Peer Community In Genomics

Genomic and transcriptomic insights into the genetic basis of anthelmintic resistance in a cyathostomin parasitic nematode

Nicolas Pollet *based on peer reviews by 2 anonymous reviewers*

Guillaume Sallé, Élise Courtot, Cédric Cabau, Hugues Parrinello, Delphine Serreau, Fabrice Reigner, Amandine Gesbert, Lauriane Jacquinot, Océane Lenhof, Annabelle Aimé, Valérie Picandet, Tetiana Kuzmina, Oleksandr Holovachov, Jennifer Bellaw, Martin K. Nielsen, Georg von Samson-Himmelstjerna, Sophie Valière, Marie Gislard, Jérôme Lluch, Claire Kuchly, Christophe Klopp (2025) Spatio-temporal diversity and genetic architecture of pyrantel resistance in *Cylicocyclus nassatus*, the most abundant horse parasite. bioRxiv, ver. 2, peer-reviewed and recommended by Peer Community in Genomics.
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Parasitic worms infect billions of animals worldwide. While parasitism is now considered a context-dependent relation along a symbiosis continuum, most of these parasitic worms, also known as helminths, can cause diseases that have a significant impact (Hopkins et al. 2017; Selzer, Epe 2021). When considering livestock animals, these impacts have a high economic cost, and therefore, prophylactic drugs are widely used (Selzer and Epe 2021). Consequently, drug resistance has become increasingly common across all parasites and concerns about drug effects on non-target organisms have been raised (de Souza and Guimarães 2022). This is why understanding the relationship between parasitic worms and their animal hosts and the diseases they cause at the genetic and molecular level is high on the agenda of parasitologists (Doyle 2022). The development of genomics resources plays a pivotal role in this agenda and is at the origin of Sallé and colleagues' article (2025).

The most common intestinal parasites in equids are helminths of the cyathostomin nematode complex. These are the primary parasitic cause of death in young horses and also exhibit a reduced sensitivity to

anthelmintic drugs. Therefore, Sallé and colleagues embarked on the arduous journey to build a reference annotated genome of the *Cylicocylus nassatus* nematode. They used cutting-edge molecular genetics methods to amplify and sequence the genome of a single individual and obtained chromosomal-level contiguity using Hi-C technology for six chromosomes and an assembly of 514.7 Mbp. Remarkably, transposable elements occupy more than half of the *C. nassatus* genome and may have led to an increase in genome size in this nematode. In parallel, the authors built a gene catalogue using transcriptomic data, reaching a BUSCO gene completion score of 94.1% with 22,718 protein-coding genes. They quantified allele frequencies based on the resequencing of nine populations, including an ancient Egyptian worm from the 19th century, indicating a recent loss of genetic diversity in European cyathostomin even if geographical sampling was limited. They also analysed transcriptomic differences between sexes and found differences linked with drug treatment. While there may be confounding effects due to global differences between sex that could explain this finding, these results will likely fuel future transcriptomic analyses investigating the response to antiparasitic drugs.

The *Cylicocylus nassatus* genome assembly obtained will be invaluable for studying nematode genome evolution and analysing the genetic and molecular basis of drug resistance in these parasites.

References:

Doyle SR (2022) Improving helminth genome resources in the post-genomic era. Trends in Parasitology, 38, 831–840. https://doi.org/10.1016/j.pt.2022.06.002

Hopkins SR, Wojdak JM, Belden LK (2017) Defensive symbionts mediate host–parasite interactions at multiple scales. Trends in Parasitology, 33, 53–64. https://doi.org/10.1016/j.pt.2016.10.003

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Selzer PM, Epe C (2021) Antiparasitics in animal health: *quo vadis*? Trends in Parasitology, 37, 77–89. https://doi.org/10.1016/j.pt.2020.09.004

de Souza RB, Guimarães JR (2022) Effects of avermectins on the environment based on its toxicity to plants and soil invertebrates–a review. Water, Air, and Soil Pollution, 233, 259. https://doi.org/10.1007/s11270-022-05744-0

Reviews

Evaluation round #2

Reviewed by anonymous reviewer 2, 07 January 2025

The reviewers have responded to all the concerns I raised and have edited their manuscript accordingly. I think the manuscript can be published in its current form.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.07.19.549683
Version of the preprint: 1

Authors' reply, 10 November 2024

Dear recommender,

Please find attached our point-by-point response to the referees and the corresponding revised version.

Best wishes,

Guillaume Sallé

Download author's reply

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Decision by Nicolas Pollet, posted 03 October 2023, validated 03 October 2023

Dear Guillaume Salé and colleagues,

Your manuscript entitled "Spatio-temporal diversity and genetic architecture of pyrantel resistance in Cylicocyclus nassatus, the most abundant horse parasite" has been reviewed by two colleagues. Globally the reviews are of high quality and very positive, and find that your study has many merits, but there are a some criticisms that you need to adress before I can finally decide on this preprint recommendation.

With my best wishes,

Nicolas Pollet

Reviewed by anonymous reviewer 1, 20 September 2023

The paper is an interesting read and makes a very valuable contribution to the equine parasitology research community. The authors are to be commended for taking a very methodical and careful approach to delivering a high-quality genome for a parasite species that has proved challenging in the past. Cyathostomins are not very tractable organisms with which to work and this manuscript reports a first success in provding a chromosomal level genome assembly. The work is very well done and is articulately described in the text. It provides interesting insights in cyathostomin biology and moves on the field significantly. The paper is generally well written and the authors provide convincing evidence for almost all of their conclusions. I have just one query for them to address:

My query centres around the section 'Transcriptomic differences across sexes upon pyrantel exposure'. The authors propose that differences in the expression profiles between males and female may be due to developing eggs in the females or sex-specific differences in their response to pyrantel. However, there could be many reasons for the differences between the sexes, gastrointestinal nematode infections are not equal in terms of the number of male and female worms residing in the gut. Female worms are often more numerous and male worms can be polyandrous by nature. This raises the prospect of males being more motile as they move from mate to mate, which is one other way to explain differences between the sexes. There will likely be many other differences between the sexes. I remain unconvinced that the difference in their data is due to sex-dependent sensitivity as stated in lines 610-611 and would prefer to see some further discussion of these data.

Minor typos and text edits

1. Line 60 'on' rather than 'to' per and livestock species
2. Line 62 'to' the brink of distinction
3. Line 65 'anthelmintic'
4. Lines 70 and 72 'S. vulgaris' and 'C. nassatus' should be in italics, if fact there are many examples throughout the text where parasite names are used and not italicised. They all need to be corrected.
5. Lines 71-73 use a comma rather than a hyphen
6. Line 75 'and' zebras

7. Line 108 ‘decreased’
8. 129 ‘Ukraine to Kentucky’
9. Line 130-132 sentence needs reworking – what does ‘in that species’ refer to?
10. Line 317 ‘end-repaired is repeated’
11. Line 423 ‘Cylicostephanus’ spelling
12. Line 504 ‘The last century affected allele frequencies over known anthelmintic drug targets’ sounds a little clumsy. Do the authors mean ‘Allele frequencies of known anthelmintic targets has altered over the last century’? Or ‘Alteration in allele frequencies over the last century are found within genomic regions encoding anthelmintic drug targets? I think this title needs rewording.
13. Line 562 Figure 3 legend a.’ The number of private SNPs for a given isolate’
14. Line 585 (reference isolate – this mean STR?)
15. Table 1 legend ‘Bold names were covered by at least one? Decisive SNP….’
16. Figure 5 The legend and citation of this figure in the text is somewhat confusing and requires clarification. Some further explanation of the relationship between FECRT and allele frequency needs to be provided.
17. Line numbers now absent – First paragraph page 21. ‘can be reproduced in another organism’ – this sentence needs clarification as to what organisms this is referring to.
18. Line 9 page 22 ‘about twice the size’ or twice as large’
19. Paragraph 3 for ‘complexifies’ substitute ‘becomes more complex, as it remains to be determined….’?

Reviewed by anonymous reviewer 2, 01 October 2023

The manuscript “*Spatio-temporal diversity and genetic architecture of pyrantel resistance in Cylicocyclus nassatus, the most abundant horse parasite*” by Sallé et al. presents a detailed study of the genome and genetic diversity of *Cylicocyclus nassatus*, the most prevalent species from the cyathostomin complex, a group of parasitic nematodes that infect horses and wild equids. The manuscript is the result of an impressive set of work: the authors have taken an important parasite from a clade of nematodes with practically no genomic data and have generated a very high-quality genome, a resequencing dataset, and sex-specific transcriptomes. They use these datasets to explore the genetic basis of pyrental resistance. The results are interesting and highlight several candidate loci that may play a role in pyrantel sensitivity. The manuscript will be of interest to those interested in parasite genetics and drug resistance in nematodes and flatworms. I commend the authors for making all data available on the relevant INSDC databases, which facilitated my review of their manuscript.

My only major comment is that the manuscript, in some places, suffers from an overly simplistic or one-sided interpretation of the results (which I detail below). In most cases, this doesn’t invalidate the major claims of the manuscript, but providing a more nuanced discussion of the results would really help readers understand the strengths and limitations of the study.

General comments

Although the reference genome is evidently of high quality, I found the presentation of the genome assembly results lacking detail in places, which led me to download the genome and annotation and calculate my own metrics. The following additions would help:

- “666,884 Kbp” would be better as 666.8 Mbp.
- The authors only quote scaffold N50 of the final assembly, but it would be good to quote the contig N50 as well (which looks to be 671 kb).

- The post-purging assembly size was 666.8 Mb, but the final assembly size was 514.7 Mb - what happened to the other ~150 Mb during Hi-C scaffolding and curation?
- The authors list BUSCO completeness which, based on the text, is based on running BUSCO on the proteome. It would be useful to also present the BUSCO completeness of the genome because gene sets may have lower completeness due to missing genes. It would also be useful to contextualise both values by comparing them to e.g. the *H. contortus* reference genome and annotation (my analysis suggests that the C. nassatus genome is more complete, at least at the genome level, than *H. contortus*).
- Adding the coverage of the HiFi data (along with whether it was derived from PacBio LI or ULI or both) would be also useful.

In my opinion, the authors' analysis of gene family evolution adds very little to the manuscript:

- When deciding which nematode species to analyse, the authors used only those with scaffold N50s above 1 Mb (presumably to avoid their analysis being influenced by poor-quality assemblies). However, this necessarily led to a very spare sampling of the tree, including just three species of strongylid. The lack of dense sampling within Strongylida means that it's hard to know if the gene families they highlight are the result of expansion in *C. nassatus* specifically, the result of expansion in the suborder Strongylina generally, or the result of contraction in *A. ceylanicum* and/or *H. contortus*. A more dense sampling, even if it included lower quality assemblies, would have provided far greater resolution (and likely far more informative results).
- The authors also comment on the expansion of a family of transposons and how that may explain the larger genome size of C. nassatus. While I have no doubt that transposon proliferation has led to an increase in genome size in *C. nassatus*, analysing individual gene families via orthology clustering is not the most appropriate analysis to do this. If the authors do want to understand genome size evolution in *C. nassatus*/strongylids (and I'm not convinced they need to, given all the other work they present), it would be far better to use repeat annotations for a variety of species and analyse which classes appear to have proliferated (or been lost) and what their relative contribution to genome size is.

I am not an expert on population genetic analyses, so other reviewers may be able to critically assess those sections better than I could, but I do have the following comments/questions:

- I was surprised not to see more discussion of the genome-wide patterns of genetic diversity in the main text, especially given the authors generated a chromosome-level reference genome. How was genetic diversity distributed through the genome? Was the pattern qualitatively similar to that reported for other nematodes (e.g. *H. contortus*)?
- As far as I can tell, the author's only evidence that present-day *C. nassatus* populations have less genetic diversity than those that existed ~200 years ago was that the single ancient Egyptian isolate has more genetic diversity than any of the present-day isolates. Without having species-wide sampling of *C. nassatus*, surely the authors cannot say whether this is due to a loss of genetic diversity in modern populations over the last century, or simply because e.g. North African populations have higher genetic diversity (both in the present-day and in the past). The authors should consider this possibility in the results and discussion and note the under/un-sampled regions of the world.

The authors used a range of techniques and approaches to sequence the *C. nassatus* genome, but it was often unclear why they used the methods they did, and how the data were used during assembly. Given their genome sequencing/assembly methods are likely to be of interest to many readers, it would be great to be clearer in the methods and results about what they did:

- The authors isolated DNA from a single worm and an aliquot of this DNA was used for REPLI-G amplification, while the rest was left unamplified. What was the amplified DNA used for?

The authors used both the PacBio LI (which doesn't involve amplification) and ULI protocol (which does use amplification). What was the reasoning for this? How were the data used during assembly? How did they compare?

- The authors mention using 1 µg of DNA for PacBio LI - how much DNA did the authors isolate in total from their worm? 1 µg is a surprisingly large amount of DNA from a single worm (although it's possible I'm underestimating how large these nematodes are).

The bias in sex ratios after pyrantel treatment is interesting. However, I am not fully convinced that the transcriptomic differences the authors identify are the result of sex-specific differences in response to pyrantel treatment:

- The authors implicitly assume that the sex ratio in the infecting population is 50/50, meaning that the bias they observe after pyrantel treatment must be due to sex-specific differences in response. Although that seems likely, is there any information on sex ratios in an adult (in vivo population) of cyathostomins or other strongyles? Is it possible that there are normally more females than males? Do females live longer than males over the course of the infection?

- The authors suggest that differences between the male and female transcriptomes transcriptome must either be the result of egg development in utero in females or transcriptomic differences due to pyrantel treatment and therefore, after removing genes that are believed to be expressed in the oocyte, germ line or embryonic lineages, all remaining differentially expressed genes are the result of pyrantel treatment. This seems overly simplistic and ignores the fact that there are likely general differences in male and female transcriptomes. Indeed, a quick scan of the literature suggests that sex-specific transcriptome analyses consistently find hundreds of genes that show sex-specific differences in expression (in the absence of an external stressor, like pyrantel).

- I think it's also important to acknowledge the lack of replication in the dataset. I realise how difficult this would be to achieve but simply adding more detail to the results about what samples were used for transcriptome sequencing would facilitate the interpretation of the results.

Minor comments

The term 'telomere-to-telomere' refers to gap-free genome assembly (e.g. the new human reference genome, or the C. elegans reference genome). There are no telomere-to-telomere strongylid genomes. Better to use 'chromosome-level' or similar.

The Hi-C plot in Figure S1 appears to be truncated.

There doesn't seem to be any information in the methods about how the *C. nassatus* genome was aligned to the *H. contortus* genome (although promer is noted in the Figure S3 caption). Also, using one-to-one orthologues, rather than promer, might reduce some of the noise seen in Figure S3.

It might just be me but I think it's better to write 19th century rather than XIXth.

The authors might want to define what they mean by 'decisive SNP'.