The immune response of *Sirex noctilio* to pathogen infection

by

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Submitted in partial fulfilment of the requirements for the degree MSc Genetics

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

2021

Declaration

I **Malehlogonolo Sophie Makua** 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.

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Preface

Over 50% of the forestry plantations in South Africa are planted with *Pinus* species. They are valued for their solid timber, pulp, paper, oils and as biofuel. Insect pests pose a threat to pine plantation forestry. One of the most significant insect pests is the woodwasp *Sirex noctilio* (Hymenoptera: Siricidae), which is estimated to have caused hundreds of millions of rands of losses in the forestry sector. Females kill pine trees by depositing their eggs, venom and its symbiotic white rot fungus, *Amylostereum aerolatum*, into the wood. *Sirex noctilio* and its symbiotic fungus are invasive in the Southern Hemisphere, including South Africa, where it is introduced. In its native range, in Europe, North Africa and parts of Asia, it is of less concern.

Due to the economic impact of this pest, means to improve its management in the Southern Hemisphere has been the focus of studies for more than a century. Currently, the most effective strategy to manage *Sirex noctilio* is through the biocontrol nematode, *Deladenus siricidicola*. This nematode has a free-living stage that reproduces in wood while feeding on *Amylostereum areolatum*. The nematode also has a parasitic phase during which it infects *Sirex noctilio* larvae and eventually also the developing eggs. As a result, infected adult female woodwasps lay packets of nematodes into trees instead of viable eggs. In some areas, such as South Africa and South America, a lower-than-expected nematode parasitism has created the need to improve the selection of effective biological control strains.

One of the possible reasons for variable infection rates is differences in the interaction between *Sirex noctilio* and *Deladenus siricidicola*, where some populations of the wasp is more resistant against the current strain of the nematode. A better understanding of the mechanisms underlying this interaction would be helpful to address this question.
The aim of the study was to characterize potential immune-related genes of *S. noctilio* and identify which of these genes are regulated by *D. siricidicola* parasitization, in comparison to infection by the entomopathogenic fungus *Beauveria bassiana* and a wounding control. We use both genome and transcriptome sequence data to explore these immunity pathways of this economically important, non-model insect.

Chapter one is a review of the literature on the mechanisms of innate immunity within Hymenoptera. Here I focus on the genes, proteins and behavioural mechanisms involved in Hymenoptera immunity. This chapter begins by illustrating how Hymenoptera deal with invading pathogens, from mechanisms recognizing the pathogens to how they are eliminated. The chapter also describes *Sirex noctilio* as an important forestry pest, which include its symbioses, life history and population control.

In Chapter two, homology-based approaches are used to investigate the composition of innate immunity orthologs of the invasive woodwasp, *S. noctilio*, in comparison with information from other Hymenoptera. The comparison with orthologs from other Hymenoptera species is said to provide clues on the composition of the conserved immune signalling pathways, as well as the more rapidly continuously evolving recognition and effector components in *S. noctilio*. Alignment tools were used to identify putative immune-related gene orthologs from the *S. noctilio* genome and protein databases. Expression patterns of the putative immune-related genes were characterized in *S. noctilio* larvae in response to *D. siricidicola* infection, *B. bassiana* infection and wounding.

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Chapter 1

Innate immunity mechanisms in Hymenoptera

1. Introduction

The Hymenoptera is amongst the most diverse insect orders known to science with various lifestyles and impacts on agriculture (Sharkey 2007). Hymenoptera include various well-known groups such as wasps, bees and ants (Aguiar *et al.* 2013). Some insects in the order are of ecological, agricultural and economic importance (Sharkey 2007). For example, bees play a crucial role in most ecosystems as pollinators of flowering plants, which is of importance for the agricultural sector (Michener 2000). Certain parasitic wasps play an integral role in pest management as they act as biological control agents of harmful insect pests (Machtinger *et al.* 2015).

There are several members of the Hymenoptera that are invasive pests, responsible for causing disastrous damage to plants worldwide. They, therefore, have significant negative impact on the economy and environment (Holway *et al.* 2002; Lach and Thomas 2008). An example of this is the invasive wasp species, *Sirex noctilio* that is considered the most harmful pest of pine trees in plantation forests (Slippers et al. 2015). These trees are amongst the dominant plantations in South Africa and elsewhere in the world (FSA 2009) (Slippers *et al.* 2015).

Insects are vulnerable to all sorts of harmful microorganisms (Gupta *et al.* 2015). Insects, including hymenopterans, are lacking an adaptive immune system, they must rely solely on innate immunological processes or external immune defences to survive (Brennan and Anderson 2004). Physical barriers, humoral responses, and cellular responses are all part of the insect's innate immune system (Lavine and Strand 2002; Kanost *et al.* 2004). Physical barriers, which include the principal passive protective barriers such as the cuticle and the peritrophic membrane in the gut, serve as the initial line of defence (Ashida and Brey 1995; Hegedus *et al.* 2009). Invading pathogens that breach these barriers are met with immediate-response defences such as phagocytic cells, phenoloxidase activity, and reactive oxygen species (Jiang *et al.* 2010; Browne *et al.* 2013; Vlisidou and Wood 2015). As a second line of defence, a potent antimicrobial immune response occurs, which is mostly based on antimicrobial peptides (AMPs), however, it also comprises

serine proteases, stress factors, and opsonization and clotting factors (Bulet *et al.* 2004; Lemaitre and Hoffmann 2007).

Pattern recognition receptors (PRRs) identify invading pathogens by recognizing conserved structural motifs in microorganisms such as meso-diaminopimelic acid (DAP) or lysine (Lys)-containing peptidoglycan (PGN) of Gram-negative or Gram-positive bacteria, respectively (Lemaitre and Hoffmann 2007). Microbe-associated molecular patterns (MAMPs) are the conserved molecular patterns of invading pathogens that are detected (Royet and Dziarski 2007). The PRRs subsequently interact with cellular signalling pathways such Toll-like receptors (TLRs) Toll, Immune Deficiency (IMD), Janus kinase (JAK)-signal transducer and activator of transcription (JAK-STAT), and c-Jun N-terminal kinase (JNK), which leads to the activation of an immune response (Ferrandon *et al.* 2007; Leulier and Lemaitre 2008).

The hymenopteran societies can be either eusocial or solitary and these lifestyles influence the properties of their immune system against pathogens. Due to their greater genetic relatedness and large population densities, eusocial hymenopterans are more likely to be cross-infected by pathogens (Cremer *et al.* 2007; Stroeymeyt *et al.* 2014; Meunier 2015; Cremer *et al.* 2018). As a result, these species have evolved a variety of defence systems, including behavioral mechanisms, to combat and limit the spread of infections within their colonies (Evans *et al.* 2006). On the other hand, solitary hymenopterans rely solely on the innate immune system to combat infections (Evans *et al.* 2006; Cremer *et al.* 2018).

This review gives an overview of the current knowledge about the mechanisms of the immune responses in Hymenoptera, these include both humoral and cellular responses to bacteria, fungi and parasites, specific receptors that recognize pathogen invasions and signalling pathways that activates genes for antimicrobial peptides synthesis. In addition, we discuss the *Sirex noctilio* insect pest and its parasitic nematode *Deladenus siricidicola*, and how a better understanding of the immune response of the wasp to the nematode can contribute to better population management.

2. Background to the Hymenoptera

Hymenoptera (sawflies, wasps, ants, and bees) is one of the four most diverse holometabolous insect orders, with over 153 000 documented species and an estimated one million undescribed extant species (Grimaldi *et al.* 2005; Aguiar *et al.* 2013). The paraphyletic Symphyta and the monophyletic Apocrita have traditionally been used to divide Hymenoptera taxa (**Figure 1.1**) (Peters et al. 2011). Symphytans are further subdivided into the sawflies and the woodwasps (Davis *et al.* 2010). The members of the symphyta have complete venation, phytophagous larvae and their adults lack a petiole. Apocrita is separated into two groups: parasitica (parasatoids) and Acuelata (acuelata) (stinging wasps, bees and ants) (**Figure 1.1**) (Davis *et al.* 2010). Apocritans are characterized by a stalk between their abdominal segment and thorax (Sharkey 2007).

The transition from an ancestral ectophytophagous lifestyle, which was retained by the majority of sawflies ("Symphyta"), to parasitoidism, a lifestyle in which a larva develops by feeding on and killing a single host, is primarily responsible for Hymenoptera diversification (Dowton and Austin 2001; Whitfield 2003; Mrinalini and Werren 2017; Peters *et al.* 2017). The Hymenoptera's diverse lifestyles necessitate not only physiological adaptations for a wide range of food sources, but also the evolution of diverse defence mechanisms hostile to other organisms (Kaltenpoth *et al.* 2014).

Hymenoptera are of ecological, agricultural and economic importance. For example parasitoid Hymenoptera are often used in biological control programs to control insect pest populations (Wilson 1971). Aculeate Hymenoptera, particularly bees, are important pollinators of flowering plants and producers of honey (Michener 2000). Ants are important decomposers, herbivores, and predators (Hölldobler and Wilson 1990). Army ants and paper wasps are important predators in the ecosystem (Hanson 2016). There are also a number of pests amongst the Hymenoptera, including woodwasps (Siricidae), some leaf feeders (Tenthredinoidea), stinging wasps (Vespidae) and bees (Apoidea) (Zhang *et al.* 2007).

3. Hymenoptera immune system and its components

The evolutionary biologist Leigh van Valen (Van Valen 1973), in his Red Queen theory proposed that evolution must continuously occur in organisms for it to sustain itself in the changing ecosphere. In terms of the host-parasite interaction, this means that in order to survive, both the insects and their pathogens must constantly improve their defence mechanisms. This coevolution, also known as the "arms race," has resulted in a plethora of interaction strategies between the invading host and the pathogen (Dawkins and Krebs 1979). Insects, including the hymenopterans have anatomical and physiological barriers that provide protection against invading pathogens. These include the exoskeleton, cuticle, tracheal tubes, and intestinal mucosa. They provide the insects with protection against infection and mechanical injury (Moussian 2010). When the physiological barriers are broken, the immune response is activated.

Hymenoptera like other insects relies on the innate defence mechanisms to recognize and clear infections (Vallet-Gely *et al.* 2008; Otti *et al.* 2014). There are two types of innate immune responses: humoral and cellular defence responses. Cellular responses rely on haemocytes, which are blood cells that can engulf invading pathogens via phagocytosis or trap them in multicellular structures known as capsules and nodules (Lavine and Strand 2002). The production of defence molecules and the prophenoloxidase cascade are examples of humoral immune responses. These defence molecules are reactive oxygen and nitrogen intermediates, as well antimicrobial peptides (AMPs) with antifungal and antimicrobial properties (Nappi and Ottaviani 2000). Insect's humoral immune responses also involve complex enzymatic cascades that control melanisation of the haemolymph. Melanin is produced at the site of injury because of the melanisation process, as well as during the nodulation and encapsulation processes. The physical barriers together with cellular and humoral immune responses are powerful tools that act synergistically to neutralize pathogens and parasites (Schmid-Hempel 2003). In summary, immune responses consist of a series of events that can be divided into three stages: 1) pathogen recognition, 2) signalling pathway activation, and 3) effector mechanisms aimed at pathogen elimination (Figure1.2) (Guzman-Novoa 2011). The recognition process, in which pathogen associated molecular patterns (PAMPs) are detected by PRRs, activates

the immune response. Core signalling pathways are activated in response, resulting in the production of effectors and receptors involved in cellular and humoral immune responses (Dubovskiy *et al.* 2016).

3.1 Recognition of pathogens

Insects' ability to fight off invading pathogens is largely dependent on pathogen recognition as non-self and then activation of the appropriate innate immune response (Yano and Kurata 2011). Thus, the detection of conserved pathogen motifs is required for the activation of innate immunity in response to pathogens (Hillyer 2016). In innate immunity, PAMPs are detected to recognize the invading pathogen. These are pathogen components that are conserved, such as lipopolysaccharides (LPS), peptidoglycan (PGN), lipoteichoic acids, 1,3 glucans, integrins, flagellin, and nucleic acids from viruses, bacteria, or fungi, and are essential for pathogen survival but are not found in higher eukaryotes (Yano and Kurata 2011; Murphy and Weaver 2016).

Recognition of the invading pathogen is mediated by receptor proteins PRRs, which can detect the conserved PAMPs. The pattern recognition receptors are produced by cells and tissues in the hemocoel (Schluns and Crozier 2009). The attachment of PRRs to the invaders' PAMPs triggers the humoral and cellular immune responses. Phagocytosis, encapsulation, opsonization, melanisation, coagulation, and the synthesis of AMPs, reactive oxygen and nitrogen species, and some proteins with lytic activities are all part of the immune system process (Schmid-Hempel 2003). Through these processes, the invading pathogen is secluded and eventually killed. Pattern recognition receptors are classified into various protein families that have been found to be evolutionarily conserved. These protein families have a high level of diversity, which could be attributed to differences in the ecology of the members of the class Insecta (Hillyer 2016). Peptidoglycan recognition proteins (PGRPs), β-1,3-glucan recognition proteins (also known as Gram-negative binding proteins), the nimrod superfamily, C-type lectins, galectins, scavenger receptors, fibrinogen-related proteins (FREPs), thioester-containing proteins (TEPs), and leucine-rich repeat-containing proteins are the different classes of pattern recognition receptors in insects (Zhang and Gallo 2016). Many pathogen

recognition protein families have expanded or contracted in different taxa. In Hymenoptera, for example, *Nasonia vitripennis*, *Apis mellifera*, *Bombus terrestris*, *Componotous floridanus*, and *Megachille rotundata* have 12, 4, 4, 4, and 2 members of the peptidoglycan recognition protein family, respectively (Evans et al. 2006; Xu and James 2009; Sackton et al. 2013; Gupta et al. 2015).

Peptidoglycan recognition proteins (PGRPs) are innate immunity molecules with a type 2 amidase domain that binds peptidoglycans. Previous research revealed that the domain is a homolog of bacteriophage and bacteria type 2 amidase (Dziarski and Gupta 2006). These proteins are classified into two types in insects, including hymenopterans: short (S) and long (L) (Dziarski 2004). Short-form PGRPs (PGRP-S) are extracellular proteins with a short length and a signal peptide. Long PGRPs (PGRP-L) are typically longer, lack a signal peptide, and are either intracellular, extracellular, or membrane-spanning proteins (Dziarski and Gupta 2006). A few PGRPs recognize and bind to Lys-type peptidoglycan and others to DAP-type peptidoglycan. The PGRP-SA, PGRP-SC1, and PGRP-SD proteins are involved in the recognition of Gram-positive bacteria that contain Lys-type peptidoglycan, which results in the activation of the Toll pathway or the melanisation process (Takehana *et al.* 2002). The PGRP-LB, PGRP-LC, and PGRP-LE recognize Gram-negative bacteria with DAP-type peptidoglycan and activate the immunodeficiency (IMD) pathway (Choe *et al.* 2002; Gottar *et al.* 2002; Rämet *et al.* 2002).

β-1,3-glucan recognition proteins (also known as Gram-negative binding proteins) are thought to be able to recognize Gram-positive bacteria and Gram-negative bacteria (Lemaitre and Hoffmann 2007)(Ma and Kanost 2000). It is thought that β-1,3-glucan recognition proteins form a complex with peptidoglycan recognition proteins (Gerardo et al. 2010). Following complex formation, β-1,3-glucan recognition proteins hydrolyze Gram-positive peptidoglycans into tiny fragments that can then be recognized by peptidoglycan recognition proteins (Lemaitre and Hoffmann 2007). This implies that an insect must have both β-1,3-glucan recognition proteins and peptidoglycan recognition proteins to detect bacteria.

Lectins are a class of sugar-binding proteins that play a role in immune-related reactions that allow organisms to distinguish between self and non-self (Gerardo et al. 2010). These proteins are characterized by various binding activities. Numerous lectins play a role in recognition in insect immunity by binding to polysaccharide chains on the surface of the invading pathogen (Tanji et al. 2006). The most common are the C-type lectins which consist of a variety of soluble and membrane-bound proteins. In several insects, lectins are involved in the activation of prophenoloxidase, nodule formation and phagocytosis (Ao et al. 2007).

Galectins are a set of lectins that are extensively distributed in insects. Insects galectins are believed to be playing a role in either recognition of pathogens through the detection of β-galactoside, or in phagocytosis (Gerardo et al. 2010). Fibrinogen-related proteins (FREPs) have a carboxyl-terminal fibrinogen-like domain linked to a variety of amino-terminal regions. In insects including the hymenopterans, fibrinogen-related proteins play a role in cell to cell interaction, detection of bacteria and antimicrobial responses (Zou et al. 2007).

Several Nimrod members appear to be phagocytosis and bacterial binding receptors (Lazzaro 2005). The Nimrod superfamily genes in insects include eater and nimrod (Gerardo et al. 2010). The Nimrod superfamily genes are distinguished by a specific EGF (epidermal growth factor) repeat and are found in *D. melanogaster* and *A. mellifera* genomes (Somogyi *et al.* 2008). Hemolin is part of the immunoglobulin superfamily. In insects, hemolin recognize and attach to lipopolysaccharides on Gram-negative bacteria and lipoteichoic acid on Gram positive bacteria, resulting in their aggregation (Daffre and Faye 1997; Yu and Kanost 2002). Lipopolysaccharides and lipoteichoic acid bind to the hemolin molecule at the same site. Hemolin act as a broad-spectrum pattern recognition receptor for infection by binding to glycolipids in bacterial cell walls (Tsakas and Marmaras 2010). Integrins are surface proteins that play a role in migration, adhesion and tissue organization (Hughes 2001). These surface proteins recognize and bind amino-acid triplet Arg-Gly-Asp (RGD motif) in extracellular matrix or soluble proteins such

as collagen, fibronectins, and laminins. Integrins play a key role in recognizing invading pathogens and initiating immune responses (Tsakas and Marmaras 2010). Binding of pattern recognition receptors to specific components of invading pathogens activates the signal transduction system (Gupta et al. 2015). This activation can be direct or occur after a series of serine proteases-mediated proteolytic events which eventually promote antimicrobial defences that include the expression of antimicrobial peptides (AMPs) (Lemaitre and Hoffmann 2007).

Pattern recognition receptors have a variety of activities in addition to their genetic diversity. For example, the nimrod gene family has a few members that code for cell surface receptors with multiple transmembrane domains, while others code for secreted proteins. Different members of the nimrod gene family also detect different pathogen compositions (Zsámboki et al. 2013; Estévez-Lao and Hillyer 2014). Some pattern recognition receptors immediately activate immune effector activities such as phagocytosis and melanisation, while others initiate intracellular signalling pathways that stimulate the production of immune effector genes, and still others activate both effector and signalling pathways (Levashina et al. 2001; Choe et al. 2002). Members of the leucine-rich repeat-containing protein family, which are usually assumed to be pattern recognition receptors, may not interact directly with pathogen-associated molecular patterns. They can also control immune responses by interacting with other host proteins directly (Fraiture et al. 2009).

3.2 Humoral immune responses

One of the earliest insect defence mechanisms found was the production of antimicrobial peptides (AMPs). When an invading pathogen is detected, a sequence of small peptides and proteins are produced and released into the haemolymph (Cao *et al.* 2015). In response to pathogen infection, AMPs are produced in large quantities, rising from nearly undetectable in uninfected animals to micromolar amounts in infected individuals' haemolymph (Imler and Bulet 2005). Although haemocytes contribute to the generation of these AMPs, they are mostly expressed in the fat body (Hoffmann 2003; Marmaras and Lampropoulou 2009; Zheng *et al.* 2016). Invading pathogens activate genes associated with immunity in the fat body, which encode antimicrobial peptides that are

released into the hemolymph after expression (Hanson *et al.* 2019; Hanson and Lemaitre 2020). In the hemolymph the AMPs act synergistically to eliminate the invading pathogen (Takov *et al.* 2020).

3.2.1 Antimicrobial immunity: Function of antimicrobial peptides (AMPs)

Insect's fat bodies, which are similar to mammalian livers, produce antimicrobial peptides in response to pathogen recognition (Bulet et al. 2004). Antimicrobial peptides are small and cationic molecules with a broad spectrum of activities against various pathogens, including fungi (Viljakainen 2015). Insect antimicrobial peptides can assume certain structures or have unique sequences and they consist of 12–50 amino acids (Hoffmann 2003; Yi *et al.* 2014; Hanson and Lemaitre 2020). As a result, cysteine-rich peptides, proline-rich peptides, glyceine-rich peptides, and -helical peptides are divided into four categories (Tsakas and Marmaras 2010).

The key features of antimicrobial peptides include: (1) broad spectrum of activity which enables them to react against various pathogen classes, (2) selective toxicity as they can react against the invading pathogen without disturbing host cells and (3) having shorter action time than the doubling time of the invading pathogen (Matsuzaki 2009). The Toll and IMD (immunodeficiency) pathways are the two primary signalling mechanisms that control the synthesis of antimicrobial peptides. These pathways, respectively, regulate the synthesis of antimicrobial peptides in response to Gram-positive and Gram-negative bacterial infections (Tanji et al. 2006). Antimicrobial peptides families can be identified only in a single insect order or even in a more restricted taxonomic group. Apidaecin, which is only found in bees (genera *Apis*, *Bombus*, *Megachila* and *Melipona*), is one of the few AMPs that are unique to the Hymenoptera (Casteels *et al.* 1989). The proline-rich peptide abaecin is found in the bees, ants, the genus *Nasonia* and other wasps (Casteels *et al.* 1990; Tian *et al.* 2010; Ratzka *et al.* 2012; Zhang and Zhu 2012). Finally, the glycine-rich peptide hymenoptaecin is exclusively found in bees, ants and the wasps in the genus *Nasonia* (Casteels *et al.* 1993).

3.2.2 Signalling pathways that activate genes encoding antimicrobial peptides

The activation of well conserved signal transduction pathways, immunodeficiency (IMD), Toll and Janus kinase/Signal Transducers, and activators of Transcription (JAK-STAT) is promoted by the detection of the invading pathogen as bacteria, fungus, or even viruses (Salcedo-Porras and Lowenberger 2019). These pathways enhance immune responses, promote the synthesis of factors with antimicrobial activity, and increase the effect of effector mechanisms. The various pathways form signal transduction cascade where they cross communicate with each other by creating a complex network. This cross-communication ultimately results in suitable response following external stimuli (Garcia-Lara et al. 2005).

The Toll pathway induces an immune response against Gram-positive bacteria and fungi (Evans et al. 2006; Lemaitre and Hoffmann 2007; Lazzaro 2008) **(Figure 1.3)**. The pathogen-associated molecular patterns of Gram-positive bacterial cell wall are Lysine (Lys)-type peptidoglycan. Toll mediated peptidoglycan recognition protein (PGRP-SA) binds to the Lys-type peptidoglycan and engages Gram-negative binding protein-1 (GNBP-1) and modular serine protease zymogen in the availability of Ca^{2+}. The Gram-positive bacteria Lys-type peptidoglycan-PGRP-SA-GNBP-1 complex then lead to the activation of the modular serine protease zymogen to active modular serine protease. Active modular serine protease enhances the conversion of peptidoglycan recognition protein (PGRP-SA) zymogen to activated SPE protease which plays a role in the cleavage of the circulating cytokine-like ligand SPAETZLE proprotein to processed SPAETZLE. The Spaetzle protein then forms a bond with the extracellular domain of the transmembrane receptor Toll. Then TUBE binds to the TOLL receptor protein though the Myeloid differentiation primary response 88 (Myd88). The TUBE protein brings PELLE protein to the TOLL protein and results in the formation of a TOLL-TUBE-PELLE complex. The PELLE protein is a protein kinase that play a role in the phosphorylation of the NF kappa B inhibitor (IκBA) CACTUS protein. Due to this, the Nf-κB-like transcription factors DORSAL and DIF (Dorsal-related immunity factor) proteins dissociate from the CACTUS protein and translocate to the nucleus. In the nucleus, the DORSAL protein (a

transcription factor) binds with DNA and results in the expression of antimicrobial peptides.

The Toll pathway is also triggered by fungal pathogen invasion. ß-1,3 glucan is a pathogen-associated molecular pattern in fungi. Fungi also produce protease virulence factors as part of the infection process. The fungal ß-1,3 glucan is recognized by Gram-negative binding protein-3 (GNBP3) or protease virulence factors are detected through the activation of Persephone gene product (Evans et al. 2006; Lemaitre and Hoffmann 2007; Lazzaro 2008; Lindsay and Wasserman 2014).

The IMD pathway is activated in insects following infection with Gram-negative bacteria (Evans et al. 2006; Gupta et al. 2015). After recognition of the bacterial peptidoglycan by the PGRPs, the 'danger' signal is transmitted into the cell by the IMD pathway (Evans et al. 2006; Lemaitre and Hoffmann 2007; Clayton et al. 2015) **(Figure 1.4)**. The pathogen-associated molecular patterns of Gram-negative bacteria are lipopolysaccharides, identified by the pattern recognition receptor PGRP-LC. The recognition of the bacterial lipopolysaccharides by PGRP-LC results in the activation of IMD receptors. The receptor has a death domain that interacts with dFADD (TAK1 activator) and DREDD (a caspase) to form IMD-dFADD-DREDD protein complex. The IMD-dFADD-DREDD protein complex activates the IAP2 protein. The IAP2 protein then associates with TAB2 and TAK1 proteins, these proteins further interact with the IKK complex. The IKK complex activates RELISH through phosphorylation, resulting in the release of RELISH from the IKK complex (Ertürk-Hasdemir et al. 2009). The RELISH protein then translocates to the nucleus, where it induces the expression of antimicrobial peptides that work against Gram-negative bacteria.

In *Drosophila melanogaster*, the IKK complex is made up of an active Ird5 subunit and kenny, a regulatory subunit. Previous studies have shown an absence of the kenny subunit in hymenopteran species including *A. mellifera, C. floridanus* and *N. vitripennis*. This demonstrated that the hymenopterans have a communal character of the IKK complex (Gupta et al. 2015). In addition to activating RELISH, the IMD signalling pathway

activates elements of the JNK signalling pathway (Agaisse et al. 2003). A protein kinase of the IMD pathway, TAK1 activates JNK pathway when active. The JNK pathway controls various developmental processes, wound healing, expression of stress proteins and cellular immune responses (Bidla et al. 2007). Genes involved in JNK pathway include *hep, kayak (kay), basket and JRA* **(Figure 1.4)** (Gerardo et al. 2010).

The JAK-STAT pathway is triggered by wounding and it plays a role in development and immunity (Evans et al. 2006; Xu and Cherry 2014; Cao et al. 2015; Clayton et al. 2015). The activation of JAK-STAT pathway lead to overproliferation of haemocytes, up-regulation of thiolester-containing proteins (TEPs), as well as antiviral response in honeybees (Evans et al. 2006). The core genes playing a role in JAK-STAT pathway include genes encoding the cytokine receptor *domeless*, *JAK tyrosine kinase* (aka *Hopscotch*), *Upd*, *negative pathway regulators SOCS* (Suppressor of cytokine signalling), *PIAS* (Protein inhibitor of activated STAT) and the *STAT92E* transcription factor (Evans et al. 2006; Gerardo et al. 2010). Steps involved in the JAK/STAT pathway are as follows (Hillyer 2017) **(Figure 1.5)**: (1) An extracellular ligand binds to domeless transmembrane receptor and induce structural change. (2) The structural change results in the self-phosphorylation of JANUS KINASE protein. (3) Activated JAK phosphorylates the DOME protein. This results in the formation of a docking site on the DOME protein for STATs (signal transducers and activators of transcription) proteins. (4) STATs then translocate into the nucleus where it allows transcription of specific genes that play a primary role in stress/viral infection response.

3.2.3 The prophenoloxidase activation cascade/ Melanisation

The biochemical pathway of the prophenoloxidase-based melanisation is summarized in **Figure 1.6**. Melanisation is a cuticle hardening, wound healing, and immunological mechanism used by insects. Melanisation is an effector mechanism in immunity that helps to eliminate bacteria, fungus, nematodes, protozoan parasites, and parasitic wasp eggs (Lavine and Strand 2003; Nappi and Christensen 2005). Nodulation or encapsulation is the term used when this process involves the aggregation of haemocytes (Lavine and Strand 2003; Nappi and Christensen 2005). The process of melanisation involves a series of reactions that include the conversion of tyrosine to melanin. The melanin is deposited

around the invading pathogen to encapsulate it or at the wound site to facilitate wound healing. Invading pathogens that have been encapsulated die as a result of oxidative stress or starvation as they become isolated from nutrient-rich parts of the insect (Cerenius and Söderhäll 2004; Nappi and Christensen 2005). Furthermore, melanisation aids in the removal of dead pathogens. Melanisation is the result of a coordinated interaction between pattern recognition receptors, serine proteases, serine protease inhibitors, and enzymes involved in melanin production (Volz et al. 2006; Hillyer 2017).

Melanisation begins when pattern-recognition proteins such as Peptidoglycan recognition proteins, β-1,3-glucan recognition proteins, C-type lectins, and Gram-negative binding proteins detect pathogen-associated molecular patterns (Cerenius and Söderhäll 2004; Wang et al. 2014). This activates prophenoloxidase activating enzymes, which cleave prophenoloxidase into its active form, phenoloxidase, through a serine protease cascade. Once activated, phenoloxidase is secreted into the hemolymph and delivered to the cuticle. By converting tyrosine to DOPA, active phenoloxidase fights pathogen infection. DOPA can then be decarboxylated to dopamine by DOPA decarboxylase (Ddc) or oxidized to dopaquinone by phenoloxidase. Both products are subsequently converted to eumelanin, which is then converted to melanin (Nappi and Christensen 2005).

When the melanisation system is activated, it produces a variety of chemicals that, if produced in excess, can be toxic to the host insect. As a result, it must be controlled under most circumstances in order to produce a local response at a specified location and for a short period of time (Cerenius and Söderhäll 2004; Nappi and Christensen 2005). Serpins suppress excess melanisation and the generation of harmful reactive oxygen species (ROS) by decreasing the activity of PO. Pacifastin, serpin27A, serpin-1, serpin-3, and serpin-6 are all common insect serpins (González-santoyo and Córdoba-aguilar 2012).

3.3 Cellular immune responses

The insect cellular immune system evolved to include haemocytes (blood cells), phagocytosis, encapsulation, and nodulation as part of its defence mechanism (Dubovskiy *et al.* 2016). These processes allow insects to isolate and neutralise invading pathogens. The physiological characteristics of insects, like the open circulatory system,

present several benefits for cellular immune reactions (Dubovskiy *et al.* 2016). For example, haemocytes can be spread much faster to provide a rapid immune response (Dubovskiy *et al.* 2013). Encapsulation and nodulation are frequently called capsule formation, and they are very crucial defence mechanisms in insects. These mechanisms ensure that the the immune response is directed to the real site of injury, allowing the disease or parasite to be killed or destroyed (Garcia *et al.* 2007; Satyavathi *et al.* 2014). Unlike the humoral responses that occur several hours after infection, cellular immune responses occur immediately after an invasion (Im et al. 2016).

3.3.1 Haemocytes

Insects have no blood vessel, and consequently there is no distinction between blood and interstitial fluid. Collectively, blood and interstitial fluids are referred to as the hemolymph. All internal tissues, organs, and haemocytes are bathed in hemolymph, which aids in the movement of nutrients, waste products, and metabolites (Tsakas and Marmaras 2010). The most common types of circulating haemocytes, in the hemolymph of insects, are granular cells, crystal cells, oenocytoids and plasmatocytes (Lavine and Strand 2003). Haemocytes are also involved in the response to external wounding by aiding in the formation of clots (Lavine and Strand 2003). Crystal cells, plasmatocytes, and lamellocytes are three types of haemocytes studied in greater detail in the model insect *Drosophila melanogaster* (Lavine and Strand 2003; Kanost et al. 2004). Crystal cells are relatively large cells that include crystalline inclusions. They are involved in the formation of prophenoloxidase, a zymogen that is triggered during the melanisation process. Melanin deposits are necessary for wound healing and encapsulation of invading microorganisms (Crozatier and Meister 2007; Hillyer 2016). Plasmatocytes are phagocytes that facilitate the process of phagocytosis which facilitates the rapid removal of dead cells, during embryogenesis and metamorphosis, as well as pathogens during infections (Tsakas and Marmaras 2010). In response to pathogen infection, plasmatocytes generate and exude antimicrobial peptides (Agaisse et al. 2003; Tanaka and Yamakawa 2011). The key defence responses that require the action of haemocytes include phagocytosis, nodulation and encapsulation (see below).

3.3.2 Phagocytosis

Phagocytosis is a generally conserved defence response in which cells recognize and bind to invading pathogens that are relatively large (Rosales 2005). The detection of the invading pathogen activates the immune cell to form a phagosome (Strand 2008). This results in signalling cascades that regulate phagosome formation phagosome and target ingestion via actin polymerization-dependent mechanisms (Hillyer and Strand 2014). Vesicle fusion events then allow the phagosome to mature into a phagolysosome, allowing effector molecules to be injected. The target is finally killed or degraded by these effector molecules (Lavine and Strand 2003). Scavenger receptors, the EGF-domain protein Eater, croquemort family members, nimrod and draper, vitellogenin, Dscam, peptidoglycan recognition protein family members and thioester-containing proteins (TEPs) are among the receptors involved in phagocytosis (Kocks *et al.* 2005; Kurucz *et al.* 2007).

3.3.3 Nodulation

When the phagocytic immune response is insufficient to suppress pathogen infections, haemocytes assist in the activation of other mechanisms such as nodulation (Lavine and Strand 2002). Haemocytes produce nodules to manage infections when there are a lot of bacteria. The formation of multicellular hemocyte aggregates that entrap vast numbers of bacteria is known as nodulation. Haemocytes first surround bacteria before joining with other haemocytes to create small aggregates (Satyavathi *et al.* 2014). More haemocytes are added to these cell aggregates, and they continue to proliferate until huge nodules appear. The nodule is eventually coated in layers of haemocytes and melanized. Bacteria are effectively isolated from the hemolymph by melanin-covered nodules. Although the process of nodule formation is not totally understood, eicosanoids, prophenoloxidase, and dopa decarboxylase (Ddc) are all important in the formation of nodules in many insect species (Gandhe et al. 2007; Satyavathi et al. 2014).

3.3.4 Encapsulation

Encapsulation is a potent defence response in which multicellular haemocytes adhere to large targets that are too large for a single cell to swallow (Strand 2008). Such large targets may be parasitoids and nematodes. Haemocytes form a multilayer capsule around the invading pathogen after attaching to it, which is followed by a melanisation process. The invading pathogen is killed either by the synthesis of reactive cytotoxic chemicals or by suffocation within the capsule (Napping 1995). During this process granulocytes interacts with the invading parasite and release chemotactic elements that will engage plasmatocytes (Hillyer 2016). The plasmatocytes promote the formation of a multi-layered capsule; within the capsule, the parasite is ultimately killed (Lavine and Strand 2003). Two components of the Rho GTPase family, Rac1 and Rac2 appear to function in this process controlling some features of cytoskeleton remodelling (Williams et al. 2005).

4. Social and Solitary Hymenoptera

Social insects are part of the most dominant and prolific life-forms on earth. The most familiar examples of social hymenopterans are ants, bees and wasps. Colonies of social insects are characterized by dense clusters of individuals who are typically closely related. These characteristics facilitate disease transmission, making social insect colonies particularly vulnerable to diseases and parasites (Meunier 2015). Additionally, social insects have evolved advanced mechanisms to inhibit pathogen spread within their colonies. Behavioral adaptations such as nest defence, nestmate recognition, and sanitary behavior such as self- and allogrooming are examples of these mechanisms. There is also an additional layer of defence in social insects called ‘social immunity’ (Cremer et al. 2007). ‘Social immunity’ is the collective immune functions that are performed by a group of individuals to counteract invading pathogen threats (Cremer et al. 2007). Some examples of social immunity include covering the nest with materials that have antimicrobial properties, allogrooming, and infected or dead individuals that are removed from the nests (Walker and Hughes 2009; Baracchi *et al.* 2012; Diez *et al.* 2012; Reavey *et al.* 2014). As a result, changes in the genetic and phenotypic traits that function

in how organisms deal with pathogen threats may occur during the evolution from solitary to eusocial lifestyles (Otani *et al.* 2016).

According to research on the genomic comparison of social and solitary insects, *A. mellifera* has fewer immune-related genes than *D. melanogaster* and the only insect genomes available at the time (Evans et al. 2006). The authors hypothesized that because of the emergence of social immunity, honeybees no longer rely solely on innate immunity like solitary insects. However, previous research on Apocrita has found that the repertoire of immune response genes, vision genes (opsins), and the GC content of Hymenoptera genomes are reduced when compared to other insect genomes (Evans *et al.* 2006; Gadau *et al.* 2012; Barribeau *et al.* 2015; Henze and Oakley 2015; Standage *et al.* 2016). According to a recent study, there is also a decrease in the diversity and abundance of transposable elements (TEs) in social Apocrita; in insects, TEs are the primary drivers of genome size evolution (Kapheim *et al.* 2015; Petersen *et al.* 2019). However, more research is needed to determine whether these characteristics are shared by all Hymenoptera or are unique to Apocrita (Oeyen *et al.* 2020).

5. Contribution of defensive symbionts to Hymenoptera defences

In addition to innate immune defences, several insect taxa, including those in the Hymenoptera, collaborate with microbial symbionts to protect themselves (Kaltenpoth 2014). When compared to solitary insects, social insects have more specialized and structured gut symbionts, according to developments in insect microbial investigations (Sabree *et al.* 2012; Engel and Moran 2013; Otani *et al.* 2014). The number of antimicrobial peptide-producing bacteria sequenced in bees is an example of this in Hymenoptera. This was performed to demonstrate that antimicrobial peptide-producing bacteria are much more common in bees than in Drosophila *(*Wong *et al.* 2011*)*. Several studies have found that gut bacteria in honeybees, such as *Snodgrasella alvi* and *Gilliamella apicola*, protect the bees from trypanosomatid parasite *Crithidia bombi (*Koch *et al.* 2012*;* Cariveau *et al.* 2014*;* Moran 2015*)*. In addition, a study of antibiotic-treated *A. mellifera* revealed that destroying their gut bacteria may make honeybees more susceptible to *Nosema* infection (Li *et al.* 2017).

Studies focusing primarily on the distinctions between social and solitary insect gut symbionts have the potential to shed light on the symbionts' role in eusociality (Otani *et al.* 2016). A CORRELATED study was carried out on the general characterization of gut symbionts in social and solitary bee species. The study reported that there are differences in gut symbionts between the social and solitary bee species. These differences were correlated to changes in diet and environmental factors (Martinson *et al.* 2011; Voulgari-Kokota *et al.* 2019). The evolution from solitary to eusociality involves a range of adaptations, and as a result defensive symbiont adaptation differ according to the host's lifestyle.

6. *Sirex noctilio*

The woodwasp, *Sirex noctilio* (Hymenoptera: Siricidae) is a member of the horntail family under the suborder Symphyta within the Hymenoptera (Bordeaux et al. 2014). This wasp is an invasive pest of pine species in numerous parts of the world including South Africa. *Sirex noctilio* in association with its fungal symbiont *Amylostereum areolatum* (Basidiomycotina: Corticiaceae) infests and kills pine trees (Fernández Ajó et al. 2015). Adult wasp species have a long cylindrical body that lacks a petiole, two sets of transparent wings, and a cornus at their tails. In its native environment, *S. noctilio* is not known to cause any considerable damage to its conifer hosts, but in the Southern Hemisphere where it is introduced, it has been causing severe damage and death to trees in commercial pine plantations. These resulted in substantial economic losses to forestry companies and the country (Slippers et al. 2012).

6.1 Symbioses and interactions

Sirex noctilio has a mutual association with a fungal symbiont, *Amylostereum areolatum*. This interaction is beneficial for both the woodwasp and the fungus. The benefits that the fungus acquire from its relationship with the woodwasp include protection and growth in the wasp's mycangia. The wasp's mycangia are bordered with glands that produce secretions that are believed to activate fungal growth. The presence of the *A. aerolatum* is important for the development of immature woodwasp. Madden (1981) showed that there is a delay in egg eclosion when conditions in the tree disrupt fungal development, and symbiotic growth is hindered by the presence of other fungi, larvae starvation is likely

to occur (Coutts and Dolezal 1965; King 1966). The development of larvae and the growth of fungi have a beneficial relationship. The fungus produces larger adults when the conditions are favorable (Madden 1981). The fungus is also capable of modifying environmental conditions. It dries up the wood substrate, creating a better micro-environment for the development of the eggs and larvae (Coutts and Dolezal 1965). The fungus degrades the wood to facilitate tunnelling of the larvae (Gilmour 1965), but most critically also ensures degradation of cellulose to sugars as nutrition for the larvae (Thompson *et al.* 2014).

The wasp's lifestyle and morphology are modified to maintain the wasp's relationship with its symbiont. Adult females carry the fungus in paired mycangia that open into the oviduct at the anterior end of the ovipositor (Boros 1968). During oviposition, the female woodwasp uses the ovipositor to inject asexual spores or arthrospores of the symbiotic fungus into the host sapwood and into a secondary drill beside the egg. Occasionally, only the fungus and the phytotoxic mucus are deposited, rather than eggs. (Coutts 1965; Coutts and Dolezal 1969). Starting from the second instar, the fungus is transferred from one larva to the next in externally opening sacs. The adult female woodwasps take it up into their mycangia when they shed their pupal skin, this ensures that the association with the fungus is continued between generations (Parkin 1941; Boros 1968).

6.2 The life history of *Sirex noctilio*

The complex life history of *S. noctilio* has been well-studied. The woodwasp goes through one generation per year, however, development can take two or more years under cold climate conditions (Ryan and Hurley 2012). Oviposition occurs in mid-summer which is mediated by the synergistic interaction between the females and their obligate fungal symbiont (*Amylostereum areolatum*). This interaction weakens the resistance of the pine trees and presents them as suitable hosts for development of the larvae (Talbot 1977). Possible oviposition sites are evaluated by the females through drilling of the bark. Several chambers are drilled into the bark, into which eggs, phytotoxic mucus, and the wood-decay fungus carried by *S. noctilio* will be injected (Haavik et al. 2015). The eggs hatch inside the chambers and the developing larvae depend on predigested wood by *A. areolatum* for nutrition (Thompson et al. 2014). The fungal associate is thus used as

external digestion of the xylem (Thompson et al. 2014). The larvae of *S. noctilio* complete a variety of developmental stages before pupation, which occurs in late spring, normally in the following year (Haavik et al. 2015). Drilling of pine trees by *S. noctilio* eventually leads to death from the combined action of the phytotoxic mucus and the wood-decaying fungus (Carnegie et al. 2005). *Sirex noctilio* favours the attack of weakened trees which can be killed in under a single season. However, healthy trees can also be killed if they suffer multiple attacks from the wasp (Madden 1968).

6.3 Population control of *Sirex noctilio*

Biological control is one of the main methods used for effective population control for *S. noctilio*. Due to the magnitude of the threat that *S. noctilio* posed to pine plantations the Australian Congress of Scientific and Industrial Research Organization and the Commonwealth Institute of Biological Control begun a worldwide campaign in the 1960s-1970s to explore natural enemies of *S. noctilio* in its native ranges (Williams and Hajek 2017). This program led to the identification of parasitoid wasps and nematodes species as biocontrol agents of *S. noctilio* (Hurley et al. 2012; Williams and Hajek 2017).

The entomophagous-mycetophagous nematode, *Deladenus siricidicola* Bedding (Nematoda: Neotylenchidae), is the main biocontrol agent used to control *S. noctilio* (Collett and Elms 2009). *Deladenus siricidicola* is an effective control agent due to its lifestyle which is almost ideal to control the woodwasp. The nematode has a complex bi-cyclic lifecycle in which there is both a fungus-feeding as well as a parasitic phase (Morris et al. 2012). The fungus-feeding phase free-living nematodes feed on *A. areolatum* (Morris et al. 2012; Slippers et al. 2012). During the parasitic phase, the nematodes can either parasitize female woodwasps, in the process sterilizing the eggs that are laid (Hurley et al. 2012; Morris et al. 2012). The emergence of the parasitized woodwasp females from the infested trees contributes to the distribution of the nematodes.

Biological control agents used against *S. noctilio* have variable levels of efficacy which threaten the future of these control strategies. There are a number of factors that influence the efficacy of the control strategy, which include environmental conditions, rearing, handling and storage of the biological control agents, biological variations, nematode

virulence, *S. noctilio* resistance and population incompatibility (Hurley et al. 2012; Morris et al. 2012). These factors or combinations of factors may affect the efficacy of *D. siricidicola*. The inconsistency in the efficacy of biological control raises a need for improvement of the current control strategies and the development of new strategies.
Deladenus siricidicola must develop and complete its lifecycle inside the *S. noctilio* host. The success of the nematode depends on its ability to evade the host immune response to survive and complete its life cycle. To date, the mechanisms that are utilized by the *D. siricidicola* nematodes to modulate the *S. noctilio* immune responses have not been studied.

Current research on insects' immune responses to nematode parasites commonly focus on entomopathogenic nematodes. Entomopathogenic nematodes are a type of roundworm that can infect and kill insects. To assist in this, these nematods have developed mutualistic associations with certain bacterial symbionts. Entomopathogenic nematodes provide shelter and serve as a vector for bacteria, allowing them to spread from one host to another. After the nematodes have infested the insect host, the bacteria are regurgitated and released within the insect hemocoel (Cooper and Eleftherianos 2016). Studies on entomopathogenic nematodes mainly focus on two genera, namely *Steinernema* (associated with *Xenorhabdus* bacteria) and *Heterorhabditis* (associated with *Photorhabdus* bacteria) (Cooper and Eleftherianos 2016). The entomopathogenic nematodes contribute shelter and act as a vector for the bacteria and transfer them from one host to another. In return, after the invasion of the insect host with the nematodes, the bacteria are regurgitated and released within the insect hemocoel. The bacteria then secrete toxins and virulence factors. The bacteria replicate rapidly in various tissues of the insect, the carcass of which is finally consumed by the nematode parasites.

Infection by nematode-bacteria complexes does not go unrecognized by the insect host. To stop the distribution of the nematode-bacteria complexes in the host, the insect innate immune system has a series of mechanisms ready to stimulate the recognition of the mutualistic partners (Castillo et al. 2011). Humoral and cellular immune responses constitute most of the insect innate immune response (Cooper and Eleftherianos 2016). These studies provide an opportunity to act as a starting point for the study of the mechanisms that are utilized by the *D. siricidicola* nematodes to modulate the *S. noctilio* immune responses.

7. Conclusions

The insects, including Hymenoptera, rely on an innate immune system. The innate immune system is made up of molecular mechanisms to defend the host insect against pathogen infections in a nonspecific manner. Therefore, insects are adapted to recognizing and responding to pathogens in a universal way. Insects are under constant threat of pathogens and the action of the pathogen infection facilitates to constant evolutionary adaptation of the insect immune system. For this reason, immune genes are a typical example of genes in which positive selection is expected to occur. Pathogens can evolve rapidly to evade the immune system of their host, resulting in a selection pressure on the host to evolve counter-adaptations. The ‘‘arms race’’ between the host and parasite/pathogen is expected to result in rapid evolution of the genes involved in the interaction.

Hymenoptera is second only to Diptera in terms of the number of sequenced genomes amongst the insect orders. The increasing availability of genetic tools and published genome sequences of Hymenoptera provide an opportunity for comparative phylogenomic analyses of the immune repertoire within Hymenoptera. Comparisons of the immune gene ‘‘repertoire’’ of different Hymenoptera could help us understand both the variation and conservation in Hymenoptera host defence mechanisms. Such an approach can be useful for non-model, but ecomically important, insects such as *Sirex noctilio* and the parasites used in its biological control.

8. References

Aguiar, A. P., A. R. Deans, M. S. Engel, M. Forshage, J. T. Huber *et al.*, 2013 Order hymenoptera. Zootaxa 3703**:** 51-62.

Ashida, M., and P. T. Brey, 1995 Role of the integument in insect defence: pro-phenol oxidase cascade in the cuticular matrix. Proceedings of the National Academy of Sciences 92**:** 10698-10702.

Baracchi, D., G. Mazza and S. Turillazzi, 2012 From individual to collective immunity: the role of the venom as antimicrobial agent in the Stenogastrinae wasp societies. Journal of insect physiology 58**:** 188-193.

Barribeau, S. M., B. M. Sadd, L. du Plessis, M. J. Brown, S. D. Buechel *et al.*, 2015 A depauperate immune repertoire precedes evolution of sociality in bees. Genome Biology 16**:** 1-21.

Brennan, C. A., and K. V. Anderson, 2004 Drosophila: the genetics of innate immune recognition and response. Annu. Rev. Immunol. 22**:** 457-483.

Browne, N., M. Heelan and K. Kavanagh, 2013 An analysis of the structural and functional similarities of insect haemocytes and mammalian phagocytes. Virulence 4**:** 597-603.

Bulet, P., R. Stöcklin and L. Menin, 2004 Anti-microbial peptides: from invertebrates to vertebrates. Immunological reviews 198**:** 169-184.

Cao, X., Y. He, Y. Hu, Y. Wang, Y. R. Chen *et al.*, 2015 The immune signalling pathways of Manduca sexta. Insect Biochem Mol Biol 62**:** 64-74.

Cariveau, D. P., J. E. Powell, H. Koch, R. Winfree and N. A. Moran, 2014 Variation in gut microbial communities and its association with pathogen infection in wild bumble bees (Bombus). The ISME journal 8**:** 2369-2379.

Casteels, P., C. Ampe, F. Jacobs and P. Tempst, 1993 Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (Apis mellifera). Journal of Biological Chemistry 268**:** 7044-7054.

Casteels, P., C. Ampe, F. Jacobs, M. Vaeck and P. Tempst, 1989 Apidaecins: antibacterial peptides from honeybees. The EMBO journal 8**:** 2387-2391.

Casteels, P., C. Ampe, L. Rivière, J. Van Damme, C. Elicone *et al.*, 1990 Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (Apis mellifera). European journal of biochemistry 187**:** 381-386.

Choe, K.-M., T. Werner, S. Stöven, D. Hultmark and K. V. Anderson, 2002 Requirement for a peptidoglycan recognition protein (PGRP) in Relish activation and antibacterial immune responses in Drosophila. Science 296**:** 359-362.

Cremer, S., S. A. Armitage and P. Schmid-Hempel, 2007 Social immunity. Curr Biol 17**:** R693-702.

Cremer, S., C. D. Pull and M. A. Fürst, 2018 Social immunity: emergence and evolution of colony-level disease protection. Annual Review of Entomology 63**:** 105-123.

Crozatier, M., and M. Meister, 2007 Drosophila haematopoiesis. Cellular microbiology 9**:** 1117-1126.

Daffre, S., and I. Faye, 1997 Lipopolysaccharide interaction with hemolin, an insect member of the Ig-superfamily. FEBS Letters 408**:** 127-130.

Davis, R. B., S. L. Baldauf and P. J. Mayhew, 2010 The origins of species richness in the Hymenoptera: insights from a family-level supertree. BMC evolutionary biology 10**:** 109.

Dawkins, R., and J. R. Krebs, 1979 Arms races between and within species. Proceedings of the Royal Society of London. Series B. Biological Sciences 205**:** 489-511.

Diez, L., J.-L. Deneubourg and C. Detrain, 2012 Social prophylaxis through distant corpse removal in ants. Naturwissenschaften 99**:** 833-842.

Dowton, M., and A. D. Austin, 2001 Simultaneous analysis of 16S, 28S, COI and morphology in the Hymenoptera: Apocrita—evolutionary transitions among parasitic wasps. Biological Journal of the linnean society 74**:** 87-111.

Dubovskiy, I., N. Kryukova, V. Glupov and N. Ratcliffe, 2016 Encapsulation and nodulation in insects. Invertebrate Survival Journal 13**:** 229-246.

Dubovskiy, I. M., M. M. Whitten, O. N. Yaroslavtseva, C. Greig, V. Y. Kryukov *et al.*, 2013 Can insects develop resistance to insect pathogenic fungi? PloS one 8.

Dziarski, R., 2004 Peptidoglycan recognition proteins (PGRPs). Molecular immunology 40**:** 877-886.

Engel, P., and N. A. Moran, 2013 The gut microbiota of insects–diversity in structure and function. FEMS microbiology reviews 37**:** 699-735.

Evans, J., K. Aronstein, Y. P. Chen, C. Hetru, J. L. Imler *et al.*, 2006 Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology 15**:** 645-656.

Ferrandon, D., J.-L. Imler, C. Hetru and J. A. Hoffmann, 2007 The Drosophila systemic immune response: sensing and signalling during bacterial and fungal infections. Nature Reviews Immunology 7**:** 862.

Gadau, J., M. Helmkampf, S. Nygaard, J. Roux, D. F. Simola *et al.*, 2012 The genomic impact of 100 million years of social evolution in seven ant species. Trends in Genetics 28**:** 14-21.

Garcia, E. S., N. A. Ratcliffe, M. M. Whitten, M. S. Gonzalez and P. Azambuja, 2007 Exploring the role of insect host factors in the dynamics of Trypanosoma cruzi–Rhodnius prolixus interactions. Journal of Insect Physiology 53**:** 11-21.

Gottar, M., V. Gobert, T. Michel, M. Belvin, G. Duyk *et al.*, 2002 The Drosophila immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature 416**:** 640-644.

Grimaldi, D., M. S. Engel and M. S. Engel, 2005 *Evolution of the Insects*. Cambridge University Press.

Gupta, S. K., M. Kupper, C. Ratzka, H. Feldhaar, A. Vilcinskas *et al.*, 2015 Scrutinizing the immune defence inventory of *Camponotus floridanus* applying total transcriptome sequencing. BMC Genomics 16**:** 540.

Guzman-Novoa, E., 2011 Genetic Basis of Disease Resistance in the Honey Bee (Apis mellifera L.).

Hanson, M. A., A. Dostálová, C. Ceroni, M. Poidevin, S. Kondo *et al.*, 2019 Synergy and remarkable specificity of antimicrobial peptides *in vivo* using a systematic knockout approach. Elife 8**:** e44341.

Hanson, M. A., and B. Lemaitre, 2020 New insights on *Drosophila* antimicrobial peptide function in host defence and beyond. Current Opinion in Immunology 62**:** 22-30.

Hanson, P. E., 2016 *Insects and other arthropods of tropical America*. Cornell University Press.

Hegedus, D., M. Erlandson, C. Gillott and U. Toprak, 2009 New insights into peritrophic matrix synthesis, architecture, and function. Annual review of entomology 54**:** 285-302.

Henze, M. J., and T. H. Oakley, 2015 The dynamic evolutionary history of pancrustacean eyes and opsins. Integrative and Comparative Biology 55**:** 830-842.

Hillyer, J. F., 2016 Insect immunology and hematopoiesis. Developmental and Comparative Immunology 58**:** 102-118.

Hillyer, J. F., and M. R. Strand, 2014 Mosquito hemocyte-mediated immune responses. Current opinion in insect science 3**:** 14-21.

Hoffmann, J. A., 2003 The immune response of *Drosophila*. Nature 426**:** 33-38.

Hölldobler, B., and E. O. Wilson, 1990 *The ants*. Harvard University Press.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui and T. J. Case, 2002 The Causes and Consequences of Ant Invasions. Annual Review of Ecology and Systematics 33**:** 181-233.

Hughes, A., 2001 Evolution of the integrin alpha and beta protein families. Journal Of Molecular Evolution 52**:** 63-72.

Jiang, H., A. Vilcinskas and M. R. Kanost, 2010 Immunity in lepidopteran insects, pp. 181-204 in *Invertebrate immunity*. Springer.

Kaltenpoth, M., T. Engl and K. Clay, 2014 Defensive microbial symbionts in Hymenoptera. Functional Ecology 28**:** 315-327.

Kanost, M. R., H. Jiang and X. Q. Yu, 2004 Innate immune responses of a lepidopteran insect, Manduca sexta. Immunological reviews 198**:** 97-105.

Kapheim, K. M., H. Pan, C. Li, S. L. Salzberg, D. Puiu *et al.*, 2015 Genomic signatures of evolutionary transitions from solitary to group living. Science 348**:** 1139-1143.

Koch, H., G. Cisarovsky and P. Schmid-Hempel, 2012 Ecological effects on gut bacterial communities in wild bumblebee colonies. Journal of Animal Ecology 81**:** 1202-1210.

Kocks, C., J. H. Cho, N. Nehme, J. Ulvila, A. M. Pearson *et al.*, 2005 Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in Drosophila. Cell 123**:** 335-346.

Kurucz, É., R. Márkus, J. Zsámboki, K. Folkl-Medzihradszky, Z. Darula *et al.*, 2007 Nimrod, a putative phagocytosis receptor with EGF repeats in Drosophila plasmatocytes. Current Biology 17**:** 649-654.

Lach, L., and M. L. Thomas, 2008 Invasive ants in Australia: documented and potential ecological consequences. Australian Journal of Entomology 47**:** 275-288.

Lavine, M., and M. Strand, 2002 Insect haemocytes and their role in immunity. Insect biochemistry and molecular biology 32**:** 1295-1309.

Lemaitre, B., and J. Hoffmann, 2007 The host defence of *Drosophila melanogaster*. Annual Review of Immunology 25**:** 697-743.

Leulier, F., and B. Lemaitre, 2008 Toll-like receptors—taking an evolutionary approach. Nature Reviews Genetics 9**:** 165.

Li, J. H., J. D. Evans, W. F. Li, Y. Z. Zhao, G. DeGrandi-Hoffman *et al.*, 2017 New evidence showing that the destruction of gut bacteria by antibiotic treatment could increase the honey bee's vulnerability to *Nosema* infection. PloS One 12**:** e0187505.

Machtinger, E. T., C. J. Geden, P. E. Kaufman and A. M. House, 2015 Use of Pupal Parasitoids as Biological Control Agents of Filth Flies on Equine Facilities. Journal of Integrated Pest Management 6**:** 16.

Marmaras, V. J., and M. Lampropoulou, 2009 Regulators and signalling in insect haemocyte immunity. Cellular signalling 21**:** 186-195.

Martinson, V. G., B. N. Danforth, R. L. Minckley, O. Rueppell, S. Tingek *et al.*, 2011 A simple and distinctive microbiota associated with honey bees and bumble bees. Molecular Ecology 20**:** 619-628.

Meunier, J., 2015 Social immunity and the evolution of group living in insects. Philos Trans R Soc Lond B Biol Sci 370.

Michener, C. D., 2000 *The bees of the world*. JHU press.

Moran, N. A., 2015 Genomics of the honey bee microbiome. Current opinion in insect science 10**:** 22-28.

Moussian, B., 2010 Recent advances in understanding mechanisms of insect cuticle differentiation. Insect Biochemistry and Molecular Biology 40**:** 363-375.

Mrinalini, W. J., and J. Werren, 2017 Parasitoid wasps and their venoms. Evolution of venomous animals and their toxins. Dordrecht: Springer**:** 187-212.

Murphy, K., and C. Weaver, 2016 *Janeway's immunobiology*. Garland science.

Nappi, A. J., and E. Ottaviani, 2000 Cytotoxicity and cytotoxic molecules in invertebrates. Bioessays 22**:** 469-480.

Napping, A. J., 1995 Superoxide anion generation in Irosophila during melanotic encapsulation of parasites. European journal of cell biology 68**:** 450-456.

Oeyen, J. P., P. Baa-Puyoulet, J. B. Benoit, L. W. Beukeboom, E. Bornberg-Bauer *et al.*, 2020 Sawfly genomes reveal evolutionary acquisitions that fostered the mega-radiation of parasitoid and eusocial Hymenoptera. Genome Biology and Evolution 12**:** 1099-1188.

Otani, S., N. Bos and S. H. Yek, 2016 Transitional complexity of social insect immunity. Frontiers in Ecology and Evolution 4**:** 69.

Otani, S., A. Mikaelyan, T. Nobre, L. H. Hansen, N. G. A. Koné *et al.*, 2014 Identifying the core microbial community in the gut of fungus-growing termites. Molecular Ecology 23**:** 4631-4644.

Otti, O., S. Tragust and H. Feldhaar, 2014 Unifying external and internal immune defences. Trends in ecology & evolution 29**:** 625-634.

Peters, R. S., L. Krogmann, C. Mayer, A. Donath, S. Gunkel *et al.*, 2017 Evolutionary history of the Hymenoptera. Current Biology 27**:** 1013-1018.

Petersen, M., D. Armisén, R. A. Gibbs, L. Hering, A. Khila *et al.*, 2019 Diversity and evolution of the transposable element repertoire in arthropods with particular reference to insects. BMC Evolutionary Biology 19**:** 1-15.

Rämet, M., P. Manfruelli, A. Pearson, B. Mathey-Prevot and R. A. B. Ezekowitz, 2002 Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli*. Nature 416**:** 644-648.

Ratzka, C., F. Förster, C. Liang, M. Kupper, T. Dandekar *et al.*, 2012 Molecular characterization of antimicrobial peptide genes of the carpenter ant Camponotus floridanus. PLoS One 7.

Reavey, C. E., L. Beare and S. C. Cotter, 2014 Parental care influences social immunity in burying beetle larvae. Ecological entomology 39**:** 395-398.

Rosales, C., 2005 *Molecular mechanisms of phagocytosis*. Springer.

Royet, J., and R. Dziarski, 2007 Peptidoglycan recognition proteins: pleiotropic sensors and effectors of antimicrobial defences. Nature Reviews Microbiology 5**:** 264-277.

Ryan, K., and B. P. Hurley, 2012 Life history and biology of Sirex noctilio, pp. 15-30 in *The Sirex Woodwasp and its Fungal Symbiont:*. Springer.

Sabree, Z. L., C. Y. Huang, G. Arakawa, G. Tokuda, N. Lo *et al.*, 2012 Genome shrinkage and loss of nutrient-providing potential in the obligate symbiont of the primitive termite Mastotermes darwiniensis. Appl. Environ. Microbiol. 78**:** 204-210.

Salcedo-Porras, N., and C. Lowenberger, 2019 The innate immune system of kissing bugs, vectors of chagas disease. Developmental & Comparative Immunology 98**:** 119-128.

Satyavathi, V. V., A. Minz and J. Nagaraju, 2014 Nodulation: an unexplored cellular defence mechanism in insects. Cell Signal 26**:** 1753-1763.

Schluns, H., and R. H. Crozier, 2009 Molecular and chemical immune defences in ants (Hymenoptera: Formicidae). Myrmecological News 12**:** 237-249.

Schmid-Hempel, P., 2003 Variation in immune defence as a question of evolutionary ecology. Proceedings of the Royal Society of London. Series B: Biological Sciences 270**:** 357-366.

Sharkey, M. J., 2007 Phylogeny and classification of Hymenoptera. Zootaxa 1668**:** 521-548.

Slippers, B., B. P. Hurley and M. J. Wingfield, 2015 Sirex woodwasp: a model for evolving management paradigms of invasive forest pests. Annu Rev Entomol 60**:** 601-619.

Somogyi, K., B. Sipos, Z. Pénzes, É. Kurucz, J. Zsámboki *et al.*, 2008 Evolution of genes and repeats in the Nimrod superfamily. Molecular biology and evolution 25**:** 2337-2347.

Standage, D. S., A. J. Berens, K. M. Glastad, A. J. Severin, V. P. Brendel *et al.*, 2016 Genome, transcriptome and methylome sequencing of a primitively eusocial wasp reveal a greatly reduced DNA methylation system in a social insect. Molecular Ecology 25**:** 1769-1784.

Stroeymeyt, N., B. Casillas-Pérez and S. Cremer, 2014 Organisational immunity in social insects. Current Opinion in Insect Science 5**:** 1-15.

Takehana, A., T. Katsuyama, T. Yano, Y. Oshima, H. Takada *et al.*, 2002 Overexpression of a pattern-recognition receptor, peptidoglycan-recognition protein-LE, activates Imd/relish-mediated antibacterial defence and the prophenoloxidase cascade in Drosophila larvae. Proceedings of the National Academy of Sciences 99**:** 13705-13710.

Takov, D. I., P. V. Ostoich, A. I. Tchorbanov and D. K. Pilarska, 2020 Order Diptera as a model in the studies of insect immunity: a review. Turkish Journal of Zoology 44**:** 481-489.

Thompson, B. M., J. Bodart, C. McEwen and D. S. Gruner, 2014 Adaptations for Symbiont-Mediated External Digestion in *Sirex noctilio* (Hymenoptera: Siricidae). Annals of the Entomological Society of America 107**:** 453-460.

Tian, C., B. Gao, Q. Fang, G. Ye and S. Zhu, 2010 Antimicrobial peptide-like genes in Nasonia vitripennis: a genomic perspective. BMC genomics 11**:** 187.

Vallet-Gely, I., B. Lemaitre and F. Boccard, 2008 Bacterial strategies to overcome insect defences. Nature Reviews Microbiology 6**:** 302.

Van Valen, L., 1973 A new evolutionary law. Evol Theory 1**:** 1-30.

Vlisidou, I., and W. Wood, 2015 Drosophila blood cells and their role in immune responses. The FEBS journal 282**:** 1368-1382.

Voulgari-Kokota, A., Q. S. McFrederick, I. Steffan-Dewenter and A. Keller, 2019 Drivers, diversity, and functions of the solitary-bee microbiota. Trends in Microbiology 27**:** 1034-1044.

Walker, T. N., and W. O. Hughes, 2009 Adaptive social immunity in leaf-cutting ants. Biology Letters 5**:** 446-448.

Whitfield, J. B., 2003 Phylogenetic insights into the evolution of parasitism in Hymenoptera. Advances in Parasitology 54**:** 69-101.

Wilson, E. O., 1971 The insect societies. The insect societies.

Wong, C. N. A., P. Ng and A. E. Douglas, 2011 Low-diversity bacterial community in the gut of the fruitfly Drosophila melanogaster. Environmental microbiology 13**:** 1889-1900.

Yano, T., and S. Kurata, 2011 Intracellular recognition of pathogens and autophagy as an innate immune host defence. The Journal of Biochemistry 150**:** 143-149.

Yi, H. Y., M. Chowdhury, Y. D. Huang and X. Q. Yu, 2014 Insect antimicrobial peptides and their applications. Appl Microbiol Biotechnol 98**:** 5807-5822.

Yu, X., and M. Kanost, 2002 Binding of hemolin to bacterial lipopolysaccharide and lipoteichoic acid. An immunoglobulin superfamily member from insects as a pattern-recognition receptor. Eur. J. Biochem 269**:** 1827-1834.

Zhang, L. J., and R. L. Gallo, 2016 Antimicrobial peptides. Current Biology 26**:** R14-19.

Zhang, R., Y. Li, N. Liu and S. D. Porter, 2007 An overview of the red imported fire ant (Hymenoptera: Formicidae) in mainland China. Florida Entomologist 90**:** 723-732.

Zhang, Z., and S. Zhu, 2012 Comparative genomics analysis of five families of antimicrobial peptide-like genes in seven ant species. Developmental & Comparative Immunology 38**:** 262-274.

Zheng, H., X. Yang and Y. Xi, 2016 Fat body remodeling and homeostasis control in *Drosophila*. Life Sciences 167**:** 22-31.

Chapter 1: Tables and figures

Table 1.1: List of abbreviations and acronyms

Functional category	Full name	Abbreviation
pathogen-associated molecular patterns (PAMPs)	lipopolysaccharide	LPS
	peptidoglycan	PGN
	β-1,3 glucans	B-glu
Recognition	peptidoglycan recognition proteins	PGRPs
	Gram-negative binding proteins (β-glucan recognition proteins)	GNBPs(βGRPs)
	fibrinogen-related proteins	FREPs
	Thioester-containing proteins	TEPs
	down syndrome cell adhesion molecule	DSCAM
	leucine-rich repeat containing proteins	LRRs
Signal transduction	Toll-like receptor protein	Toll
	Myeloid differentiation factor 88	Myd88
	TNF receptor associated factor	Traf
	Modulo serine protease	ModSP
	Wingless-type family member 11	Wnt-11
	SUMO-conjugating enzyme	UBC9
	Spaëtzle processing enzyme	SPE
	Immunodeficiency	IMD
	NF-kappa B transcription factor, Relish	Rel
	Inhibitor of apoptosis 2	IAP2
	Inhibitor of apoptosis 1	IAP1
	Fas-associated death domain protein	FADD
	Caspase-8 homolog	Dredd
	I-Kappa-B kinase 1	Ird5
	c-Jun N-terminal kinase	JNK
	Transforming growth factor activated kinase	TAK1
	Tak1-associated binding protein	TAB2
	Hemipterous	Hep
	Rho type GTPase	Rac1
	Rho type GTPase	Rho1
	Mitogen-activated protein kinase kinase kinase 4	Mekk1
	Dual specificity protein phosphatase 10	Puc
	Janus kinase/Signal transducers and activator of transcription	Jak/Stat
	Suppressor of cytokine signalling	Socs
	E3 SUMO-protein ligase	PIAS1
	Cyclin dependent kinase	Cdk
	Unpaired	Upd

	Domeless	Dome
	Janus kinase Hopscotch	Hop
	Signal transducers and activator of transcription	STAT
	p85 protein	Pi3K
	3-phosphoinositide-dependent protein	PDK1
	Serine/threonine protein kinase	Akt
	Phosphatase and tensin homolog	Pten
Effectors	Antimicrobial peptides	AMPs
	Prophenoloxidase	proPO
Oxidative Stress	Superoxide dismutase	SOD

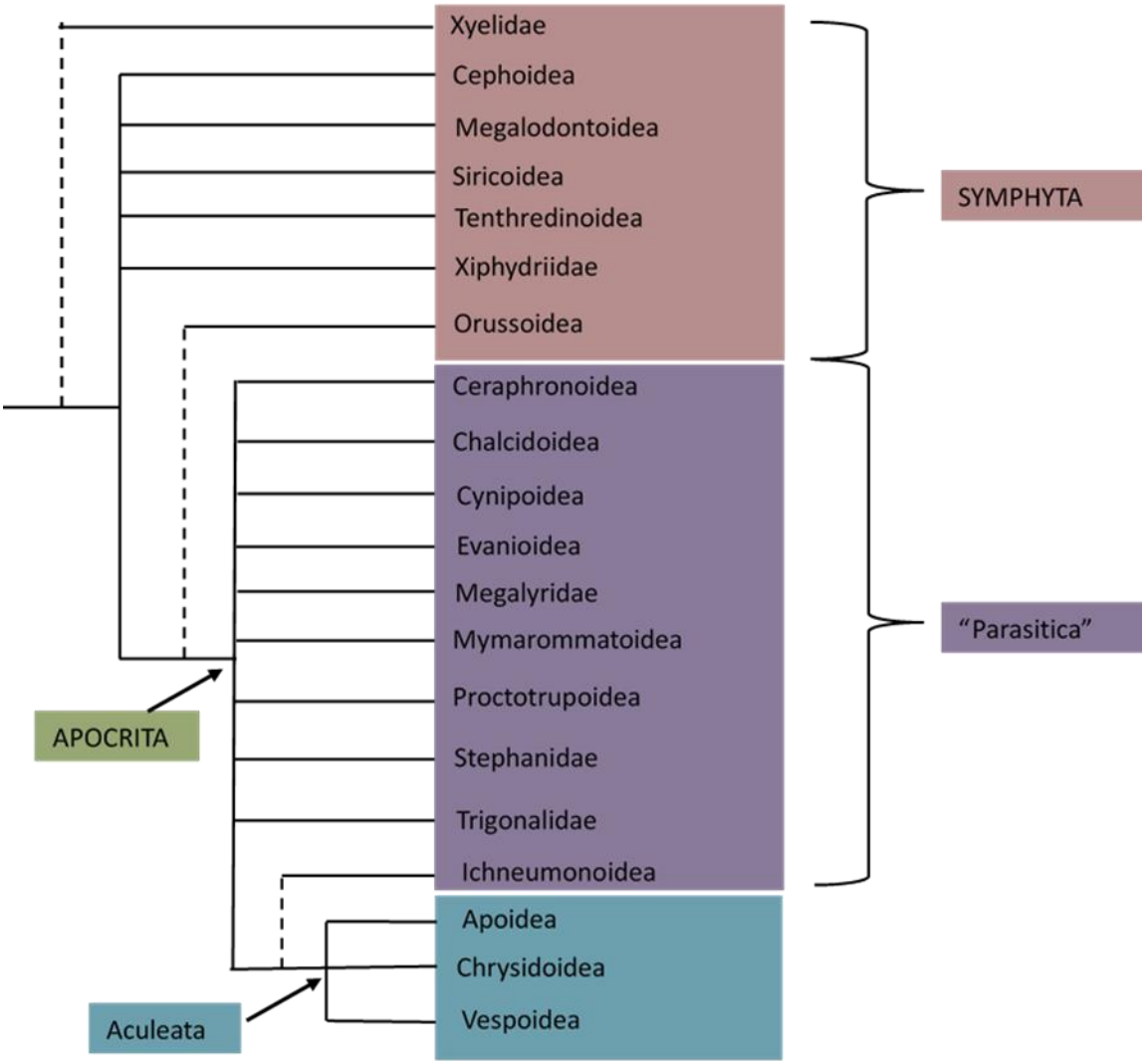


Figure 1.1: Hymenoptera classification. Symphyta (Woodwasps, saw flies, horntails) and Apocrita (Wasps, bees, and ants), the two traditional suborders of Hymenoptera, are represented in capital letters. Superfamilies are indicated by terminal taxa. Hypothesized sister group relationships are represented by dashed lines. (Adapted from Davis *et al.* 2010).

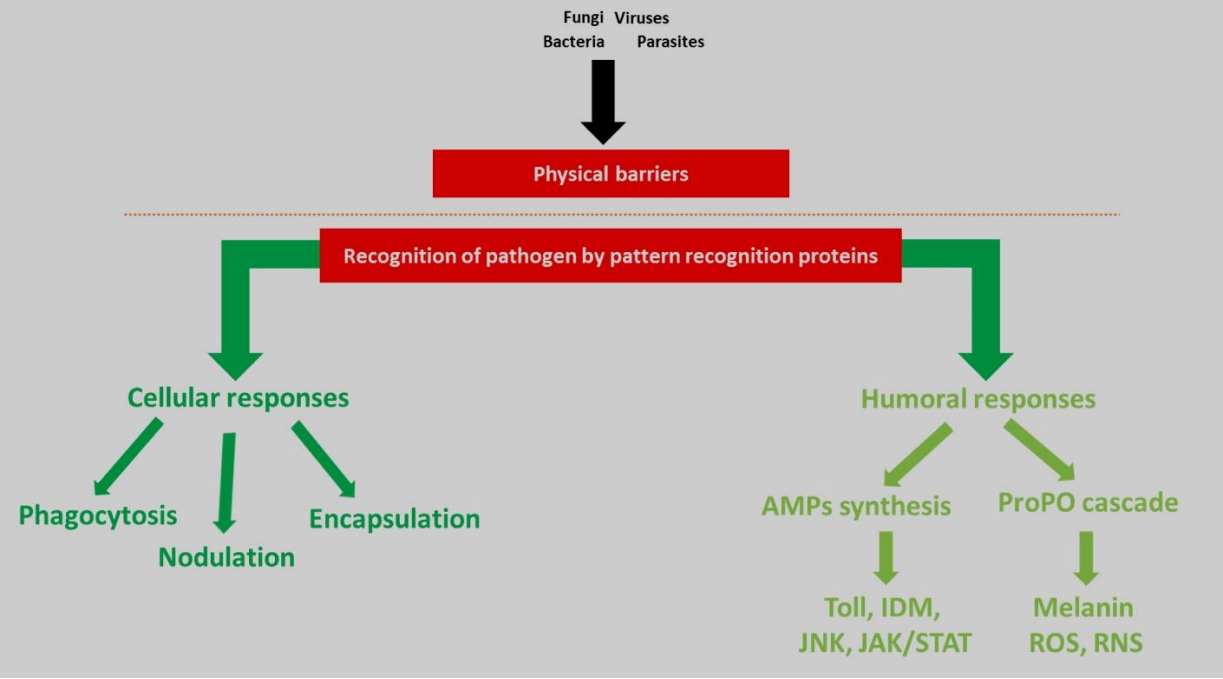


Figure 1.2: Summary of defence strategies that insects use against a various pathogens and parasites. Various pattern recognition molecules detect invading pathogens when they breach the physical barriers of the insect host. When the pathogen is successfully recognized cellular and humoral immune responses are triggered. Cellular response mechanisms such as phagocytosis, encapsulation, and nodulation are part of the immediate defense responses. Humoral responses provide sustainable defence by massively synthesizing antimicrobial peptides (AMPs) and activating the ProPO cascade, which releases other effector components. Both the cellular and humoral mechanisms release effectors that eliminate pathogens and parasites. (Adapted from Andrew *et al.* 2007, Larsen *et al*. 2019).

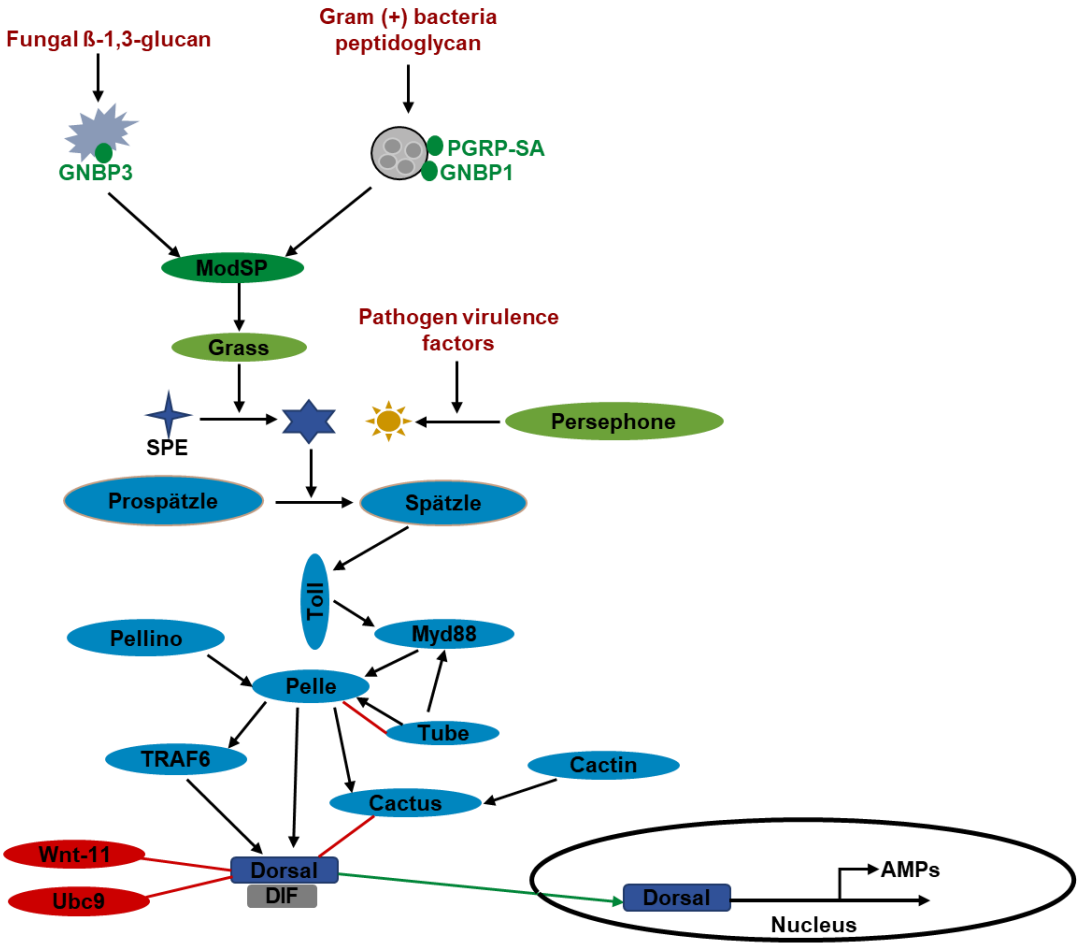


Figure 1.3: The Toll signalling pathway. Pathogen recognition receptor Gram-negative binding protein 3 recognizes the fungal cell wall component ß-1.3-glucan (GNBP3). The receptors, peptidoglycan recognition proteins PGRP-SA and GNBP1 detect peptidoglycan of Gram-positive bacteria. A protease cascade is established when an invading pathogen interacts with its respective recognition receptors. Serine protease ModSP activates the Grass protease, which then activates the Spätzle processing enzyme. Some pathogens produce virulence factors that can be recognized by the protease Persephone. When Persephone is cleaved, SPE is activated, resulting in active Spätzle. Active Spätzle is required for the activation of the transmembrane receptor Toll. Activated Toll binds to Myd88 through TIR domains. The kinase Pelle is activated by autophosphorylation. Active Pelle phosphorylates cactus, an NF-kB inhibitor. The phosphorylated cactus is marked for degradation. The NF-kB transcription factors Dorsal/DIF become free and translocate to the nucleus. In the nucleus. Antimicrobial peptide production is triggered by these transcription factor genes. (Adapted from Evans *et al.* 2006; Xu and James 2009; Gupta *et al*. 2015, Rosales 2016).

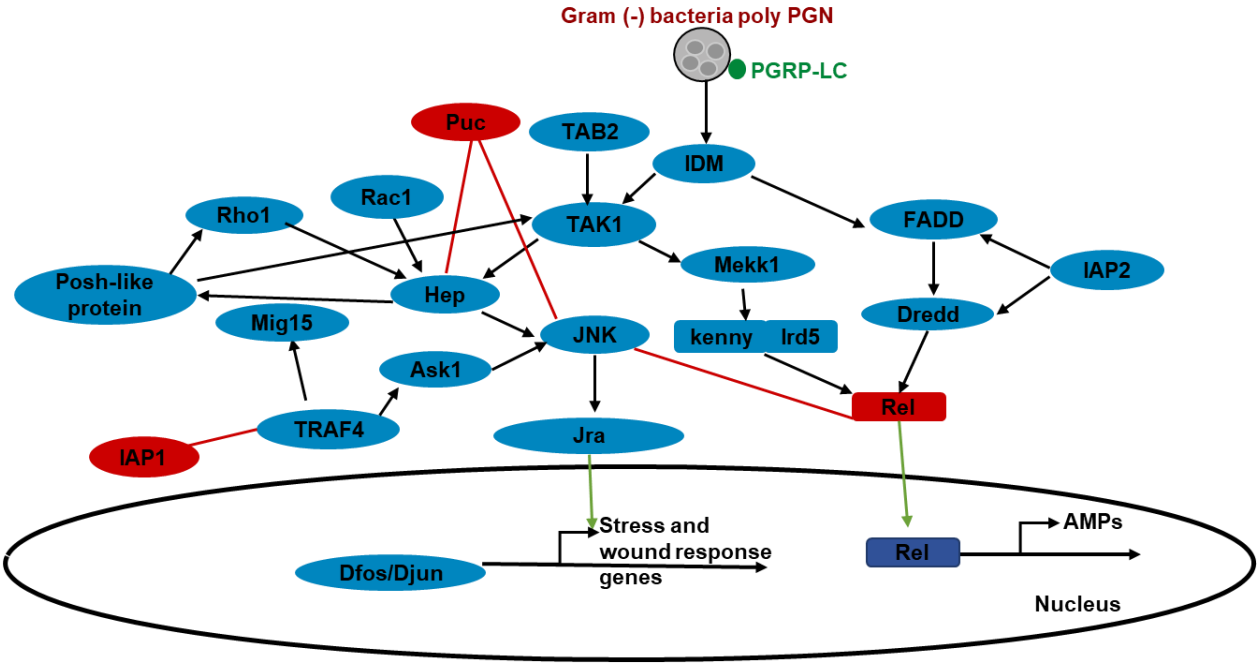


Figure 1.4: The Immune Deficiency (IDM) and c-Jun N-terminal kinase (JNK) signalling pathways. PGRP-LC recognizes DAP-type peptidoglycan (poly PGN) and activates the Imd signalling pathway. IMD interacts with FADD (Fas-associated protein with death domain), this results in the recruitment of a caspase, DREDD (FADD-death related protein. TAB2 recruits and activates TAK1, which activates the IKK complex, which then phosphorylates the NF-kB-like nuclear factor Relish. When TAK1 is activated, it also activates the JNK pathway. (Adapted from Evans *et al*. 2006; Xu and James 2009; Gupta *et al*. 2015, Rosales 2016).

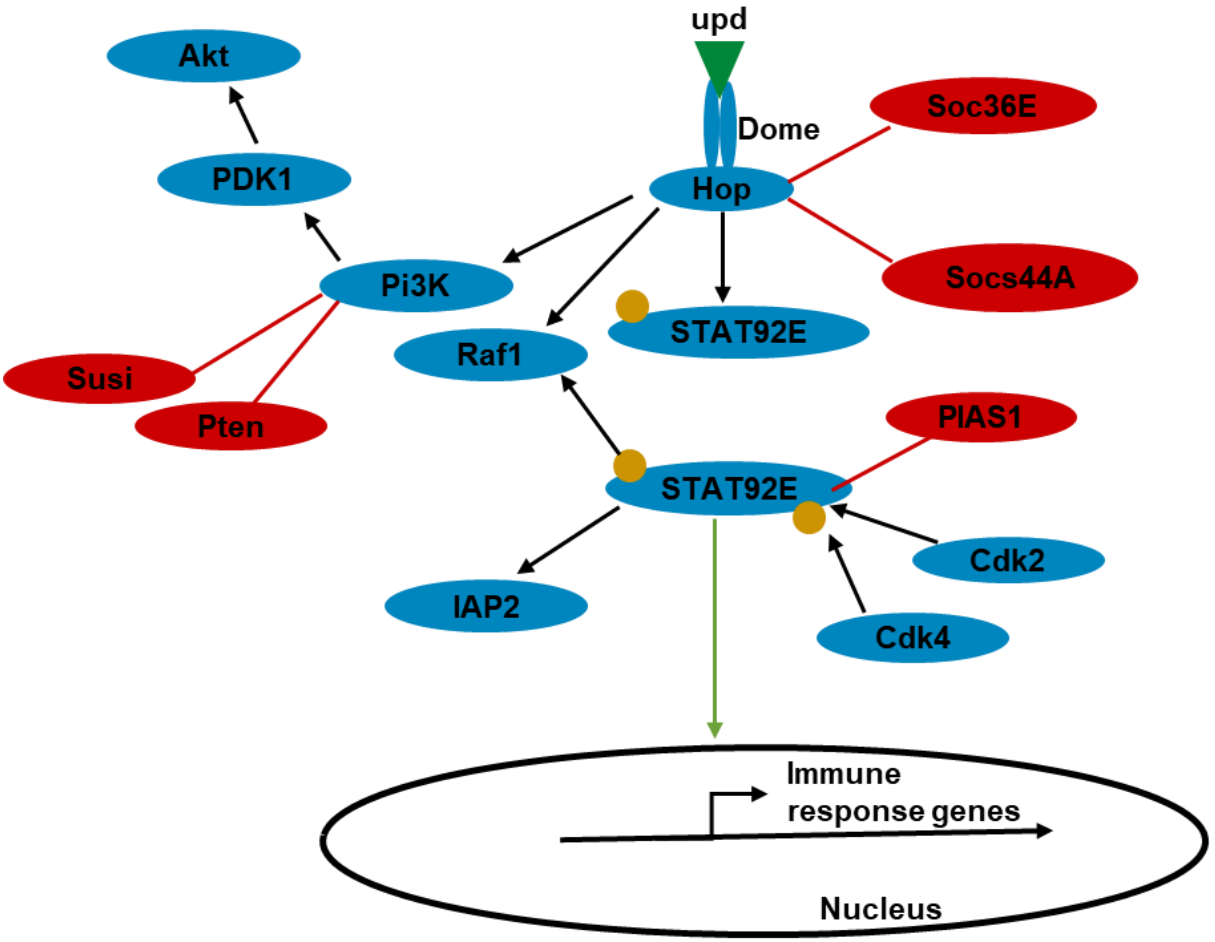


Figure 1.5: The Janus kinase-signal transducer and activator of transcription (JAK-STAT) signalling pathway. Unpaired (upd) cytokine-like proteins indicate pathogen invasion through the receptor domeless, which binds to JAK and hopscotch. Upon the activation of the receptor, hopscotch phosphorylates itself and specific tyrosine residues on the cytoplasmic part of the receptor. The phosphorylated tyrosine molecules serve as docking sites for the STAT transcription factor STAT92E. Hopscotch phosphorylates STAT92E at tyrosine residues, allowing it to dimerize and translocate to the nucleus. STAT92E binds to the promoters of their target genes in the nucleus (Adapted from Evans *et al*. 2006; Xu and James 2009; Gupta *et al*. 2015, Larsen *et al*. 2019).

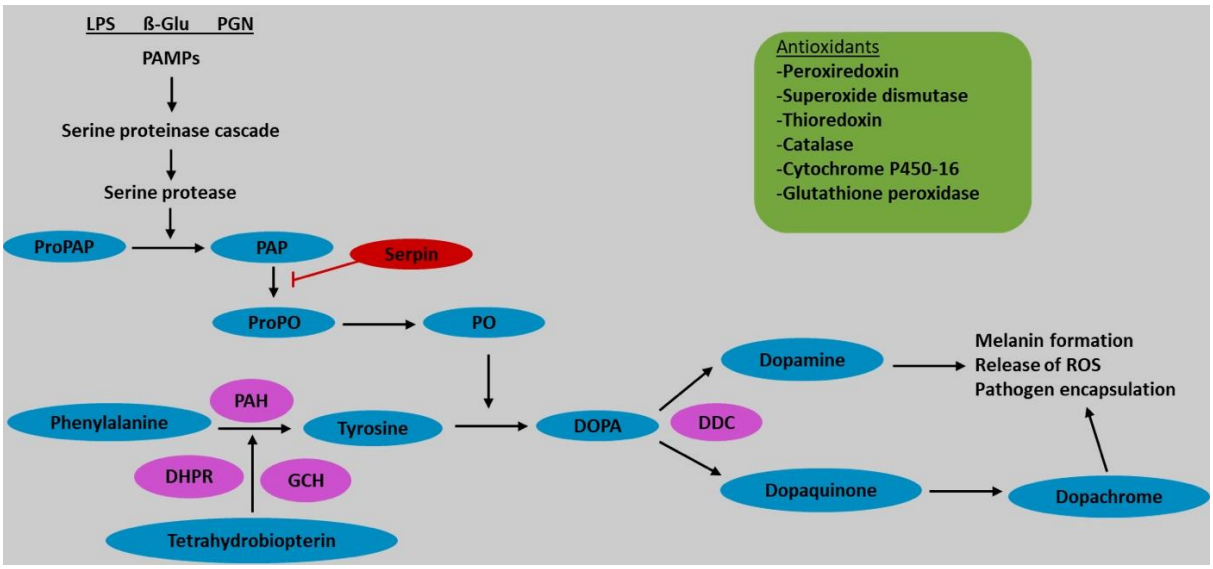


Figure 1.6: The prophenoloxidase-based melanisation biochemical process. This is a process produce melanin during wound healing, as well as in nodule and capsule formation against large invading pathogens. The enzyme phenoloxidase (PO), which is activated by a serine proteinase cascade, is the primary enzyme in this process. When pattern recognition proteins like PGRP or GRP identify certain PAMPs on the surface of an invading pathogen, the serine protease cascade is activated. The activated PO then attaches to pathogen surfaces, such as hemocyte membranes, where it starts the melanin production process. PO acts on tyrosine and converts it to dopa. Ddc can then decarboxylate dopa to produce dopamine, or PO can further oxidize it to produce dopaquinone. Both products are then further metabolized to finally produce melanin. (Adapted from Garcia *et al*. 2009, Rodriguez-Andres *et al*. 2012, Dubovdkiy *et al*. 2016).

Chapter 2

Immune-related genes activated in response to three different treatments in *Sirex noctilio*

1. Abstract

The woodwasp, *Sirex noctilio*, in association with its mutualistic fungus, *Amylostereum areolatum*, pose a threat to forest plantations in the Southern Hemisphere. This invasive pest insect is controlled with a biological control agent, a parasitic nematode, *Deladenus siricidicola*. This biological control method has previously been successful in controlling *S. noctilio* populations, achieving over 90% parasitism. Recent studies have shown that there is a variability in the efficiency of the nematode and there are some *S. noctilio* populations that are resistant to certain nematode strains. A better understanding of the immune response of the wasp during its interaction with the nematode has relevance to the evolution of immune response pathways in Hymenoptera in general and may also assist in the selection of more virulent/effective nematode strains in the future. The molecular mechanisms underlying the interaction between *S. noctilio* and *D. siricidicola* are, however, unknown. In this study, we aimed to identify the immune-related genes of this wasp. To enhance our findings, we used transcriptome analysis on immune challenged and unchallenged *S. noctilio* larvae to identify additional components implicated in immune reactions. A total of 180 immune-related genes were identified through the comparison of the *S. noctilio* genome with the genes and genomes of other Hymenoptera. Key elements of the conserved Toll, IMD, JNK and JAK-STAT signalling pathways were identified in the *S. noctilio* genome. Differential gene expression analyses performed on infected *S. noctilio* larvae demonstrated that typical wounding response mechanisms are activated by this insect in response to all the treatments. This study provides insight into the molecular pathways of innate immune processes in *S. noctilio* larvae and will serve as the foundation of future studies on the interaction between the woodwasp and pathogens/parasites.

Keywords: Hymenoptera, host-parasite interactions, immune response, *Sirex noctilio*, comparative genomics

2. Introduction

Arthropods are among the most successful life forms on earth, regarding species richness and abundance (Gupta *et al.* 2015). Like all other living organisms, they frequently encounter a wide range of pathogens. These pathogenic microorganisms include bacteria, fungi, viruses, protozoans and nematodes (Palmer and Jiggins 2015). These pathogens invade and colonize the host insect they encounter, and in most cases a successful colonization might result in harmful effects in the host (Hillyer 2016). Insects have anatomical and physiological barriers that provide a first line of defence against invading pathogens. For example, the body of the insect is covered with a single layer of epithelium (epidermis), which rests on the basal membrane. The epithelium, which is impregnated with chitin, is the foundation of the cuticle's structure (Siva-Jothy *et al.* 2005; Lundgren and Jurat-Fuentes 2012). This tough insect body coating guards against infection and mechanical injury (Moussian 2010).

When the physiological barriers are breached, the insect's immune system is switched on as a second line of defence. Insects rely on an innate immune system, although innate priming or innate memory has recently been discovered as a sort of adaptive immunity in insects (Ben-Ami *et al.* 2020). The innate immune response is made up of both humoral and cellular responses (Hoffmann 2003; Lemaitre and Hoffmann 2007). Haemocytes, which are blood cells that can engulf intruders in the process of phagocytosis or capture them in multicellular formations termed nodules or capsules, play a role in cellular immune responses (Strand 2008). The humoral immune responses involve the production of defence molecules. Reactive oxygen and nitrogen intermediates, as well as antimicrobial peptides (AMPs) are amongst the defence molecules (Aggrawal and Silverman 2007). *Drosophila melanogaster* has been the most thoroughly studied insect in terms of genetic mechanisms involved in defence reactions. In this model organism, NF-kB transcription factors activate genes encoding AMPs in response to infection via the Toll and immune deficiency (IMD) signalling pathways (Hetru and Hoffmann 2009). Other immune signalling pathways are the JNK and JAK-STAT pathways, which play a role in response to cell stress or wounding. The JAK-STAT pathway also play a role in antiviral response (Lemaitre and Hoffmann 2007).

Different pattern recognition receptors (PRRs) such as beta-1, 3-glucan recognition proteins (ßGRPs), peptidoglycan recognition proteins (PGRPs) and down syndrome cell adhesion molecules (DSCAM) detect infection and trigger the signalling cascades to activate Toll and IMD pathways (Palmer and Jiggins 2015; Zhang and Gallo 2016). Whole-genome analyses have revealed that key immune pathways and gene families are highly conserved among insect species, including hymenopterans. The Toll, IMD, JAK-STAT and JNK signalling pathways are highly conserved and are often in 1:1 orthologous relationship between species (Evans *et al.* 2006; Waterhouse *et al.* 2007; Gupta *et al.* 2015; Sackton *et al.* 2017; Zhou *et al.* 2017). However, there is still significant variation with regards to the presence/absence, copy number, and sequence divergence of genes that code for recognition and effector molecules (Sackton *et al.* 2007; Waterhouse *et al.* 2007). This could be because insects are exposed to a wide spectrum of continuously evolving pathogens, making upstream recognition and downstream effector genes targets of selection, leading to diversification (Evans *et al.* 2006; Sackton *et al.* 2007; Waterhouse *et al.* 2007).

The woodwasp, *Sirex noctilio* (Hymenoptera: Siricidae), belongs to the ancient Hymenoptera suborder Symphyta (Klopfstein *et al.* 2013). *Sirex noctilio* is native to North America, Eurasia, North Africa and Japan, where it is not considered a pest (Spradbery and Kirk 1978). *Sirex noctilio* in association with its symbiotic fungus, *Amylostereum areolatum* are globally invasive pests that attack pine species, causing significant economic and ecological damage (Tribe and Cillié 2004; Hurley *et al.* 2007; Foelker 2016). Female woodwasp infests pine trees by injecting the symbiotic fungus and phytotoxic mucus into the trees during oviposition (Bordeaux *et al.* 2014).The mucus substance weakens the pine trees and allows for the establishment of the fungus (Haavik *et al.* 2015). The fungus colonizes the wood and disrupts water flow, eventually killing the tree. The fungus participates in breaking down the cellulose which is essential for larval feeding. *Sirex noctilio* larvae do not have all the enzymes required to completely degrade cellulose, which is its principle source of carbon (Talbot 1977).

The parasitic nematode, *Deladenus siricidicola,* is the main biological control agent used against *S. noctilio*. The nematode has a bicyclic life cycle, feeding on *A. areolatum* in one part of its life cycle (mycetophagous), and infecting *S. noctilio* in the other (infective) (Morris *et al.* 2012). The infective nematodes penetrate the larvae and occupy the haemoceal, where they will develop for the duration of the life of the larvae. When the larvae pupate and the adult emerge, infective females will produce thousands of juveniles that will migrate to the developing eggs of the wasp and penetrate them. This sterilizes the female wasps. The infected eggs will serve as a vehicle for the transport of the nematodes to other trees, where they can parasitize other larvae (Hurley *et al.* 2012; Morris *et al.* 2012).

The biological control of the wasp using the nematode has been highly effective, reaching levels of >90% parasitism after inoculation (Hurley *et al.* 2007). Unfortunately, this success has not been the same throughout the regions where the wasp occurs and where the nematode is used for biological control. In various parts of South America and South Africa, parasitism levels have often been <10% (Hurley et al. 2007). One possible reason for this might be various levels of “resistance” in *S. noctilio* population to *D. siricidicola*. Bedding (1972) showed that different levels of parasitism occur when using the same nematode strain, but in different *S. noctilio* populations (Bedding 1972). Boissin et al. (2012) demonstrated that *S. noctilio* populations differ greatly between invaded regions (Boissin *et al.* 2012).

In different organisms, including non-model insects, RNA-Seq is commonly used to get transcriptomes of the organisms, tissues, or organs, to identify genes that are controlled under certain conditions, and to uncover regulatory processes (Oppenheim *et al.* 2015). In the past decade, there has been an increase in the application of RNA-Seq in biological control agents to identify the interaction mechanisms in complex parasite-host systems (Yek *et al.* 2013; de Bekker *et al.* 2015; Elya *et al.* 2018; Brettell *et al.* 2019; Lester *et al.* 2019). Transcriptome profiling of organisms under parasitisation might assist in gaining a better understanding of host responses, as well as possible effects on host growth and

development (Galetto *et al.* 2018). Many genes linked to insect's immunity has shown to be differentially expressed following an immune challenge (Salazar-Jaramillo *et al.* 2017). In this study, provide the first overview of the *S. noctilio* defence system. We first describe the putative immunity pathway models of *S. noctilio* using a homology approach with immune related genes identified in other insects. Subsequently, we use high-throughput RNA sequencing (RNA-seq) to identify which of these pathways are regulated by *D. siricidicola* parasitisation, in comparison to wounding and entomopathogenic fungal parasitisation.

3. Materials and Methods

3.1 Genome data

3.1.1 *Sirex noctilio* genome assembly and annotation

A good quality draft genome of *S. noctilio* from South Africa (unpublished data, Bernard Slippers and Alisa Postma-Smidt) was used in this study. The *de novo* genome assembly was built using VelvetOptimizer and SSPACE (Zerbino and Birney 2008; Boetzer *et al.* 2011). This resulted in the draft *S. noctilio* genome with an N50 of approximately 825 Kb, and a total size of 185 Mb. Completeness of the genome based on comparisons of conserved single copy orthologs using BUSCO v3 (Simão *et al.* 2015) was 95.4% and annotation completeness of 94%.The assembly and annotation statistics for this genome are shown in **Table 2.1**.

3.1.2 Identification and characterization of putative immune-related genes in *S. noctilio*

CLC Main Workbench 7.6.2 (https://www.qiagenbioinformatics.com/) was used to create local databases for our genome assembly and annotation data. Lists of immune-related gene sequences associated with insect's innate immunity were compiled based on literature (**Supplementary Table S2.1**). The immune-related gene sequences were then obtained from OrthoDB v8 (http://www.orthodb.org/) (Waterhouse *et al.* 2013). These immune-related gene sequences are referred to as the query sequences. As far as possible, only immune-related gene sequences that were well-annotated and functionally characterised were used as query sequences. Default BLAST parameters were used. Results identified the top scoring *S. noctilio* BLAST hit sequences for each query

sequence and the associated statistics of pairwise alignments, such as the E-values, bitscores and percentage identity.

Orthologs between the top hit *S. noctilio* protein and query protein was assigned when three criteria were met. The criteria included (a) the same putative *S. noctilio* protein sequence was identified as the top hit with the ortholog query of multiple other hymenopteran species, (b) when the E-value of a BLASTp hit was $\leq 10^{-10}$ and (c) when the bit score was above 60. The top *S. noctilio* putative innate immunity orthologs identified by local BLASTp analyses were extracted as fasta files from CLC Main Workbench 7.6.2. The extracted sequences were submitted for BLASTp analyses against the OrthoDB and NCBI databases, Hence, the percentage identity, percentage coverage, E-values, and bitscores of putative *S. noctilio* proteins could be compared with many more Hymenoptera species than in the local BLASTp analyses. In this way, the initial identification of putative *S. noctilio* innate immunity orthologs could be supported.

The selected immune-related genes were also analysed using CDD database to detect corresponding conserved domain structures (Marchler-Bauer *et al.* 2010). The function of an identified ortholog is often predicted based on the sequence similarity it shares with functionally characterised orthologs in other organisms (Pearson 2013). This is based on the concept that homologous sequences have similar secondary protein structures and are, therefore, often similar in function. The protein domains were analysed in particular as each domain is involved in specific interactions and/or functions, with evolution at these domains being more constrained than other protein regions (Bagowski *et al.* 2010). Throughout the study, we used protein sequences as queries as they allow for the detection of more distantly related sequences than DNA sequences, which is due to the degeneracy of the genetic code. These methods were employed to identify orthologs and protein domains, but also to estimate authenticity of the protein annotations.

Manual curation of the *S. noctilio* genes which passed the above-mentioned criteria were performed in WebApollo (Lee *et al.* 2013). WebApollo is a web-based JBrowse plug-in that allows the user to perform manual curation and visualisation of the changes made to the annotation. The WebApollo graphical interface was used to identify and manually curate the candidate immune-related gene orthologs. This was done by considering RNA-Seq data from the immune challenged *S. noctilio* larvae. Changes to the initial models were validated using Clustal Omega to ensure sequence is complete/correct (Sievers and Higgins 2014).

3.2 Transcriptome analysis

3.2.1 Sample preparation, RNA extraction and sequencing

Four treatments were tested for their impact on immune-related transcripts in *S. noctilio*, including a control. Ten *S. noctilio* larvae were exposed in each of the treatments. In the first, early-stage *S. noctilio* larvae were infected by *D. siricidicola* nematodes. *Deladenus siricidicola* nematodes of the strain SA107 (2013) were sub-cultured in 500 ml Erlenmeyer flasks. The flasks contained 160 g sterilised media (mix of 70% wheat and 30% brown rice), that was inoculated with *A. areolatum*, as well as 85 ml of water. The flasks were incubated, and nematodes were harvested 6-8 weeks post incubation. *Sirex noctilio* larvae were added into the flask containing harvested nematodes. In the second treatment, the immune responses in *S. noctilio* are considered following infection with the fungus *Beauveria bassiana* isolate HBD241. A volume of 3 ml of a spore suspension of the fungus was directly pipetted onto *S. noctilio* larvae. Lastly, the impact of a wounding response was assessed by pricking each individual larva twice, once at the anterior body and once at the posterior body using the tip of a disposable hypodermic needle. The samples, along with unchallenged control, were collected at 72h.

The larvae were homogenized using a mortar and pestle. Total RNA was extracted from individual larvae using a standard TRIzol method (Invitrogen, California, USA). Total RNA was re-suspended in 50µl of sterile nuclease free water. A nanodrop was used to measure the concentration of the RNA. RNA quality and integrity were assessed using a Bioanalyser. Only the RNA samples with A_{260}/A_{280} ratios in a range from 1.8 to 2.0 and

A_{260}/A_{230} >1.5 were selected for further processing. All buffers were treated with DEPC solution and autoclaved before use. Three biological replicates containing three separately prepared RNA samples each from the control or treated larvae at the 72h time point were included for RNA-Seq analysis of *S. noctilio* transcriptome (**Figure 2.1**). Separate libraries for the four experimental conditions were prepared, and the samples were sent to Beijing genomics institute (BGI) for sequencing. The libraries were labelled, pooled and sequenced using paired end libraries on the Illumina HiSeq platform.

3.2.2 Alignment of reads and coverage analysis

At one timepoint, samples representing the three infection types were collected and processed for sequencing. FASTQC and MultiQC was used to assess the quality of the raw sequencing data. Trimmomatic was used to trim and filter contaminating adapter sequences and low-quality reads from the raw Illumina RNA-Seq data (Andrews 2010; Bolger *et al.* 2014). The read alignment tool, TopHat2, was used to align the short RNA-seq reads to the *S. noctilio* reference genome for each of the sequencing datasets (Kim *et al.* 2013). The output from TopHat2 was obtained as BAM format files which contain information on the mapping position and quality of the individual reads in relation to the reference genome. Quality control was done on the BAM files using MultiQC. Subsequently, FeatureCounts was used to quantify the genetic features contained within the mapping results (Liao *et al.* 2014).

3.2.3 Differential gene expression analysis and statistical analysis

The read counts were used as input for DESeq (v1.10.1) and edgeR (v3.4.0) (Anders 2010; Robinson *et al.* 2010). Both programs are R Bioconductor packages that assess variance-mean dependency in count data from high-throughput sequencing assays, normalize count data for library sizes and dispersion, and test for differential expression using a negative binomial distribution model (Anders and Huber 2010). Compared to edgeR, DESeq algorithms are more conservative. Even though the two statistical methods may generate different significantly differentially expressed gene lists, we expect some overlap in the results. After applying significance cut-offs (adjusted p-value 0.05), the significantly differentially expressed genes were identified for multiple comparisons. BLAST2GO was used for Gene ontology (GO) analysis using the list of the significantly differentially expressed genes (www.blast2go.com). Gene ontology (GO) is an

internationally standardized functional classification system that covers three categories: biological process, molecular function, and cellular component. In our study we performed the classification of GO terms at level two in each category.

3.2.4 Primer design

Primers were designed for seven candidate genes of interest (*thioredoxin*, *integrin beta*, *epidermal growth factor receptor*, *superoxide dismutase (Mn)*, *cytochrome P450*, *glutathione peroxidase* and *dynamin*). These genes were selected as they were uniquely induced by *D. siricidicola* and are not shared with other infection models. We also designed primers for four reference genes (*GADPH*, *actin*, *alpha tubulin* and *Rp49*) that were selected based on reference genes that are already described for insects in the literature. The primer pairs were designed using Primer3 software v.0.4.0 (Rozen and Skaletsky 2000) and the Oligo-analyzer online tool from Integrated DNA Technology website (www.idtdna.com) was used to identify any secondary structures in the designed primer pairs. The criteria used for the primer design were as follows: (i) the primers had to have a minimum melting temperature (Tm) range of 40^{o}C-62^{o}C (ii) with the last base pairs not having more than 3 C's or G's (iii) they should be designed towards the 5' end.

4. Results

4.1 Identification and characterization of putative immune-related genes in *S. noctilio*

The genome of *S. noctilio* was searched for possible immune-related genes. A total of 180 immune-related genes were identified in *S. noctilio* by sequence-based protein orthology to previously published data of other Hymenoptera species (**Supplementary Table S2.1**). Genes that are involved in insect's pathogen recognition include those encoding PGRPs, βGRPs, galectins, C-type lectins, scavenger receptors (SCRs) and croquemort. Genes involved in signal transduction were grouped into pathway-related categories of Toll, IMD, JNK and JAK-STAT. The immune effector category comprised of prophenoloxidase, thioester-containing protein (TEPs) and antimicrobial peptides (AMPs). Other genes included those encoding antioxidant enzymes SOD, catalases and

peroxidases which play a role in detoxification of reactive oxygen species (ROS), as well as genes involved in phagocytosis, encapsulation, and nodulation.

Pattern recognition receptors (PRRs) mediate the identification of pathogen invasion, which is the first step in the innate immune response (PRRs) (Akira *et al.* 2006). We have identified 14 PRRs in *S. noctilio* including two PGRPs, two βGRPs, one galectin, three C-type lectins, one Scavenger receptor B subfamily member, one Drapper and one DSCAM (**Supplementary Table S2.1**). The *S. noctilio PGRP-LC* and *PGRP-SA* has a N-acetylmuramoyl-L-alanine amidase domain and peptidoglycan recognition protein domain (**Supplementary Figure S2.1 and Supplementary Figure S2.2**). One of the *S. noctilio* βGRPs genes (β-gluc1) contain the Carbohydrate binding domain (family 32) which is normally found at the N-terminus of beta-1,3-glucan-binding proteins. The two *S. noctilio* βGRPs genes contains the glycosyl-hydrolase family 16 domain (**Supplementary Figure S2.3** and **Supplementary Figure S2.4**). The domain architecture analysis indicates that the putative *S. noctilio* galectin possesses conserved galactoside binding domain (**Supplementary Figure S2.5**). The C-type lectins identified in *S. noctilio* contain carbohydrate-recognition domains (**Supplementary Figure S2.6** and **Supplementary Figure S2.7**). Croquemort and Scavenger receptor class B member 1 identified in *S. noctilio* both contain the CD36 domain (**Supplementary Figure S2.8** and **Supplementary Figure S2.9**).

In terms of the presence of orthologs in *S. noctilio*, the Toll Signalling pathway was discovered to be highly conserved (**Figure 2.2**). In *S. noctilio* three PRRs likely activating the Toll pathway are found: PGRP-SA, two beta-1,3-glucan binding proteins GNBP1 and GNBP3. A single gene encoding Dorsal was identified in *S. noctilio,* however, as in *A. mellifera* no ortholog of the dorsal-related immunity factor, Dif, was present. The IMD and JNK pathways were also conserved in *S. noctilio* (**Figure 2.3**). In *S. noctilio*, we found a single gene PGRP-LC that encodes one PRR that is involved in the activation of the IMD pathway. There is an absence of IKK complex in *S. noctilio*. The data also indicate that the JNK pathway in *S. noctilio* is like the pathway in other hymenopterans as most of the

core components of the JNK pathway of the hymenopterans have orthologs in *S. noctlio* (**Figure 2.3**).

Scavenger receptors, Croquemort family members, Nimrod, Galectins, C-type lectins, Hopscotch, Domeless, signal transducer STAT, Fibrinogen-related protein, Down syndrome cell adhesion molecular (Dscam) and Thioester containing proteins (TEPs). These are known to be involved play a role in the JAK-STAT pathway. Some of the components of the JAK-STAT pathway are present in *S. noctilio* (**Figure 2.4**). Two AMPs, Defensin and Hymenoptaecin were identified in the genome of *S. noctilio*. Immune effectors genes including a single *ProPO* gene, and one Lysozyme was identified in *S. noctilio* genome (**Figure 2.5**, **Supplementary Table S2.1**). Among signal modulation genes, a few genes encoding three Serpins and three Serine Proteases were found in the genome of *S. noctilio*. Genes encoding antioxidant enzyme were also identified in the genome of *S. noctilio*, these included two SOD genes, one was the Cu-Zn SOD and the other was the Mn-Fe SOD, two peroxidases and one catalase (**Figure 2.5**). The summary of the proposed immune signalling pathways in *S. noctilio* is shown in **Figure 2.6**.

4.2 RNA sequencing

cDNA libraries were generated RNA isolated from immune challenged (infected with *D. siricidicola* and *B. bassiana*, and wounded) and control *S. noctilio* larvae, and then sequenced using Illumina HiSeq. The average number of reads across all the libraries is 32465911 and the average GC content was calculated to be 42% (**Table 2.2**). Overall, the reads in clean data showed good mapping rates and concordant pair alignments (95.60%-97.9%) to the reference genome (**Table 2.3**).

4.3 Differential gene expression analysis

Following statistical analysis and a statistical threshold of adjusted p-value ≤ 0.05, the combination of the results from edgeR and DESeq enabled the identification of 4862 genes which were significantly differentially expressed in the immune challenged *S. noctilio* larvae compared to the control. DESeq exclusively identified a total of 540 and edgeR exclusively identified 148 significantly differentially expressed genes in the immune challenged *S. noctilio* larvae compared to the control (**Figure 2.7** and **Table 2.4**).

There was an approximately 85% overlap amongst the significantly differentially expressed genes identified through the two analysis approaches. The genes that are present in the overlap of the DESeq and edgeR are used in the analysis that follow.

RNA-Seq was used to determine the expression profiles of immune-related genes in early-stage *S. noctilio* larvae following immune challenges to show the regulation patterns of the different immune-related genes in *S. noctilio*. Out of the 4862 significantly differentially expressed genes, 1270 (608 upregulated and 662 downregulated), 1817 (902 upregulated and 915 downregulated), and 3212 (1749 upregulated and 1463 downregulated) genes that were significantly differentially expressed in *S. noctilio* after nematode infection, fungal infection, and wounding, respectively (**Table 2.4**). There were 342 significantly downregulated genes and 257 significantly upregulated genes that were commonly expressed among all the treatments **(Figure 2.8a** and **Figure2.8b**). There were 62 downregulated genes that were common between fungal infection and wounding, 170 between wounding and nematode infection and 159 between fungal infection and nematode infection, respectively (**Figure 2.8a**). Moreover, there were 103 upregulated genes that were common between fungal infection and wounding, 236 between wounding and nematode infection and 129 between fungal infection and nematode infection, respectively (**Figure 2.8b**).

The overall comparison of the differential gene expression activated by the three treatments through MA plots revealed that wounding activated large response, as expected (**Figure 2.9**). Wounding is a mechanical process that does not involve strategies to evade the host immune response, therefore, it was expected that it will show large immune activation in comparison to the response activated by the *D. siricidicola* nematode and *B. bassiana* fungus. In the PCA plot for *S. noctilio* immune response, wounding grouped differently while nematode, fungi and control grouped together (**Figure 2.10**).

4.3.1 *D. siricidicola* nematode infection, *B. bassiana* fungal infection and wounding regulates several molecular pathways and biological activities in *S. noctilio.*

In the dataset of genes that were significantly upregulated by *D. siricidicola* infection, “metabolic processes” and “biosynthetic processes” were the most common GO biological process categories. In the molecular function category, “binding” and “kinase activity” were the most abundant. In the cellular component category, “nucleus” and “cytoskeleton” represented the most abundant subcategories (**Figure 2.11**). The overall gene repertoire that was significantly downregulated by the *D. siricidicola* nematode infection, the most abundant GO biological process categories were “metabolic processes” and “biosynthetic process”. In the molecular function category, “binding” and “hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides” were the most abundant. We found that in the cellular component category “nucleus” and “mitochondrion” represented the most abundant subcategories (**Figure 2.12**).

The gene repertoire that was significantly downregulated by *B. bassiana* fungal infection, “metabolic processes” and “biosynthetic processes” were most enriched in the GO biological process category. In the category molecular function, the subcategories “binding” and “kinase activity” were the most abundant. In the category cellular component “nucleus” and “mitochondrion” represented the most abundant subcategories (**Figure 2.13**). The gene repertoire that was significantly upregulated by *B. bassiana* fungal infection, in the GO biological process category “metabolic processes” and “biosynthetic processes” were the most enriched. In the category molecular function “binding” and “transferase activity” were the most enriched subcategories. In the cellular component category, “nucleus” and “cytoskeleton” were the most enriched subcategories (**Figure 2.14**).

The gene repertoire that was significantly downregulated by wounding, “metabolic processes” and “biosynthetic processes” were most enriched in the GO biological process category. We found “binding” and “kinase activity” to be the most abundant subcategories in the molecular function category. In the cellular component category, “nucleus” and “mitochondrion” were the most enriched subcategories (**Figure 2.15**). The gene repertoire that was significantly upregulated by wounding, “metabolic processes” and “biosynthetic processes” were the most enriched in the category GO biological process. The subcategories “binding” and “transporter activity” were the most abundant in the molecular function category. In the cellular component category, the subcategories “nucleus” and “mitochondrion” were the most enriched (**Figure 2.16**).

4.3.2 Regulation of candidate immune-related genes by *S. noctilio* larvae in response to *D. siricidicola* nematode infection, *B. bassiana* fungal infection and wounding

The number of common and exclusive immunity-related differentially expressed genes (DEGs) among the treatments was determined using a Venn diagram analysis (**Figure 2.17**). There are 18 DEGs that were commonly expressed among all the treatments, while 5, 3, 12 were commonly expressed among *D. siricidicola* nematode infection and *B. bassiana* fungal infection, *B. bassiana* fungal infection and wounding, and wounding and *D. siricidicola* nematode infection, respectively. Additionally, 3, 10 and 24 DEGs were specifically expressed in *B. bassiana* fungal infection, *D. siricidicola* nematode infection and wounding, respectively (**Figure 2.17**). The immune-related differentially expressed genes in *S. noctilio* included genes involved in recognition (*B-gluc2*, *PGRP-SA*, *SRCBM1* and vigilin), Toll, IMD, JNK, and JAK-STAT signalling pathways (*SPZ*, *Toll*, *Tube*, *Rel, Fadd*, *Imd*, *STAT*), effectors, serine proteases, Prophenoloxidase and serine protease inhibitors. Other wound healing genes with chitinase activity (*Cht3*, *Cht5*) were also included.

In the immune recognition group, two genes were significantly differentially expressed, *PGRP-SA* and *β-gluc2*. *PGRP-SA* was significantly upregulated by *D. siricidicola* and *B. bassiana* infections. One gene encoding *β-gluc2* was upregulated during *B. bassiana* infection. Wounding resulted in significant upregulation of *vigilin* and downregulation of scavenger receptor *SRCBM1*. Genes that play a role in signal modulation such as serine proteases and serine protease inhibitors were also regulated in our dataset. These include *serine protease 48* and *serpin B8* which were significantly upregulated, as well as *serpin B10* that was significantly downregulated by the *D. siricidicola* infection.

In the signal transduction group the Toll pathway genes *spaetzle*, *protein spaetzle*, *tube*, *toll*, *toll6*, *toll8* and *pelle* were significantly regulated following the immune challenges. Among them *protein spaetzle* and *tube* were significantly upregulated in response to all the three infection types. *spaetzle* was significantly downregulated in response to wounding and significantly upregulated in response to *B. bassiana* infection. Wounding led to significant downregulation of *Toll* and *toll6* was significantly upregulated by *D. siricidicola* infection. The gene *pelle* was significantly upregulated by wounding. The gene *Toll8* was found to be significantly upregulated by *D. siricidicola* infection and significantly downregulated by wounding. We also found that *Tolloid-like protein 2* was significantly downregulated by wounding (**Figure 2.18**).

In the IMD pathway the following genes were regulated *IMD, dfadd*, *relish* and *ank-1*. Among these genes *Imd* and *dfadd* were siginificantly upregulated in response to all the infection types. The IMD pathway transcription factor *Relish* was significantly upregulated in response to wounding while ank-1 was significantly upregulated in response to *D. siricidicola* infection and wounding (**Figure 2.19**). In the JNK pathway, *JNK* was significantly upregulated in response to wounding, *rho1* was significantly downregulated by *D. siricidicola* and *B. bassiana* infections. *Jra* was significantly downregulated in response to *D. siricidicola* infection and wounding. The gene *puc* was significantly upregulated by *D. siricidicola* infection and wounding (**Figure 2.19**). In the JAK-STAT pathway *stat* and *pdk1* were significantly upregulated in response to all the infection

conditions. *Agrin* was significantly upregulated in response to *D. siricidicola* infection and significantly downregulated in response to wounding. Lastly, *polo* was significantly downregulated by wounding (**Figure 2.20**).

The immune effector genes such as *PPO*, *hymenoptaecin*, *defensin*, *quinone reductase* and *putative cht3* were regulated in our dataset. *PPO* and *putative cht3* were significantly upregulated in response to all the infection conditions. The gene *Quinone reductase* was significantly downregulated in response to all the infection conditions. The antimicrobial peptide gene *Hymenoptaecin* was significantly upregulated in response to wounding and *D. siricidicola* infection, and significantly downregulated in response to *B. bassiana* infection. Genes encoding enzymes that play a role in reactive oxygen species (ROS) detoxification were also induced in our dataset. These include thioredoxin, MnSOD, catalase, glutathione-S-transferase and glutathione peroxidase. The genes MnSOD, thioredoxin and glutathione peroxidase were uniquely significantly downregulated by *D. siricidicola* infection. We found catalase to be upregulated by both *D. siricidicola* infection and wounding. The antimicrobial peptide gene *Defensin* was significantly downregulated by wounding, while *B. bassiana* infection significantly upregulated its expression levels. Components of the extracellular matrix were also regulated in our gene set. These includes *paxillin, integrin beta* and *epidermal growth factor receptor*. The gene *paxillin* was significantly upregulated by all the treatment conditions. We found *integrin beta* to be significantly upregulated by both *D. siricidicola* infection and wounding. Finally, we found *epidermal growth factor receptor* to be significantly upregulated by *D. siricidicola* infection only.

We further noticed other genes to be significantly differentially expressed, these included *hexamerin*, *myosin regulatory light chain 2, myosin IA*, *myosin XV*, *cytoskeleton-associated protein 5*, *microtubule-associated protein*, *croquemort*, *structural cuticle protein*, *zinc finger protein 609*, *dynamin*, *engulfment and cell motility protein 1*, *phospholipase A2*, *phospholipase D*, *prostaglandin E synthase2*, *peroxiredoxin*, *DDC/Aromatic-L-Amino-Acid decarboxylase*, *proclotting enzyme*, *cytochrome P450-16*, *zinc transporter 2. Hexamerin* was upregulated by *D. siricidicola* infection and

downregulated by wounding. Three differentially expressed genes *myosin regulatory light chain 2*, *myosin IA*, *myosin XV* were shared between *D. siricidicola* infection, *B. bassiana* infection and wounding, all of which were upregulated. Wounding resulted in the downregulation of *cytoskeleton-associated protein 5* and upregulation of *microtubule-associated protein.* Downregulation of *croquemort* was shared between *D. siricidicola* infection and wounding. Wounding resulted in upregulated expression of *phospholipase A2*. *Phospholipase D* was significantly downregulated by all the treatment conditions. Upregulation of *prostaglandin E synthase2* was shared between *D. siricidicola* infection and wounding. *D. siricidicola* infection and *B. bassiana* infection both resulted in downregulated expression of *peroxiredoxin*. Both *D. siricidicola* infection and wounding resulted in the upregulated expression of *DDC/Aromatic-L-Amino-Acid decarboxylase,* and downregulated expression of *cytochrome P450-16*. *Proclotting enzyme* was downregulated by wounding only. The expression of *zinc transporter 2* was downregulated by *D. siricidicola* infection and upregulated by wounding. We generated heat maps to illustrate the regulation patterns of the different immune-related gene in *S.noctilio* in response to *D. siricidicola* nematode infection, *B. bassiana* fungal infection and wounding in comparison to the uninfected control (**Figure 2.21-Figure 2.23**).

4.4 Primer design

Seven significantly differential expressed genes related with the wounding and defence responses were selected to be used in the future for real-time qPCR confirmation. These genes were uniquely regulated in response to *D. siricidicola* nematode infection. Four reference gene primers were also designed. The eleven primer sequences and related information are given in **Table 2.6**.

5. Discussion

5.1 A brief overview of the *S. noctilio* immune system

We present the first view of the immune-related gene repertoire of *S. noctilio*, using sequence-based protein similarities with the previously published data of Hymenoptera species with the assumption that their roles and mechanisms of action are conserved. We found 180 immune-related genes in the genome of *S. noctilio*, and we propose models of the potential immune signalling pathways in *S. noctilio* that need to be validated

experimentally. Like other systems, the core signalling pathways seem to be more conserved, with large number of 1:1 orthologs, than the recognition and effector molecules that are often expanded and diverse (Gupta *et al.* 2015). We further evaluated the transcriptional changes that takes place following infection of *S. noctilio* larvae with the *D. siricidicola* nematode and *B. bassiana* fungus, as well as wounding. Our results indicate that there are hallmarks of a wound response that is shared amongst the infection models.

5.1.1 Pathogen recognition

When an insect comes into contact with a pathogen, it activates its innate immune system by attaching to or recognising pathogen-associated molecular patterns (PAMPs) (bacterial peptidoglycan, lipopolysaccharides, β-glucans, lipoproteins, CpG dinucleotides or flagellin) (Shelby and Popham 2012). Pattern recognition receptors (PRRs) identify these molecules. Known insect PRRs include peptidoglycan recognition proteins (PGRPs), β-1, 3-glucan recognition proteins (βGRPs), galectins, C-type lectins (CTLs) and scavenger receptors (SCRs). Peptidoglycan recognition proteins (PGRPs) are divided into two types: short (S) and long (L) (Liu *et al.* 2014; Nayduch *et al.* 2014).

In this study, we identified two PGRP genes in the genome *S. noctilio*, *PGRP-SA* and *PGRP-LC*. These are fewer than four PGRPs found in the honeybee genome and 4-6 PGRPs in the bumble bee (Evans *et al.* 2006; You *et al.* 2010). *Sirex noctilio PGRP-LC* and *PGRP-SA* has a N-acetylmuramoyl-L-alanine amidase domain and peptidoglycan recognition protein domain. This suggests that it may be able to cleave the amide bond between N-acetylmuramoyl and L-amino acids in bacterial cell walls, as well as to bind and hydrolyse peptidoglycans (PGNs) of bacterial cell walls (Xin *et al.* 1991; Cheng *et al.* 1994).

We identified two *βGRP* genes in the genome of *S. noctilio*. The first insect GRPs were discovered in *B. mori* and were linked to the activation of the prophenoloxidase (PPO) activation system and since then they have been discovered in various insects including *Drosophila*, *Anopheles* and *Tribolium*, as well as the Hymenoptera *Apis* (Ochiai and Ashida 1988; Christophides *et al.* 2002; Evans *et al.* 2006; Liu *et al.* 2014; Yokoi *et al.*

2015). βGRPs are involved in the activation of a serine protease cascade that triggers a phenoloxidase cascade and AMP gene expression in insects (Bang *et al.* 2015). The Carbohydrate binding domain is found in one of the S. noctilio GRP genes (-gluc1). One of the *S. noctilio βGRP* genes (*β-gluc1*) contain the Carbohydrate binding domain (family 32) which is generally present at the N-terminus of beta-1,3-glucan-binding proteins that are involved in pathogen recognition. This domain recognizes and attach to a triple-helical beta-1,3-glucan structure of the invader. Both genes contained the glycosyl-hydrolase family 16 domain which allows these pattern recognition receptors to form complexes with pathogen-associated beta-1,3-glucans and subsequently transduce the signals needed to activate an adequate innate immune response (Mertz *et al.* 2009; Kanagawa *et al.* 2011; Sun *et al.* 2011).

One *galectin* was identified in the genome of *S. noctilio* and possesses conserved galactoside binding domain. Galectins are a family of lectins that are characterised by the presence of evolutionary conserved family of beta-galactoside-binding proteins (Taylor and Drickamer 2003). Galectins in insects take part in the regulation of immune responses against protozoa, bacteria, and viruses (Rao *et al.* 2016; Sreeramulu *et al.* 2018). Thus, the domain in the sequence indicates a possible immune function in *S. noctilio*.

C-type lectins identified in *S. noctilio* contain carbohydrate-recognition domain. C-type lectins include a wide range of soluble and membrane-bound proteins that contain calcium-dependent carbohydrate-recognition domains (CRD) (Zhu *et al.* 2020). They play essential roles in insects' innate immunity in pattern recognition, agglutination, encapsulation, melanisation phagocytosis and ProPO activation (Zhu *et al.* 2020).

No member of the scavenger receptor A subfamily and scavenger receptor C subfamily was identified in the genome of *S. noctilio*. The scavenger receptor family has multiple domains and functions as pattern recognition receptors in innate immunity. Based on their functional domains, this family can be grouped into three subfamilies: scavenger receptor A (SCRAs), scavenger receptor B (SCRBs), and scavenger receptor C (SCRACs).

Scavenger receptors A have been found to play a role in host defense by binding polyanionic ligands like lipopolysaccharide (LPS) and lipoteichoic acid (LTA) (Lu *et al.* 2020). A scavenger receptor Cysteine-Rich domain is found in several members of the scavenger receptors A subfamily, which aids in the binding of Gram-positive and Gram-negative bacteria. Scavenger receptors C had previously been discovered to take part in phagocytosis and innate immunity as pattern recognition receptors (Kim *et al.* 2018). The absence of scavenger receptor A subfamily and scavenger receptor C subfamily members in *S. noctilio* might be due to the homologue search method that was utilized which is based on sequence similarity, it can also be possible that this gene is missing from the genome assembly. Further investigations would be needed to identify the cause.

Scavenger receptor class B member 1 and *Croquemort* were identified in *S. noctilio* as members of the scavenger receptor B subfamily. Scavenger receptors B are referred to as a novel class of scavenger receptors designated by a CD36 domain. *Croquemort* participates in phagocytosis, which allows it to efficiently absorb a wide spectrum of bacteria and fungi (Guillou *et al.* 2016).

5.1.2 Signal transduction pathways

In insects, signal transduction mechanisms involved in immune response are well-known. In insects, the Toll and Imd signalling pathways are the most well-known immune-related signalling pathways (Liu *et al.* 2014). Fungi and Gram-positive bacteria activate the Toll pathway, whereas most Gram-negative bacteria and some Gram-positive bacteria activate the IDM pathway (Lemaitre and Hoffmann 2007). JNK and JAK-STAT signalling pathways are also known to be involved in insect immunity. The signal transduction pathways are initiated following an invasive signal to produce effector molecules (Weston and Davis 2002).

Two *SPZ* genes and four *Toll receptor* genes were identified in *S. noctilio* genome. Spätzle functions as a Toll receptor ligand in the Toll pathway. *Spätzle* binds to the *Toll receptor* and activates the Toll pathway. When Toll receptors are activated, they bind with cytoplasmic MyD88 and, as a result, MyD88, tube and pelle create a complex to phosphorylate cactus, resulting in degradation of cactus and the release of Dorsal and

DIF (Dorsal-related immunity factor), which translocate to the nucleus to induce antimicrobial peptide gene expression (Evans *et al.* 2006; Roh *et al.* 2009; Hillyer 2016; Rosales 2017) . The Toll pathway genes of *tube*, *MyD88*, *pelle*, *TRAF6* and *cactus* were also identified in the genome of *S. noctilio*. Dorsal is encoded by a single gene in *S. noctilio*, however, as in *A. mellifera* and *C. floridanus* no ortholog of *DIF* was found (Evans *et al.* 2006; Gupta *et al.* 2015). This is in support of a previous suggestion that *DIF* is part of a highly derived evolutionary branch, possibly occurring only in brachyceran flies and not in other insects. As in *C. floridanus* and *A. mellifera*, *Dorsal* in *S. noctilio* appears to be a functional alternate of *DIF*.

Our results indicate that most components of the IMD pathway are present in *S. noctilio*. The Imd pathway is mainly activated by infection with Gram-negative bacteria (Rosales 2017). Genes involved in the IMD pathway including *IMD*, *Dredd*, *FADD*, *TAK1*, *TAB2*, *Relish*, but not IKK complex, were identified in the *S. noctilio* genome. A major difference is observed in *S. noctilio* IMD signalling pathway in comparison to the model species *D. melanogaster*'s IKK complex which phosphorylates the NF-B-like transcription factor Relish, activating it. The IKK complex in *Drosophila* is made up of the enzymatically active Ird5 subunit and the regulatory subunit Kenny (Gupta *et al.* 2015). However, in *S. noctilio* both the enzyme subunit and regulatory subunit are missing. Further analysis is needed to identify the cause of the missing enzyme subunit. Iterative sequence analyses have identified the lack of the Kenny subunit in several hymenopteran species, including *A. mellifera*, *N. vitripennis* and *C. floridanus* (Evans *et al.* 2006; Sackton *et al.* 2013; Gupta *et al.* 2015). This shows that the IKK complex has a common feature in Hymenoptera. It's unclear whether the absence of the Kenny subunit reflects Hymenoptera's lower immune potential or if there are other undiscovered components involved in the formation of the functional IKK complex.

JNK, *kay* and *TRAF4* genes in the JNK pathway were all identified in *S. noctilio* genome. TAK1 is a protein kinase that activates the JNK pathway in response to cell stress or injury, as well as a downstream component of Imd (Rämet *et al.* 2002). The common 1:1

orthologs of genes involved in the IMD and JNK pathways of *S. noctilio* and other insects suggest that these pathways are complete and conserved.

In the *S. noctilio* genome we have identified Domeless, one Hopscotch and one STAT as components of the JAK-STAT pathway. The JAK-STAT signalling pathway aids insect's innate immunity by inducing complement-like factors and proliferation of haemocytes. The JAK-STAT pathway has shown to be activated through cytokine-like molecules in blood cells (Hillyer 2016; Larsen *et al.* 2019). In the model insect *Drosophila melanogaster* Upd, an extracellular glycosylated protein, acts as a ligand to activate the JAK-STAT pathway, which enhances haemocyte phagocytic activity (Myllymäki *et al.* 2014). In *Drosophila*, the JAK-STAT pathway has also been demonstrated to have a role in antiviral response. JAK-STAT is made up of several components which include the cytokine receptor *domeless*, JAK tyrosine kinase (*Hopscotch*), transcription factor, two negative pathway regulators *SOCS* (suppressor of cytokine signalling) and *PIAS* (protein inhibitor of activated STAT). The genes encoding extracellular ligand proteins identified in *Drosophila*, which activates the pathway were not found in *S. noctilio*, as they were not found in other insects, including *A. mellifera* and *C. floridanus* (Evans *et al.* 2006; Gupta *et al.* 2015). The downstream effectors of the JAK-STAT pathway in *D. melanogaster* include Thioester containing proteins (TEPs) and Turadont proteins, like *A. mellifera* and *C. floridanus* Turadont proteins homologs were not identified in *S. noctilio*, but one TEP was found.

5.1.3 Immune effectors

In insects, a proPO-based melanisation reaction can be seen at the site of injury or on the invading pathogen's surface (Christensen *et al.* 2005). The activation of proPO is required for melanisation, and multiple genes encoding serine proteinases and their inhibitors (serpins), proPO-activating enzyme (PPA), proPO, and its active enzyme PO are involved (Cerenius *et al.* 2008). In our study several members of the proPO system were found in the genome of *S. noctilio*. This indicate that this system might be playing a similar role in *S. noctilio*.

In the *S. noctilio* genome we have identified several genes encoding enzymes known to be involved in detoxification of reactive oxygen species (ROS), such as catalase, superoxide dismutases, glutathione peroxidases (GPOs) and glutathione-S-transferases (GSTs). These enzymes regulate the concentration and conversion of ROS. Superoxide dismutases (SODs) convert superoxide radical (O2−) into a less toxic product, hydrogen peroxide (H_2O_2) (Oliveira *et al.* 2017). Catalases convert H2O2 to water and oxygen. H_2O_2 is converted to hydroperoxide by Peroxidases. The presence of the genes encoding catalase, SODs, GPOs and GSTs in the genome of *S. noctilio* reflect that they might be playing their roles in ROS detoxification in the woodwasp. In the genome of *S. noctilio,* two genes encoding antimicrobial peptides (AMPs), *defensin* and *hymenoptaecin* were found. Because our results were based on homologous searches, we may have missed certain AMP genes with significantly divergent sequences. The distribution of AMPs is, in general, highly complicated.

5.1.4 Cytoskeleton reorganization

Genes that encode proteins that play a role in cytoskeleton reorganization such as serine proteinase *stubble* and *myosin* were characterised in the *S. noctilio* genome. The immune system of insects is divided into two types: cellular and humoral responses. Phagocytosis, encapsulation, and nodule formation are examples of cellular responses mediated by haemocytes (Kim *et al.* 2009). Phagocytosis responses require host hemocyte cytoskeletal remodelling (Wu and Yi 2018). These proteins are important during the process of wound closure in insects. The epidermal growth factor receptor (EGFR) and other receptor tyrosine kinases are also essential for wound healing (Geiger *et al.* 2011). These also include the Ret-family receptor Stitcher (Tsarouhas *et al.* 2014). Epidermal growth factor receptor encoding gene was found in *S. noctilio*. This suggest that this gene play a role in wound healing in *S. noctilio*.

5.2 Effects of the immune challenges on the expression of immune-related genes in *S. noctilio* larvae

GO analysis using BLAST2GO indicated an effect of wounding which was supported by the fact that *Wnt* signalling pathway, small GTPases signal transduction pathway, as well as cytoskeleton organization were amongst the enriched terms in all our infection models

data sets. These pathways have been shown to be important during wound healing in vertebrates and may play comparable roles in insects including *S. noctilio* (Stramer *et al.* 2005; Lesch *et al.* 2010). The expression of *PGRP-SA* was induced in our study, *PGRP-SA* is responsible for recognizing bacterial peptidoglycan and activating the Toll pathway in response to Gram-positive bacteria (kordaczuket al 2020). In the present study we have found *PGRP-SA* expression to be induced by *D. siricidicola* and *B. bassiana* infections. The induction of *PGRP-SA* suggests that *D. siricidicola* nematodes and *B. bassiana* fungus or molecules that they produce are recognized by the Toll pathway receptor. We have also found *β-gluc2* to be significantly upregulated upon *B. bassiana* infection. This result indicates that this βGRP may be required for the immune response against the fungal infection.

In our study we found that a *spätzle* gene was induced by all the infection models. In *D. melanogaster* spätzle protein act as a ligand for Toll receptor and activate the synthesis of AMP genes (Chowdhury 2017). The infection by *D. siricidicola* nematodes and wounding induced the expression of several components of the Toll pathway. These include *Toll-6*, *Toll-8*, *Tube*, *protein spätzle* and *pelle*. Since only 72hpi samples were analysed in our study, we expect that at the later stage of infection other downstream components of the Toll pathway might also be induced to follow a sequential transcriptional regulation of this pathway. Therefore, our results suggest that our infection models lead to transcriptional regulation of the Toll pathway in *S. noctilio* larvae.

No *PGRPs* associated with the IMD pathway were induced by our infection models. However, there are downstream components of the IMD pathway that were induced. These include *Imd*, *dFadd*, *relish*, *neuron navigator 2* and *ankyrin*. This suggest that like the Toll pathway, at certain stages of the infection there are components of the IMD pathway that are activated to follow a sequential transcriptional regulation of this pathway.

The regulation of the Toll pathway and IMD pathway in response to *D. siricidicola* nematode infection which uses wounding as part of infection process is supported by a study that suggests that AMPs can be activated by bacteria-free nematodes, most likely

as a form of wounding defence (Castillo *et al.* 2013).This might also apply to the *B. bassiana* fungal infection and wounding as they also use wounding as part of their infection process and they also regulated the Toll and the IMD pathways.

The Jun N-terminal kinase (JNK) pathway was largely regulated by wounding in comparison to the *D. siricidicola* infection and *B. bassiana* infection. In insects, this pathway is involved in immune responses, wound healing, and oxidative homeostasis (Sluss *et al.* 1996; Silverman *et al.* 2003; Khoshnood *et al.* 2016; Su *et al.* 2017). This indicates that the JNK pathway is active and necessary for wound healing in *S. noctilio*.

In our results components of the melanisation pathway *ProPO*, *dopa decarboxylase*, serine proteases and their inhibitors were significantly regulated. *ProPO* and *dopa decarboxylase* were significantly upregulated by all our infection models. The expression of the serine proteases was upregulated in the *D. siricidicola* challenged larvae. This indicates that the serine proteinase cascade was activated during immune defence. The profiles of the serpins were also upregulated by *D.siricidicola*. This seem to be incompatible with their function in the regulation of the melanisation pathway. A similar result was reported in *M. domestica* in response to a bacterial challenge (Tang *et al.* 2014). This suggests that this could be a protective mechanism for host cells and tissues against the excess reactive components produced by the melanisation pathway. The results also indicate that the wounds caused during infection induced the melanisation process and they were ultimately melanized.

Our analysis showed that levels of genes encoding *superoxide dismutase*, *glutathione peroxidase* and *thioredoxin* were uniquely significantly downregulated by *D. siricidicola* infection. The expression of these enzymes must be induced in high levels for them to be able to play their roles. This suggests that the nematode might use the expression of the antioxidant enzymes in lower levels in their host as part of its immunosuppression strategy.

Two genes encoding antimicrobial peptides (AMPs), *defensin* and *hymenoptaecin* were significantly differentially expressed in response to all the infection models. *Defensin* was significantly downregulated by wounding and significantly upregulated by *B. bassiana* infection compared to controls. In insects *defensins* have antimicrobial effects against Gram-positive bacteria, however in other insects they seem to act against Gram-negative bacteria and fungi. This suggests that humoral immune responses in *S. noctilio* were active against *B. bassiana* infection. *Hymenoptaecin* was significantly upregulated by *D. siricidicola* infection and wounding, and significantly downregulated by *B. bassiana* infection. In this regard humoral immune response in *S. noctilio* were active against *D. siricidicola* infection and wounding, while *B. bassiana* seem to have a capability to suppress the humoral immune response of the woodwasp. The *hymenoptaecin* gene has been reported to be one of the most strongly induced genes following immune challenge. (Gupta *et al.* 2015).

Two chitinases were significantly upregulated by *D. siricidicola* and *B. bassiana* infections. Chitin can be found in the cell walls of bacteria and fungi, as well as in insects and the microfilarial sheaths of parasitic nematodes (Araujo *et al.* 1993; Shahabuddin and Kaslow 1994). Chitin-containing organisms utilize this polymer for protection against harsh environmental conditions and host immune responses. This suggests that a lack of chitin may result in the pathogen's death. Chitinase is an enzyme that breaks down chitin (Lee *et al.* 2011). The host produce chitinases in sufficient quantities as defence against infection with chitin-containing organisms. The latter is an attempt to destroy the infecting agent's chitin coat, thereby eliminating the pathogen (Burton and Zaccone 2007). This suggests that *D. Siricidicola* and *B. bassiana* have chitin. For this reason, *S. noctilio* produced the chitinases in significant amounts to try and degrade their chitin coat as part of its innate immune response.

Some genes linked to the extracellular matrix that were characterized in this study in *S. noctilio* was regulated following our immune challenges. These include *paxillin*, *integrin beta* and *epidermal growth factor receptor*, which were all upregulated. *Epidermal growth factor receptor* was uniquely regulated by the *D. siricidicola* infection. This provides

evidence that *D. siricidicola* nematodes, like some entomopathogenic nematodes, may include wounding as part of the infection process (Wertheim *et al.* 2005; Hallem *et al.* 2007; Arefin *et al.* 2014).

Phospholipase A2 and *phospholipase D* were induced following *D. siricidicola* infection and wounding. Phospholipases are involved in eicosanoids production and play a protective role in infection model that include wounding (Hyrsl *et al.* 2011; Stanley and Kim 2019). Eicosanoids are used by cross-talks between immune mediators as downstream signals (Sadekuzzaman and Kim 2018). They have also been found to participate in the immune response of *D. melanogaster* larvae in response to infection by *H. bacteriophora* nematodes (Hyrsl *et al.* 2011). In this study the levels of *phospholipase A2* and *phospholipase D* were significantly downregulated. There are studies that have shown that *S. carpocapsae* nematodes produce proteases and other substances that prevent clot formation, allowing them to avoid the insect's eicosanoid production and melanisation reaction (Stanley *et al.* 2012; Toubarro *et al.* 2013). The downregulated levels of the components of the eicosanoid pathway in *S. noctilio* by *D. sirircidicola* suggest that this nematode might be using the same strategy to evade the eicosanoid biosynthesis and melanisation response in the woodwasp.

Our analysis showed a significant upregulation of *vitellogenin* in response to all our infection models. *Vitellogenin* play an essential role mainly in reproduction and in wound healing, immunity, life span regulation and as an antioxidant in insects (Singh *et al.* 2013; Salmela *et al.* 2015; Park *et al.* 2018; Salmela and Sundström 2018). Our result suggests that *vitellogenin* might play similar roles in wound healing, immunity and as an antioxidant in *S. noctilio*. The possible role of vitellogenin in Hymenoptera immunity was described in a previous study which showed that *vitellogenin* is actively involved in defence reaction of *A. mellifera* towards the entomopathogenic fungus *B. bassiana (*Park *et al. 2018).* This study demonstrated that *A. mellifera vitellogenin* bound to *B. bassiana* cells, causing structural damage to the cell wall and anti-microbial activity against the fungus. The upregulated expression of *vitellogenin* in response to *B. bassiana* infection in our data indicate that *S. noctilio* might be using a similar immunity strategy against the fungus.

In addition to the known immune-related genes, we found that *hexamerin* was significantly upregulated by *D. siricidicola* infection. In general, hexamerin act as storage protein used as a source of amino acids and energy for protein synthesis during metamorphosis (Pan and Telfer 1996; Martins *et al.* 2010). *Hexamerin* has already been described to be downregulated in response to immune challenge in other insects (Gupta *et al.* 2015). Downregulation of expression and accumulation of storage proteins occur because of immune system activation, and this represents a strategy to redirect resources away from costly defense reactions (Lourenço *et al.* 2009; Gupta *et al.* 2015). Recently it was discovered that *hexamerin* involved in the activation of the prophenoloxidase system (Melanisation) (Liu *et al.* 2020). Whether *D. siricidicola* has a strategy to evade *S. noctilio* and does not cause a robust immune response or *hexamerin* in *S. noctilio* activate prophenoloxidase as an immune response against *D. siricidicola* remains to be investigated.

6. Conclusions

This is the first study to characterize immune-related genes in the Hymenopteran (Suborder Symphyta) *S. noctilio* and their regulation in larvae during parasite infection. The preliminary identification of putative immune-related genes and pathways exhibited differential expression after *D. siricidicola* infection. The results of our study provide a basic yet valuable insight that improves the general understanding of the host-parasite interaction of *Sirex-Deladenus* system. The inclusion of additional treatments, including a fungal infection by *B. bassiana* and physical wounding, gave us an opportunity to get insights into the mechanisms of *S. noctilio* response to other types of infection models. The host response to infection was strong and complex for each treatment, implying that numerous processes, including immune response, development, metabolism, and pathogenesis, are involved. Hundreds of genes' expression were either generally or specifically modulated by the three treatments. These data provide numerous candidate immune-related genes that could serve as a focus for future studies on the woodwasp immunity.

7. References

Aggrawal, K., and N. Silverman, 2007 Peptidoglycan recognition in *Drosophila*. Biochemical Society Transactions 35**:** 1496-1500.

Aguiar, A. P., A. R. Deans, M. S. Engel, M. Forshage, J. T. Huber *et al.*, 2013 Order hymenoptera. Zootaxa 3703**:** 51-62.

Akira, S., S. Uematsu and O. Takeuchi, 2006 Pathogen recognition and innate immunity. Cell 124**:** 783-801.

Anders, S., 2010 Analysing RNA-Seq data with the DESeq package. Molecular Biology 43**:** 1-17.

Anders, S., and W. Huber, 2010 Differential expression analysis for sequence count data. Genome Biology 11**:** R106.

Andrews, S., 2010 FastQC. A quality control tool for high throughput sequence data 370.

Araujo, A., T. Souto-Padrón and W. de Souza, 1993 Cytochemical localization of carbohydrate residues in microfilariae of *Wuchereria bancrofti* and *Brugia malayi*. Journal of Histochemistry & Cytochemistry 41**:** 571-578.

Arefin, B., L. Kucerova, P. Dobes, R. Markus, H. Strnad *et al.*, 2014 Genome-wide transcriptional analysis of *Drosophila* larvae infected by entomopathogenic nematodes shows involvement of complement, recognition and extracellular matrix proteins. Journal of Innate Immunity 6**:** 192-204.

Ashida, M., and P. T. Brey, 1995 Role of the integument in insect defense: pro-phenol oxidase cascade in the cuticular matrix. Proceedings of the National Academy of Sciences 92**:** 10698-10702.

Bagowski, P. C., W. Bruins and J. A. te Velthuis, 2010 The nature of protein domain evolution: shaping the interaction network. Current Genomics 11**:** 368-376.

Bang, K., S. Hwang, J. Lee and S. Cho, 2015 Identification of immunity-related genes in the larvae of *Protaetia brevitarsis seulensis* (Coleoptera: Cetoniidae) by a next-

generation sequencing-based transcriptome analysis. Journal of Insect Science 15**:** 142.

Baracchi, D., G. Mazza and S. Turillazzi, 2012 From individual to collective immunity: the role of the venom as antimicrobial agent in the Stenogastrinae wasp societies. Journal of insect physiology 58**:** 188-193.

Barribeau, S. M., B. M. Sadd, L. du Plessis, M. J. Brown, S. D. Buechel *et al.*, 2015 A depauperate immune repertoire precedes evolution of sociality in bees. Genome Biology 16**:** 1-21.

Bedding, R. A., 1972 Biology of *Deladenus Siricidicola* (Neotylenchidae) an Entomophagous-Mycetophagous Nematode Parasitic in Siricid Woodwasps. Nematologica 18**:** 482-493.

Ben-Ami, F., C. Orlic and R. R. Regoes, 2020 Disentangling non-specific and specific transgenerational immune priming components in host–parasite interactions. Proceedings of the Royal Society B 287**:** 20192386.

Boetzer, M., C. V. Henkel, H. J. Jansen, D. Butler and W. Pirovano, 2011 Scaffolding pre-assembled contigs using SSPACE. Bioinformatics 27**:** 578-579.

Boissin, E., B. Hurley, M. J. Wingfield, R. Vasaitis, J. Stenlid *et al.*, 2012 Retracing the routes of introduction of invasive species: the case of the *Sirex noctilio* woodwasp. Molecular Ecology 21**:** 5728-5744.

Bolger, A. M., M. Lohse and B. Usadel, 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30**:** 2114-2120.

Bordeaux, J. M., W. W. Lorenz, D. Johnson, M. J. Badgett, J. Glushka *et al.*, 2014 Noctilisin, a Venom Glycopeptide of *Sirex noctilio* (Hymenoptera: Siricidae), Causes Needle Wilt and Defense Gene Responses in Pines. Journal of Economic Entomology 107**:** 1931-1945.

Brennan, C. A., and K. V. Anderson, 2004 Drosophila: the genetics of innate immune recognition and response. Annu. Rev. Immunol. 22**:** 457-483.

Brettell, L. E., D. C. Schroeder and S. J. Martin, 2019 RNAseq analysis reveals virus diversity within hawaiian apiary insect communities. Viruses 11**:** 397.

Browne, N., M. Heelan and K. Kavanagh, 2013 An analysis of the structural and functional similarities of insect hemocytes and mammalian phagocytes. Virulence 4**:** 597-603.

Bulet, P., R. Stöcklin and L. Menin, 2004 Anti-microbial peptides: from invertebrates to vertebrates. Immunological reviews 198**:** 169-184.

Burton, O. T., and P. Zaccone, 2007 The potential role of chitin in allergic reactions. Trends in Immunology 28**:** 419-422.

Cao, X., Y. He, Y. Hu, Y. Wang, Y. R. Chen *et al.*, 2015 The immune signaling pathways of Manduca sexta. Insect Biochem Mol Biol 62**:** 64-74.

Cariveau, D. P., J. E. Powell, H. Koch, R. Winfree and N. A. Moran, 2014 Variation in gut microbial communities and its association with pathogen infection in wild bumble bees (Bombus). The ISME journal 8**:** 2369-2379.

Casteels, P., C. Ampe, F. Jacobs and P. Tempst, 1993 Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (Apis mellifera). Journal of Biological Chemistry 268**:** 7044-7054.

Casteels, P., C. Ampe, F. Jacobs, M. Vaeck and P. Tempst, 1989 Apidaecins: antibacterial peptides from honeybees. The EMBO journal 8**:** 2387-2391.

Casteels, P., C. Ampe, L. Rivière, J. Van Damme, C. Elicone *et al.*, 1990 Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (Apis mellifera). European journal of biochemistry 187**:** 381-386.

Castillo, J., U. Shokal and I. Eleftherianos, 2013 Immune gene transcription in *Drosophila* adult flies infected by entomopathogenic nematodes and their mutualistic bacteria. Journal of Insect Physiology 59**:** 179-185.

Cerenius, L., B. L. Lee and K. Söderhäll, 2008 The proPO-system: pros and cons for its role in invertebrate immunity. Trends in Immunology 29**:** 263-271.

Cheng, X., X. Zhang, J. W. Pflugrath and F. W. Studier, 1994 The structure of bacteriophage T7 lysozyme, a zinc amidase and an inhibitor of T7 RNA polymerase. Proceedings of the National Academy of Sciences 91**:** 4034-4038.

Choe, K.-M., T. Werner, S. Stöven, D. Hultmark and K. V. Anderson, 2002 Requirement for a peptidoglycan recognition protein (PGRP) in Relish activation and antibacterial immune responses in Drosophila. Science 296**:** 359-362.

Chowdhury, M., 2017 Regulation of Antimicrobial Peptide Genes in Insects by the Toll Pathway and NF-kB Transcription Factors, pp. University of Missouri--Kansas City.

Christensen, B. M., J. Li, C.-C. Chen and A. J. Nappi, 2005 Melanization immune responses in mosquito vectors. Trends in Parasitology 21**:** 192-199.

Christophides, G. K., E. Zdobnov, C. Barillas-Mury, E. Birney, S. Blandin *et al.*, 2002 Immunity-related genes and gene families in *Anopheles gambiae*. Science 298**:** 159-165.

Cremer, S., S. A. Armitage and P. Schmid-Hempel, 2007 Social immunity. Curr Biol 17**:** R693-702.

Cremer, S., C. D. Pull and M. A. Fürst, 2018 Social immunity: emergence and evolution of colony-level disease protection. Annual Review of Entomology 63**:** 105-123.

Crozatier, M., and M. Meister, 2007 Drosophila haematopoiesis. Cellular microbiology 9**:** 1117-1126.

Daffre, S., and I. Faye, 1997 Lipopolysaccharide interaction with hemolin, an insect member of the Ig-superfamily. FEBS Letters 408**:** 127-130.

Davis, R. B., S. L. Baldauf and P. J. Mayhew, 2010 The origins of species richness in the Hymenoptera: insights from a family-level supertree. BMC evolutionary biology 10**:** 109.

Dawkins, R., and J. R. Krebs, 1979 Arms races between and within species. Proceedings of the Royal Society of London. Series B. Biological Sciences 205**:** 489-511.

de Bekker, C., R. A. Ohm, R. G. Loreto, A. Sebastian, I. Albert *et al.*, 2015 Gene expression during zombie ant biting behavior reflects the complexity underlying fungal parasitic behavioral manipulation. BMC Genomics 16**:** 620.

Diez, L., J.-L. Deneubourg and C. Detrain, 2012 Social prophylaxis through distant corpse removal in ants. Naturwissenschaften 99**:** 833-842.

Dowton, M., and A. D. Austin, 2001 Simultaneous analysis of 16S, 28S, COI and morphology in the Hymenoptera: Apocrita—evolutionary transitions among parasitic wasps. Biological Journal of the linnean society 74**:** 87-111.

Dubovskiy, I., N. Kryukova, V. Glupov and N. Ratcliffe, 2016 Encapsulation and nodulation in insects. Invertebrate Survival Journal 13**:** 229-246.

Dubovskiy, I. M., M. M. Whitten, O. N. Yaroslavtseva, C. Greig, V. Y. Kryukov *et al.*, 2013 Can insects develop resistance to insect pathogenic fungi? PloS one 8.

Dziarski, R., 2004 Peptidoglycan recognition proteins (PGRPs). Molecular immunology 40**:** 877-886.

Elya, C., T. C. Lok, Q. E. Spencer, H. McCausland, C. C. Martinez *et al.*, 2018 Robust manipulation of the behavior of *Drosophila melanogaster* by a fungal pathogen in the laboratory. Elife 7**:** e34414.

Engel, P., and N. A. Moran, 2013 The gut microbiota of insects–diversity in structure and function. FEMS microbiology reviews 37**:** 699-735.

Evans, J., K. Aronstein, Y. P. Chen, C. Hetru, J. L. Imler *et al.*, 2006 Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology 15**:** 645-656.

Ferrandon, D., J.-L. Imler, C. Hetru and J. A. Hoffmann, 2007 The Drosophila systemic immune response: sensing and signalling during bacterial and fungal infections. Nature Reviews Immunology 7**:** 862.

Foelker, C. J., 2016 Beneath the bark: associations among *Sirex noctilio*

development, bluestain fungi, and pine host species in North America. Ecological Entomology 41**:** 676-684.

Gadau, J., M. Helmkampf, S. Nygaard, J. Roux, D. F. Simola *et al.*, 2012 The genomic impact of 100 million years of social evolution in seven ant species. Trends in Genetics 28**:** 14-21.

Galetto, L., S. Abbà, M. Rossi, M. Vallino, M. Pesando *et al.*, 2018 Two phytoplasmas elicit different responses in the insect vector *Euscelidius variegatus* Kirschbaum. Infection and Immunity 86.

Garcia, E. S., N. A. Ratcliffe, M. M. Whitten, M. S. Gonzalez and P. Azambuja, 2007 Exploring the role of insect host factors in the dynamics of Trypanosoma cruzi–Rhodnius prolixus interactions. Journal of Insect Physiology 53**:** 11-21.

Geiger, J. A., L. Carvalho, I. Campos, A. C. Santos and A. Jacinto, 2011 Hole-in-one mutant phenotypes link EGFR/ERK signaling to epithelial tissue repair in *Drosophila*. PLoS One 6**:** e28349.

Gottar, M., V. Gobert, T. Michel, M. Belvin, G. Duyk *et al.*, 2002 The Drosophila immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature 416**:** 640-644.

Grimaldi, D., M. S. Engel and M. S. Engel, 2005 *Evolution of the Insects*. Cambridge University Press.

Guillou, A., K. Troha, H. Wang, N. C. Franc and N. Buchon, 2016 The *Drosophila* CD36 homologue croquemort is required to maintain immune and gut homeostasis during development and aging. PLoS Pathogens 12**:** e1005961.

Gupta, S. K., M. Kupper, C. Ratzka, H. Feldhaar, A. Vilcinskas *et al.*, 2015 Scrutinizing the immune defence inventory of *Camponotus floridanus* applying total transcriptome sequencing. BMC Genomics 16**:** 540.

Guzman-Novoa, E., 2011 Genetic Basis of Disease Resistance in the Honey Bee (Apis mellifera L.).

Haavik, L. J., K. J. Dodds and J. D. Allison, 2015 Do Native Insects and Associated Fungi Limit Non-Native Woodwasp, *Sirex noctilio*, Survival in a Newly Invaded Environment? PLoS One 10**:** e0138516.

Hallem, E. A., M. Rengarajan, T. A. Ciche and P. W. Sternberg, 2007 Nematodes, bacteria, and flies: a tripartite model for nematode parasitism. Current Biology 17**:** 898-904.

Hanson, M. A., A. Dostálová, C. Ceroni, M. Poidevin, S. Kondo *et al.*, 2019 Synergy and remarkable specificity of antimicrobial peptides *in vivo* using a systematic knockout approach. Elife 8**:** e44341.

Hanson, M. A., and B. Lemaitre, 2020 New insights on *Drosophila* antimicrobial peptide function in host defense and beyond. Current Opinion in Immunology 62**:** 22-30.

Hanson, P. E., 2016 *Insects and other arthropods of tropical America*. Cornell University Press.

Hegedus, D., M. Erlandson, C. Gillott and U. Toprak, 2009 New insights into peritrophic matrix synthesis, architecture, and function. Annual review of entomology 54**:** 285-302.

Henze, M. J., and T. H. Oakley, 2015 The dynamic evolutionary history of pancrustacean eyes and opsins. Integrative and Comparative Biology 55**:** 830-842.

Hetru, C., and J. A. Hoffmann, 2009 NF-κB in the immune response of *Drosophila*. Cold Spring Harbor Perspectives in Biology 1**:** a000232.

Hillyer, J. F., 2016 Insect immunology and hematopoiesis. Developmental and Comparative Immunology 58**:** 102-118.

Hillyer, J. F., and M. R. Strand, 2014 Mosquito hemocyte-mediated immune responses. Current opinion in insect science 3**:** 14-21.

Hoffmann, J. A., 2003 The immune response of *Drosophila*. Nature 426**:** 33-38.

Hölldobler, B., and E. O. Wilson, 1990 *The ants*. Harvard University Press.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui and T. J. Case, 2002 The Causes and Consequences of Ant Invasions. Annual Review of Ecology and Systematics 33**:** 181-233.

Hughes, A., 2001 Evolution of the integrin alpha and beta protein families. Journal Of Molecular Evolution 52**:** 63-72.

Hurley, B. P., H. J. Hatting, M. J. Wingfield, K. D. Klepzig and B. Slippers, 2012 The influence of *Amylostereum areolatum* diversity and competitive interactions on the fitness of the *Sirex* parasitic nematode *Deladenus siricidicola*. Biological Control 61**:** 207-214.

Hurley, B. P., B. Slippers and M. J. Wingfield, 2007 A comparison of control results for the alien invasive woodwasp, *Sirex noctilio*, in the southern hemisphere.

Hyrsl, P., P. Dobes, Z. Wang, T. Hauling, C. Wilhelmsson *et al.*, 2011 Clotting factors and eicosanoids protect against nematode infections. Journal of Innate Immunity 3**:** 65-70.

Jiang, H., A. Vilcinskas and M. R. Kanost, 2010 Immunity in lepidopteran insects, pp. 181-204 in *Invertebrate immunity*. Springer.

Kaltenpoth, M., T. Engl and K. Clay, 2014 Defensive microbial symbionts in Hymenoptera. Functional Ecology 28**:** 315-327.

Kanagawa, M., T. Satoh, A. Ikeda, Y. Adachi, N. Ohno *et al.*, 2011 Structural insights into recognition of triple-helical β-glucans by an insect fungal receptor. Journal of Biological Chemistry 286**:** 29158-29165.

Kanost, M. R., H. Jiang and X. Q. Yu, 2004 Innate immune responses of a lepidopteran insect, Manduca sexta. Immunological reviews 198**:** 97-105.

Kapheim, K. M., H. Pan, C. Li, S. L. Salzberg, D. Puiu *et al.*, 2015 Genomic signatures of evolutionary transitions from solitary to group living. Science 348**:** 1139-1143.

Khoshnood, B., I. Dacklin and C. Grabbe, 2016 Urm1: an essential regulator of JNK signaling and oxidative stress in *Drosophila melanogaster*. Cellular and Molecular Life Sciences 73**:** 1939-1954.

Kim, D., G. Pertea, C. Trapnell, H. Pimentel, R. Kelley *et al.*, 2013 TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biology 14**:** R36.

Kim, G. S., M. Nalini, Y. Kim and D. W. Lee, 2009 Octopamine and 5-hydroxytryptamine mediate hemocytic phagocytosis and nodule formation via eicosanoids in the beet armyworm, *Spodoptera exigua*. Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America 70**:** 162-176.

Kim, Y., S. Ahmed, D. Stanley and C. An, 2018 Eicosanoid-mediated immunity in insects. Developmental and Comparative Immunology 83**:** 130-143.

Klopfstein, S., L. Vilhelmsen, J. M. Heraty, M. Sharkey and F. Ronquist, 2013 The hymenopteran tree of life: evidence from protein-coding genes and objectively aligned ribosomal data. PLoS One 8**:** e69344.

Koch, H., G. Cisarovsky and P. Schmid-Hempel, 2012 Ecological effects on gut bacterial communities in wild bumblebee colonies. Journal of Animal Ecology 81**:** 1202-1210.

Kocks, C., J. H. Cho, N. Nehme, J. Ulvila, A. M. Pearson *et al.*, 2005 Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in Drosophila. Cell 123**:** 335-346.

Kurucz, É., R. Márkus, J. Zsámboki, K. Folkl-Medzihradszky, Z. Darula *et al.*, 2007 Nimrod, a putative phagocytosis receptor with EGF repeats in Drosophila plasmatocytes. Current Biology 17**:** 649-654.

Lach, L., and M. L. Thomas, 2008 Invasive ants in Australia: documented and potential ecological consequences. Australian Journal of Entomology 47**:** 275-288.

Larsen, A., F. J. Reynaldi and E. Guzmán-Novoa, 2019 Fundaments of the honey bee (*Apis mellifera*) immune system. Review. Revista Mexicana de Ciencias Pecuarias 10**:** 705-728.

Lavine, M., and M. Strand, 2002 Insect hemocytes and their role in immunity. Insect biochemistry and molecular biology 32**:** 1295-1309.

Lee, C. G., C. A. Da Silva, C. S. Dela Cruz, F. Ahangari, B. Ma *et al.*, 2011 Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annual Review of Physiology 73**:** 479-501.

Lee, E., G. A. Helt, J. T. Reese, M. C. Munoz-Torres, C. P. Childers *et al.*, 2013 Web Apollo: a web-based genomic annotation editing platform. Genome Biology 14**:** R93.

Lemaitre, B., and J. Hoffmann, 2007 The host defense of *Drosophila melanogaster*. Annual Review of Immunology 25**:** 697-743.

Lesch, C., J. Jo, Y. Wu, G. S. Fish and M. J. Galko, 2010 A targeted UAS-RNAi screen in Drosophila larvae identifies wound closure genes regulating distinct cellular processes. Genetics 186**:** 943-957.

Lester, P. J., K. H. Buick, J. W. Baty, A. Felden and J. Haywood, 2019 Different bacterial and viral pathogens trigger distinct immune responses in a globally invasive ant. Scientific Reports 9**:** 1-10.

Leulier, F., and B. Lemaitre, 2008 Toll-like receptors—taking an evolutionary approach. Nature Reviews Genetics 9**:** 165.

Li, J. H., J. D. Evans, W. F. Li, Y. Z. Zhao, G. DeGrandi-Hoffman *et al.*, 2017 New evidence showing that the destruction of gut bacteria by antibiotic treatment

could increase the honey bee's vulnerability to *Nosema* infection. PloS One 12**:** e0187505.

Liao, Y., G. K. Smyth and W. Shi, 2014 featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30**:** 923-930.

Liu, C., J. Zhu, J. Ma, J. Zhang, X. Wang *et al.*, 2020 A novel hexamerin with an unexpected contribution to the prophenoloxidase activation system of the Chinese oak silkworm, *Antheraea pernyi*. Archives of Insect Biochemistry and Physiology 103**:** e21648.

Liu, Y., D. Shen, F. Zhou, G. Wang and C. An, 2014 Identification of immunity-related genes in *Ostrinia furnacalis* against entomopathogenic fungi by RNA-seq analysis. PLoS One 9**:** e86436.

Lourenço, A. P., J. R. Martins, M. M. Bitondi and Z. L. Simões, 2009 Trade-off between immune stimulation and expression of storage protein genes. Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America 71**:** 70-87.

Lu, Y., F. Su, Q. Li, J. Zhang, Y. Li *et al.*, 2020 Pattern recognition receptors in *Drosophila* immune responses. Developmental & Comparative Immunology 102**:** 103468.

Lundgren, J. G., and J. L. Jurat-Fuentes, 2012 Physiology and ecology of host defense against microbial invaders. Insect Pathology**:** 461-480.

Ma, C., and M. R. Kanost, 2000 A β1, 3-glucan recognition protein from an insect, Manduca sexta, agglutinates microorganisms and activates the phenoloxidase cascade. Journal of Biological Chemistry 275**:** 7505-7514.

Machtinger, E. T., C. J. Geden, P. E. Kaufman and A. M. House, 2015 Use of Pupal Parasitoids as Biological Control Agents of Filth Flies on Equine Facilities. Journal of Integrated Pest Management 6**:** 16.

Marchler-Bauer, A., S. Lu, J. B. Anderson, F. Chitsaz, M. K. Derbyshire *et al.*, 2010 CDD: a Conserved Domain Database for the functional annotation of proteins. Nucleic Acids Research 39**:** D225-D229.

Marmaras, V. J., and M. Lampropoulou, 2009 Regulators and signalling in insect haemocyte immunity. Cellular signalling 21**:** 186-195.

Martins, J. R., F. M. Nunes, A. S. Cristino, Z. L. Simões and M. M. Bitondi, 2010 The four hexamerin genes in the honey bee: structure, molecular evolution and function deduced from expression patterns in queens, workers and drones. BMC Molecular Biology 11**:** 1-20.

Martinson, V. G., B. N. Danforth, R. L. Minckley, O. Rueppell, S. Tingek *et al.*, 2011 A simple and distinctive microbiota associated with honey bees and bumble bees. Molecular Ecology 20**:** 619-628.

Mertz, B., X. Gu and P. J. Reilly, 2009 Analysis of functional divergence within two structurally related glycoside hydrolase families. Biopolymers: Original Research on Biomolecules 91**:** 478-495.

Meunier, J., 2015 Social immunity and the evolution of group living in insects. Philos Trans R Soc Lond B Biol Sci 370.

Michener, C. D., 2000 *The bees of the world*. JHU press.

Moran, N. A., 2015 Genomics of the honey bee microbiome. Current opinion in insect science 10**:** 22-28.

Morris, E., A. Jimenez, S. Long, D. Williams and A. Hajek, 2012 Variability in growth of *Deladenus siricidicola* on strains of the white rot fungus *Amylostereum areolatum*. BioControl 57**:** 677-686.

Moussian, B., 2010 Recent advances in understanding mechanisms of insect cuticle differentiation. Insect Biochemistry and Molecular Biology 40**:** 363-375.

Mrinalini, W. J., and J. Werren, 2017 Parasitoid wasps and their venoms. Evolution of venomous animals and their toxins. Dordrecht: Springer**:** 187-212.

Murphy, K., and C. Weaver, 2016 *Janeway's immunobiology*. Garland science.

Myllymäki, H., S. Valanne and M. Rämet, 2014 The *Drosophila* imd signaling pathway. The Journal of Immunology 192**:** 3455-3462.

Nappi, A. J., and E. Ottaviani, 2000 Cytotoxicity and cytotoxic molecules in invertebrates. Bioessays 22**:** 469-480.

Napping, A. J., 1995 Superoxide anion generation in lrosophila during melanotic encapsulation of parasites. European journal of cell biology 68**:** 450-456.

Nayduch, D., M. B. Lee and C. A. Saski, 2014 Gene discovery and differential expression analysis of humoral immune response elements in female *Culicoides sonorensis* (Diptera: Ceratopogonidae). Parasites & Vectors 7**:** 388.

Ochiai, M., and M. Ashida, 1988 Purification of a beta-1, 3-glucan recognition protein in the prophenoloxidase activating system from hemolymph of the silkworm, *Bombyx mori*. Journal of Biological Chemistry 263**:** 12056-12062.

Oeyen, J. P., P. Baa-Puyoulet, J. B. Benoit, L. W. Beukeboom, E. Bornberg-Bauer *et al.*, 2020 Sawfly genomes reveal evolutionary acquisitions that fostered the mega-radiation of parasitoid and eusocial Hymenoptera. Genome Biology and Evolution 12**:** 1099-1188.

Oliveira, J. H. M., O. A. Talyuli, R. L. Goncalves, G. O. Paiva-Silva, M. H. F. Sorgine *et al.*, 2017 Catalase protects *Aedes aegypti* from oxidative stress and increases midgut infection prevalence of Dengue but not Zika. PLoS Neglected Tropical Diseases 11**:** e0005525.

Oppenheim, S. J., R. H. Baker, S. Simon and R. DeSalle, 2015 We can't all be supermodels: the value of comparative transcriptomics to the study of non-model insects. Insect Molecular Biology 24**:** 139-154.

Otani, S., N. Bos and S. H. Yek, 2016 Transitional complexity of social insect immunity. Frontiers in Ecology and Evolution 4**:** 69.

Otani, S., A. Mikaelyan, T. Nobre, L. H. Hansen, N. G. A. Koné *et al.*, 2014 Identifying the core microbial community in the gut of fungus-growing termites. Molecular Ecology 23**:** 4631-4644.

Otti, O., S. Tragust and H. Feldhaar, 2014 Unifying external and internal immune defences. Trends in ecology & evolution 29**:** 625-634.

Palmer, W. J., and F. M. Jiggins, 2015 Comparative Genomics Reveals the Origins and Diversity of Arthropod Immune Systems. Molecular Biology and Evolution 32**:** 2111-2129.

Pan, M., and W. H. Telfer, 1996 Methionine-rich hexamerin and arylphorin as precursor reservoirs for reproduction and metamorphosis in female luna moths. Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America 33**:** 149-162.

Park, H. G., K. S. Lee, B. Y. Kim, H. J. Yoon, Y. S. Choi *et al.*, 2018 Honeybee (*Apis cerana)* vitellogenin acts as an antimicrobial and antioxidant agent in the body and venom. Developmental & Comparative Immunology 85**:** 51-60.

Pearson, W. R., 2013 An introduction to sequence similarity ("homology") searching. Current Protocols in Bioinformatics 42**:** 3.1. 1-3.1. 8.

Peters, R. S., L. Krogmann, C. Mayer, A. Donath, S. Gunkel *et al.*, 2017 Evolutionary history of the Hymenoptera. Current Biology 27**:** 1013-1018.

Petersen, M., D. Armisén, R. A. Gibbs, L. Hering, A. Khila *et al.*, 2019 Diversity and evolution of the transposable element repertoire in arthropods with particular reference to insects. BMC Evolutionary Biology 19**:** 1-15.

Rämet, M., P. Manfruelli, A. Pearson, B. Mathey-Prevot and R. A. B. Ezekowitz, 2002 Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli*. Nature 416**:** 644-648.

Rao, X.-J., P. Wu, T. Shahzad, S. Liu, L. Chen *et al.*, 2016 Characterization of a dual-CRD galectin in the silkworm *Bombyx mori*. Developmental & Comparative Immunology 60**:** 149-159.

Ratzka, C., F. Förster, C. Liang, M. Kupper, T. Dandekar *et al.*, 2012 Molecular characterization of antimicrobial peptide genes of the carpenter ant Camponotus floridanus. PLoS One 7.

Reavey, C. E., L. Beare and S. C. Cotter, 2014 Parental care influences social immunity in burying beetle larvae. Ecological entomology 39**:** 395-398.

Robinson, M. D., D. J. McCarthy and G. K. Smyth, 2010 edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26**:** 139-140.

Roh, K.-B., C.-H. Kim, H. Lee, H.-M. Kwon, J.-W. Park *et al.*, 2009 Proteolytic cascade for the activation of the insect toll pathway induced by the fungal cell wall component. Journal of Biological Chemistry 284**:** 19474-19481.

Rosales, C., 2005 *Molecular mechanisms of phagocytosis*. Springer.

Rosales, C., 2017 Cellular and Molecular Mechanisms of Insect Immunity. Insect Physiology and Ecology.

Royet, J., and R. Dziarski, 2007 Peptidoglycan recognition proteins: pleiotropic sensors and effectors of antimicrobial defences. Nature Reviews Microbiology 5**:** 264-277.

Rozen, S., and H. Skaletsky, 2000 Primer3 on the WWW for general users and for biologist programmers. Methods Molecular Biology 132**:** 365-386.

Ryan, K., and B. P. Hurley, 2012 Life history and biology of Sirex noctilio, pp. 15-30 in *The Sirex Woodwasp and its Fungal Symbiont:*. Springer.

Sabree, Z. L., C. Y. Huang, G. Arakawa, G. Tokuda, N. Lo *et al.*, 2012 Genome shrinkage and loss of nutrient-providing potential in the obligate symbiont of the primitive termite Mastotermes darwiniensis. Appl. Environ. Microbiol. 78**:** 204-210.

Sackton, T. B., B. P. Lazzaro and A. G. Clark, 2017 Rapid Expansion of Immune-Related Gene Families in the House Fly, *Musca domestica*. Molecular Biology and Evolution 34**:** 857-872.

Sackton, T. B., B. P. Lazzaro, T. A. Schlenke, J. D. Evans, D. Hultmark *et al.*, 2007 Dynamic evolution of the innate immune system in *Drosophila*. Nature Genetics 39**:** 1461-1468.

Sackton, T. B., J. H. Werren and A. G. Clark, 2013 Characterizing the infection-induced transcriptome of *Nasonia vitripennis* reveals a preponderance of taxonomically-restricted immune genes. PloS One 8**:** e83984.

Sadekuzzaman, M., and Y. Kim, 2018 Nitric oxide mediates antimicrobial peptide gene expression by activating eicosanoid signaling. PloS One 13**:** e0193282.

Salazar-Jaramillo, L., K. M. Jalvingh, A. de Haan, K. Kraaijeveld, H. Buermans *et al.*, 2017 Inter-and intra-species variation in genome-wide gene expression of *Drosophila* in response to parasitoid wasp attack. BMC Genomics 18**:** 1-14.

Salcedo-Porras, N., and C. Lowenberger, 2019 The innate immune system of kissing bugs, vectors of chagas disease. Developmental & Comparative Immunology 98**:** 119-128.

Salmela, H., G. V. Amdam and D. Freitak, 2015 Transfer of immunity from mother to offspring is mediated via egg-yolk protein vitellogenin. PLoS Pathogens 11**:** e1005015.

Salmela, H., and L. Sundström, 2018 Vitellogenin in inflammation and immunity in social insects. Inflammation and Cell Signaling 5.

Satyavathi, V. V., A. Minz and J. Nagaraju, 2014 Nodulation: an unexplored cellular defense mechanism in insects. Cell Signal 26**:** 1753-1763.

Schluns, H., and R. H. Crozier, 2009 Molecular and chemical immune defenses in ants (Hymenoptera: Formicidae). Myrmecological News 12**:** 237-249.

Schmid-Hempel, P., 2003 Variation in immune defence as a question of evolutionary ecology. Proceedings of the Royal Society of London. Series B: Biological Sciences 270**:** 357-366.

Shahabuddin, M., and D. C. Kaslow, 1994 *Plasmodium*: parasite chitinase and its role in malaria transmission. Experimental Parasitology 79**:** 85-88.

Sharkey, M. J., 2007 Phylogeny and classification of Hymenoptera. Zootaxa 1668**:** 521-548.

Shelby, K. S., and H. J. Popham, 2012 RNA-seq study of microbially induced hemocyte transcripts from larval *Heliothis virescens* (Lepidoptera: Noctuidae). Insects 3**:** 743-762.

Sievers, F., and D. G. Higgins, 2014 Clustal omega. Current Protocols in Bioinformatics 48**:** 3.13. 11-13.13. 16.

Silverman, N., R. Zhou, R. L. Erlich, M. Hunter, E. Bernstein *et al.*, 2003 Immune activation of NF-κB and JNK requires *Drosophila* TAK1. Journal of Biological Chemistry 278**:** 48928-48934.

Simão, F. A., R. M. Waterhouse, P. Ioannidis, E. V. Kriventseva and E. M. Zdobnov, 2015 BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31**:** 3210-3212.

Singh, N. K., B. C. Pakkianathan, M. Kumar, T. Prasad, M. Kannan *et al.*, 2013 Vitellogenin from the silkworm, *Bombyx mori*: an effective anti-bacterial agent. PLoS One 8**:** e73005.

Siva-Jothy, M. T., Y. Moret and J. Rolff, 2005 Insect Immunity: An Evolutionary Ecology Perspective. Advances in Insect Physiology 32**:** 1-48.

Slippers, B., B. P. Hurley and M. J. Wingfield, 2015 Sirex woodwasp: a model for evolving management paradigms of invasive forest pests. Annu Rev Entomol 60**:** 601-619.

Sluss, H. K., Z. Han, T. Barrett, R. J. Davis and Y. T. Ip, 1996 A JNK signal transduction pathway that mediates morphogenesis and an immune response in *Drosophila*. Genes & Development 10**:** 2745-2758.

Somogyi, K., B. Sipos, Z. Pénzes, É. Kurucz, J. Zsámboki *et al.*, 2008 Evolution of genes and repeats in the Nimrod superfamily. Molecular biology and evolution 25**:** 2337-2347.

Spradbery, J., and A. Kirk, 1978 Aspects of the ecology of siricid woodwasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of *Sirex noctilio* F. in Australia. Bulletin of Entomological Research 68**:** 341-359.

Sreeramulu, B., G. Arumugam, R. Paulchamy, H. Karuppiah and J. Sundaram, 2018 β-Galactoside binding lectin from caddisfly larvae, Stenopsyche kodaikanalensis with selective modes of antibacterial activity: Purification and characterization. International Journal of Biological Macromolecules 115**:** 1033-1045.

Standage, D. S., A. J. Berens, K. M. Glastad, A. J. Severin, V. P. Brendel *et al.*, 2016 Genome, transcriptome and methylome sequencing of a primitively eusocial wasp reveal a greatly reduced DNA methylation system in a social insect. Molecular Ecology 25**:** 1769-1784.

Stanley, D., E. Haas and J. Miller, 2012 Eicosanoids: exploiting insect immunity to improve biological control programs. Insects 3**:** 492-510.

Stanley, D., and Y. Kim, 2019 Prostaglandins and other eicosanoids in insects: biosynthesis and biological actions. Frontiers in Physiology 9**:** 1927.

Stramer, B., W. Wood, M. J. Galko, M. J. Redd, A. Jacinto *et al.*, 2005 Live imaging of wound inflammation in *Drosophila* embryos reveals key roles for small GTPases during in vivo cell migration. The Journal of Cell Biology 168**:** 567-573.

Strand, M. R., 2008 The insect cellular immune response. Insect Science 15**:** 1-14.

Stroeymeyt, N., B. Casillas-Pérez and S. Cremer, 2014 Organisational immunity in social insects. Current Opinion in Insect Science 5**:** 1-15.

Su, L. D., Q. L. Zhang and Z. Lu, 2017 Oxidation resistance 1 (OXR1) participates in silkworm defense against bacterial infection through the JNK pathway. Insect Science 24**:** 17-26.

Sun, Z., W. Wu and G. Zhang, 2011 Structure and expression of β-1, 3-glucan recognition proteins from the ghost moth, *Thitarodes pui* (Hepialidae), and their response to *Beauveria bassiana* infection. Journal of Insect Physiology 57**:** 1660-1669.

Takehana, A., T. Katsuyama, T. Yano, Y. Oshima, H. Takada *et al.*, 2002 Overexpression of a pattern-recognition receptor, peptidoglycan-recognition protein-LE, activates imd/relish-mediated antibacterial defense and the prophenoloxidase cascade in Drosophila larvae. Proceedings of the National Academy of Sciences 99**:** 13705-13710.

Takov, D. I., P. V. Ostoich, A. I. Tchorbanov and D. K. Pilarska, 2020 Order Diptera as a model in the studies of insect immunity: a review. Turkish Journal of Zoology 44**:** 481-489.

Talbot, P. H. B., 1977 The *Sirex-Amylostereum-Pinus* Association. Annual Review of Phytopathology 15**:** 41-54.

Tang, T., X. Li, X. Yang, X. Yu, J. Wang *et al.*, 2014 Transcriptional response of *Musca domestica* larvae to bacterial infection. PLoS One 9**:** e104867.

Taylor, M. E., and K. Drickamer, 2003 Structure–function analysis of C-type animal lectins, pp. 3-16 in *Methods in Enzymology*. Elsevier.

Thompson, B. M., J. Bodart, C. McEwen and D. S. Gruner, 2014 Adaptations for Symbiont-Mediated External Digestion in *Sirex noctilio* (Hymenoptera: Siricidae). Annals of the Entomological Society of America 107**:** 453-460.

Tian, C., B. Gao, Q. Fang, G. Ye and S. Zhu, 2010 Antimicrobial peptide-like genes in Nasonia vitripennis: a genomic perspective. BMC genomics 11**:** 187.

Toubarro, D., M. M. Avila, R. Montiel and N. Simoes, 2013 A pathogenic nematode targets recognition proteins to avoid insect defenses. PLoS One 8**:** e75691.

Tribe, G., and J. Cillié, 2004 The spread of *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) in South African pine plantations and the introduction and establishment of its biological control agents. African Entomology 12**:** 9-17.

Tsarouhas, V., L. Yao and C. Samakovlis, 2014 Src kinases and ERK activate distinct responses to Stitcher receptor tyrosine kinase signaling during wound healing in *Drosophila*. Journal of Cell Science 127**:** 1829-1839.

Vallet-Gely, I., B. Lemaitre and F. Boccard, 2008 Bacterial strategies to overcome insect defences. Nature Reviews Microbiology 6**:** 302.

Van Valen, L., 1973 A new evolutionary law. Evol Theory 1**:** 1-30.

Vlisidou, I., and W. Wood, 2015 Drosophila blood cells and their role in immune responses. The FEBS journal 282**:** 1368-1382.

Voulgari-Kokota, A., Q. S. McFrederick, I. Steffan-Dewenter and A. Keller, 2019 Drivers, diversity, and functions of the solitary-bee microbiota. Trends in Microbiology 27**:** 1034-1044.

Walker, T. N., and W. O. Hughes, 2009 Adaptive social immunity in leaf-cutting ants. Biology Letters 5**:** 446-448.

Wang, Y., M. Stata, W. Wang, J. E. Stajich, M. M. White *et al.*, 2018 Comparative Genomics Reveals the Core Gene Toolbox for the Fungus-Insect Symbiosis. MBio 9.

Waterhouse, R. M., E. V. Kriventseva, S. Meister, Z. Xi, K. S. Alvarez *et al.*, 2007 Evolutionary Dynamics of Immune-Related Genes and Pathways in Disease-Vector Mosquitoes. Science 316**:** 1738-1743.

Waterhouse, R. M., F. Tegenfeldt, J. Li, E. M. Zdobnov and E. V. Kriventseva, 2013 OrthoDB: a hierarchical catalog of animal, fungal and bacterial orthologs. Nucleic Acids Research 41**:** D358-365.

Wertheim, B., A. R. Kraaijeveld, E. Schuster, E. Blanc, M. Hopkins *et al.*, 2005 Genome-wide gene expression in response to parasitoid attack in *Drosophila*. Genome Biology 6**:** R94.

Weston, C. R., and R. J. Davis, 2002 The JNK signal transduction pathway. Current Opinion in Genetics & Development 12**:** 14-21.

Whitfield, J. B., 2003 Phylogenetic insights into the evolution of parasitism in Hymenoptera. Advances in Parasitology 54**:** 69-101.

Wilson, E. O., 1971 The insect societies. The insect societies.

Wong, C. N. A., P. Ng and A. E. Douglas, 2011 Low-diversity bacterial community in the gut of the fruitfly Drosophila melanogaster. Environmental microbiology 13**:** 1889-1900.

Wu, G., and Y. Yi, 2018 Transcriptome analysis of differentially expressed genes involved in innate immunity following *Bacillus thuringiensis* challenge in *Bombyx mori* larvae. Molecular Immunology 103**:** 220-228.

Xin, W., B. J. Wilkinson and R. K. Jayaswal, 1991 Sequence analysis of a *Staphylococcus aureus* gene encoding a peptidoglycan hydrolase activity. Gene 102**:** 105-109.

Yano, T., and S. Kurata, 2011 Intracellular recognition of pathogens and autophagy as an innate immune host defence. The Journal of Biochemistry 150**:** 143-149.

Yek, S. H., J. J. Boomsma and M. Schiøtt, 2013 Differential gene expression in *Acromyrmex* leaf-cutting ants after challenges with two fungal pathogens. Molecular Ecology 22**:** 2173-2187.

Yi, H. Y., M. Chowdhury, Y. D. Huang and X. Q. Yu, 2014 Insect antimicrobial peptides and their applications. Appl Microbiol Biotechnol 98**:** 5807-5822.

Yokoi, K., Y. Hayakawa, D. Kato, C. Minakuchi, T. Tanaka *et al.*, 2015 Prophenoloxidase genes and antimicrobial host defense of the model beetle, *Tribolium castaneum*. Journal of Invertebrate Pathology 132**:** 190-200.

You, H., H. Wan, J. Li and B. R. Jin, 2010 Molecular cloning and characterization of a short peptidoglycan recognition protein (PGRP-S) with antibacterial activity from the bumblebee *Bombus ignitus*. Developmental & Comparative Immunology 34**:** 977-985.

Yu, X., and M. Kanost, 2002 Binding of hemolin to bacterial lipopolysaccharide and lipoteichoic acid. An immunoglobulin superfamily member from insects as a pattern-recognition receptor. Eur. J. Biochem 269**:** 1827-1834.

Zerbino, D. R., and E. Birney, 2008 Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome research 18**:** 821-829.

Zhang, L. J., and R. L. Gallo, 2016 Antimicrobial peptides. Current Biology 26**:** R14-19.

Zhang, R., Y. Li, N. Liu and S. D. Porter, 2007 An overview of the red imported fire ant (Hymenoptera: Formicidae) in mainland China. Florida Entomologist 90**:** 723-732.

Zhang, Z., and S. Zhu, 2012 Comparative genomics analysis of five families of antimicrobial peptide-like genes in seven ant species. Developmental & Comparative Immunology 38**:** 262-274.

Zheng, H., X. Yang and Y. Xi, 2016 Fat body remodeling and homeostasis control in *Drosophila*. Life Sciences 167**:** 22-31.

Zhou, J., H. Y. Yu, W. Zhang, F. Ahmad, S. N. Hu *et al.*, 2017 Comparative analysis of the *Monochamus alternatus* immune system. Insect Science.

Zhu, Y., X. Yu and G. Cheng, 2020 Insect C-Type Lectins in Microbial Infections, pp. 129-140 in *Lectin in Host Defense Against Microbial Infections*. Springer.

Chapter 2: Tables and Figures

Table 2.1: The summarised assembly and annotation statistics of *Sirex noctilio* genome data used in the study.

Genome data summary								
Organism	**Assembly method**	**Number of contigs**	**Total length (Mb)**	**Nr of genes**	**Coverage**	**N50 (Kb)**	**Assembly completeness (%)**	**Annotation completeness (%)**
Sirex noctilio	Velvet and SSPACE	6215	185	20629	122x	825	95.4	94

Table 2.2: **Transcriptome data summary.** This an output of raw sequence data from BGI. The abbreviations 'GC percentage' refers to the percentage of the transcriptome that are G and C nucleotides.

Library name	Number of reads	GC content
B1F	33437346	42%
B1R	33437346	41%
B2F	30557606	42%
B2R	30557606	41%
B3F	31859999	42%
B3R	31859999	41%
C1F	36464171	42%
C1R	36464171	41%
C2F	31185398	42%
C2R	31185398	41%
C3F	33656587	42%
C3R	33656587	41%
N1F	33147709	42%
N1R	33147709	41%
N2F	33609141	42%
N2R	33609141	41%
N3F	33542102	42%
N3R	33542102	41%
W1F	32505013	42%
W1R	32505013	41%
W2F	26035750	43%
W2R	26035750	42%
W3F	33590110	43%
W3R	33590110	42%

Table 2.3: Summary of transcriptome mapping data.

Treatment group	Overall mapping rate	Concordant pair alignment
Nematode infection (N1)	95,60%	93.3%
Nematode infection (N2)	97.9%	95.6%
Nematode infection (N3)	97.8%	95.4%
Fungal infection (B1)	97.7%	95.1%
Fungal infection (B2)	97.3%	94.9%
Fungal infection (B3)	96.2%	93.9%
Wounding (W1)	97.4%	95.0%
Wounding (W2)	97.9%	95.6%
Wounding (W3)	97.6%	95.3%
Control (C1)	97.9%	95.5%
Control (C2)	97.9%	95.5%
Control (C3)	97.8%	95.3%

Table 2.4: Summary of differential expression data results obtained from DESeq and edgeR.

	Treatment/Condition					
Method	***B. bassiana* infection**		***D. siricidicola* infection**		**Wounding**	
	Up	**Down**	**Up**	**Down**	**Up**	**Down**
DESeq	707	884	1156	1181	1967	1516
edgeR	648	670	920	925	1785	1563
Both	608	662	906	915	1749	1463

Table 2.5: Information of the primers designed in this study.

Sequence Definition	Sense Primer	Tm	GC%	Anti-sense Primer	Tm	GC%
GADPH	ATCAAAGCCAAGGTCAAG	59	44,4	CATCGTCTTCGGTGTATC	58,7	50
Actin	CGGCATTCACGAGACTAC	61,7	55,6	ACGGTGTTGGCATAAAGG	61,8	50
Alpha tubulin	AGCCATCTATGACATCTG	57,2	44,4	TCCGATAAGTCTGTTGAG	57,1	44,4
Rp49	TAAGCAAGCAATGTGGATAC	60	40	CTGTAAACTGGGCGAATC	59,6	50
Thioredoxin	ACGGCAAAGAGAAAGGCATA	59,8	45	CAGATCGATCAGCAGGAACA	59,9	50
Integrin beta	CGGTGTATGCGAATGTTACG	60	50	TTCCGGATTCTCCCTTTCTT	60	45
Epidermal growth factor receptor	CTGGTCGATGCTGACGAGTA	60	55	CAACAACTCCCTGTCCCAGT	60	55
Superoxide dismutase [Mn]	CCACCGTTTGGACTAGCATT	59,9	50	CCTATCGCTGCCATCTAAGC	59,9	55
Cytochrome P450	TCCGGATCATCGAATCGT	59,9	50	ATCGAAGCACCGCAAGAG	55,5	55
Glutathione peroxidase	GGCGACAATGCTCATCCT	59,7	55,5	TCGAGGGATCCGTGTTTG	60,6	55,5
Dynamin	GTCCCTTGAGGCCTTACCTC	60	60	CGTGAAACTGGTGGATGTTG	60	50

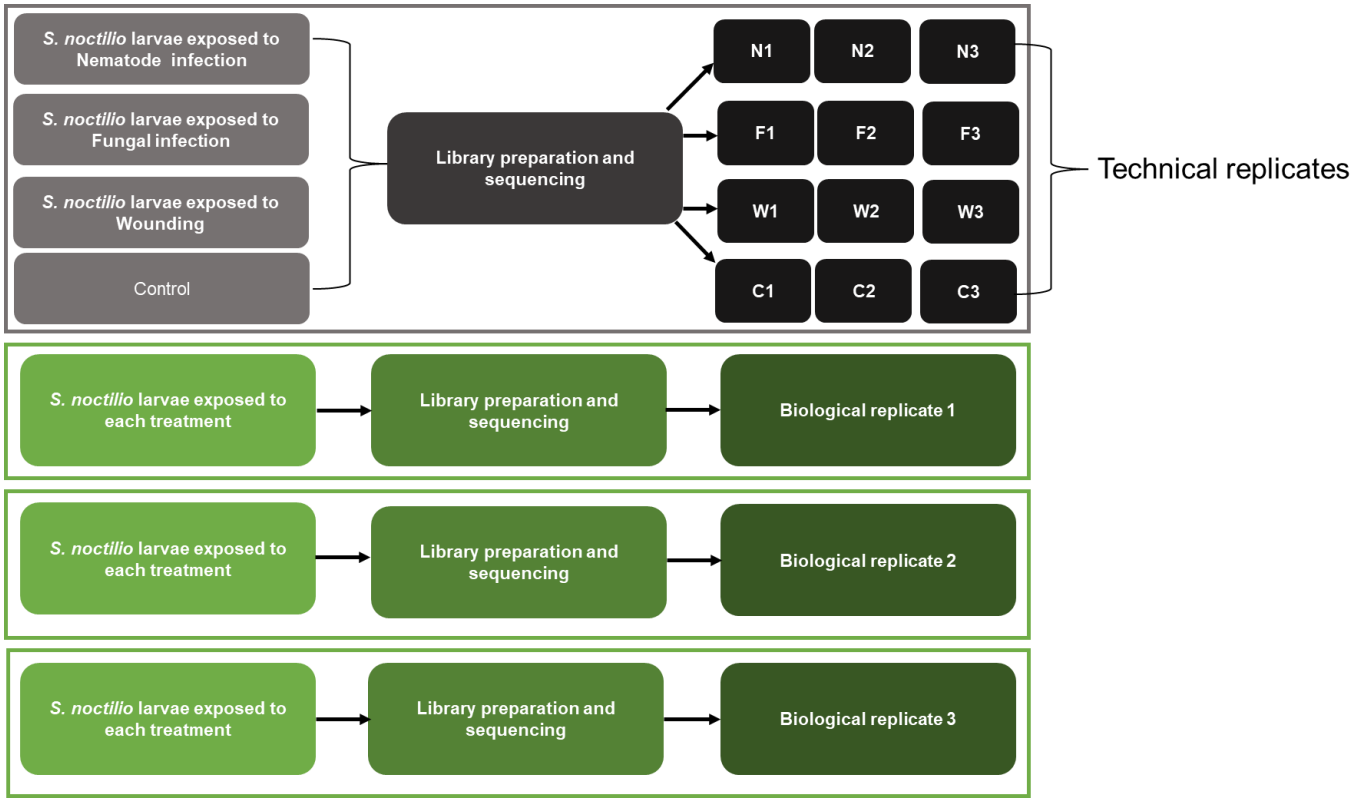


Figure 2.1: **Experimental design for the RNA-Seq experiment performed in this study.**

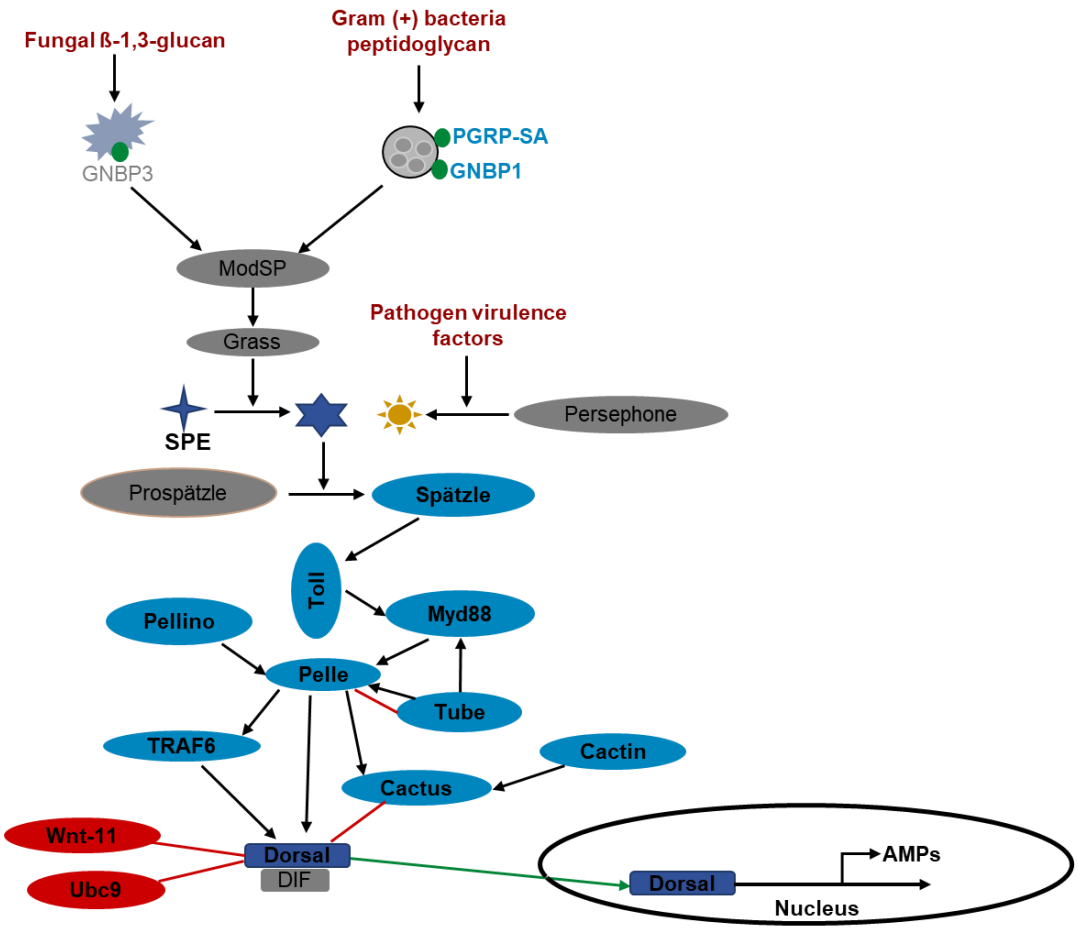


Figure 2.2: **The proposed model of the potential Toll-like receptors (Toll) signalling pathway in *S. noctilio***. *Sirex noctilio* immune-related genes are indicated with blue colour and bold text. Missing immune-related genes are shown in grey colour and normal text. The putative pathway genes for *S. noctilio* were predicted based on sequence similarities compared to other hymenopteran species and the model insect *D. melanogaster.*

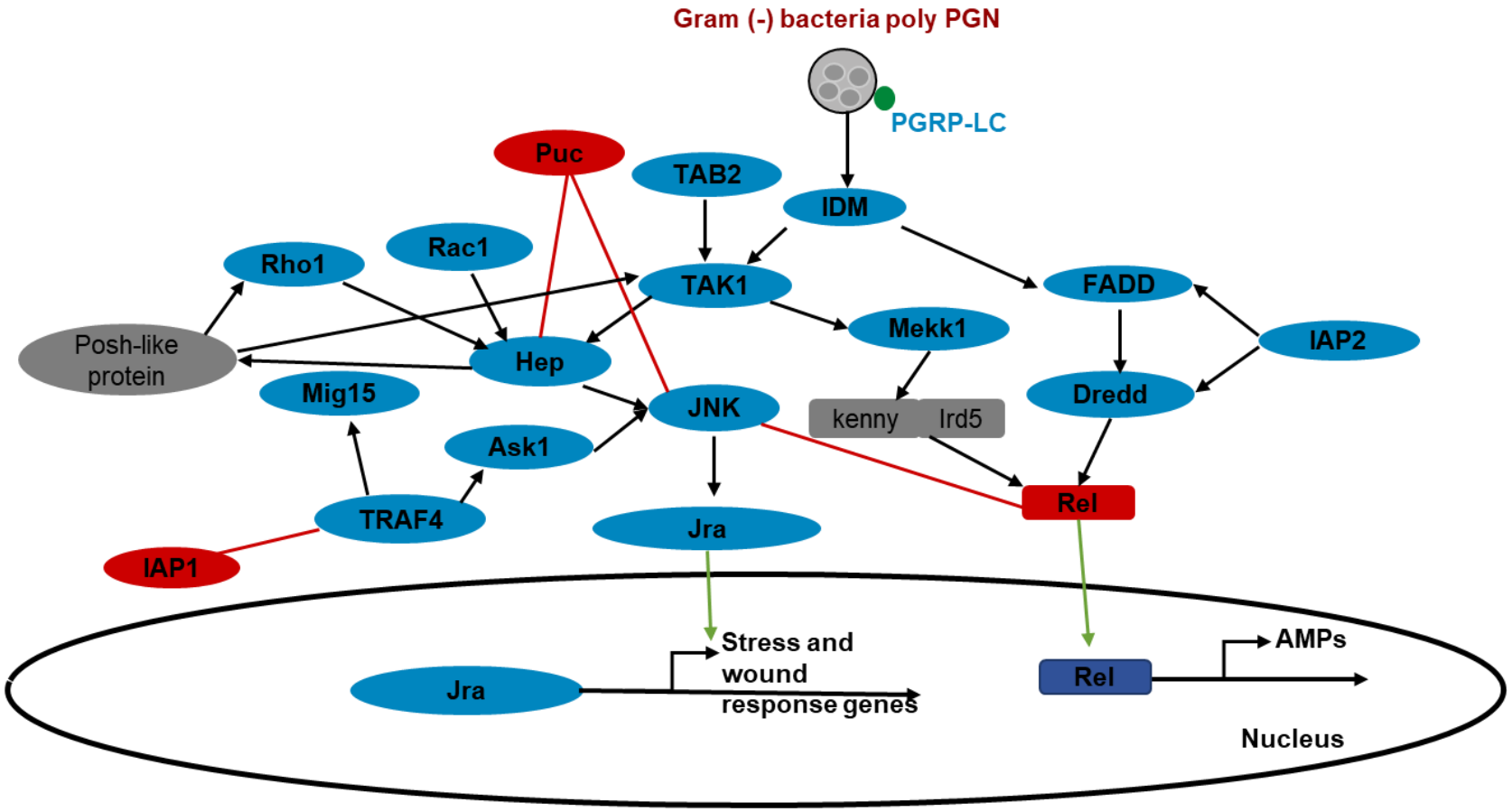


Figure 2.3: The proposed model of the potential Immune Deficiency (IMD) and c-Jun N-terminal kinase (JNK) signalling pathways in *S. noctilio*. *Sirex noctilio* immune-related genes are indicated with blue colour and bold text. Missing immune-related genes are shown in grey colour and normal text.

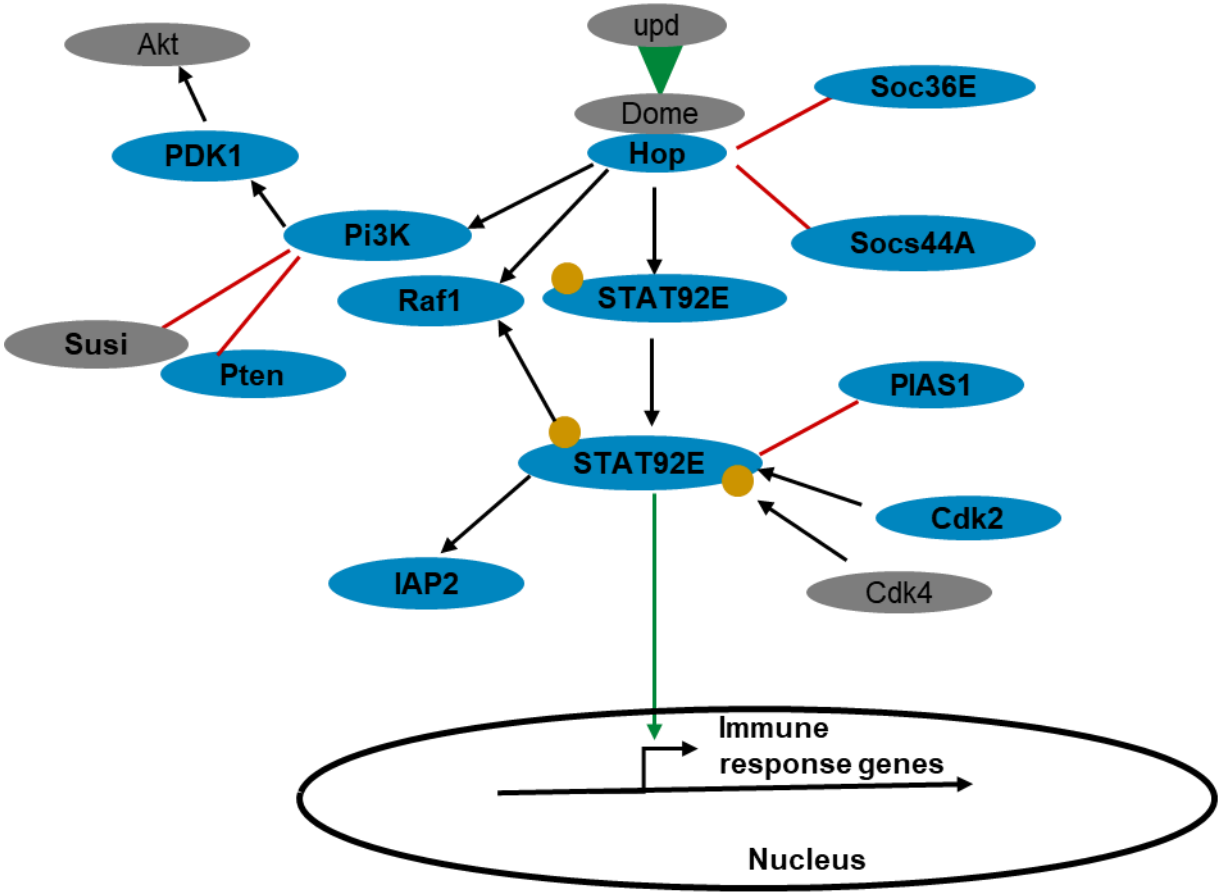


Figure 2.4: **The proposed model of the potential Janus kinase (JAK)-signal transducer and activator of transcription pathway (JAK-STAT) signalling pathways in *S. noctilio***. *Sirex noctilio* immune-related genes are indicated with blue colour and bold text. Missing immune-related genes are shown in grey colour and normal text.

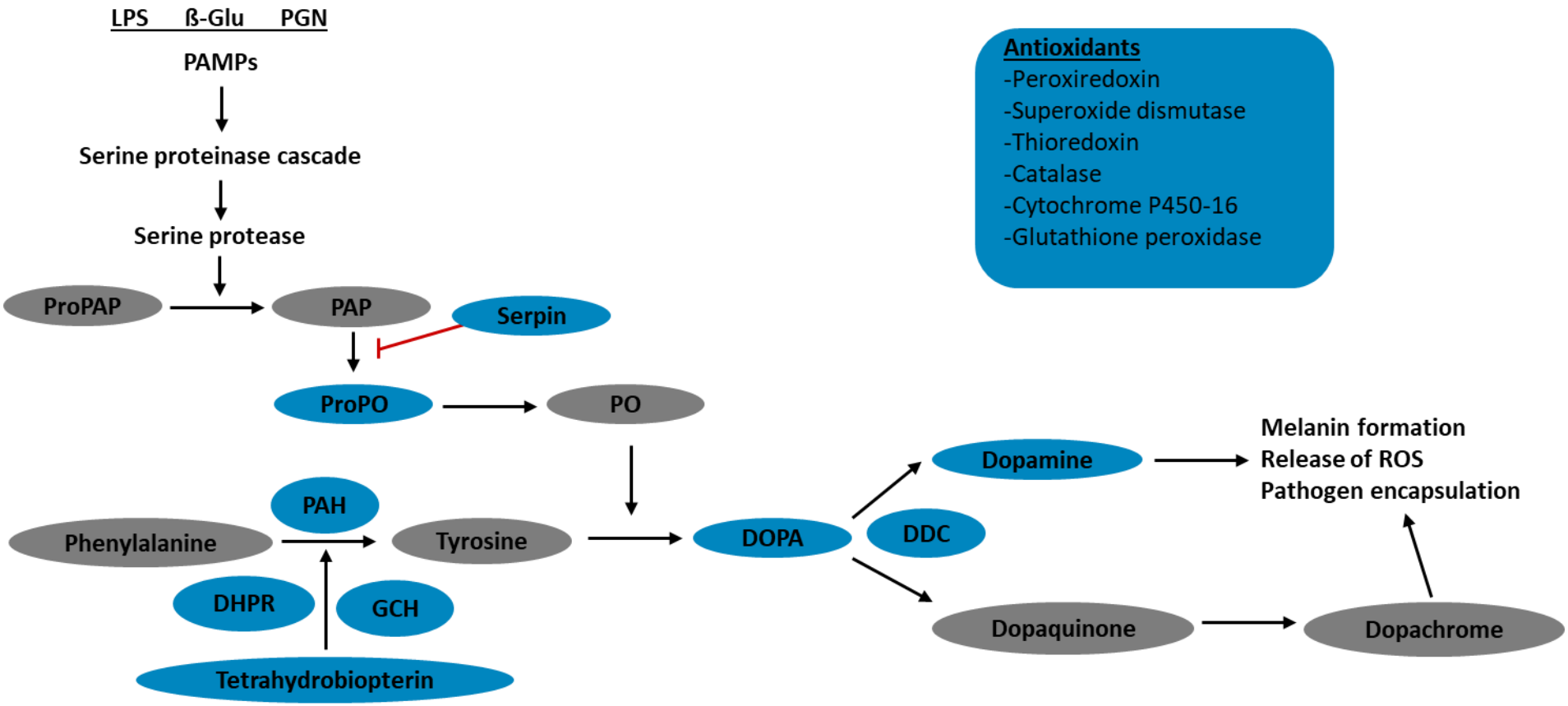


Figure 2.5: **The proposed model of the potential ProPO system in *S. noctilio***. *Sirex noctilio* immune-related genes involved in the ProPO-based melanisation are indicated with blue colour and bold text. Missing immune-related genes are shown in grey colour and normal text.

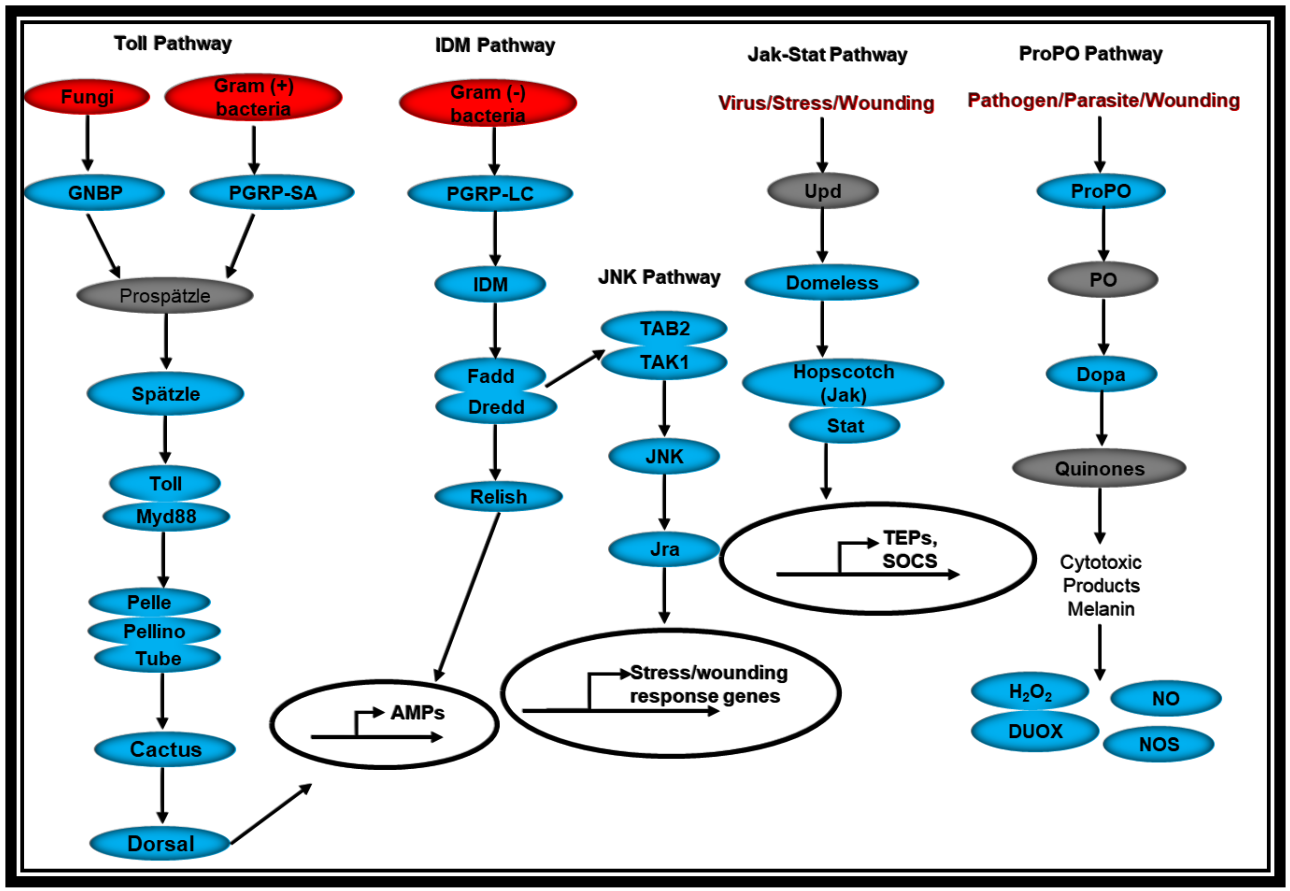


Figure 2.6: **Pathways in Summary of the overall potential immune response *S. noctilio*.**

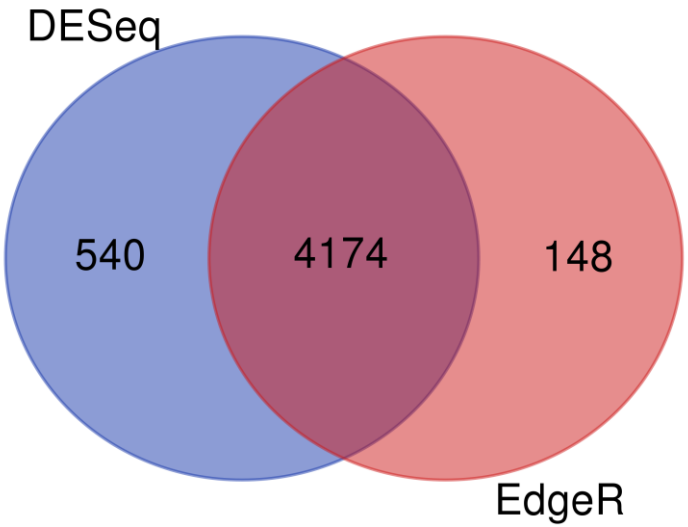
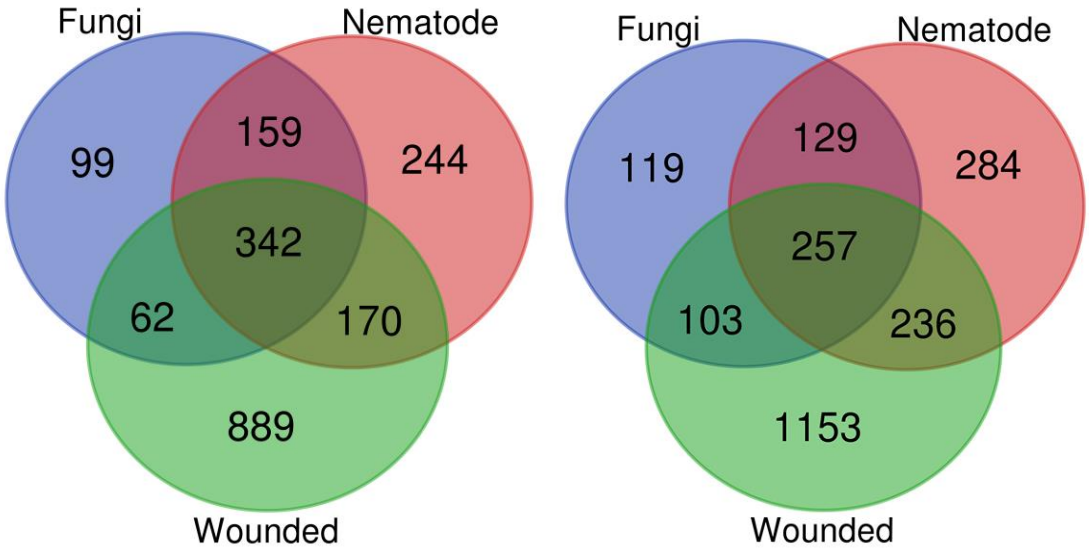


Figure 2.7: A Venn diagram analysis indicating the total number of significantly differentially expressed genes as well as the number of common and exclusive DEGs between the two programs used for the expression analysis (DESeq and edgeR).



a. Downregulated genes

b. Upregulated genes

Figure 2.8: A Venn diagram analysis indicating the number of a. downregulated genes and b. upregulated genes across all treatments.

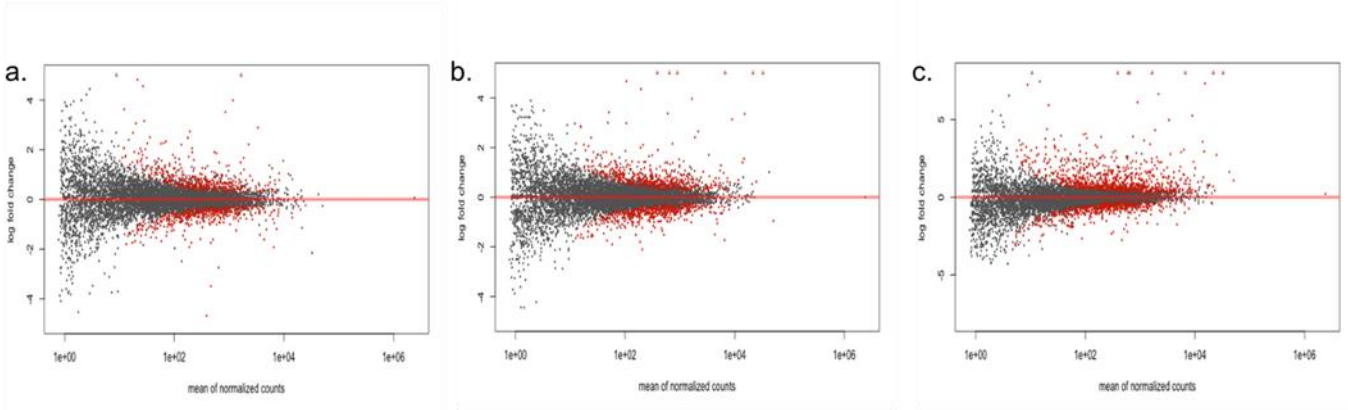


Figure 2.9: MA plots of all the expressed genes in *S. noctilio* in response to (a) *B. bassiana* fungal infection, (b) *D. siricidicola* nematode infection, (c) wounding. The x-axis represents the log fold change value of gene expression and the y-axis represent the mean of normalized counts. Each dot represents a gene, red represent significantly differentially expressed genes with genes below the vertical line being downregulated and those above the vertical line being upregulated.

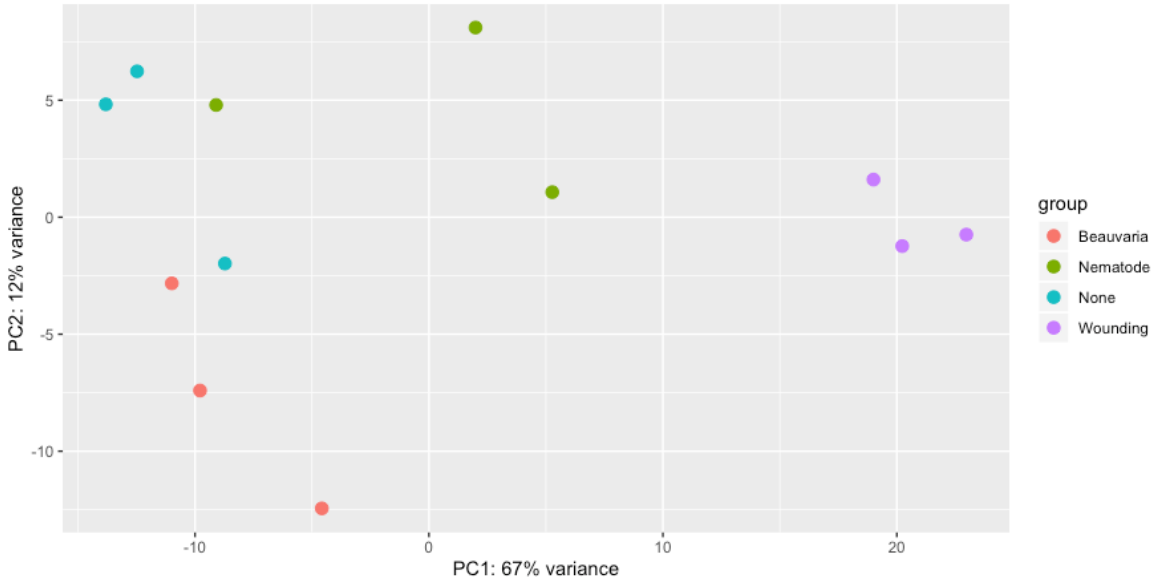


Figure 2.10: PCA plot of RNA-Seq data showing immune response of *S. noctilio* to *D. siricidicola* nematode infection, *B. bassiana* infection and wounding. Here we show how the samples are associated based on their gene expression.

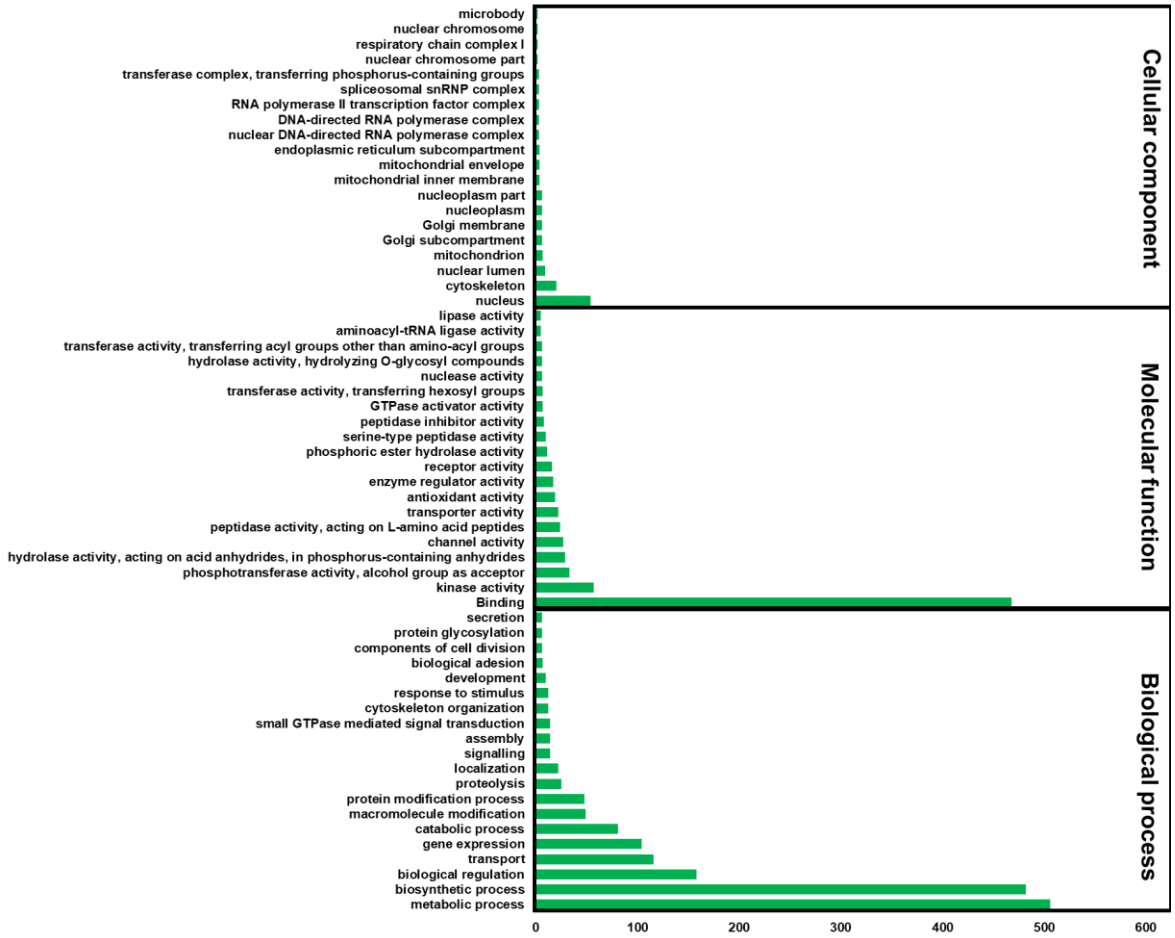


Figure 2.11: Gene ontology (GO) assignments for the enriched significantly upregulated immune-related genes following infection with *D. siricidicola* nematode.

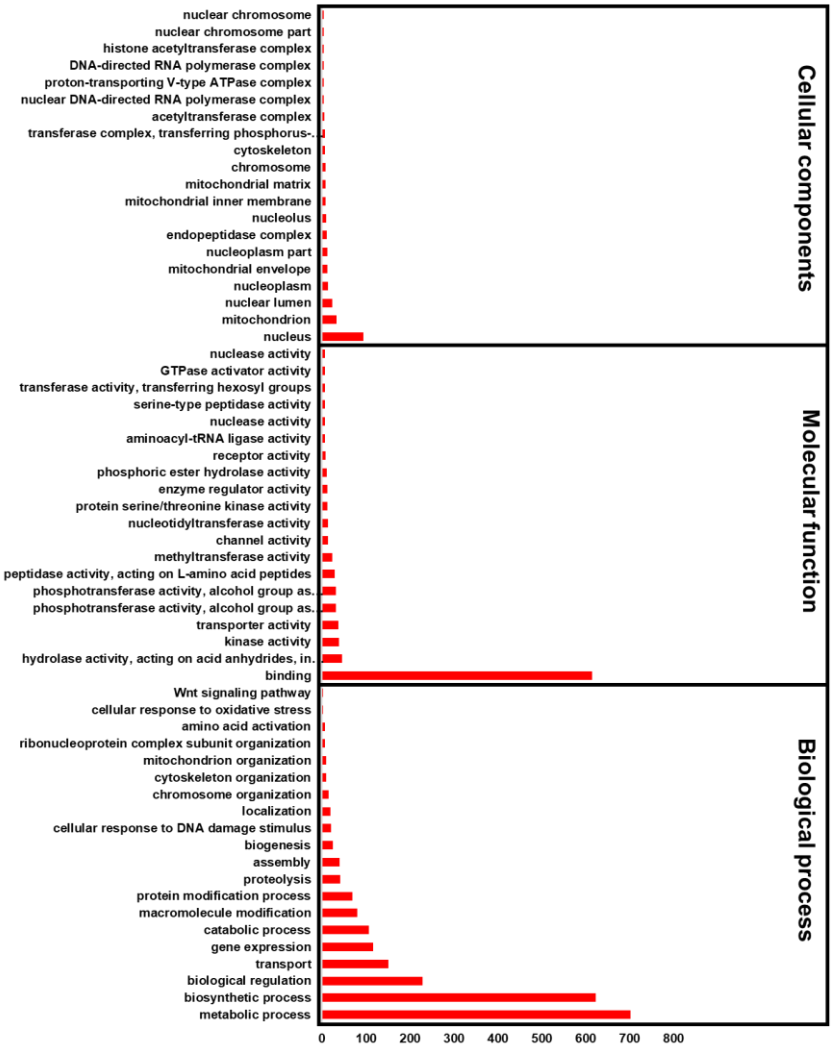


Figure 2.12: Gene ontology (GO) assignments for the enriched significantly downregulated immune-related genes following infection with *D. siricidicola* nematode.

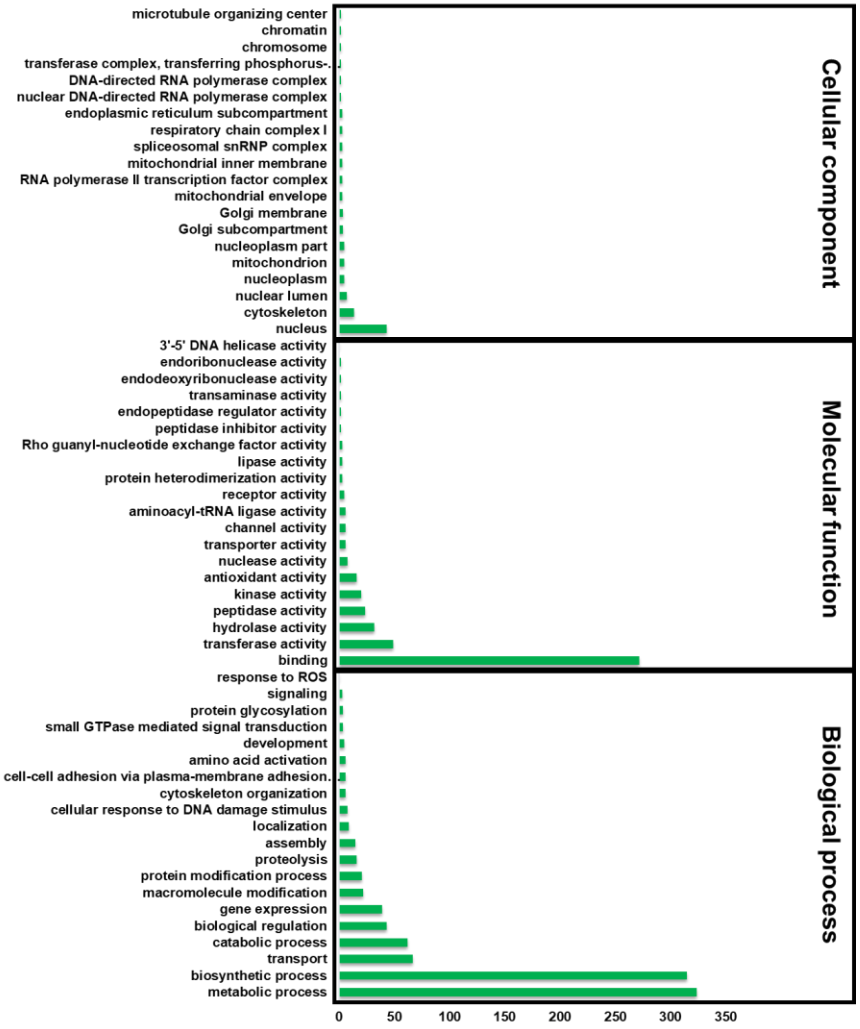


Figure 2.13: Gene ontology (GO) assignments for the enriched significantly upregulated immune-related genes following infection with *B. bassiana* fungus.

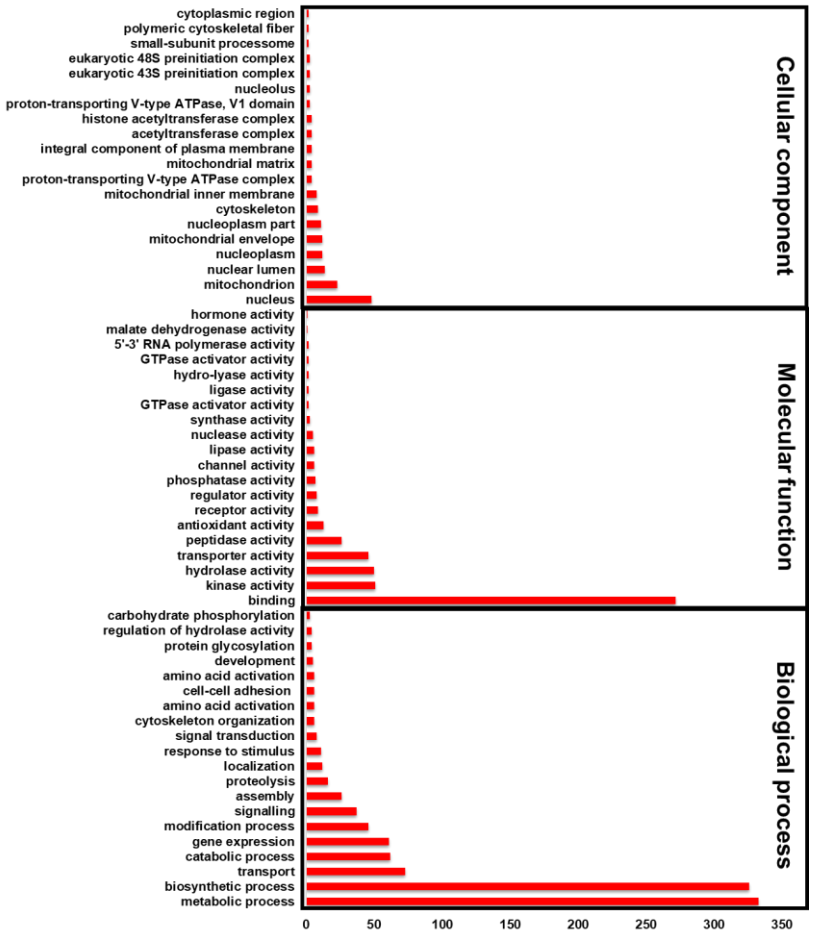


Figure 2.14: Gene ontology (GO) assignments for the enriched significantly downregulated immune-related genes following infection with *B. bassiana* fungus.

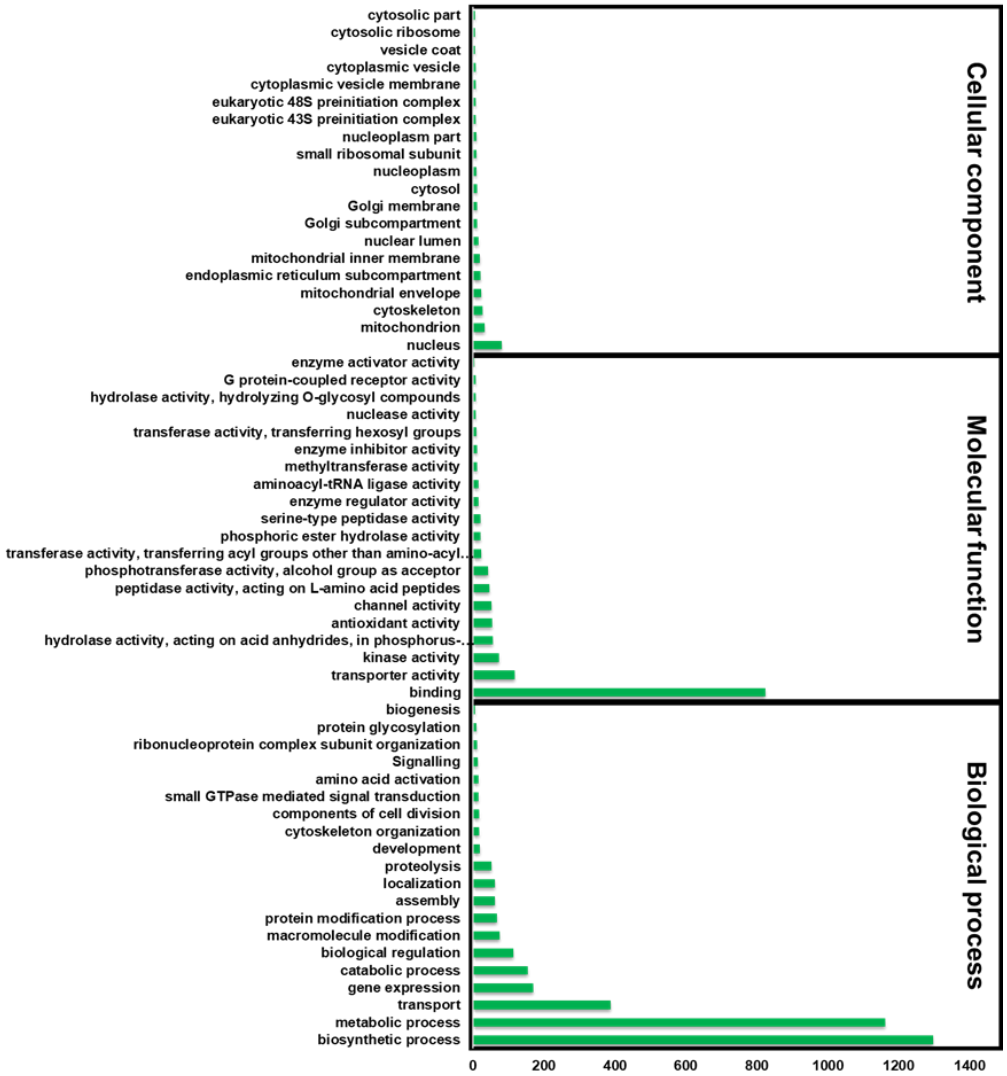


Figure 2.15: Gene ontology (GO) assignments for the enriched significantly upregulated immune-related genes following wounding.

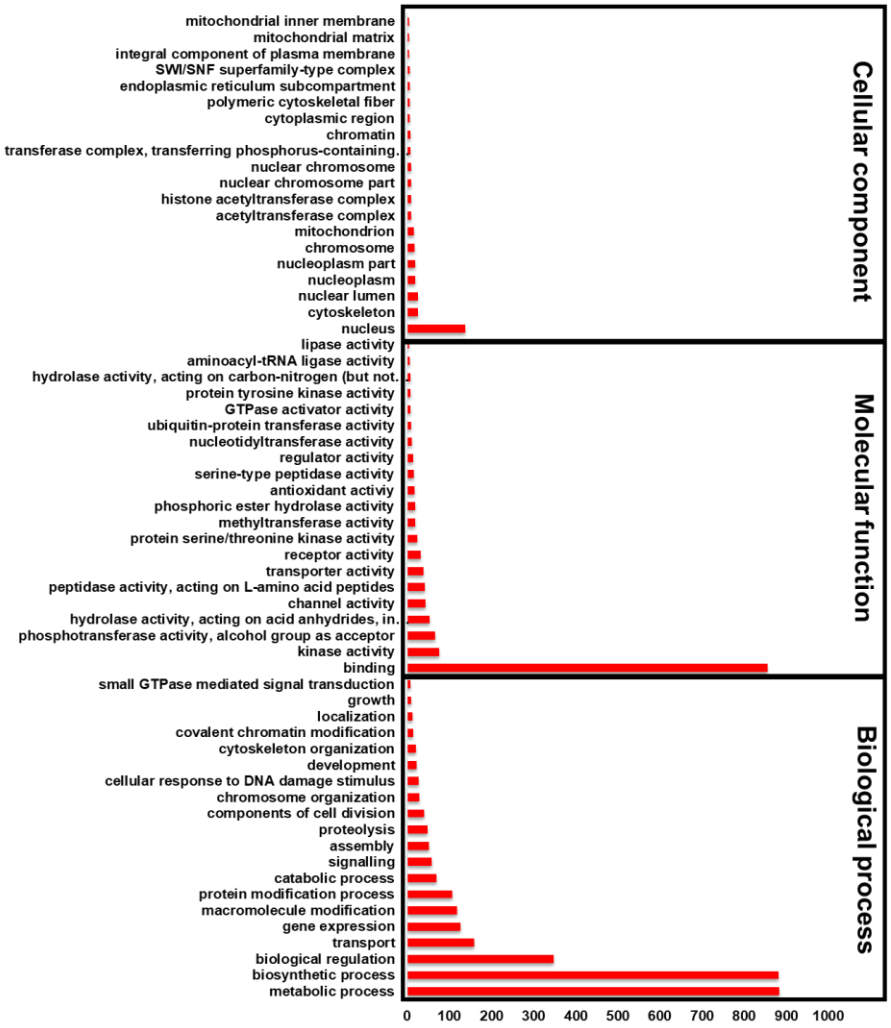


Figure 2.16: Gene ontology (GO) assignments for the enriched significantly downregulated immune-related genes following wounding.

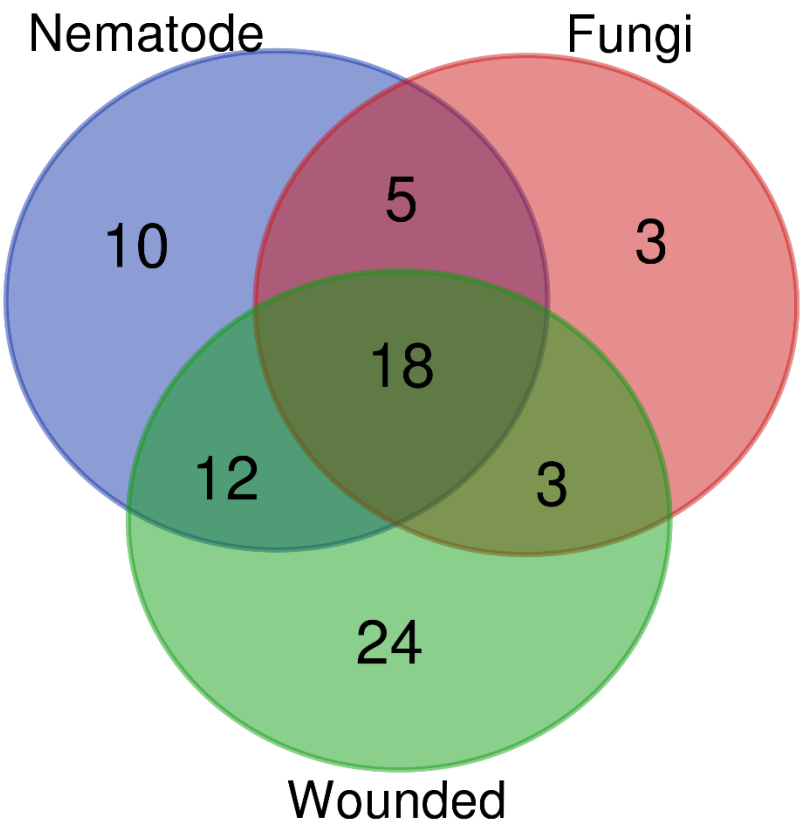


Figure 2.17: A Venn diagram of immunity-related differentially expressed genes *in S. noctilio* in response to *D. siricidicola* nematode infection, *B. bassiana* fungus infection and wounding. The numbers in each circle show immunity-related differentially expressed genes in each comparison treatment and the overlapping regions display genes that are commonly expressed among the comparison treatments.

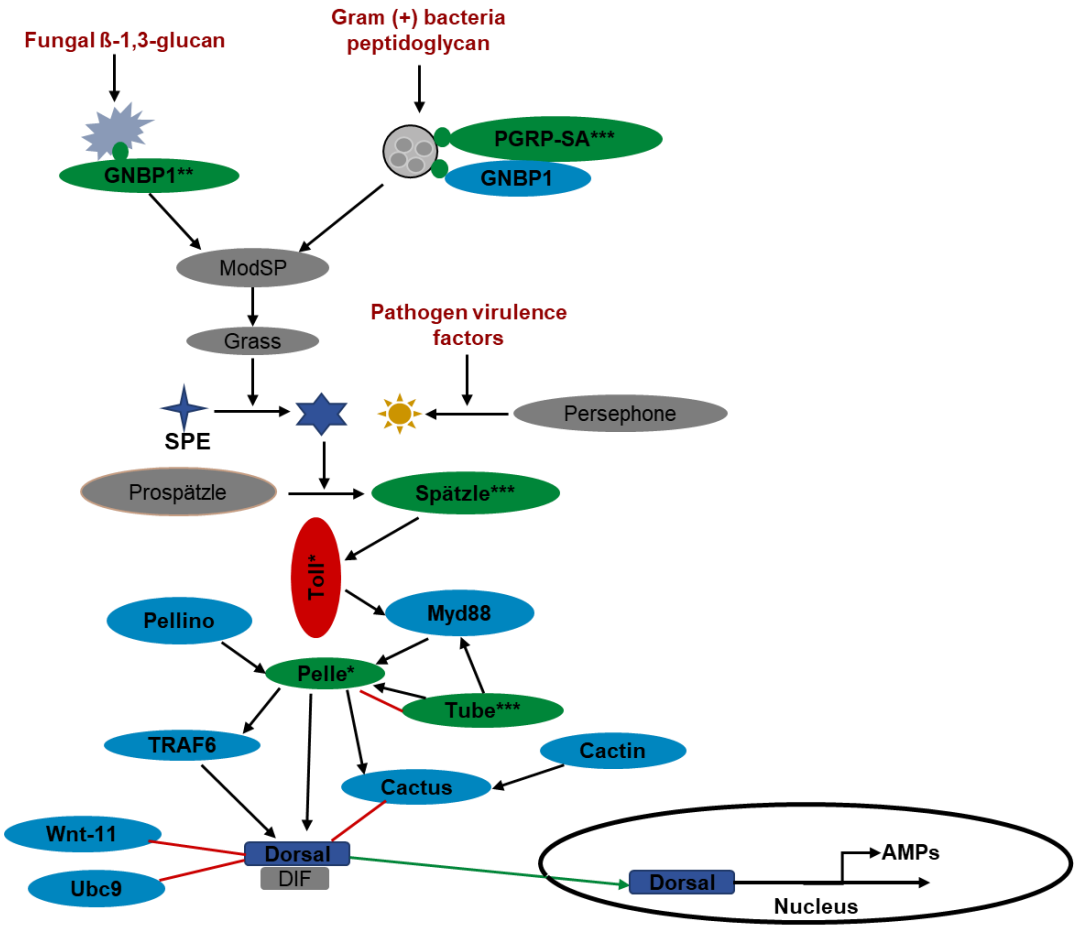


Figure 2.18: Summary of the Toll pathway DEGs identified in *S. noctilio* to be up regulated (green) and down regulated (red) after *D. siricidicola* infection, *B. bassiana* infection and wounding**.** One asterisk indicates up or down-regulated gene by one treatment, two asterisks indicate up or down-regulated gene by two treatments and three asterisks indicate up or down-regulated gene by all the three treatments.

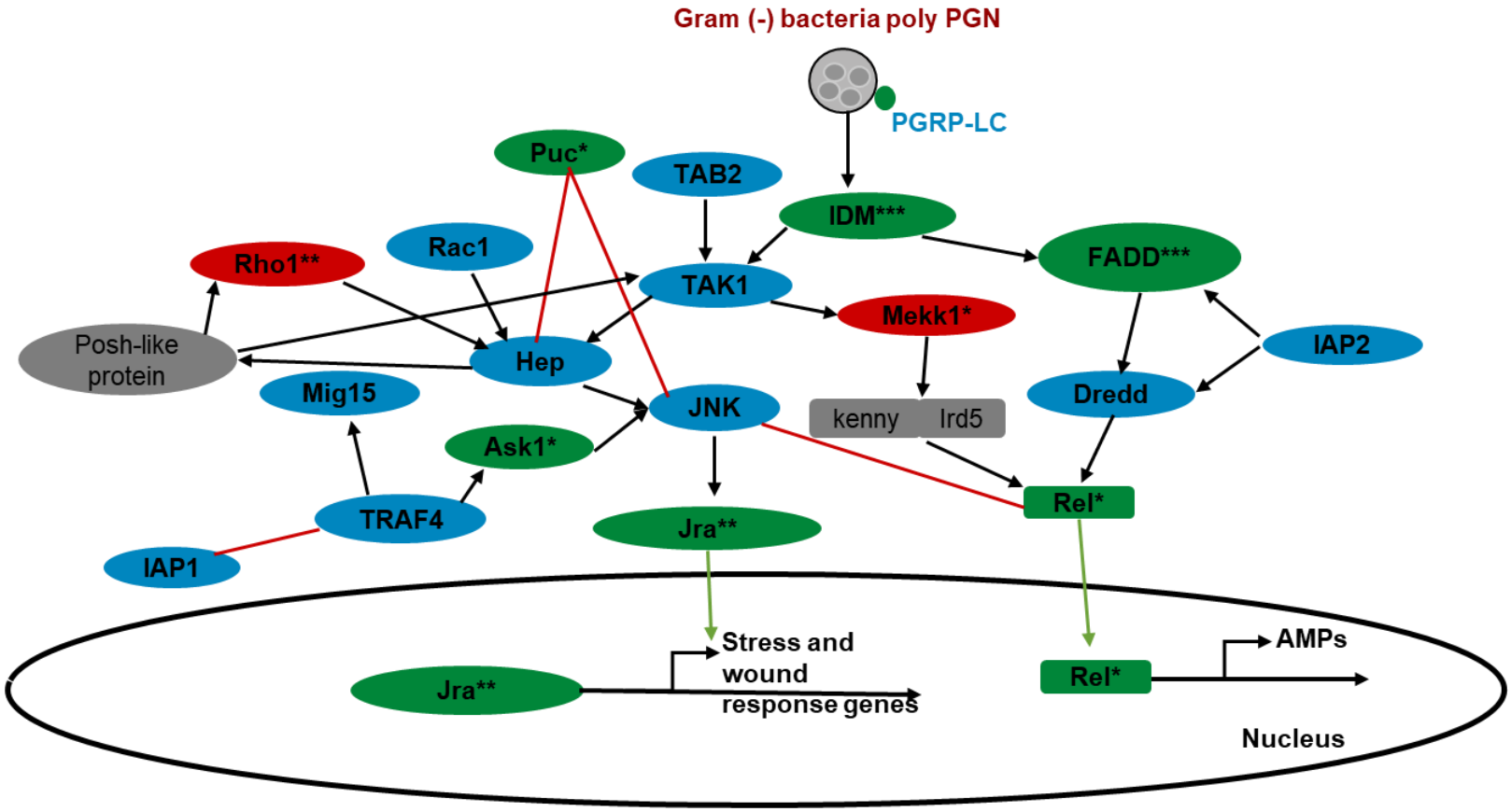


Figure 2.19: Summary of the IMD and JNK pathways DEGs identified in *S. noctilio* to be up regulated (green) and down regulated (red) after *D. siricidicola* infection, *B. bassiana* infection and wounding. One asterisk indicates up or downregulated gene by one treatment, two asterisks indicate up or down-regulated gene by two treatments and three asterisks indicate up or down-regulated gene by all the three treatments.

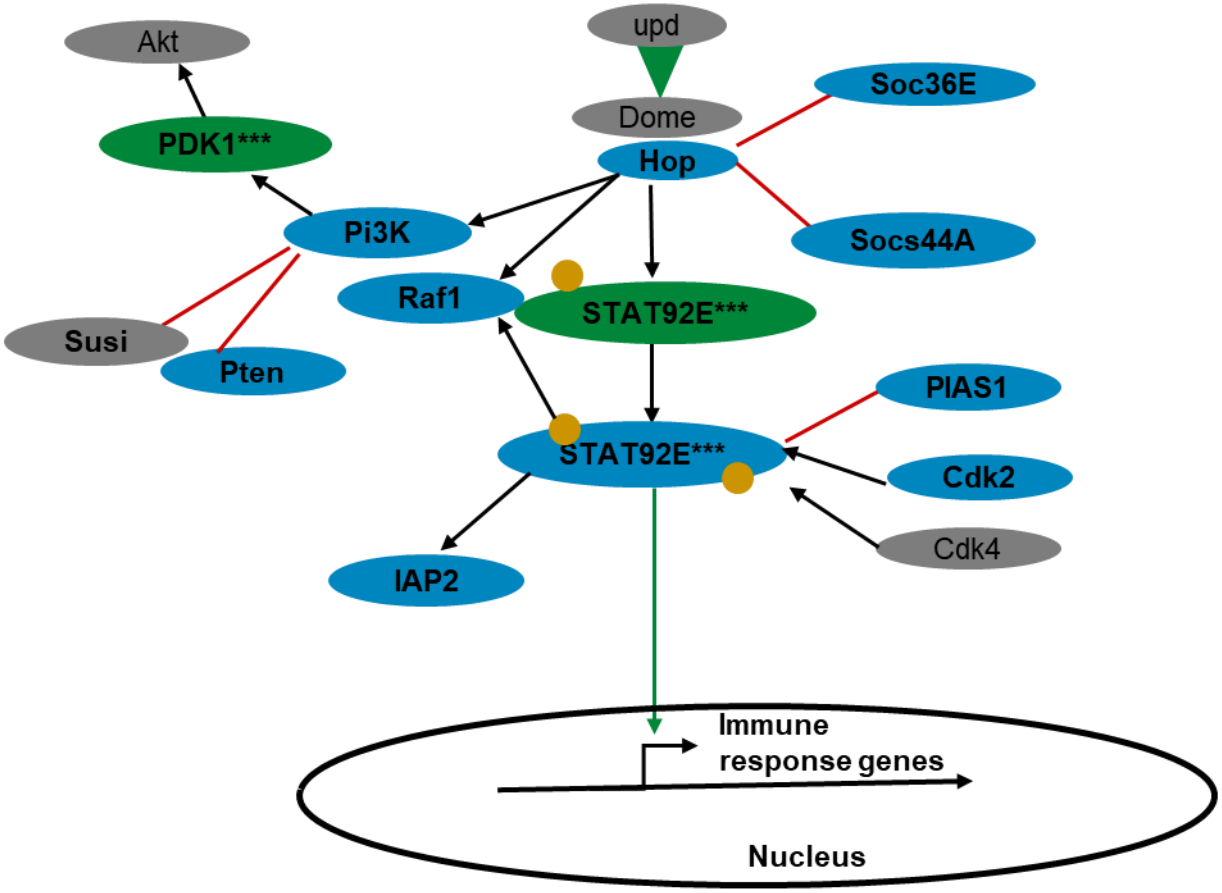


Figure 2.20: Summary of the Jak-stat pathway DEGs identified in *S. noctilio* to be up regulated (green) and down regulated (red) after *D. siricidicola* infection, *B. bassiana* infection and wounding. One asterisk indicates up or downregulated gene by one treatment, two asterisks indicate up or down-regulated gene by two treatments and three asterisks indicate up or down-regulated gene by all the three treatments.

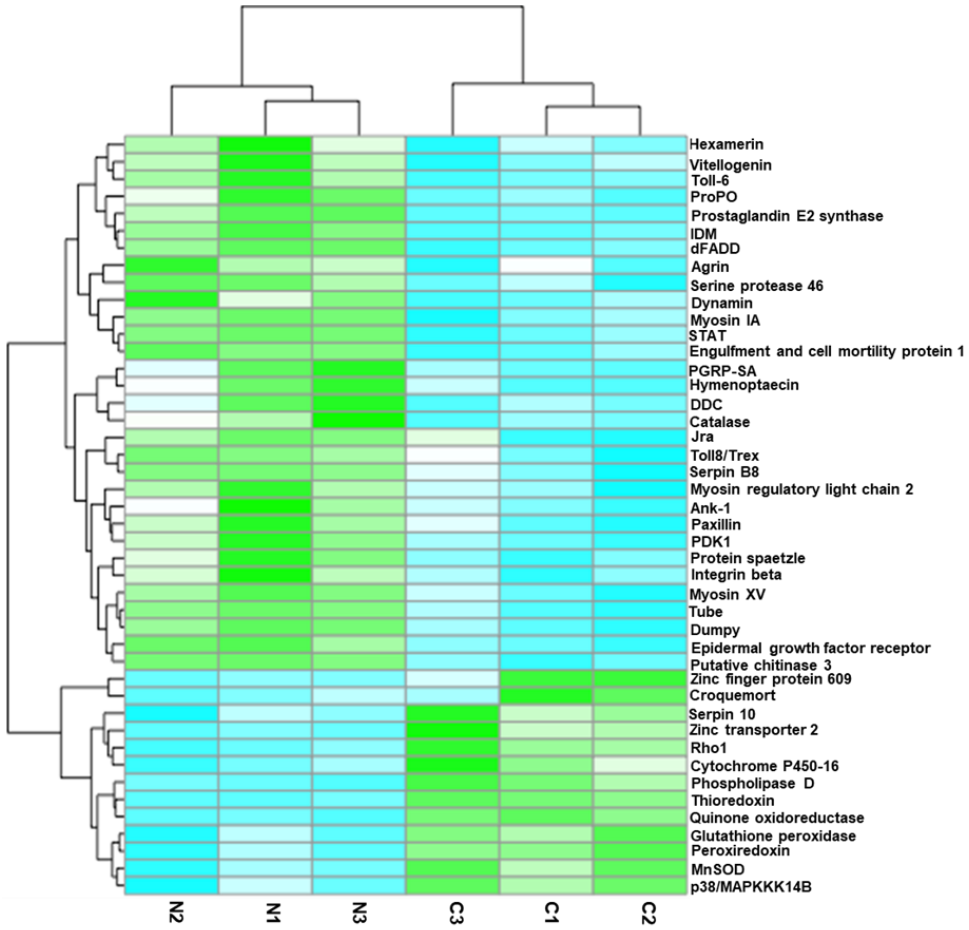


Figure 2.21: The heatmap shows significant differential expression of regulated immune-related genes 72h after infection of *S. noctilio* larvae with *D. siricidicola* nematodes of the strain SA107 (2013). Up and downregulated genes are colour coded with different shades, green shade indicate significant upregulation in the nematode vs control and blue shades indicate significant down-regulation in *D. siricidicola* nematode infection vs control.

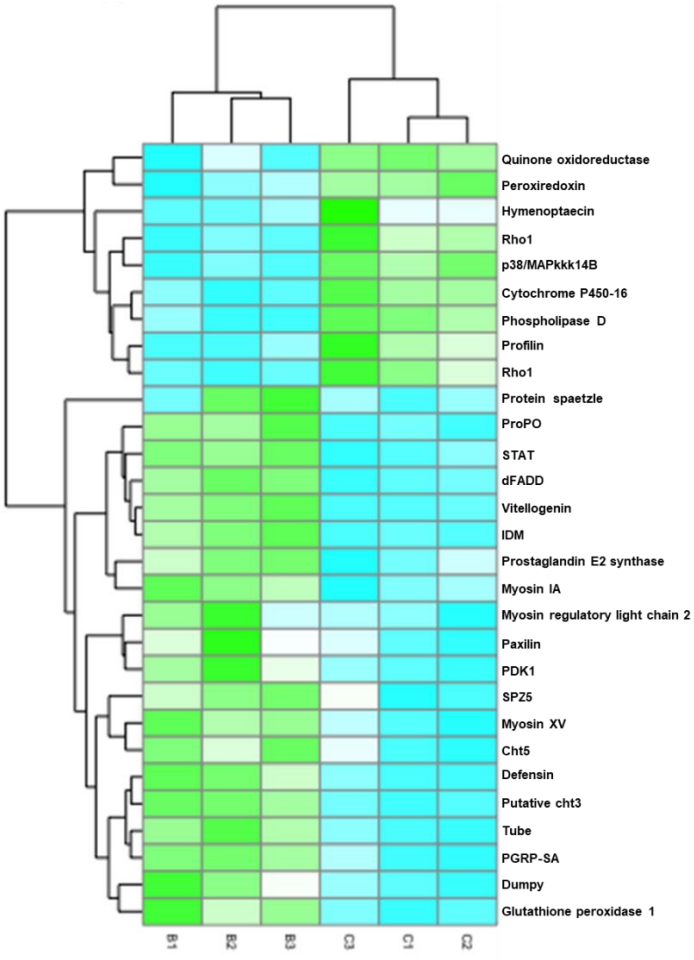


Figure 2.22: The heatmap shows significant differential expression of regulated immune-related genes 72h after infection of *S. noctilio* larvae with *B. bassiana* fungus. Up and downregulated genes are colour coded with different shades, green shade indicate significant upregulation in the fungus vs control and blue shades indicate significant downregulation in fungus vs control.

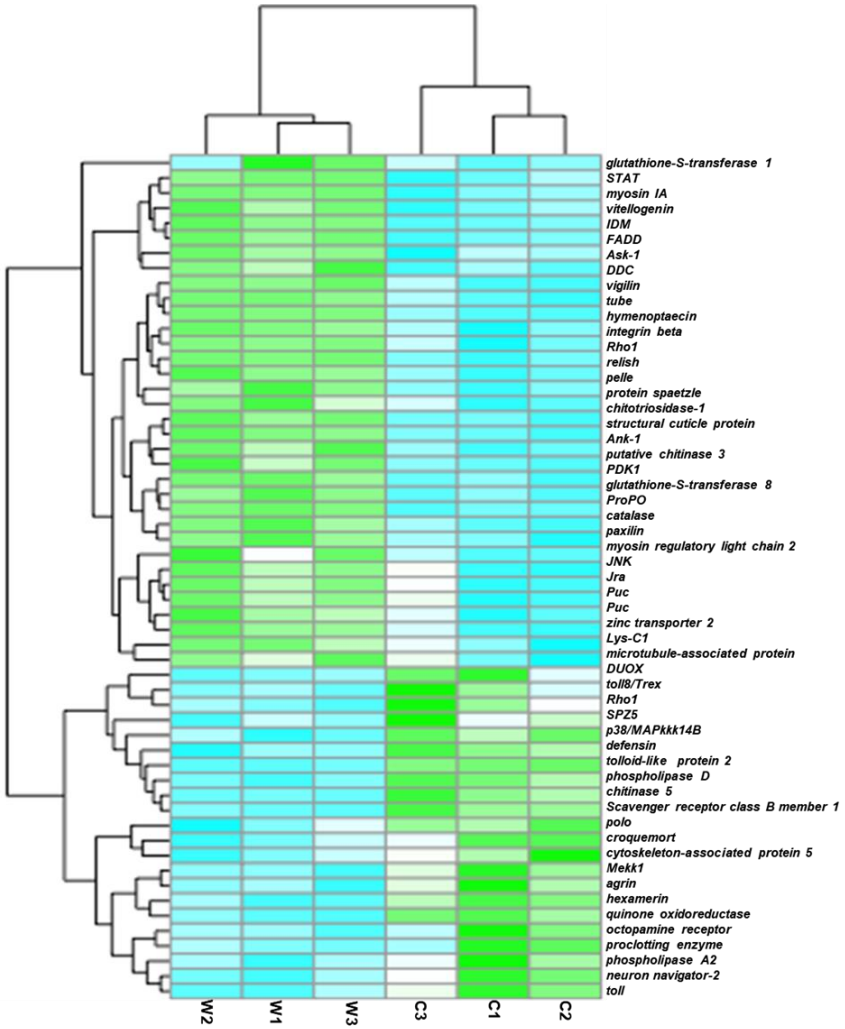


Figure 2.23: The heatmap shows significant differential expression of regulated immune-related genes 72h after *S. noctilio* larvae were wounded. Up and downregulated genes are colour coded with different shades, green shade indicate significant upregulation in the wounded vs control and blue shades indicate significant downregulation in wounded vs control.

Chapter 2: Supplementary Tables and Figures

Supplementary Table S2.1: Candidate immune-related genes identified in the genome of *S. noctilio*.

***Sirex noctilio* ID's**	**Description**	**Species top blast hit**	**E-value**	**Bit score**
SNOC_002449-RA|size2366004-augustus-gene-14.101	PGRP-lc	*A. rosae*	2,00E-136	401
SNOC_000684-RA|size3365038-exonerate_protein2genome-gene-21.89	PGRP-SA	*C. floridanum*	1,00E-44	154
SNOC_003453-RA|size2163639-processed-gene-6.129	B-gluc1	*A. rosae*	3,00E-163	476
SNOC_004654-RA|size1755071-exonerate_protein2genome-gene-1.68	B-gluc2	*H. laboriosa*	2,00E-124	369
SNOC_005303-RA|size1678705-processed-gene-2.2	Galectin-1	*E. dilemma*	2,00E-14	73
SNOC_002569-RA|size2248198-processed-gene-3.112	CTL2D3	*C. floridanus*	4,00E-119	343
SNOC_008496-RA|size1001638-augustus-gene-8.4	CTL5 precursor	*A. mellifera*	1,00E-150	421
SNOC_011061-RA|size641777-augustus-gene-3.51	C-type lectin mannose-binding isoform	*T. zeteki*	2,00E-84	253
SNOC_011163-RA|size641365-augustus-gene-5.102	Contactin	*B. impatiens*	0.0	2095
SNOC_014089-RA|size236076-augustus-gene-0.0	Titin	*A. echinatior*	5,00E-28	124
SNOC_000306-RA|size5241802-augustus-gene-33.71	Titin	*A. rosae*	0.0	3559
SNOC_003020-RA|size2204710-processed-gene-5.73	SRCBM1	*B. bifarius*	0.0	845
SNOC_007179-RA|size1086615-augustus-gene-0.157	vigilin	*C. floridanus*	0.0	2074
SNOC_012101-RA|size453568-processed-gene-3.108	DSCAM	*A. mellifera*	0.0	2040
SNOC_004888-RA|size1697473-processed-gene-2.138	Drapper	*A. mellifera*	0.0	1514
SNOC_007161-RA|size1105877-augustus-gene-8.8	dumpy	*C. floridanus*	3,00E-06	60
SNOC_002723-RA|size2248198-processed-gene-17.41	profilin	*C. cinctus*	8,00E-90	259
SNOC_006060-RA|size1504730-augustus-gene-5.117	Engulfment and cell motility protein 1	*L. niger*	0.0	1379
SNOC_014481-RA|size172559-processed-gene-0.109	NADPH oxidase (NOX)	*A. mellifera*	2,00E-104	321
SNOC_002346-RA|size2366004-augustus-gene-1.20	NOS	*C. cinctus*	0.0	2145
SNOC_011285-RA|size638645-exonerate_protein2genome-gene-2.22	Phospholipase A2	*A. mellifera*	7,00E-45	148
SNOC_011773-RA|size589636-processed-gene-1.6	Phospholipase D	*A. echinatior*	0.0	1739

SNOC_010669-RA|size687644-augustus-gene-0.19	prostaglandin E synthase2	*C. cinctus*	0.0	573
SNOC_000468-RA|size5241802-augustus-gene-51.3	prostaglandin reductase-1	*C. cinctus*	1,00E-172	488
SNOC_009869-RA|size787876-augustus-gene-3.60	Peroxiredoxin	*B. impatiens*	1,00E-132	377
SNOC_010169-RA|size734633-exonerate_est2genome-gene-5.87	Peroxiredoxin-1	*A. echinatior*	3,00E-129	365
SNOC_008502-RA|size1001638-augustus-gene-9.3	Peroxiredoxin-6	*F. arisanus*	5,00E-126	359
SNOC_004555-RA|size1760177-augustus-gene-8.207	DDC/Aromatic-L-Amino-Acid decarboxylase	*M. quadrifasciata*	0.0	894
SNOC_010713-RA|size687644-augustus-gene-5.5	Ras-related protein Rac1	*A. mellifera*	1,00E-139	391
SNOC_010309-RA|size733809-processed-gene-5.26	Ras-like GTP-binding protein RhoL	*C. floridanus*	2,00E-115	329
SNOC_008417-RA|size1001802-processed-gene-9.30	paxillin	*C. floridanus*	0.0	857
SNOC_009569-RA|size833181-augustus-gene-4.193	transglutamase	*A. mellifera*	0.0	1174
SNOC_002714-RA|size2248198-augustus-gene-16.13	Proclotting enzyme	*A. mellifera*	1,00E-62	216
SNOC_008122-RA|size1016601-processed-gene-2.32	cytochrome P450-16	*C. cinctus*	0.0	875
SNOC_009362-RA|size864392-processed-gene-6.26	Glycerol-3-dehydrogenase (NAD+) cytoplasmic	*A. echinatior*	0.0	607
SNOC_007618-RA|size1061917-augustus gene-3.129	Acylphosphatase-1	*C. cinctus*	6,00E-48	150
SNOC_006381-RA|size1469172-exonerate_est2genome-gene-4.93	Acylphosphatase-2	*A. echinatior*	1,00E-45	145
SNOC_014342-RA|size194932-augustus-gene-0.284	zinc transporter 2	*T. zeteki*	0.0	603
SNOC_012015-RA|size473363-exonerate_protein2genome-gene-3.131	Catalase	*A. rosae*	3,00E-177	505
SNOC_009935-RA|size787876-processed-gene-6.122	MnSOD	*A. mellifera*	1,00E-131	373
SNOC_015907-RA|size85242-augustus-gene-0.2	Cu-ZnSOD	*C. cinctus*	3,00E-94	273
SNOC_006558-RA|size1374663-augustus-gene-7.105	Glutathione peroxidase	*N. vitripennis*	2,00E-104	300
SNOC_000435-RA|size5241802-processed-gene-47.38	Glutathione peroxidase	*N. vitripennis*	2,00E-81	270
SNOC_010938-RA|size663648-processed-gene-2.94	Glutathione S-transferase 1	*A. mellifera*	2,00E-76	242
SNOC_014856-RA|size140371-processed-gene-0.35	Vitellogenin	*A. rosae*	0.0	1634
SNOC_006943-RA|size1170633-augustus-gene-8.0	Thioredoxin	*M. rodundata*	2,00E-82	241
SNOC_002418-RA|size2366004-augustus-gene-10.9	GST8	*C. floridanus*	1,00E-153	431
SNOC_008456-RA|size1001638-processed-gene-2.51	SPZ5	*H. laboriosa*	6,00E-15	74
SNOC_013207-RA|size336147-exonerate_protein2genome-gene-1.0	protein spaetzle	*A. compressa*	5,00E-74	236

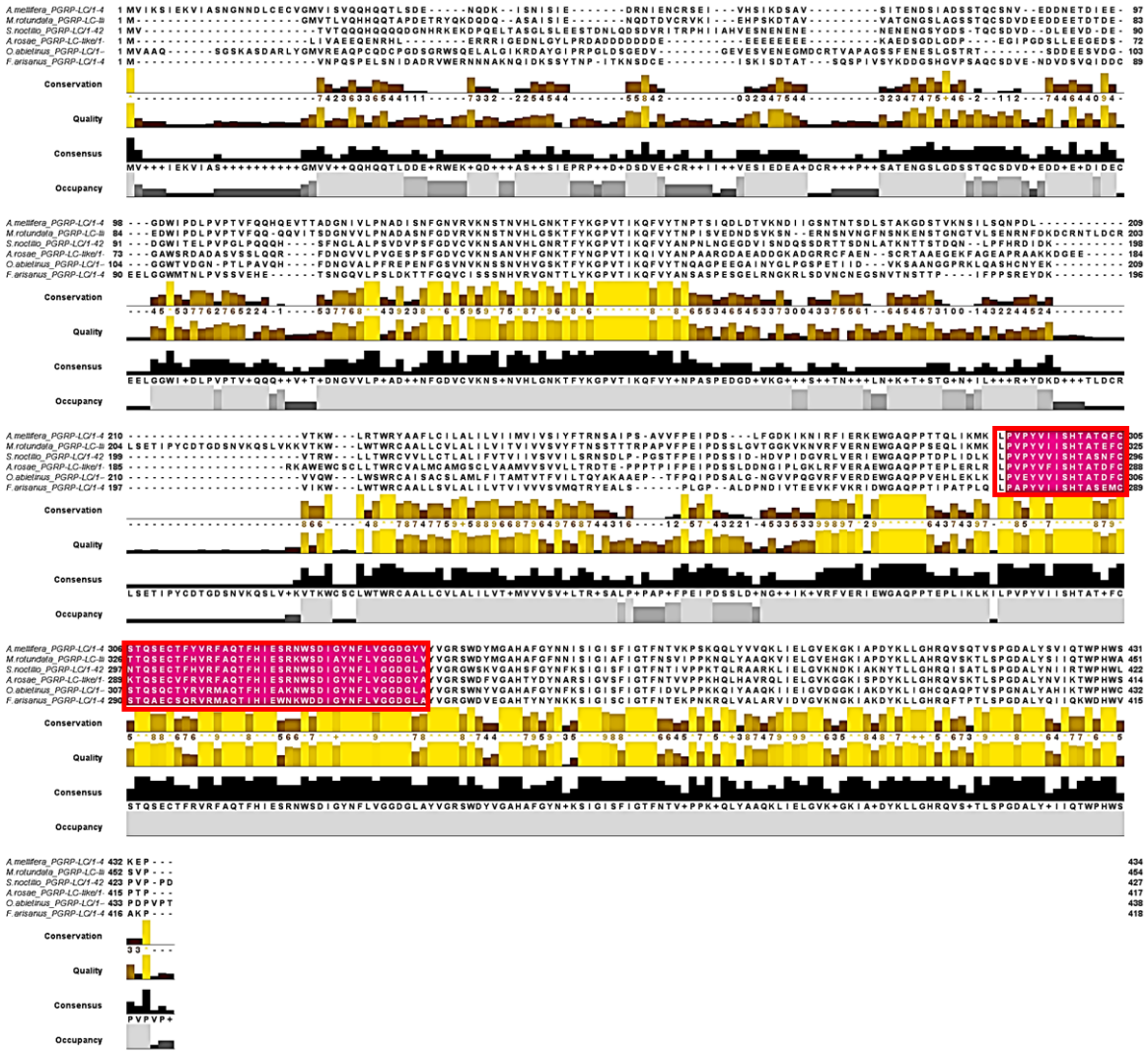
SNOC_013089-RA|size345363-augustus-gene-2.177	tube	*N. vitripennis*	1,00E-56	197
SNOC_013354-RA|size323794-augustus-gene-1.61	myd88	*C. cinctus*	9,00E-144	421
SNOC_001209-RA|size3272368-processed-gene-3.162	cactin	*B. terrestris*	0.0	1053
SNOC_008204-RA|size1016601-processed-gene-9.9	Cactus	*A. mellifera*	4,00E-27	112
SNOC_015308-RA|size109487-processed-gene-0.78	toll	*C. floridanus*	0.0	875
SNOC_011176-RA|size639514-augustus-gene-0.61	Protein toll	*N. vitripennis*	0.0	879
SNOC_016107-RA|size77364-processed-gene-0.40	toll-6	*B. terrestris*	0.0	1086
SNOC_007226-RA|size1086615-processed-gene-5.44	toll8/Trex	*A. mellifera*	0.0	2172
SNOC_010272-RA|size733809-augustus-gene-2.107	Toll-interacting protein (Tollip)	*F. arisanus*	1,00E-82	252
SNOC_005248-RA|size1690910-augustus-gene-13.3	Tolloid-like protein 2	*A. colombica*	3,00E-32	129
SNOC_006068-RA|size1504730-augustus-gene-6.156	Dorsal	*A. mellifera*	6,00E-10	61
SNOC_017776-RA|size36041-augustus-gene-0.17	Dorsal	*A. mellifera*	2,00E-09	59
SNOC_004508-RA|size1760177-augustus-gene-3.188	TNF receptor-associated factor 6 (TRAF6)	*A. colombica*	0.0	944
SNOC_010752-RA|size679134-processed-gene-3.4	pelle	*T. zeteki*	0.0	667
SNOC_009460-RA|size848173-augustus-gene-7.3	pellino	*C. floridanus*	0.0	759
SNOC_009455-RA|size848173_processed-gene-7.99	wnt11	*C. floridanus*	2,00E-68	223
SNOC_009723-RA|size825924-exonerate_protein2genome-gene-7.53	wnt4	*O. biroi*	0.0	612
SNOC_014042-RA|size238000-exonerate_protein2genome-gene-1.41	wnt6	*C. cinctus*	0.0	623
SNOC_014047-RA|size238000-exonerate_protein2genome-gene-1.133	wnt1	*A. echinatior*	0.0	763
SNOC_009307-RA|size875148-processed-gene-7.326	Ubc9-B	*T. zeteki*	2,00E-116	332
SNOC_000075-RA|size5241802-augustus-gene-7.4	Agrin	*A. mellifera*	3,00E-25	109
SNOC_006074-RA|size1504730-augustus-gene-7.0	Agrin	*A. echinatior*	0.0	904
SNOC_009698-RA|size825924-augustus-gene-5.175	Hopscotch	*A. cerana*	0.0	1524
SNOC_016115-RA|size77299-exonerate_protein2genome-gene-0.35	STAT	*M. quadrifasciata*	0.0	1414
SNOC_003768-RA|size2110464-augustus-gene-18.105	SOCS7	*A. mellifera*	0.0	1204
SNOC_004542-RA|size1760177-augustus-gene-6.83	SOCS5	*A. mellifera*	0.0	860
SNOC_007595-RA|size1061917-augustus-gene-0.3	PIAS1/PIAS2/PIAS3	*C. floridanus*	0.0	938

SNOC_001080-RA|size3354006-augustus-gene-27.11	PDK1	*A. echinatior*	0.0	885
SNOC_003086-RA|size2204710-processed-gene-13.102	IAP1	*T. longispinosus*	1,00E-88	282
SNOC_003638-RA|size2110464-augustus-gene-5.33	Pi3K	*E. mexicana*	0.0	1067
SNOC_005503-RA|size1648946-augustus-gene-1.4	Pten	*T. cornetzi*	0.0	924
SNOC_004005-RA|size2083609-augustus-gene-19.204	WNK-1	*A. echinatior*	0	1296
SNOC_000936-RA|size3354006-augustus-gene-10.0	Polo	*A. mellifera*	0.0	1001
SNOC_005625-RA|size1648946-exonerate_est2genome-gene-11.109	dRAF-1	*C. floridanus*	0.0	1133
SNOC_001102-RA|size3354006-processed-gene-29.42	TEPA	*A. mellifera*	4,00E-78	286
SNOC_003113-RA|size2204710-processed-gene-17.18	G1/S-specific cyclin-E	*L. niger*	0.0	679
SNOC_014883-RA|size139032-processed-gene-0.67	JNK	*L. niger*	0.0	742
SNOC_005663-RA|size1648946-processed-gene-15.123	Hep	*F. arisanus*	1,00E-91	294
SNOC_013417-RA|size300537-processed-gene-0.5	rho1	*C. floridanus*	5,00E-18	78
SNOC_011816-RA|size561864-augustus-gene-1.89	rho1	*A. mellifera*	1,00E-16	74
SNOC_011815-RA|size561864-processed-gene-1.17	rho1	*A. mellifera*	3,00E-18	79
SNOC_004278-RA|size2040173-processed-gene-17.5	mig-15	*C. costatus*	0.0	1129
SNOC_007289-RA|size1086615-exonerate_est2genome-gene-9.63	Jra	*A. cerana cerana*	2,00E-155	437
SNOC_015850-RA|size87683-augustus-gene-0.22	kay	*A. colombica*	4,00E-172	486
SNOC_008836-RA|size937237-processed-gene-1.29	EGFR	*A. mellifera*	0.0	2377
SNOC_019443-RA|size7153-processed-gene-0.7	puc	*T. zeteki*	1,00E-93	277
SNOC_018859-RA|size15935-processed-gene-0.0	puc	H. laboriosa	4,00E-61	191
SNOC_004508-RA|size1760177-augustus-gene-3.188	traf4	*A. echinatior*	0.0	944
SNOC_015436-RA|size102882-processed-gene-0.35	IDM	*A. mellifera*	3,00E-55	177
SNOC_008185-RA|size1016601-processed-gene-8.142	dFadd	*P. gracilis*	2,00E-55	181
SNOC_008204-RA|size1016601-processed-gene-9.9	Relish	*A. dorsata*	0.0	563
SNOC_009039-RA|size925038-augustus-gene-5.3	dredd	*F. arisanus*	2,00E-62	211
SNOC_005717-RA|size1620047-processed-gene-3.142	Tak1	*H. laboriosa*	2,00E-44	170
SNOC_005734-RA|size1620047_processed-gene-5.42	Tab	*E. mexicana*	5,00E-171	498
SNOC_003087-RA|size2204710-augustus-gene-13.64	IAP2	*C. floridanus*	7,00E-129	394

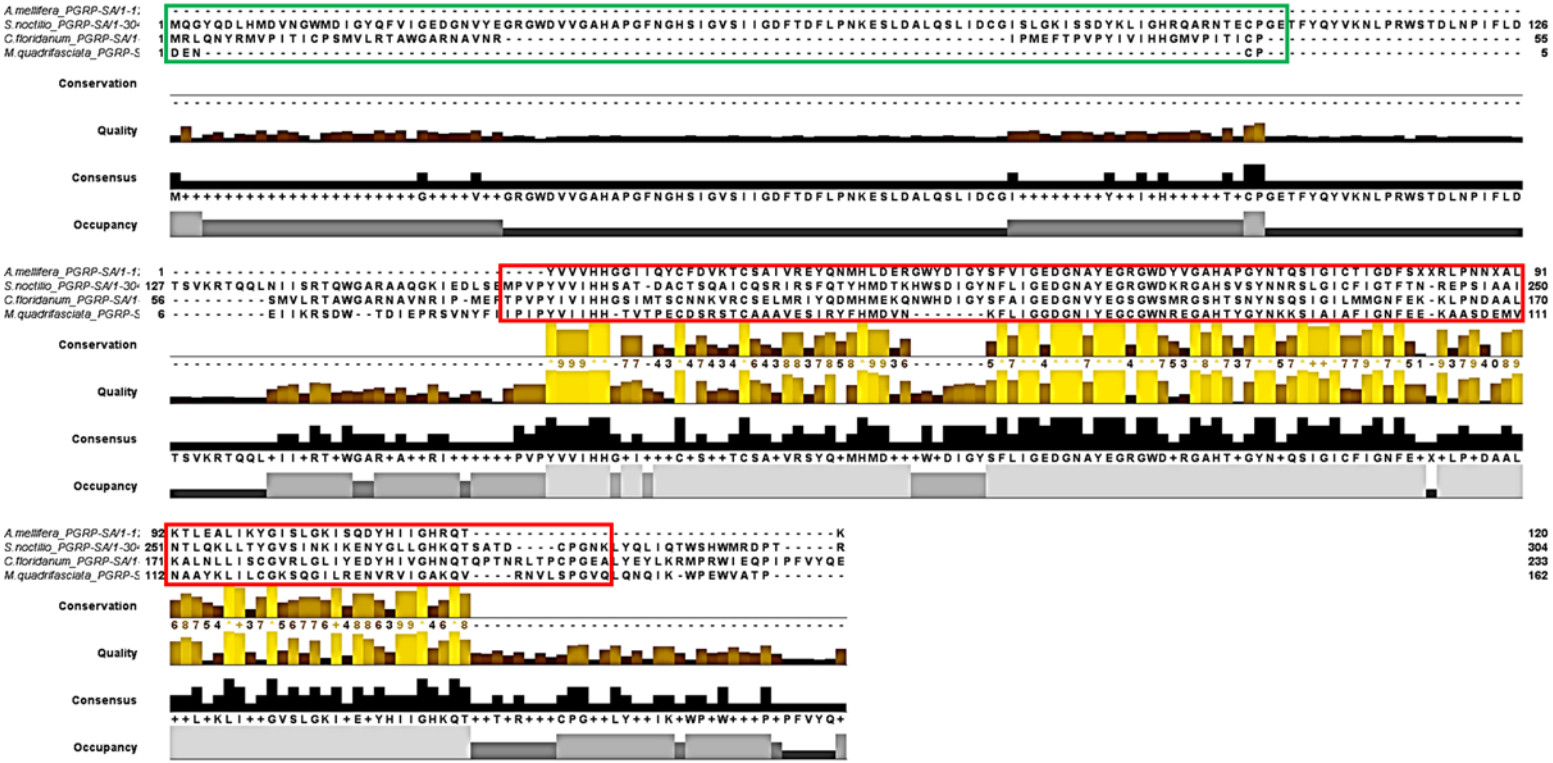
SNOC_002344-RA|size2366004-augustus-gene-0.76	IAP5	*C. floridanus*	0.0	866
SNOC_008224-RA|size1006308-augustus-gene-1.65	mylip	*C. floridanus*	0.0	866
SNOC_001426-RA|size3272368-processed-gene-21.0	Neuron navigator-2	*A. cerana cerana*	0.0	1508
SNOC_012564-RA|size395255-processed-gene-2.165	Ank-1	*A. mellifera*	4,00E-89	288
SNOC_014797-RA|size142934-processed-gene-0.34	Ank-1	*A. mellifera*	6,00E-59	186
SNOC_013989-RA|size242859-augustus-gene-1.16	Caspase-1	*N. vitripennis*	2,00E-173	484
SNOC_008044-RA|size1031301-processed-gene-7.43	DUOX	*A. echinatior*	0.0	2656
SNOC_008045-RA|size1031301-processed-gene-8.116	Dual oxidase maturation factor 1	*T. septentrionalis*	0.0	632
SNOC_013220-RA|size336147-processed-gene-1.126	mekk1	*B. impatiens*	0.0	1641
SNOC_000911-RA|size3354006-exonerate_protein2genome-gene-6.51	p38/MAPkkk14B	*P. gracilis*	1,00E-104	317
SNOC_004935-RA|size1697473-processed-gene-6.22	Ask1	*A. echinatior*	0.0	2472
SNOC_013715-RA|size277051-processed-gene-1.151	MAPk1	*O. biroi*	0.0	681
SNOC_003094-RA|size2204710-augustus-gene-14.3	Lic	*C. floridanus*	0.0	1697
SNOC_007016-RA|size1131650-processed-gene-4.1	Slpr	*A. colombica*	0.0	2353
SNOC_002447-RA|size2366004-processed-gene-14.87	MAPkkk12	*D. novaeangliae*	0.0	1440
SNOC_005316-RA|size1678705-augustus-gene-2.66	Ptr	*H. laboriosa*	0.0	2034
SNOC_003596-RA|size2110464-processed-gene-0.40	defensin	*H. laboriosa*	3,00E-10	64
SNOC_000231-RA|size5241802-processed-gene-25.8	hymenoptaecin	*A. mellifera*	4,00E-39	140
SNOC_015770-RA|size91327-exonerate_protein2genome-gene-0.7	chitotriosidase-1	*C. floridanus*	2,00E-89	309
SNOC_015773-RA|size91327-exonerate_protein2genome-gene-0.3	chitotriosidase-1	*H. laboriosa*	6,00E-62	205
SNOC_000429-RA|size5241802-augustus-gene-46.13	ProPO	*A. mellifera*	0.0	1231
SNOC_006415-RA|size1469172-processed-gene-8.167	Phenyalanine hydroxylase	*P. vicina*	0.0	775
SNOC_009108-RA|size890669-processed-gene-3.0	Quinone oxidoreductase	*A. echinatior*	0.0	572
SNOC_007999-RA|size1031301-processed-gene-4.130	Octopamine receptor	*M. rodundata*	0.0	937
SNOC_012162-RA|size446351-augustus-gene-3.22	Dopamine receptor D1	*D. novaeangliae*	0.0	659
SNOC_002117-RA|size2667483-augustus-gene-3.50	Protein yellow	*C. cinctus*	1,00E-58	206
SNOC_001848-RA|size2854333-processed-gene-1.51	hexamerin	*C. floridanus*	0.0	705
SNOC_016310-RA|size70023-processed-gene-0.3	hemocytin	*D. novaeangliae*	0.0	2771

SNOC_012198-RA|size445744-augustus-gene-3.193	apolipophorin	*A. cerana*	0.0	5162
SNOC_010927-RA|size663648-augustus-gene-0.0	apolipophorin D	*O. biroi*	7,00E-106	347
SNOC_004316-RA|size1849273-processed-gene-0.12	Limulus clotting factor C	*A. echinatior*	1,00E-54	204
SNOC_006608-RA|size1374663-augustus-gene-11.4	Cht5	*T. longispinosus*	0.0	670
SNOC_007633-RA|size1061917-augustus-gene-4.46	putative cht3	*H. laboriosa*	0.0	1149
SNOC_006328-RA|size1493491-exonerate_protein2genome-gene-13.79	prp3	*C. floridanus*	1,00E-30	129
SNOC_000972-RA|size3354006-exonerate_est2genome-gene-14.33	Lys-c1	*A. echinatior*	6,00E-65	199
SNOC_007069-RA|size1131650-processed-gene-8.10	AK	*B. impatiens*	0.0	702
SNOC_006103-RA|size1504730-processed-gene-9.39	ferritin	*T. longispinosus*	1,00E-88	263
SNOC_003856-RA|size2083609-processed-gene-5.81	stubble	*L. niger*	5,00E-55	198
SNOC_006740-RA|size1209655-processed-gene-2.28	neurotrypsin	*D. novaeangliae*	0.0	1853
SNOC_002853-RA|size2226856-augustus-gene-8.13	snake	*O. biroi*	3,00E-98	300
SNOC_013659-RA|size284868-processed-gene-1.243	venom serine protease 34	*O. biroi*	4,00E-91	290
SNOC_009542-RA|size833181-exonerate_protein2genome-gene-1.62	serine protease 48	*A. cerana*	1,00E-37	145
SNOC_012665-RA|size386173-augustus-gene-1.39	serpin B8	*D. novaeangliae*	2,00E-10	64
SNOC_017955-RA|size31723-processed-gene-0.3	Serpin 10	*L. niger*	2,00E-95	302
SNOC_014061-RA|size236911-processed-gene-0.71	Serpin I2	*H. saltator*	1,00E-52	182
SNOC_005495-RA|size1678705-augustus-gene-15.125	Hsp90	*P. puparum*	0.0	1202
SNOC_013373-RA|size323794-processed-gene-2.61	Hsp90	*P. puparum*	0.0	1317
SNOC_007524-RA|size1067625-augustus-gene-7.20	Hsp60	P. puparum	0.0	1020
SNOC_013304-RA|size332486-exonerate_protein2genome-gene-2.60	Hsp70	*T. chilonis*	0.0	1177
SNOC_005147-RA|size1690910-processed-gene-5.86	Hsp70	*E. mexicana*	0.0	755
SNOC_013373-RA|size323794-processed-gene-2.61	Hsp83	*N. vitripennis*	0.0	1326
SNOC_005617-RA|size1648946-augustus-gene-11.140	Hsc3	*A. echinatior*	0.0	1241
SNOC_004730-RA|size1755071-processed-gene-7.42	Hsc5	*L. niger*	0.0	1247
SNOC_001611-RA|size2909140-processed-gene-4.21	Hsc70	*P. vicina*	0.0	1229
SNOC_012217-RA|size441294-exonerate_protein2genome-gene-0.53	myosin regulatory light chain 2	*A. echinatior*	1,00E-83	249
SNOC_018102-RA|size28649-augustus-gene-0.0	myosin IA	*C. floridanus*	0.0	1805

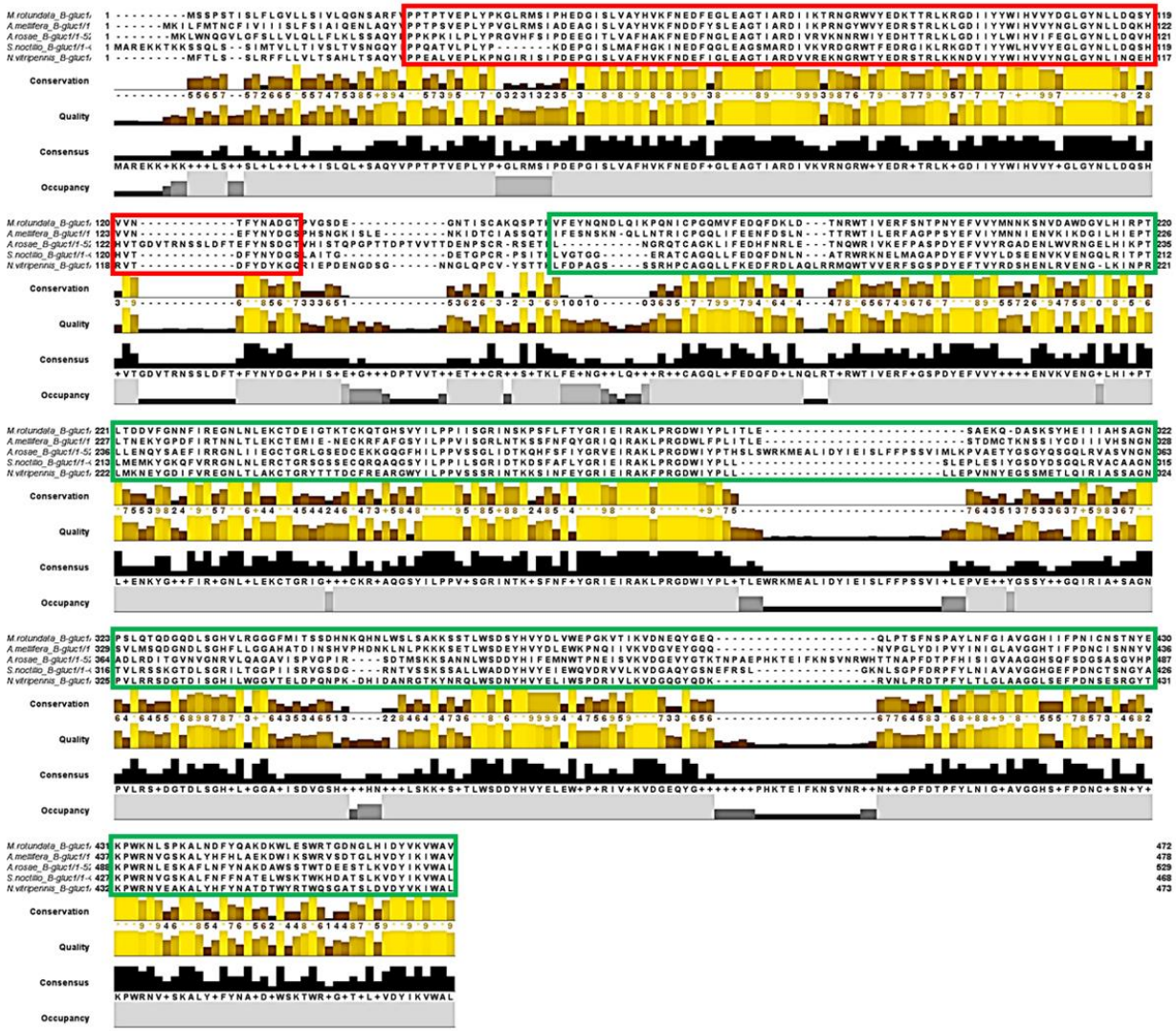
SNOC_009808-RA|size824638-processed-gene-5.106	myosin XV	*H. saltator*	0.0	4088
SNOC_010624-RA|size696552-augustus gene-5.343	Transmembrane protein 179	*L. niger*	6,00E-131	371
SNOC_000492-RA|size3365038-processed-gene-1.1	Nephrin	*H. laboriosa*	0.0	669
SNOC_008109-RA|size1016601-augustus-gene-1.3	Cytoskeleton-associated protein 5	*A. rosae*	0.0	2734
SNOC_013223-RA|size336147-exonerate_protein2genome-gene-2.300	Microtubule-associated protein	*A. mellifera*	4,00E-120	378
SNOC_014234-RA|size214696-processed-gene-1.4	Croquemort	*A. mellifera*	0.0	609
SNOC_010613-RA|size696552-processed-gene-4.155	Larval cuticle protein 8	*C. floridanus*	1,00E-56	176
SNOC_012504-RA|size410872-processed-gene-3.18	Larval cuticle protein a2b	*D. novaeangliae*	4,00E-50	172
SNOC_011877-RA|size524835-processed-gene-4.139	Structural cuticle protein	*A. mellifera*	2,00E-75	224
SNOC_007953-RA|size1052808-processed-gene-9.35	integrin beta	*A. echinatior*	0.0	1424
SNOC_005950-RA|size1562650-processed-gene-11.29	integrin alpha PS-2	*T.zeteki*	0.0	1594
SNOC_007498-RA|size1067625-augustus-gene-5.117	metap2	*A. mellifera*	0.0	804
SNOC_008470-RA|size1001638-processed-gene-3.73	Akirin	*M. quadrifasciata*	1,00E-117	334
SNOC_007413-RA|size1067625-augustus-gene-0.25	sno	*T. cornetzi*	0.0	2249
SNOC_014561-RA|size161172-processed-gene-0.29	apterous	*A. echinatior*	2,00E-166	484
SNOC_010857-RA|size670485-augustus-gene-0.0	carboxypeptidase B	*T.longispinosus*	9,00E-84	281
SNOC_007633-RA|size1061917-augustus-gene-4.46	zinc finger protein 609	*H. saltator*	0.0	1149
SNOC_010871-RA|size670485-processed-gene-2.96	protein scabrous	*T. cornetzi*	0.0	897
SNOC_001546-RA|size3272368-processed-gene-31.66	Dynamin	*C. biroi*	0.0	1655
SNOC_008398-RA|size1001802-processed-gene-7.90	Dorsal-ventral patterning protein sog	*E. Mexicana*	0.0	1311



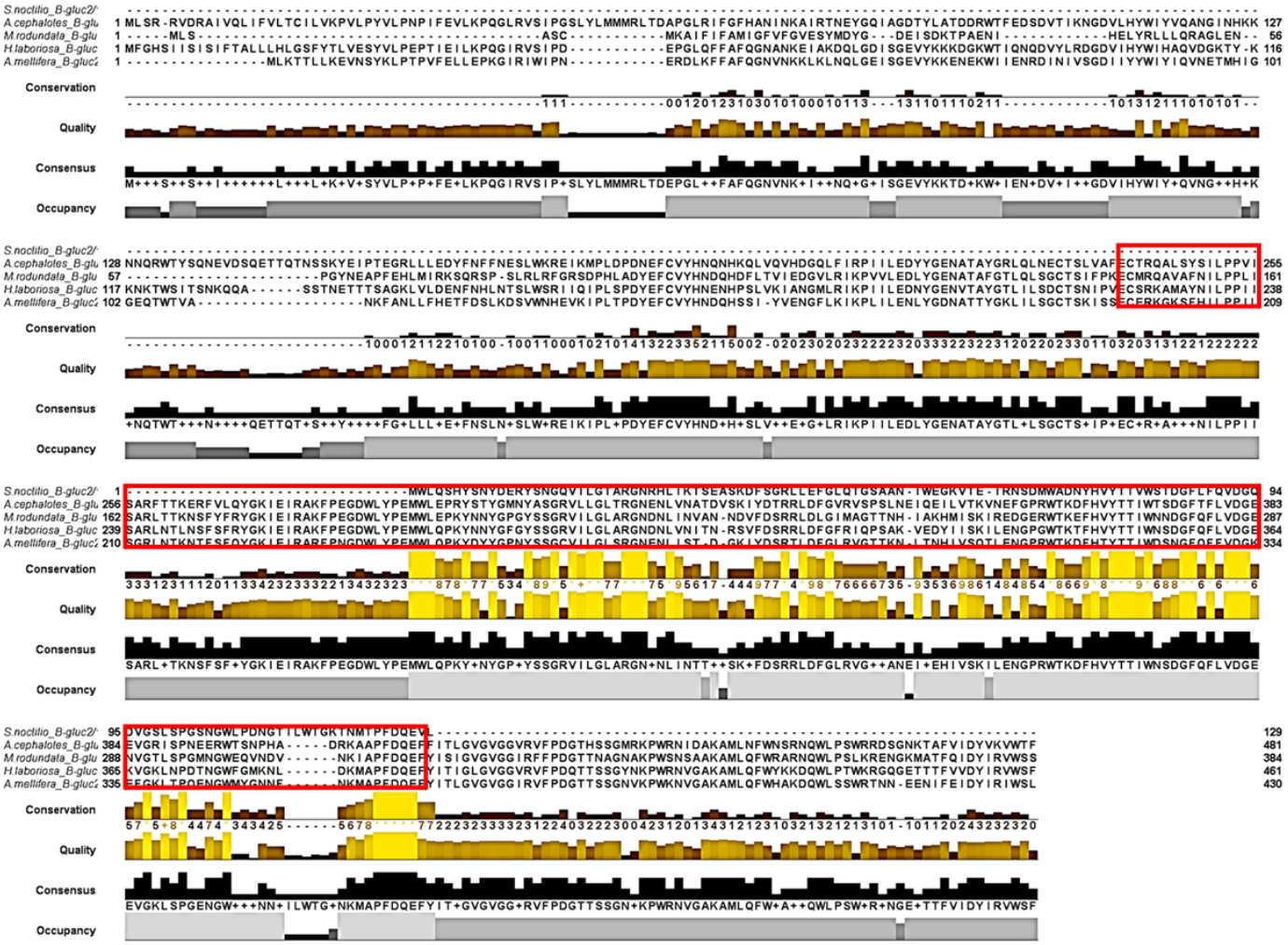
Supplementary Figure S2.1: Multiple sequence alignment for PGRP-Lc. The red boxes indicate the acetylmuramoyl-L-alanine amidase domain and the Highlighted text indicate the Peptidoglycan recognition protein domain.



Supplementary Figure S2.2: Multiple sequence alignment for PGRP-SA. The green box indicates the acetylmuramoyl-L-alanine amidase domain and the red box indicate the Peptidoglycan recognition protein domain.

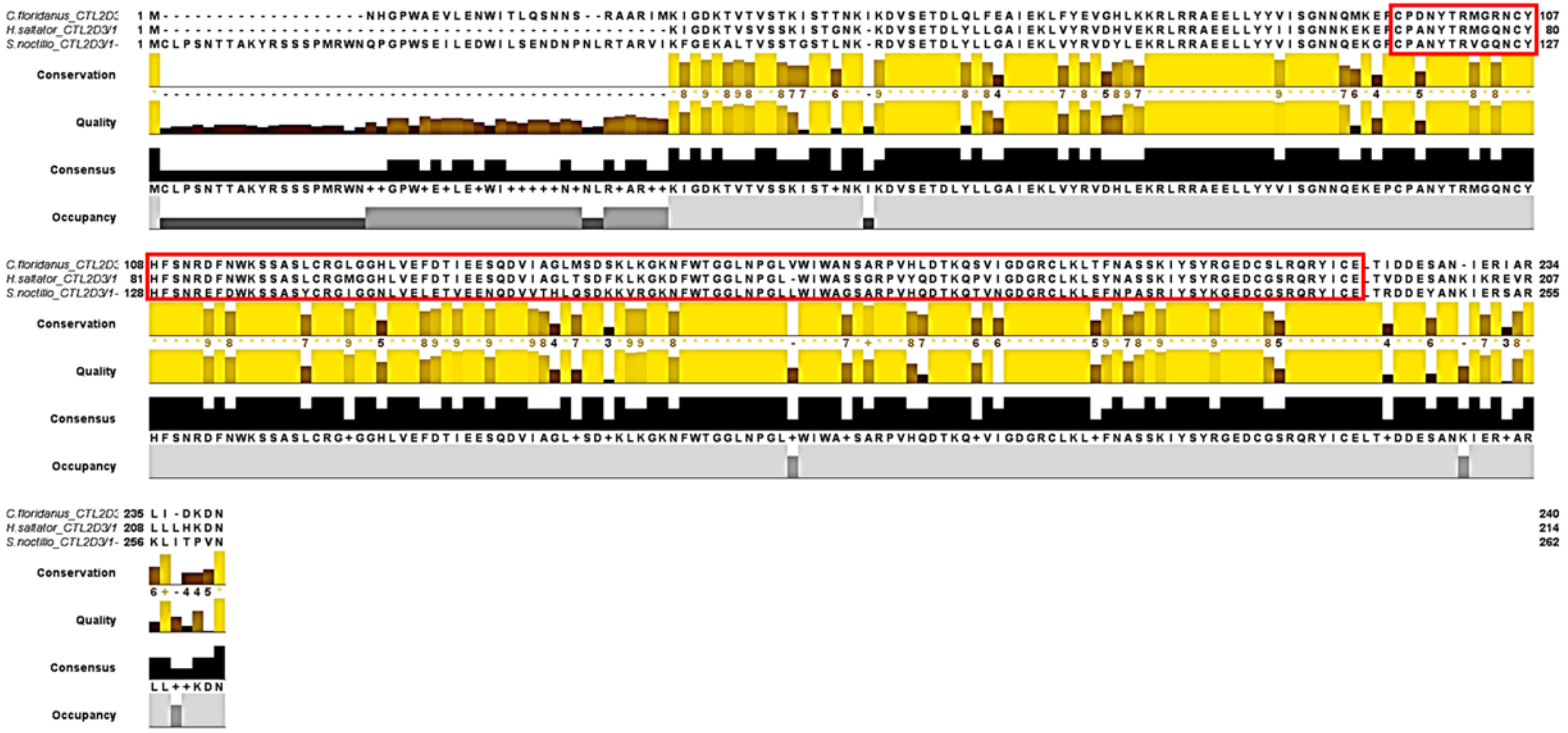


Supplementary Figure S2.3: Multiple sequence alignment for β-gluc1 (Gram-negative bacteria-binding protein 1-2). The red boxes indicate the Carbohydrate binding domain (family 32), and the green boxes indicate the glycosyl hydrolase family 16 domain.

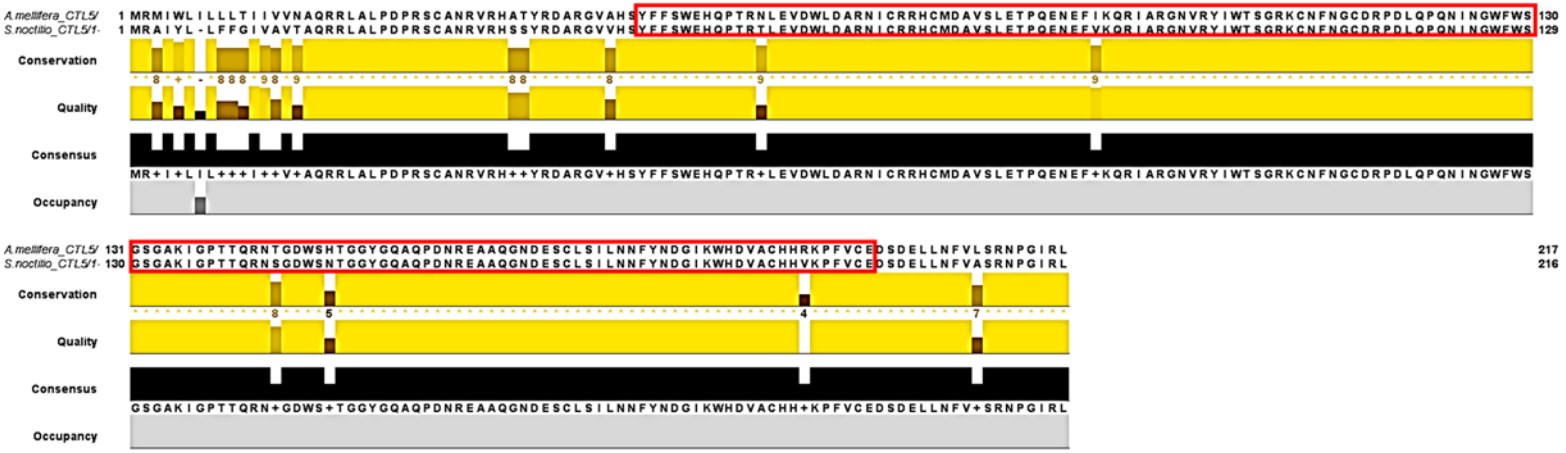


Supplementary Figure S2.4: Multiple sequence alignment for β-gluc2 (Gram-negative bacteria-binding protein 1-1). The red boxes indicate the glycosyl hydrolase family 16 domain.

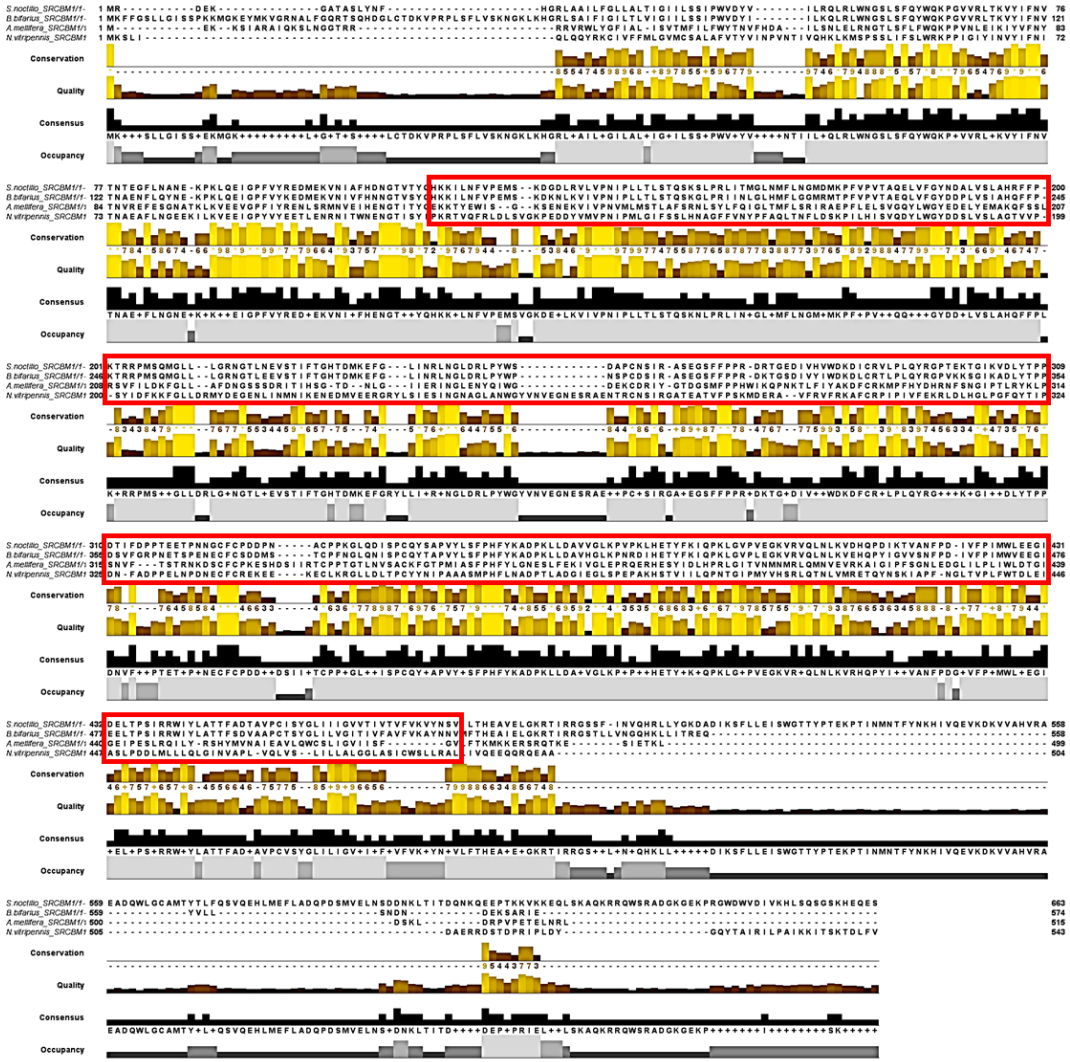
Supplementary Figure S2.5: Multiple sequence alignment for galectin. The red boxes indicate the galactoside binding domain.



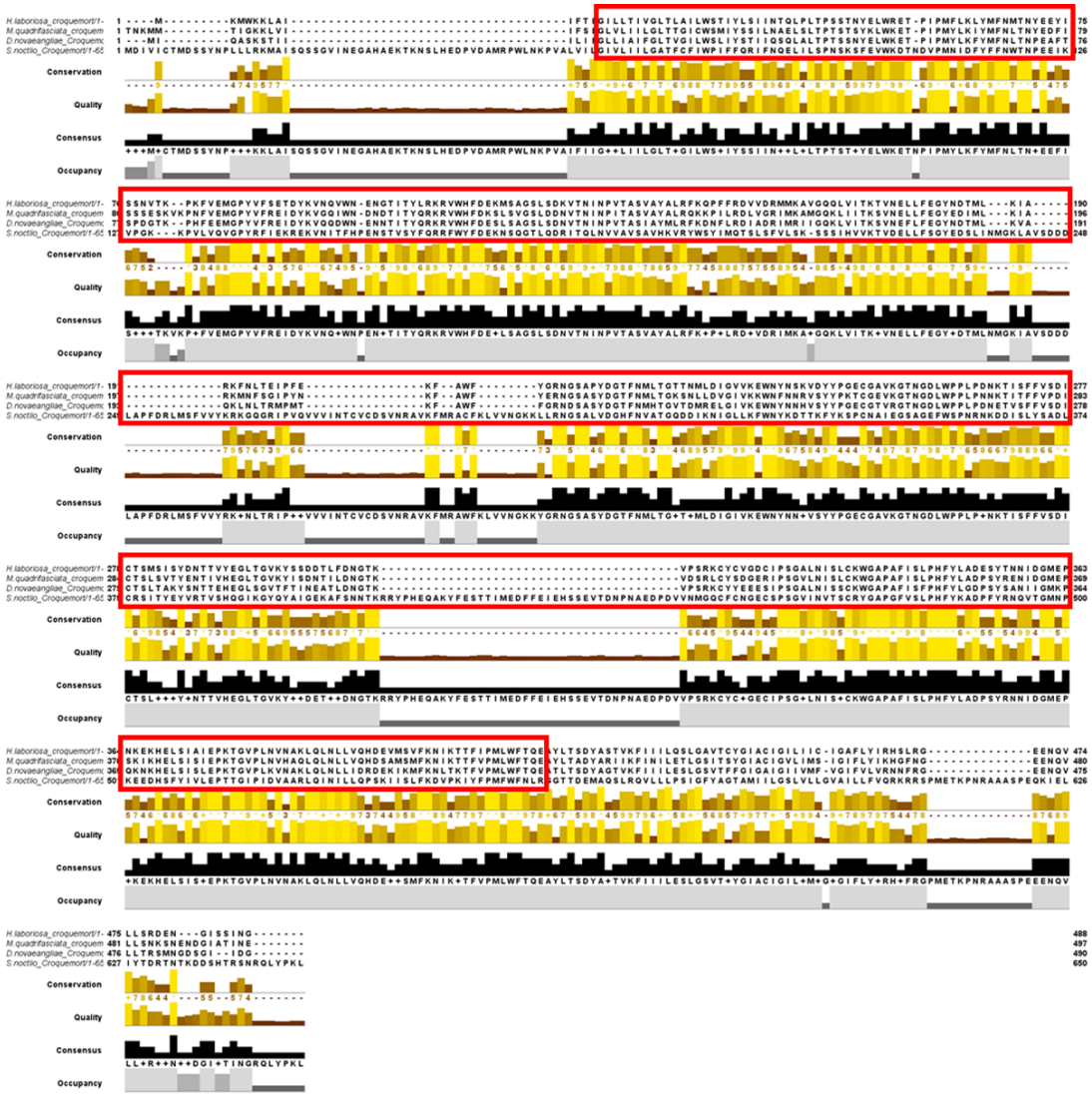
Supplementary Figure S2.6: Multiple sequence alignment for C-type lectin domain family 2 member D3. The red boxes indicate the carbohydrate-recognition domain (CRD).



Supplementary Figure S2.7: Alignment of S. noctilio and A. mellifera C-type lectin 5 precursor. The red box red boxes indicate the carbohydrate-recognition domain (CRD).



Supplementary Figure S2.8: Multiple sequence alignment for scavenger receptor class B member 1. The red boxes indicate the CD36 family domain.



Supplementary Figure S2.9: Multiple sequence alignment for croquemort. The red boxes indicate the CD36 family domain.

Summary

In this study we characterized immune-related genes of *S. noctilio*. We provide the first comparative overview of the *S. noctilio* defence system and describe the putative immunity pathway models of this woodwasp. An increasing number of genome-wide analyses have contributed to the identification of immune-related genes and gene families in various insect species. These studies, and our study presented in this dissertation, show that the core signalling pathways are conserved among insects, including in *S. noctilio*. Furthermore, we characterised and analysed the immune-related genes of *S. noctilio* in response to nematode infection, fungal infection, and physical wounding, in comparison to uninfected controls, to better understand the regulation of these immune-related genes. We compared the RNA expression profiles in *S. noctilio* during these infections and control only at one-time point (72 h). The comparison of control with the three treatments performed was sufficient to show dynamic changes of differentially expressed genes (DEGs) in *S. noctilio*. Future studies will involve gene expression profiling at different time points to improve our understanding of the *Sirex-Deladenus* interactions. A control will be compared with two different nematode strains (more virulent and less virulent) at three-time point of the larval infection stages using RNA-Seq and DGE methods. Functional tests will be included for the candidate genes that shows unique results utilizing CRISPR to perform gain-of-function or loss-of-function analysis. Our findings not only shed more light on the immunogenetics of *S. noctilio* in response to parasite infection, but it will also improve general understanding of this system's host-pathogen interactions.