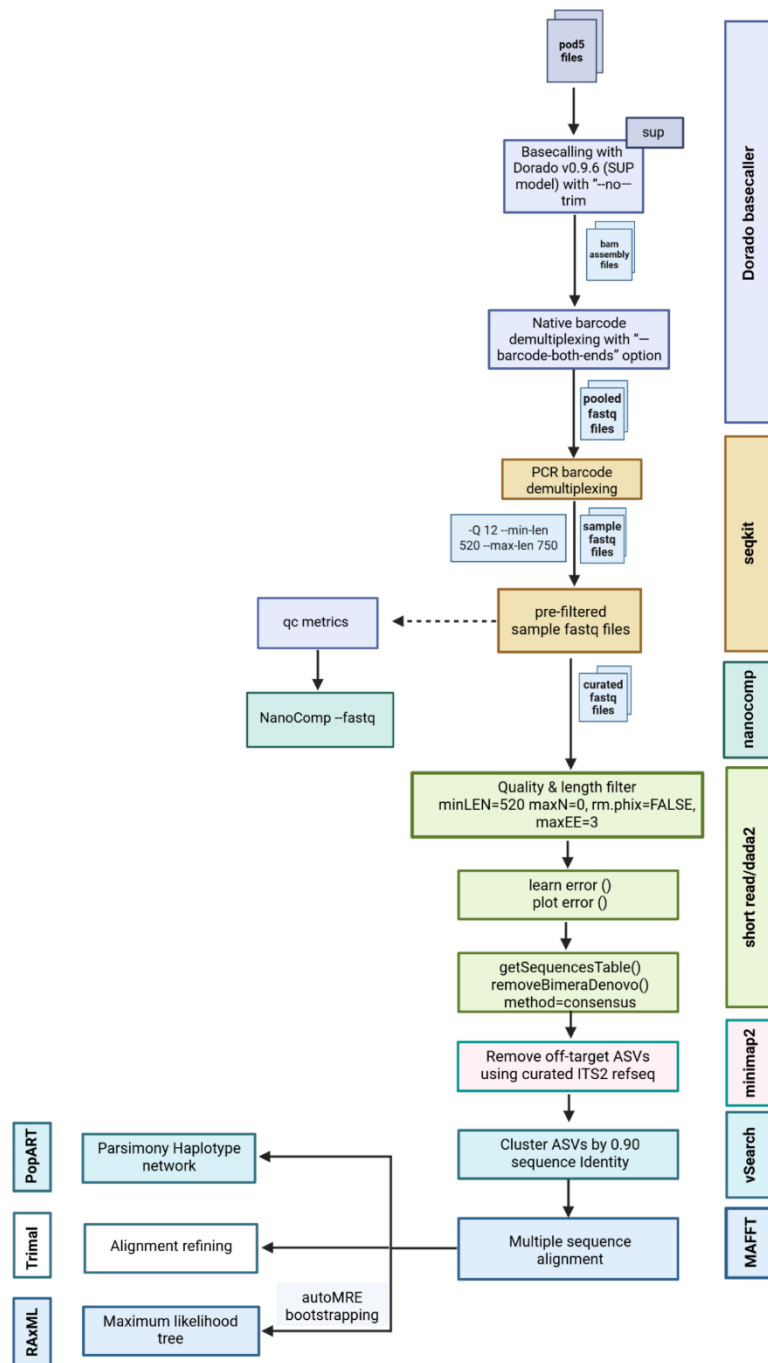
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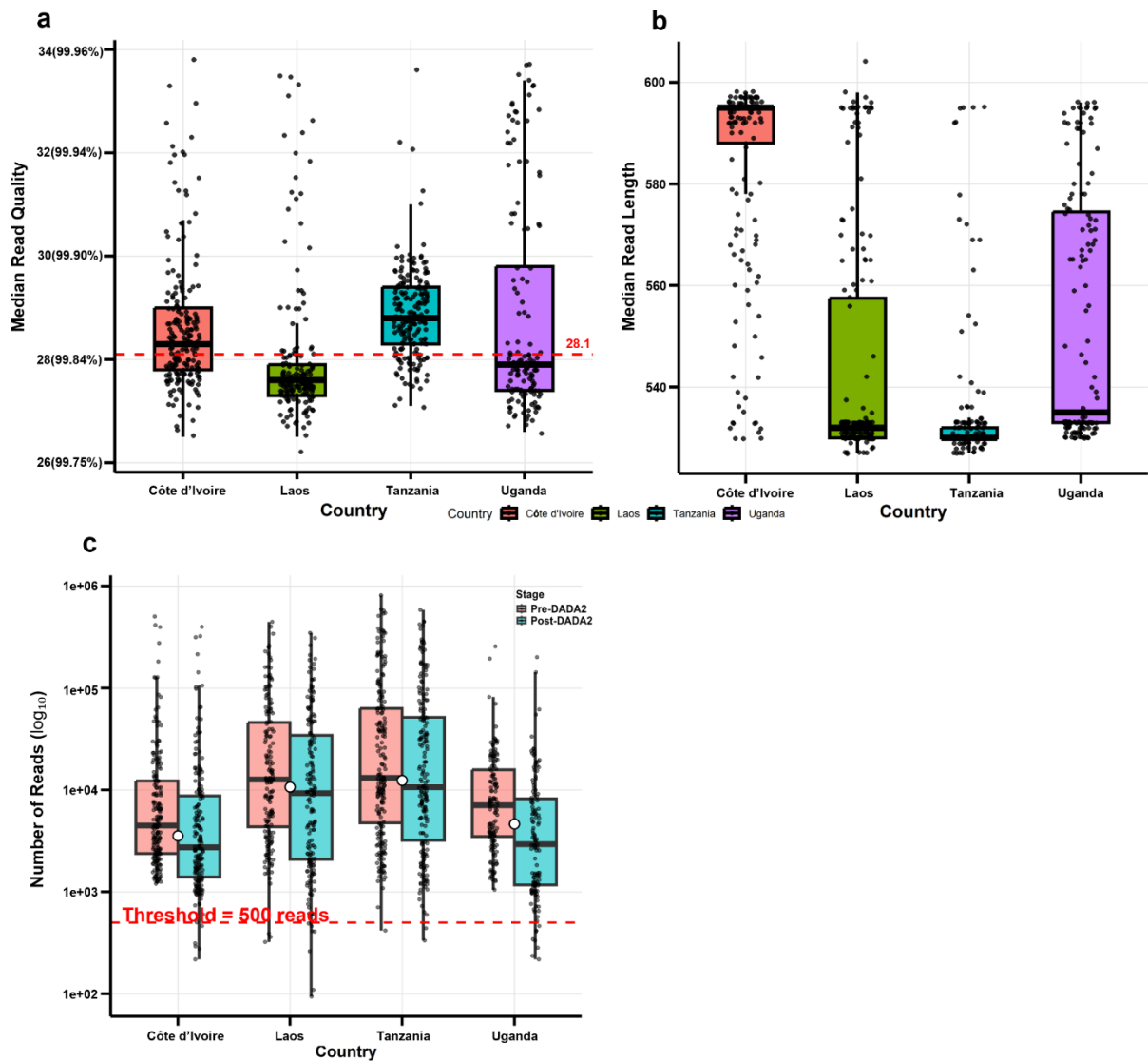
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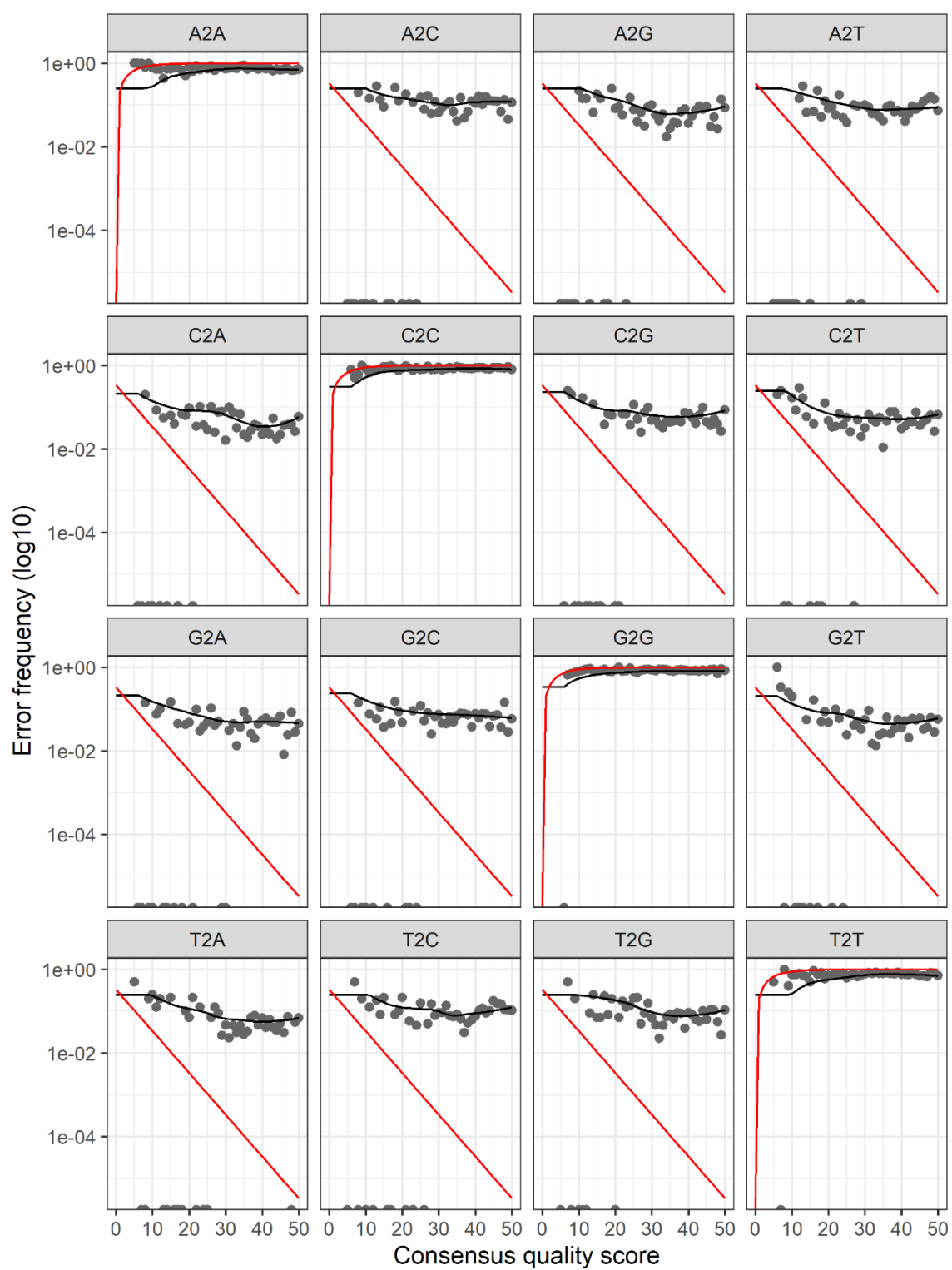
Supplementary Appendix 1. DNA extraction protocol for ethanol-preserved Faecal samples



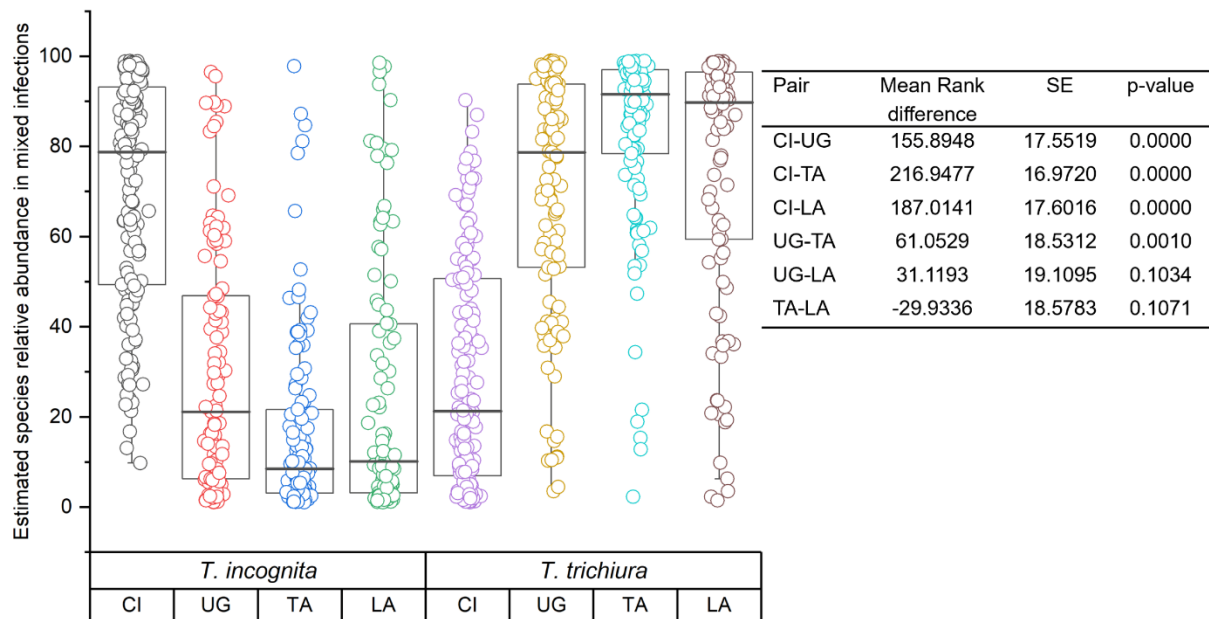
Supplementary Figure 1. Overview of the bioinformatics workflow for a genetic diversity study using ONT sequencing data. Softwares used are shown on the right and left ends. Created in BioRender. Pierre, S. (2025) <https://BioRender.com/63bfqzo>



Supplementary Figure 2. Overview of the quality run characteristics of sequences and reads generated before and after filtering steps with a stringent DADA2 analysis from Côte d'Ivoire ($n = 189$); Laos ($n = 173$); Tanzania ($n = 190$); UG ($n = 135$); *T. trichiura* worm control ($n = 4$); *T. incognita* worm control ($n = 4$). **a** Median read quality (red line showing the average read quality across all samples sequenced). **b** Median read length (bp) **c**. Reads generated before and after DADA2 processing (red line showing the threshold of reads cutoff after a stringent quality filtering approach); Quality (error probability in %), bp = basepairs



Supplementary Figure 3. Error plot generated from DADA2 denoising process run of sequence analyzed



Supplementary Figure 4. Estimated relative abundance of *Trichuris incognita* and *T. trichiura* in mixed infections across countries. Boxplots show the relative abundance distribution of each species among individuals with mixed infections in Côte d'Ivoire (CI; $n = 182$), Uganda (UG; $n = 122$), Tanzania (TA; $n = 189$), and Laos (LA; $n = 164$). Pairwise comparisons of mean ranks between countries for *T. incognita* are summarized in the embedded table using post-hoc testing (p -values adjusted). Significant differences were observed between CI and all other countries, and between UG and TA.

Supplementary Appendix 1. DNA extraction protocol for ethanol-preserved Faecal samples

1. Mix the ethanol samples with a disposable spatula (homogeneous suspension)
2. Transfer an aliquot of approximately 500 µl of the 96% ethanol-preserved stool sample into an Eppendorf tube (NB: This volume corresponds with the 0.15 – 0.25g of stool recommended by the manufacturer)
3. Centrifuge at 15000 x g for 5 min, and discard the supernatant (ethanol)
4. Wash the pellet by adding 1 ml of phosphate buffer saline (PBS), and mix by vortexing briefly
5. Centrifuge at 15000 x g for 1 min, and discard the supernatant (PBS)
6. Resuspend pellet in 200 µl of PBS
7. Freeze pellet on dry ice for 30 mins
8. Thaw-boil in a preheated thermoblock at 100°C for 10 mins
9. Vortex briefly with the vortex adapter for 3 mins
10. Centrifuge the Powerpro bead tubes at 15000 x g for 1 min
11. Transfer the pellet to Powerpro bead tubes
12. Add 800 µl of Solution CD1 and vortex briefly to mix for 15-20 min
13. Centrifuge at 15000 x g for 2 min
14. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube. Transfer around 800 – 850 µL of supernatant (depending on the nature and volume of the pellet)
15. Add 200 µl of Solution CD2 and vortex for 5 s
16. Centrifuge it at 15000 x g for 1 min. Avoid the pellet, transfer up to 800 µL of supernatant (depending on the volume of your pellet) to a clean 2 mL Microcentrifuge Tube (provided)
18. Add 600 µl of Solution CD3 and vortex for 5 seconds
19. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15000 x g for 1 min
20. Discard the flow-through and repeat step 19 to ensure that all of the lysate has passed through the MB Spin Column
21. Place the MB Spin Column into a clean 2 ml Collection Tube and add 500 µl of Solution EA
22. Centrifuge at 15000 x g for 1 min and discard the flow-through
23. Add 500 µl of Solution C5 and centrifuge at 15000 x g for 1 min
24. Place the MB Spin Column into a clean 2 ml Microcentrifuge Tube and centrifuge at 20000 x g for 3 min
25. Place the MB Spin Column into a new 1.5 ml Elution Tube and add 80 µl of Solution C6
26. Centrifuge at 15000 x g for 1 min and discard the MB Spin Column. The DNA is now ready for downstream applications