




## Forum

### SchistoCyte Atlas: A Single-Cell Transcriptome Resource for Adult Schistosomes

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**Schistosomes cause untold disease and disability in the developing world. Here, we introduce SchistoCyte Atlas, a web-based platform for exploring gene expression at single-cell resolution in adult *Schistosoma mansoni*. Similar resources accessible to non-specialists across the globe will expedite our ability to understand the biology of these devastating parasites.**

Schistosomes are among the world's most successful pathogens, infecting roughly 240 million of the world's poorest people [1,2]. While these parasitic flatworms claim the lives of ~250 000 people annually [2], perhaps the biggest tragedy is the extent of chronic morbidities associated with infection that rob millions of the opportunity to live healthy and productive lives. These parasites have a complicated life cycle that involves both mammalian definitive and snail intermediate hosts. Humans become infected when exposed to water contaminated with larval schistosomes, which then penetrate the skin and enter the blood – where the parasites mature over the course of several weeks to either male or female adult worms. Once mature, adults lay hundreds of eggs per day. Those not voided from the body become trapped in the viscera, resulting in the inflammation and fibrosis that drives disease pathology.

Although schistosomiasis is second only to malaria in terms of the devastation caused by a parasitic disease, treatment has relied on a single drug for nearly 40 years, and recent work suggests that mass drug administration efforts with praziquantel are likely insufficient to achieve the goal of elimination [3]. Compared with the resources invested in other parasitic diseases, we would argue that the commitment of the research community to understanding schistosome biology and developing new therapies is not remotely commensurate with the scale of the problem. Therefore, it is critical that we expand our understanding of these parasites and use this knowledge to advance new strategies to combat this disease.

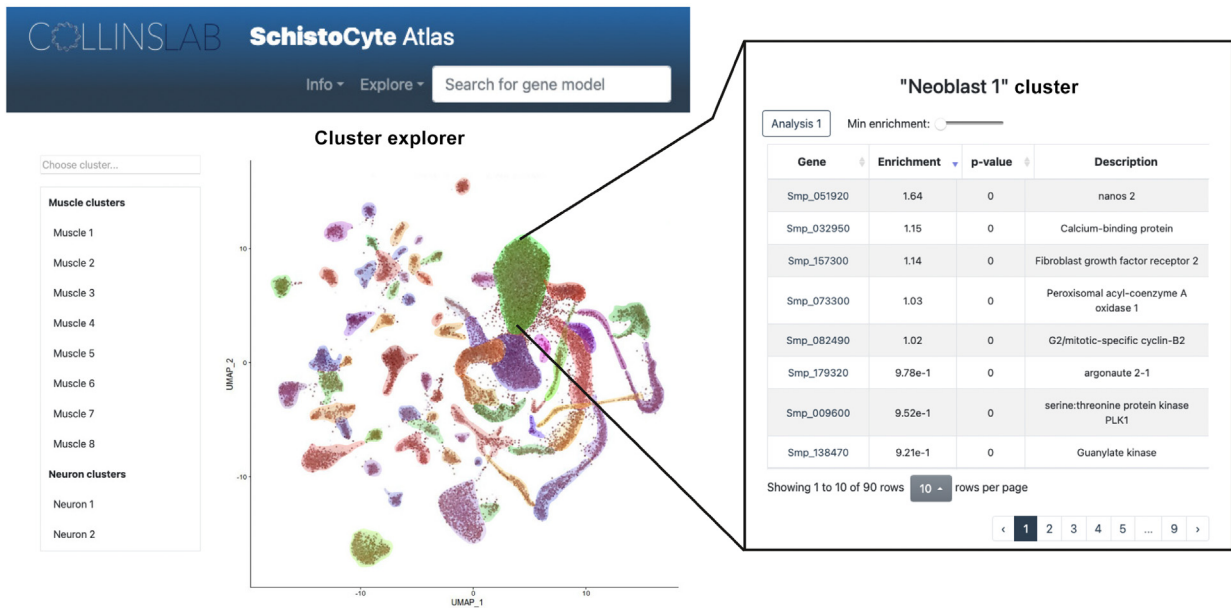
Unlike many of the most experientially tractable and heavily studied parasitic organisms (*Toxoplasma*, *Plasmodium*, *Trypanosoma brucei*, etc.), schistosomes are metazoans with several complicated organ systems that sustain their ability to thrive and lay eggs in the blood for decades. Clearly, a deeper molecular understanding of the anatomical 'nuts and bolts' of these parasites would provide a rich knowledge base by which to begin dismantling their complex and unique biology, suggesting new therapies. With this in mind, several groups in the past decade have combined gene-expression profiling (i.e., microarray and next-generation sequencing) with various tissue-isolation approaches (organ isolation [4,5], dissection [6,7], and dissociation/fluorescence-activated cell sorting [8]) to characterize parasite organ systems on a molecular level. More recently, the promise of this approach has expanded exponentially with the implementation of single-cell RNA sequencing (scRNAseq) approaches. Studies have used scRNAseq to characterize cells from the parasite's intramolluscan stage [9], larval [10] and juvenile [11] intramammalian forms, and adult male and female parasites [12]. In total, these studies provide the most

comprehensive molecular description of the parasite's cells, tissues, and organ systems to date. Given this massive amount of new information the field will have its work cut out for itself unwrapping these data for years to come.

Since scRNAseq experiments generate such voluminous datasets, a major challenge is making sense of the data. This is compounded by the fact that there is no standard analysis and visualization platform, requiring biologists (like us) to dive into complicated programs to fully exploit the data. With this in mind, we have created SchistoCyte Atlas<sup>i</sup> that presently includes the scRNAseq atlas made from over 40 000 cells from adult male and both sexually mature and immature female parasites [12]. This web-based platform allows users to query any gene in the *S. mansoni* genome and evaluate its expression across 68 molecularly distinct cellular clusters present in the adult parasites, including the sexual organs, 31 populations of neurons, eight populations of muscle cells, somatic stem cells, tegument (skin), parenchyma, and excretory cells (Figure 1A). Not only can users examine the expression of genes across sexes and between worms at distinct stages of sexual maturation, but all genes are linked to WormBase ParaSite<sup>ii</sup> [13]. By directly interfacing with WormBase ParaSite, users can acquire sequences for genes of interest and also determine whether homologous genes are present in various flatworm species, including other schistosomes such as *Schistosoma haematobium* and *Schistosoma japonicum*. This will give researchers working in these other flatworm systems an opportunity to make hypotheses regarding what genes might be cell-type-specific markers in their model.

We have also incorporated two 'explorer' features – 'Gene Explorer' and 'Cluster Explorer' – that allow users to explore the data in more depth. Gene Explorer allows users to search for genes based on either

(A)



(B)



Gene Ontology terms or Interpro domains, and then examine where these genes are expressed in the worm. This feature will allow users interested in specific classes of proteins (e.g., kinases) or cellular processes (e.g., mitosis) to examine what cell types these proteins or processes might function in. The newly added Cluster Explorer feature allows users to click on cell clusters of interest and explore what transcripts are significantly enriched in these clusters. This feature will provide users with an opportunity to make connections between genes based on cell-type-specific coexpression that were previously not possible using non-single-cell-based gene-expression approaches. In addition to providing a user-friendly portal for accessing scRNAseq data, we have designed the platform to be modular to include gene-specific information that is challenging to fit either into supplemental materials or in standard databases. In particular, SchistoCyte contains over 100 videos depicting RNA interference phenotypes from a recent large-scale RNAi screen [14] (Figure 1B).

Although single-cell transcriptional studies have the potential to transform our understanding of these parasites, they are not without their limitations. Depending on the technology utilized to generate single-cell transcriptomes, there are tradeoffs between the number of cells that can be analyzed in a single experiment and the sensitivity to capture transcripts over a large dynamic range. Thus, lowly expressed transcripts can be overlooked in some analyses. Also, since some cells/tissues are either rare or challenging to purify from the worm, scRNAseq-based analyses may not provide a complete

picture of the cellular repertoire of the parasite. Finally, there are a myriad of analysis platforms, and even more analysis parameters in these platforms, all of which can shape the outcome of the final scRNAseq product. Therefore, it is essential that data from a variety of sources (both computational and experimental validation) are brought together to define the 'true' cellular make-up of the worm. Thus, we look forward to incorporating experimental validation (e.g., *in situ* hybridization data) and expanding the site to include not only additional scRNAseq atlases but reanalysis of our and other scRNAseq datasets.

Paramount to developing new strategies for combating schistosomiasis is a continued community-wide effort to gain deeper understanding of the parasite's biology. This includes not just those of us working at major western research institutions, but also our colleagues working in endemic regions. Therefore, a sustained effort to make our research widely accessible is critical. We hope that resources such as SchistoCyte and other scRNAseq resources for schistosomes<sup>iii</sup> will complement existing community-wide resources (e.g., Wormbase ParaSite<sup>ii</sup>).

#### Acknowledgments

This work was supported by the National Institutes of Health (NIH) grants R01AI150776 (J.J.C.), R01AI121037 (J.J.C.), R01AI150715 (M.L.R.), the National Science Foundation (NSF) MCB1553334 (M.L.R.), and the Welch Foundation I-1948-20210327 (J.J.C.).

#### Declaration of Interests

The authors declare no competing interests.

#### Resources

<sup>i</sup>[www.collinslab.org/SchistoCyte](http://www.collinslab.org/SchistoCyte)

<sup>ii</sup><https://parasite.wormbase.org/index.html>

<sup>iii</sup>[www.schistosomulacellatlas.org/](http://www.schistosomulacellatlas.org/)

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<https://doi.org/10.1016/j.pt.2021.04.010>

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#### References

- Chitsulo, L. *et al.* (2000) The global status of schistosomiasis and its control. *Acta Trop.* 77, 41–51
- van der Werf, M.J. *et al.* (2003) Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Trop.* 86, 125–139
- Colley, D. (2020) Contributions of the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) to Schistosomiasis control and elimination: key findings and messages for future goals, thresholds, and operational research. *Am. J. Trop. Med. Hyg.* 103, 125–134
- Lu, Z. *et al.* (2017) A gene expression atlas of adult *Schistosoma mansoni* and their gonads. *Sci. Data* 4, 170118
- Hahnel, S. *et al.* (2013) Whole-organ isolation approach as a basis for tissue-specific analyses in *Schistosoma mansoni*. *PLoS Negl. Trop. Dis.* 7, e2336
- Wilson, R.A. *et al.* (2015) The schistosome esophagus is a 'hotspot' for microexon and lysosomal hydrolase gene expression: implications for blood processing. *PLoS Negl. Trop. Dis.* 9, e0004272
- Wang, J. and Cols, J.J., III (2016) Identification of new markers for the *Schistosoma mansoni* vitelline lineage. *Int. J. Parasitol.* 46, 405–410
- Wendt, G.R. *et al.* (2018) Flatworm-specific transcriptional regulators promote the specification of tegumental progenitors in *Schistosoma mansoni*. *eLife* 7, e33221
- Wang, B. *et al.* (2018) Stem cell heterogeneity drives the parasitic life cycle of *Schistosoma mansoni*. *eLife* 7, e35449
- Diaz Soria, C.L. *et al.* (2020) Single-cell atlas of the first intra-mammalian developmental stage of the human parasite *Schistosoma mansoni*. *Nat. Commun.* 11, 6411
- Li, P. *et al.* (2021) Single-cell analysis of *Schistosoma mansoni* identifies a conserved genetic program controlling germline stem cell fate. *Nat. Commun.* 12, 485
- Wendt, G. *et al.* (2020) A single-cell RNA-seq atlas of *Schistosoma mansoni* identifies a key regulator of blood feeding. *Science* 369, 1644–1649
- Howe, K.L. *et al.* (2017) WormBase ParaSite – a comprehensive resource for helminth genomics. *Mol. Biochem. Parasitol.* 215, 2–10
- Wang, J. *et al.* (2020) Large-scale RNAi screening uncovers therapeutic targets in the parasite *Schistosoma mansoni*. *Science* 369, 1649–1653

**Figure 1. The SchistoCyte User Interface.** (A) Screen shot showing the Cluster Explorer feature where users can click on any one of 68 clusters and pull up a gene whose expression is enriched in the cluster. (B) Screen shot showing view for gene *Smp\_096640*, its expression across tissues, and a link to a video showing its RNAi phenotype (left).