The genetic basis of adaptive evolution in parasitic environment from the Angiostrongylus cantonensis genome

Lian Xu¹,²,³*, Meng Xu¹⁴, Xi Sun¹,²,³, Junyang Xu⁴, Xin Zeng¹,²,³, Sha Shan⁴, Dongjuan Yuan¹,²,³, Ping He¹,²,³, Weiming He⁴, Yulan Yang⁴, Shiqi Luo¹,²,³, Jie Wei¹,²,³, Xiaoying Wu², Zhen Liu¹,²,³, Xiaomin Xu⁴, Zhensheng Dong⁴, Langui Song¹,²,³, Beibei Zhang¹,²,³, Zilong Yu¹,²,³, Lifu Wang¹,²,³, Chi Zhang⁴, Xiaodong Fang⁴, Qiang Gao⁴*, Zhiyue Lv¹,²*, Zhongdao Wu¹,²*

¹ Department of Parasitology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China, ² Key Laboratory for Tropical Diseases Control of the National Ministry of Education, Sun Yat-sen University, Guangzhou, China, ³ Provincial Engineering Technology Research Center for Disease and Vector Control, Guangdong, Guangzhou, China, ⁴ BGI Genomics, BGI-Shenzhen, Shenzhen, China

* These authors contributed equally to this work.
* gaoqiang@genomics.cn (QG); lvzhiyue@mail.sysu.edu.cn (ZL); wuzhd@mail.sysu.edu.cn (ZW)

Abstract

Angiostrongylus cantonensis (rat lungworm) is the etiological agent of angiostrongyliasis, mainly causing eosinophilic meningoencephalitis in human. Although the biology of A. cantonensis is relatively well known, little is understood about the mechanisms of the parasite’s development and survival in definitive hosts, or its adaptation to a broad range of snail intermediate hosts. Here, we generate a high-quality assembly of a well-defined laboratory strain of A. cantonensis from Guangzhou, China, by using Illumina and PacBio sequencing technologies. We undertake comparative analyses with representative helminth genomes and explore transcriptomic data throughout key developmental life-cycles of the parasite. We find that part of retrotransposons and gene families undergo multiple waves of expansions. These include extracellular superoxide dismutase (EC-SOD) and astacin-like proteases which are considered to be associated with invasion and survival of the parasite. Furthermore, these paralogs from different sub-clades based on phylogeny, have different expression patterns in the molluscan and rodent stages, suggesting divergent functions under the different parasitic environment. We also find five candidate convergent signatures in the EC-SOD proteins from flukes and one sub-clade of A. cantonensis. Additionally, genes encoding proteolytic enzymes, involved in host hemoglobin digestion, exhibit expansion in A. cantonensis as well as two other blood-feeding nematodes. Overall, we find several potential adaptive evolutionary signatures in A. cantonensis, and also in some other helminths with similar traits. The genome and transcriptomes provide a useful resource for detailed studies of A. cantonensis-host adaptation and an in-depth understanding of the global-spread of angiostrongyliasis.
Author summary

*Angiostrongylus cantonensis*, rat lungworm, is a common pathogen that causes human eosinophilic meningitis via eating contaminated food. Human angiostrongyliasis has been reported globally. This worm has a complex life-cycle, which includes an especially wide range of snails as intermediate hosts, making it more difficult to eradicate. In this study, we sequenced the genome and transcriptome, and performed comparative analyses to study the potential genetics of its biology using short-read and long-read sequencing technologies. We revealed some potential adaptive evolution in the genome, such as the expansion of retrotransposons and gene families encoding antioxidant enzymes, invasion, migration and digestion related proteases. Specifically, we found a potential clue suggesting convergent evolution of EC-SODs in *Angiostrongylus* and flukes, all of which require snails as intermediate hosts. These results provide an abundant data resource to study the biology and evolution of *A. cantonensis* and showed some potential targets against *A. cantonensis* and helminths with similar traits.

Introduction

*Angiostrongylus cantonensis* (rat lungworm) is a parasitic roundworm (nematode) of the superfamily Metastrongyloidea, with a complicated life cycle via a gastropod intermediate host [1]. More than twenty species of *Angiostrongylus* have been discovered in rodents, carnivores and insectivores, and two of them *A. cantonensis* and *A. costaricensis* are human parasites [1]. *A. cantonensis* is the most common infectious cause of eosinophilic meningitis in humans, causing central nervous system (CNS) angiostrongyliasis [2]. Since the first human CNS angiostrongyliasis case reported in 1945 [3], other clinical symptoms including ocular disease, encephalitis and fever of unknown origin have been reported for this disease [4–6]. While most cases were reported in Asia, the Pacific Basin and Australia, human angiostrongyliasis has been found emerging worldwide in the past decades, including USA, France and the UK [7–10] (Figure S1 in S1 Supporting Information).

The life cycle of *A. cantonensis* involves a molluscan intermediate host (various species) and a definitive rodent host *(cf. review [11], Fig 1)*. Briefly, the first-stage larvae (L1) are swallowed by an intermediate host, they molt twice into third-stage larvae (L3). The infective L3 are ingested by a definitive host, then they migrate to the brain and molt twice into young adults (L5). Eventually, the L5 migrate to the lungs where develop to sexual maturity and lay eggs. The eggs embryonate, develop and hatch to L1 and they are excreted in host feces, restarting the life cycle. This worm can infect a very wide range of intermediate hosts, comprising at least 160 species belonging to 44 families of freshwater and land gastropods [12]. The two available assemblies are highly fragmented in nature which has posed as an obstacle to detailed biological and evolutionary investigations [13, 14].

In the present study, we sequenced and assembled a high-quality reference genome of a well-defined laboratory strain of *A. cantonensis* from Guangzhou, China. Through analyses of comparative genomics and transcriptome, we explored potential molecules regarding the nematode survival in intermediate host and/or definitive host.
Methods

Ethics statement

Procedures involving animals and their care described here were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University (Permit No: 2016–055) and followed the National Guidelines for Experimental Animal Welfare (MOST, China, 2006).

Sample preparation and sequencing

The life-cycle of *A. cantonensis* was established and maintained in the Department of Parasitology, Zhongshan School of Medicine. L1 larvae were separated from feces of rats. L3 were isolated from experimentally infected snails using the method previously described by Zeng [15]. Sprague Dawley rats were challenged with L3 (200 per animal) via intragastric administration. Procedures for animal care described herein were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University. Other developmental worms (L4, L5 and mature adults, including female and male) were harvested from rats at 21, 28, and 48 days post-infection (dpi) respectively. Genomic DNA was extracted from ten adult worms. Seven paired-end and mate-pair whole-genome shotgun libraries (250bp, 500bp, 800bp, 2kb, 5kb, 10kb, and 20kb, Table S1 in S2 Supporting Information) were constructed and then sequenced using the Illumina HiSeq 2000 platform. Another 20 kb library for PacBio sequencing was constructed and sequenced using RSII. RNA was extracted from different developmental stages of *A. cantonensis* (L1, L3, L4, L5 and mature adults, the *Pomacea canaliculata* used as an intermediate host), respectively. Seven cDNA libraries were sequenced using the Illumina Hiseq 2000 platform.
platform. Another four cDNA libraries (L3 and L4, the *Biomphalaria glabrata* used as an intermediate host) were constructed and sequenced using the Illumina HiSeq 4000 platform.

**Genome assembly and annotation**

We employed a hybrid assembly strategy by combining Illumina and PacBio data (Figure S3 in S1 Supporting Information). Illumina reads were first assembled into contigs using the *Platynus* [16] (v1.2.4) with default parameters. The resulting Illumina contigs and PacBio sub-reads were further used to assemble with DBG2OLC [17] pipeline (release Jun 2015). Then, correction of the assembly was performed twice with Pilon [18] (v1.22) by using Illumina reads. To further link the corrected contigs, the corrected PacBio reads and Illumina mate-pair reads were employed to extend and link into scaffolds using SSPACE-LongRead [19] (v1–1) and SSPACE [20] (v2.0). Remaining gaps within these scaffolds were filled with GapCloser available in SOAPdenovo2 [21]. CEGMA (Core Eukaryotic Genes Mapping Approach) [22] (v2.4), BUSCO (benchmarking universal single-copy orthologues) [23] (v3.0.1), and *de novo* assembled transcripts with Trinity [24] (v2.0.6) were used to assess the completeness of the assembly.

Protein-coding gene models were predicted using a strategy to combine the homology-based prediction and RNA-seq data as previously described [25] (cf. S1 Supporting Information, Supplementary Methods and Results sections). The functional annotation of protein-coding genes was performed using BLASTP alignment to databases: Swiss-Prot (release Jun 2019), TrEMBL (release Jun 2019), NCBI NR (release Sep 2017) and KEGG (release 89). InterPro domains and GO terms were assigned with InterProScan [26] (release 5.3).

Repetitive elements (REs) in the assembly were identified using a combination of homology- and *ab initio*-based approaches. RepeatMasker and RepeatProteinMask (http://www.repeatmasker.org/, version open-4-0-5) were applied to detect homologous REs in the RepBase database (v20.04). PILER [27] (v1.0), RepeatScout [28] (v1.0.5), and LTR-Finder [29] (v1.0.6) were used to build a *de novo* repeat library. RepeatMasker was run against the *de novo* library. The same pipeline was employed to predict REs in seven other nematode genomes (*A. suum* [30], *Brugia malayi* [31]; *Caenorhabditis elegans* [32], *Haemonchus contortus* [33, 34], *Necator americanus* [35], *Meloidogyne hapla* [36]). For RTE-RTE transposable elements, proteins of RTE-RTEs deposited in RepeatPep (RepeatMasker-open-4.0.6) were collected and used to search in eight nematode genomes using homology-based prediction pipeline as delineated in gene prediction, except with an alignment rate of more than 50%. Amino acid sequences encoding a reverse transcriptase (RT, PF00078) domain were aligned using MUSCLE [37] (v3.8.31) and then were constructed a phylogenetic tree using FastTree [38] (v2.1).

**Genome evolution**

The OrthoMCL [39] pipeline was used to determine orthologous groups in *A. cantonensis* and seven other represented nematode genomes (*A. suum, B. malayi; C. elegans, H. contortus, N. americanus, M. hapla* and *T. spiralis*, related data was downloaded from the WormBase [40] (version 246). 788 single-copy orthologous genes were extracted to build a phylogenetic tree. Sequences from each single copy orthologs were aligned using MUSCLE and then filtered with trimAl [41] (v1.2) with default parameters except “-gt 0.5”. RAxML [42] (v8.2) was used to construct gene tree with “GTR+GAMMA” model. Finally, ASTRAL [43] (v5.6.1) was used to construct species tree based on 788 gene trees. Phylogenetic relationship among eight nematodes and six flatworms (*Schistosoma japonicum* [44], *S. mansoni* [45], *S. haematobium* [46], *Opisthorchis viverrini* [47], *Clonorchis sinensis* [48] and *Schmidtea mediterranea* [49], related data was downloaded from WormBase Parasite [50], WBPS5) was resolved using the method...
described above based on 173 single-copy orthologous genes. Species divergence time was estimated using MCMCTREE, which is part of the PAML package [51] (v4.5). Published times for *T. spiralis* and *C. elegans* (~428 million years ago, mya), and *B. malayi* and *C. elegans* (~241 mya) divergence were used to calibrate divergence time [52]. We investigated the expansion and contraction of gene families using the CAFÉ [53] (Computational Analysis of gene Family Evolution, v2.1), which infers the dynamics of the gene family under a stochastic birth and death model.

**Identification, evolution and expression of specific gene or gene families**

To avoid systematic biases, for example, different methods in the annotation of the previously published helminth draft genomes, we adopted a uniform strategy to re-annotate and check candidate genes screened from the above comparative analysis. Generally, protein sequences of superoxide dismutase (SOD) genes, astacin-like genes, and several hemoglobin digestion proteases of nematodes deposited in Swiss-Prot or MEROPS [54] (download in Nov 2016) databases were downloaded and mapped to the genomes using the homology-based gene prediction. We also manually checked these putative genes and compared with the original gene annotation (The associations of the gene IDs used in this study and the gene IDs in Wormbase are listed in Table S9 in S2 Supporting Information). Phylogenomic analyses of the gene families studied herein were based on protein sequences. The best model of amino acid replacement was estimated using ProtTest [55] (v3.4.2) software. The phylogenetic trees of these genes were constructed using PhyML [56] (v3.0) software, respectively. For EC-SODs, we also reconstructed the phylogenetic trees using RAxML and IQ-TREE [57] (v1.6.5), and conducted a hypergeometric test site by site at amino acid level to detect the potential convergent evolution [25] between the genus *Angiostrongylus* and flukes in a broad range of 141 EC-SODs from 62 species (43 nematodes and 19 platyhelminths, Table S10 in S2 Supporting Information).

For RNA-seq analysis, we mapped RNA-seq reads to the genome with Tophat2 [58] (v2.0.8). We quantitated the gene expression level using uniquely mapped reads and measure in reads per kilobase per million reads (RPKM). The expression of EC-SOD and MTP-1 subclade I and II genes were validated using real-time PCR (qPCR, primers are shown in Table S8 in S2 Supporting Information), with pooled larvae/adults isolated from multiple hosts for each developmental stage. β-actin was used as an internal control. The relative changes in gene expression were calculated by equation 2−ΔΔCT, where ΔΔCT=CT(target)−CT(Actin)Time x−(CT(Target)−CT(Actin))Time 0. Time x is any time point and Time 0 represents the 1× expression of the target gene normalized to β-actin [59].

**Results**

**Genome assembly and annotation**

The genome of *A. cantonensis* was sequenced using the Illumina HiSeq 2000 and PacBio RSII platforms, yielding a total of ~267-fold and ~41-fold coverage, respectively (estimated genome size: 290 Mb; Figure S2 in S1 Supporting Information and Table S1 in S2 Supporting Information). The final genome assembly included 282 Mb in 816 contigs and 425 scaffolds, with a contig N50 of 993 kb and a scaffold N50 of 1.8Mb (Table 1). The assembly covered more than 95% (coverage ≥ 70%) of the assembled RNA-seq transcripts, indicating that the gene region was well represented (Table S2 in S2 Supporting Information). In addition, both the CEGMA and BUSCO methods were used, and the results showed that the assembly in this study was more complete than the published fragment assemblies of *A. cantonensis* in the protein-coding region (Table 1). Taken together, the results showed that the present genome assembly of *A. cantonensis* represented a substantial part of the whole genome. Combined homology-
based and RNA-seq methods, we predicted 13,473 protein-coding gene models (5.16% of the assembly, spanning ~15 Mb) in *A. cantonensis* genome. Of these genes, 13,114 (97%) were supported by RNA-seq data (RPKM ≥ 1 at least one sample), and 12,407 (92.1%) genes either had homologues in public databases (Swiss-Prot, KEGG, and NCBI NR).

Transposable elements (TEs) represented 54.61% of the assembled genome, representing a greater percentage than most parasitic nematode genomes characterized to date (3.4–32.9% using the same bioinformatics pipeline; Table S3 in S2 Supporting Information). RTE-RTE retrotransposons, belonging to the long interspersed elements (LINEs), were the most abundant group in *A. cantonensis* genome, representing 39.2% of the genome and 72% of repeats respectively (Table S4 in S2 Supporting Information), a markedly higher percentage than the genomes of other studied nematodes (0~5.1%; Table S3 in S2 Supporting Information). Sequence divergence of extant RTE-RTE copies compared to the repeat consensus showed at least two periods of element expansion in *A. cantonensis* (Fig 2a). The phylogeny showed that the RTE-RTEs expanded independently in *A. cantonensis*, *N. americanus*, and *H. contortus*, but the *A. cantonensis* displayed substantially higher divergence and abundance than the other two strongyloids (Fig 2).

## Genome evolution

To better understand the evolution of *A. cantonensis* genome and to infer genes or gene families associated with parasitism, we performed a comparative analysis with seven other nematode genomes representing clades I, III, IV and V (*A. suum, B. malayi; C. elegans, H. contortus, N. americanus, M. hapla and T. spiralis*). We identified 788 one-to-one orthologous genes in all eight nematodes and assessed the phylogenetic relationships using a coalescent-based method [43]. The phylogenetic analysis showed that *A. cantonensis* was genetically closer to *H. contortus* (Figure S5 in S1 Supporting Information), which was the same as the study of the 50 Helminths Genome Project [13]. We inferred that 26 and 119 gene families respectively underwent significant expansion and contraction in the *A. cantonensis* lineage (Viterbi $P_{≤0.05}$; Tables S6 and S7 in S2 Supporting Information, Figure S6 in S1 Supporting Information). Expanded genes included protease (neprilysin-1 and legumain), transporter (sodium-dependent high-affinity dicarboxylate transporter 3), receptor (acetylcholine receptor) and ancylostoma secreted protein. Based on the OrthoMCL cluster result, we also identified 454 genes (159 groups; Table S5 in S2 Supporting Information) that appeared to be unique in *A. cantonensis*, which were significantly enriched in GO terms of "Superoxide dismutase activity".

### Table 1. Statistics of the *A. cantonensis* assemblies.

|                      | This study | Yong et al [14] | Avril et al [13] |
|----------------------|------------|----------------|-----------------|
| Assembly size (Mb)   | 283        | 260            | 253             |
| Scaffold number $^a$ | 425        | 16,326         | 18,635          |
| Gaps (bp)            | 1,480,142  | 25,114,564     | 4,505,497       |
| Contig N50 (kb); Scaffold N50 (kb) | 993; 1,815 | 1.7;42.2       | 27.1;43.9       |
| GC content %         | 41.7       | 41.2           | 41.5            |
| Complete BUSCOs [Duplicated]$^b$ | 84.3%[1.2%] | 62.5%[0.8%] | 70.0%[1.2%] |
| Fragmented BUSCOs    | 7.80%      | 12.30%         | 10.20%          |
| Missing BUSCOs       | 7.90%      | 25.20%         | 19.80%          |
| CEGMA completeness   | 97.98%     | 80.24%         | 82.66%          |

$^a$, length cut-off: 500bp;  
$^b$, Nematoda_odb9 dataset was used

https://doi.org/10.1371/journal.pntd.0007846.t001
(GO:0004784) and metallopeptidase activity” (GO:0008237) (adjusted P-value < 0.05, Figure S4 in S1 Supporting Information). These “unique” genes included astacin-like metalloproteinase (M12A), Aspartic protease (A01) and Extracellular superoxide dismutase (EC-SOD) which are likely related to the invasion, migratory and digestive processes, innate immune of A. cantonensis. Those expanded or specific genes may provide novel clues to A. cantonensis adaptation to hosts.

**Expansion of EC-SOD genes related to host adaptation in A. cantonensis**

Reactive oxygen species (ROS), such as oxygen radicals and superoxide, are generated by phagocytes (vertebrates) or haemocytes (invertebrates), and represent an innate defense system against pathogens [60–62]. Helminth parasites secrete antioxidant enzymes for defense against host-generated ROS for survival in the host [60]. Extracellular superoxide dismutase (EC-SOD, also known as SOD3) belongs to the SOD gene family, which converts superoxide radical into hydrogen peroxide and represents the first step in the antioxidant enzyme system to reduce ROS [60].

Eleven tandem EC-SOD genes were identified in the A. cantonensis genome, which were also confirmed by PCR using genomic DNA from A. cantonensis (Figure S9 in S1 Supporting Information), which was four times more than the number in the other seven nematodes studied herein (1–2 copies, Fig 3b and Figure S7 in S1 Supporting Information). Phylogenetic analysis revealed that paralogous EC-SOD genes in A. cantonensis might likely arise via two evolutionary events (Fig 3b), reflected by three paralogs (cluster I) in one clade with other nematodes and eight paralogs in another clade (cluster II, Fig 3b and Figure S7 in S1 Supporting Information). In addition, transcription analysis revealed two expression patterns occurred in these two clusters (Fig 3b). The genes in cluster I were relatively highly upregulated in the...
mammalian stage of *A. cantonensis* (L4 and L5 in Fig 3b), indicating these genes may be related to defend against definitive host-derived ROS. We examined the gene expression level of EC-SOD in *A. cantonensis* recovered from its nonpermissive host (mice) and permissive host (rat) using qPCR. We observed significantly higher transcription of the gene in cluster I
Proteases related to hemoglobin and tissue digestion in *A. cantonensis*

Adults of *A. cantonensis* dwell in the pulmonary arteries of the definitive host, where worms mature and lay eggs. The parasite digests blood and other tissue components of the host for major protein synthesis [64] (Fig 4a). Hematophagous nematodes employ an ordered pathway with distinct proteases to degrade host hemoglobin or other serum proteins [65] (Fig 4a). Through annotation and comparative analysis of eight nematode genomes, we found that genes encoding proteases such as nematode-specific aspartic proteases (e.g., necropsin-1), cathepsin B-like, legumain (*Lgmn*) and nephrilysin (e.g., NEP-1), inferred to be involved in hemoglobin digestion, are expanded in *A. cantonensis* (Fig 4 and Figures S13–16 in S1 Supporting Information). For instance, necropsin-1 (known as APR-2 in *N. americanus*) belongs to the aspartic proteases (MEROPS: A01A), and it is likely involved in the initial cleavage of hemoglobin [66]. Cysteine peptidases (including cathepsin B-like proteases) are likely involved in the second step of digestion. In addition, *A. cantonensis* has at least six genes encoding legumain, which likely activate cysteine proteases by specific hydrolysis of peptide bonds following asparagine residues [67]. In the final digestion step, metalloproteases, such as NEP-1, likely degrade small peptide fragments to dipeptides. Interestingly, the proteases necropsin-1, cathepsin B-like, legumain and NEP-1 are also expanded in *N. americanus* and/or *H. contortus* with respect to the five other nematodes studied herein (Fig 4, Figures S13–16 in S1 Supporting Information).

**Expansion of astacin-like genes in *A. cantonensis***

Astacin-like metalloproteases are involved in molting, feeding and/or host tissue penetration in nematodes [68–70]. The number of genes encoding astacin-like protease (M12A,
metalloproteases) in *A. cantonensis* (n = 75) was greater than that in *C. elegans* (n = 40). One subfamily, with the highest sequence similarity to the MTP-1 of *Ancylostoma caninum* [71], had only one gene in *C. elegans*, but had 63 genes in *A. cantonensis*. The expanded MTP-1 genes of *A. cantonensis* separated into two large subclades (subclade I: n = 22; subclade II: 

---

Fig 4. Phylogenomic analysis of three proteases related to hemoglobin digestion in eight nematodes. (a), H&E stained longitudinal section of the digestive tract from a female adult (on the upper left) shows the presence of red blood cells. The possible hemoglobin digestion pathway in the nematodes is illustrated on the right panel [65] with subfamily of enzymes (aspartic protease, cysteine protease and metalloprotease) related to hemoglobin and/or tissue digestion highlighted in yellow background. Specifically, the expanded subfamilies of enzymes from *A. cantonensis* are highlighted in red. Maximum likelihood phylogenies of *nep-1* (b), *Lgmn* (c) and *Nep-1* (d) show expansion of these proteases in *A. cantonensis*, *N. americanus* and/or *H. contortus*, all of which are blood-feeding nematodes.

https://doi.org/10.1371/journal.pntd.0007846.g004
n = 41; Fig 5a) and ten amino acid sites that were relatively specifically between the two sub-clades (Figure S19 in S1 Supporting Information). Additionally, genes in sub-clade I were mainly expressed in the L1 or L3, which was also supported by qPCR (Fig 5b and Figure S18 in S1 Supporting Information). The genes in sub-clade II had relatively low transcription compared with sub-clade I (Fig 5 and Figure S18 in S1 Supporting Information). MTP-1 has been reported to be associated with tissue migration in *A. caninum* [71], suggesting that the expanded MTP-1s may be related to the survival and/or infectivity of *A. cantonensis*. Additionally, the sequence divergence and distinct RNA expression of these two sub-clades suggest that *A. cantonensis* may have acquired multiple related abilities. A study has shown that an expansion of astacin-like genes was also discovered in *Strongyloides* and *Parastrongyloides* species [72] (Clade IV). But our phylogenetic analysis showed that the expanded astacin-like genes in *S. ratti* formed a single and distinct clade that diverged from the above mentioned MTP-1 clade (Figure S20 in S1 Supporting Information). Recently, another study of comparative...
analysis of the major parasitic worms also identified the expansion of astacin-like genes in the clades IVa, Vc and Vb [13] (including *A. cantonensis*), which is consistent with our findings.

**Discussion**

Parasite adaptation to the host is a key factor for its success [73]. The increasing genomic data for parasitic worms provides resources to explore biological and genetic differences between free-living and parasitic nematodes of plants and animals, shedding light on genomic adaptations [74]. These resources also offer unique opportunities to explore the fundamental biology of parasitic helminths and to identify potential interventions for diseases caused by worms.

Here we sequenced and assembled a high-quality reference genome of the Guangzhou strain of *A. cantonensis*, which were superior in quality to previous drafts for this species [13, 14] and published draft genomes for other strongylid nematodes [33–35]. We also employed transcriptomic data from multiple different developmental stages to reliably predict protein-coding genes and to underpin the subsequent analyses. We found that some of the genomic elements experienced multiple waves of expansion in *A. cantonensis*, including non-coding regions (e.g., RTE-RTE retrotransposons) and protein coding genes (e.g. EC-SOD and astacin-like genes). The paralogs of EC-SODs and astacin-like genes from different sub-clades, have different expression patterns in the molluscan stage and mammalian stage. Thus, the results may partly explain the adaptive evolution of the complex life cycle of *A. cantonensis*, such as the two different parasitic environments (mollusc and definitive rodent host).

Extracellular/secreted SOD of helminth parasite is one of the main components in excretory-secretory (ES) and plays a key role in fighting against host-produced ROS [60]. Our study showed that the cluster I EC-SODs of *A. cantonensis* mainly expressed in the mammalian stage, and expressed higher in the permissive host (rat) than in non-permissive host (mice). A previous investigation showed the high activity of EC-SOD in ES from rat-originated *A. cantonensis* [75]. And another study showed the higher SOD activity in *Heligmosomoides polygyrus* (mice is non-permissive hosts) than in *Nippostrongylus brasiliensis* (mice is non-permissive hosts) when they infected the mice [76]. These results suggested that some of parasitic nematode EC-SOD may be important for its survival in permissive mammalian hosts.

In contrast, the cluster II EC-SODs of *A. cantonensis* showed significant higher expression in the L3 (mollusc-dwelling). Moreover, the cluster II EC-SODs may experience convergent evolution at several amino acids with the EC-SODs of flukes. In *Fasciola hepatica*, EC-SOD was also identified in ES products from intra-molluscan larval stages [77]. The SOD showed the most significant differential expression patterns of three antioxidant enzymes (SOD, glutathione peroxidase, glutathione-S-transferase) in *S. mansoni* recovered from the susceptible snail than that from resistant snail [78]. Taken together, sequence convergence and expression similarity suggested the EC-SODs from gastropod-borne helminths might be related to their survival in gastropod species. Further, we observed that the liver flukes (*O. viverrini*: the family Bithyniidae and *C. sinensis*: the family Thiaridae and Bithyniidae) [79] with 3–4 copies of EC-SODs have a relatively broad spectrum of intermediate snail hosts than blood flukes (*S. japonicum*: *Oncomelania hupensis*, *S. haematobium*: the genus Bulinus and *S. mansoni*: the genus *Biomphalaria*) [63] with 1–2 copies of EC-SODs. While *A. cantonensis* has 7 paralogs in the GBH clade and has the broadest spectrum of snail hosts (the order Gastropod) [12] among the six species in this study. The largest copy number and diverged EC-SODs in *A. cantonensis* may provide more resources as well as possibilities for it to escape from host immune attack, which may therefore be a potential explanation for its survival in a variety of intermediate hosts. We also discovered some proteases involved in Hb digestion that were expanded in *A. cantonensis* and the other two blood-feeding nematodes. This result reveals a comparable
spectrum of essential proteases involved in hemoglobin and possible tissue digestion among three haematophagous nematodes (i.e. *A. cantonensis*, *N. americanus* and *H. contortus*). These two instances merit further investigations as they may provide clues to broad-spectrum intervention to not only *A. cantonensis* control but also other parasites control. The high-quality genome and abundant transcriptomes of *A. cantonensis* should provide a deeper exploration of the co-evolution in the complex life cycles and host adaptability for helminths, which can be used as a resource to identify regions of genetic diversity in this species and help to deeply understand the global-spread of angiostrongyliasis in order to explore novel anthelmintic agents and/or vaccines.

**Supporting information**

S1 Supporting Information. File containing all supporting figures and detailed methods.
(DOC)

S2 Supporting Information. File containing all supporting tables.
(XLSX)

**Acknowledgments**

The authors acknowledge Prof. Robin B. Gasser (the University of Melbourne) for valuable suggestions and revision of the manuscript; Dr Bill Chobotar from Andrews University, Prof. Barry Koehler (University of British Columbia), Prof. Ming-Chiu Fung (The Chinese University of Hong Kong) and Prof. Jerome H.L. Hui (The Chinese University of Hong Kong) for suggestions regarding the manuscript.

**Author Contributions**

**Conceptualization:** Xi Sun, Qiang Gao, Zhongdao Wu.

**Data curation:** Xin Zeng, Zhongdao Wu.

**Formal analysis:** Lian Xu, Meng Xu, Junyang Xu, Xin Zeng, Dai Shan, Dongjuan Yuan, Yulan Yang, Chi Zhang, Zhiyue Lv, Zhongdao Wu.

**Funding acquisition:** Zhongdao Wu.

**Investigation:** Jie Wei, Xiaoying Wu, Zhen Liu, Lifu Wang, Qiang Gao, Zhongdao Wu.

**Methodology:** Lian Xu, Meng Xu, Junyang Xu, Xin Zeng, Dai Shan, Weiming He, Yulan Yang, Chi Zhang, Xiaodong Fang.

**Project administration:** Zhongdao Wu.

**Resources:** Xi Sun, Zhongdao Wu.

**Supervision:** Junyang Xu, Zhensheng Dong, Zhongdao Wu.

**Validation:** Dai Shan, Dongjuan Yuan, Ping He, Shiqi Luo, Beibei Zhang, Zilong Yu, Zhongdao Wu.

**Visualization:** Lian Xu, Meng Xu, Dai Shan, Weiming He, Yulan Yang, Zhongdao Wu.

**Writing – original draft:** Lian Xu, Meng Xu, Xi Sun, Zhiyue Lv, Zhongdao Wu.

**Writing – review & editing:** Lian Xu, Meng Xu, Xi Sun, Junyang Xu, Xin Zeng, Dongjuan Yuan, Xiaomin Xu, Langui Song, Zhiyue Lv, Zhongdao Wu.
References

1. Spratt DM. Species of Angiostrongylus (Nematoda: Metastrongyloidea) in wildlife: A review. Int J Parasitol Parasites Wildl. 2015; (4):178–89. https://doi.org/10.1016/j.ijppaw.2015.02.006 PMID: 25853051

2. Fierling T, Ovarnstrom Y, Nih J, Devincenzo JP, Madhi A, Bagga B, et al. Angiostrongylus cantonensis Eosinophil Meningitis in an Infant, Tennessee, USA. Emerg Infect Dis. 2017; 23(10):1756–8. Epub 2017/09/21. https://doi.org/10.3201/eid2310.170978 PMID: 28930003

3. Beaver PC, Rosen L. Memorandum on the First Report of Angiostrongylus in Man, by Nomura and Lin, 1945. Am J Trop Med Hyg. 1964; 13:589–90. Epub 1964/07/01. https://doi.org/10.4269/ajtmh.1964.13.589 PMID: 14196058

4. Foster CE, Nicholas EG, Chun AC, Gharfeh M, Anvari S, Seeborg FO, et al. Angiostrongylus cantonensis Infection: A Cause of Fever of Unknown Origin in Pediatric Patients. Clin Infect Dis. 2016; 63(11):1475–8. Epub 2016/09/01. https://doi.org/10.1093/cid/ciw006 PMID: 27578821.

5. Peng Y, Liu X, Pan S, Xie Z, Wang H. Anti-N-methyl-D-aspartate receptor encephalitis associated with intracranial Angiostrongylus cantonensis infection: a case report. Neurol Sci. 2017; 38(4):703–6. Epub 2016/10/26. https://doi.org/10.1007/s10072-016-2718-3 PMID: 27778112.

6. Sinawat S, Sanguansak T, Angkawijwong T, Ratanapakorn T, Intapan PM, Sinawat S, et al. Ocular angiostrongyliasis: clinical study of three cases. Eye (Lond). 2008; 22(11):1446–8. Epub 2008/06/07. https://doi.org/10.1038/sote.2008.135 PMID: 18535614.

7. Al Hammoud R, Nayes SL, Murphy JR, Heresi GP, Butler UJ, Perez N. Angiostrongylus cantonensis Menigitis and Myelitis, Texas, USA. Emerg Infect Dis. 2017; 23(6):1037–8. Epub 2017/05/19. https://doi.org/10.3201/eid2306.161683 PMID: 28618035.

8. Morassutti AL, Thiengo SC, Fernandez M, Sawanyawisuth K, Graefe-Teixeira C. Eosinophilic meningitis caused by Angiostrongylus cantonensis: an emergent disease in Brazil. Mem Inst Oswaldo Cruz. 2014; 109(4):399–407. Epub 2014/07/31. https://doi.org/10.1590/0074-0276140023 PMID: 25075779.

9. Nguyen Y, Rossi B, Argy N, Baker C, Nickei B, Marti H, et al. Autochthonous Case of Eosinophilic Meningitis Caused by Angiostrongylus cantonensis, France, 2016. Emerg Infect Dis. 2017; 23(6):1045–6. Epub 2017/05/19. https://doi.org/10.3201/eid2306.161199 PMID: 28518042.

10. Wang QP, Lai DH, Zhu XQ, Chen XG, Lun ZR. Human angiostrongyliasis. Lancet Infect Dis. 2008; 8 (10):621–30. Epub 2008/10/17. https://doi.org/10.1016/S1473-3099(08)70229-9 PMID: 18922484.

11. Barratt J, Chan D, Sandaradura I, Malik R, Spielerman D, Lee R, et al. Angiostrongylus cantonensis: a review of its distribution, molecular biology and clinical significance as a human pathogen. Parasitology. 2016; 143(9):1087–118. Epub 2016/05/27. https://doi.org/10.1017/S0031182016000652 PMID: 27258800.

12. Kim JR, Hayes KA, Yeung NW, Cowie RH. Diverse gastropod hosts of Angiostrongylus cantonensis, the rat lungworm, globally and with a focus on the Hawaiian Islands. PLoS One. 2014; 9(5):e94969. Epub 2014/05/03. https://doi.org/10.1371/journal.pone.0094969 PMID: 24788772.

13. International Helminth Genomes C. Comparative genomics of the major parasitic worms. Nat Genet. 2019; 51(1):163–74. Epub 2018/11/07. https://doi.org/10.1038/s41588-018-0262-1 PMID: 30397333.

14. Yong HS, Eamsobhana P, Lim PE, Razali A, Aziz FA, Rosli NS, et al. Draft genome of neurotropic nematode parasite Angiostrongylus cantonensis, causative agent of human eosinophilic meningitis. Acta Trop. 2015; 148:1–7. Epub 2015/04/26. https://doi.org/10.1016/j.actatropica.2015.04.012 PMID: 25910624.

15. Zeng X, Wei J, Wang J, Wu F, Fung F, Wu X, et al. Angiostrongylus cantonensis: scanning electron microscopic observations on the cuticle of molting larvae. Korean J Parasitol. 2013; 51(6):633–6. Epub 2014/02/12. https://doi.org/10.3347/kjp.2013.51.6.633 PMID: 24516266.

16. Kajitani R, Toshimoto K, Naguchi H, Toyoda A, Ogura Y, Okuno M, et al. Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res. 2014; 24 (8):1384–95. Epub 2014/04/24. https://doi.org/10.1101/gr.170720.113 PMID: 24755901.

17. Ye C, Hill CM, Wu S, Ruan J, Ma ZS. DBG2OLC: Efficient Assembly of Large Genomes Using Long Read Errorless Reads of the Third Generation Sequencing Technologies. Sci Rep. 2016; 6:31900. Epub 2016/08/31. https://doi.org/10.1038/srep31900 PMID: 27573208.

18. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sathikumar S, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One. 2014; 9(11):e112963. Epub 2014/11/20. https://doi.org/10.1371/journal.pone.0112963 PMID: 25409509.

19. Boetzer M, Pirovano W. SSPACE-LongRead: scaffolding bacterial draft genomes using long read sequence information. BMC Bioinformatics. 2014; 15:211. Epub 2014/06/22. https://doi.org/10.1186/1471-2105-15-211 PMID: 24950923.
20. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics. 2011; 27(4):578–9. Epub 2010/12/15. https://doi.org/10.1093/bioinformatics/btq683 PMID: 21149342.

21. Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience. 2012; 1(1):18. Epub 2012/01/01. https://doi.org/10.1186/2047-217X-1-18 PMID: 23587118

22. Parra G, Bradnam K, Korf I. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics. 2007; 23(9):1061–7. Epub 2007/03/03. https://doi.org/10.1093/bioinformatics/btm071 PMID: 17332020.

23. Simao FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015; 31(19):3210–2. Epub 2015/06/11. https://doi.org/10.1093/bioinformatics/btv578 PMID: 26059717.

24. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 2011; 29(7):644–52. Epub 2011/05/17. https://doi.org/10.1038/nbt.1883 PMID: 21572440.

25. Gao G, Xu M, Bai C, Yang Y, Li G, Xu J, et al. Comparative genomics and transcriptomics of *Chrysopelea* provide insights into the evolution of complex plumage coloration. Gigascience. 2018; 7(10). Epub 2018/09/08. https://doi.org/10.1093/gigascience/giy113 PMID: 30192940.

26. Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, et al. InterProScan 5: genome-scale protein function classification. Bioinformatics. 2014; 30(9):1236–40. Epub 2014/01/24. https://doi.org/10.1093/bioinformatics/btu152 PMID: 24451626.

27. Edgar RC, Myers EW. PILER: identification and classification of genomic repeats. Bioinformatics. 2005; 21 Suppl 1:i52–8. Epub 2005/06/18. https://doi.org/10.1093/bioinformatics/bti1003 PMID: 15961452.

28. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol. 2009; 26(7):1641–50. Epub 2009/04/21. https://doi.org/10.1093/molbev/msp077 PMID: 19370759.

29. Li L, Stoeckert CJ Jr., Roos DS. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res. 2003; 13(9):2178–89. https://doi.org/10.1101/gr.1224503 PMID: 12952885

30. Xie Y, Li Z, Luo R, Liu J, Zeng G, Huang W, et al. SOAP2: An improved short读 de novo assembler. Genome Biol. 2011; 12(3):R32. Epub 2011/03/16. https://doi.org/10.1186/gb-2011-12-3-r32 PMID: 21434475.
41. Capella-Gutierrez S, Silia-Martinez JM, Gabaldon T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics. 2009; 25(15):1972–3. Epub 2009/06/10. https://doi.org/10.1093/bioinformatics/btp348 PMID: 19505945

42. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014; 30(9):1312–3. Epub 2014/01/24. https://doi.org/10.1093/bioinformatics/btu033 PMID: 24451623

43. Mirarab S, Reaz R, Bayzid MS, Zimmermann T, Swenson MS, Warnow T. ASTRAL: genome-scale coalescent-based species tree estimation. Bioinformatics. 2014; 30(17):i541–8. Epub 2014/08/28. https://doi.org/10.1093/bioinformatics/btu462 PMID: 25161245

44. Zhou Y, Zheng H, Chen X, Zhang L, Wang K, Guo J et al. The Schistosoma japonicum genome reveals features of host-parasite interplay. Nature. 2009; 460(7253):345–51. Epub 2009/07/17. https://doi.org/10.1038/nature08140 PMID: 19606140

45. Berriman M, Haas BJ, LoVerde PT, Wilson RA, Dillon GP, Cerqueira GC, et al. The genome of the blood fluke Schistosoma mansoni. Nature. 2009; 460(7253):352–8. Epub 2009/07/17. https://doi.org/10.1038/nature08160 PMID: 225161245

46. Young ND, Jex AR, Li B, Liu S, Yang L, Xiong Z, et al. Whole-genome sequence of Schistosoma hematobium. Nat Genet. 2012; 44(2):221–5. Epub 2012/01/17. https://doi.org/10.1038/ng.1065 PMID: 22246508.

47. Young ND, Nagarajan N, Lin SJ, Korhonen PK, Jex AR, Hall RS, et al. The Opisthorchis viverrini genome provides insights into life in the bile duct. Nat Commun. 2014; 5:4378. Epub 2014/07/10. https://doi.org/10.1038/ncomms5378 PMID: 25007141

48. Wang X, Chen W, Huang Y, Sun J, Men J, Liu H, et al. The draft genome of the carcinogenic human liver fluke Clonorchis sinensis. Genome Biol. 2011; 12(10):R107. Epub 2011/10/26. https://doi.org/10.1186/gb-2011-12-10-r107 PMID: 22023798

49. Robb SM, Gotting K, Ross E, Sanchez Alvarado A. SmedGD 2.0: The Schmidtea mediterranea genome database. Genesis. 2015; 53(8):535–46. Epub 2015/07/04. https://doi.org/10.1002/dvg.22872 PMID: 26138588

50. Howe KL, Bolt BJ, Shafie M, Kersey P, Berriman M. WormBase ParaSite—a comprehensive resource for helminth genomics. Mol Biochem Parasitol. 2017; 215:2–10. Epub 2016/12/03. https://doi.org/10.1016/j.molbiopara.2016.11.005 PMID: 27899279

51. Yang Z. PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol. 2007; 24(8):1586–91. Epub 2007/05/08. https://doi.org/10.1093/molbev/msm088 PMID: 17483113.

52. Hedges SB, Dudley J, Kumar S. TimeTree: a public knowledge-base of divergence times among organisms. Bioinformatics. 2006; 22(23):2971–2. Epub 2006/10/06. https://doi.org/10.1093/bioinformatics/btl505 PMID: 17021158.

53. De Bie T, Cristiani N, Demuth JP, Hahn MW. CAFE: a computational tool for the study of gene family evolution. Bioinformatics. 2006; 22(10):1269–71. Epub 2006/03/18. https://doi.org/10.1093/bioinformatics/btl097 PMID: 16543274.

54. Rawlings ND, Barrett AJ, Finn R. Twenty years of the MEROPS database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Res. 2016; 44(D1):D343–50. Epub 2015/11/04. https://doi.org/10.1093/nar/gkv1118 PMID: 26527717

55. Abascal F, Zardoya R, Posada D. ProtTest: selection of best-fit models of protein evolution. Bioinformatics. 2005; 21(9):2104–5. Epub 2005/01/14. https://doi.org/10.1093/bioinformatics/bti263 PMID: 15647292.

56. Guindon S, Delsuc F, Dufayard JF, Gascuel O. Estimating maximum likelihood phylogenies with PhyML. Methods Mol Biol. 2009; 537:113–37. Epub 2009/04/21. https://doi.org/10.1007/978-1-59745-251-9_6 PMID: 19378142.

57. Nguyen LT, Schmidt HA, von Haeseler A, Minh BO. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015; 32(1):268–74. Epub 2014/11/06. https://doi.org/10.1093/molbev/msv300 PMID: 25371430

58. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcripts in the presence of insertions, deletions and gene fusions. Genome Biol. 2013; 14(4):R36. Epub 2013/04/27. https://doi.org/10.1186/gb-2013-14-4-r36 PMID: 23618408

59. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4):402–8. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609.

60. Chiumiento L, Bruschi F. Enzymatic antioxidant systems in helminth parasites. Parasitol Res. 2009; 105(3):593–603. Epub 2009/05/23. https://doi.org/10.1007/s00436-009-1483-0 PMID: 19462181.
61. Hahn UK, Bender RC, Bayne CJ. Involvement of nitric oxide in killing of Schistosoma mansoni sporocysts by hemocytes from resistant Biomphalaria glabrata. J Parasitol. 2001; 87(4):778–85. Epub 2001/09/06. https://doi.org/10.1645/0022-3395(2001)087[0778:ONOKI]2.0.CO;2 PMID: 11534641.

62. Hahn UK, Bender RC, Bayne CJ. Killing of Schistosoma mansoni sporocysts by hemocytes from resistant Biomphalaria glabrata: role of reactive oxygen species. J Parasitol. 2001; 87(2):292–9. Epub 2001/04/25. https://doi.org/10.1645/0022-3395(2001)087[0292:KOSMSB]2.0.CO;2 PMID: 11318558.

63. Giannelli A, Cantacessi C, Coella V, Dantas-Torres F, Otranto D. Gastropod-Borne Helminths: A Look at the Snail-Parasite Interplay. Trends Parasitol. 2016; 32(3):255–64. Epub 2016/01/08. https://doi.org/10.1016/j.pt.2015.12.002 PMID: 26740470.

64. Huttermann M, Schmahl G, Mehlhorn H. Light and electron microscopic studies on two nematodes, Angiostrongylus cantonensis and Trichuris muris, differing in their mode of nutrition. Parasitol Res. 2007; 101 Suppl 2:S225–32. Epub 2008/01/19. https://doi.org/10.1007/s00436-007-0698-1 PMID: 17823832.

65. Williamson AL, Brindley PJ, Knox DP, Hotze PJ, Loukas A. Digestive proteases of blood-feeding nematodes. Trends Parasitol. 2003; 19(9):417–23. Epub 2003/09/06. PMID: 12957519.

66. Williamson AL, Brindley PJ, Abbenante G, Datu BJ, Prociv P, Berry C, et al. Hookworm aspartic protease, Na-APR-2, cleaves human hemoglobin and serum proteins in a host-specific fashion. J Infect Dis. 2003; 187(3):484–94. Epub 2003/01/29. https://doi.org/10.1086/367708 PMID: 12552433.

67. Oliver EM, Skuce PJ, McNair CM, Knox DP. Identification and characterization of an asparaginyl proteinase (legumain) from the parasitic nematode, Haemonchus contortus. Parasitology. 2006; 133(Pt 2):237–44. Epub 2006/05/03. https://doi.org/10.1017/S003118200600229 PMID: 16650340.

68. Jing Y, Toubarro D, Hao Y, Simoes N. Cloning, characterisation and heterologous expression of an astacin metalloprotease, Sc-AST, from the entomoparasitic nematode Steinernema carpocapsae. Mol Biochem Parasitol. 2010; 174(2):101–8. Epub 2010/07/31. https://doi.org/10.1016/j.molbiopara.2010.07.004 PMID: 20670659.

69. Park JO, Pan J, Mohrlen F, Schupp MO, Johnsen R, Baillie DL, et al. Characterization of the astacin family of metalloproteases in C. elegans. BMC Dev Biol. 2010; 10:14. Epub 2010/01/30. https://doi.org/10.1186/1471-213X-10-14 PMID: 20109220.

70. Stepek G, McCormack G, Page AP. Collagen processing and cuticle formation is catalysed by the astacin metalloprotease DPY-31 in free-living and parasitic nematodes. Int J Parasitol. 2010; 40(5):533–42. Epub 2009/11/04. https://doi.org/10.1016/j.ijpara.2009.10.007 PMID: 19883650.

71. Williamson AL, Lustgorman S, Oksov Y, Deumic V, Pleskatt J, Mendez S, et al. Ancylostoma caninum MTP-1, an astacin-like metalloprotease secreted by infective hookworm larvae, is involved in tissue migration. Infect Immun. 2006; 74(2):961–7. Epub 2006/01/24. https://doi.org/10.1128/IAI.74.2.961-967.2006 PMID: 16428741.

72. Hunt VL, Tsai LJ, Coghlan A, Reid AJ, Holroyd N, Foth BJ, et al. The genomic basis of parasitism in the Strongyloides clade of nematodes. Nat Genet. 2016; 48(3):299–307. Epub 2016/02/02. https://doi.org/10.1038/ng.3495 PMID: 26829753.

73. Lively CM, Dybdahl MF. Parasite adaptation to locally common host genotypes. Nature. 2000; 405(6787):679–81. Epub 2000/06/23. https://doi.org/10.1038/35015069 PMID: 10864323.

74. Zarowiecki M, Berriman M. What helminth genomes have taught us about parasite evolution. Parasitology. 2015; 142 Suppl 1:S85–97. Epub 2014/12/04. https://doi.org/10.1017/S0031182014001449 PMID: 25482650.

75. Morassutti AL, Pinto PM, Dutra BK, Oliveira GT, Ferreira HB, Graeff-Teixeira C. Detection of anti-oxidant enzymatic activities and purification of glutathione transferases from Angiostrongylus cantonensis. Exp Parasitol. 2011; 127(2):365–9. Epub 2010/09/03. https://doi.org/10.1016/j.exppara.2010.08.018 PMID: 20807531.

76. Smith NC, Bryant C. The role of host generated free radicals in helminth infections: Nippostrongylus brasiliensis and Nematodirospoides dubius compared. Int J Parasitol. 1986; 16(6):617–22. Epub 1986/12/01. https://doi.org/10.1016/0160-7511(86)90029-8 PMID: 3804571.

77. Gourbal BE, Guillou F, Mitta G, Sibile P, Theran A, Pointier JP, et al. Excretory-secretory products of larval Fasciola hepatica investigated using a two-dimensional proteome approach. Mol Biochem Parasitol. 2008; 161(1):63–6. Epub 2008/06/17. https://doi.org/10.1016/j.molbiopara.2008.05.002 PMID: 18556074.

78. Zelck UE, Von Janowsky B. Antioxidant enzymes in intramolluscan Schistosoma mansoni and ROS-induced changes in expression. Parasitology. 2004; 128(Pt 5):493–501. Epub 2004/06/08. https://doi.org/10.1017/s0031182004004089 PMID: 15180317.

79. Murell K, Pozio EJGWPP, Part. The liver flukes: Clinorchis sinensis, Opisthorchis spp, and Metorchis spp. 2017; 3.