Characterisation of *Deladenus siricidicola* genes potentially involved in parasitism and host immunomodulation

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Declaration of originality

I, Zorada Swart declare that the dissertation, which I hereby submit for the degree MSc (Genetics) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature Date

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Preface

Nematodes often associate with insects. Usually, it is the nematode that benefits from this interaction as the insect serves either as vector – for many plant- and vertebrate-parasitic nematodes (VPN) – or as the target host in the case of insect-parasitic or entomopathogenic nematodes (EPN). Parasitic nematodes are frequently responsible for chronic, debilitating diseases in millions of people, as well as crop losses, damage to plantations, and loss of livestock. By contrast, EPN used in the biological control of insect pests provide beneficial alternatives to costly and potentially hazardous chemical pesticides. The Steinernematidae and Heterorhabditidae coevolved with symbiotic bacteria and are the EPN families most widely used and well-studied as biocontrol agents of a variety of insect pests. These nematodes have evolved different strategies with which to overcome the robust immune response of the host. Although not as well studied, the nematode *Deladenus siricidicola* is of particular interest to pine growers, especially in the Southern Hemisphere, where it is the main biocontrol agent of the invasive woodwasp *Sirex noctilio*. In this study, I apply the available knowledge of immunomodulation in *Steinernema* spp. and *Heterorhabditis* spp. to better understand the interaction between *D. siricidicola* and the immune response of *S. noctilio*.

Chapter 1 reviews the current knowledge on the immune interactions between parasitic nematodes and their insect hosts. The review provides an overview of the insect immune response, especially in response to nematode invasion. Nematode biology related to host infection and the strategies aimed at overcoming the host immune response is discussed with reference to two well-known nematode-insect interactions. The first interaction is that of the biocontrol EPN of the genera *Steinernema* and *Heterorhabditis*, followed by the plant-parasitic pinewood nematode (PWN) *Bursaphelenchus xylophilus* and its beetle vector *Monochamus alternatus*. The relevance of and possible approaches for dissecting this interaction between the pine woodwasp, *S. noctilio,* and its biocontrol nematode, *D. siricidicola*, is proposed.

In Chapter 2, I identify and characterise genes and gene families in the genome of *D. siricidicola* which are potentially involved in parasitism and immunomodulation during the infection of *S. noctilio*. Sequence similarity searches with known virulence genes in *Steinernema* and other nematodes are performed against the genome of *D. siricidicola* using local BLAST, protein clustering, and hidden Markov model (HMM) searches. The identified

genes are characterised functionally by comparison with functional databases. The expression of the genes are analysed with transcriptome data from both free-living nematodes and an early infection of *S. noctilio* larvae by *D. siricidicola*.

The third chapter of this dissertation considers the relevance of studying the interactions between nematodes and insects for human disease. Research on parasitic nematodes and their vertebrate hosts is challenging and costly. However, simpler organisms can be used as models to study complex biological and pathological processes. Although insects lack the sophisticated adaptive immune responses of mammals and rely only on an innate immune system, I argue that the similarities between the interactions of nematodes, insects, and bacteria in EPN and VPN systems, provide opportunities to explore additional and alternative treatment options for challenging human conditions. This chapter is structured as published in the journal Pathogens and Global Health in November 2021.

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The EPN covers its body surface with molecules mimicking host antigens or sequestered from the host haemolymph. The host's pathogen recognition system fails to identify the nematode as non-self and no immune response is activated. **Immune suppression –** EPN release symbiotic bacteria and immunosuppressive molecules. The excreted/secreted products of EPN contain proteases and protease inhibitors, which impair the function of haemocytes and pathogen recognition proteins (PRP), and reduce prophenoloxidase activity and subsequent melanisation. **Nematode development, reproduction –** Toxins, antimicrobials and enzymes released by symbiotic bacteria contribute to killing the insect host and digesting host tissues to create a suitable environment for nematode maturation. The next generation of IJ exit the depleted cadaver in search of a new host. (Adapted from: Brivio & Mastore, 2018)

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Figure 1.4 The bicyclic life style of *D. siricidicola*. **Free-living cycle (left) –** Nematodes feed on *A. areolatum*, the symbiotic fungus of *S. noctilio*. Feeding, maturation and reproduction take place within the host tree. **Parasitic cycle (right) –** Juvenile nematodes develop into infective form adults in response to the high CO_2 levels and a low pH micro-environment in the vicinity of *Sirex* larvae. Infective males and females mate before females penetrate the wasp larvae. Absorption of nutrients, fertilisation and hatching of eggs take place within the host. As the adult wasp leaves the tree, next generation juvenile nematodes migrate to the reproductive organs of the host. Infection of male wasps represents a "dead-end" for *D. siricidicola* as male wasps do not spread the nematode. Conversely, female wasps are sterilised by the nematode but continues oviposition of the fungus and juvenile nematodes into new host trees.

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Higher resolution versions of the images in Chapter 1 are available at https://drive.google.com/drive/folders/1F-lgsmMsD9cfRdk_j7qS8zc1Xor3RGBG?usp=sharing.

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Higher resolution versions of the images in Chapter 2 are available at https://drive.google.com/drive/folders/1KBI8sqdlMvRrWSrK6fKXu72hxbIIKKhe?usp=sharing.

Chapter 1.

The immune interactions between parasitic nematodes and their insect hosts

Abstract

Insects interact with nematodes in a variety of ways – as vectors to vertebrate- and plant-parasitic nematodes and as hosts to insect-parasitic nematodes. Here I focus on entomopathogenic nematodes (EPN) that coevolved with bacterial symbionts to become virulent pathogens of insects. The ability of the nematode-bacterial complexes to infect and kill insects is harnessed in the biological control of insect pests, providing a valuable alternative to chemical pesticides. Successful biocontrol EPN modulate the insect host immune response to ensure their own survival and development. These parasitic nematodes, insect hosts or vectors, and symbiotic bacteria represent important models for the study of pathogenic and symbiotic interactions. In this review, I summarise the cellular and humoral mechanisms of the insect innate immune system, especially in response to nematode invasion, and outline how nematodes evade or suppress the host immune response. Host-parasite mechanisms are best described in *Steinernema* and *Heterorhabditis*, but I propose possible approaches for and the relevance of unravelling this interaction between the pine woodwasp, *Sirex noctilio*, and its biocontrol nematode, *Deladenus siricidicola*.

Keywords – biocontrol, *Deladenus siricidicola*, entomopathogenic nematode, immunomodulation, insect immune response, *Sirex noctilio*

1.1 Introduction

Nematodes are adapted to a variety of natural habitats and feeding habits, including both free-living and parasitic life styles (Blaxter et al., 1998, 2003). Given their nearly universal presence, free-living nematodes contribute to the equilibrium of ecosystems by decomposing organic matter, degrading toxins and competing with pathogens (Bongers & Ferris, 1999; Ferris & Bongers, 2006). Parasitic nematodes affect millions of people by causing diseases such as lymphatic filariasis (elephantiasis) (Bockarie et al., 2009; James et al., 2018; Taylor et al., 2010), malnutrition and bowel obstruction (O'lorcain & Holland, 2000), eye, and even brain infections (Lukiana et al., 2006; Pinder, 1988). By infecting livestock (Besier et al., 2016; Waller, 2006), agricultural crops (Elling, 2013; Nicol et al., 2007) and plantations (Kim et al., 2020; Mamiya, 1976), animal and plant parasitic nematodes affect human populations indirectly.

Nematodes and insects are common associates with a wide range of interactions (Figure 1.1). In most parasitic infections of vertebrates and plants, nematodes rely on an insect as vector or intermediate host to reach the target host (Katiyar & Singh, 2011; Ryss et al., 2005). Filarial nematodes, for instance, are transmitted to their human hosts through the bite of certain mosquito species and black flies. In addition to being vectors, insects are also the hosts for parasitic nematodes known as entomopathogenic- or insect-parasitic nematodes (EPN) (Lewis & Clarke, 2012; Torres-Barragan et al., 2011; Zhang et al., 2008). The ability of EPN to infect and kill insects is harnessed in the biocontrol of insect pests (Gaugler, 2002; Grewal et al., 2005; Noosidum et al., 2021; Salari et al., 2021). Species from the genera *Heterorhabditis* and *Steinernema* are the EPN most commonly studied as they are frequently used in biocontrol (Dillman & Sternberg, 2012; Kaya & Gaugler, 1993).

Both vertebrate and insect parasitic nematodes are often associated with symbiotic bacteria. The nematode species responsible for the majority of human filariasis all depend on the intracellular bacteria *Wolbachia* for survival and reproduction (Taylor et al., 2005, 2010). The bacterial symbionts of EPN play an active role in killing the insect host and preventing invasion by other microorganisms (Brivio et al., 2005). The associations between EPN and their bacterial symbionts are typically species-specific. For example, *Heterorhabditis bacteriophora*

is associated with the bacterium *Photorhabdus luminescens* and *Steinernema carpocapsae* with *Xenorhabdus nematophila* (Adams et al., 2006; Boemare, 2002).

Parasitic nematodes, insect hosts and bacterial symbionts provide valuable opportunities to study the biological processes related to interspecies relationships (Hallem et al., 2007; Stock, 2005). For example, because the nematode and its bacterial symbiont can be cultured separately, manipulated and re-associated, they can be used as study systems to investigate factors influencing mutualism (Ciche & Sternberg, 2007; Ruby, 2008). Furthermore, insects susceptible to EPN provide models to study host-parasite interactions, such as immune responses or the genetic mechanisms involved in infection.

The close evolutionary relationship of vertebrate- and invertebrate parasitic nematodes means that insect-nematode interactions can be used as models to study human disease (Blaxter & Koutsovoulos, 2015; Hallem et al., 2007; Lu et al., 2017). This can be useful because financial, logistical and ethical challenges restrict the study of parasitic nematodes in vertebrate hosts. By contrast, EPN are cheaper, safer and easier to culture as they do not pose an infection threat to humans and require insects instead of mammals as hosts. The use of insect hosts also simplifies ethical clearance (Lu et al., 2017). Entomopathogenic nematodes and insects therefore provide simpler experimental systems to study pathogenesis, host immune responses, and even drug efficacy (Chamilos et al., 2007).

Insect-parasitic nematodes provide an important alternative to chemical insecticides in the control of arthropod pests (Benseddik et al., 2021; Coppel & Mertins, 1977). These biological control agents are generally safer for humans and the environment, and carry a smaller risk of resistance developing in non-target organisms than broad-spectrum insecticides (Bathon, 1996). Despite the wide usage of nematodes in biocontrol, efficacy in the field lacks consistency (Hurley et al., 2007; Lu et al., 2016). Investigations into the mechanisms underlying host-parasite interactions, in addition to environmental conditions and nematode handling methods, have the potential to reveal modifiable factors that could help improve biocontrol systems.

The genome sequences of several EPN are available (Bai et al., 2013; Dillman et al., 2015; Postma et al., 2019). Access to genomic data increases the potential for genetic improvement of biocontrol agents (Lu et al., 2016). Genomic data could, for instance, shed light on gene

variants associated with beneficial traits so that these traits can be enhanced through artificial selection (genomics-assisted breeding) and molecular gene editing tools. Studies using transcriptome data are providing valuable insight into host-parasite interactions by identifying differentially expressed genes implicated in desirable traits, trait deterioration and immune interactions (Adhikari et al., 2009; Vadnal et al., 2017).

To take advantage of the available EPN genomic and transcriptomic resources for the improvement of biocontrol systems or the study of human disease, insight into the mechanisms involved in parasite-host relationships is needed. This review provides a synthesis of current knowledge of the insect immune system, especially in response to nematode invasion, and an outline as to how nematodes overcome the host immune response. Although host-parasite mechanisms are best described in *Steinernema* and *Heterorhabditis*, the relevance of and possible approaches for unravelling this interaction between the pine woodwasp, *Sirex noctilio*, and its biocontrol nematode, *Deladenus siricidicola*, are proposed.

1.2 The insect immune system

All multicellular organisms are armed with a genetically encoded, innate immune system (Brivio & Mastore, 2018; Müller et al., 2008). The innate immune system takes effect if the first lines of defence, namely the exoskeleton and epithelial tissue lining the respiratory, digestive and genital tracts, fail to counter pathogen invasion (Ferrandon et al., 1998; Tzou et al., 2000). In mammals, immune defence is enhanced by the acquired or adaptive immune response. Lacking the antigen-specific molecules or sophisticated immunological memory of acquired immunity, insects rely solely on the cellular and humoral effector processes of the innate immune system (Figure 1.2).

1.2.1 THE CELLULAR IMMUNE RESPONSE

Cellular immunity is the protection offered by cells resembling human white blood cells (Meister & Lagueux, 2003). These blood cells, or haemocytes, circulate through the insect haemolymph performing functions such as phagocytosis and encapsulation.

Phagocytosis involves the engulfment of microscopic particles such as yeasts, bacteria, viruses, and even abiotic bodies by a single haemocyte (Evans et al., 2003; Lanot et al., 2001; Lavine & Strand, 2002). Following engulfment, the pathogen or foreign body is contained in a vesicle known as the phagosome, within the haemocyte cytoplasm (Eley & Beatty, 2009). Cellular organelles – such as lysosomes and endosomes – containing catabolic enzymes merge with the phagosome to digest the phagocytosed foreign particle (Stuart & Ezekowitz, 2005; Wei & Wang, 2019). The haemocytes involved in phagocytosis are referred to as plasmatocytes in *Drosophila* and granulocytes in other insects (Strand, 2008).

Encapsulation targets larger invading organisms such as nematodes (Brivio & Mastore, 2018; Mastore & Brivio, 2008; Strand, 2008). During encapsulation, layers of haemocytes adhere to the surface of a pathogen or foreign body. Aggregates of bacteria can also be encapsulated by haemocytes in the process referred to as nodulation (Ratcliffe & Gagen, 1976, 1977). Encapsulated pathogens die from asphyxiation and the release of harmful compounds into the capsule, e.g., antimicrobial peptides and products of the melanisation cascade (Nappi & Christensen, 2005). Lamellocytes in *Drosophila*, and plasmatocytes in other insects, are flattened cells that initiate encapsulation by adhering to the target and activate other blood cells to form subsequent layers.

1.2.2 THE HUMORAL IMMUNE RESPONSE

The humoral immune response consists of soluble molecules in the haemolymph that contribute to the recognition and elimination of foreign microorganisms (Brivio & Mastore, 2018). Antimicrobial peptides (AMP), digestive enzymes (e.g., lysozyme), and melanisation form part of humoral immunity.

Antimicrobial peptides are a diverse group of small proteins consisting of fewer than a hundred amino acids (Bulet & Stöcklin, 2005; Lazslo Otvos, 2000). The most common mechanism of action of AMP is destruction of the pathogen membrane either by disrupting its phospholipid layer, or by preventing membrane formation (Shai, 1999). The insect fat body (similar to the liver in mammals) produces the majority of AMP with a small amount being secreted by haemocytes and epithelial tissue (Dimopoulos, 2003). With the rise of antimicrobial resistance, AMP have been gaining interest as a potential source of antimicrobial drug discovery (Bulet &

Stöcklin, 2005). Insects tolerating high levels of AMP suggests that systemic treatments derived from AMP will also be well tolerated. However, the cost of producing AMP and their sensitivity to proteases are challenges yet to be overcome.

Melanisation is a key component of the innate immune response in insects due to its efficacy and quick onset of action (usually faster than cellular mechanisms) (Brivio et al., 1996; Cerenius et al., 2008; Dimopoulos et al., 2001). Phenoloxidase (PO) is a humoral protein known for its role in the prophenoloxidase activating system (proPO-AS), the end-product of which is the brown-black pigment melanin. The proPO-AS is a complex, highly reactive enzymatic cascade consisting of recognition proteins, phenoloxidase, proteases and protease inhibitors (Cerenius et al., 2008). Melanin limits the spread of an infection by being deposited at wounding sites and on the surface of foreign bodies. As part of "humoral encapsulation", melanin is deposited on the surface of larger invading organisms (including nematodes), followed by layers of lamellocytes or plasmatocytes (Liu et al., 1998; Schmidt et al., 2001; Vey, 1993). In addition to the direct toxic effect of melanin, the chemically active by-products of the proPO-AS, e.g. quinones and reactive oxygen species (ROS), further contribute to killing invading microorganisms (Kanost & Gorman, 2008; Zhao et al., 2007).

1.2.3 REGULATION OF THE INSECT IMMUNE RESPONSE

Widespread, indiscriminate deployment of defence mechanisms, e.g. melanisation, would be lethal to the host insect (Cerenius & Söderhäll, 2004; Eleftherianos & Revenis, 2010; Gubb et al., 2010; Söderhäll & Cerenius, 1998). Instead, accurate pathogen recognition by one or more receptors, followed by the activation of relevant signalling pathways, ensure that immune responses are directed appropriately (Pal & Wu, 2009).

Pattern recognition proteins (PRP) are extracellular receptors responsible for detecting foreign organisms. By binding to microbe/pathogen-associated molecular patterns (MAMP/PAMP), PRP distinguish foreign material (non-self) from the host's own tissue (self) (Castillo et al., 2011; Kim et al., 2008; Müller et al., 2008). Microbe-associated molecular patters are usually cell-wall components, e.g. lipopolysaccharides and peptidoglycans associated with bacteria, or fungal ß-1,3-glucans (Brookman et al., 1989; Pal & Wu, 2009). Pathogen recognition proteins are categorised according to the MAMP they interact with and include peptidoglycan-

recognition proteins, ß-glucan receptors, and glucan-binding proteins (Hoffmann, 2003; Kanost et al., 2004).

For nematodes to be recognised, proteins or carbohydrate motifs in the nematode cuticle interact either with receptors on the insect haemocyte surface directly, or with opsonins (Castillo et al., 2011). Opsonins are molecules that coat the surface of a pathogen, making the pathogen more susceptible to phagocytosis or encapsulation. The specificity with which PRP differentiate between self and non-self is an important first step in regulating both cellular and humoral responses.

Some PRP can have direct catalytic effects on a pathogen, but all trigger a signal transduction pathway to produce the desired immune response (Pal & Wu, 2009). The Toll pathway responds to fungal and Gram-positive bacterial infections (Rutschmann et al., 2002). Pathogen recognition triggers an intracellular proteolytic cascade which results in the expression of AMP specific to fungi or Gram-positive bacteria, respectively (Bulet et al., 1999). Similarly, the immune-deficiency (Imd) pathway is activated in response to Gram-negative bacteria and consequently produces Gram-negative AMP.

In addition to accurate pathogen recognition and specific signalling pathways, immune factors exist as inactive enzymes (zymogens) in the haemolymph. These require activation by other enzymes before performing their functions and further contribute to the control of immune responses. For example for melanin to be produced, the proPO-AS has to be activated, either by pathogen recognition, mechanical wounding and basement membrane disruption, or by defective cells (Brennan & Anderson, 2004; Bulet et al., 1999; Galko & Krasnow, 2004). Pathogen recognition triggers the c-Jun N-terminal kinase (JNK) intracellular signalling pathway, causing the relevant haemocytes to secrete inactive PPO into the haemolymph (Bidla et al., 2007). Extracellularly, pathogen recognition initiates a serine protease cascade which cleaves proPO to form active phenoloxidase (Cerenius et al., 2008). Similarly, serine protease inhibitors control the extent and duration of action of serine proteases (Kanost et al., 2004; Ligoxygakis et al., 2002; Nappi et al., 2005).

The cellular and humoral systems do not function in isolation but act simultaneously and influence one another. The interaction between PRP and MAMP can trigger more than one signalling pathway to produce both cellular and humoral immune responses against a single

infection (Cerenius et al., 2008). Haemocytes secrete humoral proteins such as prophenoloxidase and small amounts of AMP (Stanley et al., 2012). In turn, humoral proteins – including products of the prophenoloxidase system, prostaglandins and eicosanoids – appear to influence cellular functions such as phagocytosis and AMP production (Cerenius et al., 2008). The balance between sensitive recognition of non-self and specific signalling pathways leading to potent downstream response mechanisms, provides insects with robust resistance against microbial and parasitic infections. The efficacy of the innate immune system enables insects to not only survive, but also become established as pests and disease vectors despite considerable exposure to pathogens.

1.3 Nematode infection of insects

Nematodes survive within insects despite the robust innate immune system of the host (Li, Cowles, et al., 2007). Two well-known nematode-insect interactions will be used here to illustrate nematode biology related to host infection and the survival strategies nematodes employ to overcome host immune responses. The nematodes are EPN from the Steinernematidae and Heterorhabditidae families – used in the biological control of several insect pests – and the plant-parasitic pinewood nematode (PWN) *Bursaphelenchus xylophilus* with its beetle vector *Monochamus alternatus*.

1.3.1 *STEINERNEMA* AND *HETERORHABDITIS*

1.3.1.1 Biology and biological control

Entomopathogenic nematodes limit the spread of target insects by killing, sterilising or reducing the overall fitness of the insect. The use of EPN as biological control agents against insect pests reduces the need for expensive and potentially hazardous chemical pesticides (Burnell & Stock, 2000; Coppel & Mertins, 1977; Vega et al., 2012). In these aspects, the success of the *Steinernema* and *Heterorhabditis* genera, make them the most widely used and best studied biological control nematodes (Brivio et al., 2005; Cooper & Eleftherianos, 2016; Kaya & Gaugler, 1993).

Steinernema spp. and *Heterorhabditis* spp. share basic features in terms of development and host infection (Poinar, 1993). Both are obligate parasites requiring an insect host to develop

and reproduce. Both also form mutualistic associations with specific genera of Gram-negative bacteria, namely *Xenorhabdus* with *Steinernema*, and *Photorhabdus* with *Heterorhabditis* (Kaya & Gaugler, 1993).

The life cycle of *Steinernema* and *Heterorhabditis* is characterised by six life stages, starting with eggs (Lewis & Clarke, 2012). After hatching, the juvenile nematode moults four times. The final moult marks the transition from juvenile or larval stage to early adult. The nematodes continue to develop and reproduce inside the host cadaver until it has been depleted of nutrients. *Heterorhabditis* spp. reproduce by self-fertilisation or crossing since either hermaphrodites, or males and females are produced depending on environmental factors (Kahel-Raifer & Glazer, 2000; Koltai et al., 1995). The majority of *Steinernema* spp. are gonochoristic and therefore at least one male and one female have to infect the same insect simultaneously to permit sexual reproduction (Lewis & Clarke, 2012). The hermaphroditic, *S. hermaphroditum*, first isolated in Indonesia, is the exception and has a reproductive cycle similar to that of *Heterorhabditis* spp. (Stock et al., 2004).

Only nematodes in the third juvenile stage are adapted to surviving outside the host, resembling the dauer stage of *Caenorhabditis elegans* (Lewis & Clarke, 2012). Known as infective juveniles (IJ), these are responsible for finding and infecting a new insect host when resources from the current host have been exhausted (Womersley, 1993). Chemotaxis attracts IJ to higher concentrations of carbon dioxide (CO_2) and the excretory products surrounding potential hosts. Upon finding the insect, IJ use a variety of strategies to gain entry into the host haemocoel. Depending on the species of nematode and insect, IJ enter through the mouth (Cui et al., 1993), anus (Renn, 1998), spiracles (Georgis & Hague, 1981), or directly through the insect cuticle (Bedding & Molyneux, 1982; Wang & Gaugler, 1998).

Infective juveniles release their symbiotic bacteria – by defecation in the case of *S. carpocapsae* and *X. nematophila*, and regurgitation for *H. bacteriophora* and *P. luminescens* – once they have reached the insect haemocoel (Ciche & Ensign, 2003; Snyder et al., 2007). Once released, *Photorhabdus* and *Xenorhabdus* secrete toxins which kill the insect within a few days (An et al., 2009; ffrench-Constant & Bowen, 2000; Khandelwal & Banerjee-Bhatnagar, 2003; Rodou et al., 2010). Before the host is killed, bacteria also release compounds that interfere with the host immune response, e.g. by suppressing the phenoloxidase system (Eleftherianos et al., 2007; Song et al., 2011). Bacterial proteases and lipases digest host tissues as source of

nutrition for the nematodes (ffrench-Constant et al., 2007; Lewis & Clarke, 2012). To fend off competing organisms from the insect cadaver, symbiotic bacteria secrete antimicrobial compounds which deter other bacteria (Boemare et al., 1992; Gualtieri et al., 2009), fungi (Chen et al., 1994; Gualtieri et al., 2009) and ants (Zhou et al., 2002).

1.3.1.2 Immunomodulation

In order to develop and reproduce within the host, nematodes use a number of strategies to overcome the host immune response. Some nematodes overwhelm the immune response by the sheer number of nematodes infecting a single host (Dowds & Peters, 2002). This is characteristic of *Steinernema* spp. where both males and females have to infect the same insect for bisexual reproduction but applies to hermaphroditic nematodes as well. Nematodes also have more specialised survival strategies, which fall broadly into two categories, namely evasion and suppression (Table 1.1, Figure 1.3).

The goal of immune evasion systems in parasitic nematodes is to avoid recognition by the host, thereby preventing the host immune response from being activated. Parasitic nematodes often prefer younger hosts whose immature immune systems are associated with lower levels of circulating blood cells, less sensitive pathogen recognition and consequently less-virulent immune responses (Gardiner & Strand, 2000; Khafagi & Hegazi, 2004). A more specific evasion strategy is molecular mimicry. Parasites express molecules on the body-surface that mimic the antigens of the host (Bayne et al., 1987; Damian, 1964; Dissous et al., 1990). These molecules serve as camouflage, tricking the pathogen recognition system of the host. Alternatively, with molecular disguise, parasites cover themselves with proteins sequestered from the host haemolymph (Brivio et al., 2005).

The nematode body surface plays an important role in the host-parasite interaction, both as antigenic trigger for the pathogen recognition system and as immune-evasion mechanism (Blaxter et al., 1992; Castillo et al., 2011; Politz & Philipp, 1992). Lipid compounds in the cuticle of *S. feltiae* interact with humoral proteins of the wax moth, *Galleria mellonella* forming a coat around the nematode (Brivio et al., 2004; Dunphy & Webster, 1987; Mastore & Brivio, 2008). This molecular coat disguises *S. feltiae* as "self", preventing haemocyte pathogen recognition proteins (PRP) from binding to the body surface of the nematode and from initiating cellular encapsulation. By depleting the haemolymph of humoral components, the

nematode interferes with the activation of prophenoloxidase and consequently prevents melanisation (Brivio et al., 2002). The presence of nematode cuticular lipids also suppresses the production of AMP when *G. mellonella* is co-infected by *S. feltiae* and *X. nematophila* (Brivio et al., 2006). Overall, *S. feltiae* relies strongly on its cuticle to overcome both humoral and cellular immune mechanisms when infecting *G. mellonella*.

Immunosuppression is the active interference with the host immune response by excreted/secreted molecules intended to damage immunocompetent cells and neutralise humoral factors (Cooper & Eleftherianos, 2016; Simões & Rosa, 1996). Immunosuppressive molecules interfere with the cellular immune response by impairing the production or functioning of surface PRP, by impeding the spreading and adhesive properties of the cells, or by direct cytotoxicity (Brivio et al., 2005; Ribeiro et al., 1999). Specific protein-encoding genes involved in suppression of cellular defence mechanisms have been sequenced. For instance, ScKU4 is a serine protease inhibitor that is up-regulated in *S. carpocapsae* juveniles during invasion and early infection of *G. mellonella*. ScKU4 interacts with PRP thereby interrupting pathogen recognition, the activation and aggregation of blood cells, and ultimately preventing encapsulation (Toubarro *et al*., 2013).

Similar to the protection against host humoral responses that symbiotic bacteria gain from the *S. feltiae* cuticle, *X. nematophila* associating with *S. carpocapsae* are protected from AMP directed at Gram-negative bacteria by immunosuppressive molecules from the nematode (Götz et al., 1981). The quick onset and lethality of melanisation make the proPO-AS a key humoral target for immunosuppression. Several excreted/secreted proteins that inhibit prophenoloxidase or melanisation have been described in *S. carpocapsae*. The chymotrypsin serine protease (ScCHYM) disrupts prophenoloxidase activation *in vitro* and prevents cellular encapsulation and melanisation *in vivo* (Balasubramanian et al., 2009). A trypsin-like serine protease also inhibits prophenoloxidase activity and impairs the ability of haemocytes to spread (Balasubramanian et al., 2010). Mastore et al. (2015) demonstrated how live *S. carpocapsae* nematodes caused a decrease in phenoloxidase activity of the red palm weevil, *Rhynchophorus ferrugineus*. Cold-killed nematodes or nematode cuticles did not have the same inhibitory effect, suggesting that immunosuppressive molecules are actively secreted in contrast to the passive role played by the nematode cuticle.

1.3.2 *BURSAPHELENCHUS XYLOPHILUS*

1.3.2.1 Biology and pest status

The pinewood nematode (PWN), *B. xylophilus*, and pine sawyer beetle, *Monochamus* spp. represent a nematode-insect relationship of particular concern to pine forests and pine growers in the northern hemisphere (Mota & Vieira, 2008). Pinewood nematodes are responsible for pine wilt disease, a fatal infection in Asia and Europe (Mamiya, 1976; Rutherford et al., 1990). This plant-parasitic nematode depends on beetle vectors to reach and infect susceptible *Pinus* spp. (Mamiya & Enda, 1972). Beetles from several genera carry the PWN but association between *B. xylophilus* and *Monochamus* spp. occur most frequently and with the highest mean number of nematodes transmitted per beetle (Aikawa, 2008; Linit et al., 1983; Wingfield & Blanchette, 1983). In the United States, where pine wilt disease is endemic and most trees resist or tolerate the PWN, *M. carolinensis* and *M. scutellatus* are the most important species responsible for nematode transmission (Linit, 1990; Pimentel et al., 2014), compared to *M. alternatus* in Japan and China (H. Li et al., 2007; Matsushita, 2020), and *M. galloprovincialis* in Portugal (Firmino et al., 2017; Sousa et al., 2001).

Adult *M. alternatus* beetles feed on the young branches of healthy trees, enabling PWN to enter the tree through the beetle feeding wounds (primary transmission) (Linit, 1990; Luzzi et al., 1984). As nematodes feed on the epithelial lining of resin canals and fungi around the pupal chambers, oleoresin flow is impeded, causing the tree to wilt. Susceptible trees will start to show symptoms of disease three to four weeks after infection. Stressed or diseased trees and recently cut logs attract mature adult beetles to mate and oviposit, introducing more nematodes into the tree (secondary transmission) (Futai, 2013).

Nematodes locate beetle pupae in the tree by chemotaxis (Linit, 1988). Attractants include terpenes and CO_2 produced by the developing beetles (Zhao et al., 2014). As soon as young adult beetles emerge from the pupa, but before leaving the tree, dauer (dispersal) juveniles climb onto the body surface of the beetle. While some nematodes remain on the beetle body surface, most move to the insect's tracheal system via the spiracles, specifically in the thoracic region (Kobayashi et al., 1984; Linit et al., 1983; Wingfield & Blanchette, 1983).

1.3.2.2 Beetle immune response

Although insect immune defences have mainly been studied in response to insect-parasitic nematodes, Zhou, et al. (2018) described the reaction of the vector to the plant-parasitic nematode *B. xylophilus*. These nematodes cause ROS to accumulate in the epidermis and trachea of the *M. alternatus* beetle. However, within the trachea, higher levels of peroxidase and superoxide dismutase detoxify the ROS, creating a more favourable environment for the nematodes. Up-regulation of Toll receptors are responsible for the increased expression of antioxidative enzymes.

In addition to the vector-pathogen relationship with *B. xylophilus*, *M. alternatus* also plays host to the insect-parasitic nematode, *Howardula phyllotretae*. In contrast to the relative immune tolerance of *Monochamus* beetles to the pinewood nematode, *H. phyllotretae* infection triggers humoral defence mechanisms, especially melanisation, and reduces the beetle's antioxidative capabilities (Zhou et al., 2018). Increased oxidative stress and melanisation inhibit the survival of the PWN in beetles co-infected with *H. phyllotretae* and reduces the lifespan and reproductive potential of the beetle itself. Even though the vector remains alive, *H. phyllotretae* therefore has some role to play in the biological control of pine wilt disease.

1.4 Opportunities to study nematode-insect interactions in the *Deladenus-Sirex* system

The insect-parasitic nematode *D. siricidicola* is an important biological control agent of the woodwasp, *S. noctilio*. Little is known about the host-parasite mechanisms involved in the *Deladenus-Sirex* system, but the relevance of unravelling this interaction and a number of possible approaches for doing so are proposed.

1.4.1 *DELADENUS SIRICIDICOLA* IN THE BIOLOGICAL CONTROL OF *SIREX NOCTILIO*

Pines, especially in the Southern Hemisphere, are threatened by the woodwasp, *S. noctilio* (Carnegie et al., 2006; Haugen & Underdown, 1990; Hurley et al., 2007). In its native range (Europe, Asia and northern Africa), *S. noctilio* is not considered a significant pest (Spradbery

& Kirk, 1978), but accidental introductions throughout the twentieth century are responsible for established *Sirex* infestations in New Zealand and Australia, South America, and South Africa (Hurley et al., 2007).

During oviposition, *S. noctilio* females inject the fungal symbiont *Amylostereum areolatum*, as well as a phytotoxic mucus into susceptible trees (Coutts & Dolezal, 1969; Madden, 1974). Although the fungus is not pathogenic when inoculated in isolation, the combination of fungus and mucus cause severe physiological stress in the tree (Coutts, 1969a; Vaartaja & King, 1964; Wong & Crowden, 1976). Symptoms of *Sirex* infection include yellowing, followed by copper-brown discolouration of the foliage, needle wilting, and visible oviposition tunnels and resin blobs on the bark surface (Coutts, 1969b; Neumann et al., 1987). The large volume of mucus produced could explain the ability of *S. noctilio* to infect and kill healthy trees (Spradbery, 1973, 1977).

Early *S. noctilio* control consisted of cutting down and burning infested trees to remove the source of infection (Neumann et al., 1987). Due to the high cost and low success of these measures, focus has been shifted to biological control, initially in the form of parasitic wasps (Hanson, 1939; Nuttall, 1989). In the early 1960s, however, parasitic nematodes were discovered inside the ovaries and eggs of a *S. noctilio* female in New Zealand (Zondag, 1962, 1969). The nematode was subsequently described as *Deladenus* sp. (Bedding, 1967). Research into the biology of the seven different *Deladenus* species across the distribution of Siricid woodwasps demonstrated the potential of *D. siricidicola* to control *S. noctilio* infestations (Bedding & Akhurst, 1974; Zondag, 1969, 1971, 1979). *Deladenus siricidicola* is now the main biological control agent used against *S. noctilio* in the Southern Hemisphere (Bedding, 2009; Carnegie et al., 2005; Slippers et al., 2015).

1.4.2 BIOLOGY

Deladenus siricidicola nematodes boast adult polymorphism which results in both free-living and parasitic life cycles (Bedding, 1967, 1972) (Figure 1.4). Free-living *D. siricidicola* feed on *A. areolatum*, the symbiotic fungus of *S. noctilio* and other Siricid woodwasps. Juvenile nematodes produced during the free-living, mycetophagous stage develop into adults of either form depending on environmental conditions but free-living cycles may continue indefinitely

(Bedding, 1993; Zondag, 1969). The mycetophagous cycle simplifies mass-rearing for use in biocontrol as the nematodes can be cultured on any medium that supports growth of the fungus (Bedding & Akhurst, 1974). In the wild, the nematode's specificity for *A. areolatum* limits its spread to areas where the wasp occurs.

The high CO_2 levels and low pH surrounding *Sirex* larvae prompt nematode juveniles to develop into infective form adults (Bedding, 1993). The low pH micro-environment can be simulated by allowing nematode eggs to develop on a growth medium containing 0,2 % lactic acid (Bedding, 1984; Mlonyeni et al., 2018). Although Mlonyeni et al. (2018) were unable to reproduce the results under elevated CO_2 conditions alone, Bedding (1972) described increased conversion rates on old plates, possibly due to the accumulation of CO_2 which lowers the pH of the media.

After mating, infective females penetrate the cuticle of *S. noctilio* larvae using a large stylet characteristic of this form (Bedding, 1967, 1972). Entry– or attempted entry wounds become visible as light to dark brown spots on the larval surface as a result of melanisation. Multiple infectives may enter a single female host larva (Zondag, 1962). The female nematode grows rapidly thanks to microvilli on the outer body surface allowing direct absorption of nutrients from the host haemocoel (Riding, 1970). However, development of the nematode reproductive system – including the fertilisation of oocytes – only takes place around the start of host pupation (Bedding, 1972, 1993).

Juvenile nematodes emerge into the host haemocoel a few days after hatching inside the parent and migrate to the reproductive organs of the host (Bedding, 1972; Bedding & Akhurst, 1974). In male hosts, nematodes only reach the testes after spermatozoa have moved to the seminal vesicles, and therefore have no effect on fertility and cannot be transmitted to *Sirex* females during copulation (Bedding, 1972; Zondag, 1962). Conversely, infection with *D. siricidicola* causes sterility in female wasps as the host ovaries and developing eggs are invaded by nematodes (Bedding, 1972). Oviposition remains unaffected, but instead of viable eggs, eggs containing up to 200 juvenile nematodes are injected into susceptible trees (Zondag, 1962). Not only does the female wasp contribute to the spread of its own biocontrol agent, but it also provides the nematodes with a fungal food source by co-injecting symbiotic *A. areolatum* at each oviposit site (Bedding & Akhurst, 1974; Zondag, 1969).

1.4.3 POPULATION VARIATION AND BIOCONTROL SUCCESS

After the discovery of *D. siricidicola* in New Zealand, and studies to determine the most suitable strain in terms of virulence and selectivity, the nematodes have been introduced to *S. noctilio* infested plantations across the non-native range of the woodwasp (Bedding & Akhurst, 1978; Hurley et al., 2007). Unfortunately, not all instances have been equally successful and parasitism rates continue to vary in various areas where it is used for biocontrol, including in South Africa (Hurley et al., 2007, 2008). Various factors likely contribute to the unpredictable success of *D. siricidicola* as biocontrol agent (Slippers et al., 2012). These include environmental conditions, variation in the populations of the wasp, the nematode and the fungus, and interactions between the various factors.

Biocontrol programs of *S. noctilio* in the southern hemisphere all rely on the same strain of *D. siricidicola*, namely the Kamona strain (Hurley et al., 2007). This strain was first collected in the Tasmanian forest, Kamona in 1991 (Bedding & Iede, 2005). It was distributed to Australia to replace the earlier Sopron strain which lost its infectivity towards the end of the 1980s (Haugen & Underdown, 1993), and eventually also to other southern hemisphere countries (Hurley et al., 2007). The introduction of a single nematode strain to different areas, combined with repeated genetic bottle-necks and inbreeding from continuous laboratory rearing, resulted in extreme homozygosity of the southern hemisphere population of *D. siricidicola* (Fitza et al., 2019; Mlonyeni et al., 2011). Lack of genetic diversity might limit the nematode's potential to adapt to different environmental conditions and variations or changes in the population of *S. noctilio* (Dlugosch & Parker, 2008). Despite the low genotypic variation, different isolates of nematodes still perform differently when the ability to convert to infectives or growth on different fungal strains are compared (Mlonyeni et al., 2018).

1.4.4 HOST-PARASITE INTERACTIONS BETWEEN *SIREX* AND *DELADENUS*

Some fluctuation in the virulence of a parasite and resistance to infection of a host are to be expected in biological interactions (Slippers et al., 2012). Continuous sub-culturing may select for nematodes incapable of producing infectives. This has been proposed as an explanation for the *S. noctilio* outbreak of 1987 in Australia (Haugen et al., 1990; Haugen & Underdown, 1990, 1993). In the field, a less infective parasite may become dominant in keeping with the trade-

off hypothesis where killing too many hosts will lead to the collapse of a virulent parasite population (Alizon et al., 2009). This mechanism applies to classical biocontrol systems – and therefore possibly also the *Deladenus-Sirex* interaction – where the target host contributes to the dispersal of the parasite. Evolutionary changes in the immune system of a host could cause variable success of a biocontrol agent. The same applies to changes in the pathogen's immunomodulatory mechanisms or ability to withstand the host immune response.

In contrast to other insect biocontrol systems, the interactions between *D. siricidicola* and the immune response of *S. noctilio* have not been studied. For the time being, knowledge of virulence and immunomodulatory mechanisms in better-studied EPN such as *Steinernema* spp. and *Heterorhabditis* spp., can be used to investigate the immunological aspects of the *Deladenus-Sirex* interaction. For instance, proteases isolated from the excretory-secretory products of other EPN are presumably involved in crossing into the haemocoel and modulating host immune responses such as melanisation and encapsulation (Brivio & Mastore, 2018; Cooper & Eleftherianos, 2016). Although *Deladenus* uses a stylet to enter the host haemocoel and therefore supposedly relies less on proteases, it still has to evade and possibly suppress *Sirex* immune responses such as encapsulation and melanisation inside the haemocoel.

With access to the genome of *D. siricidicola* (Postma et al., 2019), its immunomodulatory mechanisms can be compared with that of other EPN by searching for homologues of the sequenced EPN proteases in the genome of *D. siricidicola*. The polymorphism of *D. siricidicola* adults also provide an opportunity to compare the expression of potential immunomodulatory genes between the free-living and infective life cycles. Differentially expressed genes identified in this manner could shed light on how immune-related genes are switched on and off and predict how the juveniles of mass-cultured free-living nematodes would perform as parasites after release into plantations.

The relatively distant evolutionary relatedness between *D. siricidicola* and other EPN, may limit the extent to which genomic data can be translated from one species to the other. *Deladenus siricidicola* has a closer phylogenetic relationship with plant-parasitic nematodes, including *Bursaphelenchus,* and the insect-parasitic *Howardula phyllotretae* than with *Steinernema* spp. for example (Schoch et al., 2020). This shortcoming can be addressed by including the growing number of genomes of various nematodes of different lifestyles that are

more closely related to either *Deladenus* or *Steinernema* and that can help bridge this evolutionary gap.

1.5 Conclusion

Insects are significant role-players across the many different lifestyles of nematodes – both as vectors to vertebrate- and plant-parasitic nematodes, and as hosts to insect-parasitic nematodes. Entomopathogenic nematodes coevolved with bacterial symbionts to become virulent complexes infecting insect pests (Chapuis et al., 2009). Bacteria kill and digest the host and protect the insect cadaver from invasion by other micro-organisms. Even so, without the nematode the bacteria are without means of dispersal. Entomopathogenic nematodes evade and modulate the host immune response to ensure their own survival and development. In addition, the release of the symbiotic bacteria is timed to only occur after the nematode has reached the host haemocoel and evaded recognition. Several proteins have been isolated and sequenced that appear to be involved in these immune modulatory mechanisms. While the characterisation of these proteins is providing some insight into the molecular mechanisms underlying the host-parasite interactions between insects and nematodes, much clearly remains to be discovered about the process.

The insect-parasitic nematode *D. siricidicola* is particularly well suited to the biological control of the woodwasp, *S. noctilio*. The nematode exhibits both parasitic and free-living cycles. Free-living nematodes feed on the symbiotic fungus of *Sirex*, namely *A. areolatum*. In the lab, *D. siricidicola* can therefore be cultured and mass-reared on any medium that supports growth of the fungus. In the wild, the specificity of *D. siricidicola* for *A. areolatum* limits the spread of the nematode to areas infested by *S. noctilio*. By sterilising instead of killing the insect host, *D. siricidicola* is spread by its very target insect. The female wasp also provides nematode juveniles with a food source when *Amylostereum* is co-injected with egg-containing nematodes during oviposition. These characteristics theoretically make *D. siricidicola* the ideal biocontrol agent but unfortunately, actual biocontrol success is variable.

Several factors that contribute to biocontrol success of *D. siricidicola* have been investigated, including environmental conditions, handling methods and population variation. However, little is known about the interaction between *D. siricidicola* and the immune response of *S.*

noctilio and the impact immunomodulation has on parasite virulence. Despite the differences between *Steinernema* spp. or *Heterorhabditis* spp. and *D. siricidicola* in terms of feeding habits and the outcome of host infection, the immunomodulation mechanisms described in model EPN, and the availability of nematode genomes, provide valuable tools to study the interspecific interactions in other systems, including the *Deladenus*-*Sirex* interaction. For example, the sequences of *Steinernema* immunomodulatory genes can be used as queries to search the *D. siricidicola* genome for genes with similar functions. Insight into the genetics underlying *D. siricidicola* modulation of *S. noctilio* immune response could reveal desirable traits to be selected for during breeding. At the very least, it will contribute to a better understanding of the biology and evolution of the highly specific *Deladenus*-*Sirex* interaction.

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Chapter 1. Tables

Table 1.1 Immunomodulatory strategies used by entomopathogenic nematodes to overcome the host immune response

Characteristic	Immune evasion	Immune suppression
Outcome	Recognition by host immune system is avoided (preventing immune activation)	Host immune responses are suppressed (following immune activation)
Mechanism	– Molecular mimicry: pathogen expresses molecules on its body surface that mimic host antigens – Molecular disguise: pathogen covers itself with circulating host proteins	Secreted/excreted products
Effect on cellular immune responses	(Not activated)	– Impaired PRP production and function – Impaired cell spreading and -adhesion – Direct cytotoxicity
Effect on humoral immune responses	Depletion of humoral proteins prevents activation of prophenoloxidase	– Protection of symbiotic bacteria against AMP – Reduced prophenoloxidase activity and subsequent melanisation
Example	*S. feltiae* infection of *G. mellonella* (Brivio et al., 2006)	*S. carpocapsae* infection of *R. ferrugineus* (Mastore et al., 2015)

AMP – antimicrobial peptides; PRP – pathogen recognition proteins

Chapter 1. Figures

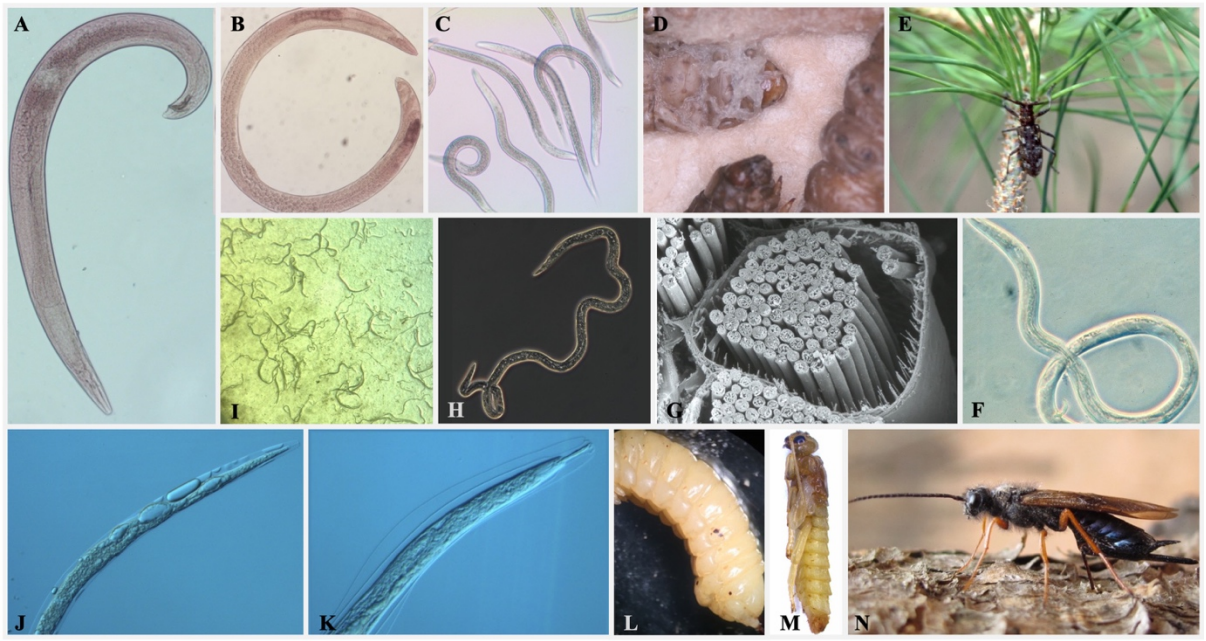

Figure 1.1 Parasitic nematodes often rely on insects, either as hosts, or as vectors to reach the target host. **A –** *Steinernema bertusi* (adult male); **B –** *Steinernema bertusi* (adult female); **C –** *Heterorhabditis zealandica* (infective juveniles); **D –** *Steinernema fabii* (infective juveniles) exiting the cadavers of *Galleria mellonella* larvae [Image A-D, courtesy of Agil Katumanyane]; **E –** *Monochamus carolinensis* beetle feeding on young branches of *Pinus* sp. (maturation feeding); **F –** *Bursaphelenchus xylophilus* (adult), the pinewood nematode; **G –** SEM of *B. xylophilus* within the tracheal branch of a beetle [Image E-G, courtesy of Michael J. Wingfield]; **H –** *Deladenus siricidicola* (free-living adult); **I –** *Deladenus siricidicola* (free-living) on culture medium **J –** Head of *D. siricidicola* (free-living) adult female; **K –** Head of *D. siricidicola* (parasitic) adult female showing well-developed stylet [Image H, J, K, courtesy of X. Osmond Mlonyeni]; **L –** *Sirex noctilio* larva with penetration/attempted penetration wounds (black spots); **M –** *Sirex noctilio* pupa [Image M, courtesy of Leandri Klynsmith]; **N –** *Sirex noctilio* (adult female) [Image N, courtesy of Katrin N.E. Fitza]

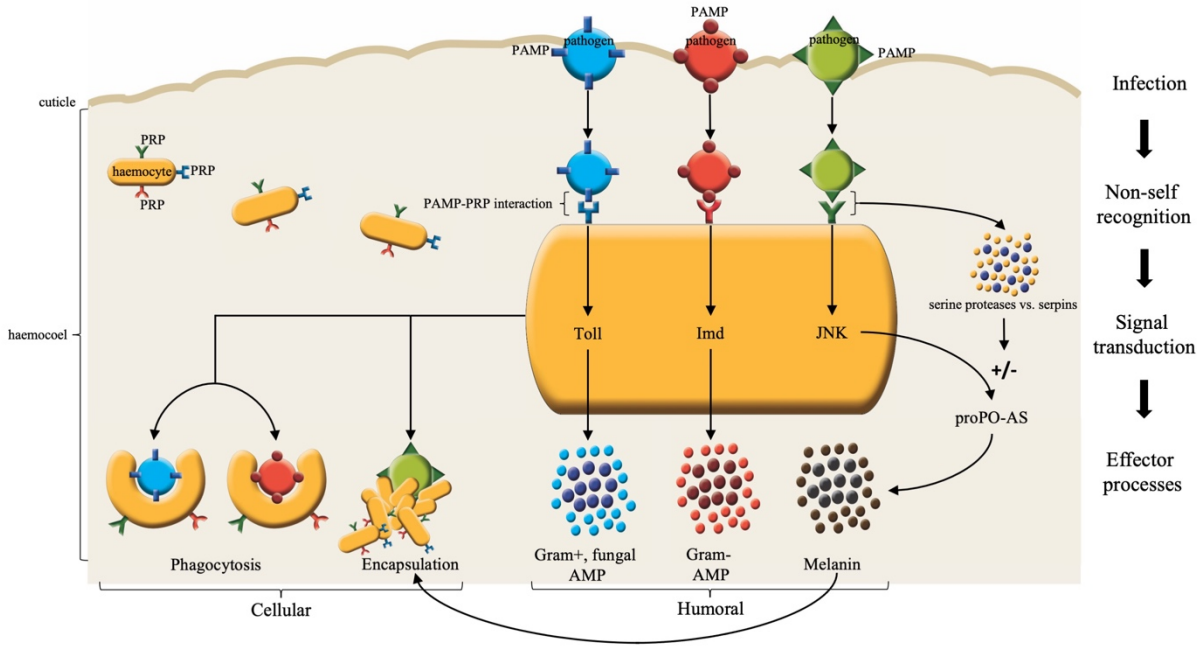

Figure 1.2 The innate immune response of insects. Pathogens overcome the first line of defence (exoskeleton/cuticle/epidermis) to cause infection. Pattern recognition proteins (PRP) interacting with pathogen-associated molecular patterns (PAMP) detect infection. Non-self-recognition activates haemocytes responsible for the cellular immune responses, phagocytosis and encapsulation. Simultaneously, intracellular signal transduction pathways are triggered leading to the production of antimicrobial peptides (AMP) and prophenoloxidase (proPO). Serine proteases and serine protease inhibitors (serpins) control the extent of proPO activation. The end-product of the proPO activating system (proPO-AS) is melanin which is incorporated into the cellular capsule.

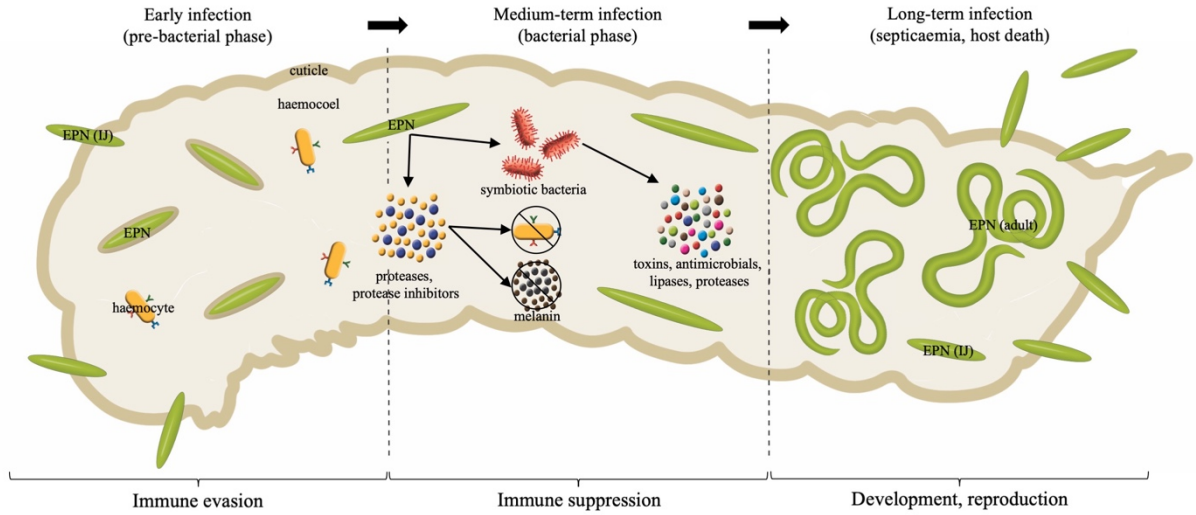

Figure 1.3 Immunomodulation strategies of entomopathogenic nematodes (EPN). **Immune evasion –** Infective juveniles (IJ) enter the host through the mouth, anus or cuticle. The EPN covers its body surface with molecules mimicking host antigens or sequestered from the host haemolymph. The host's pathogen recognition system fails to identify the nematode as non-self and no immune response is activated. **Immune suppression –** EPN release symbiotic bacteria and immunosuppressive molecules. The excreted/secreted products of EPN contain proteases and protease inhibitors, which impair the function of haemocytes and pathogen recognition proteins (PRP), and reduce prophenoloxidase activity and subsequent melanisation. **Nematode development, reproduction –** Toxins, antimicrobials and enzymes released by symbiotic bacteria contribute to killing the insect host and digesting host tissues to create a suitable environment for nematode maturation. The next generation of IJ exit the depleted cadaver in search of a new host. (Adapted from: Brivio & Mastore, 2018)

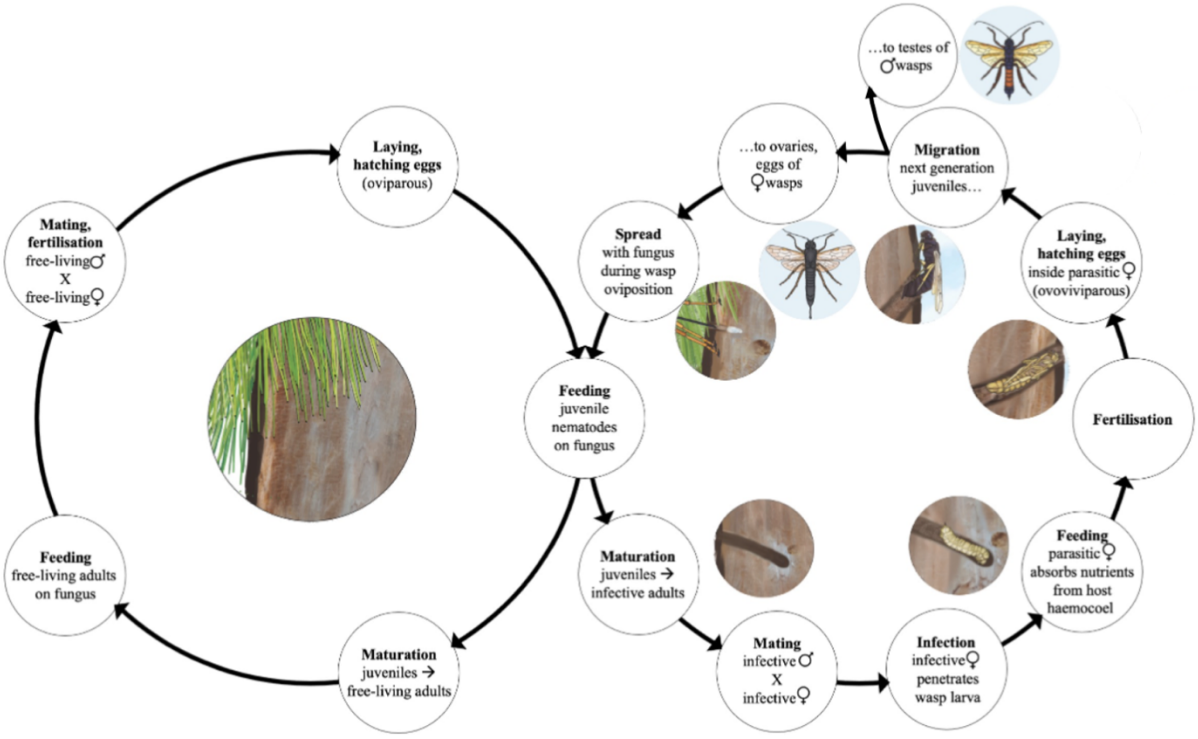

Figure 1.4 The bicyclic life style of *D. siricidicola*. **Free-living cycle (left) –** Nematodes feed on *A. areolatum*, the symbiotic fungus of *S. noctilio*. Feeding, maturation and reproduction take place within the host tree. **Parasitic cycle (right) –** Juvenile nematodes develop into infective form adults in response to the high CO_2 levels and a low pH micro-environment in the vicinity of *Sirex* larvae. Infective males and females mate before females penetrate the wasp larvae. Absorption of nutrients, fertilisation and hatching of eggs take place within the host. As the adult wasp leaves the tree, next generation juvenile nematodes migrate to the reproductive organs of the host. Infection of male wasps represents a "dead-end" for *D. siricidicola* as male wasps do not spread the nematode. Conversely, female wasps are sterilised by the nematode but continues oviposition of the fungus and juvenile nematodes into new host trees.

Chapter 2.

Identification and characterisation of *Deladenus siricidicola* genes potentially associated with parasitism and immunomodulation in the infection of *Sirex noctilio*

Abstract

Entomopathogenic nematodes (EPN) are parasites that infect and kill insects and are therefore valuable for use as biological control agents against a variety of insect pests. To survive within the insect, EPN must overcome a robust host immune response. Mechanisms enabling the nematode to evade or suppress the host immune response ensure the success of EPN as parasites and biocontrol agents. In this study, I explored genome and transcriptome data of *Deladenus siricidicola* – the biocontrol nematode of the invasive woodwasp, *Sirex noctilio* – for genes and gene families potentially involved in the host-parasite interaction. I used sequence similarity of parasitism and immunomodulatory genes characterised in model EPN *Steinernema* spp. to guide our analysis of the genome. Transcriptome data from free-living *D. siricidicola* as well as nematode-exposed *S. noctilio* larvae were used to determine the expression profile of the putative parasitism and immunomodulation genes. With the use of local BLAST searches, protein clustering, and hidden Markov model (HMM) searches, I identified 23 *D. siricidicola* genes that are potentially involved in the infection process in *S. noctilio*. Based on comparison with functional databases, the majority of these genes are involved in proteolysis, but regulation of endopeptidase activity and glycolysis are additional biological processes implicated in manipulation of the host immune response. All 23 genes were expressed in free-living nematodes. During infection of *S. noctilio* larvae, only seven genes had detectable transcript levels, which could be explained by the low recovery rate of nematode RNA from the nematode-exposed woodwasp larvae. This is the first attempt to characterise the genes underlying infection and immunomodulation processes in *D. siricidicola*. While this study is preliminary in nature, it provides a valuable foundation for future characterisation of infection and immunomodulation processes that might be considered in selection in this biological control agent.

Keywords – biocontrol agent, *Deladenus siricidicola*, entomopathogenic nematode, immunomodulation, immunosuppression, insect immune response, parasitism, *Sirex noctilio*

2.1 Introduction

Entomopathogenic nematodes (EPN) are parasitic nematodes that kill, sterilise or reduce the overall fitness of their insect hosts (Lewis & Clarke, 2012; Torres-Barragan et al., 2011; Zhang et al., 2008). As a result, EPN are used in the biological control of insect pests and provide an alternative to costly and potentially hazardous chemical pesticides (Gaugler, 2002; Noosidum et al., 2021; Salari et al., 2021). Species from the genera *Steinernema* and *Heterorhabditis* are the most widely used biocontrol nematodes (Brivio et al., 2005; Cooper & Eleftherianos, 2016; Kaya & Gaugler, 1993).

When EPN infect a host, they encounter a robust host immune response. Pattern recognition proteins (PRP) associated with the host blood cells interact with proteins or carbohydrate motifs on the surface of the nematode to alert the host immune system of foreign invasion (Castillo et al., 2011; Kim et al., 2008; Müller et al., 2008). By binding to the pathogen-associated molecular patterns (PAMP) on the surface of nematodes (and other pathogens), PRP trigger intracellular signal transduction pathways that elicit the desired immune response (Pal & Wu, 2009).

The insect's innate immune responses can be categorised into cellular – involving host blood cells (haemocytes) – and humoral mechanisms – consisting of molecules circulating in the haemolymph (Brivio & Mastore, 2018; Meister & Lagueux, 2003). The cellular response to nematode infection entails encapsulation where flattened haemocytes adhere to the nematode surface forming a multilayer capsule (Brivio & Mastore, 2018; Mastore & Brivio, 2008; Strand, 2008). The encapsulated nematode dies from asphyxiation and from harmful compounds being released into the capsule (Nappi & Christensen, 2005). Molecules of the humoral immune response include antimicrobial peptides, digestive enzymes and melanin (Brivio & Mastore, 2018).

The prophenoloxidase activating system (proPO-AS), or melanisation, is a key component of the innate immune response in insects as it is efficient and of quick onset (Brivio et al., 1996; Cerenius et al., 2008; Dimopoulos et al., 2001). The proPO-AS is a highly reactive enzymatic cascade that leads to the production of melanin. Pathogen recognition, mechanical wounding and defective cells trigger an intracellular signalling pathway which prompts haemocytes to

secrete the zymogen, prophenoloxidase (proPO) into the haemolymph (Bidla et al., 2007; Brennan & Anderson, 2004; Bulet et al., 1999; Galko & Krasnow, 2004). Prophenoloxidase is cleaved into active phenoloxidase by serine proteases (Cerenius et al., 2008). In turn, serine protease inhibitors control the extent and duration of action of serine proteases (Kanost et al., 2004; Ligoxygakis et al., 2002; Nappi et al., 2005). Melanin has a direct toxic effect on pathogens but is also incorporated into the cellular capsule around larger invaders ("humoral encapsulation"). The melanisation cascade further produces chemically active by-products such as quinones and reactive oxygen species to counter the invasion (Kanost & Gorman, 2008; Zhao et al., 2007).

Entomopathogenic nematodes have to overcome the host immune responses to be successful parasites and biocontrol agents (Li et al., 2007). The strategies that EPN employ to survive within the host are an important part of the host-parasite interaction and continue to be the subject of investigation in model species such as *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* (Bai et al., 2013; Blaxter et al., 1998; Kenney et al., 2021; Lu et al., 2016).

Nematodes can overwhelm the insect immune system by infecting the host in large numbers (Dowds & Peters, 2002; Simões et al., 2000). More specialised strategies of modulating the host immune response involve immune evasion and immune suppression. Parasitic nematodes evade the host immune response by covering their body surface with molecules either resembling the antigens of the host (molecular mimicry) or sequestered from the host haemolymph (molecular disguise). The host's pathogen recognition system fails to detect the nematodes as non-self, consequently, no immune response is activated (Bayne et al., 1987; Brivio et al., 2005; Damian, 1964; Dissous et al., 1990). Alternatively, parasitic nematodes actively excrete/secrete molecules which damage immunocompetent cells and neutralise humoral factors as part of immune suppression (Cooper & Eleftherianos, 2016; Simões & Rosa, 1996).

Several molecules presumably involved in immune suppression have been characterised in *Steinernema* spp. (Balasubramanian et al., 2009, 2010; Toubarro, Avila, Hao, et al., 2013; Toubarro, Avila, Montiel, et al., 2013). These molecules are mostly proteases except for two protease inhibitors and a glycolytic enzyme. Genes encoding the immunosuppressive molecules are either upregulated during the initial parasitic stage of the nematode, or only

expressed during the parasitic stage. The molecules typically target humoral encapsulation by impairing the activity of haemocytes, decreasing proPO/PO activity, and by preventing the incorporation of melanin into the haemocyte capsule.

The nematode *Deladenus siricidicola* is the main biological control agent of the woodwasp *Sirex noctilio* (Bedding, 2009; Carnegie et al., 2005; Slippers et al., 2015). *Sirex noctilio* with its symbiotic fungus, *Amylostereum areolatum*, cause extensive damage in pine plantations, especially in the Southern Hemisphere (Carnegie et al., 2006; Haugen & Underdown, 1990; Hurley et al., 2007). In contrast to other insect biocontrol systems using EPN (in particular *Steinernema*), the interactions between *D. siricidicola* and *S. noctilio* remain to be studied at the molecular level.

In this study, I explored the recently published genome of *D. siricidicola* (Postma et al., 2019) for genes and gene families potentially involved in parasitism of *S. noctilio* with a specific focus on those involved in the modulation of the host immune response. This was achieved by performing sequence similarity searches of known virulence genes in *Steinernema* and other nematodes against the genome of *D. siricidicola.* Furthermore, I used transcriptome data from both free-living nematodes and an early infection of *S. noctilio* larvae by *D. siricidicola* to determine the expression of the genes of interest.

2.2 Materials and methods

2.2.1 GENE IDENTIFICATION

The sequences of twelve genes previously described as being involved in parasitism and immunomodulation by EPN (Table 2.1, supplementary File S2.1) were used as queries to find orthologues in the genome of *D. siricidicola*. Local BLAST searches, protein clustering, and hidden Markov model (HMM) searches were applied to identify homologues of these genes in *D. siricidicola*.

2.2.1.1 Local BLAST

For basic sequence comparison and database searching, local BLAST searches (Altschul et al., 1990) were performed. Local BLAST databases were constructed from the genome and proteome of *D. siricidicola* on CLC Main Workbench (QIAGEN, Aarhus, Denmark). BLASTn (cDNA to DNA database), tBLASTx (translated cDNA to translated DNA database), BLASTx (translated cDNA to protein database), BLASTp (protein sequence to protein database) and tBLASTn (protein sequence to translated DNA database) were performed using either nucleotide or amino acid sequences from the reference genes as queries against the appropriate databases.

The default BLAST parameters in CLC Main Workbench and a low complexity filter were used for all BLAST searches. In the case of tBLASTx and tBLASTn, the standard genetic code was used for translation. A word size of 3 and the BLOSUM62 matrix were used for all protein-related searches (tBLASTx, BLASTx, tBLASTn, BLASTp). Gaps were assigned a cost of 11 for existing and 1 for extending in the case of BLASTx, tBLASTn and BLASTp. For BLASTn, word size was 11. Matches scored 2 each and mismatches -3. The cost of gap existence and extension was 5 and 2, respectively, for BLASTn.

In cases where the best result was a genomic region instead of a gene ID (produced by BLASTn, tBLASTx or tBLASTn), the relevant scaffold was exported and submitted for online gene prediction with AUGUSTUS version 3.3.3 (Stanke et al., 2008; http://bioinf.uni-greifswald.de/augustus/submission.php). *Caenorhabditis elegans* was selected as model organism with genes to be reported on both strands and without allowing alternative transcripts. The predicted amino acid sequence spanning the scaffold region in question was validated and curated as described in section 2.2.1.4.

2.2.1.2 Protein clustering using OrthoFinder

To find all possible orthologues of each query sequence in the genome of *D. siricidicola*, the program OrthoFinder version 2.2.0 (Emms & Kelly, 2015) was used. The gene sets of *D. siricidicola* and 16 other nematode species were used as input data for the analysis. These nematodes were selected to represent different nematode families and lifestyles (Table 2.2). The amino acid sequences for each nematode were obtained from WormBase ParaSite (Howe et al., 2017; https://parasite.wormbase.org/index.html) in FASTA format (supplementary File

S2.2). Orthology clustering was carried out on the 17 FASTA files (one file per nematode species) using the default parameters (see supplementary File S2.3 for OrthoFinder commands). For each query sequence the following was recorded: orthogroup the query sequence was assigned to, *D. siricidicola* genes assigned to the same orthogroup (if any), the orthologues in the species pair of interest (*S. carpocapsae-D. siricidicola* and *S. glaseri-D. siricidicola*).

2.2.1.3 HMMER searches

Hidden Markov models were used to expand the search for serine proteases and serpins beyond those identified with local BLAST searches and protein clustering. Two searches were performed on the Identical Protein Groups database of GenBank (Benson et al., 2013; https://www.ncbi.nlm.nih.gov/genbank/) using the search terms "serine protease nematodes" and "serine protease inhibitor nematodes". Less stringent parameters were used for initial sequence collection, followed by more stringent approaches to filter the results. The sequences obtained for each of these searches were downloaded and aligned separately using an online version of MAFFT version 7 (Katoh et al., 2019; https://mafft.cbrc.jp/alignment/server/) with default parameters. Two HMMs were built from the alignments using the hmmbuild function of the HMMER package version 3.1b2 (Eddy, 2011; http://hmmer.org/) (see supplementary File S2.3 for HMMER commands). These HMMs were then used to search the *D. siricidicola* proteome for possible serine proteases and serine protease inhibitors with HMMER's hmmsearch function; recognizing that not all genes identified in this manner necessarily indicate orthology, which would require further verification. The default inclusion threshold of E-value < 0,01 per sequence and per domain was applied.

2.2.1.4 Validation and manual curation

The *D. siricidicola* genes identified using the above-mentioned approaches were manually curated on a web-based version of Apollo (Dunn et al., 2019; http://apollo.bi.up.ac.za:8080/webapollo/annotator/index). RNA-seq reads were used as supporting evidence where available. Each *D. siricidicola* sequence was aligned with its query sequence and/or orthologues on MAFFT online and in MEGAX version 10.1.8 (Kumar et al., 2018; Stecher et al., 2020). Changes to the original gene models were evaluated by performing BLASTp searches on the NCBI non-redundant database

(https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm that the curated *D. siricidicola* genes contained the same conserved domain as the query sequence.

2.2.2 GENE CHARACTERISATION

2.2.2.1 Functional analysis

The software InterProScan version 5.29 was used to search the InterPro database (Blum et al., 2021; https://www.ebi.ac.uk/interpro/) for protein domains, superfamilies and gene ontology (GO) terms associated with the amino acid sequences of the curated *D. siricidicola* genes (see supplementary File S2.3 for InterProScan scripts). InterPro combines protein signatures from several member databases into a single resource. The following member databases were used in our analysis: CATH-Gene3D, PANTHER, Pfam, PROSITE profiles, SMART and SUPERFAMILY.

2.2.2.2 Phylogenetic analysis

Sequence selection – BLASTp with default settings was performed with *D. siricidicola* aspartic proteases, enolase, serine proteases, and serine protease inhibitors, respectively, against the NCBI non-redundant protein database. The top 200 hits according to percent identity were selected for each set, except for enolase where there were only 100 hits.

Multiple sequence alignment (MSA) – The resulting BLAST hit sequences were downloaded and aligned with the relevant sequences from *D. siricidicola* and *Steinernema* spp. using the online version of MAFFT with default settings.

Phylogenetic trees – A maximum likelihood (ML) analysis was conducted on each MSA separately using IQ-TREE version 1.6.12 available on the web server (Nguyen et al., 2015; http://iqtree.cibiv.univie.ac.at/). The best substitution model was automatically selected in IQ-TREE by making use of ModelFinder (Kalyaanamoorthy et al., 2017) and the ultrafast bootstrap analysis was used to evaluate branch support (Hoang et al., 2018). The best ML tree for each gene family was midpoint rooted and annotated in FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/).

2.2.3 GENE EXPRESSION

2.2.3.1 Free-living stage *D. siricidicola*

Nematode culture – Two different strains of *D. siricidicola*, SA757b(2013) and SA1109(2019), were cultured on *A. areolatum* strain SA14(2013) (CMW number 46043) on malt extract agar (MEA) plates for 14 days. The two different nematode strains were selected to represent nematodes isolated several years apart (2013 and 2019, respectively) in order to determine whether continuous subculturing has an effect on the expression of putative parasitism genes. Genetic changes associated with selection for free-living nematodes during continuous subculturing were proposed to be the cause of the reduced parasitism success of *D. siricidicola* in the 1980's (Haugen & Underdown, 1993). Nematodes were washed off the MEA plates with sterile water and transferred to microcentrifuge tubes. Six plates of each nematode strain were used, constituting six technical replicates per strain. Tubes were centrifuged and excess water removed by pipetting

RNA extraction and sequencing – RNA was extracted from the nematodes using the RNA Purification from Tissue Protocol of the NucleoSpin® RNA XS kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). RNA was eluted in 16 µl of RNase-free water. The concentration and quality of the RNA were evaluated with spectrophotometry and gel electrophoresis. RNA sequencing was performed on the Illumina Hiseq platform at the Agricultural Research Council Biotechnology Platform, South Africa to generate paired end reads of 151bp. Trimming (eight bases from the start of the read) and filtering (of reads shorter than 80 bases) were performed with Trimmomatic version 0.36 (Bolger et al., 2014) (see supplementary File S2.3 for Trimmomatic script).

2.2.3.2 Parasitic stage *D. siricidicola* nematodes

Nematode culture – *Deladenus siricidicola* nematodes of strain SA107(2013) were cultured in three 500 ml Erlenmeyer flasks containing a growth medium of wheat and brown rice pre-inoculated with *A. areolatum* as described by Bedding & Akhurst (1974). Ten *S. noctilio* larvae were added to each flask after six to eight weeks of incubation and removed following 72 hours of nematode exposure.

RNA extraction and sequencing – Total RNA was extracted from the nematode-exposed *S. noctilio* larvae using the TRIzol reagent (Invitrogen, California, USA) and resuspended in 50 µl RNase-free water. Three technical replicates were performed for each flask of larvae exposed to nematodes. Spectrophotometry was used to measure RNA concentration and Bioanalyzer was used to evaluate RNA integrity. Paired end Illumina sequencing was performed at the Beijing Genomics Institute (BGI) on the Illumina HiSeq platform. Paired end reads of 49 bp were generated. Trimming (3 bases from the start of the read and 3 bases from the end) were performed with Trimmomatic.

2.2.3.3 RNA-seq data analysis

Quality of the RNA-seq data was assessed with FastQC version 0.11.7 (Andrews, 2010) and quality control reports generated with MultiQC version 1.6 (Ewels et al., 2016). HISAT2 version 2.1.0 (Kim et al., 2015) was used to map RNA-seq reads to the genome of *D. siricidicola* with default parameters. Counting of mapped reads was performed with featureCounts (Liao et al., 2014). StringTie version 1.3.4 (Pertea et al., 2015) was used for transcript assembly and quantification. The R package, DESeq2 (Love et al., 2014) was used to identify differentially expressed genes between the two free-living *D. siricidicola* strains (isolated in 2013 and 2019, respectively). Genes with an adjusted p-value < 0,05 were classified as being significantly differentially expressed. Scripts for the bioinformatics analyses are available in supplementary File S2.3.

2.3 Results

2.3.1 GENE IDENTIFICATION

2.3.1.1 Local BLAST

BLAST searches with the 12 EPN parasitism/immunomodulation sequences from literature against the genome and proteome of *D. siricidicola* identified 12 *D. siricidicola* genes (Table 2.3). For most query sequences, BLASTp and BLASTx produced the same best result. For ScKU4, however, tBLASTx produced the best hit with the result from BLASTp and BLASTx having the second highest bit score. For one of the query sequences (ScSP1), two genes were identified, one with BLASTp and another with BLASTx. As the bit scores were comparable, both gene IDs were included for further analysis.

2.3.1.2 Protein clustering

OrthoFinder assigned two thirds (66,2 %) of the 381 773 proteins from the 17 nematode species into 17 404 orthogroups of two or more genes, while a third of genes remained unassigned. The 12 *Steinernema* genes known to be involved in parasitism and immunomodulation were assigned to eight different orthogroups, five of which also contained genes from *D. siricidicola* (Table 2.4). Four of these orthogroups contained *Steinernema* query genes and *D. siricidicola* genes that are orthologues of one another, while the *S. carpocapsae* query gene (ScAST) in the fifth group (OG0000066) did not have any *D. siricidicola* orthologues (Table 2.5). The OrthoFinder analysis confirmed the orthology of four of the *D. siricidicola* local BLAST hits, namely DSIR_07099 for ScASP110 and ScASP155, DSIR_04158 for ScASP113, DSIR_03412 for ScSP1, and DSIR_00839 for SgENOL. It also identified six *D. siricidicola* proteins that were not detected with local BLAST.

2.3.1.3 HMMER searches

Keyword searches for serine proteases and serine protease inhibitors in nematodes on the GenBank Identical Protein Groups database yielded 255 and 125 matched sequences, respectively (supplementary Table S2.1 and Table S2.2). The two HMMs built from these sequences detected ten serine proteases and five serine protease inhibitors, respectively, in the *D. siricidicola* proteome (Table 2.6 and Table 2.7). Four of these serine proteases, but none of the serine protease inhibitors had been detected by local BLAST and/or OrthoFinder analyses.

2.3.1.4 Validation and manual curation

In total, 27 *D. siricidicola* genes possibly involved in parasitism and immunomodulation were identified by local BLAST, protein clustering and HMM searches (Table 2.8). Manual curation of all predicted gene models was performed and included the addition and/or removal of exons and adjustment to gene and intron-exon boundaries (Table 2.9). Four pairs of genes were merged into four combined gene models. This brought the number of *D. siricidicola* genes potentially involved in immunomodulation and parasitism to 23. The amino acid sequences of the curated parasitism genes can be found in supplementary Table S2.3 and File S2.4.

2.3.2 GENE CHARACTERISATION

2.3.2.1 Functional analysis

Each of the 23 genes was assigned at least one GO term and contained at least one conserved domain allowing its classification into a gene family/superfamily. By cross-referencing GO and InterPro entries, additional GO terms could be derived so that most genes are described in terms of at least biological process and molecular function (Table 2.10, supplementary Table S2.4).

Most genes (15 of 23) contribute to the biological process proteolysis (supplementary Figure S2.1). Of these, eight exhibit serine-type endopeptidase activity as molecular function, six aspartic-type endopeptidase activity, and one metallo-endopeptidase activity together with zinc ion binding (supplementary Figure S2.2). Gene families and protein domains associated with proteolysis are the peptidase S1 PA clan with a serine protease trypsin domain, the aspartic peptidase A1 family with a peptidase family A1 domain, and the metallopeptidase catalytic domain superfamily containing peptidase M12A, peptidase metallopeptidase, and ShKT domains.

Two genes – DSIR_04361 and DSIR_13362 – are predicted to be capable of serine-type endopeptidase inhibitor activity. Although no biological process was specified, the GO ancestor chart for serine-type endopeptidase inhibitor activity shows that it forms part of the biological process, negative regulation of endopeptidase activity. Both DSIR_04361 and DSIR_13362 contain pancreatic trypsin inhibitor Kunitz domains, confirming their homology to the Kunitz-type serpin in *S. carpocapsae*, ScKU4. Only the cellular component, extracellular space was specified for DSIR_00450, a homologue of the *S. carpocapsae* serpin ScSRP (supplementary Figure S2.3). However, given the presence of a serpin domain in this gene, its serine-type endopeptidase inhibitor activity and contribution to endopeptidase regulation are assumed, as is the extracellular site of action for the two pancreatic trypsin inhibitors.

The genes detected with the serpin HMM – DSIR_05889, DSIR_05892, DSIR_13365, and DSIR_10164 – are capable of protein binding. Each of these four genes contain one or more Kazal domains. The biological function and cellular component were not specified for these genes. In addition to protein binding, DSIR_10164 is capable of calcium ion, collagen, and extracellular matrix binding, contributing to the biological process, signal transduction. This

gene belongs to the "secreted protein acidic rich in cysteine" (SPARC) family with conserved domains involved in calcium binding such as SPARC/Testican calcium binding and EF-hand. InterPro lists the collagen-containing extracellular matrix as cellular component associated with SPARC family genes and confirms their calcium and collagen binding capability.

The third biological process in the gene set is the glycolytic process, which was assigned to DSIR_00839, orthologue of the *S. glaseri* enolase, SgENOL. The molecular functions assigned to DSIR_00839 are phosphopyruvate hydratase activity – also known as enolase – and magnesium ion binding. Enolase forms part of an intracellular structure, the phosphopyruvate hydratase complex. Not surprisingly, DSIR_00839 belongs to the enolase family containing domains such as enolase N-terminal and enolase C-terminal TIM barrel.

2.3.2.2 Phylogenetic analysis

Enolases – The BLASTp search with *D. siricidicola* enolase, DSIR_00839, produced 100 hits on the NCBI database. These sequences share a high sequence similarity with DSIR_00839 with percent identity ranging from 74,39 % to 85,25 % and all matches having an E-value of 0,00. The phylogenetic analysis and subsequent ML enolase tree constructed with IQ-TREE consisted of DSIR_00839, SgENOL, and the 100 sequences from NCBI (Figure 2.1). Homologues of DSIR_00839 were found in a variety of phyla other than Nematoda, including Chordata, Cnidaria, Echinodermata, and Arthropoda.

Aspartic peptidases – The six *D. siricidicola* aspartic peptidases had a total of 242 unique BLASTp matches with percent identity from 28,75 % to 56,82 % and E-values from 6,02E-125 to 5,00E-03. The phylogenetic analysis of aspartic peptidases consisted of 200 NCBI aspartic peptidases, six *D. siricidicola*, and three *S. carpocapsae* aspartic peptidases (supplementary Figure S2.4). The phylogenetic distribution of the *D. siricidicola* aspartic peptidases were in keeping with the orthology inferred by OrthoFinder with DSIR_10876 closely related to ScASP110, and ScASP113 and ScASP155 clustering together and in the same clade as the remaining *D. siricidicola* aspartic peptidases.

Serine proteases – 701 unique serine proteases with sequence similarity to the eight *D. siricidicola* serine proteases were found on NCBI. Percent identity ranged from 22,08 % to 62,63 % and E-value from 7,44E-166 to 4,90E-02. The phylogenetic analysis of serine

proteases consisted of 200 NCBI serine proteases, eight *D. siricidicola*, and six *S. carpocapsae* serine proteases (supplementary Figure S2.5). The six *S. carpocapsae* serine proteases formed a cluster within the same clade as seven of the *D. siricidicola* serine proteases and serine proteases from a number of other nematode species. DSIR_03388 fell in its own clade consisting of sequences representing organisms from diverse phyla.

Serine protease inhibitors – The serpin DSIR_00450, Kunitz-type serine protease inhibitors DSIR_04361 and DSIR_13362, and four Kazal-type serine protease inhibitor genes were all used in the serine protease inhibitor phylogenetic analysis. These seven *D. siricidicola* sequences had 628 unique BLASTp matches on NCBI with percent identity between 19,25 % and 89,62 %, and E-values between 9,37E-148 and 4,9E-02. The seven *D. siricidicola*, two *S. carpocapsae*, and 200 NCBI protease inhibitors were included in the analysis (supplementary Figure S2.6). Surprisingly, the two Kunitz-type serine protease inhibitors detected in *D. siricidicola* were not grouped together but at the bases of two separate clades, namely DSIR_13362 in the clade containing the Kunitz-type inhibitor from *S. carpocapsae* (ScKU4) and a *D. siricidicola* Kazal-type serine protease inhibitor (DSIR_05892), and DSIR_04361 in the clade with two other *D. siricidicola* Kazal-type inhibitors (DSIR_05889 and DSIR_13365). The serpin domain containing DSIR_00450 were the closest relative to its *S. carpocapsae* homologue, ScSRP6.

2.3.3 GENE EXPRESSION

2.3.3.1 Free-living stage *D. siricidicola*

Three of the 2013 RNA samples and four of 2019 samples passed quality control and were subsequently sequenced. The number of reads ranged between 17 and 43 million reads per sample (average 28,2 million). The average GC content across samples was 50 % (Table 2.11, supplementary File S2.5).

2.3.3.2 Parasitic stage *D. siricidicola*

The RNA sequences from *S. noctilio* larvae parasitised by *D. siricidicola* were between 33 and 34 million reads per sample. The average GC content across samples were 42 % (Table 2.12, supplementary File S2.5).

2.3.3.3 RNA-seq data analysis

The average proportion of sequenced reads aligning with the reference genome of *D. siricidicola* was 91 % for RNA obtained from free-living nematodes with reads from the 2013 samples mapping slightly better than the 2019 samples (mean of 94 % and 89 %, respectively) (Figure 2.2 and Figure 2.3, supplementary File S2.5). For RNA obtained from nematode-infected larvae, 2 % of reads from one of the samples aligned to the nematode genome, but only 0,02 % of reads from the other two samples aligned to the genome.

All 23 identified genes were expressed during the free-living life stage of *D. siricidicola* (Table 2.13). Based on the mean transcripts per million (TPM) values for each strain, aspartic peptidase DSIR_04158 was the most abundant of the set, followed by enolase DSIR_00839 in both the 2013 and 2019 strains. Astacin-like metallopeptidase DSIR_00361 was the gene with the lowest average TPM in the 2019 strain. In the 2013 strain, one of the serine proteases DSIR_13363 had the lowest expression.

Regarding expression during parasitism of *S. noctilio* larvae, seven of the 23 identified genes had TPM values > 0 in the sample with 2 % alignment to the *D. siricidicola* genome (sample N1). In one of the samples with 0,02 % mapping (sample N3), three aspartic peptidases had detectable expression levels, one of which (DSIR_04158) was also expressed in sample N1, but at a much lower level (4 TPM in N1 compared with 611 in N3). None of the genes appeared to be expressed in the remaining parasitic sample and this sample was therefore excluded from further calculations (sample N2). Overall, DSIR_04158 had the highest transcript level of the gene set during the parasitic stage, followed by another aspartic peptidase, DSIR_07099 and enolase DSIR_00839 in third place. Of the detectably expressed genes, aspartic peptidase DSIR_13366 transcripts were the least abundant, followed by serine protease DSIR_08439.

Considering the expression of each gene family as a whole, the aspartic peptidases remained the most expressed in both free-living strains and the parasitic stage, followed by enolase (Table 2.14). The metallopeptidase, DSIR_00361 was the least expressed across strains/stages. In free-living nematodes, serine protease transcripts were more abundant than those of the serpin and Kunitz serine protease inhibitors, but less expressed than Kazal domain-containing genes. In the parasitic stage, only one member of each gene family was expressed, except for

four aspartic peptidases with detectable expression levels. During this life stage, all protease inhibitors were expressed at higher levels than the serine protease.

Differential expression – Gene expression varied across the two free-living strains with eight genes being differentially expressed based on p-values of ≤ 0,05 (Table 2.15). DSIR_10164, DSIR_13362, DSIR_13363, and DSIR_13366 were upregulated in the 2019 strain. DSIR_05528, DSIR_07880, DSIR_08439, and DSIR_13364 were downregulated in the 2019 strain.

2.4 Discussion

This is the first study to characterise genes in *D. siricidicola* that potentially play a role in parasitism and host immunomodulation during the infection of *S. noctilio*. I identified and characterised 23 such genes in the genome of *D. siricidicola* based on sequence similarity with immunomodulation and parasitism genes characterised in other parasitic nematodes. Comparisons with functional databases suggest that 15 of these genes contribute to proteolysis, three to the regulation of endopeptidase activity, and one each to the glycolytic process and signal transduction. Three genes were only characterised as protein binding. The complete set of genes are expressed during the free-living phase of *D. siricidicola*, but transcripts of only nine were observed during parasitism of *S. noctilio* larvae. When comparing nematodes subcultured since 2013 with nematodes subcultured since 2019, eight of the genes are differentially expressed (p-value < 0,05), half of which are upregulated in the 2019 strain.

2.4.1 PROTEOLYSIS

The putative *D. siricidicola* parasitism and immunomodulation genes are mostly proteases. Proteolytic enzymes feature prominently in the host-parasite interaction, both as part of the host immune response – e.g., serine proteases activate proPO in the melanisation pathway (Cerenius et al., 2008) – and as virulence factors of the parasite. In *S. carpocapsae*, the excreted/secreted products (ESP) of virulent nematode strains have higher proteolytic activity than low virulence strains (Simões et al., 2000). Proteases are classified according to catalytically important residues in the active site, namely, cysteine, serine, metallo- and aspartic

proteases (Yang et al., 2015). The latter three were amongst the set characterised in this study of *D. siricidicola*.

Eight of the *D. siricidicola* proteases contain a serine protease trypsin domain and belong to the chymotrypsin family of serine proteases (MEROPS family S1). The chymotrypsin family is divided into trypsin-like, chymotrypsin-like and elastase-like subfamilies based on substrate cleavage specificity (Yang et al., 2015). Serine proteases in parasitic nematodes are mostly digestive enzymes, responsible for metabolising nutrients and degrading host tissue as part of invasion. Two chymotrypsin-like serine proteases in *S. carpocapsae*, ScSP1 (Toubarro, 2010) and ScSP3 (Toubarro et al., 2009), facilitate host invasion through the intestinal wall by causing detachment of epithelial cells from the basal lamina and by inducing apoptosis of epithelial cells, respectively. The ability of the elastase-like serine protease ScELA to digest elastin (a protein of connective tissue) is also presumed to aid invasion and migration (Hao et al., 2009). Similarly, elastases in the ESP of the filarial nematode *Onchocerca volvulus* are associated with tissue penetration in their human hosts (Haffner et al., 1998).

In addition to host invasion, serine proteases also play a role in overcoming the host immune response. The chymotrypsin-like ScCHYM and trypsin-like serine protease (designated xScTSP1) suppress proPO activity, preventing melanisation and cellular encapsulation in *Galleria mellonella* larvae (Balasubramanian et al., 2009, 2010). The effect on the cellular immune response is due to disruption of the haemocyte cytoskeleton which changes the shape and ability of the cell to spread and adhere to other haemocytes. Serine proteases that target host immune responses are not exclusive to EPN. The filarial nematode *Brugia malayi* secretes serine proteases that suppress the complement system of the host, thereby interrupting complement-mediated cellular immune responses (Rees-Roberts et al., 2010). To prevent expulsion from the mouse gastrointestinal tract, *Trichuris muris* nematodes secrete serine proteases that degrade mucin, a component of the mucus barrier in the host intestine (Drake et al., 1994).

I identified six aspartic peptidases in the genome of *D. siricidicola*, all belonging to the aspartic peptidase A1 family. Three aspartic proteases – ScASP110, ScASP113, and ScASP155 – have also been characterised in *S. carpocapsae*. Their specific functions were not determined, but based on the expression of ScASP113 and ScASP155 in L3 nematode larvae in the host gut, their role in host invasion is suspected (Balasubramanian, Nascimento, et al., 2012;

Balasubramanian, Toubarro, et al., 2012). In contrast to the expression of ScASP113 and ScASP155 during early infection, ScASP110 is upregulated in L4 and adult nematodes, suggesting a role in nutrition by digestion of host tissue once infection has been established (Balasubramanian & Simões, 2013). In other parasites, including *Plasmodium falciparum*, *Schistosoma* spp., and haematophagous nematodes such as *Necator americanus, Haemonchus contortus* and *Strongyloides stercoralis*, aspartic proteases contribute to the digestion of host haemoglobin (Banerjee et al., 2002; Brindley et al., 2001; Williamson et al., 2003). The digestion of humoral defence proteins by aspartic peptidases has been observed in *Taenia solium* (White et al., 1992). As *D. siricidicola* does not feed on blood yet expresses high levels of aspartic peptidases during both life cycles, these genes could contribute to nutrition in the free-living stage and neutralising humoral response mechanisms in the parasitic stage.

The remaining *D. siricidicola* protease detected in this study is an astacin-like metallopeptidase belonging to the peptidase M12A family and homologous to ScAST in *S. carpocapsae*. ScAST is upregulated during the parasitic stage of *S. carpocapsae*, and is active against protease substrates, suggesting a role in parasitism (Jing et al., 2010). A metalloprotease secreted by *S. stercoralis* has elastase activity, which enables the infective juvenile to penetrate host skin and subcutaneous tissues (McKerrow et al., 1990). Metallocarboxypeptidase, a related enzyme secreted by *Trichinella spiralis* nematodes, plays a similar role (although less pronounced) in the interruption of complement-mediated cellular host immune response as serine proteases in *B. malayi* (Rees-Roberts et al., 2010). During the mycetophagous stage of *D. siricidicola*, the expression of metallopeptidase is much lower than any of the other curated proteases, and not detected at all in the parasitic samples. The relative sparsity of metallopeptidase in the transcriptome of *D. siricidicola* could argue against a significant role in parasitism.

I detected twice the number of aspartic peptidases in the genome of *D. siricidicola* than what has been described in *S. carpocapsae*. The ratio of *D. siricidicola* homologues detected for each gene described in *S. carpocapsae* was 1,6:1 for the serine proteases (trypsin domain-containing) and 1:1 for most of the other genes in this study, keeping in mind that HMMER searches were used in addition to local BLAST and protein clustering for the detection of serine proteases and serine protease inhibitors but not aspartic peptidases. Regarding transcriptome analysis, aspartic peptidases were expressed at higher levels relative to all other genes – including other proteases – in all three strains examined. The large number of aspartic peptidases in *D. siricidicola* and relatively high expression in parasitic nematodes, could

indicate that members of the aspartic peptidase family are integral to parasitism and immunosuppression by *D. siricidicola*.

Proteases that facilitate invasion are important virulence factors of parasites that enter through or reside in the gastrointestinal tract of the host. The infective form of *D. siricidicola* does not reach the *S. noctilio* haemocoel through the gut, but directly through the larval cuticle using a spear-shaped stylet (Bedding, 1972). No secretions have been observed during penetration suggesting mechanical rather than enzymatic host invasion. After emerging from the infective female nematode, juvenile nematodes do, however, migrate to the reproductive organs of the adult host and into the developing eggs of female wasps and proteases could facilitate this movement through host tissue (Bedding, 1972; Bedding & Akhurst, 1974). Whereas most EPN digest host tissues as food source, the parasitic *D. siricidicola* female develops microvilli on the body surface, allowing the absorption of nutrients directly from host haemolymph, thus reducing the need for digestive enzymes to be excreted/secreted (Riding, 1970). Furthermore, other biocontrol EPN kill their insect hosts within days, but *D. siricidicola* and *S. noctilio* live in relative metabolic harmony which will not be the case if widespread host tissue degradation was taking place (Hajek & Morris, 2021). Proteases therefore probably play a limited role in nutrition and host invasion in the *Deladenus*-*Sirex* interaction but could influence the success with which the nematode sterilises female wasps.

I propose that *D. siricidicola* proteases are more likely involved in suppressing the immune response of *S. noctilio*, than in invasion or nutrition. Encapsulation and melanisation are indispensable tools in the immune response of insects such as *S. noctilio* to metazoan parasites such as *D. siricidicola* (Brivio & Mastore, 2018; Wang et al., 1995). Several components of the proPO-AS have been detected in the genome of *S. noctilio*, together with genes involved in the cellular responses – phagocytosis, encapsulation and nodulation (Sophie Makua, MSc dissertation, 2021). Consequently, without mechanisms allowing evasion or suppression of humoral encapsulation, *D. siricidicola* would not be able to survive, reproduce or spread in the woodwasp. Proteases that neutralise immune-related proteins and disarm immunocompetent cells, as has been observed in other parasitic nematode systems (Cooper & Eleftherianos, 2016; Simões & Rosa, 1996), could explain the molecular mechanism behind the *D. siricidicola* survival strategy.

2.4.2 REGULATION OF ENDOPEPTIDASE ACTIVITY

I identified potential regulators of endopeptidase activity in the genome of *D. siricidicola*, namely the protein inhibitors of proteases. Three *D. siricidicola* genes were detected based on sequence similarity with two serine protease inhibitors characterised in *S. carpocapsae*. Another four genes matched a serine protease inhibitor HMM. Endogenous protease inhibitors are one of the mechanisms that prevents widespread, indiscriminate digestion by proteases within an organism (Laskowski & Qasim, 2000). Parasites secrete/excrete protease inhibitors to deactivate harmful digestive and immune-related proteases in the host organism (Toubarro, Avila, Hao, et al., 2013; Toubarro, Avila, Montiel, et al., 2013) making protease inhibitors a second category of potential immunomodulation and parasitism genes during the infection of *S. noctilio*. Based on their mechanisms of inhibition, protease inhibitors can be classified into canonical inhibitors (standard mechanism), non-canonical inhibitors and serpins (Krowarsch et al., 2003). The canonical inhibitors include the Kunitz bovine pancreatic trypsin inhibitor and Kazal families and these form reversible interactions with the target protease. The serpin (serine protease inhibitor) family belongs to the "other" inhibitors and are characterised by irreversible inhibition (Olson & Gettins, 2011). According to this classification, the *D. siricidicola* protease inhibitors detected in this study consist of six canonical inhibitors and a serpin.

The genome of *D. siricidicola* contain two canonical protease inhibitors that have a Kunitz pancreatic trypsin inhibitor domain similar to that of ScKU4 of *S. carpocapsae*. ScKU4 interferes with the recognition, cellular and humoral aspects of the host immune response (Toubarro, Avila, Montiel, et al., 2013). The binding of ScKU4 to humoral proteins participating in pathogen recognition could explain the mechanism underlying *S. carpocapsae*'s evasion of various insects' immune responses. Furthermore, ScKU4 interferes with encapsulation by inhibiting haemocyte aggregation, delays proPO activation, and disrupts the structure of clotting fibres necessary for the entrapment of foreign bodies. One of the *D. siricidicola* Kunitz-type inhibitors was expressed during parasitism of *S. noctilio* suggesting a similar role in overcoming the host immune response as ScKU4.

The four serine protease inhibitors identified with the serine protease inhibitor HMM search, all belong to the Kazal domain superfamily, but are only assigned protein-binding as a

molecular function and not serine-type endopeptidase inhibitor activity as expected. DSIR_10164 is the exception with molecular functions of calcium ion, collagen and extracellular matrix binding being specified, as well as its classification into the SPARC family. Kazal-type inhibitors are the most studied of the canonical protease inhibitors (Laskowski & Kato, 1980). This would explain why all the serine protease inhibitors detected by HMM search contain a Kazal domain as the sequences obtained from the GenBank database to build the HMM model contained a high proportion of Kazal-type inhibitors. The high relative expression of this gene family in *D. siricidicola* is mainly due to the high transcript count of DSIR_10164. In addition to its Kazal domain, DSIR_10164 contains several conserved sites related to calcium ion binding. The binding of a calcium ion causes conformational change in the conserved motif which activates or deactivates the target protein and supports the presumed importance of this Kazal-type serine protease inhibitor in overcoming the *S. noctilio* immune response (Yap et al., 1999).

The serpin identified in this study, DSIR_00450, is homologous to ScSRP6. In parasitic *S. carpocapsae*, ScSRP6 protects juvenile nematodes in the insect gut by neutralising host digestive enzymes (Toubarro, Avila, Hao, et al., 2013). Serpins from *Ascaris* human and pig intestinal nematodes exhibit a similar protective mechanism (Martzen et al., 1985). ScSRP6 also binds to clotting proteins, thereby impairing the incorporation of melanin into clots. Clot formation and proPO activation are required for encapsulation and both are regulated by the balance between serine proteases and serpins (Li et al., 2002). Given that parasitic *D. siricidicola* nematodes occupy the haemocoel rather than the intestine of *S. noctilio*, serpins are more likely to be involved in reducing clotting and melanisation.

2.4.3 GLYCOLYSIS

The best conserved of the putative *D. siricidicola* immunomodulation genes is DSIR_00839, an orthologue of the *S. glaseri* enolase SgENOL. Enolase is a cytosolic enzyme involved in the glycolytic process (Canback et al., 2002). Cell surface-associated enolases can also mediate the host-pathogen interaction by serving as surface proteins and receptors (Pancholi, 2001). When enolase binds to host plasminogen (the inactive precursor of an enzyme in the fibrinolytic pathway, plasmin), proteolytic activity of the pathogen increases, which, as discussed above, is crucial for host invasion (Saksela & Rifkin, 1988). In *S. glaseri*, SgENOL is found in the

cuticle and surface coat and detectable in host haemolymph after nematodes have reached the haemocoel (Liu et al., 2012). SgENOL reduces phagocytic activity in the insect host and increases the lethality of *S. glaseri*'s bacterial symbiont, *Xenorhabdus poinarii* and an entomopathogenic fungus, *Metarhizium anisopliae,* in their relevant hosts. As SgENOL alone does not kill the host, it appears to serve as complementary and broad-spectrum suppressor of host immune responses. "Broad-spectrum" and "non-lethal" would be an ideal immunomodulation tool for *D. siricidicola* which requires immune suppression without damage to the host.

The abundance of DSIR_00839 transcripts in free-living nematodes (in expression second only to that of the aspartic peptidase DSIR_04158 in all strains and higher than the average TPM per aspartic peptidase), could be explained by the presumed expression of this gene in the nematode cuticle/surface coat. The nematode cuticle is an important component of the host-pathogen interaction. Firstly, molecules expressed on the surface of the pathogen provides the antigenic material for recognition of non-self by the host immune system (Pal & Wu, 2009; Sato et al., 2019). This mechanism is conserved well enough that cell-surface enolase in *Ascaris suum* and other parasites are being investigated as potential vaccine candidates (Chen et al., 2012; Mangalam et al., 2016). The cuticle is therefore also an important locus of immune evasion strategies. Mechanisms to avoid recognition involving the body surface/cuticle include shedding of the surface coat, switching of surface antigens, and reorganisation of the coat structure (Page et al., 1992; Shamseldean et al., 2007; Smith et al., 2000). Secondly, as evidenced by SgENOL, components of the surface coat can also actively suppress the cellular and humoral immune responses. Immunosuppressive surface coat components include proteins, glycoproteins and lipids (Liu et al., 2012).

The enolases are highly conserved in plants, yeasts and animals, being more than 50 % identical across these diverse groups (Van der Straeten et al., 1991). Despite being present in organisms across a wide range of phyla, representing a variety of habitats and lifestyles, all enolases incorporated in the phylogenetic analysis of DSIR_00839 had an E-value of 0 and a higher average percent identity than any of the other gene families examined. The high level of conservation and slow rate of evolution are characteristic of glycolytic enzymes such as enolase and could be explained by their role in carbohydrate metabolism (Fothergill-Gilmore, 1986).

In nematodes, enolases appear to be limited to three suborders of Rhabditida, namely Rhabditina, Tylenchina, and Spirurina based on a recently published nematode phylogeny (Smythe et al., 2019). Most of the nematodes with enolase genes are plant or animal (including insect) parasites. Surprisingly, the Trichinellida, which include important human and animal pathogens *Trichuris suis* and *T. muris*, are not represented in the set. By contrast, free-living nematodes *Caenorhabditis* spp., *Diploscapter pachys*, *Aphelenchus avenae*, and the facultative parasite, *Halicephalobus* sp., do have enolases. However, when comparing the secretomes of 19 parasitic nematodes (including *Trichuris* spp.) with that of *C. elegans*, two glycolytic enzymes – enolase and aldolase – were secreted by all parasitic species and not by free-living *C. elegans* (Tritten et al., 2021). Apart from parasitic nematodes, enolases have also been implicated in the virulence of a bacterial insect pathogen *Paenibacillus larvae*, parasitoid *Aphidius ervi*, and multiple vector-borne pathogens including viruses, bacteria, protozoans and nematodes (Antúnez et al., 2011; Falabella et al., 2009; Mangalam et al., 2016). The role of cell-surface and secreted enolases in host invasion, immune evasion and immune suppression thus clearly supports its importance in parasitism, in addition to its role in carbohydrate metabolism.

2.5 Conclusion

This is the first study to characterise genes in *D. siricidicola* that are potentially involved in parasitism and virulence during the infection of *S. noctilio*. I characterised genes linked to proteolysis (endopeptidase activity), the regulation of endopeptidase activity and glycolysis. While limited in scope, the study opens an important door into the study of the molecular mechanisms underlying the virulence of this important biological control nematode. It also has implications for understanding nematode-insect interactions more broadly, as most previous studies have been on *Steinernema* and *Heterorhabditis* systems. *Deladenus siricidicola* thus offers a valuable point of comparison.

One limitation to this study was the lack of comparable transcriptome data from free-living and parasitic *D. siricidicola* life stages. Future studies could analyse the expression of genes identified here in more life stages by using qRT-PCR to compensate for the low recovery of parasitic *D. siricidicola* RNA from *S. noctilio* transcriptome studies. Comparing gene expression in cultures subcultured for different lengths of time and from different *Sirex*

populations could reveal differentially expressed genes possibly linked to virulence and the effect of continuous subculturing.

In this study, I focused on homologues of genes already described and not genes characterised from changing expression profiles during the infection process. Whole transcriptome data from infection studies and different life stages could enable the discovery of parasitism genes unrelated to or not yet described in other EPN. Functional studies are needed for further functional characterisation of the putative parasitism genes. Regarding genes identified in this study, further investigation into the evolution and function of the highly conserved enolase in nematodes and specifically parasitic nematodes, could be of particular interest.

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Chapter 2. Tables

Table 2.1 Parasitism and immunomodulation genes characterised in entomopathogenic nematodes in the genera *Steinernema*

Sequence ID	GenBank definition	GenBank accession no.	Species	Reference
ScASP110	aspartic protease	AFP21684	S. carpocapsae	(Balasubramanian & Simões, 2013)
ScASP113	aspartic protease	ADJ94115	*S. carpocapsae*	(Balasubramanian, Toubarro, et al., 2012)
ScASP155	aspartic protease	ADQ44147	*S. carpocapsae*	(Balasubramanian, Nascimento, et al., 2012)
ScAST	astacin protease	ACZ98149	*S. carpocapsae*	(Jing et al., 2010)
ScCHYM	trypsin-like serine protease	ABY74341	*S. carpocapsae*	(Balasubramanian et al., 2009)
ScELA	elastase [a]	ABI18379.1	*S. carpocapsae*	(Hao et al., 2009)
ScKU4	serpin	ADN06882	*S. carpocapsae*	(Toubarro, Avila, Montiel, et al., 2013)
ScSP1	chymotrypsin-like serine protease	AAT27470	*S. carpocapsae*	(Toubarro, 2010)
ScSP3	chymotrypsin-like serine protease	ACJ22854	*S. carpocapsae*	(Toubarro et al., 2009)
ScSRP6	serpin	ADN06885	*S. carpocapsae*	(Toubarro, Avila, Hao, et al., 2013)
SgENOL	enolase	ACA34974	*S. glaseri*	(Liu et al., 2012)
xScTSP1	trypsin-like serine protease	ACO35737	*S. carpocapsae*	(Balasubramanian et al., 2010)

[a] – precursor

Table 2.2 Nematode species included in the OrthoFinder analysis. Species were selected to represent diverse nematode families and lifestyles.

Species name	Abbreviation	Lifestyle	Description
Ascaris suum	ASUU	parasitic	large roundworm of pigs
Brugia malayi	BMAL	parasitic	causes human lymphatic filariasis
Bursaphelenchus xylophilus	BXYL	parasitic	pinewood nematode
Caenorhabditis elegans	CELE	free-living	model organism
Dirofilaria immitis	DIMM	parasitic	dog heartworm nematode
Deladenus siricidicola	DSIR	biphasic	biocontrol of Sirex woodwasp
Dictyocaulus viviparus	DVIV	parasitic	bovine lungworm
Globodera pallida	GPAL	parasitic	potato cyst nematode
Haemonchus contortus	HCON	parasitic	barber pole worm
Heterorhabditis bacteriophora	HBAC	parasitic	EPN – biocontrol of insect pests
Loa loa	LLOA	parasitic	eye worm
Meloidogyne hapla	MHAP	parasitic	northern root-knot nematode
M. incognita	MINC	parasitic	southern root-knot nematode
Panagrellus redivivus	PRED	free-living	
Steinernema carpocapsae	SCAP	parasitic	EPN – biocontrol of insect pests
S. glaseri	SGLA	parasitic	EPN – biocontrol of insect pests
Trichinella spiralis	TSPI	parasitic	causes human trichinosis

EPN – entomopathogenic nematode

Table 2.3 Local BLAST results of *Steinernema* parasitism/immunomodulation genes against the genome and proteome of *D. siricidicola*. In only one instance, ScKU4, tBLASTx produced a better result than BLASTp or BLASTx. For ScSP1, BLASTp and BLASTx produced different results.

Query sequence	Best hit	Bit score	E-value	Search type
ScASP110	DSIR_07099	229,57	5,35E-68	BLASTp=x
ScASP113	DSIR_04158	312,77	2,63E-103	BLASTp=x
ScASP155	DSIR_07099	136,73	3,59E-37	BLASTp>x
ScAST	DSIR_00361	192,2	5,47E-56	BLASTx>p
ScCHYM	DSIR_03408	44,67	7,21E-06	BLASTp=x
ScELA	DSIR_03388	43,13	4,39E-05	BLASTp>x
ScKU4	scaffold211_192518[b]	65,67	1,01E-15	tBLASTx
	DSIR_04361	60,46	2,32E-11	BLASTp>x
ScSP1	DSIR_05528	47,37	1,16E-06	BLASTp
	DSIR_03412	46,40	9,75E-07	BLASTx
ScSP3	DSIR_03411	55,07	1,14E-09	BLASTp=x
ScSRP6	DSIR_00450	119,78	6,12E-31	BLASTp=x
SgENOL	DSIR_00839	683,72	0	BLASTp=x
xScTSP1	DSIR_03408	55,07	1,98E-09	BLASTp=x

[b] – start: 22194, end: 22319

Table 2.4 Orthogroups containing *Steinernema* parasitism/immunomodulation genes

Steinernema gene ID	Orthogroup	Number of *Steinernema* genes	Number of *D. siricidicola* genes
ScASP110, ScASP113, ScASP155	OG0000032	21	11
ScCHYM, ScSP3, ScELA	OG0000049	57	2
ScAST	OG0000066	10	5
ScKU4	OG0001148	16	0
SgENOL	OG0001997	2	1
ScSRP6	OG0003428	10	0
ScSP1	OG0010274	3	2
xScTSP1	OG0017056	1	0

Table 2.5 *Deladenus siricidicola* orthologues of *Steinernema* parasitism/immunomodulation genes as inferred by OrthoFinder

Steinernema gene ID	Orthogroup	*D. siricidicola* orthologue(s)
ScASP110	OG0000032	DSIR_10876[c]
ScASP113, ScASP155	OG0000032	DSIR_04158, DSIR_04159[c], DSIR_07099, DSIR_08072[c], DSIR_08073[c], DSIR_08074[c]
ScCHYM, ScELA, ScSP3	OG0000049	DSIR_03411
ScAST	OG0000066	n/a
ScKU4	OG0001148	n/a
SgENOL	OG0001997	DSIR_00839
ScSRP6	OG0003428	n/a
ScSP1	OG0010274	DSIR_03410[c], DSIR_03412
xScTSP1	OG0017056	n/a

[c] – not detected with local BLAST; n/a – no orthologues detected in *D. siricidicola*

Table 2.6 Serine proteases detected by HMMER search of the *D. siricidicola* genome. E-value < 0,01 was used as inclusion threshold.

D. siricidicola gene ID	Score [d]	E-value [d]
DSIR_03388	91,2	8,9E-26
DSIR_03408	45,7	6,4E-12
DSIR_03410	15,9	8,1E-03
DSIR_03411	25,8	7,9E-06
DSIR_03412	20,1	4,3E-04
DSIR_03413 [e]	24,3	2,3E-05
DSIR_04569 [e]	20,1	4,4E-04
DSIR_05528	39,7	4,5E-10
DSIR_07880 [e]	33,4	3,8E-08
DSIR_08439 [e]	17,0	3,8E-03

[d] – for full sequence (includes all domains); [e] – not detected with local BLAST or OrthoFinder

Table 2.7 Serine protease inhibitors detected by HMMER search of the *D. siricidicola* genome. E-value < 0,01 was used as inclusion threshold.

D. siricidicola gene ID	Score [d]	E-value [d]
DSIR_05889 [e]	54,8	9,5E-15
DSIR_05890 [e]	18,2	0,0013
DSIR_05891 [e]	32,3	6,8E-08
DSIR_05892 [e]	38,4	9,4E-10
DSIR_10164 [e]	75,8	3,8-21

[d] – for full sequence (includes all domains); [e] – not detected with local BLAST or OrthoFinder

Table 2.8 *Deladenus siricidicola* genes potentially involved in parasitism and immunomodulation

D. siricidicola gene ID	Source		
	Local BLAST	OrthoFinder	HMM
DSIR_00361	ScAST	n/a	n/a
DSIR_00450	ScSRP6	n/a	n/a
DSIR_00839	SgENOL	SgENOL	n/a
DSIR_03388	ScELA	n/a	serine protease
DSIR_03408	ScCHYM, xScTSP1	n/a	serine protease
DSIR_03410	n/a	ScSP1	serine protease
DSIR_03411	ScSP3	ScCHYM	n/a
DSIR_03412	ScSP1 [2]	ScSP1	serine protease
DSIR_03413	n/a	n/a	serine protease
DSIR_04158	ScASP113	ScASP113, ScASP155	n/a
DSIR_04159	n/a	ScASP113, ScASP155	n/a
DSIR_04361	ScKU4 [2]	n/a	n/a
DSIR_13362	ScKU4 [1]	n/a	n/a
DSIR_04569	n/a	n/a	serine protease
DSIR_05528	ScSP1 [1]	n/a	serine protease
DSIR_05889	n/a	n/a	serpin
DSIR_05890	n/a	n/a	serpin
DSIR_05891	n/a	n/a	serpin
DSIR_05892	n/a	n/a	serpin
DSIR_07099	ScASP110, ScASP155	ScASP113, ScASP155	n/a
DSIR_07880	n/a	n/a	serine protease
DSIR_08072	n/a	ScASP113, ScASP155	n/a
DSIR_08073	n/a	ScASP113, ScASP155	n/a

Table 2.8 (continued)

Gene ID	local BLAST	OrthoFinder	HMM
DSIR_08074	n/a	ScASP113, ScASP155	n/a
DSIR_08439	n/a	n/a	serine protease
DSIR_10164	n/a	n/a	serpin
DSIR_10876	n/a	ScASP110	n/a

[1] – best hit for query sequence; [2] – second best hit for query sequence; n/a – not applicable; serpin – serine protease inhibitor

Table 2.9 Manual curation of putative parasitism and immunomodulation genes in *D. siricidicola*

Gene ID	Locus	Strand	Length (aa)	Exons	Change(s) to original model
DSIR_00839	scaffold1_389578: 378744-381452	+	434	7	add UTR
DSIR_03388	scaffold15_222767: 272-1935	-	296	6	adjust 5' end adjust intron-exon boundaries
DSIR_03408	scaffold15_222767: 148006-153290	+	381	8	find start codon add exon adjust intron-exon boundaries find stop codon add UTR
DSIR_13363	scaffold15_222767: 154459-157537	+	312	8	combine DSIR_03410 and DSIR_03411 adjust intron-exon boundaries
DSIR_13364	scaffold15_222767: 159356-164146	+	254	8	combine DSIR_03412 and DSIR_03413 adjust intron-exon boundaries delete 1 exon
DSIR_00361	scaffold27_539869: 299880-303637	+	589	9	add exon merge 2 exons, adjust intron-exon boundaries delete UTR
DSIR_00450	scaffold47_518607: 450022-452407	+	379	7	find start codon add exon move last exon upstream add UTR
DSIR_04569	scaffold83_190529: 98753-103681	+	623	8	find start codon add 5 exons adjust intron-exon boundaries
DSIR_05889	scaffold164_156356: 104678-107927	-	504	5	(unable to find start codon) add exon adjust intron-exon boundaries find stop codon
DSIR_13365	scaffold164_156356: 108138-115932	-	561	8	combine DSIR_05890 and DSIR_05891 find start codon add 3 exons (unable to find stop codon)

Table 2.9 (continued)

Gene ID	Locus	Strand	Length	Exons	Change(s) to original model
DSIR_05892	scaffold164_156356: 116082-119785	-	360	7	find start codon split 1 exon add 3 exons (unable to find stop codon)
DSIR_04361	scaffold211_192518: 2466-7853	-	390	7	find start codon find stop codon add UTR
DSIR_13362	scaffold211_192518: 19407-23519	+	369	7	(use AUGUSTUS *ab initio* model) delete 8 exons adjust 3' end
DSIR_04158	scaffold213_201625: 23216-25993	-	469	6	adjust intron-exon boundaries
DSIR_04159	scaffold213_201625: 28327-31132	+	501	7	find start codon find stop codon add UTR
DSIR_08072	scaffold218_116733: 79276-81984	+	493	6	adjust intron-exon boundaries delete UTR
DSIR_13366	scaffold218_116733: 83220-86056	-	463	6	combine DSIR_08073 and DSIR_08074 adjust intron-exon boundaries
DSIR_08439	scaffold250_109343: 30185-33404	+	656	8	add 2 exons adjust intron-exon boundaries
DSIR_05528	scaffold362_167960: 43129-46054	+	332	8	find start codon add 3 exons
DSIR_07099	scaffold389_136094: 116508-119398	+	455	6	find start codon delete 10 exons
DSIR_10164	scaffold541_78565: 17702-19674	-	272	6	delete untranslated 1st exon adjust 5' end
DSIR_10876	scaffold567_64778: 10294-16211	-	393	7	find start codon add 2 exons adjust intron-exon boundaries
DSIR_07880	scaffold673_120591: 99653-106885	+	628	11	add 3 exons delete 7 exons adjust intron-exon boundaries add UTR

aa - amino acid residues

Table 2.10 Functional classification of putative parasitism and immunomodulation genes in *D. siricidicola*

Biological process	proteolysis		
Molecular function	aspartic-type endopeptidase *A*	metallo-endopeptidase *A* Zn^{2+} binding	serine-type endopeptidase *A*
Cell component	n/a	n/a	n/a
Conserved domain(s)	aspartic peptidase, active site pepsin-like *D* peptidase family A1 *D*	astacin-like metallopeptidase *D* peptidase M12A *D* peptidase, metallopeptidase *D* ShKT *D*	serine-proteases, trypsin *D*
Family	aspartic peptidase A1 *F*	n/a	n/a
Clan/Homologous superfamily	aspartic peptidase domain *H*	metallopeptidase, catalytic domain *H*	n/a
D. siricidicola gene ID(s)	DSIR_04158 DSIR_04159 DSIR_07099 DSIR_08072 DSIR_10876 DSIR_13366	DSIR_00361	DSIR_03388 DSIR_03408 DSIR_04569 DSIR_05528 DSIR_07880 DSIR_08439 DSIR_13363 DSIR_13364
Steinernema spp. gene ID(s)	ScASP110 ScASP113 ScASP155	ScAST	ScCHYM ScELA ScSP1 ScSP3 xScTSP1

A – activity; *D* – domain; *F* – family; *H* – (homologous) superfamily; Zn^{2+} – zinc ion

Table 2.10 (continued)

Biological process	regulation of endopeptidase *A*		n/a	signal transduction	glycolytic process
Molecular function	serine-type endopeptidase inhibitor *A*		protein binding	Ca^{2+} binding collagen binding extracellular matrix binding	phosphopyruvate hydratase *A* Mg^{2+} binding
Cell component	extracellular space		n/a	collagen-containing extracellular matrix	phosphopyruvate hydratase complex
Conserved domain(s)	pancreatic trypsin inhibitor Kunitz *D* proteinase inhibitor I2, Kunitz, *S* thyroglobulin type 1 *H*	serpin *D* serpin *S*	Kazal *D*	osteonectin-like, *S* EF-hand *D* EF-hand 1, calcium-binding site SPARC/Testican calcium binding *D*	enolase, *S* enolase, C terminal TIM barrel enolase, N-terminal
Family	n/a	serpin *F*	n/a	SPARC *F*	enolase *F*
Clan/Homologous superfamily	pancreatic inhibitor Kunitz domain *H*	serpin *H*	Kazal *H*	EF-hand domain pair *H*	enolase-like, C-terminal domain *H* enolase-like N-terminal *H*
D. siricidicola gene ID(s)	DSIR_04361 DSIR_13362	DSIR_00450	DSIR_05889 DSIR_05892 DSIR_10164 DSIR_13365	DSIR_10164	DSIR_00839
Steinernema spp. gene ID(s)	ScKU4	ScSRP6	n/a	n/a	SgENOL

A – activity; Ca^{2+} – calcium ion; *D* – domain; *F* – family; *H* – (homologous) superfamily; Mg^{2+} – magnesium ion; *S* – conserved site; SPARC – secreted protein acidic rich in cysteine

Table 2.11 RNA-seq data obtained from free-living *D. siricidicola* nematodes

Nematode strain	Sample name	Sequencing type	Library name	Library type	Read length (bp)	Number of reads	GC content (%)
SA757b(2013)	2013.1	Illumina 1.9	Alisa_2013_1_1.fastq	PE	128	24621004	51
			Alisa_2013_1_2.fastq	PE	127	24621004	51
	2013.3	Illumina 1.9	Alisa_2013_3_1.fastq	PE	129	25559651	50
			Alisa_2013_3_2.fastq	PE	127	25559651	50
	2013.4	Illumina 1.9	Alisa_2013_4_1.fastq	PE	131	35266131	50
			Alisa_2013_4_2.fastq	PE	129	35266131	50
SA1109(2019)	2019.1	Illumina 1.9	Alisa_2019_1_1.fastq	PE	128	42819461	50
			Alisa_2019_1_2.fastq	PE	126	42819461	50
	2019.2	Illumina 1.9	Alisa_2019_2_1.fastq	PE	135	21873279	51
			Alisa_2019_2_2.fastq	PE	133	21873279	51
	2019.3	Illumina 1.9	Alisa_2019_3_1.fastq	PE	131	17451375	51
			Alisa_2019_3_2.fastq	PE	129	17451375	51
	2019.4	Illumina 1.9	Alisa_2019_4_1.fastq	PE	134	29733775	50
			Alisa_2019_4_2.fastq	PE	132	29733775	50

bp – base pairs; PE – paired end

Table 2.12 RNA-seq data obtained from *S. noctilio* larvae exposed to *D. siricidicola*

Nematode strain	Sample name	Sequencing type	Library name	Library type	Read length (bp)	Number of reads	GC content (%)
SA107(2013)	N1	Illumina 1.5	FCC6104ACXX-RDWHSIRiwuEAAGRAAPEI-219_L8_1.fq	PE	49	33147709	42
			FCC6104ACXX-RDWHSIRiwuEAAGRAAPEI-219_L8_2.fq	PE	49	33147709	41
	N2	Illumina 1.5	FCC6104ACXX-RDWHSIRiwuEAAHRAAPEI-220_L8_1.fq	PE	49	33609141	42
			FCC6104ACXX-RDWHSIRiwuEAAHRAAPEI-220_L8_2.fq	PE	49	33609141	41
	N3	Illumina 1.5	FCC6104ACXX-RDWHSIRiwuEAAIRAAPEI-221_L8_1.fq	PE	49	33542102	42
			FCC6104ACXX-RDWHSIRiwuEAAIRAAPEI-221_L8_2.fq	PE	49	33542102	41

bp – base pairs; PE – paired end

Table 2.13 Expression levels of putative parasitism and immunomodulation genes in *D. siricidicola* during free-living and parasitic stages in transcripts per million (TPM). TPM values are shaded from highest (dark red) to lowest (dark blue) per strain. Transcript levels in the parasitic stage are from sample N1 unless indicated otherwise.

Gene ID	SA1109(2019) [F]	SA757b(2013) [F]	SA107(2013) [P]
DSIR_04158	3589,15	4004,44	610,86[N3]
DSIR_00839	1407,26	1403,35	277,11
DSIR_04159	887,83	1051,14	176,68[N3]
DSIR_07099	870,11	1033,91	331,62[N3]
DSIR_13366	1271,82	619,84	1,69
DSIR_08072	468,75	388,15	0
DSIR_10164	310,25	147,66	20,91
DSIR_00450	159,71	111,30	58,05
DSIR_03408	47,87	64,77	0
DSIR_04361	83,64	58,70	20,45
DSIR_13364	22,86	51,19	0
DSIR_05528	17,99	30,29	0
DSIR_03388	15,80	21,72	0
DSIR_13362	66,75	15,98	0
DSIR_05889	13,44	15,66	0
DSIR_04569	12,69	11,34	0
DSIR_10876	12,07	9,36	0
DSIR_07880	3,94	7	0
DSIR_05892	6,67	5,73	0
DSIR_13365	6,07	5,25	0
DSIR_08439	3,38	5,23	2,29
DSIR_00361	2,20	2,15	0
DSIR_13363	10,90	0,56	0

[F] – free-living; [P] – parasitic

Table 2.14 Expression of putative parasitism and immunomodulatory gene families in *D. siricidicola* during free-living and parasitic stages in transcripts per million (TPM). TPM values are shaded from highest (dark red) to lowest (dark blue) per strain. Transcript levels in the parasitic stage are from sample N1 unless indicated otherwise.

Gene family	SA1109(2019) [F]	SA757b(2013) [F]	SA107(2013) [P]
Aspartic peptidases	7099,73	7106,84	1120,85[N3]
Enolase [f]	1407,26	1403,35	277,11
Kazal	646,68	321,98	41,81
Serine proteases	135,44	192,10	2,29
Serpin [g]	159,71	111,30	58,05
Kunitz	150,39	74,67	20,45
Metallopeptidase [h]	2,20	2,15	0
Protease inhibitors [i]	956,77	507,95	120,31

[f] – DSIR_00839; [F] – free-living; [g] – DSIR_00450; [h] – DSIR_00361; [i] – Kazal, Kunitz, and serpin; [P] – parasitic

Table 2.15 Differential gene expression of putative parasitism and immunomodulation genes from the sequenced transcriptomes of *D. siricidicola* cultures isolated in 2019 and 2013, respectively. Log2FoldChange values are shaded from highest (dark red) to lowest (dark blue) with corresponding P-values of < 0,05 shaded yellow.

Gene ID	log2FoldChange	p-value
DSIR_13363	3,18	5,66E-06
DSIR_13362	2,05	4,60E-04
DSIR_10164	0,97	4,59E-03
DSIR_13366	0,80	2,81E-02
DSIR_05892	0,26	0,72
DSIR_00450	0,24	0,49
DSIR_13365	0,24	0,67
DSIR_10876	0,17	0,68
DSIR_00361	0,07	0,91
DSIR_04361	0,04	0,94
DSIR_08072	-0,06	0,88
DSIR_04569	-0,09	0,88
DSIR_00839	-0,40	0,34
DSIR_04158	-0,44	0,28
DSIR_05889	-0,55	0,17
DSIR_04159	-0,60	0,14
DSIR_07099	-0,63	0,15
DSIR_03388	-0,92	0,17
DSIR_08439	-0,96	3,46E-02
DSIR_03408	-1,03	0,23
DSIR_07880	-1,10	1,16E-02
DSIR_05528	-1,22	1,06E-02
DSIR_13364	-1,61	1,80E-03

log2FoldChange > 0 – gene upregulated in 2019 strain

log2FoldChange < 0 – gene downregulated in 2019 strain

p-value < 0,05

Chapter 2. Figures

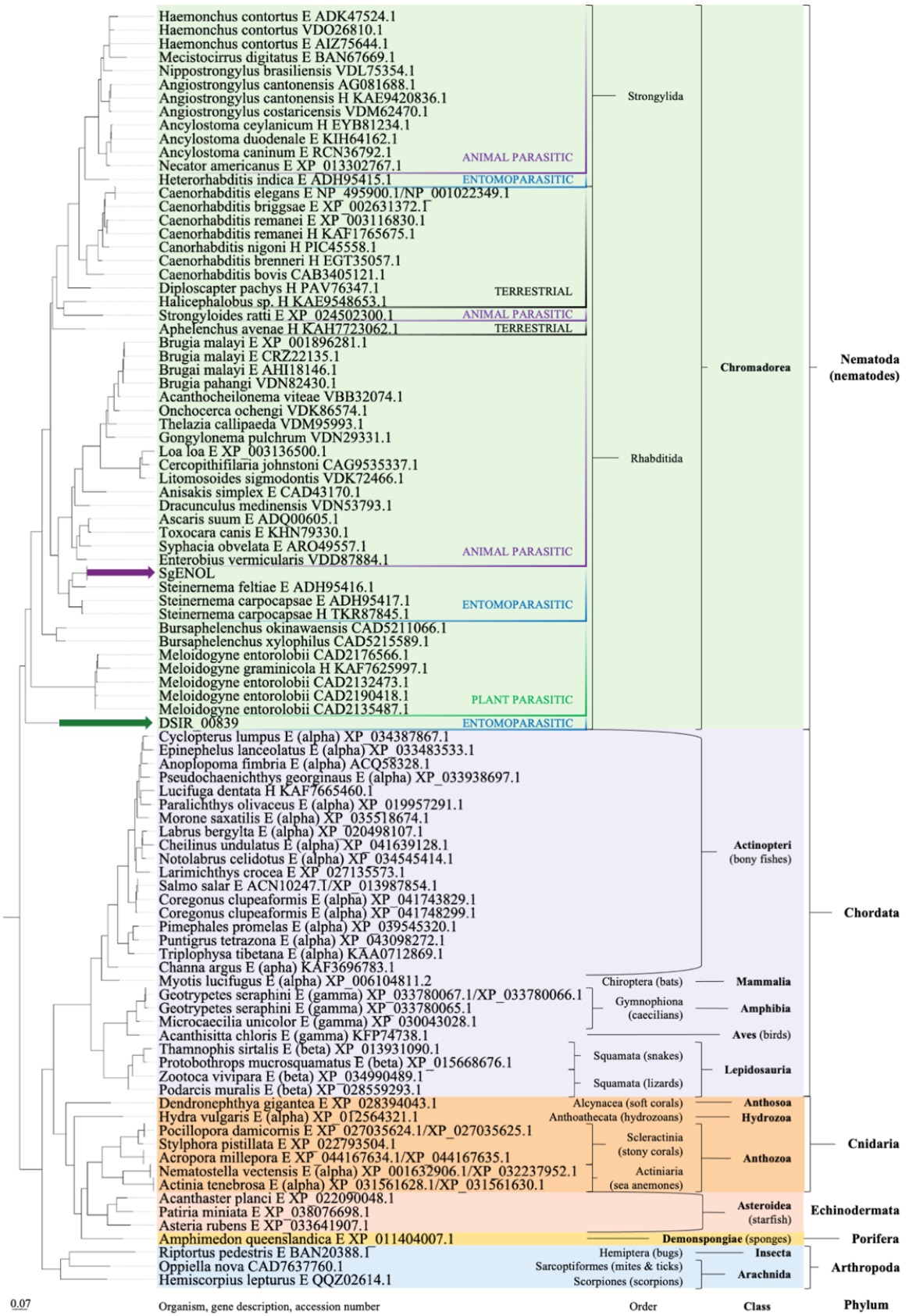

Figure 2.1 Maximum likelihood analysis comparing *D. siricidicola* enolase (DSIR_00839, green arrow) with its

top 100 BLAST hits on NCBI, including *S. glaseri* SgENOL (purple arrow). Taxonomic classification is indicated according to NCBI Taxonomy; NCBI common names are given in brackets. Nematode lifestyle is indicated as "terrestrial" for free-living nematodes and according to host for parasitic nematodes. Enolases with high sequence similarity to DSIR_00839 were found in a wide range of phyla, but only in the nematode orders Strongylida and Rhabditida. Enolase is mostly present as a single copy gene. E – enolase; H – hypothetical protein; no letter after species name – unnamed protein product.

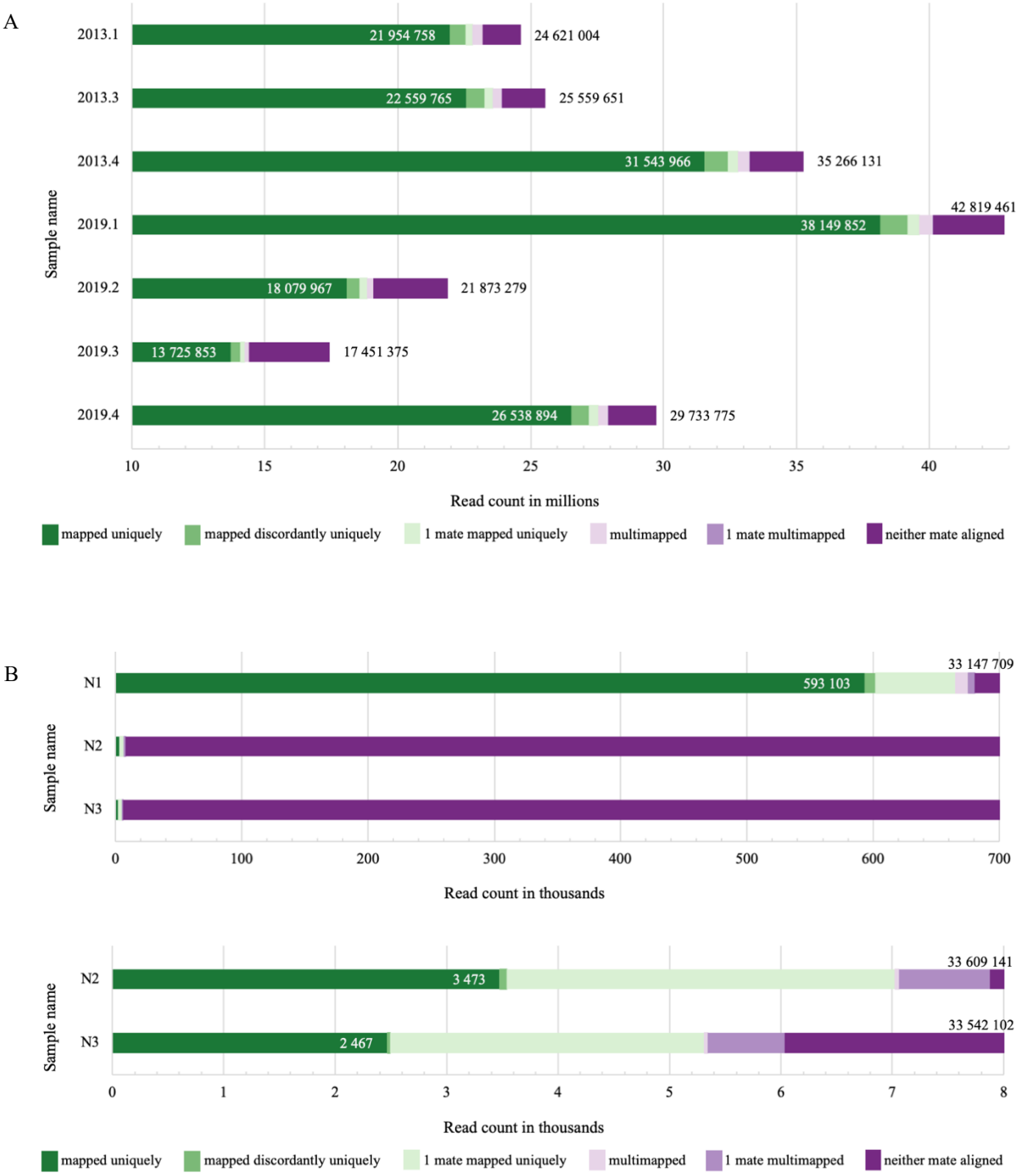

Figure 2.2 Alignment of RNA-seq reads with the genome of *D. siricidicola*. The number of paired end reads that mapped uniquely are indicated in white, the total number of reads appear in black next to/above the bar. **A** – Alignment of RNA from free-living nematodes; **B** – larger magnifications of RNA alignment from nematode-exposed *S. noctilio* larvae

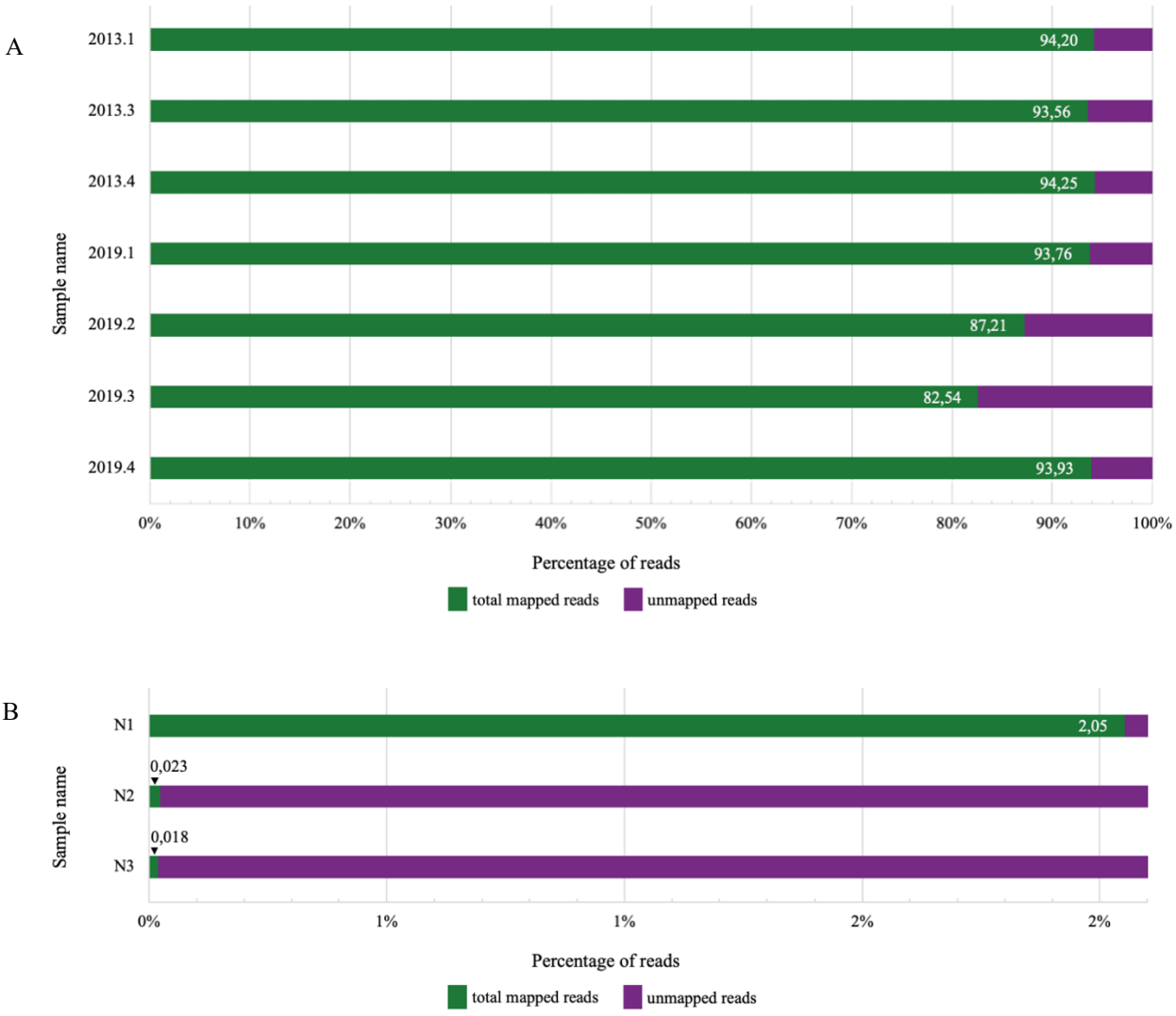

Figure 2.3 Ratio of mapped versus unmapped reads. The percentage of mapped reads are indicated on or above the bar. **A –** Alignment of RNA from free-living nematodes; **B –** larger magnifications of RNA alignment from nematode-exposed *S. noctilio* larvae

Chapter 2. Supplementary tables

Table S2.1 Nematode sequences used to build the serine protease HMM

Accession number	Description	Organism
AAK16516.1	serine protease precursor	*Trichinella spiralis*
AAK16520.1	newborn larvae-specific serine protease SS2	*Trichinella spiralis*
AAR36900.1	newborn larvae-specific serine protease SS2-1	*Trichinella spiralis*
AAT27470.1	chymotrypsin-like serine protease	*Steinernema carpocapsae*
ABY60756.1	putative serine protease, partial	*Trichinella spiralis*
ABY60757.1	putative serine protease	*Trichinella spiralis*
ABY60758.1	putative serine protease	*Trichinella spiralis*
ABY60759.1	putative serine protease	*Trichinella spiralis*
ABY60760.1	putative serine protease	*Trichinella spiralis*
ABY60761.1	putative serine protease, partial	*Trichinella spiralis*
ABY60762.1	putative serine protease	*Trichinella spiralis*
ABY60763.1	putative serine protease	*Trichinella spiralis*
ABY73337.1	serine protease SP-1	*Trichinella pseudospiralis*
ACA28930.1	serine protease	*Trichinella spiralis*
ACA28931.1	serine protease	*Trichinella spiralis*
ACA28932.1	serine protease	*Trichinella spiralis*
ACA30304.1	serine protease	*Gnathostoma spinigerum*
ACJ22854.1	chymotrypsin-like serine protease precursor	*Steinernema carpocapsae*
ACJ22855.1	chymotrypsin-like serine protease precursor	*Steinernema carpocapsae*
ACO35737.1	trypsin-like serine protease	*Steinernema carpocapsae*
ACO35738.1	trypsin-like serine protease	*Steinernema carpocapsae*
ACO35739.1	elastase-like serine protease	*Steinernema carpocapsae*
ACO35740.1	elastase-like serine protease	*Steinernema carpocapsae*
ADY41315.1	Serine protease	*Ascaris suum*
ADY43728.1	Serine protease pcp-1	*Ascaris suum*
ADY43812.1	Serine protease pcp-1	*Ascaris suum*
ADY44052.1	Serine protease, partial	*Ascaris suum*
ADY44374.1	Serine protease	*Ascaris suum*
ADY47395.1	Serine protease 55	*Ascaris suum*
ADY48991.1	Serine protease	*Ascaris suum*
ADY49767.1	Serine protease, partial	*Ascaris suum*
AEH42099.1	trypsin-like serine protease	*Ascaris suum*
AEV21546.1	serine protease, partial	*Trichinella pseudospiralis*
AGW99258.1	serine protease, partial	*Heterodera avenae*
CBX25712.1	putative serine protease, partial	*Trichinella spiralis*
CBX25719.1	putative serine protease	*Trichinella spiralis*
CDW52068.1	serine protease	*Trichuris trichiura*

Table S2.1 (continued)

Accession number	Description	Organism
CDW53123.1	transmembrane serine protease 8	*Trichuris trichiura*
CDW55047.1	transmembrane serine protease 8, partial	*Trichuris trichiura*
CDW55589.1	Serine protease, partial	*Trichuris trichiura*
CDW55844.1	newborn larvae specific serine protease ss2	*Trichuris trichiura*
CDW56064.1	serine protease HTRA2, mitochondrial	*Trichuris trichiura*
CDW57252.1	Serine protease	*Trichuris trichiura*
CDW57253.1	newborn larvae specific serine protease SS2 1	*Trichuris trichiura*
CDW57390.1	serine protease	*Trichuris trichiura*
CDW57543.1	Serine protease	*Trichuris trichiura*
CDW57632.1	newborn larvae specific serine protease SS2 1	*Trichuris trichiura*
CDW57755.1	transmembrane serine protease coagulation factor ix	*Trichuris trichiura*
CDW59132.1	serine protease	*Trichuris trichiura*
CDW59468.1	Serine protease	*Trichuris trichiura*
CDW59497.1	transmembrane serine protease 8	*Trichuris trichiura*
CDW60413.1	serine protease	*Trichuris trichiura*
CDW61271.1	Serine protease	*Trichuris trichiura*
CRZ25617.1	BMA-PCP-5	*Brugia malayi*
D3GGZ8.1	Kunitz-type protein bli-5	*Haemonchus contortus*
KFD51747.1	hypothetical protein	*Trichuris suis*
KHN74292.1	Mannan-binding lectin serine protease 1	*Toxocara canis*
KHN74579.1	putative serine protease K12H4.7, partial	*Toxocara canis*
KHN74592.1	putative serine protease K12H4.7, partial	*Toxocara canis*
KHN77544.1	putative serine protease F56F10.1	*Toxocara canis*
KHN82658.1	putative serine protease pcp-1	*Toxocara canis*
KHN82660.1	putative serine protease pcp-1	*Toxocara canis*
KHN87475.1	putative serine protease K12H4.7	*Toxocara canis*
KRX16162.1	Serine protease HTRA2, mitochondrial	*Trichinella nelsoni*
KRX22602.1	Serine protease 27	*Trichinella nelsoni*
KRX23231.1	putative serine protease K12H4.7	*Trichinella nelsoni*
KRX23232.1	putative serine protease K12H4.7	*Trichinella nelsoni*
KRX25899.1	Serine protease 30	*Trichinella nelsoni*
KRX25900.1	Serine protease 30	*Trichinella nelsoni*
KRX25901.1	Serine protease 30	*Trichinella nelsoni*
KRX25902.1	Serine protease 30	*Trichinella nelsoni*
KRX39158.1	Serine protease 27	*Trichinella murrelli*
KRX41412.1	Serine protease 28, partial	*Trichinella murrelli*
KRX47703.1	Serine protease 30	*Trichinella murrelli*
KRX47705.1	Serine protease 30	*Trichinella murrelli*
KRX47707.1	Serine protease 30	*Trichinella murrelli*
KRX47708.1	Serine protease 30	*Trichinella murrelli*

Table S2.1 (continued)

Accession number	Description	Organism
KRX47709.1	Serine protease 30	*Trichinella murrelli*
KRX47710.1	Serine protease 30	*Trichinella murrelli*
KRX47713.1	Serine protease 30	*Trichinella murrelli*
KRX47714.1	Serine protease 30	*Trichinella murrelli*
KRX48412.1	putative serine protease K12H4.7	*Trichinella murrelli*
KRX50056.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella murrelli*
KRX50057.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella murrelli*
KRX50059.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella murrelli*
KRX50373.1	Serine protease 27	*Trichinella murrelli*
KRX52954.1	Serine protease 30, partial	*Trichinella sp. T6*
KRX58852.1	putative serine protease F56F10.1	*[Trichinella sp. T9*
KRX60932.1	Serine protease 27	*Trichinella sp. T9*
KRX61476.1	Serine protease 28	*Trichinella sp. T9*
KRX61805.1	Serine protease HTRA2, mitochondrial	*Trichinella sp. T9*
KRX62546.1	Serine protease 30	*Trichinella sp. T9*
KRX62547.1	Serine protease 30	*Trichinella sp. T9*
KRX62548.1	Serine protease 30	*Trichinella sp. T9*
KRX62549.1	Serine protease 30	*Trichinella sp. T9*
KRX62550.1	Serine protease 30	*Trichinella sp. T9*
KRX62551.1	Serine protease 30	*Trichinella sp. T9*
KRX62553.1	Serine protease 30	*Trichinella sp. T9*
KRX62554.1	Serine protease 30	*Trichinella sp. T9*
KRX62555.1	Serine protease 30	*Trichinella sp. T9*
KRX66431.1	putative serine protease K12H4.7	*Trichinella sp. T9*
KRX70457.1	Serine protease 38, partial	*Trichinella sp. T6*
KRX71534.1	Serine protease 27, partial	*Trichinella sp. T6*
KRX72571.1	Serine protease 48	*Trichinella sp. T6*
KRX73773.1	Serine protease 28	*Trichinella sp. T6*
KRX73774.1	Serine protease 28	*Trichinella sp. T6*
KRX77104.1	Serine protease 30	*Trichinella sp. T6*
KRX77106.1	Serine protease 30	*Trichinella sp. T6*
KRX77111.1	Serine protease 30	*Trichinella sp. T6*
KRX77112.1	Serine protease 30	*Trichinella sp. T6*
KRX77113.1	Serine protease 30	*Trichinella sp. T6*
KRX82853.1	Serine protease 27	*Trichinella sp. T6*
KRX84203.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella sp. T6*
KRX84204.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella sp. T6*
KRX84452.1	putative serine protease K12H4.7, partial	*Trichinella sp. T6*
KRX86075.1	Serine protease 30, partial	*Trichinella pseudospiralis*
KRX88974.1	Serine protease 33, partial	*Trichinella pseudospiralis*

Table S2.1 (continued)

Accession number	Description	Organism
KRX91662.1	Serine protease 48	*Trichinella pseudospiralis*
KRX95583.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella pseudospiralis*
KRX95584.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella pseudospiralis*
KRX95585.1	Serine protease HTRA2, mitochondrial	*Trichinella pseudospiralis*
KRX99150.1	Serine protease 30	*Trichinella pseudospiralis*
KRY01032.1	putative serine protease K12H4.7	*Trichinella pseudospiralis*
KRY08169.1	Brain-specific serine protease 4, partial	*Trichinella patagoniensis*
KRY12405.1	Serine protease 28	*Trichinella patagoniensis*
KRY16272.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella patagoniensis*
KRY16273.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella patagoniensis*
KRY19108.1	Serine protease 27	*Trichinella patagoniensis*
KRY19130.1	Serine protease 42, partial	*Trichinella patagoniensis*
KRY19703.1	Serine protease 27, partial	*Trichinella patagoniensis*
KRY23474.1	putative serine protease K12H4.7	*Trichinella patagoniensis*
KRY23475.1	putative serine protease K12H4.7	*Trichinella patagoniensis*
KRY23476.1	putative serine protease K12H4.7	*Trichinella patagoniensis*
KRY23879.1	Serine protease 30	*Trichinella patagoniensis*
KRY23880.1	Serine protease 30	*Trichinella patagoniensis*
KRY24040.1	Serine protease 42, partial	*Trichinella britovi*
KRY34982.1	Serine protease 30	*Trichinella spiralis*
KRY34983.1	Serine protease 30	*Trichinella spiralis*
KRY34987.1	Serine protease 30	*Trichinella spiralis*
KRY34988.1	Serine protease 30	*Trichinella spiralis*
KRY34989.1	Serine protease 30	*Trichinella spiralis*
KRY34990.1	Serine protease 30	*Trichinella spiralis*
KRY34991.1	Serine protease 30	*Trichinella spiralis*
KRY34992.1	Serine protease 30	*Trichinella spiralis*
KRY36415.1	Serine protease 38, partial	*Trichinella spiralis*
KRY36798.1	Serine protease 28	*Trichinella spiralis*
KRY38568.1	Serine protease HTRA2, mitochondrial	*Trichinella spiralis*
KRY39237.1	Serine protease 27	*Trichinella spiralis*
KRY43526.1	putative serine protease K12H4.7	*Trichinella spiralis*
KRY49897.1	Serine protease 28	*Trichinella britovi*
KRY51447.1	Serine protease 27	*Trichinella britovi*
KRY53646.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella britovi*
KRY53647.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella britovi*
KRY57281.1	Serine protease 27	*Trichinella britovi*
KRY58836.1	Serine protease 30	*Trichinella britovi*
KRY58837.1	Serine protease 30	*Trichinella britovi*
KRY58838.1	Serine protease 30	*Trichinella britovi*

Table S2.1 (continued)

Accession number	Description	Organism
KRY58839.1	Serine protease 30	*Trichinella britovi*
KRY58840.1	Serine protease 30	*Trichinella britovi*
KRY58841.1	Serine protease 30	*Trichinella britovi*
KRY58842.1	Serine protease 30	*Trichinella britovi*
KRY58845.1	Serine protease 30	*Trichinella britovi*
KRY60857.1	putative serine protease K12H4.7	*Trichinella britovi*
KRY70627.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella pseudospiralis*
KRY70628.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella pseudospiralis*
KRY75326.1	putative serine protease K12H4.7	*Trichinella pseudospiralis*
KRY77580.1	Serine protease 30	*Trichinella pseudospiralis*
KRY79540.1	Serine protease 30	*Trichinella pseudospiralis*
KRY79541.1	Serine protease 30	*Trichinella pseudospiralis*
KRY80867.1	Serine protease 30	*Trichinella pseudospiralis*
KRY84623.1	Serine protease 53, partial	*Trichinella pseudospiralis*
KRY88307.1	Serine protease 30	*Trichinella pseudospiralis*
KRY88615.1	putative serine protease K12H4.7	*Trichinella pseudospiralis*
KRY90559.1	Serine protease HTRA2, mitochondrial	*Trichinella pseudospiralis*
KRZ04702.1	Serine protease 30, partial	*Trichinella pseudospiralis*
KRZ09637.1	Serine protease 27, partial	*Trichinella zimbabwensis*
KRZ12429.1	putative serine protease K12H4.7	*Trichinella zimbabwensis*
KRZ18687.1	Serine protease 30	*Trichinella zimbabwensis*
KRZ18690.1	Serine protease 30	*Trichinella zimbabwensis*
KRZ18691.1	Serine protease 30	*Trichinella zimbabwensis*
KRZ29407.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella pseudospiralis*
KRZ29408.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella pseudospiralis*
KRZ29409.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella pseudospiralis*
KRZ32609.1	putative serine protease K12H4.7	*Trichinella pseudospiralis*
KRZ32610.1	putative serine protease K12H4.7	*Trichinella pseudospiralis*
KRZ35842.1	Serine protease 48	*Trichinella pseudospiralis*
KRZ38478.1	Serine protease HTRA2, mitochondrial	*Trichinella pseudospiralis*
KRZ40840.1	putative serine protease K12H4.7	*Trichinella pseudospiralis*
KRZ40841.1	putative serine protease K12H4.7	*Trichinella pseudospiralis*
KRZ43044.1	Serine protease 30	*Trichinella pseudospiralis*
KRZ43045.1	Serine protease 30	*Trichinella pseudospiralis*
KRZ43046.1	Serine protease 30	*Trichinella pseudospiralis*
KRZ43047.1	Serine protease 30	*Trichinella pseudospiralis*
KRZ43049.1	Serine protease 30	*Trichinella pseudospiralis*
KRZ43051.1	Serine protease 30	*Trichinella pseudospiralis*
KRZ43052.1	Serine protease 30	*Trichinella pseudospiralis*
KRZ47260.1	Serine protease 38, partial	*Trichinella nativa*

Table S2.1 (continued)

Accession number	Description	Organism
KRZ51251.1	Serine protease 27	*Trichinella nativa*
KRZ52763.1	Serine protease 28, partial	*Trichinella nativa*
KRZ52847.1	Serine protease 48	*Trichinella nativa*
KRZ57189.1	putative serine protease K12H4.7	*Trichinella nativa*
KRZ57612.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella nativa*
KRZ57613.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella nativa*
KRZ57614.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella nativa*
KRZ59608.1	Serine protease 30	*Trichinella nativa*
KRZ59611.1	Serine protease 30	*Trichinella nativa*
KRZ59612.1	Serine protease 30	*Trichinella nativa*
KRZ59613.1	Serine protease 30	*Trichinella nativa*
KRZ59614.1	Serine protease 30	*Trichinella nativa*
KRZ75033.1	putative serine protease K12H4.7	*Trichinella papuae*
KRZ78281.1	putative serine protease K12H4.7	*Trichinella papuae*
KRZ78420.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella papuae*
KRZ78421.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella papuae*
KRZ79027.1	Serine protease 30	*Trichinella papuae*
KRZ79028.1	Serine protease 30	*Trichinella papuae*
KRZ79029.1	Serine protease 30	*Trichinella papuae*
KRZ79030.1	Serine protease 30	*Trichinella papuae*
KRZ79033.1	Serine protease 30	*Trichinella papuae*
KRZ79035.1	Serine protease 30	*Trichinella papuae*
KRZ79037.1	Serine protease 30	*Trichinella papuae*
KRZ92993.1	Serine protease 27	*Trichinella sp. T8*
KRZ94985.1	putative serine protease K12H4.7	*Trichinella sp. T8*
KRZ95397.1	Serine protease HTRA2, mitochondrial, partial	*Trichinella sp. T8*
KRZ96347.1	Serine protease 30	*Trichinella sp. T8*
KRZ96348.1	Serine protease 30	*Trichinella sp. T8*
KRZ96352.1	Serine protease 30	*Trichinella sp. T8*
KRZ96353.1	Serine protease 30	*Trichinella sp. T8*
KRZ96354.1	Serine protease 30	*Trichinella sp. T8*
KRZ96355.1	Serine protease 30	*Trichinella sp. T8*
KRZ96356.1	Serine protease 30	*Trichinella sp. T8*
KRZ96357.1	Serine protease 30	*Trichinella sp. T8*
KRZ96358.1	Serine protease 30	*Trichinella sp. T8*
KRZ97606.1	Serine protease 27	*Trichinella sp. T8*
NP_001294837.1	Putative serine protease F56F10.1	*Caenorhabditis elegans*
NP_498688.1	Putative serine protease pcp-1	*Caenorhabditis elegans*
NP_498758.2	Putative serine protease K12H4.7	*Caenorhabditis elegans*
NP_501379.2	Serine protease svh-1	*Caenorhabditis elegans*

Table S2.1 (continued)

Accession number	Description	Organism
NP_505421.3	Trypsin-like protease try-5	*Caenorhabditis elegans*
WP_017980025.1	hypothetical protein	*Sphingomonas melonis*
XP_001892869.1	Serine protease Z688.6 precursor	*Brugia malayi*
XP_001892870.1	Serine protease Z688.6 precursor	*Brugia malayi*
XP_003366826.1	serine protease, trypsin family	*Trichinella spiralis*
XP_003368555.1	serine protease SP24D	*Trichinella spiralis*
XP_003369429.1	putative serine protease	*Trichinella spiralis*
XP_003370990.1	putative serine protease UNQ9391/PRO34284-like protein, partial	*Trichinella spiralis*
XP_003374478.1	putative serine protease	*Trichinella spiralis*
XP_003375204.1	peptidase, S1 family	*Trichinella spiralis*
XP_003376868.1	transmembrane serine protease 8	*Trichinella spiralis*
XP_003378705.1	putative serine protease pcp-1	*Trichinella spiralis*
XP_003379580.1	serine protease hepsin	*Trichinella spiralis*
XP_003382019.1	hypothetical protein, partial	*Trichinella spiralis*

Table S2.2 Nematode sequences used to build the serine protease inhibitor HMM

Accession number	Description	Organism
AAD51334.1	Kunitz type serine protease inhibitor, partial	*Ancylostoma ceylanicum*
AAD51336.1	ascaris type serine protease inhibitor, partial	*Ancylostoma ceylanicum*
AAF09189.1	serine protease inhibitor TCI	*Trichuris suis*
AAF09190.1	serine protease inhibitor CEI	*Trichuris suis*
AAY56748.1	serine protease inhibitor, partial	*Caenorhabditis remanei*
AAY67786.1	serine protease inhibitor 1	*Onchocerca volvulus*
AAY67787.1	serine protease inhibitor 2	*Onchocerca volvulus*
AAZ38760.1	serine protease inhibitor 1	*Brugia malayi*
AAZ38762.1	serine protease inhibitor 1	*Dirofilaria immitis*
AAZ38763.1	serine protease inhibitor 1	*Litomosoides sigmodontis*
AAZ38765.1	serine protease inhibitor 1, partial	*Dirofilaria immitis*
ACP43576.1	serine protease inhibitor	*Haemonchus contortus*
ADK37592.1	serine protease inhibitor precursor	*Steinernema carpocapsae*
ADN06882.1	serine protease inhibitor precursor	*Steinernema carpocapsae*
ADN06883.1	serine protease inhibitor precursor	*Steinernema carpocapsae*
ADN06884.1	serine protease inhibitor precursor	*Steinernema carpocapsae*
ADN06885.1	serine protease inhibitor precursor	*Steinernema carpocapsae*
ADN06886.1	serine protease inhibitor precursor	*Steinernema carpocapsae*
ADT91439.1	putative serine protease inhibitor, partial	*Wuchereria bancrofti*
ADZ39076.1	Kunitz type serine protease inhibitor 1	*Ancylostoma duodenale*
ADZ39077.1	Kunitz type serine protease inhibitor 2	*Ancylostoma duodenale*
ADZ39078.1	Kunitz type serine protease inhibitor 3	*Ancylostoma duodenale*
ADZ39079.1	Kunitz type serine protease inhibitor 4	*Ancylostoma duodenale*
AEH42098.1	serine protease inhibitor	*Ascaris suum*
AEO72144.1	Kazal-type serine protease inhibitor 1 serpin	*Trichinella pseudospiralis*
AEO72145.1	serine protease inhibitor 1 serpin	*Trichinella pseudospiralis*
AEO72146.1	serine protease inhibitor 2 serpin	*Trichinella pseudospiralis*
AEO72148.1	Kazal-type serine protease inhibitor 2, partial	*Trichinella pseudospiralis*
CDW52552.1	Squash family serine protease inhibitor	*Trichuris trichiura*
CDW53616.1	kazal type serine protease inhibitor	*Trichuris trichiura*
CDW55271.1	Serine protease inhibitor family protein	*Trichuris trichiura*
CDW58606.1	Serine protease inhibitor 2	*Trichuris trichiura*
CDW60605.1	Serine protease inhibitor family protein	*Trichuris trichiura*
D3GGZ8.1	Kunitz-type protein bli-5	*Haemonchus contortus*
EJW81976.1	kazal-type serine protease inhibitor domain-containing protein	*Wuchereria bancrofti*
EPB68717.1	Kazal-type serine protease inhibitor domain protein, partial	*Ancylostoma ceylanicum*
EPB70310.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma ceylanicum*
EPB73598.1	squash family serine protease inhibitor	*Ancylostoma ceylanicum*
EPB73599.1	squash family serine protease inhibitor	*Ancylostoma ceylanicum*

Table S2.2 (continued)

Accession number	Description	Organism
EPB76561.1	Kazal-type serine protease inhibitor domain protein, partial	*Ancylostoma ceylanicum*
EPB78951.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma ceylanicum*
KFD51747.1	hypothetical protein	*Trichuris suis*
KHJ41241.1	Kazal-type serine protease inhibitor domain protein	*Trichuris suis*
KHJ48489.1	Kazal-type serine protease inhibitor domain protein, partial	*Trichuris suis*
KHJ86342.1	Kazal-type serine protease inhibitor domain protein, partial	*Oesophagostomum dentatum*
KHJ92890.1	squash family serine protease inhibitor	*Oesophagostomum dentatum*
KHJ94185.1	squash family serine protease inhibitor	*Oesophagostomum dentatum*
KHJ96620.1	squash family serine protease inhibitor	*Oesophagostomum dentatum*
KHJ97570.1	Kazal-type serine protease inhibitor domain protein	*Oesophagostomum dentatum*
KHJ98156.1	squash family serine protease inhibitor	*Oesophagostomum dentatum*
KHJ98158.1	squash family serine protease inhibitor	*Oesophagostomum dentatum*
KHJ98159.1	squash family serine protease inhibitor	*Oesophagostomum dentatum*
KHJ98160.1	squash family serine protease inhibitor	*Oesophagostomum dentatum*
>KHJ98782.1	Kazal-type serine protease inhibitor domain protein	*Oesophagostomum dentatum*
KHJ98783.1	Kazal-type serine protease inhibitor domain protein	*Oesophagostomum dentatum*
KIH44785.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma duodenale*
KIH46688.1	Kazal-type serine protease inhibitor domain protein, partial	*Ancylostoma duodenale*
KIH49606.1	Kazal-type serine protease inhibitor domain protein, partial	*Ancylostoma duodenale*
KIH53694.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma duodenale*
KIH62681.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma duodenale*
KIH62683.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma duodenale*
KIH64340.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma duodenale*
KJH39800.1	Kazal-type serine protease inhibitor domain protein, partial	*Dictyocaulus viviparus*
KJH41802.1	Kazal-type serine protease inhibitor domain protein	*Dictyocaulus viviparus*
KRX14877.1	Serine protease inhibitor Kazal-type 4	*Trichinella nelsoni*
KRX25209.1	Serine protease inhibitor Kazal-type 4	*Trichinella nelsoni*
KRX43266.1	Serine protease inhibitor Kazal-type 4	*Trichinella murrelli*
KRX80539.1	Serine protease inhibitor Kazal-type 4	*Trichinella sp. T6*
KRX81768.1	Serine protease inhibitor Kazal-type 4	*Trichinella sp. T6*
KRX95181.1	Serine protease inhibitor Kazal-type 4	*Trichinella pseudospiralis*
KRY17236.1	Serine protease inhibitor Kazal-type 4	*Trichinella patagoniensis*
KRY23759.1	Serine protease inhibitor Kazal-type 4	*Trichinella patagoniensis*
KRY31272.1	Kunitz-type serine protease inhibitor	*Trichinella spiralis*
KRY34094.1	Serine protease inhibitor Kazal-type 4	*Trichinella spiralis*
KRY38614.1	Serine protease inhibitor Kazal-type 4	*Trichinella spiralis*
KRY68191.1	Serine protease inhibitor Kazal-type 4	*Trichinella pseudospiralis*
KRZ05113.1	Serine protease inhibitor Kazal-type 4	*Trichinella zimbabwensis*
KRZ12928.1	Serine protease inhibitor Kazal-type 4	*Trichinella zimbabwensis*
KRZ19844.1	Serine protease inhibitor Kazal-type 4	*Trichinella pseudospiralis*
KRZ35908.1	Serine protease inhibitor Kazal-type 4, partial	*Trichinella pseudospiralis*

Table S2.2 (continued)

Accession number	Description	Organism
KRZ54140.1	Four-domain proteases inhibitor	*Trichinella nativa*
KRZ69162.1	Serine protease inhibitor Kazal-type 4	*Trichinella papuae*
KRZ87041.1	Serine protease inhibitor Kazal-type 4	*Trichinella sp. T8*
NP_505346.1	Serine protease inhibitor swm-1	*Caenorhabditis elegans*
NP_872050.1	Serine protease inhibitor 1 protein	*Caenorhabditis elegans*
O77416.1	Serine protease inhibitor 1	*Anisakis simplex*
O77417.1	Serine protease inhibitor 2	*Anisakis simplex*
O77418.1	Serine protease inhibitor 3	*Anisakis simplex*
OUC42312.1	Kazal-type serine protease inhibitor domain protein, partial	*Trichinella nativa*
OUC42990.1	Kazal-type serine protease inhibitor domain protein	*Trichinella nativa*
OUC44371.1	Kazal-type serine protease inhibitor domain protein, partial	*Trichinella nativa*
OUC44374.1	Kazal-type serine protease inhibitor domain protein, partial	*Trichinella nativa*
OUC44775.1	Kazal-type serine protease inhibitor domain protein	*Trichinella nativa*
OUC48929.1	Kazal-type serine protease inhibitor domain protein	*Trichinella nativa*
OUC49154.1	Kazal-type serine protease inhibitor domain protein	*Trichinella nativa*
OUC49318.1	squash family serine protease inhibitor	*Trichinella nativa*
OUC49341.1	squash family serine protease inhibitor, partial	*Trichinella nativa*
OZC11189.1	Kazal-type serine protease inhibitor domain protein	*Onchocerca flexuosa*
PIO54985.1	Kazal-type serine protease inhibitor domain protein, partial	*Teladorsagia circumcincta*
PIO63181.1	Kazal-type serine protease inhibitor domain protein	*Teladorsagia circumcincta*
PIO69137.1	Kazal-type serine protease inhibitor domain protein	*Teladorsagia circumcincta*
PIO71527.1	Kazal-type serine protease inhibitor domain protein	*Teladorsagia circumcincta*
QBF38545.1	serine protease inhibitor 1	*Dirofilaria repens*
RCN33206.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma caninum*
RCN33283.1	Kazal-type serine protease inhibitor domain protein, partial	*Ancylostoma caninum*
RCN51060.1	Kazal-type serine protease inhibitor domain protein	*Ancylostoma caninum*
VIO94387.1	Kazal-type serine protease inhibitor domain containing protein	*Brugia malayi*
XP_001892192.1	Kunitz/Bovine pancreatic trypsin inhibitor domain containing protein	*Brugia malayi*
XP_001900126.1	Kazal-type serine protease inhibitor domain containing protein	*Brugia malayi*
XP_001900127.1	Kazal-type serine protease inhibitor domain containing protein	*Brugia malayi*
XP_001900128.1	Kazal-type serine protease inhibitor domain containing protein	*Brugia malayi*
XP_001902444.1	Kazal-type serine protease inhibitor domain containing protein, partial	*Brugia malayi*
XP_003373875.1	putative kazal-type serine protease inhibitor domain protein	*Trichinella spiralis*
XP_003374153.1	serine protease inhibitor Kazal-type 4	*Trichinella spiralis*
XP_003374469.1	putative kazal-type serine protease inhibitor domain protein	*Trichinella spiralis*
XP_003375404.1	squash family serine protease inhibitor	*Trichinella spiralis*
XP_003377380.1	serine protease inhibitor family protein	*Trichinella spiralis*
XP_003379899.1	putative kazal-type serine protease inhibitor domain protein	*Trichinella spiralis*
XP_003380011.1	putative kazal-type serine protease inhibitor domain protein	*Trichinella spiralis*
XP_003381353.1	serine protease inhibitor Kazal-type 4	*Trichinella spiralis*

Table S2.2 (continued)

Accession number	Description	Organism
XP_013292646.1	Kazal-type serine protease inhibitor domain protein	*Necator americanus*
XP_013292647.1	Kazal-type serine protease inhibitor domain protein, partial	*Necator americanus*
XP_013295543.1	Kazal-type serine protease inhibitor domain protein	*Necator americanus*
XP_013306703.1	Kazal-type serine protease inhibitor domain protein, partial	*Necator americanus*
XP_013308686.1	squash family serine protease inhibitor	*Necator americanus*

Table S2.3 Amino acid sequences of putative *D. siricidicola* parasitism and immunomodulatory genes

Gene ID	Length (aa)	Amino acid sequence
DSIR_00361	589	MNPHQLGNRTRKKFSLILISTFLSVFTIGECVKRLAHKDQFLLYDANLPDYETVLTPSDFEMASEIND YPSQKNTIDGDDAWDSDAMYSPDKFEGDIANDLNASTVAMFIRGAPTDGDSNHAQYTQFEIVGNYGQM DEYHMRLAVSTAATRSVIASAMQEYSKSTCIQWSPRGANDRDYIYIVPDRGCYSLVGRTGGRQTLSLG NGCIQKIIIHEMMHAVGFFHEQSRTDRDDFITIMWHNILPGMQQFEKYTTATVQTLGTEYDYGSIHYG PNAFTRNGMPTIVPKRRASIGQRSGFSKIDSYKINTLYDCPNYNSGSGLGTVVGGAIAVPTARPAASL VAATTSQPSAVASTASTRWAPLPSSGLNGGVVSLCRNTRPDCEQLAKQWCKRNSGWMRDNCPLACGLC SVTVGNSAAGIVPITTRPVLPDTLPLLPSNAGGSGGFCEDLRVDCAELAKRRYCITAQSFTRVYCARS CGFCFVPPVTEIPEVAAGNRNNSIYGSVVNKAVTARPIENGKIPLVTFWPTLRPSATEAPPSTVSRLG ESGTQSPSQCRDRKHFCVSWKKSGFCQGIFTNYMKKNCPMACGWC
DSIR_00450	379	MELTVAQADFAFNLLREIVADEPDTSLVCSPVSVSMALSMVLAGARGKTAEELTSVLGGGVKASAAIHNH FAALTEVMNAHNDKFTLSAANRAYVDAQFPLLDSFREIIDAKYAGQFQSVDFKQTLETVKAVNAFVSEKT AGKITDLLMPEDVRDTVLLLVNALHFKGRWASEFYKENTKMEMFYASADKQREVEMMQQTQDCDYYEDEQ LQCVALPYEGHLINAHFLLPKARHGLREVLDAVTGEAFVTKILGGMRREKVDVKLPKFRVESKHDLEEIL ENMGVASIFGAGADLSGISKPPKNGEKLKVGGVIQKAVVQTDEKGSEAAAATAISVSITGCMMSAPPRVH AFHADHAFVFAITHARTQAILFGGVIRKE
DSIR_00839	434	MSIQRVFARQIYDSRGNPTVEVDLTTDKGVFRAAVPSGASTGIYEALELRDGDKKVHLGKGVLKAVDNIN SKIGPALVAKGINVTQQKEIDEFMIQLDGTENKSNFGANAILGVSLAVCKAGAVHKGLPLYKYIAELSGT SKVILPVPAFNVINGGSHAGNKLAMQEFMILPTGASTFKQAMQIGAEVYHNLKTVIKKRYGLDATAVGDE GGFAPNIQDNKEALNLIKEAIELAGYTGKVVIGMDVAASEFYKDGKYCLDFKSDKPDPSTIISGEELSNL YKSFIAEYPIVSIEDAFDQDDWEHWSQFLASTNTQLVGDDLTVTNPKRIKTAIEKKACNCLLLKVNQIGS VTESIEAANISRKNGWGVMVSHRSGETEDTFIADLVVGLATGQIKTGAPCRSERLAKYNQLLRIEEELGA DAVYAGQKFRNPLA
DSIR_03388	296	MLGLLIFMLAVNIASTLGPSTNSSCGVADVNKDFSRFDMLTPEHRLVGGIESTPHAWPWTGQLLMKDKHH CGCALIDREIALTAAHCFAKSRKPSTYKILLGGHEIFSGKKFFVTTISIHPLYQTQASAFDVALIRIFPP ATLGANISTICLPSTPPPFNRMCVVTGWGKLTEDGERARALREIHIPIIPPFICNNMRHYAGRIQASSMM CAGYNNGRIDSCQGDSGGPLQCQNVHGAWELQGVVSWGIGCAQPDFPGVYSKIFVMVGWIHFQMGAMKKT LNRADVEDESPRHLAA
DSIR_03408	381	MKNRKIWKIFFPLFVGHLINVNLALAVIYPNQRLDPRYLGPIFDVNIDASAGPNIDDKNFSQNNDGENIG ENSGENIAANNGENTDNGPMPDTIYYELPQPQQISDSDMDCGISSMGLPTRRILNGKDVIDGDWPWAVYL SSGCTGSIVSSRHVLSAAHCAFEDTRNGTRRLSSGTVVIGRDRNVARSVHDLRLQMAKIFVHPQYDFHDF ASPDIAIFELAKNVTFSQKAQKICLPADYREYYGQIAVIVGWGADNRTDKSNKAQFAQETMIPLNAPEDC DKHFKNISYGIDFNEKEFICGGGTLRGTEGGDSGGPLMVNERGRWFQVGITSFGEMGKSPRGYYDVGAYL RVAEHCDWILRVTNGEVKCLDMYKFPHRMRH
DSIR_04158	469	MFANALSLLFLAALAALAAAAVHKMQLNHVESRFETAIRQGKLKEFRAAQEFSRLYHKYAMRAKGKKGKI VAQTVNDYNDFEYVGNISIGKPEQSFSVVLDTGSANLWVPDSSCSTKAKCNLNCDDPDYCYQMCDDYCCN GGGGGDDDDSDDSGDDSDDDSYVYEDDEAKMKLQKKKSSSKLTCQMKQQFHSNQSKSYVADSRAFNIEYG IGFAAGFLGTDTVRFGAPGTAQLVVPKTTFGQATTMSKDFQHSPADGILGLAFTSLAVDGVDPPLINAIN QKLLDQPIFTVFLDTHGEKKTDRPGGVFTYGGLDTENCEDVTGYAPLSSATYYQFTVSGIKVGKKFKTSK SMEAVSDTGTSLLMGPEKVVKKIADAVGATFYQDYGLYLIKCNAKYDPITLKINHKHYKLTKDVMTVDLG IGQGMCVFGALGADLGNIDWILGDPFLRAYCNVHDIGQSRMGFAPVKKQ

Table S2.3 (continued)

Gene ID	Length (aa)	Amino acid sequence
DSIR_04159	501	MAQNTTLCGLSLALIIVALAMGQTLGAVHKMALDRVEGGFIKAIREGRAVEYRNMLNYARLYNKYAKLTG SNRNAYSQGVTDYDDLQYLGNITIGTPEQTFRVVLDTGSANLWVPDSSCGPNVKCDKNCADAEYCSEYCD EYCCEGGPHGNDGDDDSNSSSDSDDDGGGGHGGHGGGGASSSGDDYYYNDLTVRGRRSVMKAVNSNAAFN RNTAKTTCQMKNQFNSKKSSTYKKNGQEFQISYGTGFADGILGVDTVRFGGPGEPQLTVPKTTFGQAKTM SKFFRSQAVDGILGLAFSALAADDVEPPLIRAINKNLLDEPIFTVYMGTHPKTKQRGGVFTYGGLDTENC QDVMGYAPLTSNTYYQFKIDGVRVGKKYKNTKSANVISDTGTSLIIGPEDVVKKLAAAAGANYVENYGVY MIKCDAKYPPVGFTINGKLYRLSREVLTIDVNTGNECLFGIAGGNFGGGIDWILGDPFIRAYCNIHDIGK SQMGFAPIKKH
DSIR_04361	390	MHFSLVQLLLLIAVRFTQGVSKNFERKPGPCTFGTHSDSAQYKCADNGYFEVRQCLRDYCVCVDPRSGHE APATRTTSSKVEPRCGKCHLEMARLLASNSPKQLQCEPNTGHFQPKQCQSGQSCVCVNRETGEPISGANA RVETRSNADLACGISASIDSRPISTSSAAKSAASQRPPASSARMDANHPPPPPPTASSATTTDASSSLAD DFPPVGNAACRLPKDAGRLCGDGRTPASVQWYFDVESFECLAFRHNGCGGNENHFYSVSDCWRQCKLADF GSCAGLKTPAKDAKGENIVCYSHNGGDDAAKCPPNYRCTMLAFFGVCCHEETQELYKRNYLPSCADRRQT PKRIESGGVPMTLIGKSCEDDFCPRNTTCHKKEIFAHCCQ
DSIR_04569	623	MMRISALLFLFLAEFAVVGDAEERIALSNMLLPLSAKEAAEISAKCAGQNEATYRVEGGEHSKVPIPHAA VLISQETETHCSAVFISKRHLISSTECFGIREAKFLRLAAGGICYRSSSTCGGGDTAAAAAPDMVDLEID LVVYDKSAHRSATTIAIIQLKKSVPFEYASRISFACLPKKDAPLPDFLTIAGWGSASANEWISLSSLKKV DLVTGSSANECKEEFLFCAYGPTNEPLASAAFGDVGAGAVDMKNNKNVLYGIVEDVGVKSTISPHKRYTR FVDVRKIVDDLCALLQFDVCRKSEDTVSLKQSVFVPNAVFFKRPYFYRDFRPSFRISRPTAIRECVADSF SVYPPFAATLVNNDKVQFCSAIFITMRHLITTRKCIVEHRNRAERLPSNVAQLHFTVEGGGSCLASSNLA GTCEETNKTQTYQVQFVAYDDEDVGWESANLAIIELADEAGPRVFPACSTLLRHNYAFYQVNGRKAEARF FENDAELNLQLLKQSKIHIGGMKNNVLYTVPPTAQTRNRDRGAAVFDSDDGELVGMLLGWNGTTNLILPL NAVEDALCIYLDRCDFPRKTHYNVREFAVFDKIFRPNEQLVKSDLELRETARINMQINPHNIN
DSIR_05528	332	MWRSDCAAAIFLVLVVRAVVASAASSVDDVEAGHFVYVKGGISRRMSRLSAQQNRQLHSMCGHAGEFPRR YLSYAHYAHRGQFPWTVSLAIDNINMCGGTLISDRHVLSAAHCFFKYKDGSVPCRGPSFIGKRIEKIEVK YGGVCLRESILCKRKQVTMKSALIKRLVISIRFNDRECLYGDDYAIVELEQPVSFTPFTQPICLRSMNES DIPMRSGDSPKVDRVRTLGWGRTEYGESSPELRYLELKVMSIYEIFIKTISTSYEGGICSGDSGGTLQAR TLDTHRHVLIGIHSNGPDCEKLPAGEASSVYMPAILPIICHFTGICPPALGE
DSIR_05889	504	DGGAEDAAVCDSLGRSHQTRCLFDVARCRAQKIAHVNLTEVACERLARYGERAASSKLQQRDYSSLAAEN FQIGANKIAAKANTNRSHSGSETRTAAAAAAATAAFSERISAHAGATAASVESTPAPTGEIAAPVIRTTA PIGGIAAPERRIAAPVVRTAVQTSTESTQWSTERSESLQNLSNWHSSTISPIANSQISASANFVMMGRQL TTATLQRDEKVVNSGDTKFNANLEEIFAISSAISSANSGANDHTDMDNANFGSANPPKSLAANHKANSTA NSLAKTANCSNVYEPICGSDLVTYANKCHFQLALASALAAERQLVLPIGANANTTNDLILSSHNSDNDND DSDNGGENEMLKSLTILHAGECCALTICSSTDYAPVCDNRGDTHLNLCWFGRRRCVLERFALANLTVVAY DMCAEAKLRCESPANCPPDYDPVCASDGATFTNKCNLARNACALAVGGGARPTAISAHYYGSAPIRFAHY GNCRKKSQTERRRR
DSIR_05892	360	MKFKFAAYFIIRFFPFFISSVDGQISCDCGRSFKPVCGSDSQTYDNHCLLGCYREHNSDLKLEYTGTCCP SLELCADYSDPLCDDRGKTHENECHFNYHKCIEAKRANTVIRIASRGKCGVDADSGTTVVDDNYSQTTTS TVSSSAPERRPSPTVVRRIPLSDSYNSTGIVSKLINSTVRGAKKTATTNNNNGVELARRDASGRYCSFFC EETVEPLCDSSGHTHKNPCKFELANCRLLSRGLPAPTVAKLGECDEADAPADVFSSRLRSAQLAYGRSSR EKSARKNNSTDDDGSSESVDGGDHRSSSSSMTVVESLASTLPFDAIENEAKFLANASDSSSPTLLTPSAA TVLSADRISS

Table S2.3 (continued)

Gene ID	Length	Amino acid sequence
DSIR_07099	455	MNAYFVLVLAALAALSVAAVHKMQLDHIESGFRKALKQGKVKEYRAAQEFSRLHHKYAIRAGAKGKVTAQ TVYDYSDFEYVGNITVGTPGQSFSVILDTGSADLWIPDSSCSTKSKCHKNCDNKDYCNHICDRYCCVDGD SDDDSDQDGSDDSASFEFNSTMAVEVRVKKVTCRSKRQFYSNMSKSYVNDSRSFSVEYGLAFAGGFLGTD TVRLGAAGTTQLVVPKTTFGQATTMSKDFQQSDTDGILGLALKSLTVDGVIPPIVNAINQNLLDQPIFTV YLDTQRKKKTSRAGGLDTDNCKDVVGYAPFSSDSYYQFTMNGIKVGKKFKTSKKMEVISGSGTPYIIGPK KVVANIANAVGAKYSMDYRVYLIECDAEYDPVTLKINNKHYKLTKDVLTMELGIGKGMCVFGMLGADFGG AGMDWILGNPFLRAYCNVHDIGKSRMGFAPSKKHH
DSIR_07880	628	MYLLIFVGVLLAQARTSRGVGFGFSPVLKRLSYNEMFEIVLACQVLTQEEKRLSVDRVMNGQPTQPGVYR HAAVLTYRGEDTGFCGATFISWRHLVTARHCVSNVSEIVKDGIPVLKAGGVCVSKATADCEADADAMVEL EYDLIAFESDKPDFMTGMHPNDIAILQLRQPVPDNLRHRIDFACISSMSEDGEVELAGWGRLTDHTLPTR LHHTPLFISITSEPPWTELKLCNLSLFLCGYTPSWGFANNTYTGSNFGDSGSGVVRVKGRTRTGQTRYNV IGVVSGSNALNDTNIRTVIIPIARFTNDICYYLNVCPRGNTITEDPSLLKLDSLIMARQKRSDFKAIAPF RMISDSAELKIVDWCSSRKQIEMPSPETFAWTAVFVDAEDDAVKCGATLLTSSHLLTSRGCLKAFFTDAA LGDTTAAAARNLLRRRVRLFGGGTCAICHFSDLDIQLIAWPTSHDRLADVAIIQLAADVTNEAFAGNRFA PACMSAVASGDNVTRLTSYGFLTDDAPGARLDRLHSREVAVYKPDTFDETENPCSMDAFICTQRDEGVAP FEPGGGVLDERGARLIAVHSGRREPRLDVYLRVEYFFDEFCALLNRCVGGGGAPRLLRARHTYTILQF
DSIR_08072	493	MLTNAYCLLVLAALAALAASAIHKVHFHHDESRLQWAIRQGKMKEYHATRELSRLYHKYAALDDSPANGE RKAKSTGKVISQTIQDYGDFEYVANITAGTPEQSFSVILDTGSGDVWLPDSSCSSESKCAVKCTTNLDYC NKMCDNYCCTSGADNSNDDSDDSDDSEDSDDSDDSDDSDDSDGSDDRKNNKNIKDSGDSTITCQLKRQFY SNMSKTYVKDSRVFAAELDRGVGFAEGFLGKDTVRFGAAGTKQLVIPKTIFGQATTISRDFRDSNADGVF GLARKAANGVDSPILHAIKKNFLELPIFTVYLDTQGQTESNRAGGVFTFGGFDRVNCEDVVGYAPFSSDT RYQFTISAVEVCVNFRTNKRTEAITSTATPFLIGPQNVVKKIAKAAGASFSADYGLYVIRCSIYYNPLTL KINHKHYTLTQDVLTLGHWRTRDGSCVFGAQASEFLSSDVDWVLGAPFQRAYCTVHDIGSSRIGFAPVKQ HNN
DSIR_08439	656	MYYFQLFVLNLIPQIATSYLPYPTPAFAGIETHLAKLTAAEANEIRDFCSNSGLRQPQNRIIGGAKVGGV VPYAAVFVSNGLAQCGATFISTRHLLSARHCFKKLEQSGAVYRLLAGGTCLRDSPHCEATTDGKADMVEL EFELIIYDERDVEDMSATSTDFAIIQLKNAIPSELRDRIEFACLPSKDAKLPAIVTVNGWGMIVDSNSVS ALIPALKQINLTVESAPPTTSSAAENCQPAWNSFCANPLRLGERSAQGWLPFDHMPFSGDSGSGAVRLAQ RDRRAVLYGILSMDRRSHANIHVVFVDLRKFLDDLCLQLSLCAKGAVAKMVNFRPTALYFSRRDQSHFGQ SAFQSIQSFQDDEYRRKCDEQRTSKTFPPRIGWAAAVVDAFGDFVCAATFVTRRHLVTTRSCFTDRIFAR IQKGHFKSYRDAFFVAAGGACLANPNNASAGNCDASSADTTVQMDVGLVAWTSAHRGDWLADIAIIELRR DNFMPNSSSRVFPACINTDLDSVWRNEAMLHAYAWSGTDTVNEDDKRNLLRRVMKVDLWHTENRQLYYTD TTSTTGALPQAMTPHDQGTALVDPESGEMAGVFLKTDSAYDMYNIVYPAEYLEDLLCIYLNRCARKKNFY SLTPFHRLDIIVQPFINLSNTAFNDF
DSIR_10164	272	MARIAFFLFVIALLQCAQAKKRAEFEELESILEDIDDTKKVRDLSKHHEPVQREDPSVCDTHVCGWGKEC VVQKGRPVCECISRCPLLDNDPLDQVCASNNQTYASLCELYRQRCVCKKRSGFESECEHPSNSKIHLEYL GACKELDTCTDELMAQFPQRMSDWLFQVMKELKKRRELHGLEWEELVSEAEGDDEKKHVYPVIWKFCDLD IKPHDKHVSHHELIPITAPVIPMESCIKPFLEACDADKDGSITIKEWGKCLGLKEGEIQERC

Table S2.3 (continued)

Gene ID	Length (aa)	Amino acid sequence
DSIR_10876	393	MNFLYTFASLFWILQFQSQLQLRYVLTKIVRLELRSTGSLRTRLVKENRWRETVDGIRNNLAVGGQPFLD YFDGYYLFNISIGTPPQNFTIAIDTGSSYFWVVDAERCVQDACRGHKLSRYHXLSSTSTLENRRFVVEYR SGNVSGGYVKDYIHLANLQIVGEIGLVVEVPHIIALQPFDGAMGFAWPHFTKERTKSPIFGMLQQMDEPI FTVWIDRRRPLWMGGSGGLVTFGGRDEKNCFPEWNYVPLSSRSLWQFYIQEFQIGKYRSWRRRIAIADTG TSLLGGPYGQFSIIISEVGARYSVEDDFYTVNCDSFQNLSDIVFIIEKVKYRIPPIDYVLNMENGICVLG IFGMFASGFGVDWILGDTFLNSYCNLYDIGNGRMGFAKARDAI
DSIR_13362	369	MPMPMPMLIFLSISLTASASLFTAATAAGVNLNNDPNPPFEFDGAASAETQMFRDSVRRAAAARPAILQQ PAVAEGDAPPGINPLAARVQLDSKCTEPLERGNGSAEMARFFFDAKNATCRKFTYTGDGGNENNFETELD CLNDCYTGVGEGEPVHEEDVVFIGRSTDDQADFSRREMAAASPDCLLPKDVGNCTGSVQNFYFLPDWQSC VAFKYTGCGGNANRFASRIECEQSCLVADGSVCRGPSGAAMPLQSGADCASTVCPSGTVCAMGFGAPECC EAAEAELLTQVYAPTCANGAQAGGHFGEYFVATFATACADLECAPGERCALHGAGEHAFAKCCQHDKQPQ LLLLRQPKSLLPVISERRV
DSIR_13363	312	MLLIAATITTAVVGVVLCQSPTQPPQDFSECGITSMTLPDDRVIAGKNVTAGDWPFLVKIVAGAEMFCTG SIISEVDVLTAAHCAPVFEQANVFVYAGTSHIYANGSFDGYTVGTEFHKARPTGVAVGIKRAQIHPTYRP AANGAPSAGLDVAVLELKQALVFNENIRKVCLAADFTEMRGHIGFVVGWGYDEKAHEARPDNAREDTIPI MSPSTCENSILYYNRTESLCAGGTNRGTQSGDSGGPLMANFRGRFFQVGVVSNGFTDKSTTGQKLDYGKY LRISQTCDFLKSATNGRANCMEMRSVQYARMH
DSIR_13364	254	MHSLLAIVVSTAVVLCGGSQAPKDFSECGTPGMTLPGDRVIAGKNVTAGDWPFLVKIASGAKSFCTGSII SESHILTAAHCVPIFKRKNVLVYAGTTPISGNGEPLQFNENVRKICLPSQFAEIRGQIGYIVGWGFDQPG GEAMPETAREDIVPIEQPFICENELRHYNRTISICAGGTNRGTQVGDSGGPLMFNYNGRYFQIGVVSSGY TEDSNTGEKLDYGNYVRTTMTCSFIKSVTDGSAECIEMNSQSVI
DSIR_13365	561	MMQISANSTTRGENSTTTTTTSDAARGGTPAAANATTTTVVERFVVEPPLKIVHYGGCGRRRNTRRREPT PSTGARKWRAGRNEDALGAPSGAYTLEKCAALCTDDGGGGGGAIYAVTDAPVAVCDNANRTHQSICLFAV WNCERRVRGEEARVLVHIGACQIVSPIFNLSAEVCPYKCSKQYKPVCDTNNLTHLNLCAFQLLNCYQRKK GVANYGWLKRLSACVDENGATIAPVGGEKFIRSENALMPSLASVIAMDAVAPAAAARNASALDVGAARFD ECPQVRRDCDNDAHEELSDIERQRRAPRRNGTRRQKRRICDNFGNLHKNECEFARAHCLAARQGRTLRIV HENACDAAPNSAEKFAAADVDDASRADNYAPKPAPIEGERVESSTATTAQQQAAAAANSGEIAKSPTRNF DCADISAVNCSYGGGFDAYCGSDFVTYANFCIFQKARCRNAALEILFRGECERCLHSPCPVIESAAAAAD DALFVCDQGGESKSKCEFEMLRCIYEAKFGYNITIAYEGKCCPSAEACVSQLFYAAEGGGDGGAEDAAVC D
DSIR_13366	463	MSASAYSLLVFAALATLAASAIHKVEFYHGESRLENAIRQGKMKEYHAKRELSRLYHKSKDKGKGKVISQ NVRDFDDFEYIANITVGTPDQSFSVVVDTGSAELWLPDSSCSSESKCNKRCIDDLKYCNYVCDNFCCADD TDDGEDSDDNDDSNVSFYFDRYTAVRAQSKPKKHAVTCNWKLKFYSNMSRTYVNDSRDFAAGFDIGIGFA EGFLGKDTVRFGAAGTKQLVIPKTIFGQATTISRDFRNSNADGVFGLARKAANGVDSPIMNAINQNLLDQ PIFTVYLDRQAPEGSSRAGGTFTFGGLDNDNCHGVVGYAPFSSDTRYQFTISGVKVGKKFKQSRKMDAIV STGTPFLLGPHKLIKKIGDSVGAMYEPQTRLYVIECNLKYDPITLKINGKHYQLTKREMTLSYQIRPGIC VFGALDSDYVGAGVDWVLGTPFQRAYCNVHDIGNSRIGFAPVK

aa – amino acid residues

Table S2.4 Functional analysis of putative *D. siricidicola* parasitism and immunomodulatory genes

Gene ID	Domain analysis	Gene ontology		
		Biological process	Molecular function	Cellular component
DSIR_00361	metallopeptidase, catalytic domain *H* astacin-like metallopeptidase *D* peptidase M12A *D* (x 4) peptidase, metallopeptidase *D* ShKT *D* (x 3)	proteolysis	metallo-endopeptidase *A* metallopeptidase *A* zinc ion binding	n/a
DSIR_00450	serpin *F* serpin *D*	(regulation of endopeptidase *A*)	(serine-type endopeptidase *A*)	extracellular space
DSIR_00839	enolase-like *H* enolase-like, C-terminal domain *H* enolase-like, N-terminal domain *H* enolase *F* enolase, C-terminal TIM barrel *D* enolase, N-terminal *D* enolase, conserved *S*	glycolytic process	phosphopyruvate hydrate *A* metal ion binding magnesium ion binding	phosphopyruvate hydratase complex
DSIR_03388	peptidase S1, PA clan peptidase S1A, chymotrypsin *F* serine proteases, trypsin *D* serine proteases, trypsin family, histidine active *S* serine proteases, trypsin family, serine active *S*	proteolysis	serine-type endopeptidase *A*	n/a
DSIR_03408	peptidase S1, PA clan peptidase S1A, chymotrypsin *F* serine proteases, trypsin *D* serine proteases, trypsin family, histidine active *S* serine proteases, trypsin family, serine active *S*	proteolysis	serine-type endopeptidase *A*	n/a
DSIR_04158	aspartic peptidase domain *H* aspartic peptidase A1 *F* peptidase family A1 *D* (x2) pepsin-like *D* aspartic peptidase, active *S* (x2)	proteolysis	aspartic-type endopeptidase *A*	n/a
DSIR_04159	aspartic peptidase domain *H* aspartic peptidase A1 *F* peptidase family A1 *D* (x2) pepsin-like *D* aspartic peptidase, active *S* (x2)	proteolysis	aspartic-type endopeptidase *A*	n/a

Table S2.4 (continued)

Gene ID	Domain analysis	Biological process	Molecular function	Cellular component
DSIR_04361	thyroglobulin type-1 *H* pancreatic trypsin inhibitor Kunitz domain *H* pancreatic trypsin inhibitor Kunitz *D* thyroglobulin type-1 *D* (x2) proteinase inhibitor I2, Kunitz, conserved *S*	(regulation of endopeptidase *A*)	serine-type endopeptidase inhibitor *A*	(extracellular space)
DSIR_04569	peptidase S1, PA clan serine proteases, trypsin *D*	proteolysis	serine-type endopeptidase *A*	n/a
DSIR_05528	peptidase S1, PA clan peptidase S1A, chymotrypsin *F* serine proteases, trypsin *D* serine proteases, trypsin family, histidine active *S*	proteolysis	serine-type endopeptidase *A*	n/a
DSIR_05889	Kazal domain *H* Kazal *D* (x4)	n/a	protein binding	n/a
DSIR_05892	Kazal domain *H* Kazal *D* (x3)	n/a	protein binding	n/a
DSIR_07099	aspartic peptidase domain *H* aspartic peptidase A1 *F* peptidase family A1 *D* (x2) pepsin-like *D* aspartic peptidase, active *S*	proteolysis	aspartic-type endopeptidase *A*	n/a
DSIR_07880	peptidase S1, PA clan peptidase S1A, chymotrypsin *F* serine proteases, trypsin *D*	proteolysis	serine-type endopeptidase *A*	n/a
DSIR_08072	aspartic peptidase domain *H* aspartic peptidase A1 *F* peptidase family A1 *D* (x2) pepsin-like *D*	proteolysis	aspartic-type endopeptidase *A*	n/a
DSIR_08439	peptidase S1, PA clan peptidase S1A, chymotrypsin *F* serine proteases, trypsin *D*	proteolysis	serine-type endopeptidase *A*	n/a

Table S2.4 (continued)

Gene ID	Domain analysis	Biological process	Molecular function	Cellular component
DSIR_10164	Kazal domain *H* SPARC *F* Kazal *D* EF-hand *D* EF-hand domain pair follistatin-like, N-terminal *D* follistatin/osteonectin EGF *D* SPARC/Testican calcium binding *D* EF-hand 1, calcium-binding *S* osteonectin-like, conserved *S*	signal transduction	protein binding calcium ion binding collagen binding extracellular matrix binding	collagen-containing extracellular matrix
DSIR_10876	aspartic peptidase domain *H* aspartic peptidase A1 *F* peptidase family A1 *D* pepsin-like *D* aspartic peptidase, active *S*	proteolysis	aspartic-type endopeptidase *A*	n/a
DSIR_13362	pancreatic trypsin inhibitor Kunitz domain *H* pancreatic trypsin inhibitor Kunitz *D* proteinase inhibitor I2, Kunitz, conserved *S*	(regulation of endopeptidase *A*)	serine-type endopeptidase inhibitor *A*	(extracellular space)
DSIR_13363	peptidase S1, PA clan peptidase S1A, chymotrypsin *F* serine proteases, trypsin *D* serine proteases, trypsin family, histidine active *S* serine proteases, trypsin family, serine active *S*	proteolysis	serine-type endopeptidase *A*	n/a
DSIR_13364	peptidase S1, PA clan peptidase S1A, chymotrypsin *F* serine proteases, trypsin *D* (x2) serine proteases, trypsin family, histidine active *S* serine proteases, trypsin family, serine active *S*	proteolysis	serine-type endopeptidase *A*	n/a
DSIR_13365	Kazal domain *H* Kazal *D* (x5)	protein binding	n/a	n/a
DSIR_13366	aspartic peptidase domain *H* aspartic peptidase A1 *F* peptidase family A1 *D* (x2) pepsin-like *D* aspartic peptidase, active *S*	proteolysis	aspartic-type endopeptidase *A*	n/a

aa – amino acid residues; *A* – activity; *D* – domain; *F* – family; *H* – (homologous) superfamily; *S* – site; gene ontology terms in brackets represent derived characteristics

Chapter 2. Supplementary figures

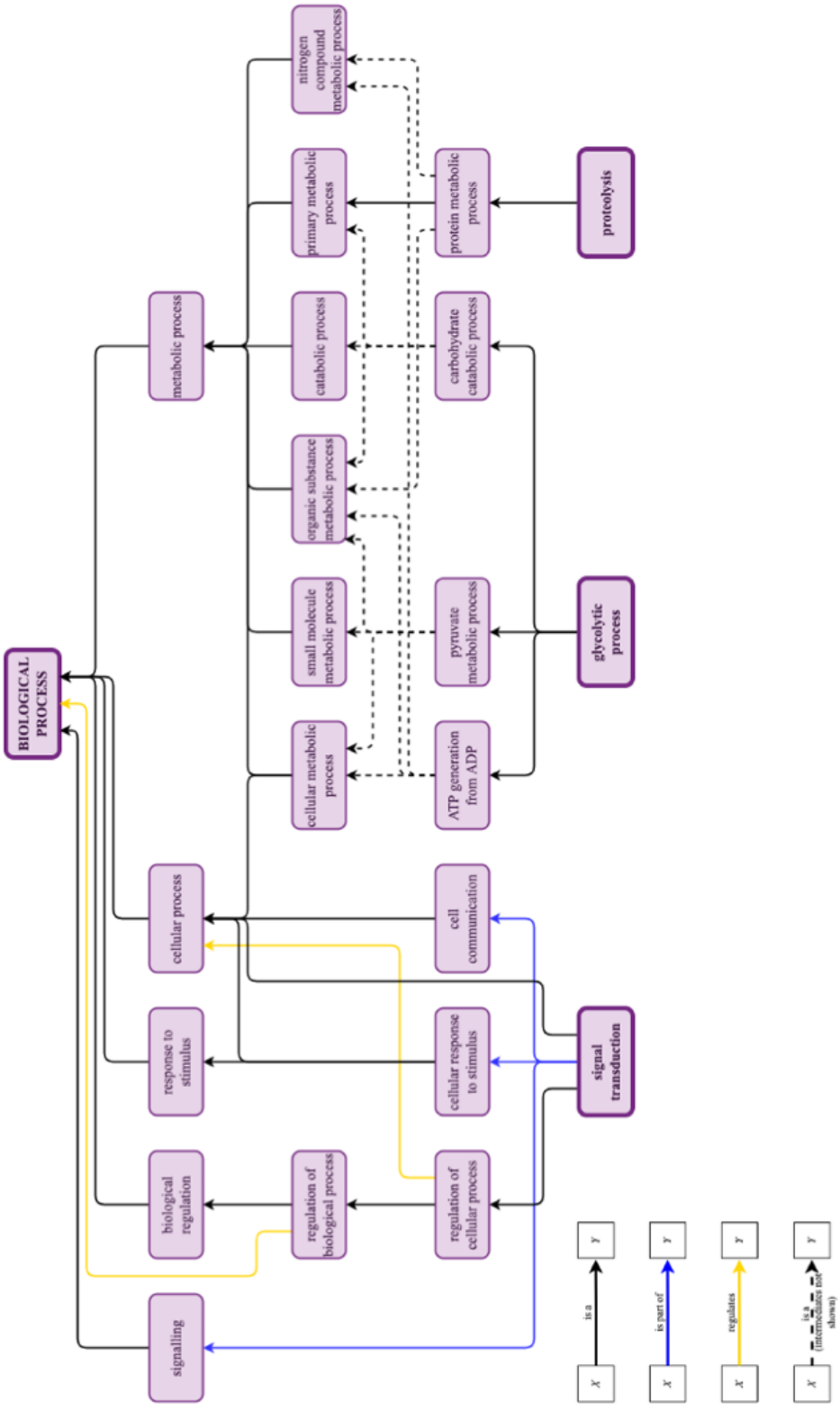

Figure S2.1 Gene ontology chart of the biological processes associated with the *D. siricidicola* putative parasitism and immunomodulation genes, namely signal transduction, glycolytic process and proteolysis.

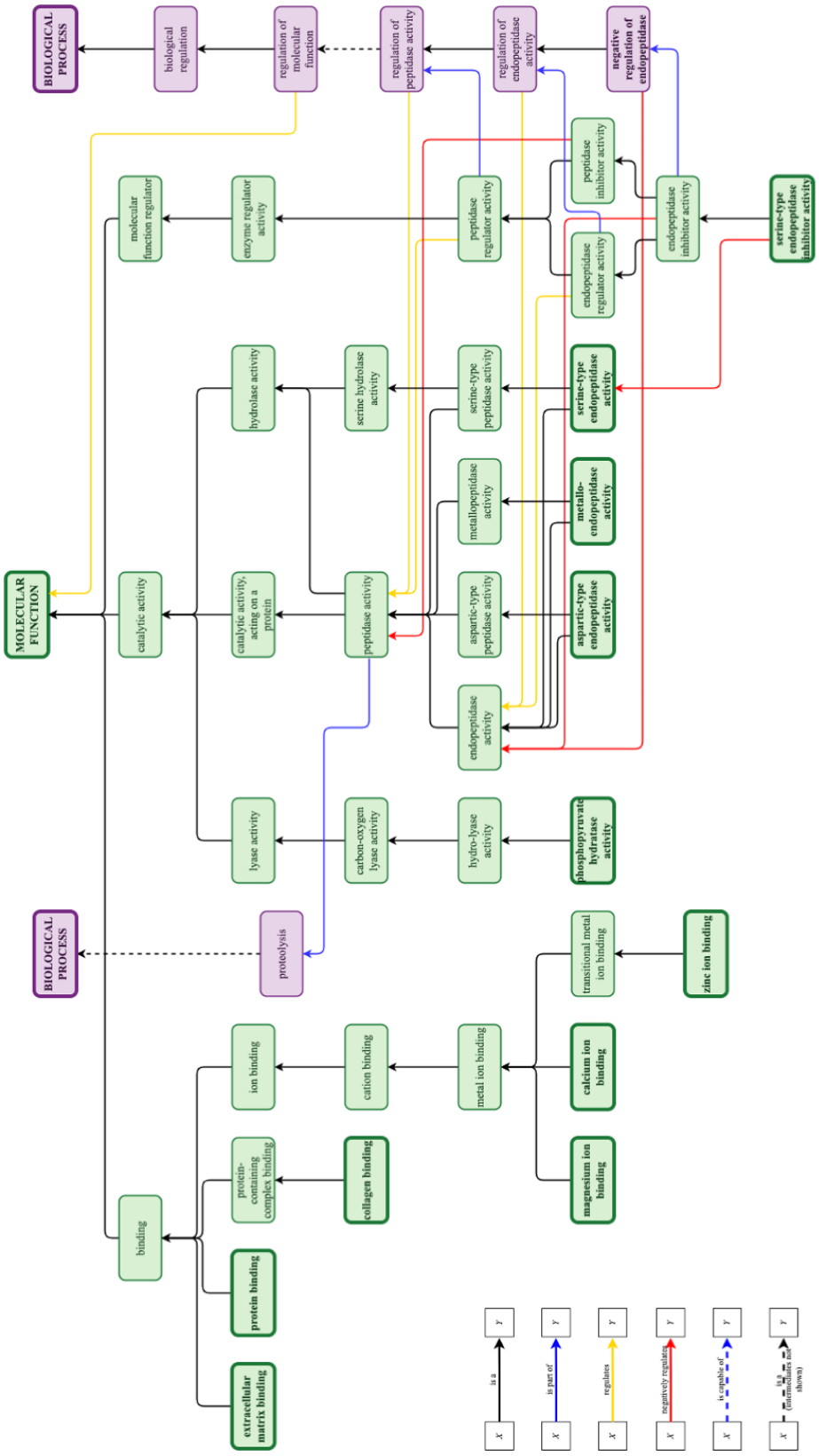

Figure S2.2 Gene ontology chart of the molecular functions (green boxes) associated with the *D. siricidicola* putative parasitism and immunomodulation genes and how these are connected to the biological processes (purple boxes).

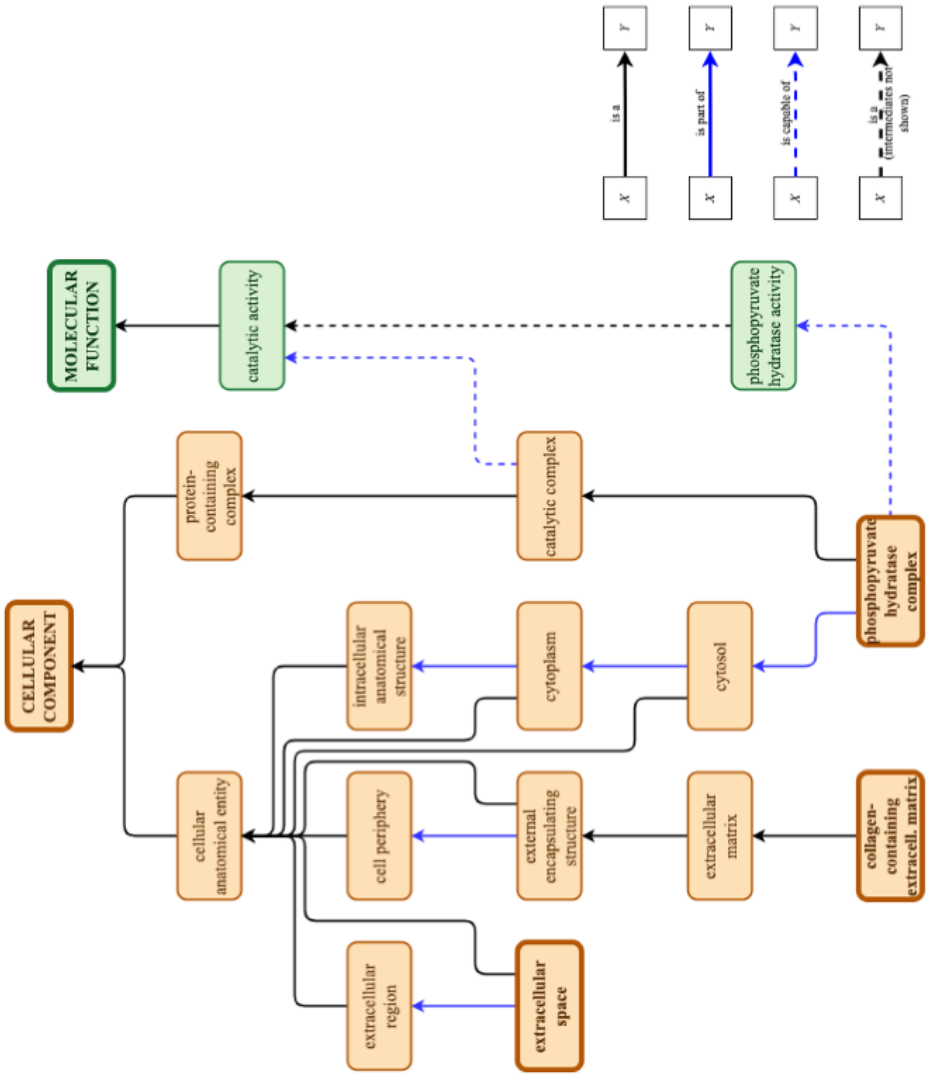

Figure S2.3 Gene ontology chart of the cellular components (orange boxes) associated with the *D. siricidicola* parasitism genes and how these are connected to the molecular functions (green boxes).

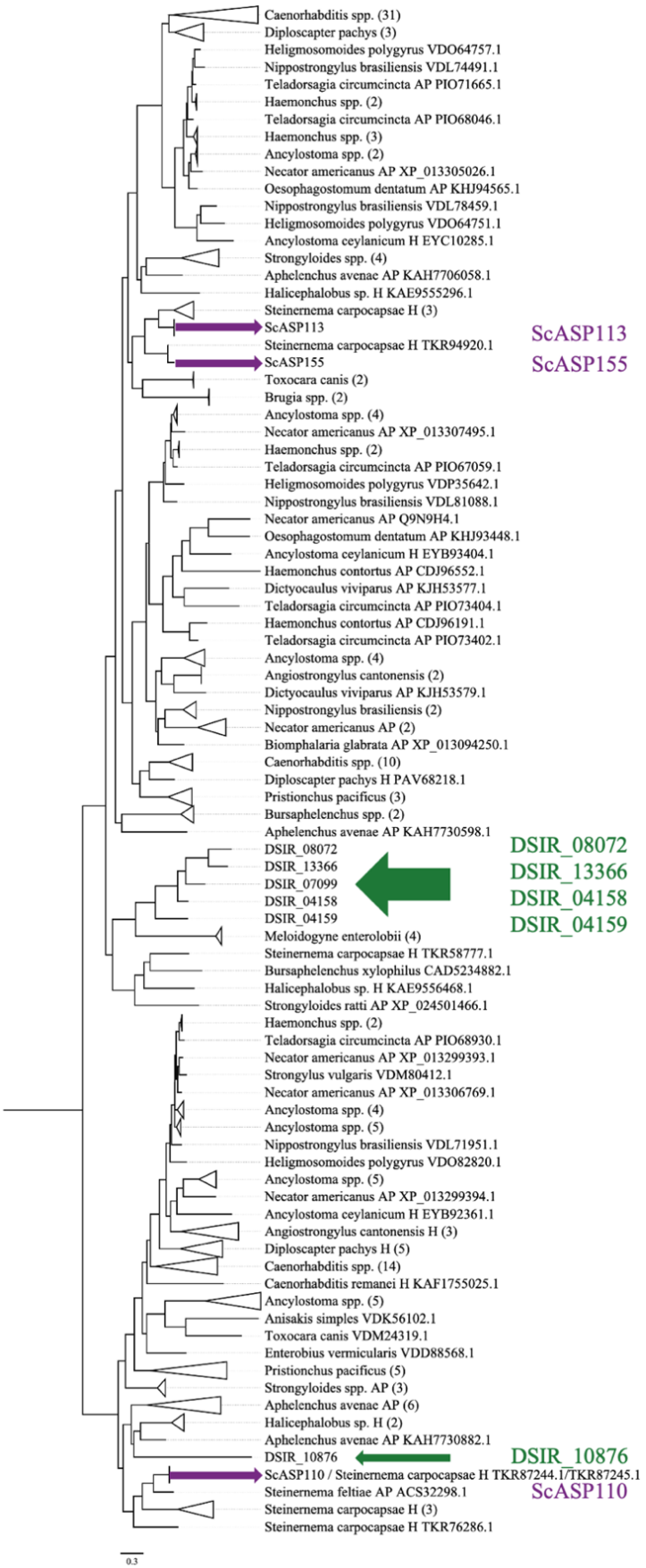

Figure S2.4 Maximum likelihood analysis comparing six *D. siricidicola* aspartic peptidases (green arrows) with

three aspartic peptidases from *S. carpocapsae* (purple arrows) and 200 NCBI BLASTp hits. Clades containing > 1 taxon from the same genus have been collapsed. Five of the *D. siricidicola* aspartic peptidases cluster together and fall in the same larger clade as orthologues ScASP113 and ScASP155, although only distantly. DSIR_10876 forms another clade with ScASP110. AP – aspartic peptidase; H – hypothetical protein; no letter after species name – unnamed protein product; (number) – number of taxa in the collapsed node.

Figure S2.5 Maximum likelihood analysis comparing eight *D. siricidicola* serine proteases (green arrows) with

six *S. carpocapsae* serine proteases (purple arrows) and 200 NCBI BLAST hits. Clades containing > 1 taxon from the same genus have been collapsed. DSIR_03388 falls in the larger of two clades (marked A), separate from the other *D. siricidicola* serine proteases and those from *S. carpocapsae*. Clade A is highly diverse with sequences from various species in the phylum Nematoda, as well as Annelida, Arthropoda, Chordata, Cnidaria, Echinodermata, Mollusca, and Platyhelminthes. Clade B (branch at node B not visible at this magnification but position indicated with dashed arrow) can be divided into two subclades (B_1 and B_2) with four of the *D. siricidicola* serine proteases grouping with serine proteases from a number of nematode species and one crustacean, *Pollicipes pollicipes* (*). In the group marked B_2, the six *S. carpocapsae* query sequences cluster together forming a sister clade to the remaining three serine proteases of *D. siricidicola*, 7 *Aphelenchus avenae*, and one *Bursaphelenchus xylophilus* serine proteases (orange text). SP – serine protease; H – hypothetical protein; no letter after species name – unnamed protein product; (number) – number of taxa in the collapsed node.

Figure S2.6 Maximum likelihood analysis comparing seven *D. siricidicola* serine proteases inhibitors (green

arrows) with two *S. carpocapsae* serine proteases inhibitors (purple arrows) and 200 NCBI BLAST hits. Clades containing > 1 taxon from the same genus have been collapsed. The Kazal serine protease inhibitor, DSIR_05892 is grouped with a hypothetical protein from the oomycete, *Phytophthora aleatoria* (*) and the Kunitz-type serine protease inhibitor of *S. carpocapsae* ScKU4. One of the *D. siricidicola* Kunitz-type serine protease inhibitors, DSIR_13362 is basal to this subclade (marked A_1). The other *D. siricidicola* Kunitz-type inhibitor, DSIR_04361 forms the base of a sister clade to A_1 (marked A_2) which also contains the two Kazal-type serine protease inhibitors DSIR_13365 and DSIR_05889. The Kazal/SPARC ("secreted protein acidic rich in cysteine") DSIR_10164 is in its own subclade (B_1) with SPARC sequences from various other organisms. The two serpin domain containing genes, DSIR_00450 and ScSRP6 are one another's closest relatives and cluster with an unnamed protein product of *Thelazia callipaeda*. SPI – serine protease inhibitor; H – hypothetical protein; no letter after species name – unnamed protein product; (number) – number of taxa in the collapsed node.

Chapter 2. Supplementary files

Supplementary material including nucleotide and amino acid sequences, commands and scripts, and higher resolution images are available at the following link: https://drive.google.com/drive/folders/18xqbdaZqqEnf_96A3l2QSjWlmgKSZvNJ?usp=sharing.

File S2.1 *Steinernema* parasitism and immunomodulation genes used as query sequences in local BLAST searches and protein clustering – Sequences/Steinernema_sequences/

File S2.2 Nematode protein sequences used in the OrthoFinder analysis – Sequences/WormBase_sequences/

File S2.3 Commands and scripts used for *in silico* analyses – /Commands_scripts/

File S2.4 Amino acid sequences of putative *D. siricidicola* parasitism genes after manual curation – Sequences/Deladenus_sequences/

File S2.5 Quality control reports for RNA sequencing data – Results/DSIR_RNAseq_MultiQC/

Chapter 3.

The relevance of studying insect-nematode interactions for human disease*

*Swart, Z., Duong, T. A., Wingfield, B. D., Postma, A., & Slippers, B. (2021) The relevance of studying insect-nematode interactions for human disease. *Pathogens and Global Health*, 116(3), 140-145.

Abstract

Vertebrate-parasitic nematodes cause debilitating, chronic infections in millions of people worldwide. The burden of these so-called “neglected tropical diseases” is often carried by poorer socio-economic communities in part because research on parasitic nematodes and their vertebrate hosts are challenging and costly. However, complex biological and pathological processes can be modelled in simpler organisms. Here I consider how insight into the interactions between entomopathogenic or insect-pathogenic nematodes (EPN), their insect hosts and bacterial symbionts may reveal novel treatment targets for parasitic nematode infections. I argue that a combination of approaches that target the nematodes, as well as the interaction of the pathogens with insect vectors and bacterial symbionts, offer potentially effective, but underexplored opportunities.

Keywords – alternative treatment options, entomopathogenic nematodes, inter-species interactions, model organisms, vector control, vertebrate-parasitic nematodes

3.1 Introduction

Vertebrate-parasitic nematodes (VPN) infect millions of people worldwide (James et al., 2018; WHO, 2007). These nematode infections are usually chronic and target a variety of organ systems – from the skin to the gastrointestinal tract (Blaxter 1998). Some of the most deleterious are the filarial nematodes that damage the lymphatic system – known as lymphatic filariasis – causing debilitating limb oedema (elephantiasis) and even renal damage (Bockarie et al., 2009; Taylor et al., 2010).

From a public health perspective, the main treatment strategy for filariasis is preventative chemotherapy in the form of Mass Drug Administration (MDA) (CDC, 1993; Molyneux & Zagaria, 2002; WHO, 2019a). Mass Drug Administration programmes administer antiparasitic medication to all members of a community at risk without first testing individuals for an infection. Mathematical models predicted that these regimens are not sufficient to keep the disease controlled in areas with high prevalence (Michael et al., 2004). Among the challenges to MDA is compliance as successful elimination requires at least five doses of anti-filarial drugs and one or more doses are often missed (Burgert-Brucker et al., 2020; de Souza et al., 2020). Only two combinations of three different drugs are available to treat filariasis and all three target the larval stage of the nematodes only, leaving adult worms unaffected (Ottesen et al., 1997; WHO, 2011).

The wide-spread use of a limited number of anti-filarial medications also carry the risk of drug resistance developing in these nematodes (Michael et al., 2004; Schwab et al., 2005). With antimicrobial resistance on the WHO's list of top ten threats to global health, it is crucial to optimise additional/alternative treatment options (Friedrich, 2019; WHO, 2019b). The interactions between nematodes, insects and bacteria – concerning both nematodes responsible for human disease and insect-parasitic nematodes – provide opportunities to explore such alternatives.

3.2 Nematode-insect-bacterial interactions

Nematodes capable of infecting and killing insects are known as entomopathogenic nematodes (EPN) (Lewis & Clarke, 2012; Torres-Barragan et al., 2011; Zhang et al., 2008).

Entomopathogenic nematodes are used as beneficial biological control agents of insect pests, providing an alternative to expensive, broad-spectrum, chemical insecticides (Benseddik et al., 2021; Coppel & Mertins, 1977; Vega et al., 2012). Nematodes from the *Heterorhabditis* and *Steinernema* genera are frequently used in biocontrol and are therefore the EPN most commonly studied (Dillman & Sternberg, 2012; Kaya & Gaugler, 1993). In addition to playing host to nematodes, insects are often involved in the nematode life cycle as intermediate hosts or vectors (Katiyar & Singh, 2011; Ryss et al., 2005). Dispersal by an insect vector is a characteristic of many animal and some plant-parasitic nematodes.

Bacteria often play a role in nematode-insect interactions. *Heterorhabditis* and *Steinernema* coevolved with bacteria in the genera *Photorhabdus* and *Xenorhabdus* to become virulent insect pathogens (Adams et al., 2006; Boemare, 2002). The bacteria contribute to a suitable environment for nematode development and multiplication by killing and digesting the insect host and preventing other micro-organisms from colonising the carcass (Brivio et al., 2005). In the case of parasitic nematodes causing human diseases such as filariasis, an intracellular endosymbiont, *Wolbachia*, is involved in the nematode's survival and reproduction (Taylor et al., 2005, 2010). Furthermore, *Wolbachia* also influences many insects' biology, either as mutualist or as pathogen (Hughes & Rasgon, 2012; Werren et al., 1995).

The relative ease with which insects and nematodes can be cultured and manipulated – with or without their bacterial symbionts – make them useful models for observing interspecies relationships (Hallem et al., 2007; Stock, 2005). Host-parasite interactions such as the insect's immune response to invasion and how the nematode overcomes the immune response can be investigated through studying parasitic nematodes and their insect hosts and vectors (Brivio & Mastore, 2018; Cooper & Eleftherianos, 2016). Parasitic nematodes and symbiotic bacteria also provide opportunities to study factors influencing mutualism, such as the evolution of biochemical communication between host and symbiont (Ciche & Sternberg, 2007; Ruby, 2008).

The value of studying the interactions between insects and their parasites extends beyond gaining insight into the particular pest or biocontrol management system. These investigations may also reveal novel treatment strategies for challenging human conditions. For instance, the insect vectors and bacterial symbionts of parasitic nematodes present promising targets for combatting these infections in humans. Additionally, the systems that insect parasitic

nematodes use to evade and even suppress the host immune response are increasingly well studied (Angeles et al., 2020; White et al., 2020). This not only enables the nematodes to survive and cause disease within the host, but to influence co-existing infections as well as non-infectious conditions of the host. The close phylogenetic relatedness amongst human and insect infecting nematodes, as well as the presence of orthologous genes involved in virulence and defence, mean the organisms involved in entomopathogenic nematode parasitism (nematode parasite, insect host, bacterial symbiont), can be used as simpler models to study nematode infections in humans.

3.3 Insect and bacterial options to manage nematode infections

3.3.1 TARGET THE INSECTS

Filarial nematodes are transmitted to their vertebrate hosts by mosquitoes of different genera (Bockarie et al., 2009). Consequently, transmission can be interrupted by targeting the insect vector. Vector control usually consists of spraying insecticides inside homes and distributing netting material impregnated with long-lasting insecticides (Pedersen & Mukoko, 2002; WHO, 1984). Other vector control strategies target the source of mosquitoes, for instance, polystyrene beads that form floating layers on potential breeding sites such as pit latrines and water tanks suffocate mosquito larvae, leading to a drastic decline in the adult mosquito population (Curtis et al., 2002; Maxwell et al., 1990, 1999). Combined vector control and MDA suppress the transmission of filariasis more effectively and with less resurgence than MDA alone. A focus on integrated vector management in addition to MDA was therefore included in the strategic plan for 2010-2020 of the Global Programme to Eliminate Lymphatic Filariasis (WHO, 2010).

Effective vector control also impacts diseases that co-exist with filariasis, for instance malaria and dengue fever which are transmitted by the same mosquitoes (Manga, 2002). Unfortunately, wherever chemicals are used, the risk of resistance developing exists and resistance to a number of insecticides have been documented (Rodriguez et al., 1993; WHO, 1984). Similarly, the use of polystyrene beads is not fool-proof as all the potential mosquito breeding sites in a community have to be identified and treated, its use is limited to smaller bodies of still-standing water, and it is not effective for all mosquito species. Flooding of pits containing these

polystyrene beads leads to unsightly pollution and loss of larvicidal function (Curtis et al., 2002).

With an increasing number of insect genomes being sequenced and made available in public databases, together with the development of advanced gene-editing tools, gene modification provides an alternative to traditional chemical or environmental vector control measures (Kim & Kim, 2014; Yin et al., 2016). Genetically-modified mosquitoes are already being released to control mosquito populations responsible for the spread of dengue fever, for example (Carvalho et al., 2015; Lacroix et al., 2012). Releasing transgenic organisms is of course not without risks. Modified genes might be transmitted to the wild-type population and changes in the wild-type population could affect the virulence of the vector-borne pathogen. Molecular insight into the interactions between parasite, vector and bacterial symbiont are therefore important, not only to discover additional treatment targets, but also to ensure the safety of existing and developing control measures (Castillo et al., 2011).

3.3.2 TARGET THE BACTERIA

The nematode species responsible for the majority of filariasis all rely on an intracellular bacterium for development and reproduction (Taylor, Bandi, et al., 2005; Taylor et al., 2010). The bacterial symbiont, *Wolbachia*, belongs to the order Rickettsiales – the same order containing *Rickettsia* species associated with tick-bite fever and other spotted fevers. The drugs used to treat rickettsia infections, especially doxycycline, successfully suppress filarial infections (Mand et al., 2012; Taylor, Makunde, et al., 2005). Unfortunately, a course of treatment with doxycycline lasts six to eight weeks and cannot be used in pregnant women or children.

By targeting the bacteria and its molecular pathways, instead of the eukaryotic pathways of nematodes, drugs with potentially fewer adverse reactions on humans can be developed. The combination of high-throughput assays and bioinformatics tools facilitate the screening of millions of compounds for desirable properties (Clare et al. 2015). One such study identified five compounds with potential fast-acting anti-*Wolbachia* activity (Clare 2019). These compounds can now be tested in animal and clinical trials.

Wolbachia bacteria also colonise many insect species and other arthropods, either as mutualists or pathogens (Hughes & Rasgon, 2012; Jeyaprakash & Hoy, 2000; Werren et al., 1995). *Wolbachia* endosymbionts influence the host insect's reproductive fitness and can increase the fertility of infected females or cause sterility in males (Miller et al., 2010). Artificial infection of previously uninfected insects can be lethal or reduce the capability to vector certain pathogens (Moreira, Iturbe-Ormaetxe, et al., 2009; Moreira, Saig, et al., 2009; Suh et al., 2009). The ability of *Wolbachia* to alter insect reproduction, earns them a place in vector control and these bacteria are already being investigated for use against malaria, dengue fever and lymphatic filariasis (Brelsfoard et al., 2008; Hughes et al., 2011; Turley et al., 2009). As in the case of insect vectors and nematode parasites, the molecular mechanisms underlying these interspecific interactions are in need of further investigation (Hughes & Rasgon, 2012).

3.4 The use of nematodes and insects as mini-host models

3.4.1 MODELS FOR NEMATODE INFECTIONS IN HUMANS

Nematode infections of humans are regarded as "neglected tropical diseases" (WHO, 2019a). Especially poorer socio-economic communities carry the burden of the filarial diseases (Brelsfoard et al., 2008; O'lorcain & Holland, 2000; WHO, 2007). In order to "Ensure healthy lives and promote well-being for all at all ages", the eradication of neglected tropical diseases forms part of the 2030 Agenda for Sustainable Development (UN, 2015). Research into filariasis is, however, hampered by the cost and complexity of studying infections in their vertebrate hosts.

The use of simpler organisms to study complex biological and pathological processes is not new. The free-living nematode, *Caenorhabditis elegans* has since the 1960s been put to use in the investigation of human conditions ranging from neurological degeneration and aging to metabolic diseases and cancer (Markaki & Tavernarakis, 2010; Tissenbaum, 2015). Genes involved in the pathogenicity of medically important fungi, including *Candida* spp. and *Cryptococcus* spp., play similar roles when infecting and killing model invertebrates such as *Drosophila melanogaster* and *C. elegans* (Chamilos et al., 2007). Subsequently, susceptible invertebrates present the opportunity to study fungal virulence mechanisms and even test antifungal treatment without exposing patients to added risks.

Both VPN and EPN suppress the immune responses of their host (McSorley et al., 2013; Navarro et al., 2013). As VPN and EPN are closely related phylogenetically (Bai et al., 2013; Blaxter & Koutsovoulos, 2015), orthologues of genes associated with host immunosuppression can be found in both types of nematodes (Lu et al., 2017). Insect-pathogenic *Heterorhabditis bacteriophora* shares ancestral traits with free-living *C. elegans* but is phylogenetically positioned closer to the mammal-parasitic nematodes. *Heterorhabditis bacteriophora*, therefore, represents a "bridge" species to translate existing knowledge of molecular pathways in *C. elegans* and other EPN, to VPN (Bai et al., 2013). Compared to mammalian parasites, EPN culturing requires fewer resources in terms of laboratory equipment and personal protection, as well as host animals. As a result, entomopathogenic nematodes and their insect hosts offer an alternative option to study nematode infections of humans and other mammals.

3.4.2 Models for bacterial infections in humans

Knowledge on interspecies interactions gained from studying EPN systems is not limited to the field of nematode infections. The symbiotic bacteria of EPN represent as important models to study bacteria-host interactions, as nematode-host interactions (Lewis & Clarke, 2012). Bacteria from the genera *Photorhabdus* and *Xenorhabdus* (the symbionts of *Heterorhabditis* and *Steinernema*, respectively) form part of the Enterobacteriaceae (Tailliez et al., 2010). Other members of this family include the common human pathogens, *Escherichia coli*, *Salmonella* spp., *Yersinia* spp. and *Proteus* spp. In fact, *Proteus mirabilis* – one of the most common causative agents of urinary tract and hospital-acquired infections (Armbruster & Mobley, 2012; Chen et al., 2012) – is the closest phylogenetic relative to *Photorhabdus* and *Xenorhabdus*. Therefore, an understanding of pathogenicity in the entomopathogenic bacteria can contribute to a search for similarities in the human pathogen. The discovery of such orthologous virulence pathways could reveal strategies for the prevention and treatment of *P. mirabilis* infection in humans.

3.5 Conclusion

Insight into the interactions at play within one multi-species system will benefit the improvement or control of the system in question, but could also prove applicable in other

settings. The current treatment strategies that only target the nematodes responsible for human infection are unlikely to relieve the burden of chronic, debilitating disease in areas with high prevalence (Stolk et al., 2018). However, a combination of approaches that also control or manipulate the interaction with insect vectors and bacterial symbionts, has a better chance of being effective and well-tolerated.

Insect- and vertebrate-parasitic nematodes both suppress the host immune response, but EPN are much easier, safer, and cheaper to culture than human pathogens. Although nematode-insect models may not mimic human diseases in every respect, simpler systems do make the application of genetic and molecular techniques easier in order to dissect pathogen-host interactions (Markaki & Tavernarakis, 2010).

3.6 Disclosure statement

No potential conflict of interest was reported by the authors.

3.7 Funding

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3.8 References

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Summary

Nematodes often rely on insects – as hosts for entomopathogenic nematodes, or as vectors to reach the target plant or vertebrate host. In either case, the nematode faces a robust host immune response which it must withstand/overcome in order to survive in the host. The immunomodulatory mechanisms of parasitic nematodes include immune evasion and immune suppression. Immune evasion aims to avoid recognition by the host immune system, preventing the subsequent activation of immune responses. The nematode cuticle plays an important role in immune evasion as molecules on the surface of the nematode can either resemble antigens of the host, or are sequestered from the host haemolymph to prevent the nematode from being recognised as "non-self". Immune suppression involves the excretion/secretion of molecules that actively interfere with the host immune responses. Immunosuppressive molecules may damage immunocompetent cells and cell-surface pathogen recognition proteins and/or neutralise humoral immune factors. A number of genes encoding parasitism and immunosuppressive molecules have been characterised in *Steinernema carpocapsae* and *S. glaseri*. Humoral encapsulation is an important defence mechanism against eukaryotic parasites such as nematodes. As such, many immunosuppressive molecules interfere with humoral encapsulation by hindering the function of host blood cells responsible for encapsulation, by inhibiting prophenoloxidase to reduce the production of melanin, by preventing the incorporation of melanin into the capsule, or by a combination of these mechanisms.

I was able to identify and characterise 23 genes in the genome of *D. siricidicola* that are potentially involved in parasitism and immunosuppression of the host, *S. noctilio*. Based on the functional classification of these genes, three biological processes appear to be of significance for parasitism, namely proteolysis (endopeptidase activity), regulation of endopeptidase activity, and glycolysis. Expression of all 23 genes have been confirmed during the free-living stage of *D. siricidicola* but further studies are needed to compare expression between free-living and parasitic conditions with greater accuracy.

Entomopathogenic nematodes, insect hosts, and bacterial symbionts represent helpful models for the study of interspecific interactions. Insight into these interactions are not only of importance for the biocontrol system in question, but could also be used to unravel complex

biological and pathological processes associated with nematode infections in humans and other vertebrate hosts.
