

Genomic and morphologic characterization of *Strongyloides nasua* causing fatal strongyloidiasis in coatis (*Nasua nasua*)

Benjamin G. Dewals

bgdewals@uliege.be

University of Liège

Dominika Lastik

University of Bath

Huan Zhao

James Cook University

Ophélie Piedfort

University of Liège

Meriem Flih

Hoja Nueva

Françoise Maréchal

University of Liège

Michela Deiana

Ospedale Sacro Cuore Don Calabria

Edward Morris

University of Bath

Oliwier K Maziarski

University of Bath

Philippe Compère

University of Liège

Dora Buonfrate

Ospedale Sacro Cuore Don Calabria

Richard S. Bradbury

James Cook University

Vicky L. Hunt

University of Bath

Keywords:

Posted Date: June 15th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9890174/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: Competing interest reported. Richard S. Bradbury is a editor in chief of npj Parasitology. Vicky L. Hunt is an associate editor at npj Parasitology. No other conflict of interest to disclose on the manuscript itself.

Abstract

The parasitic nematode *Strongyloides* sp. infects a large range of host species, including ungulates, carnivores, non-human primates, and humans, causing mostly asymptomatic chronic infections. *Strongyloides nasua* represents a previously understudied and poorly described species, which is capable of causing fatal hyperinfection in its host. We report the first genomic description and comprehensive morphological analysis of *S. nasua*, which caused fatal strongyloidiasis in two coatis (*Nasua nasua*) at a wildlife rescue centre in Peru, South America. The two animals were housed in the centre for a few months prior to clinical signs occurring, with infection ultimately leading to a fatal outcome. Post-mortem examination showed cachectic animals, inflamed intestine with enlarged Peyer's patches and mesenteric lymph nodes. Histological analysis revealed hyperinfection, with severe parasitic enteritis with the presence of numerous nematodes at various developmental stages entrapped in the intestinal mucosa. Direct microscopic examination of the nematodes revealed key morphological characteristics of parasitic female *S. nasua*. Combining 18S rRNA and *cox1* genotyping together with short- and long-read whole genome sequencing, our data revealed that *S. nasua* represents a member of a potentially new group in the genus *Strongyloides*, closely related to *S. stercoralis* and *S. procyonis*. The *S. nasua* genome was associated with expansion of acetylcholinesterases, embryonic development and developmental processes and contraction of orthogroups associated with immune and antimicrobial responses, indicating potential diversification or specialisation of associated processes in *S. nasua*.

Introduction

The genus *Strongyloides* contained a number of species of soil-transmitted gastrointestinal parasitic nematodes infecting a wide range of vertebrates, including mammals, birds, amphibians, and reptiles¹. Many species of *Strongyloides* are generally host specific in their parasitic cycle. Among described *Strongyloides* species, *S. stercoralis* is by far the most studied species of the genus as it infects humans, as well as their closest companion animals, dogs and cats². Although referred to as one species, molecular tools have allowed the identification of two main lineages of *S. stercoralis* either restricted to canids or infecting canids, felids, non-human primates and humans³. Recent data have further demonstrated that *S. stercoralis* is originally a parasite of canids, which then mostly evolved separately in canids and humans, but without becoming separate species². *S. fuelleborni* infects non-human primates but has been reported to be prevalent in humans in parts of Asia and Africa, and apparently undergoes only human to human transmission in New Guinea⁴. Another *Strongyloides* species, related to *S. ransomi* of pigs, has been reported infecting infants in New Guinea². In addition, other *Strongyloides* species infect livestock, such as *S. papillosus*, which infects ruminants, as well as *S. procyonis* which infects several procyonid species like badgers, raccoons, raccoon dogs, and weasels.

The life cycle of *Strongyloides* alternates between free-living and parasitic generations. The parasitic stage is represented by the parthenogenetic female only⁵, which lives in the small intestine of its host where it produces eggs. These eggs are either passed in faeces and hatch in the environment, or hatch

within the intestine (vis. *S. stercoralis*, *S. procyonis*, *S. felis*)⁶ and are passed as rhabditiform first stage larvae (rL1). Only *Strongyloides* species with intestinal hatching larvae are capable of internal auto-infection leading to hyperinfection and systemic strongyloidiasis². These rhabditiform offspring develop either directly into infective third-stage infective filariform larvae (iL3s) or into a sexually reproducing rhabditiform adult generation, including both male and female nematodes. These sexually reproducing free-living adults' progeny develop either into iL3s or further rhabditiform generations. The number of free-living rhabditiform generations is dependent upon both the species of *Strongyloides* involved, and environmental conditions. When iL3s penetrate the skin of a host they either migrate to the gut directly, or via the pulmonary or alternative routes^{7,8}, where they develop into parasitic adults⁹. In some species, the L3 will engage in an erratic tissue migration phase into other organs such as the mammary gland, where they enter a hypobiotic state¹⁰. Thus, two genetically identical but morphologically different adult female stages exist, one obligate and parasitic and one facultative and free-living.

Most cases of strongyloidiasis are reported in tropical and subtropical countries, and associated with a lower socioeconomic status¹¹. In immunocompetent adult hosts, strongyloidiasis is often asymptomatic but in younger or immunocompromised individuals, infection can result in severe hyperinfection and disseminated disease^{10,12}. The female parasite nematodes reside deep in the intestinal crypts and can release many larvae which are excreted in the faeces but can also engage in cause an autoinfection with erratic tissue migration leading to disseminated disease¹¹. Such scenarios of hyperinfection caused by autoinfection are often fatal and are characterized by proliferation of parasites within the host, causing bacterial sepsis due to disruption of the intestinal barrier, and disseminated disease, where massive numbers of larvae migrate through various organs and tissues.

Diagnosis of strongyloidiasis in human and veterinary medicine has historically been based on the morphological identification of larvae or embryonated eggs in faecal samples, an approach suffering low sensitivity¹³. Adult parasitic females are extremely thin and not easily found during post-mortem examinations^{13,14}. Although molecular diagnostic tools such as real-time PCR (qPCR) are becoming more widely used, they often lack sufficient specificity for reliable species-level identification^{13,15}. Precise detection of *Strongyloides* species other than *S. stercoralis* in humans and companion animals typically depends on detailed morphological examination of cultured free-living adult stages or on molecular genotyping techniques¹⁶. Both methods are technically demanding and time-consuming, increasing the risk of underdiagnosis or erroneous attribution to *S. stercoralis*, given its prominent clinical relevance.

Comparative omics approaches have become important for understanding evolution, diversification and characterisation of parasitic nematodes¹⁷. There are currently publicly available genome assemblies for only four *Strongyloides* species: *S. stercoralis*, *S. ratti* and *S. venezuelensis*, parasites of rodents, and *S. papillosus* found in ruminants. These genome assemblies are derived from laboratory strains. Improving the availability of genomic data for *Strongyloides* species from wild hosts will expand our repertoire of data and improve our understanding of genomic diversity and parasite biology for *Strongyloides* sp.¹⁸.

Research efforts have traditionally prioritized *S. stercoralis*, while other *Strongyloides* species of possible human and veterinary importance remain underexplored. We report here two fatal cases of strongyloidiasis in ring-tailed coatis (*Nasua nasua*) in a tropical area of Peru, South America. Coatis are members of the family *Procyonidae* from which *Strongyloides* infection has been reported in a study from the early 20th century where the morphology of female parasites and free-living forms of *Strongyloides* sp. found in coatis was reported¹⁹. The species was referred to as *S. nasua*, whereas some confusion of this species with *S. stercoralis* had also been reported²⁰.

Results

Strongyloides sp. infection in coatis in a rescue centre in Peru.

In March and April 2024, two juveniles ($\pm$ 4 month-old) male coatis housed at the Hoja Nueva rescue centre (Department of Madre de Dios, Peru, South America) were presented to the veterinarians working at the rescue centre for poor appetite, weight loss and lethargy. Within 48 hours, their health declined rapidly. The animals became anorectic, severely dehydrated, had watery diarrhoea and died after about 24 hours of hospitalization despite receiving support therapies such as broad-spectrum antibiotics, parenteral fluidic therapy, gastro-protectant, antiemetic, and vitamins.

At post-mortem examination, both animals showed cachexia (Fig. 1a), and signs of watery diarrhoea. At necropsy, significant gastro-enteritis with enlarged Peyer's patches and mesenteric lymph nodes were observed (Fig. 1b). Upon opening of the small intestine, macroscopic examination revealed a significant thickening of the mucosa (Fig. 1c-d). A direct smear of the intestine to collect the intestinal content was performed for microscopic examination, revealing the presence of a high number of nematodes. Although field microscopic examination was performed at 400 $\times$ magnification with no scaling (Fig. 1e-f), the observation of adult females compatible with *Strongyloides* sp. parasites could be made. No additional macroscopic lesion could be observed.

Samples from several tissues, including small intestine were collected in formalin 10%. Histological examination of the intestinal tissue of both animals revealed generalized and severe lymphocytic and eosinophilic enteritis, with a severe loss of the structure of the intestinal villi and the presence of numerous nematode stages in the mucosal layer (Fig. 2a-e). Nematodes within the intestinal wall were compatible with *Strongyloides* sp. developmental stages, based on the observation on cross-sections of a paired genital tract, a relatively large intestine, and a small size of the parasites (Fig. 2e-f)²¹. Adult female nematodes were found at high number from the collected intestinal content and preserved in 70% ethanol for further microscopic and molecular characterization.

Morphological analysis.

Adult worms were preserved in 70% ethanol, measurements listed hereafter are approximate only. The deterioration of the worms means that phasmids and papillae were not clearly discernible in available material, and the finer details of the oral cavity region were difficult to observe using classical

microscopic examination (Fig. 3a-h). The L1 and L2 rhabditiform larvae were too deteriorate to undertake comprehensive morphological analysis and very few, deteriorated, L3 filariform larvae were available. Scanning electron microscopy (SEM) examination of two specimen allowed a detailed morphological characterization of the nematode (Fig. 4a-g), which were used to generate morphological diagrams (Fig. 5).

The parasitic female nematodes are serpentine, slender, filiform and cylindrical with a translucent body, measuring approximately 1.7–2.3 mm in length and 25–35 μm at the widest point and tapering slightly towards the anterior region (Fig. 3a and Fig. 5g). The cuticle is thin and has fine 1 μm wide transverse striations (Fig. 4d). Lateral chords are present but not conspicuous, extending slightly into the pseudocoel. The muscle structure is meromyarian and platymyarian (Fig. 2f). The mouth is dorsoventrally elongated and surrounded by a low circumoral elevation (Fig. 4a). In *en face* view, the stoma is elongate-hexagonal, measuring approximately 3 μm long by 1 μm wide (Fig. 4b and Fig. 5a). The circumoral elevation is divided into six lobes, comprising paired subdorsal, lateral, and subventral lobes; a deep lateral sulcus is present on each side of the median stomal region (Fig. 3b, Fig. 3c, and Fig. 5a). The oesophagus is long and filariform and takes up approximately 40% of the entire length of the body (Fig. 5b). A nerve ring is found approximately 10% along the body from the anterior, at approximately one quarter of the length of the oesophagus. This nerve ring demarcates a muscular anterior from the glandular posterior region. The oesophageal-intestinal junction is subtle rather than sharply demarcated. The intestine is straight. The reproductive system is didelphic-amphidelphic (Fig. 2e and f, Fig. 5c) with reflexed ovaries running parallel to the intestine (Fig. 5c). Developing oocytes are arranged in single or double rows. The uteri are straight, paired, and converge towards the vulva. The vulva subtly protrudes and is placed slightly posterior to the mid-body (Fig. 4d). The vagina is very short, T shaped, with distal arm entering into the uterus being longer and slightly wider than the proximal arm (Fig. 5d). A small lateral pore-like structure was observed 12 μm anterior to the tip of the tail and lateral and posterior to the anal opening (Fig. 4f). The body tapers abruptly distal to the rectum, into a rounded conical posterior tip with a blunt ending (Fig. 4e and f, Fig. 5e), consistent with the “bulbous tail” described by Darling et al. in 1911¹⁹.

Eggs (Fig. 5f) are often seen in mucoid strings containing 3 to 4 eggs. Up to three eggs were seen in the uteri of the three gravid parasitic adult females examined. They are distinctly ovoid to elliptical, thin shelled, averaging approximately 52 μm long by 32 μm wide. Each egg observed contained a developing rhabditiform larva upon ejection from the vulva. The rhabditiform larvae has a rhabditiform oesophagus and measures approximately 230 μm long by approximately 14 μm wide (Fig. 3e).

Fresh faecal material was further collected from an additional healthy coati (7 month-old, male), housed in a fenced enclosure, and which was in indirect contact with one of the fatal cases described above. A modified Baermann apparatus was performed to collect potential free-living forms of this *Strongyloides* sp. (**SI Fig. S1**). A significant number of nematodes were collected and observed by microscopic examination (original magnification = 400 $\times$), revealing the presence of free-living male and females

nematodes, sharing several characteristics with free-ranging nematodes of *Strongyloides* sp²¹. Such a discovery supports the hypothesis of a severe infection of the coatis housed in the rescue centre.

Free-living adult stages conform to adult female *Strongyloides* morphology, both having a spindle shaped body tapering towards the anterior and a rhabditiform oesophagus^{21,22}. Free-living forms were only seen in the field, and measurements were not possible. Among differentiating features of the **free-living female** (Fig. 5h), the post-vulval narrowing is less than 10% of the total body width and the angle of rotation of the vulval opening is between 90° and 100° to the lateral axis of the body. Very few gravid females were available for analysis (**Fig. S1c**). One gravid female had four to five eggs in the uterus, but only half of the uterus was filled, and the eggs were in a very early stage of development. The true number of eggs in a fully gravid female could not be ascertained. **The free-living male** (Fig. 5i) has a rhabditiform oesophagus, the gubernaculum is distinct, having a bluntly rhomboid base leading into a concave spicule (**Fig. S1d**). The majority of the body is made up of the testes.

Genotype sequence analysis reveals distinct *Strongyloides* species.

To identify the *Strongyloides* species infecting the coatis, intestinal content from one of the two cases was preserved in 70% ethanol. DNA was extracted and genotyping PCR targeting the *18S rRNA* HVR-IV and cytochrome c oxidase subunit I gene (*cox1*) were performed and sequenced. Sequencing of the PCR amplicons revealed one *18S rRNA* sequence, and two OTUs for *cox1*; which branched together between *S. stercoralis* lineages A and B, and clade of sequences of *S. procyonis* and *Strongyloides* sp. found in racoons, racoon dogs, Japanese badgers and a Siberian weasel (Fig. 6a). Together, these data confirmed that the parasites found in coatis are *Strongyloides* sp., distinct from any known species. Together with the morphometric examination in Fig. 3, we can confidently conclude that the hyperinfection syndrome seen in these coatis was caused by *S. nasua*.

Genomic sequence characteristics of *S. nasua*.

The genome of *S. nasua* was sequenced using short-read Illumina paired end reads and long-read Oxford Nanopore Technology. In total, 624,000 base-called reads were obtained from long-read sequencing, with a median quality Q15.7 (**SI Fig. S2**) and N50 of 7,043 bp. After removal of long-reads assigned as bacterial contamination, the remaining 4,311 (N50 6,081) reads were used to scaffold the short-read assembly. Illumina NovaSeq (PE150) sequencing yielded ~ 35.5 Gb of pair-end reads, which were further filtered to remove bacterial aligning reads (**SI Fig. S3a-b**). The final assembly comprised 2,130 contigs with a N50 of 54,526 and mean sequence length of 19,395 bp (S.D. = 31,952), with an assembly coverage of 99.62% and a BUSCO completeness score of 96.3%. Overall, 78% of the assembled genome is in the top 500 longest scaffolds (**SI Fig. S3c**). The genome is estimated to be 41.9 Mb long, similar to other *Strongyloides* sp.⁵.

Of the 13,225 genes predicted (14,217 including predicted transcript variants) in the *S. nasua* genome, 1.5% were assigned to species-specific orthogroups and 97.2% of genes were assigned to orthogroups also containing genes from other *Strongyloides* sp. (**SI Table 1**). Of these orthogroups, 76 and 317 had

expanded and contracted, respectively, where the *S. nasua* and *S. stercoralis* subclade diverged from other *Strongyloides* species (Fig. 6b). At this shared *S. nasua* – *S. stercoralis* node, an expansion was observed in orthogroups that include genes enriched for GO terms such as phosphatase and acetylcholinesterase (Fig. 6c), which has previously been associated with a role in *Strongyloides* parasitism²³. There were also expansions of orthogroups involved in hatching, oxidoreductase activity and hormone metabolic processes, suggesting specialization of development associated regulation processes. Contracted gene families (Fig. 6d) included those associated with immune defence, including defence against bacterium, antimicrobial response and innate immune response, and gene enrichment of KEGG pathways involved in protein digestion and absorption (**SI Fig. S4a-b**).

A further 235 orthogroups had expanded and 328 had contracted specifically in the *S. nasua* genome. The 235 expanded *S. nasua* orthogroups also included genes enriched for GO terms involved in muscle tissue development, embryonic development, acetylcholine activity, response-related and hormone related terms (Fig. 6e), and an enrichment of KEGG pathways involved in metabolism and metabolites and adsorption (**SI Fig. S4c**). Interestingly, the 328 contracted orthogroups were enriched for GO terms involved in hatching and emergence from protective structure, phosphorylation and phosphatase activity and protein digestion and adsorption pathways, suggesting that there could be unique features associated with these processes in *S. nasua* (Fig. 6f, **SI Fig. S4d**). GO enrichment of genes associated with hatching, protein digestion and absorption pathways was observed in orthofamilies specifically expanded in *S. stercoralis* (**SI Fig. S5a-b**). Contracted orthofamilies in *S. stercoralis* include genes enriched for GO terms involved in acetylcholinesterase activity and processes (**SI Fig. S4c-d**), suggesting further species-specific specialization.

Discussion

Rescue and rehabilitation of wildlife that has fallen victim to the illegal pet trade and deforestation is a global concern, especially in some regions in South America where wildlife and human populations coexist in close proximity^{24,25}. Veterinarians and volunteers in these rescue centres are committing with day-to-day care and face the challenge of releasing healthy wild animals, together with dealing with potential zoonotic risks. In Peru, the Hoja Nueva rescue centre faced several fatal cases in ring-tailed coatis, which were initially reported as septic shock, but took the attention of the attending veterinarian. We report here two fatal cases that were associated with severe strongyloidiasis, caused by a not yet characterized species of the genus *Strongyloides*, namely *S. nasua*.

We provide unprecedented, detailed description of *S. nasua*, at both morphological and molecular levels. Our data revealed that *S. nasua* demonstrates classical morphology of the genus, belonging to the sub-group of *Strongyloides* having straight ovaries, including *S. stercoralis*, *S. procyonis*, *S. myopotami*, *S. dasypodis*, *S. spearei*, and *S. rattii*. The oesophagus to body length percentage (~ 40%) is high for the genus, differentiating it from *S. spearei* (20%), *S. stercoralis*, *S. fuelleborni*, *S. cebus*, *S. myopotami*, *S. venezuelensis*, and *S. procyonis* (~ 25%); as well as *S. rattii*, *S. ardeae*, *S. physali* (30%), but consistent with *S. lutrae*, *S. dasypodis*, *S. gulae*, and *S. serpentis* (40%)^{26,27}. The tail morphology is very close to that of *S.*

stercoralis and *S. procyonis*, but distinct from other *Strongyloides* species^{26,27}. The hexagonal stoma with six lobes of the circumoral elevation is morphologically most similar to *S. stercoralis* and *S. procyonis*^{26,27}. The subtly protruding vulva at the mid-body is most reminiscent of *S. fuelleborni*²⁷. The vulval morphology of the free-living adult is reminiscent of *S. stercoralis* in having a 90°-100° vulval angle but differs in having a < 10% post vulval constriction. Although no freshly passed faecal samples were available for analysis, the presence of L1 rhabditiform larvae in intestinal wall sections and hatched L1 rhabditiform larvae in intestinal contents, alongside a straight ovary *Strongyloides*, indicates that hosts will likely pass larvae rather than eggs in faeces.

We provide the first genome assembly of a wild *Strongyloides* species, combining both short- and long-read sequencing. The high BUSCO completeness score of 96.3% and orthology shared with other *Strongyloides* species, suggests a highly complete assembly with strong conservation of *Strongyloides* biology within the genus. An expansion of acetylcholinesterase-associated orthogroups was identified at the shared *S. stercoralis*-*S. nasua* ancestral node, plus a further expansion of these gene families at the *S. nasua* node. The acetylcholinesterase gene family is generally expanded in *Strongyloides* species compared to other nematodes but appears to have further expanded in *S. nasua*²³. Acetylcholinesterase genes are well described in parasitic nematodes and have been implicated in host-parasite interactions^{28,29,23}, particularly in inhabiting mucosal environments where they may hydrolyse host acetylcholine and modulate local physiological or immune responses^{29,30}. However, in *Strongyloides* many of the genes in the expanded acetylcholinesterase families are non-canonical with altered active sites, and their role in parasitism is unclear. The relative high gene expression levels and presence as proteins during the adult *Strongyloides* parasitic life cycle stage supports that they have a role during infection²³. The lineage-specific divergence in *S. stercoralis* and *S. nasua* may represent an adaptive mechanism within the subclade, potentially reflecting differences in hosts, transmission or host-parasite interactions.

Contraction of orthogroups associated with the immune response and antimicrobial genes at the shared *S. stercoralis*-*S. nasua* node could reflect difference in the response to the microbial environment of the host intestine. It would be interesting in future work to compare such observations to other wild collected species to establish if host (or host microbiome) diversity drives evolution of gene family expansion and contractions, compared to parasites maintained in genetically similar laboratory hosts. Indeed, loss of acetylcholinesterase gene families at the *S. stercoralis* node, whereas expanded genes involved in muscle development and acetylcholine metabolism at the *S. nasua* node could result from the fact that *S. stercoralis* genome was obtained from laboratory strains and could be driven by environmental adaptation rather than species adaptation. Differences between the *S. stercoralis*-*S. nasua* node and *S. ratti*, *S. venezuelensis* and *S. papillosus* may also reflect differences in reproductive strategies i.e. the presence of larvae as well as eggs released in the host intestine. Further investigations are required to better understand what we can learn from global genomic data on *Strongyloides* sp. biology and parasitism.

In conclusion, we provide for the first time a comprehensive description of *S. nasua*, a parasite of coatis, forming a separate clade within the *Strongyloides* genus, in terms of morphology and genomic sequence. This species likely undergoes autoinfection within its host animal species and is here described causing fatal hyperinfection in ring tailed coatis. While infections of human populations globally by the threadworm *S. stercoralis* have recently gained significant attention¹¹, this study highlights the importance of *Strongyloides* spp. as potentially fatal pathogens in animals, and in particular in wildlife and wild animals housed in rescue centres.

Materials and methods

Rescue center

The cases occurred at Hoja Nueva (<https://hojanueva.org/>), a rescue center focused on the rehabilitation and reintroduction of wildlife species in the Peruvian Amazon.

Histology

Organ explants were fixed in 10% buffered formalin and embedded in paraffin blocks. Five-micron sections were then stained with hematoxylin and eosin (H&E) prior to microscopic examination performed using a DM2000 LED microscope (Leica) equipped with a DF450 C camera (Leica). Images were acquired using the Leica Application Suite 4.2 software (Leica).

Digital microscopy

Images from single nematodes were obtained from diluting the intestinal content of the dead coati preserved in 70% ethanol, and selecting the most intact nematodes to be placed on a microscopic slide. Morphological examination of the parasite structures was performed using a Keyence VHX-7000 digital microscope.

Scanning electron microscopy

Nematode specimens preserved in 70% ethanol were completely dehydrated through a graded ethanol series (70, 90–100%). Nematodes were taken in ethanol drops, deposited and left to air-dry on glass slides. Subsequently, the samples were sputter-coated with gold using a Balzers sputtering unit SCD 004 (BAL-TEC, Lichtenstein). Scanning electron micrographs of the gold-coated samples were obtained using a FEG-SEM TESCAN Clara under high vacuum conditions, with the Everhart-Thornley (ET)-secondary electron detector at a 40 mm working distance and 10 kV accelerating voltage.

DNA extraction

DNA was extracted from intestinal content preserved in 70% ethanol. Ethanol was washed by centrifugation at $8,500 \times g$ for 30 seconds, the pellet thoroughly suspended in sterile phosphate-buffered solution (PBS) by vortexing, and the sample was left overnight to equilibrate at 4°C. Samples were centrifuged at $8,500 \times g$ for 30 seconds, supernatant removed and the tube containing the pellet was placed at -80°C for 30 min, then thawed on a heat-block at 100°C for 1 min. DNA was extracted using the DNeasy PowerSoil Pro kit (Qiagen) according to the manufacturer's recommendation and using the TissueLyser II (Qiagen).

PCR and genotyping

To amplify HVR-IV 18S ribosomal rRNA and COX1 genomic regions, the following primers were used : Nextera_HVR_IV_F (5'-TCGTCGGCAGCGTCAGATG TGTATAAGAGACAGCGGGCCGGACACTATAAGG-3') Nextera_HVR_IV_R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCTCTAAACAGGAACATAATGATCACTAC-3') ; or Nextera_SSP_COX1_F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTGATCCTAGTTCTGGTGGTAATCC-3'), and Nextera_SSP_COX1_R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTAGCAGCAGTAAAATAAGCACGAGA-3'). PCR products were further purified and processed for Illumina sequencing, as described. Sequencing was performed at the GIGA Genomics platform (ULiege).

Phylogenetic analysis

The raw Illumina sequencing data were analysed using a custom workflow implemented in Geneious Prime (<https://www.geneious.com>), as previously described^{4,16}. The analytical pipeline incorporated sequence quality filtering, read trimming, contig assembly, and haplotype assignment. Bayesian phylogenetic inference was conducted in MrBayes using MUSCLE-aligned *cox1* sequences generated with MUSCLE³¹. Phylogenetic reconstruction was performed under the general time-reversible nucleotide substitution model.

Illumina gDNA extraction

Between 1 to 30 nematodes were taken from ethanol preserved samples, transferred into Eppendorf tubes and washed with cold 1x PBS three times to remove ethanol, followed by an incubation at 4°C overnight. Nematodes were transferred to a Biomasher II tube (Nippi) and NEB's Monarch Spin gDNA Extraction Kit was used to extract gDNA according to the manufacturer's instructions, with the addition of the following step. During lysis, 200 µL of lysis buffer was added along with 10 µL Proteinase K before manual homogenisation with a pestle and then incubated on a shaking thermomixer at 56°C. Nematodes were checked under the microscope every 30 minutes until fully dissolved. The elute was concentrated using DNA Clean and Concentrator-5 (Zymo) and resuspended in 6 µL of dd water. Samples were quantified using a Qubit (Thermo Fisher Scientific) and sent to Novogene for mRNA library

preparation and Illumina NovaSeq X Plus paired-end sequencing. A total of three samples were used for Illumina sequencing.

Oxford Nanopore Technology (ONT) gDNA preparation

Single nematodes were pipetted into a 96 well plate using a glass pipette and checked for quality. The nematodes were washed three times with cold 1x PBS, followed by an incubation at 4°C overnight. NEB's Monarch Spin gDNA Extraction Kit was followed as before, with nematodes being checked under the microscope every hour until they were fully lysed. The elute was concentrated using a SpeedVac for 45 minutes at 30°C and then resuspended in 6 µL of dd water. The REPLI-G Midi kit was used for gDNA amplification and gDNA quantities checked using a BR dsDNA kit on a Qubit (Thermo Fisher Scientific). 1.5 µg of amplified gDNA was used for T7 Endonuclease I digestion (NEB). The samples were incubated at 20°C for 30 minutes and 65°C for 30 minutes in thermal cycler, then quantified and stored at -20°C.

ONT gDNA sequencing

The ONT Ligation Sequencing gDNA Native Barcoding kit (SQK-NBD114.23) was used according to the manufacturer's protocol for library preparation of six nematode samples, using 400 ng of starting material from each. A Qubit and TapeStation were used after end-prep to calculate molarity for barcoding and normalised to the lowest molarity sample. The prepared library was loaded onto a single Flow Cell (R10.4.1), sequenced using the GridION (Oxford Nanopore Technologies) and basecalled in MinKNOW.

Genome Assembly

Short-read Illumina paired-reads were trimmed using trim-galore³² and checked for quality with FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Non-*Strongyloides* contamination was identified and removed using Kraken2³³. K-mers and repeat content was estimated using Jellyfish³⁴ and GenomeScope³⁵. The long ONT reads were assessed for length and quality using NanoPlot³⁶ and reads over Q7 kept. Nematode reads were identified using Diamond blastx³⁷ and retained. Contamination was visualised using Blobtools before and after removal of contaminant sequences³⁸. The genome was assembled using Spades³⁹ using the --nanopore flag to combine short- and long-read data and correct the assembly. Contigs under 500 bp were removed and coverage and depth were analysed using samtools⁴⁰. Genome assembly and raw sequence data are available on NCBI (<https://www.ncbi.nlm.nih.gov/search/all/?term=PRJNA1435085>, Accession number: PRJNA1435085).

Gene model and orthogroup annotation

BRAKER2 (v. 3.0.8)⁴¹ was used for gene annotations, run with default parameters and eukaryota.fa from orthodb for protein evidence. AGAT (v. 1.5.1)⁴² was used to keep only the longest transcript variant using the -agat_sp_keep_longest_isoform.pl script and the gff3 file from BRAKER3. This was carried out for all *Strongyloides* genomes in the analysis (*S. venezuelensis*, *S. stercoralis*, *S. ratti*, *S. papillosus* and *S. nasua*). Gffread (v. 0.12.7)⁴³ was used to extract CDS from all genomes using the longest isoform containing gff

files generated by AGAT. Using default parameters, OrthoFinder3 (OF3) (v. 3.1.0)⁴⁴ was used to generate orthogroups across the five *Strongyloides* species (**SI Table 1**). CAFE5⁴⁵ was used to infer gene family expansion/contraction at each node of the tree rooted tree generated from OF3 (**SI Table 2**).

Functional annotation with Egnog and GO enrichment

To assess functional characteristics of CAFE5-detected expanded and contracted gene families, Gene Ontology (GO) term and KEGG pathway enrichment analyses were performed on the longest isoforms from each gene, using the hypergeometric test from clusterProfiler⁴⁶ in R v 4.4.3. (**SI Tables 3–8**).

Isoform GO and KEGG terms were provided via eggNOG-mapper v2.1.8⁴⁷ and eggnog database v5.0.2⁴⁸ on “very-sensitive” mode.

Declarations

Acknowledgements

The authors are grateful to the staff at the Hora Nueva Rescue Centre for providing samples, as well as to the Centre for Applied Research and Education in Microscopy (CAREM, ULiège), Prof. E. Parmentier and J. Campisi for providing access and assistance to the microscopic equipment at FOCUS. We also thank J. Javaux and S. Leemans for technical assistance. We are also grateful to the FARAH histology facility and assistance of M. Sarlet.

Funding Declaration

D.L. and V.H. are funded by BBSRC grant BB/X016676/. B.D. is a programme investigator at the WEL Research Institute grant X.1515.24. H.Z. is supported by an Australian Government Research Training Program scholarship.

References

1. Viney, M. *Strongyloides* spp. *WormBook* <https://doi.org/10.1895/wormbook.1.141.1> (2007) doi:10.1895/wormbook.1.141.1.
2. Zhao, H., Constantinoiu, C. & Bradbury, R. Human, canine and feline strongyloidiasis: beyond *Strongyloides stercoralis*. *Parasitology* 1–9 (2025) doi:10.1017/S0031182025100644.
3. Bradbury, R. S. & Streit, A. Is strongyloidiasis a zoonosis from dogs? *Phil. Trans. R. Soc. B* 379, 20220445 (2024).
4. Zhao, H. et al. Insights into Infant Strongyloidiasis, Papua New Guinea. *Emerg. Infect. Dis.* 31, (2025).
5. Hunt, V. L. et al. The genomic basis of parasitism in the *Strongyloides* clade of nematodes. *Nat Genet* 48, 299–307 (2016).
6. Sato, H., Suzuki, K., Osanai, A., Kamiya, H. & Furuoka, H. Identification and characterization of the threadworm, *Strongyloides procyonis*, from feral raccoons (*procyon lotor*) in Japan. *Journal of*

- Parasitology* 92, 63–68 (2006).
7. Schad, G. A., Aikens, L. M. & Smith, G. *Strongyloides stercoralis*: is there a canonical migratory route through the host? *J Parasitol* 75, 740–749 (1989).
 8. Dörken, S. et al. Experimental Infection of Mice with the Parasitic Nematode *Strongyloides ratti*. *JoVE* 67533 (2025) doi:10.3791/67533.
 9. Viney, M. E., Matthews, B. E. & Walliker, D. Mating in the nematode parasite *Strongyloides ratti*: proof of genetic exchange. *Proc. R. Soc. Lond. B* 254, 213–219 (1993).
 10. Thamsborg, S. M., Ketzis, J., Horii, Y. & Matthews, J. B. *Strongyloides* spp. infections of veterinary importance. *Parasitology* 144, 274–284 (2017).
 11. Gordon, C. A. et al. Strongyloidiasis. *Nat Rev Dis Primers* 10, (2024).
 12. Herbert, D. R., Stoltzfus, J. D. C., Rossi, H. L. & Abraham, D. Is *Strongyloides stercoralis* hyperinfection induced by glucocorticoids a result of both suppressed host immunity and altered parasite genetics? *Molecular and Biochemical Parasitology* 251, 111511 (2022).
 13. Buonfrate, D. et al. Chapter One - The diagnosis of human and companion animal *Strongyloides stercoralis* infection: Challenges and solutions. A scoping review. in *Advances in Parasitology* (eds Rinaldi, L. & Cringoli, G.) vol. 118 1–84 (Academic Press, 2022).
 14. Speare, R. Studies on the taxonomy of *Strongyloides* (Nematoda; Strongyloididae). (James Cook University of North Queensland, 1986). doi:10.25903/5E1BF2068703B.
 15. Buonfrate, D., Bradbury, R. S., Watts, M. R. & Bisoffi, Z. Human strongyloidiasis: complexities and pathways forward. *Clinical Microbiology Reviews* 36, e00033-23 (2023).
 16. Barratt, J. L. N. et al. A global genotyping survey of *Strongyloides stercoralis* and *Strongyloides fuelleborni* using deep amplicon sequencing. *PLoS Negl Trop Dis* 13, e0007609 (2019).
 17. Al-Jawabreh, R. et al. Advancing *Strongyloides* omics data: bridging the gap with *Caenorhabditis elegans*. *Phil. Trans. R. Soc. B* 379, 20220437 (2024).
 18. Al-Jawabreh, R. et al. *Strongyloides* questions—a research agenda for the future. *Phil. Trans. R. Soc. B* 379, 20230004 (2024).
 19. Darling, S. T. STRONGYLOIDES INFECTIONS IN MAN AND ANIMALS IN THE ISTHMIAN CANAL ZONE. *Journal of Experimental Medicine* 14, 1–24 (1911).
 20. Moraes, M. F. D., Da Silva, M. X., Tebaldi, J. H. & Hoppe, E. G. L. Parasitological assessment of wild ring-tailed coatis (*Nasua nasua*) from the Brazilian Atlantic rainforest. *International Journal for Parasitology: Parasites and Wildlife* 9, 154–158 (2019).
 21. Speare, R. Identification of species of *Strongyloides*. in *Strongyloidiasis: A major roundworm infection of man* (Grove, DI) 11–84 (Taylor and Francis, London, 1989).
 22. Zhao, H., Constantinoiu, C. & Bradbury, R. S. Taxonomy of *Strongyloides* in humans, dogs and cats: a comprehensive review from morphology to molecular and population genetics. *Parasitology* 153, 505–530 (2026).

23. Hunt, V. L., Tsai, I. J., Selkirk, M. E. & Viney, M. The genome of *Strongyloides* spp. gives insights into protein families with a putative role in nematode parasitism. *Parasitology* 144, 343–358 (2017).
24. Mendoza, A. P. et al. Diversity and prevalence of zoonotic infections at the animal-human interface of primate trafficking in Peru. *PLoS ONE* 19, e0287893 (2024).
25. Garland-Lewis, G., Whittier, C., Murray, S., Trufan, S. & Rabinowitz, P. M. Occupational Risks and Exposures Among Wildlife Health Professionals. *Ecohealth* 14, 20–28 (2017).
26. Little, M. D. Seven new species of *Strongyloides* (Nematoda) from Louisiana. *J Parasitol* 52, 85–97 (1966).
27. Little, M. D. Comparative morphology of six species of *Strongyloides* (Nematoda) and redefinition of the genus. *J Parasitol* 52, 69–84 (1966).
28. Ogilvie, B. M. et al. Acetylcholinesterase secretion by parasitic nematodes—I. Evidence for secretion of the enzyme by a number of species. *International Journal for Parasitology* 3, 589–597 (1973).
29. Selkirk, M. E., Lazari, O., Hussein, A. S. & Matthews, J. B. Nematode acetylcholinesterases are encoded by multiple genes and perform non-overlapping functions. *Chemico-Biological Interactions* 157–158, 263–268 (2005).
30. Ndjim, M. et al. Tuft cell acetylcholine is released into the gut lumen to promote anti-helminth immunity. *Immunity* 57, 1260–1273.e7 (2024).
31. Huelsenbeck, J. P. & Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755 (2001).
32. Krueger, F. et al. FelixKrueger/TrimGalore: v0.6.10 - add default decompression path. Zenodo <https://doi.org/10.5281/ZENODO.7598955> (2023).
33. Wood, D. E., Lu, J. & Langmead, B. Improved metagenomic analysis with Kraken 2. *Genome Biol* 20, 257 (2019).
34. Li, Y. et al. Chromosome-level reference genome of the jellyfish *Rhopilema esculentum*. *GigaScience* 9, giaa036 (2020).
35. Vurture, G. W. et al. GenomeScope: fast reference-free genome profiling from short reads. *Bioinformatics* 33, 2202–2204 (2017).
36. De Coster, W. & Rademakers, R. NanoPack2: population-scale evaluation of long-read sequencing data. *Bioinformatics* 39, btad311 (2023).
37. Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using DIAMOND. *Nat Methods* 12, 59–60 (2015).
38. Laetsch, D. R. & Blaxter, M. L. BlobTools: Interrogation of genome assemblies. *F1000Res* 6, 1287 (2017).
39. Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A. & Korobeynikov, A. Using SPAdes De Novo Assembler. *CP in Bioinformatics* 70, e102 (2020).
40. Li, H. et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079 (2009).

41. Gabriel, L. et al. BRAKER3: Fully automated genome annotation using RNA-seq and protein evidence with GeneMark-ETP, AUGUSTUS, and TSEBRA. *Genome Res.* 34, 769–777 (2024).
42. Jacques Dainat et al. NBISweden/AGAT: AGAT v1.6.1. Zenodo <https://doi.org/10.5281/ZENODO.3552717> (2026).
43. Pertea, G. & Pertea, M. GFF Utilities: GffRead and GffCompare. *F1000Res* 9, 304 (2020).
44. Emms, D. M., Liu, Y., Belcher, L., Holmes, J. & Kelly, S. OrthoFinder: scalable phylogenetic orthology inference for comparative genomics. Preprint at <https://doi.org/10.1101/2025.07.15.664860> (2025).
45. Mendes, F. K., Vanderpool, D., Fulton, B. & Hahn, M. W. CAFE 5 models variation in evolutionary rates among gene families. *Bioinformatics* 36, 5516–5518 (2021).
46. Yu, G., Wang, L.-G., Han, Y. & He, Q.-Y. clusterProfiler: an R Package for Comparing Biological Themes Among Gene Clusters. *OMICS: A Journal of Integrative Biology* 16, 284–287 (2012).
47. Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P. & Huerta-Cepas, J. eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. *Molecular Biology and Evolution* 38, 5825–5829 (2021).
48. Huerta-Cepas, J. et al. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Research* 47, D309–D314 (2019).

Figures

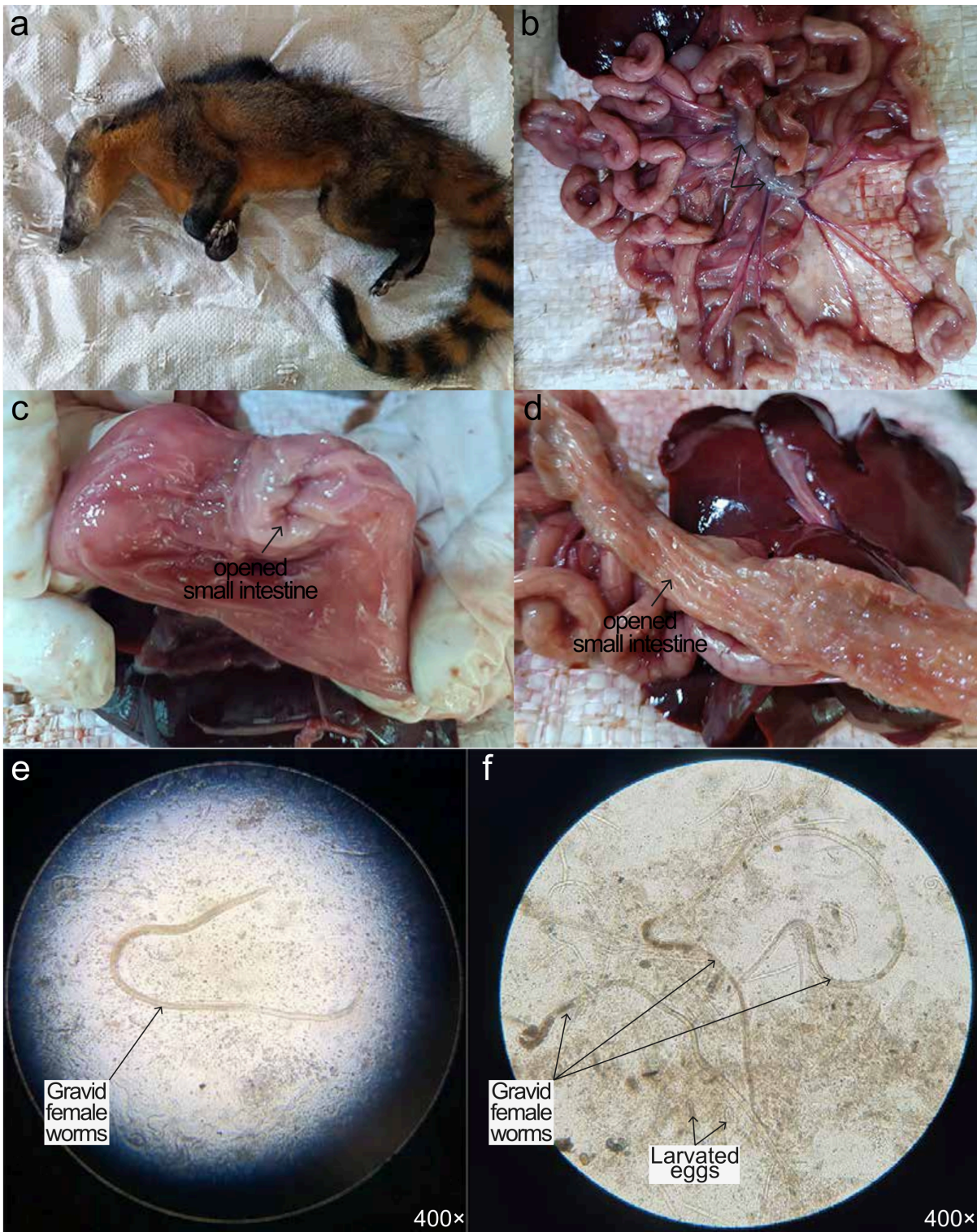


Figure 1

Figure 1

Post-mortem examination reveals strongyloidiasis in coatis. Representative gross lesions observed during post-mortem examination of the affected coatis. (a) Photograph of one of the two male coatis, which presented with clinical signs; (b) small intestine congestion and enlarged mesenteric lymph nodes (arrows); (c, d) thickened intestinal wall, typical of inflammation; (e-f) direct microscopic field

examination of the intestinal content, revealing the presence of *Strongyloidessp.* female nematodes and larvated eggs.

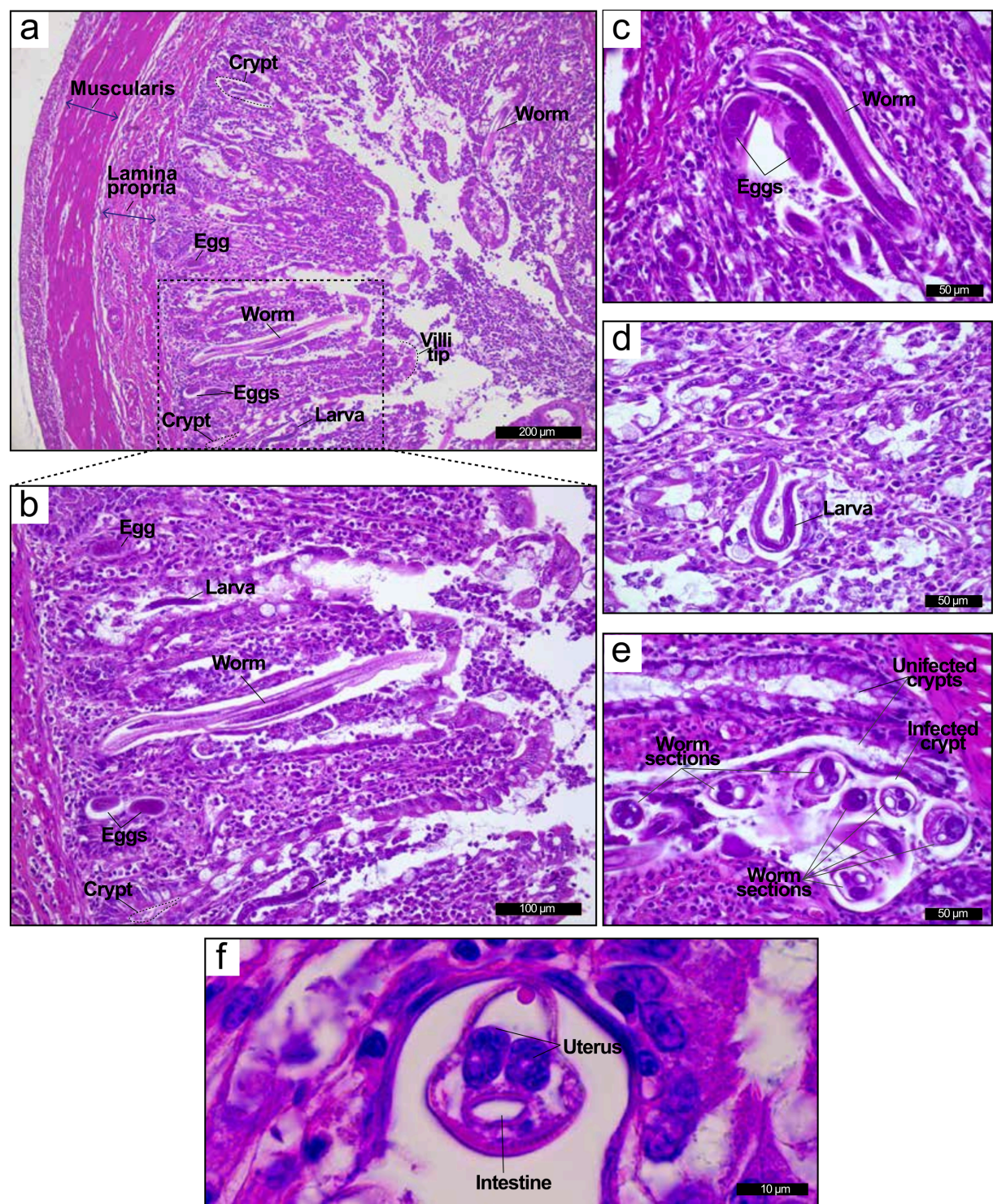


Figure 2

Figure 2

Histological examination reveals *Strongyloidessp.* hyperinfection. (a-b) Cross-section of the intestine tissue with loss of typical structure of the intestinal wall, severe inflammation, and presence of

numerous nematodes at various development stages. (c-f) Examination of intestinal tissue sections under high magnification reveals the presence of cross and longitudinal sections of juvenile and adult nematodes, and eggs, intricated in the lumen of the intestine and crypts. H&E stain.

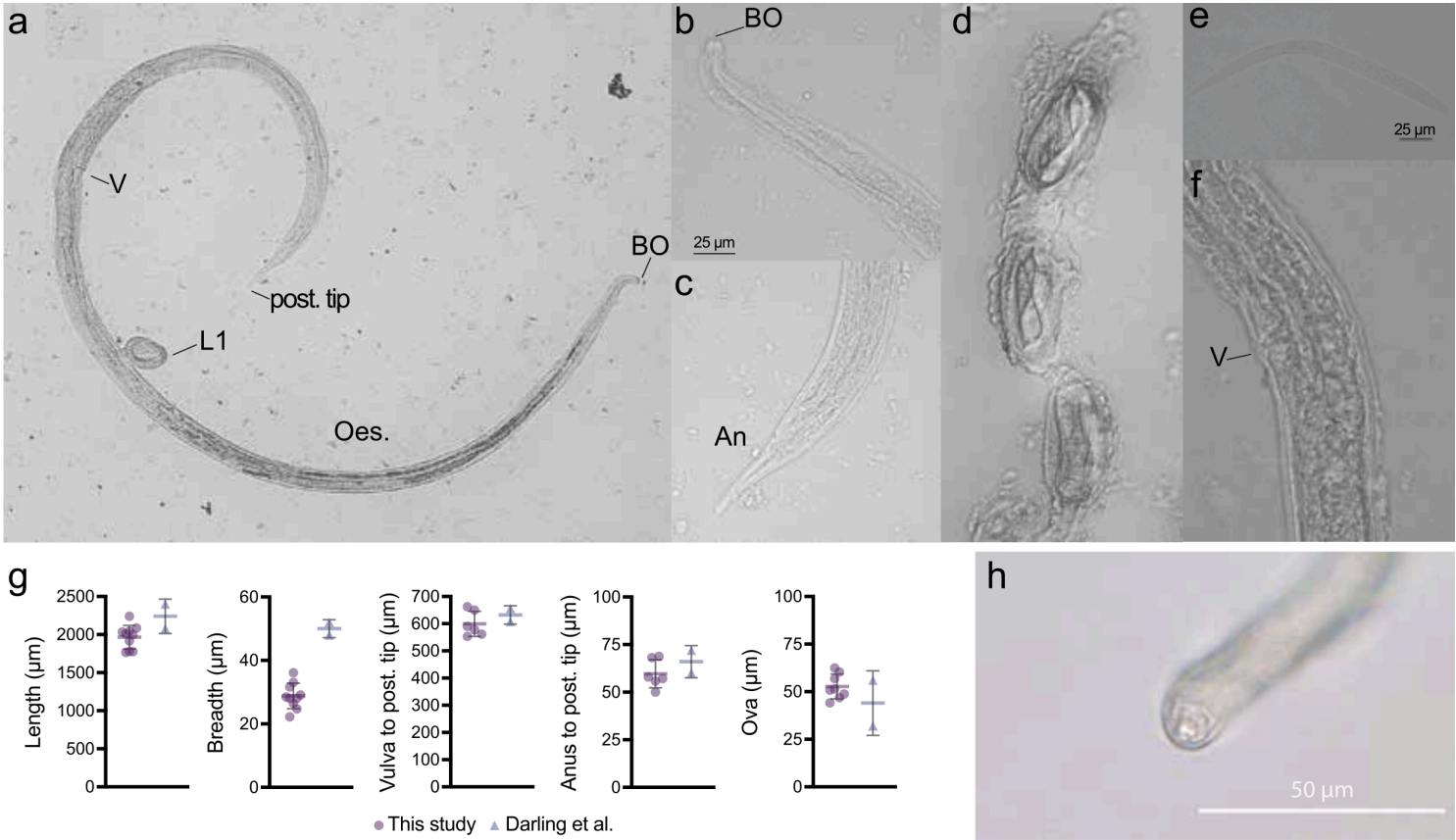


Figure 3

Figure 3

Light microscopy examination of *Strongyloides nasua*. Nematodes sampled from intestinal content were imaged by digital microscopy. (a) Representative image of an adult female parasite highlighting the buccal orifice (BO), oesophagus (Oes.), vulva (V), tail, and a L1 stage larvae; (b) magnification on the anterior extremity, showing the buccal orifice (BO); (c) magnification of the posterior extremity showing the tail and anus (An); (d) stripe of three eggs containing L1 stage larvae; (e) stage 1 rhabditiform larvae; (f) magnification of the region of the vulva (V). (g) Measurements of the indicated features from specimen of *S. nasua*, as compared to the early study by Darling et al. in 1911¹⁹. (h) Magnified view of the buccal orifice of one female nematode.

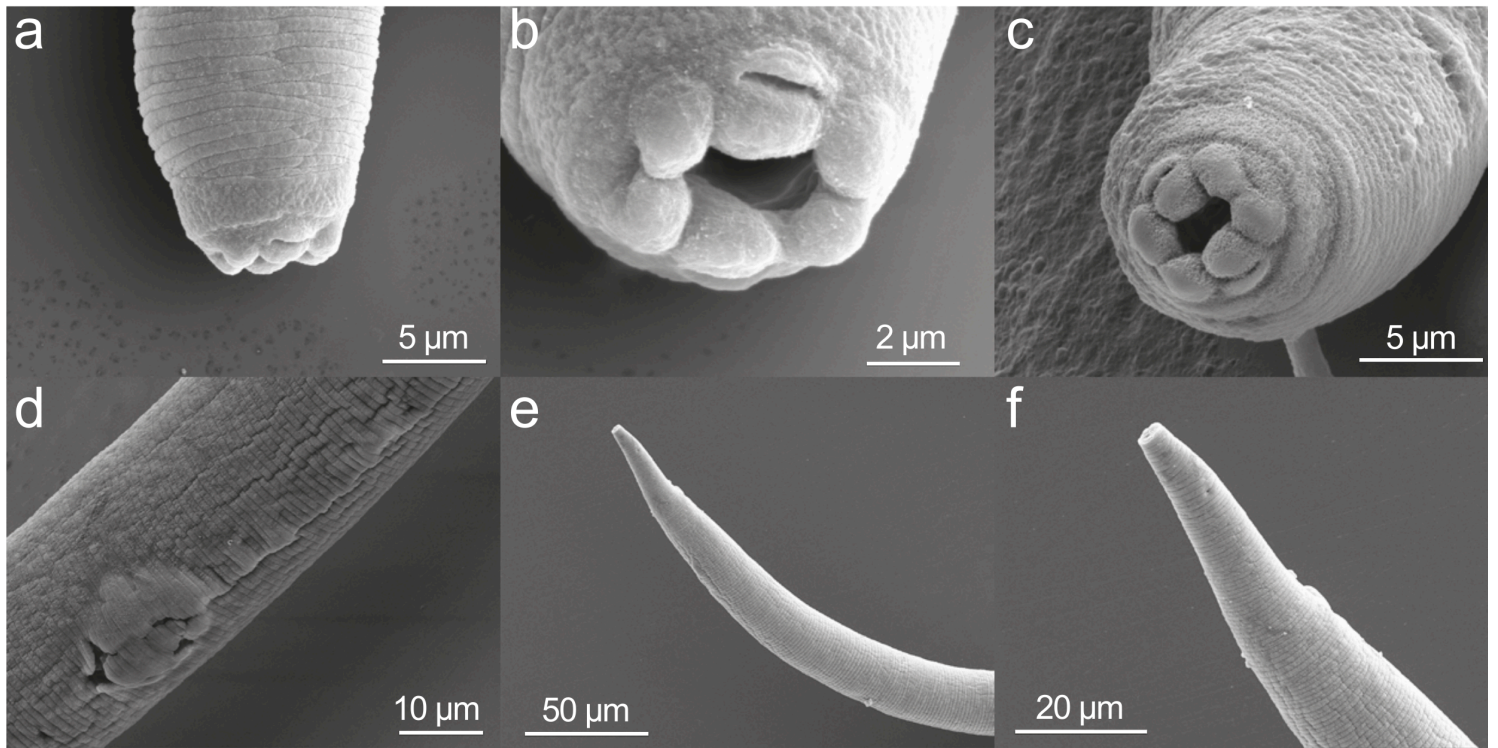


Figure 4

Figure 4

Scanning electron micrograph examination of adult parasitic *Strongyloides nasua*. Adult parasitic female anterior showing circumoral elevation (a) consisting of six lobes with a deep lateral sulcus (b) and a hexagonal stoma (c). The mid body demonstrates a protruding vulva and fine transverse striations (d). The posterior region rapidly tapers post-anally to a blunt termination at the tail (e) and has a small pore-like structure anterior to the tip, but lateral and posterior to the anal opening (f).

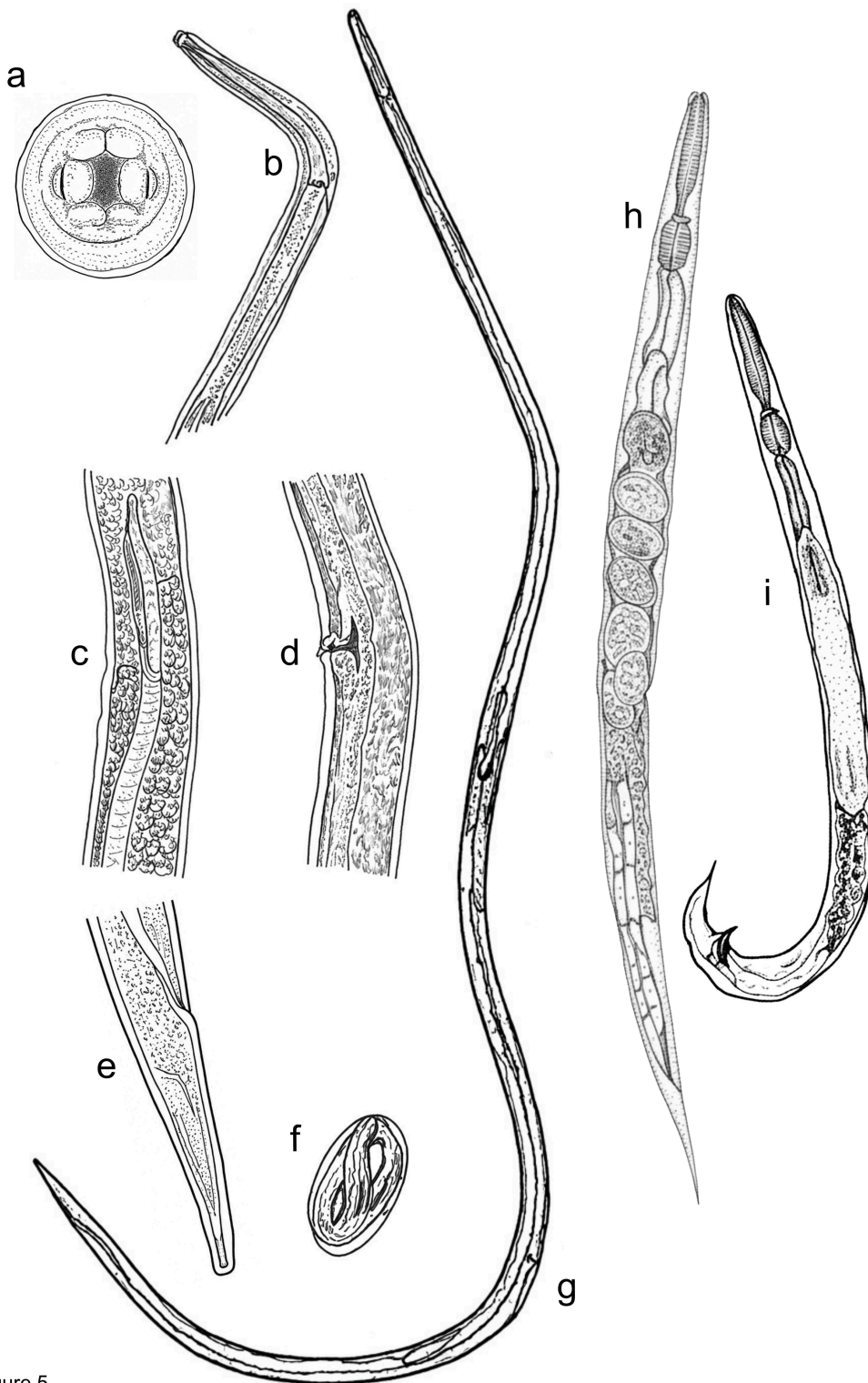


Figure 5

Figure 5

Anatomical drawings of adult parasitic *Strongyloides nasua*. The buccal region *en face* has a hexagonal stoma with six lobes and deep lateral sulcus (a). The anterior portion of the worm including the nerve ring and oesophageal-intestinal junction (b). The worm mid body region with a straight, reflected ovary (c) and protruding vulval opening with T shaped vagina (d). Posterior region with marked post-anal tapering towards a blunt tail (e). Eggs contain a visible rhabditiform larvae (f). The entire worm is long

and filariform with the gastrointestinal tract clearly visible throughout the length of the body (g). (h) free-living female with a spindle shaped body tapering towards the anterior and a rhabditiform oesophagus. (i) free-living male gubernaculum is distinct, with a bluntly rhomboid base leading into a concave spicule, and testes taking most of the body.

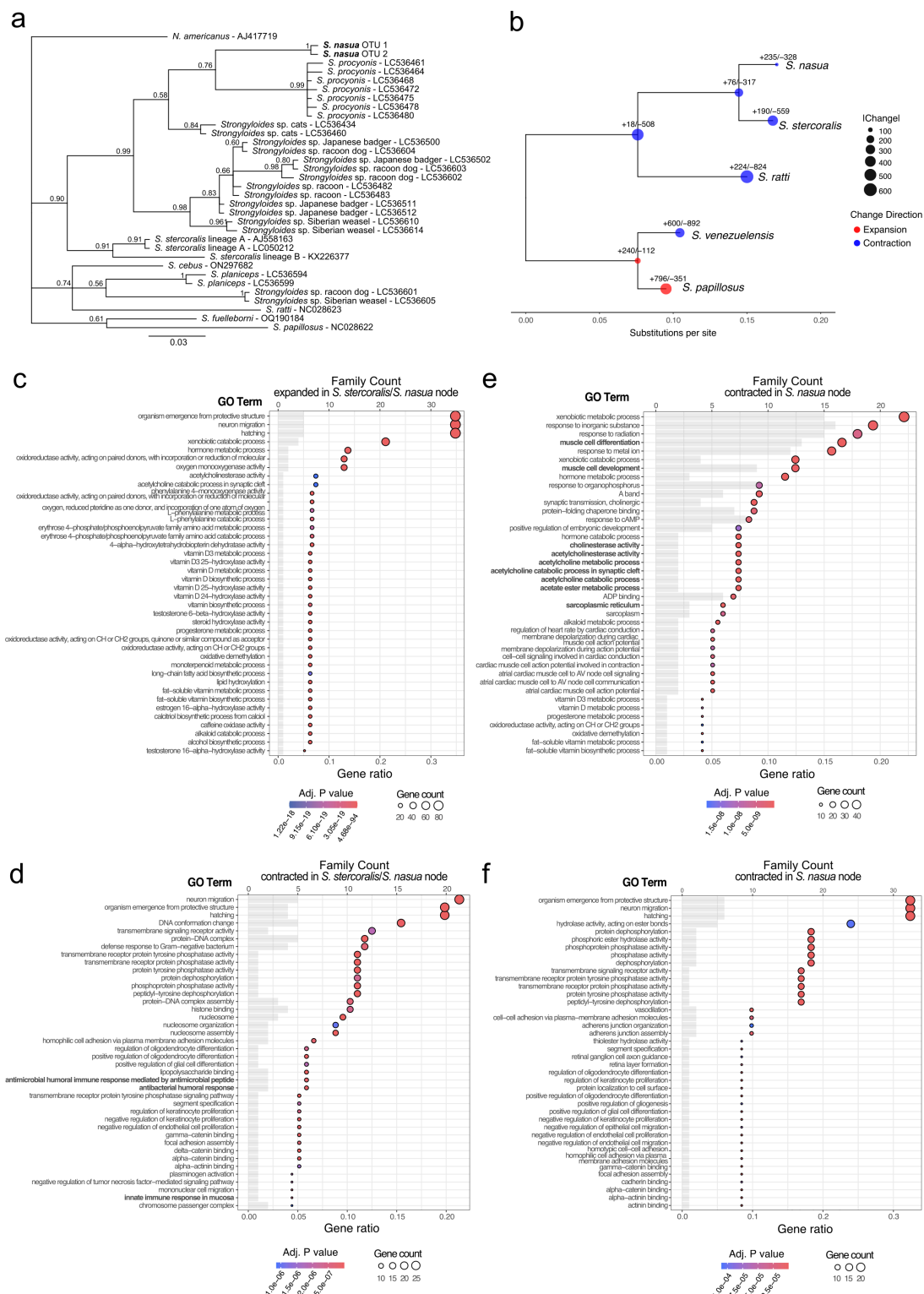


Figure 6

Figure 6

Genotypic and genomic analysis.

(a) Phylogenetic tree obtained by Bayesian analysis of *cox1* genotyping. (b) Summary of expanded and contracted gene families in *S. nasua* and four other *Strongyloides* sp., from whole genome sequencing. Branch lengths are substitution per site. (c) Gene Ontology (GO) enrichment of orthogroups expanded at the shared *S. nasua* - *S. stercoralis* node, (d) GO enrichment of orthogroups contracted at the shared *S. nasua* - *S. stercoralis* node, (e) GO enrichment of orthofamilies expanded in *S. nasua*, and (f) GO enrichment of orthofamilies contracted in *S. nasua*. Gene ratio represents the proportion of genes with a give GO term. Family count is the number of orthogroups containing genes with a given GO term.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigureandTablelegends.docx](#)
- [SIFigureS01.pdf](#)
- [SIFigureS02.pdf](#)
- [SIFigureS03.pdf](#)
- [SIFigureS04.pdf](#)
- [SIFigureS05.pdf](#)
- [SITables.xlsx](#)