Title:

Application of Oxford Nanopore sequencing in transcriptomics profiling of *Schistosoma* sp. life cycle stages, and development of molecular diagnostics

Authors:

Piyas Mukherjee[1,2,3], Youssef Hamway[1,2,3], Arne Jacobs[4], Poppy Lamberton[4,6], Clarissa Prazeres da Costa[1,2,3]

Affiliations:

[1]Institute for Medical Microbiology, Immunology and Hygiene, TUM School of Medicine and Health, Technical University of Munich (TUM), Munich, Germany

[2]Center for Global Health, TUM School of Medicine and Health, Technical University of Munich (TUM), Munich, Germany

[3]German Centre for Infection Research (DZIF), Germany

[4]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK

[5]British Society for Parasitology, UK

Abstract (4000 characters)

Introduction

Schistosomiasis, caused by blood flukes of the genus *Schistosoma*, affects over 240 million people worldwide and remains a major Neglected Tropical Disease, particularly in sub-Saharan Africa, Southeast Asia, and parts of South America[1]. Chronic infection in the human definitive host leads to substantial morbidity driven by complex host-parasite interactions[2]. The parasite undergoes an elaborate life cycle, transitioning through distinct environmental and host niches, each requiring tightly regulated stage-specific genomic and transcriptomic programs[3]. Indeed, the parasite exhibits complex gene regulation and stage-specific transcription throughout its life cycle, enabling adaptation, modulate host-pathogen immune responses, and reproduction[3-5]. A key mechanism underlying this regulation is alternative splicing, which expands transcript diversity by generating multiple isoforms, that may influence parasite development, pathogenicity, and potentially drug responsiveness[6]. Although widespread alternative splicing has been suggested in schistosomiasis, it remains poorly characterized[7]. Previous short-read transcriptomic studies have revealed stage- and sex-specific gene expression patterns related to host adaptation and development[3,8,9], but such approaches lack the resolution to accurately capture alternative splicing events that may drive phenotypic diversity, and even drug resistance, such as TRPM channels, other receptor families, and PZQ resistance-associated isoforms[10], offering both new drug targets and diagnostic biomarkers. To address this limitation, we aim to apply Oxford Nanopore Technologies (ONT) long-read sequencing to profile full-length

transcripts across key life-cycle stages of *Schistosoma mansoni*. This approach enables comprehensive isoform identification, improved transcriptome annotation, and precise mapping of alternative splicing events. By defining stage-specific gene isoforms, we seek to establish a lifecycle-resolved molecular "fingerprint" of schistosome infection, supporting the discovery of novel diagnostic markers and therapeutic targets while expanding the application of long-read transcriptomics in schistosomiasis research.

Methodology

Parasite material was obtained from the established *Schistosoma mansoni* (NMRI strain) life cycle, including cercariae, miracidia, eggs, schistosomula, and adult worms, maintained at TUM (Prof. C. Prazeres da Costa). Total RNA was extracted and quality assessed (RIN) prior to preparation of Oxford Nanopore Technologies (ONT) full-length cDNA libraries, as per guidance from Dr. Arne Jacobs. The ONT transcriptomic data will be analyzed to profile Schistosoma parasites across key life-cycle stages to define alternative splicing events, under the expert guidance of Dr. Jacobs as well. Raw ONT reads will be filtered for quality and aligned to the *S. mansoni* reference genome, followed by transcript assembly and isoform annotation. Stage-specific gene expression and alternative splicing events will be quantified to assess differential transcript and isoform usage across life-cycle stages. Statistical analyses will be utilized to identify significantly regulated genes and splice variants using sound correction methods for multiple testing. Finally, the observations can be extrapolated to highly alternatively spliced praziquantel target gene, *Sm.TRPM*$_{PZQ}$, analyzed as a case study to characterize stage-dependent isoform diversity and potential functional implications.

Inference

Full-length transcriptome sequencing using the Oxford Nanopore platform enables high-resolution identification of stage-specific transcripts and novel isoforms across the *Schistosoma mansoni* life cycle. This strategy facilitates systematic characterization of transcriptional regulation and biomarker expression dynamics, supporting the discovery of life-stage–specific diagnostic candidates and therapeutic targets. Collectively, these findings provide a molecular "fingerprint" framework to enhance stage-specific diagnosis and inform targeted intervention strategies in schistosomiasis.

References

1. World Health Organization, Global report on neglected tropical diseases 2023, (2023), https://www.who.int/publications/i/item/9789240067295
2. Kokaliaris, C., Garba, A., Matuska, M., Bronzan, R. N., Colley, D. G., Dorkenoo, A. M., Ekpo, U. F., Fleming, F. M., French, M. D., Kabore, A., Mbonigaba, J. B., Midzi, N., Mwinzi, P. N. M., N'Goran, E. K., Polo, M. R., Sacko, M., Tchuem Tchuenté, L. A., Tukahebwa, E. M., Uvon, P. A., Yang, G., … Vounatsou, P. (2022). Effect of preventive chemotherapy with praziquantel on schistosomiasis among school-aged children in sub-Saharan Africa: a spatiotemporal modelling study. *The Lancet. Infectious diseases*, *22*(1), 136–149. https://doi.org/10.1016/S1473-3099(21)00090-6
3. Jolly, E.R., Chin, CS., Miller, S. *et al.* Gene expression patterns during adaptation of a helminth parasite to different environmental niches. *Genome Biol* **8**, R65 (2007). https://doi.org/10.1186/gb-2007-8-4-r65
4. Gobert, G. N., Tran, M. H., Moertel, L., Mulvenna, J., Jones, M. K., McManus, D. P., & Loukas, A. (2010). Transcriptional changes in Schistosoma mansoni during early schistosomula development and in the presence of erythrocytes. *PLoS neglected tropical diseases*, *4*(2), e600. https://doi.org/10.1371/journal.pntd.0000600
5. Buddenborg, S.K., Lu, Z., Sankaranarayan, G. *et al.* The stage- and sex-specific transcriptome of the human parasite *Schistosoma mansoni*. *Sci Data* **10**, 775 (2023). https://doi.org/10.1038/s41597-023-02674-2
6. Matlin, A. J., Clark, F., & Smith, C. W. (2005). Understanding alternative splicing: towards a cellular code. *Nature reviews. Molecular cell biology*, *6*(5), 386–398. https://doi.org/10.1038/nrm1645
7. Nahum, L. A., Mourão, M. M., & Oliveira, G. (2012). New frontiers in schistosoma genomics and transcriptomics. *Journal of parasitology research*, *2012*, 849132. https://doi.org/10.1155/2012/849132
8. Wangwiwatsin, A., Protasio, A. V., Wilson, S., Owusu, C., Holroyd, N. E., Sanders, M. J., Keane, J., Doenhoff, M. J., Rinaldi, G., & Berriman, M. (2020). Transcriptome of the parasitic flatworm Schistosoma mansoni during intra-mammalian development. *PLoS neglected tropical diseases*, *14*(5), e0007743. https://doi.org/10.1371/journal.pntd.0007743
9. Cheng, S., Zhu, B., Luo, F., Lin, X., Sun, C., You, Y., Yi, C., Xu, B., Wang, J., Lu, Y., & Hu, W. (2022). Comparative transcriptome profiles of Schistosoma japonicum larval stages: Implications for parasite biology and host invasion. *PLoS neglected tropical diseases*, *16*(1), e0009889. https://doi.org/10.1371/journal.pntd.0009889
10. Le Clec'h, W., Chevalier, F. D., Mattos, A. C. A., Strickland, A., Diaz, R., McDew-White, M., Rohr, C. M., Kinung'hi, S., Allan, F., Webster, B. L., Webster, J. P., Emery, A. M., Rollinson, D., Djirmay, A. G., Al Mashikhi, K. M., Al Yafae, S., Idris, M. A., Moné, H., Mouahid, G., LoVerde, P., … Anderson, T. J. C. (2021). Genetic analysis of praziquantel response in schistosome parasites implicates a transient receptor potential channel. *Science translational medicine*, *13*(625), eabj9114. https://doi.org/10.1126/scitranslmed.abj9114