Trends in Parasitology

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REVIEWS

Review

Transcriptomic Resources for Parasitic Nematodes of Veterinary Importance

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Parasitic nematodes are important pathogens of animals, causing diseases that impact on agricultural production worldwide. Research on these worms has been constrained by a lack of genetic and genomic tools. Nonetheless, over the past decade this field has made substantial advances, many of which have been led by transcriptomic sequencing. The present review summarises major transcriptomic studies of veterinary parasitic nematodes in recent years, and comments on overarching themes stemming from this work that inform our understanding of parasitism. Finally, we comment on current, state-of-the-art informatic tools for the analysis of complex worm transcriptomes to extract maximum the molecular information from them.

Highlights

Transcriptomic RNA sequencing provides a powerful suite of tools for exploring parasitic worms.

Many transcriptomic studies are now published for a wide range of veterinary parasitic nematodes.

These studies provide insight into stage-specific development, reproduction, infection, host–parasite interactions, and many aspects of the biology of these and other parasites.

Informatics approaches have developed significantly over the last decade, and numerous user-friendly resources are now available for researchers interested in applying transcriptomic studies to their research.

Significance of Veterinary Parasitic Nematodes and Their Transcriptomic Study

Parasitic nematodes cause globally destructive diseases of humans, animals, and plants. Soil-transmitted helminths, for instance, infect >1 billion people, costing ~5 million disability-adjusted life years (**DALYs**) (see Glossary) [1]. Plant-parasitic nematodes affect most major food crops, resulting in >$80 billion in economic losses annually [2]. Parasitic worms have a significant impact on wildlife which, though sparsely measured, affects their sustainability. Treatment costs alone for parasitic worms of domesticated or livestock animals exceed $3.3 billion per year [3]. Control of parasitic nematodes requires integrated management and relies on few anthelmintic drugs [4]. Anthelmintic resistance is widespread [5], most notably in parasites of sheep and other ruminants. Although efforts to repurpose existing or develop novel drug classes are ongoing, they benefit from a detailed understanding of the molecular biology of parasitic worms. Furthermore, gaining insight into fundamental biological functions and host–parasite interactions can yield new discoveries about infection, pathogenesis, cellular biology, host specificity, and immune responses. Next-generation sequencing (**NGS**) has transformed knowledge of these obstinate parasites. This review seeks to summarise and, where possible, stimulate efforts toward expanding knowledge of parasitic nematodes of veterinary importance through 'omics technologies, with a focus on transcriptomic studies.

Order Ascaridida

Transcriptomic studies are available for taxa including *Anisakis* spp. (humans, fish, and piscivores), *Ascaris suum* (humans and pigs), *Parascaris equorum/Parascaris univalens* (horses), and *Toxocara* spp. (dogs, cats, and, zoonotically, humans) (Figure 1) (see also Table S1 in the supplemental information online).

Baird *et al*. [6] explored pooled L3 larvae of *Anisakis pegreffi* and *Anisakis simplex* sensu stricto from mackerel to identify parasite-derived allergens secreted into food-fish species; some *Anisakis* proteins are major contributors to fish allergies [7]. Using known allergens, these authors [6] identified 36 and 29 putative allergens in *A. simplex* s.s. and *A. pegreffi* L3s,

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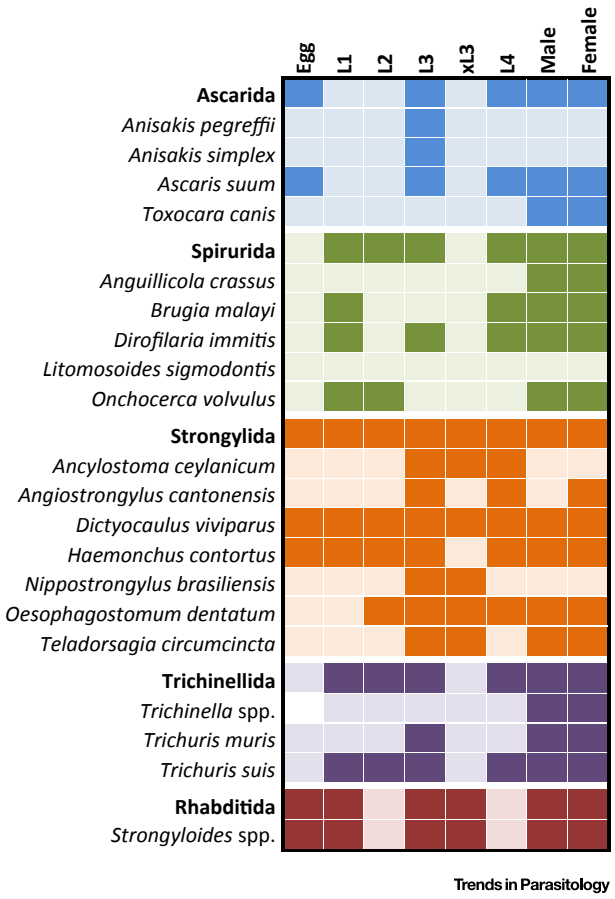

Figure 1. Block Schematic of Transcriptomic Datasets Available for Each Major Veterinary Parasitic Nematode Group by Lifecycle Stage. Blocks are coloured by nematode order. Dark shading represents available RNA-seq data sets per order and species. Additional information for each dataset is provided in Data S1 in the supplemental information online.

respectively, including previously unidentified cyclophilins and ABA-1 domain-like proteins, and proposed their use for diagnosis and research of fish allergies. Cavellero *et al*. [8] also studied larval *A. simplex* s.s for mechanisms for host-tissue degradation and pathogenesis. In addition to identifying numerous pharyngeal-enriched transcripts, including metalloproteases and **SCP-TAPs** proteins [9], Cavellero *et al*. [8] found proteins that encoded ShK-toxin and astacin (M12A) protease domains, which, as the authors noted, have an immunomodulatory role in *Toxocara canis* [10].

Jex *et al*. [11] characterized the draft genome of *A. suum* and explored transcriptional changes during its hepatopulmonary migration (HPM) in pigs and the development of larvae to adult stages. HPM coincided with an upregulation of secreted proteases and immunomodulators, including *alt-1*, *cpi-2*, and *mif-4*. The study [11] found an upregulation of genes associated with volatile chemotaxis during liver migration and aerotaxis upon reaching the lung, suggesting that chemosensation might be required for HPM in *Ascaris*. Jex *et al*. [11] examined differences between reproductive and muscle tissues in adult male and female worms, identifying genes involved in sexual dimorphism or reproduction. This work was followed by a tissue-specific

Glossary

ASPR: *Ancylostoma*-secrete proteins are a subset of SCP-TAPS secreted by species of hookworms (and related nematodes) with immunogenic and immunomodulatory properties.
CRISPR: a functional genomic technology that uses complimentary guide RNAs to target and manipulate customized genetic sequences. Typically used to knock-out, mutate, tag, or suppress genes of interest.
DALYs: a metric used to consider the impact of a disease accounting for years of life lost due to poor health, disability, or deaths.
L1–L4: nematodes moult their cuticle through development. Each larval moult following formation of the larvae in the egg is given a number based on its moulting phase. L1 = first larvae, L2 = larvae after the first cuticular moult, etc.
NGS: next-generation sequencing platforms are high-throughput methods that have been developed since the advent of capillary-based sequencing. Current examples include Illumina, PacBIO, and Oxford Nanopore-based sequencing.
Raw read counts: an absolute count of the number of RNA-seq reads mapping to a gene or other genomic feature of interest.
RNA-seq: a mode of NGS that allows direct RNA sequencing without cDNA synthesis. This approach retains quantitative capacity and strand-specificity (i.e., sense vs. antisense) and is widely used to study mRNA or noncoding RNA abundance.
RNAi: a functional tool that allows custom-designed RNAs to be incorporated into a target cell to reversibly suppress translation of its complimentary mRNA or noncoding RNA.
RPKM and FPKM: common transcriptomic metrics that scale raw read (or read-pairs; i.e., 'fragments') counts by the length of each transcript as a proportion of the total million reads sequenced.
SCP-TAPs: a family of secreted proteins expanded in many parasitic nematode species and described in a variety of animal toxins/venoms (e.g., spiders, snakes, and cone snails).
Th1: the proinflammatory arm of the T cell-mediated immune response.

transcriptomic study by Ma *et al*. [12] of infective egg stages, embryos, and testes, and ovaries dissected from adult worms, which identified homologues of major sperm proteins and meiotic regulators, and also implicated tyrosine phosphorylation in spermatogenesis. Wang *et al*. [13] studied the transcriptional effects of the loss of 45 Mb (~20%) of germline chromatin in somatic tissues (*aka* chromatin diminution) in *A. suum* during development. Genes lost during chromatin diminution had tissue-specific transcription associated with testes, ovaries, and embryos, and predicted functions in spermatogenesis, oogenesis, and embryogenesis, suggesting that chromatin diminution acts to silence germline genes and to control sexual dimorphism [13]. This fascinating form of genetic regulation that, as noted by Wang *et al*. [13], has been described for other metazoans [14], was later similarly explored at a transcriptomic level in *P. univalens* and *T. canis* [15].

Zhu *et al*. [16] produced a draft genome for *T. canis* and characterised transcriptomic changes during its lifecycle. This study [16] showed that *T. canis* L3s were enriched, relative to adults, for transcripts associated with neuronal signalling, cuticle formation and shedding, and immunomodulation. By contrast, upregulated transcription in adults was associated with metabolic activity and sexual development/dimorphism. These observations were subsequently explored in adult *T. canis* [17], which identified transcripts encoding homologues of sperm transmembrane protein 9, serine/threonine phosphatase PP1 and PP2, among 1467 male-enriched transcripts. Additionally, Zhou *et al*. [17] characterised 321 female-enriched transcripts, encoding homologues of the E3-ubiquitin ligase, MARCH2, aquaporin-8, vitellogenins, and lipid-binding proteins; they noted that many of these sex-enriched transcripts have homologues in other species that are so far unstudied.

References the observation that parasitic nematode infections typically correspond to a suppressed Th1 and more Th2 (eosinophil/granulocyte mediated) biased response.

TPM: a transcriptomic metric that first scales raw read (or read-pairs; i.e., 'fragments') by the length of each transcript and then expresses this as a proportion of all the transcripts in the dataset (inferred from the sum of all of its reads per transcript length values). By expressing transcript levels as a proportion of total transcription rather than total reads, TPM values retain greater comparability between samples than RPKM or FPKM.

xL3: some nematode species, for example, species of Strongylida, retain their L2 cuticle as an external sheath during the L3 stage. This provides environmental protection and is shed upon infection of a host. Following shedding of the cuticle, these larvae are 'exsheathed' (i.e., xL3).

Order Strongylida

The order Strongylida includes nematodes of major human health and veterinary/agricultural relevance, including lungworms, nodule worms, hookworms, and large and small strongyles, representatives of which have been studied at the genomic and/or transcriptomic levels.

Lungworms

Lungworms include species of *Aelurostrongylus, Dictyocaulus*, and *Protostrongylus*, as well as *Angiostrongylus vasorum* and *An. cantonensis*. Ansell *et al*. [18] undertook a transcriptomic study of *An. vasorum* from the pulmonary arteries and right heart ventricle from infected dogs. The study assembled 20 033 transcript contigs encoding a large repertoire of proteases, kinases, phosphatases, receptors/transporters, and molecules involved in metabolism or signalling. Subsequently, Yu *et al*. [19] undertook a transcriptomic study of *An. cantonensis*, comparing late-stage **L4** ('L5′) larvae with adult females, coinciding with larval invasion of the brain.

Of ruminant lungworms, *Dictyocaulus filaria* and *D. viviparus* are most relevant. McNulty *et al*. [20] generated a genomic assembly for *D. viviparus* and studied the differential transcriptomic of the complete lifecycle. The study [20] noted abundant transcripts associated with chemotaxis/signalling, transcriptional regulation, and oxygen transport in free-living **L1s**–L3s; these transcripts shifted to protein folding, redox pathways, and carbohydrate metabolism with parasitism (L4 to adult). Sexual dimorphism and maturation related to an upregulation of protein phosphatases, neurotransmitters, and glycolytic pathways in males, and transcriptional regulators, chitin metabolism, and signalling pathways in females [20]. Transcriptomes of L4 and hypobiotic/preadult stages of *D. viviparus* were similar; however, a detailed analysis found an upregulation of multiple transcription factors, proteolysis, and lipid metabolism in the latter stages. Additional transcriptomic studies of *Dictyocaulus* include 454-based comparisons of

larval L3s and male and female adults [21], and Illumina-based comparisons of mixed adults of *D. filaria* and *D. viviparus* [22].

Hookworms

The most complete transcriptomic study of an ancylostomatoid ('hookworm') of veterinary importance, to date, was published by Schwarz *et al*. [23], who, in addition to characterizing the draft *Ancylostoma ceylanicum* genome, explored changes in transcript abundance from infective L3s through to adulthood. Schwarz *et al*. [23] compared transcriptomes of infectious L3s incubated for 24 h in culture medium with those harvested from experimentally infected hamsters 24 h after inoculation, identifying 240 and 942 transcripts upregulated in the former and latter, with only 141 transcripts commonly enriched in both conditions. Transcripts upregulated only in infected hamsters included a suite of SCP-TAPs and **ASPR** proteins, highlighting their importance in hookworm infection and the difficulty in using *in vitro* data to understand the *in vivo* biology of obligate parasites. Schwarz *et al*. [23] followed transcriptomic changes in *Anc. ceylanicum* as it developed to adulthood in hamsters. From 24 h to 5 days after inoculation, which corresponds to the development of L4s, *Anc. ceylanicum* upregulated a previously unrecognized family of proteins (named strongylid L4 proteins; SL4Ps) with homologues in blood-feeding strongylids including *Haemonchus contortus*, *Angiostrongylus cantonensis*, and *Necator americanus*. The study noted a similar upregulation, 12 days after inoculation (which coincides with sexual maturation and early blood-feeding), of a previously unrecognised family of secreted proteins with homology across clade V nematodes (Strongylida and Rhabditida), and named them secreted clade V proteins (SCVPs). Other studies of hookworms have explored transcription in the intestines of male adult *Anc. ceylanicum* [24], in infective and serum-stimulated L3 larvae and male and female adults of *Ancylostoma caninum* [25], and in eggs, L3, L4, and adult stages of *N. brasiliensis* [26,27].

Strongyles (Large Intestine)

Important representatives of this superfamily are nodule worms (i.e., *Oesophagostomum* spp.) of primates, ruminants, and pigs, as well as large (i.e., *Strongylus vulgaris*) and small (i.e., cyathostomes) strongyles of horses. Two transcriptomic studies investigated *Oesophagostomum dentatum* of pigs [28,29]. Tyagi *et al*. [29] presented a draft genome of *O. dentatum* and followed its transcription through larval development (L2 to L4) and maturation. Transition from free-living L3s to parasitic L4s in the pig intestine was marked by upregulation of transcripts encoding excretory/secretory cysteine, metallo- and serine proteases, aprotinin and subtilisin-like protease inhibitors, SCP-TAPs and transthyretin-like proteins. These remained elevated through adulthood and were noted as common changes in parasitic nematodes upon host infection [29]. Parasitism is also marked in *O. dentatum* by upregulation of secreted eiconsanoids, which are immunomodulatory [29]. Cwiklinski *et al*. [30] have published the only small strongyle transcriptome to date, with a study of *Cylicostephanus goldi* (a cyathostomin of equids). Additional study of these small strongyles, particularly with respect to their dormant larval stages and activation of these stages, would be intriguing.

Trichostrongyles (Stomach and Small Intestine)

Gastrointestinal trichostrongyles include species of *Haemonchus*, *Trichostrongylus*, *Teladorsagia*, and *Cooperia* of ruminants, which are highly significant in terms of disease, financial impact, and drug resistance. Transcriptomes are available for *Cooperia oncophora* [31], *Ostertagia ostertagi* [31,32], *Teladorsagia circumcincta* [33], and *Trichostrongylus colubriformis* [34], but most work has focused on *H. contortus*.

Draft assemblies of the *H. contortus* genome were copublished in independent studies of the MHco3(ISE).N1 [35] and McMaster [36] strains of *H. contortus*. Laing *et al*. [35] determined RNA-seq support for 17 483 coding genes, 11 295 of which were differentially transcribed throughout the lifecycle. Egg-enriched transcripts associated with redox activity, apoptosis, morphogenesis, embryonic development, and chromatin regulation. L1-enriched transcripts associated with muscle development and motor activity. With the moult to L4 and initiation of blood-feeding, Laing *et al*. [35] observed increased transcription associated with lipid and sugar metabolism, cuticle development, collagen production, and growth. Development to adulthood related to an upregulation of transcripts involved in redox activity, sperm production and growth in males, and oogenesis, embryogenesis, germ-line replication and cell-cycle regulation in females. Laing *et al*. [35] also explored transcription in the intestine of *H. contortus*, identifying an upregulation of cysteine-type peptidases and peptidase inhibitors, thought to be involved in blood-feeding. Similar observations were made by Schwarz *et al*. [36], who, additionally, recorded an upregulation in L1 stages of class A and SR-type G-protein-coupled receptors (typically associated with chemosensation, mechanosensation, osmosensation, and proprioception in *Caenorhabditis elegans*). Transcriptional activity was largely stable from L1 to **L2** stages, which are free-living. The change from L3 to L4 and the activation of blood-feeding coincided with an upregulation of ~120 peptidases [36]. Schwarz *et al*. [36] noted, with male development, an upregulation of genes involved in spermatogenesis, including homologues of *C. elegans alg-4* [tag-76], *cyk-4, fer-1, hsp-12.2, hsp-12.3, spe-15, vab-1,* and *vpr-1*.

Recently, Ma *et al*. [37] studied transcriptional regulation in *H. contortus* L3, **xL3**, and L4 stages. Consistent with earlier studies [35,36], these authors [37] found that ~5–10% of coding genes are differentially transcribed in each stage, with signalling, chemosensation, and transcriptional regulation in L3s, giving way to metabolic activity, peptidases, SCP-TAPS proteins, galectins, and other immunomodulators in L4s. Interestingly, these authors [37] proposed a potential role for translational repression in larval development in *H. contortus* (a phenomenon by which mRNA transcripts are held in stasis for later translation). Translational repression has an important regulatory role in *C. elegans* [38], but, to our knowledge, it has not been studied in parasitic nematodes.

Order Spirurida

Key species include *Brugia pahangi* and *Spirocerca lupi* and *Dirofilaria immitis* (infecting cats, dogs, or other animals). Transcriptomic data are available for *B. pahangi* microfilariae [39], but broad studies are limited to *D. immitis*. Prior studies of *D. immitis* include presentation of its draft genome and transcriptome, as well as that of its mutualistic *Wolbachia* (alpha-proteobacteria) symbionts [40,41]. These symbionts can be targeted with doxycycline to support anthelmintic control, including for canine heartworm [42].

Luck *et al*. [43] undertook an extensive quantitative transcriptomic study of *D. immitis* larvae (microfilaria [L1], L3 and L4) and adults and found that 70% of *D. immitis* genes were stage-enriched. Adult-enriched genes were associated with nematode dimorphism, including spermatogenesis and protein phosphorylation in males. and serpin activity, collagen formation, cuticle turnover and oogenesis/embryogenesis in females. Microfilariae (mff) were enriched in ciliary functions, which the authors related to the ciliated chemosensory organs of nematodes (amphids), chemosensation, and the migration of mff in the host blood [43]. The authors also identified upregulated GABA signalling in mff and suggested a link to the microfilaricidal potency of ivermectin [43]. Their study identified also L3-enriched transcripts involved in redox activity, carbohydrate metabolism, and cuticular and larval development, and L4-enriched transcripts related to reproductive and nervous system development, meiosis, and protein complex

formation [43]. Further, Luck *et al*. [43] found that, like *Brugia* spp. [44], *alt-1* and *alt-2* ('abundant larval transcripts 1 and 2') were amongst the most abundant transcripts in larval *D. immitis*, with *Bm*-ALT-2 being a known **Th1** suppressor and immunomodulator [45]. Lastly, Luck *et al.* [43] studied transcription of endosymbiotic *Wolbachia* within *D. immitis* and found an overall enrichment in *Wolbachia*-associated metabolic and biosynthetic pathways in mff relative to all other stages; these included pathways associated with haeme, riboflavin, folate and peptidoglycan synthesis, purine/pyrimidine metabolism and Sec translocases, which are essential components of *Wolbachia* secretion systems.

Order Enoplida

Species of *Trichinella* (trichina), *Trichuris* (whipworms) and *Capillaria* (hairworms) are the primary enoplidans of veterinary or human health importance. Genomes and transcriptomes are available for *Trichinella* [46–50] and *Trichuris* [51–54], but (to our knowledge) not for *Capillaria*.

Trichinella includes numerous species of veterinary relevance, most notably *T. spiralis*, a zoonotic parasite with broad host-specificity. Two transcriptomic studies [46,49] followed the sequencing of the *T. spiralis* draft genome [50]. Liu *et al*. [49] compared newborn L1s (NBLs), infective L1s in muscle (ML), and mixed adults (Ad) in pigs, finding that roughly one-third of *T. spiralis* genes were uniformly transcribed in all stages, with the rest showing stage-specificity. Many differentially transcribed genes related to excretory/secretory proteins, including serine proteases and DNase II enzymes, noted by Liu *et al*. [49] as important in *Trichinella* infection and host immunomodulation, respectively [50,55]. Gao *et al*. [46] studied DNA methylation in transcriptional regulation of *T. spiralis*. The study [46] noted that *T. spiralis* encodes homologues of a vertebrate DNA methyltransferase and tRNA methyltransferase not identified in other nematodes, which suggested a greater role for methylation in this species. Gao *et al*. [46] undertook further **RNA-seq** characterisation of NBL, ML, and mixed adult stages from pigs, and found that transcription negatively correlated with DNA methylation, supporting this role. This was further supported by the observation that methylation density was higher in promoter regions of weakly transcribed genes. Intriguingly, DNA methylation was enriched at exon splice boundaries and methylation hotspots were associated with alternative transcript splicing, particularly exon skipping [46].

Trichuris suis [51,52] of pigs, *Trichuris trichiura* of humans [53,54], and *Trichuris muris* [54] of mice have also been explored extensively through genomic and transcriptomic studies. Jex *et al*. [52] generated draft assemblies of male and female *T. suis* genomes and undertook transcriptomic analyses of larvae (L1/L2, L3, and L4 stages) and adults (male vs. female), and tissue-specific comparisons of the absorptive stichosome embedded within the host intestinal epithelium, relative to the luminal posterior body of adult males and females [52]. Parasitic L1/L2s were enriched for porins, ion/sugar transporters, redox enzymes, and a suite of known host immunomodulators [45], including cystatins, serpins, and a putative TGF-β mimic. Jex *et al*. [52] noted upregulation in L1/L2s of lactosylceramide synthesis, particularly for β-glucosylceramide. β-Glucosylceramide is anti-inflammatory and can block symptoms associated with inflammatory bowel disease (IBD) [56]. Aside from its relevance as a veterinary parasite, *T. suis* is a potential immunomodulator in humans and provides insight into treatment of inflammatory, autoimmune disorders, including, principally, IBD [57,58]. At L4, 28 days postinfection (dpi), Jex *et al*. [52] noted a peak in transcription of *Ts*-CEI, which is a chymotrypsin/elastase inhibitor that blocks neutrophil-mediated tissue remodelling and prevents host inflammatory responses [59]; neutrophils reach peak activity ~28 dpi in *T. suis*-infected pigs (cf. [60]). Trypsin-like secreted serine proteases were prominent among larval and stichosome-enriched transcripts for *T. suis* [52]. These proteases are implicated in degrading the host mucus barrier during the initial stages of *T. suis* infection [61].

The transition to adulthood in *T. suis* corresponded to an upregulation of sex-specific genes for gonadal development, sex-determination, gamete production, and mating behaviour/chemosensation [52]. The study explored transcripts in adult parasites associated with the stichosome (the worm's highly modified and absorptive pharynx/oesophagus), which, like tissue-embedded larval *T. suis*, were enriched for transcripts associated with immunomodulation, porins, secreted trypsin-like proteases, and ion/sugar transporters [52]. The study [52] also revealed an upregulation of glucose and lipid metabolism in this tissue, indicating that initial digestion may occur in the stichosome.

Order Rhabditida

Species of *Strongyloides* (e.g., *S. stercoralis*) are of particular veterinary and human health relevance [62]. The life-cycle of *Strongyloides* involves a complete free-living phase and an obligate, female-exclusive parasitic phase. Transcriptomic studies have focused on human-infective species and the switch between free-living and parasitic stages [63–67].

A key feature of the *Strongyloides* lifecycle and transmission centres around its ability to form resting environmental stages in the environment [62], which have been compared to the dauer larvae of free-living worms, such as *C. elegans* [68]. Stoltzfus *et al*. [65] undertook the first detailed quantitative transcriptomic study of *S. sterocoralis* L3 stages during environmental arrest and activation. The study [65] found overlaps in *S. sterocoralis* arrest and dauer formation in *C. elegans*, particularly in cyclic-GMP and TGF-ß signalling [69]. However, Stoltzfus *et al*. [65] found little evidence for upregulation of genes for dafachronic acid (DAF) synthesis (a key step in dauer formation in *C. elegans*; [70]) and suggested that although arresting stages had similar developmental phenotypes, they exhibited notable differences in their genetic mechanisms. This observation was followed more recently by a study [71] that explored activation and transcriptional changes in larval *S. stercoralis* on exposure to DAF, which found that, despite these differences, DAFs, particularly DAF-12, play key roles in larval activation and developmental arrest in *S. stercoralis*.

Hunt *et al*. [72] presented the landmark 'omics contribution to this field, with the publication of four *Strongyloides* genomes (*S. sterocoralis*, *S. ratti*, *S. venezuelensis*, and *S. papillosus*), comparing these with closely related free-living (*C. elegans*), invertebrate parasitic (*Rhabditophanes* sp. KR3021), and facultative vertebrate parasitic (*Parastrongyloides trichosuri*) nematodes. They identified ~1075 gene families acquired/expanded in parasitic species, including expansions of astacin proteases, SCP-TAPs, and tyrosine phosphatases [72]. Importantly, comparing free-living to parasitic phases of the lifecycle showed that these expanded gene families were upregulated with active parasitism [72]. Many of these parasitism-associated genes encode predicted secreted proteins and correlate with transcriptomic studies of parasitic stages across the phylum. These findings have been further explored in *S. papillosus* and *S. ratti* [73], which similarly find an upregulation of SCP-TAPs and astacin proteases with parasitism.

Further Work on the Helminth Secretomes, the Pathogenesis of Disease, and Immunomodulation

Until now, it has been a crucial task of parasitic nematode transcriptomics to identity and catalogue proteins that may enable infection. Future work must go beyond categorization and define the function of gene sets implicated in parasitism, both to devise new therapies against infection and to capture the possible medical benefits of dampening the host immune system. Many protease and other secreted protein families (e.g., SCP-TAPs) are expanded in parasitic nematode genomes and transcriptionally upregulated with infection. SCP-TAPs provides an example of the challenges that will be encountered when dissecting gene function in parasitic nematodes with advanced technologies.

SCP-TAPs proteins are one of the first classes of secreted molecules identified in *Anc. caninum* [74,75], and are expanded in hookworms and related nematodes [23,72,76]. The *in vivo* functions of SCP-TAPs proteins are unknown. In a few instances, individual SCP-TAPs proteins have been shown to block blood clotting [77] or suppress B cell receptor signalling [78]; both activities could enable infection by blood-feeding worms. A fundamental clue to their function arose from budding yeast, in which the SCP-TAPs Pry1 and Pry2 are required for efficient transportation of sterols across the cell membrane [79]. Remarkably, mutants in budding yeast can be transgenically rescued by the human homologue CRISP2 [79], the filarial homologue BmVAL-1 [80], or the strongylid worm homologue HpVAL-4 [81]. Meanwhile, other work has demonstrated that SCP-TAPs proteins may have a more general ability to bind other lipids, such as fatty acids [80,82].

Phylogenetically, SCP-TAPs in parasitic nematodes have a conserved core gene set and a highly expanded shell. The conserved core includes orthologs found in *C. elegans* that mediate core functions, such as regulating body size through TGF-ß signalling [83,84]. The expanded shell consists of genes that are not shared with *C. elegans*, that have duplicated and diverged; in *N. americanus* and *Anc. ceylanicum* there are 128 and 432 SCP-TAPs, respectively, versus 35 in *C. elegans* [23]. This large expansion, in which any one gene is likely to have little effect on fitness, is consistent with the 'gray pawn' hypothesis [85]: such diverse gene families may reflect a requirement for survival under unpredictably variable conditions (e.g., for parasitic nematodes, the diverse immune systems of their outbred mammalian hosts).

Defining clear biological functions of SCP-TAPs and other expanded protein families will require technologies adapted from *C. elegans* [86]. SCP-TAPs likely act either through transporting biologically active lipids, or by binding host protein ligands, or both. Defining small-molecule and host protein ligands, requires application of high-resolution mass spectrophotometry on individually tagged proteins extracted from infections. This has been used on model nematodes to expand the network of metabolic and signalling small molecules [87,88] and to define native protein–protein interactions [89], but has only begun to be applied to parasitic helminths. Tagging of SCP-TAPs might be achieved using specific antibodies; but given the large number of genes encoding them, a more scalable approach will probably be to epitope-tag proteins using **CRISPR** mutagenesis, which has been shown to work in *Strongyloides* [90], or similar methods. Having identified SCP-TAPs protein ligands, and inferred possible biological functions from them, it will be necessary to test such inferences by inactivating their genes *in vivo*. This, in turn, will likely require either CRISPR mutagenesis or **RNAi** [90].

Transcriptomics can guide this post-transcriptomic work in several ways. Long-read RNA-seq can be used to generate full-length cDNA structures for parasitic nematode genes [91], which will allow more accurate definition of their exon/intron organization – essential for CRISPR mutagenesis, RNAi, and other forms of genomic manipulation. Comparative analysis of expression patterns across gene families is already practicable with existing RNA-seq data, and will provide clues about cotranscribed genes that may act as partially redundant functional units.

Although we have delineated a possible strategy for functionally characterizing SCP-TAPs genes, this is merely one instance of many different categories of parasitic nematode genes that will all require post-transcriptomic analysis: proteases, protease inhibitors, a melange of diverse immunomodulators, and still-uncharacterized parasite-specific gene families such as SL4Ps and SCVPs. A recent analysis of over 50 parasitic nematode genomes [92] shows that dozens of such novel parasite-specific gene families remain to be understood.

State-of-the-art in Transcriptome Study Design, Sequencing and Informatics

NGS has transformed understanding of veterinary parasitic nematodes, but challenges remain. Even within species, multiple transcriptomic studies employ a mixture of pregenomic and post-genomic datasets and unstable gene models, using disparate NGS platforms, study designs, and informatic approaches. This limits comparisons among studies within and among species and stages. Further, although numerous groups now publish regularly in this field, new researchers or those with expertise in classical parasitology may find it difficult to determine how to incorporate transcriptomics technologies into their own studies. We discuss these challenges and recommend current approaches for robust transcriptomic analyses (Figure 2; see Data S1 in the supplemental information online), with a primary focus on Illumina-based methods.

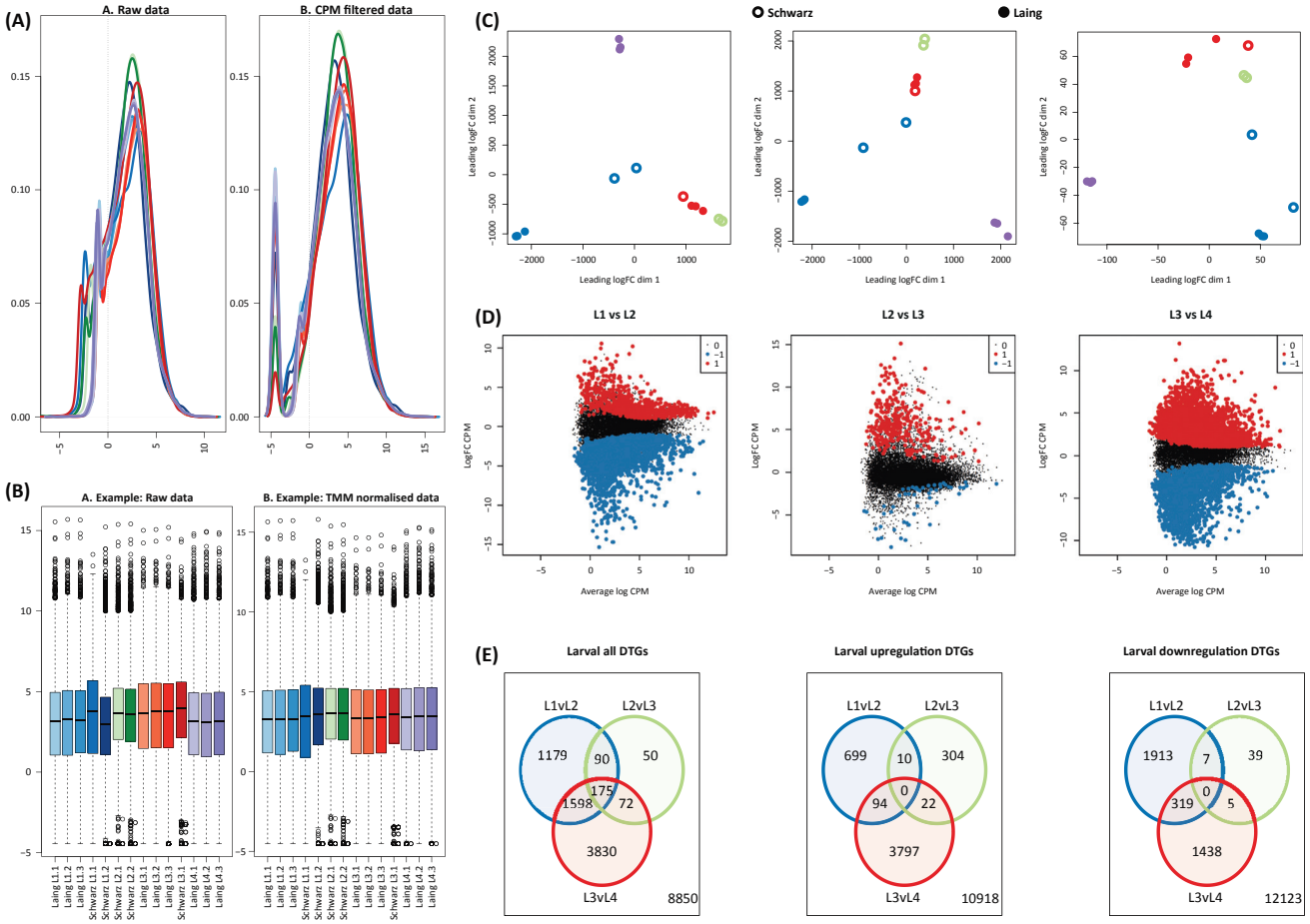

Figure 2. RNA-seq Analysis of *Haemonchus contortus* Larval Stages of Data Combined from Two Separate Studies (Laing *et al*. [35] and Schwarz *et al*. [36]) Using edgeR, limma and voom. (A) Filtering of low-abundance transcripts (<1 count per million (CPM) in at least five samples) to reduce false discovery rate through multiple pair-wise testing. (B) Using trimmed-mean of M-values (TMM) normalization to scale between and among all samples for relative differences in library size and minor batch effects. (C) Multidimensional scaling plot of raw (left), TMM-normalization (middle), and voom batch corrected (right) CPM data for all larval samples from Laing *et al*. (closed circles) and Schwarz *et al*. (open circles) for L1 (blue), L2 (green), L3 (red), and L4 (purple) stages. Note, post-voom the L2 replicate data from Schwarz *et al*. now sit between the L1 and L3 stage data. (D) MD plot showing differentially transcribed genes (DTGs) following voom batch correction; blue = downregulated in comparison, red = upregulated in comparison. (E) Venn diagram comparing overlap of DTGs between each larval stage using post-voom data, showing all (left), upregulated (middle), and downregulated (right) transcripts. The unix commands and an R-script required to run this analysis and generate these and additional quality control figures are provided in Data S1 in the supplemental information online.

Genomes are available for many veterinary parasitic nematodes and can be accessed through WormBase Parasite [93]. These provide standardized gene models allowing more reproducible, orthology-based comparisons of transcription among stages and species. Where genomes are unavailable, *de novo* transcriptomic assembly has significantly improved, with specialist transcript and isoform assemblers available [94]. Further, until now, due to the costs of sequencing and the difficulty in collecting sufficient material, many transcriptomic studies rely on single biological replicates per stage or condition. Modern analytical methods require multiple biological replicates to identify differentially transcribed genes reliably [95]. Falling sequencing costs and improvements in library barcodes (allowing sample multiplexing) should allow replicate sequencing for any study and is strongly recommended.

Comprehensive benchmarking studies are available for RNA-seq alignment and quantitation (e.g., [96]). Postalignment normalization of **raw read counts** is an important consideration. Strategies include scaling methods that adjust for sequencing depth to detailed statistical approaches that consider sample variance and technical biases (reviewed in [97]). Two common methods for scaling transcript abundance data are Reads Per Kilobase per Million reads (**RPKM**) or Fragments Per Kilobase per Million reads (**FPKM**); these methods consider transcript lengths and total number of reads generated per library. They retain some biases in estimating transcript abundance, and thus have been somewhat superseded by metrics such as Transcripts Per Million (**TPM**). However, most analytical tools accept raw or adjusted read counts directly and provide a variety of robust normalization methods. Packages such as voom [98] can reduce common technical artefacts, such as sample batch effects and support comparing RNA-seq among studies (Figure 2). Quantitative-PCR remains a common approach to test RNA-seq results.

A variety of packages are available for assessing differential transcription [96,98]. Most use pairwise t-test or Fischer's exact test with a *P*-value cut-off (e.g., <0.05). However, it is critical to account for multiple testing of the many thousands of transcripts in a given study, which will lead to numerous incorrect calls (i.e., false discoveries). This can be reduced by filtering low-abundance transcripts prior to testing [97]. Further, *P*-values for each test must be adjusted to limit false discoveries [99]. Limma [98] uses a linear or generalized linear model to make differential calls that are more robust to low replicate studies (e.g., most transcriptomic studies), false discovery, and technical artefacts. Complete protocols are available for packages such as edgeR/limma [98] and the HISAT suite [100] to assist in all of these analytical steps.

Concluding Remarks

Transcriptomic sequencing of parasitic nematodes of human and veterinary importance has explored changes associated with development and maturation to adulthood as well as a shift to parasitism during the lifecycle and over evolutionary time. However, major gaps remain. There is currently no central repository for all transcriptomes of parasitic nematodes. Most studies have deposited raw reads in EMBL ENA or NCBI's SRA databases, but many entries lack important metadata (including stage and source/host information) needed to make use of them, and a consensus on minimal information for these raw data is needed. NCBI Gene Expression Omnibus (GEO) provides a more standardized repository for many parasitic nematode transcriptomes. Excitingly, Wormbase ParaSite will include RNA-seq data in its planned release 11 (W. Bazant, pers. comm.). This will allow users to access and undertake a variety of intraspecies and interspecies analyses of published data using standardized pipelines.

Further biological questions can be explored using these approaches (see Outstanding Questions). With a centralized dataset, future efforts can include filling in gaps in current 'omics

Outstanding Questions

What is the centralized and standardized repository for parasite nematode RNA-seq and its analysis?

To what extent should the field focus on new parasite transcriptomes versus adding replication and exploring host variation for species that have already be explored?

How do parasitic worms alter their transcription and that of their host during the host immune response?

How does this differ with parasite species, mode of infection, and prior host exposure?

How do parasitic worms transcriptionally respond to anthelmintic drug exposure, and how does this compare to other forms of stress response and with drug resistance?

How are these transcriptional changes regulated, and what can they tell us about key aspects of parasite biology, including development, metabolism, reproduction, parasitism, host–parasite interactions and site-selection and immunomodulation?

data, including unstudied stages or tissues of a variety taxa, increased replication of existing datasets, or studies focused on changes with host genetics. Of additional particular interest, in our view, are studies focused on how parasitic nematodes differ in their stimulation and modulation of the host immune response using dual or targeted immune-cell population-specific transcriptomics, and how this differs with parasite species, mode and site of infection, and host exposure and nutrition. Further studies may harness these methods to explore how parasitic worms respond to sublethal anthelmintic drug exposure, how this differs from or overlaps with other types of stress responses, and how these responses change with drug resistance. Indications that translational repression may play a role in nematode biology should stimulate additional interest in understanding how parasitic nematodes regulate specific transcriptional responses during infection, mating, drug exposure, and host immune responses. Transcriptomics is not the only step down this path of discovery, but it is a mighty one. Nevertheless, as with any enabling technology, these methods stimulate as many new and interesting questions as they answer. Scientists studying parasitic nematodes will not be unemployed any time soon.

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Supplemental Information

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References

1. Pullan, R.L. *et al.* (2014) Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit. Vectors* 7, 37
2. Nicol, J.M. *et al.* (2011) Current nematode threats to world agriculture. In *Genomics and Molecular Genetics of Plant–Nematode Interactions*, pp. 21–43, Springer
3. Evans, T. and Chapple, N. (2002) *The Animal Health Market,* Nature Publishing Group
4. Brunsdon, R. (1980) Principles of helminth control. *Vet. Parasitol.* 6, 185–215
5. Kaplan, R.M. (2004) Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitol.* 20, 477–481
6. Baird, F.J. *et al.* (2016) The *Anisakis* transcriptome provides a resource for fundamental and applied studies on allergy-causing parasites. *PLoS. Negl. Trop. Dis.* 10, e0004845
7. Sharp, M.F. and Lopata, A.L. (2014) Fish allergy: in review. *Clin. Rev. Allergy Immunol.* 46, 258–271
8. Cavallero, S. *et al.* (2018) Tissue-specific transcriptomes of *Anisakis simplex* (sensu stricto) and *Anisakis pegreffii* reveal potential molecular mechanisms involved in pathogenicity. *Parasit. Vectors* 11, 31
9. Cantacessi, C. *et al.* (2009) A portrait of the 'SCP/TAPS' proteins of eukaryotes – developing a framework for fundamental research and biotechnological outcomes. *Biotechnol. Adv.* 27, 376–388
10. Maizels, R.M. (2013) *Toxocara canis*: molecular basis of immune recognition and evasion. *Vet. Parasitol.* 193, 365–374
11. Jex, A.R. *et al.* (2011) *Ascaris suum* draft genome. *Nature* 479, 529–533
12. Ma, X. *et al.* (2011) Comparative transcriptome sequencing of germline and somatic tissues of the *Ascaris suum* gonad. *BMC Genomics* 12, 481
13. Wang, J. *et al.* (2012) Silencing of germline-expressed genes by DNA elimination in somatic cells. *Dev. Cell* 23, 1072–1080
14. Smith, J.J. (2018) Programmed DNA elimination: keeping germline genes in their place. *Curr. Biol.* 28, R601–R603
15. Wang, J. *et al.* (2017) Comparative genome analysis of programmed DNA elimination in nematodes. *Genome Res.* 27, 2001–2014
16. Zhu, X.Q. *et al.* (2015) Genetic blueprint of the zoonotic pathogen *Toxocara canis*. *Nat. Commun.* 6, 6145
17. Zhou, R.Q. *et al.* (2017) Comparative transcriptomic analyses of male and female adult *Toxocara canis*. *Gene* 600, 85–89
18. Ansell, B.R. *et al.* (2013) Insights into the immuno-molecular biology of *Angiostrongylus vasorum* through transcriptomics – prospects for new interventions. *Biotechnol. Adv.* 31, 1486–1500

19. Yu, L. *et al.* (2017) Comparative transcriptomic analysis of two important life stages of *Angiostrongylus cantonensis*: fifth-stage larvae and female adults. *Genet. Mol. Biol.* 40, 540–549
20. McNulty, S.N. *et al.* (2016) *Dictyocaulus viviparus* genome, variome and transcriptome elucidate lungworm biology and support future intervention. *Sci. Rep.* 6, 20316
21. Cantacessi, C. *et al.* (2011) Deep insights into *Dictyocaulus viviparus* transcriptomes provides unique prospects for new drug targets and disease intervention. *Biotechnol. Adv.* 29, 261–271
22. Mangiola, S. *et al.* (2014) Analysis of the transcriptome of adult *Dictyocaulus filaria* and comparison with *Dictyocaulus viviparus*, with a focus on molecules involved in host–parasite interactions. *Int. J. Parasitol.* 44, 251–261
23. Schwarz, E.M. *et al.* (2015) The genome and transcriptome of the zoonotic hookworm *Ancylostoma ceylanicum* identify infection-specific gene families. *Nat. Genet.* 47, 416–422
24. Wei, J. *et al.* (2016) The hookworm *Ancylosoma ceylanicum* intestinal transcriptome provides a platform for selecting drug and vaccine candidates. *Parasit. Vectors* 9, 518
25. Wang, Z. *et al.* (2010) Characterizing *Ancylostoma caninum* transcriptome and exploring nematode parasitic adaptation. *BMC Genomics* 11, 307
26. Sotillo, J. *et al.* (2014) Secreted proteomes of different developmental stages of the gastrointestinal nematode *Nippostrongylus brasiliensis*. *Mol. Cell. Proteom.* 13, 2736–2751
27. Eccles, D. *et al.* (2018) *De novo* assembly of the complex genome of *Nippostrongylus brasiliensis* using MinION long reads. *BMC Biol.* 16, 6
28. Gasser, R.B. *et al.* (2007) *Oesophagostomum dentatum*: potential as a model for genomic studies of strongylid nematodes, with biotechnological prospects. *Biotechnol. Adv.* 25, 281–293
29. Tyagi, R. *et al.* (2015) Cracking the nodule worm code advances knowledge of parasite biology and biotechnology to tackle major diseases of livestock. *Biotechnol. Adv.* 33, 980–991
30. Cwiklinski, K. *et al.* (2013) Transcriptome analysis of a parasitic clade V nematode: comparative analysis of potential molecular anthelmintic targets in *Cylicostephanus goldi*. *Int. J. Parasitol.* 43, 917–927
31. Heizer, E. *et al.* (2013) Transcriptome analyses reveal protein and domain families that delineate stage-related development in the economically important parasitic nematodes, *Ostertagia ostertagi* and *Cooperia oncophora*. *BMC Genomics* 14, 118
32. Abubucker, S. *et al.* (2009) The transcriptomes of the cattle parasitic nematode *Ostertagia ostartagi*. *Vet. Parasitol.* 162, 89–99
33. Menon, R. *et al.* (2012) An analysis of the transcriptome of *Teladorsagia circumcincta*: its biological and biotechnological implications. *BMC Genomics* 13 (Suppl. 7), S10
34. Cantacessi, C. *et al.* (2010) First transcriptomic analysis of the economically important parasitic nematode, *Trichostrongylus colubriformis*, using a next-generation sequencing approach. *Infect. Genet. Evol.* 10, 1199–1207
35. Laing, R. *et al.* (2013) The genome and transcriptome of *Haemonchus contortus*, a key model parasite for drug and vaccine discovery. *Genome Biol.* 14, R88
36. Schwarz, E.M. *et al.* (2013) The genome and developmental transcriptome of the strongylid nematode *Haemonchus contortus*. *Genome Biol.* 14, R89
37. Ma, G. *et al.* (2018) Molecular alterations during larval development of *Haemonchus contortus in vitro* are under tight post-transcriptional control. *Int. J. Parasitol.* 48, 763–772
38. Lau, N.C. *et al.* (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 294, 858–862
39. Kariuki, M.M. *et al.* (2010) Differential transcript expression between the microfilariae of the filarial nematodes, *Brugia malayi* and *B. pahangi*. *BMC Genomics* 11, 225
40. Godel, C. *et al.* (2012) The genome of the heartworm, *Dirofilaria immitis*, reveals drug and vaccine targets. *FASEB J.* 26, 4650–4661
41. Fu, Y. *et al.* (2012) Novel insights into the transcriptome of *Dirofilaria immitis*. *PLoS One* 7, e41639
42. Bazzocchi, C. *et al.* (2008) Combined ivermectin and doxycycline treatment has microfilaricidal and adulticidal activity against *Dirofilaria immitis* in experimentally infected dogs. *Int. J. Parasitol.* 38, 1401–1410
43. Luck, A.N. *et al.* (2014) Concurrent transcriptional profiling of *Dirofilaria immitis* and its *Wolbachia* endosymbiont throughout the nematode life cycle reveals coordinated gene expression. *BMC Genomics* 15, 1041
44. Choi, Y.J. *et al.* (2011) A deep sequencing approach to comparatively analyze the transcriptome of lifecycle stages of the filarial worm, *Brugia malayi*. *PLoS. Negl. Trop. Dis.* 5, e1409
45. Hewitson, J.P. *et al.* (2009) Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. *Mol. Biochem. Parasitol.* 167, 1–11
46. Gao, F. *et al.* (2012) Differential DNA methylation in discrete developmental stages of the parasitic nematode *Trichinella spiralis*. *Genome Biol.* 13, R100
47. Korhonen, P.K. *et al.* (2016) Phylogenomic and biogeographic reconstruction of the *Trichinella* complex. *Nat. Commun.* 7, 10513
48. Liu, J.Y. *et al.* (2016) Proteomic analysis of differentially expressed proteins in the three developmental stages of *Trichinella spiralis*. *Vet. Parasitol.* 231, 32–38
49. Liu, X. *et al.* (2012) Global gene expression analysis of the zoonotic parasite *Trichinella spiralis* revealed novel genes in host parasite interaction. *PLoS Negl. Trop. Dis.* 6, e1794
50. Mitreva, M. *et al.* (2011) The draft genome of the parasitic nematode *Trichinella spiralis*. *Nat. Genet.* 43, 228–235
51. Cantacessi, C. *et al.* (2011) The transcriptome of *Trichuris suis* – first molecular insights into a parasite with curative properties for key immune diseases of humans. *PLoS One* 6, e23590
52. Jex, A.R. *et al.* (2014) Genome and transcriptome of the porcine whipworm *Trichuris suis*. *Nat. Genet.* 46, 701–706
53. Santos, L.N. *et al.* (2016) *De novo* assembly and characterization of the *Trichuris trichiura* adult worm transcriptome using Ion Torrent sequencing. *Acta Trop.* 159, 132–141
54. Foth, B.J. *et al.* (2014) Whipworm genome and dual-species transcriptome analyses provide molecular insights into an intimate host–parasite interaction. *Nat. Genet.* 46, 693–700
55. Liu, M.-f. *et al.* (2008) The functions of Deoxyribonuclease II in immunity and development. *DNA Cell Biol.* 27, 223–228
56. Zigmond, E. *et al.* (2007) β-glucosylceramide: a novel method for enhancement of natural killer T lymphoycte plasticity in murine models of immune-mediated disorders. *Gut* 56, 82–89
57. Summers, R.W. *et al.* (2005) *Trichuris suis* therapy in Crohn's disease. *Gut* 54, 87–90
58. Summers, R.W. *et al.* (2003) *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. *Am. J. Gastroenterol.* 98, 2034–2041
59. Rhoads, M.L. *et al.* (2000) *Trichuris suis*: A secretory serine protease inhibitor. *Exp. Parasitol.* 94, 1–7
60. Kringel, H. *et al.* (2006) A time course study of immunological responses in *Trichuris suis* infected pigs demonstrates induction of a local type 2 response associated with worm burden. *Int. J. Parasitol.* 36, 915–924
61. Hasnain, S.Z. *et al.* (2012) Serine protease(s) secreted by the nematode *Trichuris muris* degrade the mucus barrier. *PLoS Negl. Trop. Dis.* 6, e1856
62. Thamsborg, S.M. *et al.* (2017) *Strongyloides* spp. infections of veterinary importance. *Parasitology* 144, 274–284
63. Jaleta, T.G. *et al.* (2017) Parasitological and transcriptomic comparison of *Strongyloides ratti* infections in natural and in suboptimal permissive hosts. *Exp. Parasitol.* 180, 112–118
64. Nagayasu, E. *et al.* (2013) Transcriptomic analysis of four developmental stages of *Strongyloides venezuelensis*. *Parasitol. Int.* 62, 57–65

65. Stoltzfus, J.D. *et al.* (2012) RNAseq analysis of the parasitic nematode *Strongyloides stercoralis* reveals divergent regulation of canonical dauer pathways. *PLoS Negl. Trop. Dis.* 6, e1854
66. Marcilla, A. *et al.* (2012) The transcriptome analysis of *Strongyloides stercoralis* L3i larvae reveals targets for intervention in a neglected disease. *PLoS Negl. Trop. Dis.* 6, e1513
67. O'Meara, H. *et al.* (2010) Response of the *Strongyloides ratti* transcriptome to host immunological environment. *Int. J. Parasitol.* 40, 1609–1617
68. Golden, J.W. and Riddle, D.L. (1984) The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. *Dev. Biol.* 102, 368–378
69. Fielenbach, N. and Antebi, A. (2008) *C. elegans* dauer formation and the molecular basis of plasticity. *Genes Dev.* 22, 2149–2165
70. Kimura, K.D. *et al.* (1997) daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277, 942–946
71. Albarqi, M.M. *et al.* (2016) Regulation of life cycle checkpoints and developmental activation of infective larvae in *Strongyloides stercoralis* by dafachronic acid. *PLoS Pathog.* 12, e1005358
72. Hunt, V.L. *et al.* (2016) The genomic basis of parasitism in the *Strongyloides* clade of nematodes. *Nat. Genet.* 48, 299–307
73. Baskaran, P. *et al.* (2017) Duplications and positive selection drive the evolution of parasitism-associated gene families in the nematode *Strongyloides papillosus*. *Genome Biol. Evol.* 9, 790–801
74. Hawdon, J.M. *et al.* (1996) Cloning and characterization of *Ancylostoma*-secreted protein. A novel protein associated with the transition to parasitism by infective hookworm larvae. *J. Biol. Chem.* 271, 6672–6678
75. Hawdon, J.M. *et al.* (1999) *Ancylostoma* secreted protein 2: cloning and characterization of a second member of a family of nematode secreted proteins from Ancylostoma caninum. *Mol. Biochem. Parasitol.* 99, 149–165
76. Tang, Y.T. *et al.* (2014) Genome of the human hookworm *Necator americanus*. *Nat. Genet.* 46, 261–269
77. Del Valle, A. *et al.* (2003) Isolation and molecular cloning of a secreted hookworm platelet inhibitor from adult *Ancylostoma caninum*. *Mol. Biochem. Parasitol.* 129, 167–177
78. Tribolet, L. *et al.* (2015) Probing of a human proteome microarray with a recombinant pathogen protein reveals a novel mechanism by which hookworms suppress B-cell receptor signaling. *J. Infect. Dis.* 211, 416–425
79. Choudhary, V. and Schneiter, R. (2012) Pathogen-Related Yeast (PRY) proteins and members of the CAP superfamily are secreted sterol-binding proteins. *Proc. Natl. Acad. Sci. U. S. A.* 109, 16882–16887
80. Darwiche, R. *et al.* (2018) Crystal structure of *Brugia malayi* venom allergen-like protein-1 (BmVAL-1), a vaccine candidate for lymphatic filariasis. *Int. J. Parasitol.* 48, 371–378
81. Asojo, O.A. *et al.* (2018) *Heligmosomoides polygyrus* Venom Allergen-like Protein-4 (HpVAL-4) is a sterol binding protein. *Int. J. Parasitol.* 48, 359–369
82. Darwiche, R. *et al.* (2017) The pathogen-related yeast protein Pry1, a member of the CAP protein superfamily, is a fatty acid-binding protein. *J. Biol. Chem.* 292, 8304–8314
83. Morita, K. *et al.* (2002) A *Caenorhabditis elegans* TGF-beta, DBL-1, controls the expression of LON-1, a PR-related protein, that regulates polyploidization and body length. *EMBO J.* 21, 1063–1073
84. Cantacessi, C. *et al.* (2009) A portrait of the 'SCP/TAPS' proteins of eukaryotes – developing a framework for fundamental research and biotechnological outcomes. *Biotechnol. Adv.* 27, 376–388
85. Thomas, J.H. and Robertson, H.M. (2008) The *Caenorhabditis* chemoreceptor gene families. *BMC Biol.* 6, 42
86. Ward, J.D. (2015) Rendering the intractable more tractable: tools from *Caenorhabditis elegans* ripe for import into parasitic nematodes. *Genetics* 201, 1279–1294
87. Schroeder, F.C. (2015) Modular assembly of primary metabolic building blocks: a chemical language in *C. elegans*. *Chem. Biol.* 22, 7–16
88. Artyukhin, A.B. *et al.* (2018) Metabolomic 'dark matter' dependent on peroxisomal beta-oxidation in *Caenorhabditis elegans*. *J. Am. Chem. Soc.* 140, 2841–2852
89. Fonslow, B.R. *et al.* (2014) Mass spectrometry-based shotgun proteomic analysis of *C. elegans* protein complexes. *WormBook* 1–18
90. Gang, S.S. *et al.* (2017) Targeted mutagenesis in a human-parasitic nematode. *PLoS Pathog.* 13, e1006675
91. Magrini, V. *et al.* (2018) Improving eukaryotic genome annotation using single molecule mRNA sequencing. *BMC Genomics* 19, 172
92. Coghlan, A. *et al.* (2017) Comparative genomics of the major parasitic worms. *bioRxiv* 236539
93. Howe, K.L. *et al.* (2015) WormBase 2016: expanding to enable helminth genomic research. *Nucleic Acids Res.* 44, D774–D780
94. Wang, S. and Gribskov, M. (2017) Comprehensive evaluation of *de novo* transcriptome assembly programs and their effects on differential gene expression analysis. *Bioinformatics* 33, 327–333
95. Schurch, N.J. *et al.* (2016) How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? *RNA* 22, 839–851
96. Engstrom, P.G. *et al.* (2013) Systematic evaluation of spliced alignment programs for RNA-seq data. *Nat. Methods* 10, 1185–1191
97. Finotello, F. and Di Camillo, B. (2015) Measuring differential gene expression with RNA-seq: challenges and strategies for data analysis. *Brief. Funct. Genomics* 14, 130–142
98. Law, C.W. *et al.* (2016) RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. *F1000Research* 5, 1408
99. Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Methodol.* 57, 289–300
100. Pertea, M. *et al.* (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat. Protoc.* 11, 1650–1667