Title: Technical cooperation for the identification and characterisation of specific molecular markers to diagnose and control of dioctophymosis.

Final Technical Report

Introduction

Dioctophyme renale, the giant kidney worm is the largest parasitic nematode of terrestrial vertebrates described so far and is distributed worldwide (1). It develops in, and completely destroys mammalian kidneys, and is thereby a debilitating and potentially lethal parasite of humans, domestic animals and endangered wildlife. Among domestic animals it is particularly pathogenic and common in dogs (2-5). Particularly in South America, it severely affects wild: bush dog (*Speothosvenaticus*) (6), southern two-toed sloth (*Choloepus didactylus*) (7), crab-eating fox (*Cerdocyonthous*) (8) and maned wolf (*Chrysocyonbrachyurus*) (9,10). The latter is the largest canid in South America, is listed as a CITES Appendix II species (11), and dioctophymatosis is now included among threats to its wild population (12, 13). Additionally, there are sporadic reports in other mammals as atypical definitive hosts, such as cattle, horses, swine and rats (14-17). Regarding human infections, *D. renale* is a zoonotic disease since unequivocal cases in human beings have been documented, including fatal cases and in relation with renal carcinoma (18-21). Due to changes in climatic conditions, environmental degradation, deforestation, and compromised sanitation many natural places considered biodiversity hotspots have undergone severe habitat fragmentation forcing wild species to move and face different threats and hence becoming more vulnerable to infections caused by parasites. During these migrations wild species interact with domestic animals spreading and increasing the risk of infections of both and also to humans in the case of zoonosis (22). Despite the importance and threat for humans of this parasitic disease there is little information about the molecular bases of this organism (e.g. genome and proteome). Data of this kind could improve the designing of immunological methods for identifying exposed or infected animals and hence have a better knowledge of the prevalence of this parasite in different regions of South America.

During their life cycle, the adults of *D. renale* locate in renal pelvis of one kidney (usually the right one) of their definitive hosts destroying the parenchyma of this organ and leaving a thin capsula that content the parasites. Usually the unaffected kidney compensate in function and presents hypertrophy (10) so in most cases there are no clear clinic signs of infection. Eggs are dispersed via urine in the environment, where an annelid (oligochaete) ingests the embrionated eggs allowing them to develop to L3 stage (23). Finally the definitive host ingests the oligochaete or any of the possible paratenic hosts (frogs, fishes or rodents). Recently, L3 larvae have been detected in fresh water turtles (*Trachemys dorbigni*) that are restricted to South America (Brazil, Uruguay and Argentina) (24) suggesting that this organism presents a wide range of possibilities of spreading the disease that is still to know. In South America, *D. renale* is found mostly in dogs that live close to rivers and the infection is diagnosed only by urine analysis, ultrasonography, surgery, or at necropsy. Although the parasite is usually located in one of the kidneys, worms may also develop to adulthood in sites other than these organs, such as the abdominal cavity, the uterus, ovary, mammary gland, urethra,

subcutaneous tissues of the inguinal region and mesenteric lymph nodes (2, 25). Current diagnostic methods are difficult to handle due to heavy equipment or are of poor sensitivity, frequently giving false negative results. In this regard, recently published data show the existence of soluble antigens from the esophagous of *D. renale* that may help to determine infections in dogs (26). Additionally, people living in poverty, poor sanitary conditions and of poor educational backgrounds, consume the potential paratenic hosts, increasing the risk of infecting themselves as well as contributing to the persistence of the parasite's life cycle by feeding their dogs with raw material from the same paratenic hosts. Given the above reasons, the main concerns about Dioctophymatosis include the lack of more sensitive and specific diagnostic techniques in cases where: 1) there are adults of only one sex in the kidneys, 2) ectopically locations of the worms inside the host, and 3) prepatent asymptomatic incubation periods of the disease.

Relevant to both immunodiagnosis and probably also to how the parasites persist in hosts, are the abundant proteins they synthesize. Preliminary data obtained in our laboratory showed that pseudocelomic body fluid (PCF) of *D. renale* contains two highly abundant proteins, one of 44 kDa (P44) in size that bind lipids, and another of 17 kDa (P17) in both males and females. Our preliminary N-terminal sequencing of these proteins analysed by Edman degradation provided no apparent protein homologues in public protein or nucleotide databases, placing them as good candidates for diagnostic markers. Additionally, we found P44 and other comoponents of PCF to be targets of strong antibody responses in naturally infected dogs (27). The relevance of lipid binding proteins lies in the fact that nematode parasites produce and secrete an unexpectedly wide range of novel lipid binding proteins (LBPs), many of which are structurally distinct from those of their hosts and are therefore highly immunogenic (28). The acquisition and transport of several lipid molecules is crucial to these organisms, and the proteins and receptors involved in lipid transport and exchange provide potential targets for chemo- and immunotherapy. Some of the hypothetical roles of helminth LBPs are internal functions typical of all multicellular organisms; specialized external functions, including acquisition and distribution of lipids; and/or modulation of the host's local tissue environment and it's innate and acquired immune systems. In this proposal we aim at identifying and characterising P44 and other possible LBPs from *D. renale* using biochemical and biophysical techniques, and to explore their value as diagnostic markers. Additionally we plan to initiate genomic analysis of this parasite in order to provide a new platform for the development of immune- and chemotherapies against this pathogen.

Results

1. Analysis of somatic and pseudocoelomic fluid (PCF) proteins of adult *D. renale*

PCF and soluble proteins of body wall, intestine, testis, and ovary were separated by SDS PAGE (**Fig. 1**). The protein profile of PCF is dominated by two notably abundant proteins, one of Mr 17,000 (designated P17) and another of Mr 44,000 (P44) in both males and females. The proteins of body wall, intestine, testis, and ovary showed little overlap with each other except for the common presence of a protein of similar Mr to P44. The protein profile of *D. renale* PCF is different from those of the only other parasitic nematodes large and accessible enough for PCF

to be recovered easily, namely the adults of large ascaridids such as *Ascaris suum* and *Toxocara canis* [29, 30]. The most abundant proteins in adult ascaridid PCFs are approximately 14.4 kDa monomers of the nematode polyprotein allergens (NPAs) [31, 32, 33], and a globin of approximately 43 kDa [34, 35, 36].

PCF was fractionated by size exclusion gel chromatography through a Superdex 75 column (**Fig. 2a**). The two major proteins were readily separable, as seen from the accompanying SDS-PAGE of the resulting fractions (Fig. 2b). The sizes of these proteins as calculated relative to the time of elution of standard calibration proteins approximated the values obtained from SDS-PAGE under reducing conditions (Fig. 1), indicating that both proteins eluted from the column as monomers, and may therefore exist as such in PCF. Protein mass spectrometry of the two provided molecular masses of 44,460 Da for P44 and 16,622 Da for P17 (data not shown). The P17 protein is intensely red, and retains its colour in gel filtration and in native non-reducing PAGE, but not under SDS-PAGE. The red chromophore was proven to be non-covalently attached and presents a mass spec profile according to a haem prosthetic group like those found in proteins such as vertebrate globins (haemoglobin, myoglobin, neuroglobin). P17's molecular mass is similar to that of the 'nemoglobins' that have been described from nematodes [36], but which are not related to vertebrate globins. The nematodes from which nemoglobins have been described are in clades (Clades II to V) that are phylogenetically remote from Clade I in which *D. renale* is now placed [37, 38], so P17's evolutionary relationship, if any, to nemoglobins remains to be established.

Figure 1

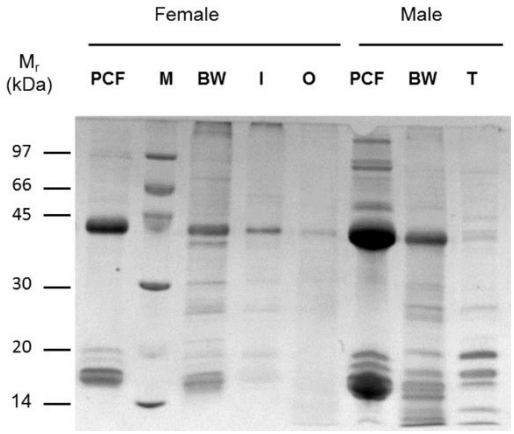

Figure 1. Protein profiles of tissues and pseudocoelomic fluid (PCF) of adult D. renale. SDS-PAGE of soluble protein extracts from the PCF and tissues of adult female and male adult worms run under reducing conditions and stained with Coomassie Blue. PCF, pseudocelomic fluid; BW, body wall; I, intestine; O, ovary; T, testis. Other gel analysis showed that the protein profile of male and female intestines were similar (not shown). M, reference proteins with masses given in kiloDaltons (kDa). Mr, relative mobility.

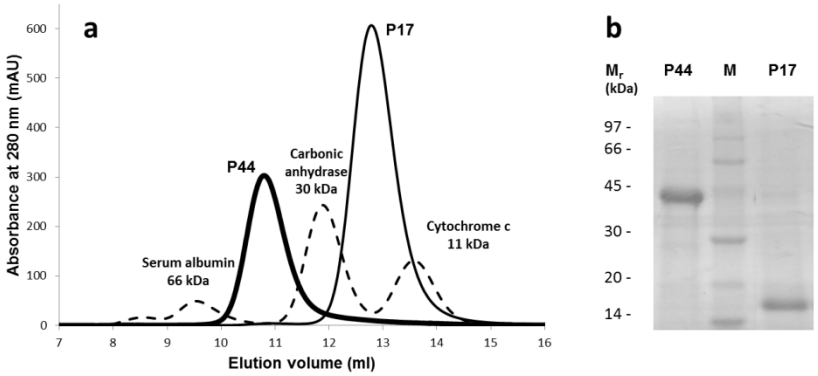

Figure 2. Purification of the abundant P17 and P44 proteins of adult D. renale pseudocoelomic fluid. (A) Pure major gel filtration peaks of D. renale PCF correspond to the P17 and P44 proteins (solid lines), mass spectrometry of which yielded masses of 16,622 Da and 44,460 Da, respectively (see main text). Absorbance peaks from the three standard proteins are overlain (dashed lines). (B) SDS-PAGE analysis of the different fractions after gel filtration. Coomassie Blue-stained. M, reference proteins with masses given in kiloDaltons (kDa). Mr, relative mobility.

2. Protein-associated lipids in *D. renale* PCF

The most abundant proteins found in animal blood or haemolymph are often respiratory or lipid-carrier proteins. In order to determine the lipid classes present in PCF and associated with P17 or P44, Folch lipid extractions from PCF and the two purified proteins were carried out and analysed by TLC. As seen in Fig. 3, the lipids found in unfractionated PCF were heterogeneous and similar in type and relative concentrations to those of rat liver. In contrast, lipids found in association with P44 (and to a minor extent with P17) were fatty acids. **Figure 3.**

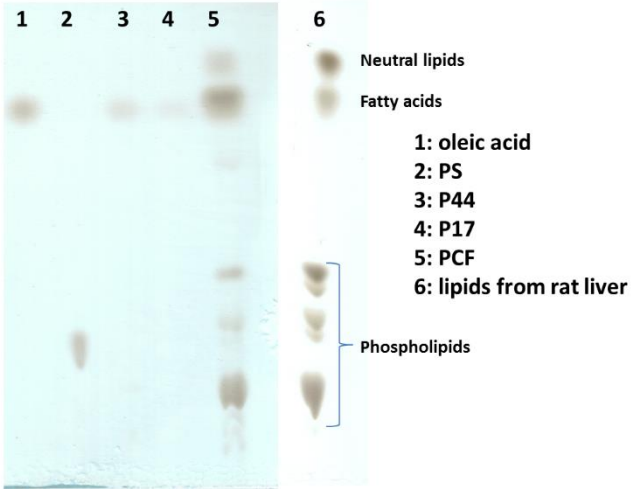

Figure 3. Lipids associated with the abundant P17 and P44 proteins of D. renale pseudocoelomic fluid. Lipids were extracted from purified P44, P17 or unfractionated PCF as described in Materials and Methods, and separated by TLC to resolve polar lipids. Rat liver lipids were included for comparison. PCF contains similar lipids to rat liver but P44 appeared to associate selectively with fatty acids. P17 shows trace association with fatty acids

3. Spectrofluorometric analysis of lipid-binding by P44

We further characterised the ligand-binding properties of P44 using environment-sensitive fluorescent probes and P44 from which any resident lipid had been removed by reverse phase HPLC performed as previously described [39]. We first used 8-anilinonaphthalene-1-sulfonic acid (ANS), a non-specific probe of hydrophobic sites in proteins, which exhibited a dramatic

enhancement of fluorescence emission in the presence of P44 (**Fig. 4a**). Addition of oleic acid to preformed P44: ANS complexes resulted in displacement of ANS from the protein. We then used DAUDA, the environment-sensitive dansyl fluorophore that exhibits low florescence in water and that is markedly enhanced and blue-shifted in emission when in an apolar environment such as a protein binding site [40].DAUDA's fluorescence emission was dramatically enhanced when mixed with de-lipidated P44, and its peak wavelength of emission underwent a dramatic blue shift from 544 nm to 470 nm (**Fig. 4b**). When oleic acid was added to preformed DAUDA:P44 complexes there was a clear displacement of the probe from the protein, which is indicative of the binding site being preferential for fatty acids and that the attached dansyl fluorophore is probably irrelevant to binding.

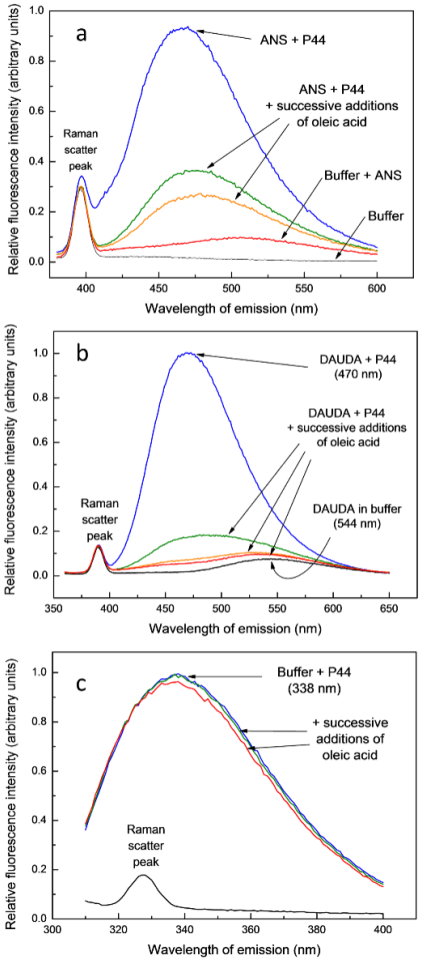

Figure 4. Hydrophobic ligand binding by D. renale P44. (A) Superdex 75-purified and delipidated P44 (1.3 μM) was added to the non-specific hydrophobic probe ANS (3.3 μM) in PBS causing a dramatic increase in the probe's fluorescence emission indicative of association with an exposed hydrophobic surface or a ligand-binding pocket in the protein. Successive additions of 0.5 μL of 1 mM oleic acid partially reversed the fluorescence enhancement. Excitation wavelength = 350 nm. (B) P44 (1.3 μM) added to the dansyl fluorophore-tagged fatty acid DAUDA (0.3 μM) in buffer yielding a dramatic increase in fluorescence intensity and blue shift in DAUDA's peak wavelength of emission from 544 nm to 470 nm. This was reversed with successive additions of 0.5 μL of 1 mM oleic acid solution indicating competitive displacement of DAUDA from its binding site in P44. Control experiments with a well-characterised lipid-binding protein of nematodes, recombinant ABA-1A allergen from *Ascaris suum*, produced similar spectral change in DAUDA emission and competitive displacement by oleic acid (not shown). Similar experiments using *D. renale* P17 revealed no binding of DAUDA (not shown). Excitation wavelength = 345 nm. (C) Intrinsic (predominantly tryptophan) protein fluorescence emission by P44. P44 in buffer was excited at 295 nm producing peak fluorescence emission at 338 nm. Successive additions of 0.5 μl of 1 mM oleic acid solution in

ethanol to 300 µl protein in PBS produced only very slight if any changes in emission intensity and wavelength of peak emission – peak searching for each of the three spectra using ORIGIN software provided peak wavelength of emission values of 338 or 337 nm, and correcting for dilutions upon addition of protein or oleic acid did not change these values. The spectrum of buffer alone (shown) was subtracted from each of the P44 intrinsic fluorescence emission spectra.

4. Mass spectrometry analysis and amino-acid sequence analysis

From its size and ligand-binding characteristics, P44 appears to be a novel type of lipid-binding protein of nematodes. To investigate whether P44 exhibits any relationship to known proteins from nematodes, we obtained N-terminal and several internal peptide sequences (Fig. 5). Database searching revealed that the peptide sequences all align reasonably well with the sequence of a protein from *Trichinella spiralis* that is described as a poly-cysteine and histidine-tailed protein (PCHTP; Fig. 5) [41]. The similarities are collectively convincing given the alignments of cysteines and bulky hydrophobic amino acids such as tryptophan and phenylalanine. A similar degree of similarity was found with PCHTPs predicted for other species of Trichinella and also *Trichuris trichiura*, all of which are, like *D. renale*, Clade I nematodes. An N-terminal amino acid sequence was also obtained from P17 (TQNKPLLTAQMDXIHADA; single amino-acid code), but alignments even with putative globin-like proteins from Clade I nematodes (Trichinella and Trichuris species) in current databases were not convincing.

a

P44 peptide	Amino acid sequence	As edited or combined for alignment
D.renale_1	DQPDCPAFGEWKPWTDQCL	DQPDCPAFGEWKPWTDQCL
D.renale_2	TEWSQCLXF	TEWSQCLWFPLSXMAT
D.renale_3	WSQCLWFPLSXMAT	
D.renale_4	CGFCSFSVR	CGFCSFSVR
D.renale_5	DVCTLTPHPK	SCGKDEHCETCGDVCTLTPHPK
D.renale_6	XSCGKDEHCETCGDV	
D.renale_7	DYADILWYLPXA	DYADILWYLPXA

b

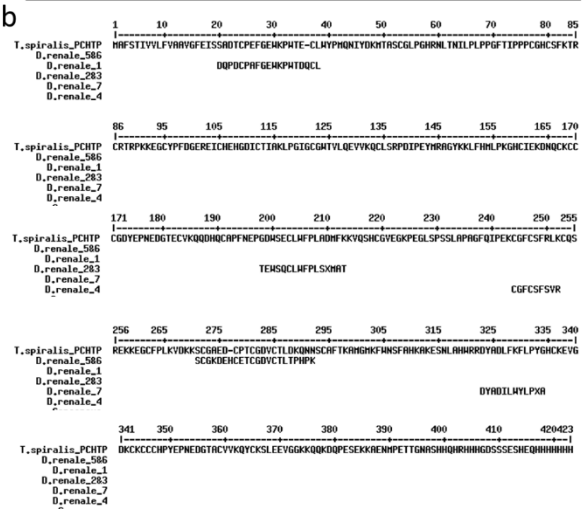

Figure 5. Putative orthologues of D. renale P44. (A) Peptide sequences from P44 and how they were combined or edited for the alignment. Sequences are given using the single letter code for amino acids. (B) Alignment between the peptides obtained from P44 and the full predicted amino acid sequence of the poly-cysteine and histidine-tailed protein isoform 2 from Trichinella spiralis (Ts-PCHTP; NCBI accession AEQ29641). The alignment was made using MultAlin set for the default blosum62 substitution matrix. Though not shown, the alignment was similarly good with sequences from other species of Trichinella, and with a PCHTP sequence from Trichuris trichiura (NCBI accession CDW54015). The first 21 amino acids in the T. spiralis sequence are predicted to be a cleavable signal peptide by SignalP (www.cbs.dtu.dk/services/SignalP/), the predicted mature protein therefore beginning close to where the D. renale P44 N-terminal peptide sequence begins (D.renale_1).

5. Immunoreactivity of P44 and P17

On October 2017, Lic. A. Nahili Giorello spent two weeks at the laboratory of Dr. Peddrassani and Dr. Rosangela Zacarias Machado placed at Department of Veterinary Pathology, FCAV/UNESP, Jaboticabal, SP, Brazil. During this stay Lic. Giorello acquired the skills to work with dog sera and immunoassays. She worked on P44 and P17 as well as whole pseucelomic fluid. Both proteins were studied by immunoassays in comparison with esophagus proteins to test their potential as diagnostic markers. Interestingly, while P44 was found to be immunogenic in the dog sampled, P17 was not Figures 6 and 7.

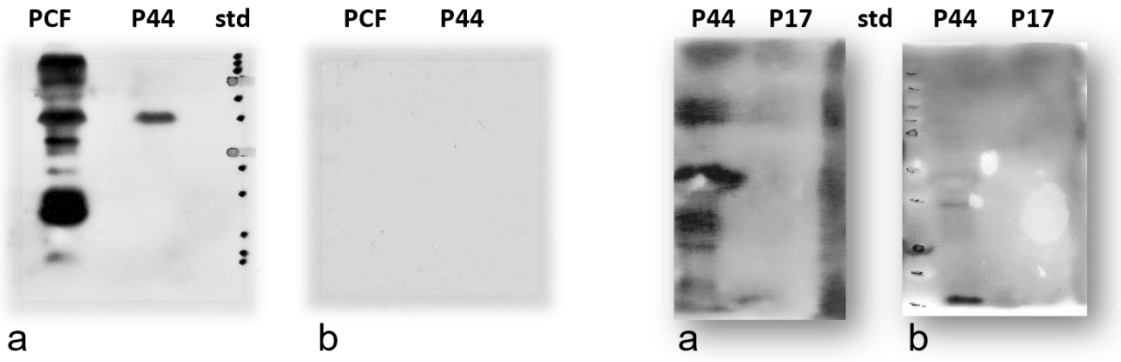

Figure 6. Western blots where PCF P44 and P17 were incubated with a) with serum from infected dogs and b) serum from non infected dogs.

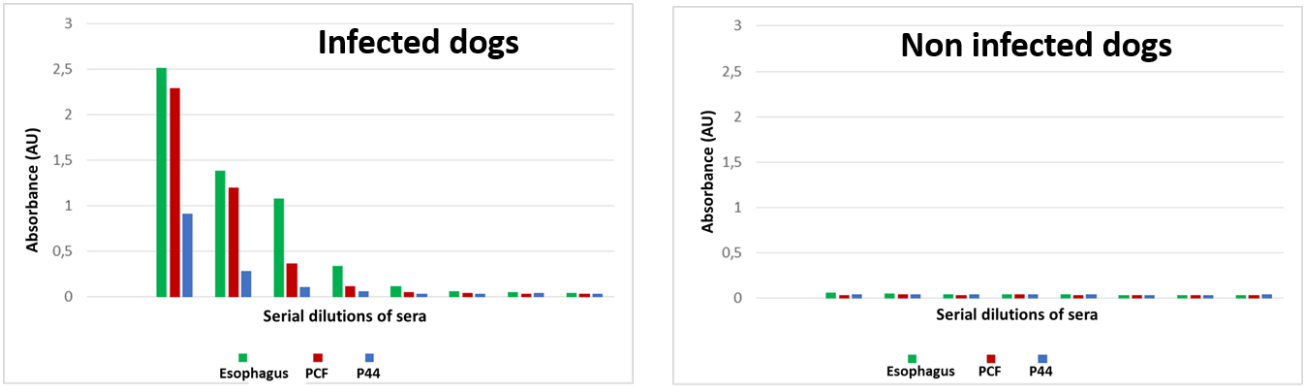

Figure 7. ELISA using PCF, esophagus, proteins, or P44 with serum from infected and uninfected dogs. Esophagus has previously been proposed as a good immunodiagnostic (Pedrassani D, do Nascimento AA, André MR, Machado RZ. Improvementofan enzyme immunosorbent assay for detecting antibodies against *Dioctophyme renale*. VetParasitol. 2015;212:435–8.)

6. Genomic studies on *D. renale*

We have initiated genomic analysis of *D. renale* working in collaboration with Professor Mark Blaxter originally leader of the “959 Nematode Genomes” initiative at the Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, UK. Now, Professor Blaxter is working at Sanger Institute, Hinxton, UK as leader of the Tree of life initiative. Dr. Lucas L. Maldonado working as a postdoc in this project spent one month (August2018) at Dr. Blaxter´s lab at the University of Edinburgh. Briefly, he extracted DNA from male tissues and constructed two Paired-End libraries of 350 bp fragments. These fragments were sequenced using HiSeqX

Illumina producing high-coverage short reads. Reads were filtered by quality and we performed an assembly with heterozygosity-aware tools (Platanus toolkit). The first version of draft genome presented a high repetitive content including new species-specific repetitive sequences. To overcome the fact of a high repetitive content we changed the sequencing strategy and isolated DNA again from male tissues. This high molecular weight DNA material was sequenced using PacBio SEQUEL platform and generated long reads (>10 kb) with a ~20X coverage. The draft assembly of *D. renale* has 80% of completness in BUSCO database. We are now running the gene annotation pipeline.

7. Phylogenetic studies on *D. renale*.

Forty-six adults of *D. renale* were collected from interventions in veterinary clinics and from necropsies of wild fauna in different locations in northeastern Argentina and southern Brazil. DNA was extracted from each sample and three molecular markers of COX1 were amplified by PCR. One of them previously reported by Tokiwa, et al. 2011, of 74 nt (**Fig. 8**) and another two (of 259 nt and 635 nt) that were designed using information available from the *D. renale* draft genome project under development. Bayes and ML-phylogeny suggests that *D. renale* populations were in South America long enough to develop local genetic variants. They could arrive from the north of the continent and dispersed between Brazil and Argentina on more than one occasion. Finally, the same variants can infect and be transmitted between domestic and wild mammals. New markers based on the nuclear genome will be designed to complement the analyzes and to improve the sensibility in determination of genetic differences.

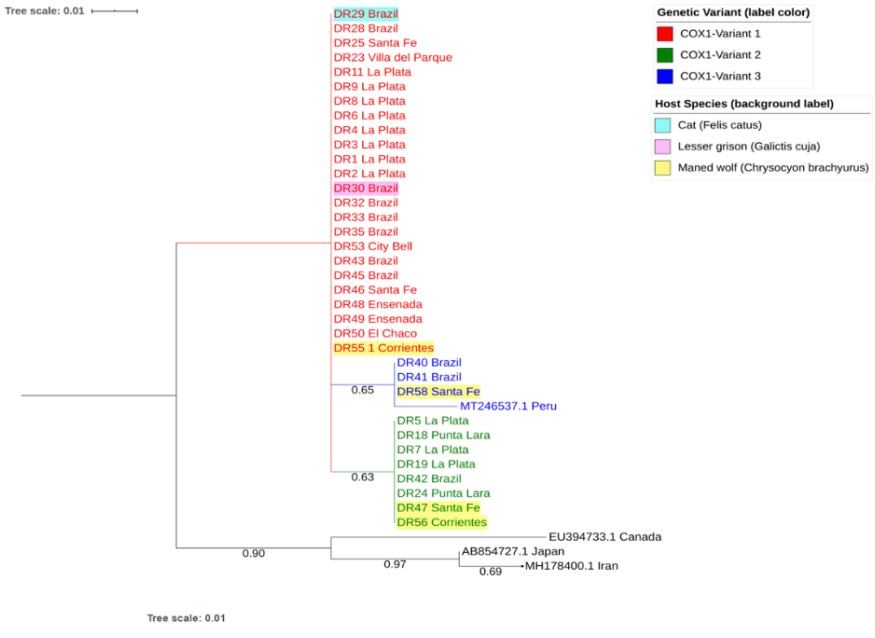

Figure 8. Phylogenetic tree based on COX1_short [42] showing three genetic variants red, green and blue. Samples were identified geographically and those from wild fauna.

8. Transcriptomic studies

This has also been performed by Dr. Lucas L. Maldonado during his stay at Dr. Mark Blaxter´s lab during 2018. RNA has been isolated from dissected tissues of *D. renale* (oesophagus, intestine, body wall and ovary/testes). Samples of *D. renale* were washed with sterile

phosphate buffered saline pH 7.4 (PBS) and dissected. RNA extraction has been performed using Trizol, followed by several freeze-thaw cycles. Followed by a cleaning step with chloroform. RNA was precipitated with isopropanol and successive washes with 70 % ethanol. Final pellet was resuspended in RNAse free water. Extractions were done at least three times for each sex.Pure RNA was sent for sequencing University of Edinburgh.

Perspective: RNA sequencing data analysis. High quality RNA-seq datasets will complement genomic data with improved gene annotations using BRAKER software [43]. Moreover, RNA sequencing data will help to quantify gene expression**.** The availability of high-quality RNA datasets from different tissues and organs from *D. renale* not only will enhanced analyses of the protein-coding genome, but it will also help to identify and quantify non-coding RNAs [44].These data will be treated independently, and an assembly will be obtained using Trinity [45, 46] . Lastly, a whole proteome will be predicted base on the obtained gene models according to Maldonado et al., 2017 [47]. We will further functionally annotate the predicted proteome using domain and other analysis tools. We will compare the genome and predicted proteome with those of other nematodes, particularly those of the (relatively-distantly) related *Trichinella spiralis* and *Trichuris trichura*, and the recent draft genome of *Sobolibophyme baturini* (another Dioctophymatid species). We will also include if needed *Caenorhabditis elegans*. All these genomes are available at free access databases.

9. Proteomic analysis of *D. renale*

A complete set of tissues from three adult females and three adult males of *D. renale*were sent for proteomic analysis to CEQUIBIEM facility located at School of Exact and Natural Sciences, University of Buenos Aires. These samples were sent by November 2019. Unfortunately, due to pandemic situation we are still waiting for the results.

Protein samples preparation: *D. renale* is a large nematode that yields large quantities of easily-dissectable tissues. Freshly collected parasites were washed several times with sterile PBS and placed in a Petri dish. Pseudocelomic fluid (PCF)was collected by punction with a syringe in the region between esophagus and the start of digestive duct and extract as much liquid as possible without taking other organs. PCF will be filtered (0.22 µm pore size) and store at -20 °C. The rest of the parasites' bodies were dissected into bodywall, intestine and gonads (testis and ovary). These tissues were homogenized using a mechanical tissue grinder. All samples will be in PBS buffer containing a cocktail of protease inhibitors and stored at -20 °C until analysis. Protein content were estimated using the Bradford assay. Sample integrity will also be evaluated using SDS-PAGE. Homogenates were loaded on SDS-PAGE gels and allowed to run until all proteins from the samples are inside the polyacrylamide mesh. The gel were sliced and stored until analysis.

Proteomic analysis: Protein preparations were sent to the Proteomics Core Facility of CEQUIBIEM at the University of Buenos Aires.

Perspective: Data will be analysed with Proteome Discoverer 2.1 software (Thermo Scientific, Germany) for identification and area quantitation of each protein using selected databases. In this case we will use *D. renale* draft genome or reference genomes from related nematodes

named before.

10. *In vitro* culture of *D. renale* and analysis of Excretion/Secretion products

As stated before, two major proteins were described in the PCF of *D. renale*, P17 and P44, present in both male and females. N-terminal and internal peptide amino-acid sequences of P44 indicate a relationship with a cysteine- and histidine-rich protein of unknown function from *Trichinella spiralis* and *Trichuris muris* which are found to be the major component of E/S products [48,49] . Importantly, P43 (secreted protein from *T. muris*) have proven to bind IL-13, though it might be important in the attenuation of host´s immune response. Available draft genome of *D. renale* confirmed that P44 is effectively an orthologue of the described proteins found in Trichinella and *Trichuris muris*. Recently, we were able to keep *D. renale* in culture conditions and collect the E/S products confirming that P44 is secreted, see Figure 9.

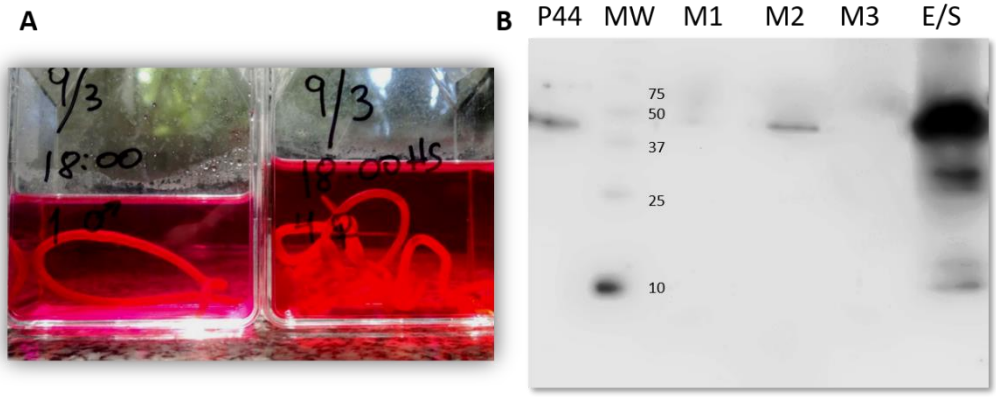

Figure 9. Search for P44 using Western blot in E/S products: A. Culture of *D. renale* in RPMI medium with high glucose and antibiotics for 24 hs. B. Western Blot using antibody anti P44 produced in our laboratory. P44: pure P44; MW: molecular weight standard (kDa); M1: sample obtained from liquid sorrounding worms inside kidney 1; M2: inner fluid from renal cyst containing *D. renale* worms; M3: sample obtained from kidney2; E/S: excretory-secretory products collected *in vitro*.

Publications of the group related to the project during the period of the grant

- Identification and characterization of the major pseudocoelomic proteins of the giant kidney worm, Dioctophyma renale. A. Nahili Giorello, Malcolm W. Kennedy, Marcos J.Butti, Nilda E. Radman, BetinaCórsico& Gisela R. Franchini. (2017) Parasit Vectors 10(1):446. doi: 10.1186/s13071-017-2388-x.
- *Dioctophyme renale* in a domestic cat (Felis catus): Renal location and nephrectomy. M Butti, M I Gamboa, J Terminiello, G Franchini, N Giorello, L Maldonado, L Kamenetzky, M Florencia Luna, M Lopez Merlo, N Radman. (2019) Vet Parasitol Reg Stud Reports. 2019 Dec;18:100339. doi: 10.1016/j.vprsr.2019.100339.

Other publications during the period:

- As-p18, an extracellular fatty acid binding protein of nematodes, exhibits unusual structural features. M Ibanez-Shimabukuro, M F Rey-Burusco, M Gabrielsen, G Franchini, A RiboldiTunnicliffe, A Roe, K Griffiths, A Cooper, B Corsico, M Kennedy,

and B Smith. Biosci Rep. (2019) Jul 23;39(7). pii: BSR20191292. doi: 10.1042/BSR20191292.

- Fatty Acid and Retinol-Binding Protein: Unusual Protein Conformational and Cavity Changes Dictated by Ligand Fluctuations. Barletta GP, Franchini G, Corsico B, Fernandez-Alberti S. 3 (2019) J Chem Inf Model. 59(8):3545-3555.

- Fatty acid binding proteins in Echinococcus spp.: the family has grown. Jorge L. Pórfido, Michaela Herz, Ferenc Kiss, Laura Kamenetzky, Klaus Brehm, Mara C. Rosenzvit, BetinaCórsico, Gisela R. Franchini. (2020). Parasitolgy Research, 119(4):1401-1408. doi: 10.1007/s00436-020-06631-5.

- Application of target repositioning and in silico screening to exploit fatty acid binding proteins (FABPs) from *Echinococcus multilocularis* as possible drug targets. Bélgamo JA, Alberca LN, Pórfido JL, Romero FNC, Rodriguez S, Talevi A, Córsico B, Franchini GR. (2020) J Comput Aided Mol Des. 34(12):1275-1288. doi: 10.1007/s10822-020-00352-8.

Poster presentations

- Arce, Lucas F; Facelli Fernández, Florencia; Giorellio, Nahili; Butti, Marcos; Maldonado, Lucas L; Arrabal, Juan P; Natalini, María B; Kowalewski, Martín; Pedrassani, Daniela; Zilli, Florencia; Franchini, Gisela R; Beldomenico, Pablo M; Kamenetzky, Laura. Genetic Diversity of *Dioctophyma renale* in Northeast Argentina and Southern Brazil. Molecular Parasitology Meeting XXXI, USA, Virtual, 21 al 25 de Septiembre 2020
- Lucas L. Maldonado, Nahili Giorello, Marcos Butti, Dominik R. Laetsch, Nilda A. Radman, Malcolm W. Kennedy, Betina Córsico, Gisela Franchini, Mark Blaxter, Laura Kamenetzky. Genome project of the giant kidney worm (Dioctophyme renale). 5th Ecology, Evolution, and Genomics of C. elegans and other Model Nematodes, Hixton, Cambrige, 2018
- Nahili Giorello, Marcos Butti, Lucas Maldonado, Laura Kamenetzky, Dominik R. Laetsch, Mark Blaxter, Nilda A. Radman, Betina Córsico y Gisela Franchini. Identificación y caracterización de antígenos para su uso en el desarrollo de métodos diagnósticos de helmintiasis huérfanas. IV Congreso Argentino de Microbiología Agrícola y Ambiental I Jornada de Microbiología General, 2018
- Kamenetzky L. Helminth parasite genomes: new tools for the study of the host-parasite relationship. Biotechnology Programme for Latin America and the Caribbean, Webinar 1-15 December 2020.
- Kamenetzky L. Genomics of parasitic helminths. 1st Latin American Congress of Women in Bioinformatics and Data Sciences. Virtual edition 21-24 September 2020.
- Characterization of the major pseudocoelomic proteins of the giant kidney worm, Dioctophymerenale. N. Giorello, D. Pedrassani, M. Andre, R. Zacarias Machado,

M.Butti, N. A. Radman, M. W. Kennedy, B Corsico and G. Franchini. Molecular and Cellular Biology of Helminths IX. 2-7 September 2018. Hydra, Greece.

- Fatty acid binding proteins (FABP) of parasitic cestodes: functional studies and evaluation as novel therapeutic targets. J. Belgamo, J.Porfido, U.Koziol, E. Castillo, M. Herz, M. Perez, M. Rosenzvit, K. Brehm, B. Corsico and G. Franchini. Molecular and Cellular Biology of Helminths IX. 2-7 September 2018. Hydra, Greece.
- First approximation to the study of a tandemly repeated protein: the case of ABA-1A from Ascaris suum. Rodriguez S, Bélgamo J, Kennedy MW, Córsico B, Franchini G. XLVII Reunión Anual SAB, 5 – 7 Diciembre 2018. La Plata, Argentina.
- Initial studies of fatty acid binding proteins (FABP) from parasitic cestodes as novel therapeutic targets. Bélgamo J, Pórfido JL, Rodriguez S, Pérez M, Rosenzvit M, Brehm K, Córsico B, Franchini G. XLVII Reunión Anual SAB, 5 – 7 Diciembre2018. La Plata, Argentina.
- Avances en el estudio molecular del nematode parásito *Dioctophymerenale*. Nahili Giorello, Marcos Butti, Lucas Maldonado, Laura Kamenetzky, Dominik R. Laetsch, Mark Blaxter, Nilda A. Radman, Betina Córsico y Gisela Franchini. VIII Congreso Argentino de Parasitología. Corrientes,Argentina 24-27 de abril de2019.
- *Caenorhabditis elegans* como modelo para el estudio de la proteína que une ácidos grasos y retinol del nematode parásito *Necator americanus* (Na-FAR-1). Jose F Lombardo, M Laura Migliori, Betina Córsico y Gisela Franchini. VIII Congreso Argentino de Parasitología. Corrientes,Argentina 24-27 de abril de2019.
- Functional analysis of fatty acid and retinol binding proteins (FAR) in Caenorhabditis elegans". Jose F. Lombardo; Betina Corsico; Collette Britton and Gisela Franchini. 2nd LAWM, Rosario, Argentina, 19-21 de Febrero de 2020.
- Genetic Diversity of *Dioctophyma renale* in Northeast Argentina and Southern Brazil. Arce, LF; Facelli Fernández, F; Giorello, N; Butti, M; Maldonado, LL; Arrabal, JP; Natalini, MB; Kowalewski, M; Pedrassani, D; Zilli, F; Franchini, GR; Beldomenico, PM; Kamenetzky, L. Molecular Parasitology Meeting XXXI. 2020. pág 57.

PhD Thesis related to the project defended during the period of the grant

1. **A. NahiliGiorello.** Title: Identification of antigens for their use in serological diagnosis of Dioctofimosis. In progress. SchoolofExactSciences, Universityof La Plata Argentina. PhD supervisor: Gisela R. Franchini.
2. **Lucas Arce.**Title: Molecular epidemiology of Dioctophyme renale and species from Echinococcus genus in domestic and wild fauna from North Argentina . In progress. School of Exactand Natural Sciences, UniversityofBuenos Aires, Argentina. PhD supervisor: Dr. Laura Kamenetzkyand Dr. Gisela R. Franchini.

Literature

1. Acha, P.N., Szyfres, B., (1989). Zoonosis y Enfermedades Transmisibles Comunes al Hombre y a los Animales, third ed. Organización Panamericano de la Salud, Washington, pp.265-267

2. Nakagawa TL1, Bracarense AP, dos Reis AC, Yamamura MH, Headley SA. (2007). Giant kidney worm (Dioctophyma renale) infections in dogs from Northern Paraná, Brazil. Vet Parasitol. 145(3-4):366-70.

3. Ferreira VL et al., (2010). Vet Parasitol. 168:151-5.

4. Mesquita LR, Rahal SC, Faria LG, Takahira RK, Rocha NS, Mamprim MJ, Oliveira HS. (2014). Pre- and post-operative evaluations of eight dogs following right nephrectomy due to Dioctophyma renale. Vet Q. 34(3):167-71. doi: 10.1080/01652176.2014.924166.

5. Pereira BJ, Girardelli GL, Trivilin LO, Lima VR, Nunes Lde C, Martins (2006). The occurrence of dioctophymosis in dogs from Municipality of Cachoeiro do Itapemirim in the State of Espírito Santo, Brazil, from May to December of 2004 IV.Rev Bras Parasitol Vet. 15(3):123-5. .

6. Proença C. Sobre dois casos de Dioctophyme renale em cão no Rio de Janeiro. Bol Vet. Exerc. 1935:2:50-1.

7. Rocha UF, Serra RG, Grechi R. (1965). Parasitismo por *Dioctophyma renale* (Goeze, 1782) em "preguiça" *Choloepus didactyus*, Linnaeus, 1758. Rev Farm Bioquim Univ Sao Paulo 3:325-34.

8. Ribeiro CT, Verocai GG, Tavares LE. Dioctophyme renale (Nematoda, Dioctophymatidae) infection in the crab-eating fox (*Cerdocyon thous*) from Brazil. J Wildl Dis. 2009;45:248- 50.

9. Giovannoni M, Molfi A. O Dioctophyma renale (Goeze, 1782) no Brasil. In: Anais da Faculdade de Medicina da Universidade Federal do Paraná; 1960, Curitiba. Curitiba: Universidade Federal do Paraná; 1960. p.99-104.

10. Duarte J,. et al. (2013). Parasitism by Dioctophyme renale (Goeze, 1782) in Maned Wolf (Chrysocyon brachyurus) Brazil. Vet. e Zootec. 20(1): 52-56.

11. Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendices I, II & III (5/02/2015) – p. 7

12. Maia, O.B. & Gouveia, A.M.G. (2002). Birth and mortality of maned wolf Chrysocyon brachyurus (ILLIGER, 1811) in captivity. Brazilian Journal of Biology, 62(1):25-32.

13. Deem, D.V.M.S.L. & Emmons, L.H. (2005). Exposure of free-ranging maned wolves (Chrysocyon brachyurus) to infectious and parasitic disease agents in the noel kempff Mercado national park, Bolivia. Journal of Zoo and Wildlife Medicine, 36(2):192-7.

14. Beaver PC, Theis JH. (1979). Diocytophymatid larval nematode in a subcutaneous nodule from man in California. Am J Trop Med Hyg. 28(2):206-12.

15. Kommers, G.D., Ilha, M.R.S., Barros, C.S.L.. (1999). Dioctofimose em ca˜es: 16 casos. Cieˆncia Rural 29, 517–522.

16. Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., (1998). Parasitologia Veterina´ ria, second ed. Guanabara Koogan, Sa˜o Paulo, p. 88.

17. Verocai, G.G., Measures, L.N., Azevedo, F.D., Correia, T.R., Fernandes, J.I., Scott, F.B., (2009). Dioctophyma renale (Goeze, 1782) in the abdominal cavity of a domestic cat from Brazil. Vet. Parasitol. 161, 342–344.

18. Gang Li, Caigang Liu, Fang Li, Maoyi ZhouI, Xiangyong Liu, and Yuanjie Niu. (2010). Fatal bilateral Dioctophymatosis. The Journal of Parasitology. 96, 1152-1154.

19. Katafigiotis I et al., (2013). Parasitol Int. 62(5):459-60.

20. Venkatrajaiah N, Kalbande SH, Rao GV, Reddy VC, Reddy SH, Rao PR, Babu K, Keerthi A. (2014). Dioctophymatosis renalis in humans: first case report from India. J Assoc Physicians India. 62(10):70-3.

21. Kuehn J, Lombardo L, Janda WM, Hollowell CM. (2016). Giant kidney worms in a patient with renal cell carcinoma. BMJ Case Rep. pii: bcr2015212118. doi: 10.1136/bcr-2015-212118.

22. Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; Fonseca, G.A.B. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature, 403:853-8.

23. Pedrassani D1, Lux Hoppe EG, Avancini N, do Nascimento AA. (2009). Morphology of eggs of Dioctophyme renale Goeze, 1782 (Nematoda: Dioctophymatidae) and influences of temperature on development of first-stage larvae in the eggs. Rev Bras Parasitol Vet. 18(1):15-9.

24. Mascarenhas CS1, Müller G1. (2015) Third-stage larvae of the enoplid nematode Dioctophyme renale (Goeze, 1782) in the freshwater turtle Trachemys dorbigni from southern Brazil. J Helminthol. 89(5):630-5. doi: 10.1017/S0022149X14000364.

25. Georgi, J.R., Georgi, M.E., (1991). Canine Clinical Parasitology. Lea & Febiger, Pennsylvania, pp. 183–184.

26. Pedrassani D, do Nascimento AA, André MR, Machado RZ. (2015). Improvement of an enzyme immunosorbent assay for detecting antibodies against Dioctophyma renale. Vet Parasitol. 212(3-4):435-8.

27. Giorello N, Butti M, Radman NA, Kennedy MW, Corsico B and G Franchini. Identification and characterization of lipid binding proteins from the parasitic nematode *Dioctophyma renale*. Molecular and Cellular Biology of Helminths IX. 31 August - Saturday 5 September 2015. Hydra, Greece. Poster presentation.

28. Franchini GR, Pórfido JL, Ibáñez Shimabukuro M, Rey Burusco MF, Bélgamo JA, Smith BO, Kennedy MW, Córsico B. (2015). The unusual lipid binding proteins of parasitic helminths and their potential roles in parasitism and as therapeutic targets. Prostaglandins Leukot Essent Fatty Acids. 93:31-6. doi: 10.1016/j.plefa.2014.08.003.

29. Kennedy MW, Qureshi F, Fraser EM, Haswell-Elkins MR, Elkins DB, Smith H V. Antigenic relationships between the surface-exposed, secreted and somatic materials of the nematode parasites *Ascaris lumbricoides*, *Ascaris suum*, and *Toxocara canis*. Clin Exp Immunol. 1989;75:493–500.

30. Kennedy MW, Qureshi F, Haswell-Elkins M, Elkins DB. Homology and heterology between the secreted antigens of the parasitic larval stages of Ascaris lumbricoides and Ascaris suum. Clin Exp Immunol. 1987;67:20–30.

31. Xia Y, Spence HJ, Moore J, Heaney N, McDermott L, Cooper A, et al. The ABA-1 allergen of *Ascaris lumbricoides*: sequence polymorphism, stage and tissue-specific expression, lipid binding function, and protein biophysical properties. Parasitology. 2000;120:211–24. http://www.ncbi.nlm.nih.gov/pubmed/10726282.

32. Kennedy MW. The nematode polyprotein allergens/antigens. Parasitol Today. 2000;16:373–80. http://www.ncbi.nlm.nih.gov/pubmed/10951596.

33. Kennedy MW, Qureshi F. Stage-specific secreted antigens of the parasitic larval stages of the nematode Ascaris. Immunology. 1986;58:515–22. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1453479&tool=pmcentrez&rendertype=abstract. Accessed 12 Apr 2014.

34. Davenport HE. The haemoglobins of Ascaris lumbricoides. Proc R Soc London Ser B, Biol Sci. 1949;136:255–70. http://www.ncbi.nlm.nih.gov/pubmed/18152153. Accessed 27 Mar 2017.

35. Goldberg DE. The enigmatic oxygen-avid hemoglobin of Ascaris. BioEssays. 1995;17:177–82.

36. Blaxter ML. Nemoglobins: divergent nematode globins. Parasitol Today. 1993;9:353–60. http://www.ncbi.nlm.nih.gov/pubmed/15463668. Accessed 9 Jan 2017.

37. Blaxter M, Koutsovoulos G. The evolution of parasitism in Nematoda. Parasitology. 2014;:1–14. doi:10.1017/S0031182014000791.

38. Koehler AV, Hoberg EP, Torres-Pérez F, Cook JA. A molecular view of the superfamily dioctophymatoidea (Nematoda). Comp Parasitol. 2009;76:100–4. http://digitalcommons.unl.edu/parasitologyfacpubs. Accessed 14 Mar 2017.

39. Rey-Burusco MF, Ibanez-Shimabukuro M, Gabrielsen M, Franchini GR, Roe AJ, Griffiths K, et al. Diversity in the structures and ligand-binding sites of nematode fatty acid and retinol-binding proteins revealed by Na-FAR-1 from *Necator americanus*. Biochem J. 2015;471:403–14. doi:10.1042/BJ20150068.

40. Wilton DC. The fatty acid analogue 11-(dansylamino)undecanoic acid is a fluorescent probe for the bilirubin-binding sites of albumin and not for the high-affinity fatty acid-binding sites. Biochem J. 1990;270:163–6.

41. Prior A, Jones JT, Blok VC, Beauchamp J, McDermott L, Cooper A, et al. A surface-associated retinol- and fatty acid-binding protein (Gp-FAR-1) from the potato cyst nematode Globodera pallida: lipid binding activities, structural analysis and expression pattern. Biochem J. 2001;356 Pt 2:387–94.

42. Tokiwa T, Harunari T, Tanikawa T, Akao N, Ohta N. Dioctophyme renale (Nematoda: Dioctophymatoidea) in the abdominal cavity of Rattus norvegicus in Japan. Parasitol Int. 2011 Sep;60(3):324-6. doi: 10.1016/j.parint.2011.03.003. Epub 2011 Mar 17. PMID: 21419863.

43. Hoff KJ, Lange S, Lomsadze A, Borodovsky M, Stanke M. BRAKER1: Unsupervised RNA-Seq-Based Genome Annotation with GeneMark-ET and AUGUSTUS. Bioinformatics. 2016 Mar 1;32(5):767-9. doi: 10.1093/bioinformatics/btv661. Epub 2015 Nov 11. PMID: 26559507; PMCID: PMC6078167.

44. Cucher M, Macchiaroli N, Kamenetzky L, Maldonado L, Brehm K, Rosenzvit MC. High-throughput characterization of Echinococcus spp. metacestode miRNomes. Int J Parasitol. 2015; 45(4):253–67. https://doi.org/10.1016/j.ijpara.2014.12.003 PMID: 25659494

45. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 2011 May 15;29(7):644-52. doi: 10.1038/nbt.1883. PMID: 21572440; PMCID: PMC3571712.

46. Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD, Ott M, Orvis J, Pochet N, Strozzi F, Weeks N, Westerman R, William T, Dewey CN, Henschel R, LeDuc RD, Friedman N, Regev A. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc. 2013 Aug;8(8):1494-512. doi: 10.1038/nprot.2013.084. Epub 2013 Jul 11. PMID: 23845962; PMCID: PMC3875132.

47. Maldonado LL, Assis J, Araújo FM, Salim AC, Macchiaroli N, Cucher M, Camicia F, Fox A, Rosenzvit M, Oliveira G, Kamenetzky L. The Echinococcus canadensis (G7) genome: a key knowledge of parasitic platyhelminth human diseases. BMC Genomics. 2017 Feb 27;18(1):204. doi: 10.1186/s12864-017-3574-0. PMID: 28241794; PMCID: PMC5327563.

48. Radoslavov G, Jordanova R, Teofanova D, Georgieva K, Hristov P, Salomone-Stagni M, Liebau E, Bankov I. A novel secretory poly-cysteine and histidine-tailed metalloprotein (Ts-PCHTP) from Trichinella spiralis (Nematoda). PLoS One. 2010 Oct 13;5(10):e13343. doi: 10.1371/journal.pone.0013343. PMID: 20967224; PMCID: PMC2954182.

49. Tritten L, Tam M, Vargas M, Jardim A, Stevenson MM, Keiser J, Geary TG. Excretory/secretory products from the gastrointestinal nematode Trichuris muris. Exp Parasitol. 2017 Jul;178:30-36. doi: 10.1016/j.exppara.2017.05.003. Epub 2017 May 19. PMID: 28533110.