Instituto de Biociências

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

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UNIVERSIDADE ESTADUAL PAULISTA

"Julio de Mesquita Filho"

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

IMPACTO DE INSETICIDAS SINTÉTICOS (PIRIPROXIFEN E SULFOXAFLOR) NA REPRODUÇÃO DO INSETO NÃO ALVO *Ceraeochrysa claveri* (NAVÁS, 1911) (NEUROPTERA: CHRYSOPIDAE): ESTUDO MOLECULAR E MORFOLÓGICO

Jefferson Fogaça Tomacheski

Profa. Dra. Daniela Carvalho dos Santos

Dr. Rafael Takahiro Nakajima

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Tese apresentada ao Instituto de Biociências, Campus de Botucatu, UNESP, para obtenção do título de Doutor no Programa de Pós-Graduação em Biologia Geral e Aplicada, Área de concentração em *Biologia Celular Estrutural e Funcional.*

Orientadora: *Profa. Dra. Daniela Carvalho dos Santos*
Coorientador: *Dr. Rafael Takahiro Nakajima*

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Impacto potencial desta pesquisa

Impacto científico

O conhecimento dos efeitos subletais dos inseticidas sintéticos (piriproxifen e sulfoxaflor) na reprodução do inseto não-alvo *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae) é de extrema importância para a garantia da manutenção deste inimigo natural de pragas em ambientes onde se faz uso de controle químico de pragas , pois, a caracterização das respostas celulares dos testículos e ovários dos insetos benéficos expostos as diferentes dosagens dos inseticidas fornece informações valiosas sobre possíveis alterações no processo de espermatogênese e ovogênese na idade adulta dos insetos que foram expostos ainda na fase larval, que é a fase predadora em campo. Desta forma, os resultados gerados contribuem para o manejo integrado de pragas (MIP), favorecendo a manutenção de populações de insetos não-alvos benéficos, como *C. claveri* e contribuindo para uma melhor seletividade dos inseticidas utilizados.

Impacto econômico e social:

Com o aumento da produção de culturas cultivadas, os insetos considerados pragas também aumentam sua população. Com isto, o uso de agrotóxicos (inseticidas) para o controle destes insetos se faz necessário. Através do uso do manejo integrado de pragas (MIP), que também usa insetos predadores de pragas como controle natural das pragas em conjunto com o uso de inseticidas, este estudo traz resultados importantes sobre o impacto de inseticidas não somente sobre os insetos pragas, mas também sobre os insetos benéficos que coexistem no mesmo ambiente, o que pode interferir no controle natural das pragas. Desta forma, conhecer os efeitos dos inseticidas sobre insetos benéficos pode contribuir para melhorar estratégias em campo no sentido de diminuir o uso de inseticidas e assim promover uma agricultura mais sustentável, com menor impacto de toxicidade ao meio ambiente e ao homem, tanto diretamente (melhor qualidade de vida aos agricultores, quanto indiretamente (alimentação com produtos com menor exposição à agrotóxicos).

Potential impact of this research

Scientific impact:
Understanding the sublethal effects of synthetic insecticides – specifically pyriproxyfen and sulfoxaflor – on the reproduction of the non-target insect *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae) is crucial for ensuring the preservation of this natural pest control agent in environments where chemical pest management is employed. By characterizing how these insecticides affect the cellular responses in the testes and ovaries of beneficial insects exposed to different doses, this research provides valuable insights into potential changes in spermatogenesis and oogenesis during adulthood by insects that have been exposed during their larval, which is the predatory stage in the field. The findings contribute to integrated pest management (IPM) strategies by supporting the maintenance of beneficial non-target insect populations like *C. claveri* and helping to improve the selectivity of the insecticides used.

Economic and social impact:
As crop production increases, so does the number of insects considered pests, making the use of pesticides necessary for control. However, through IPM – which combines the use of natural predators with chemical control – this study offers important insights into how insecticides impact not only pest insects but also beneficial insects that coexist in the same environment. Understanding these effects can help refine field strategies to reduce insecticide use, promoting more sustainable agriculture. This approach can lead to lower environmental toxicity and benefits for human health – improving farmers' quality of life and providing consumers with food products that have less pesticide exposure.

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE JEFFERSON FOGAÇA TOMACHESKI, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA GERAL E APLICADA, DO INSTITUTO DE BIOCIÊNCIAS - CÂMPUS DE BOTUCATU.

Aos 31 dias do mês de julho do ano de 2025, às 8h30min, por meio de Videoconferência, realizou-se a defesa de TESE DE DOUTORADO de JEFFERSON FOGAÇA TOMACHESKI, intitulada **Impacto de inseticidas sintéticos (Piriproxifen e Sulfoxaflor) na reprodução do inseto não alvo *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae): estudo molecular e morfológico**, sob orientação da Profa. Dra. Daniela Carvalho dos Santos. A Comissão Examinadora foi constituída pelos seguintes membros: Profa. Dra. DANIELA CARVALHO DOS SANTOS (Orientador(a) - Participação Virtual) do(a) Departamento de Biologia Estrutural e Funcional / Instituto de Biociências de Botucatu Unesp, Profa. Dra. SHEILA MICHELE LEVY (Participação Virtual) do(a) Departamento de Histologia / Universidade Estadual de Londrina - UEL, Profª. Drª. ELAINE CRISTINA MATHIAS DA SILVA ZACARIN (Participação Virtual) do(a) Departamento de Biologia / Universidade Federal de São Carlos (UFSCar) , Prof. Associado RICARDO DE OLIVEIRA ORSI (Participação Virtual) do(a) Departamento de Produção Animal e Medicina Veterinária / Faculdade de Medicina Veterinária e Zootecnia, Unesp, Câmpus de Botucatu, Prof. Dr. ELTON LUIZ SCUDELER (Participação Virtual) do(a) Departamento de Biologia Geral e Aplicada / Instituto de Biociências de Rio Claro - SP - UNESP. Após a exposição pelo doutorando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final: APROVADO. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

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Busquem conhecimento.
ET Bilú

SUMMARY

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RESUMO

O manejo de pragas de insetos geralmente depende da otimização e combinação do uso de inseticidas seletivos e controle biológico por inimigos naturais. Inseticidas, como piriproxifen e sulfoxaflor, apresentam vários efeitos sobre pragas (insetos-alvo) e predadores naturais (insetos não-alvo), buscando exercer efeitos de baixa toxicidade sobre artrópodes benéficos. A tolerância a inseticidas é uma característica desejável para insetos não-alvo, uma vez que a conservação e manutenção de insetos benéficos podem controlar o crescimento populacional de pragas e/ou o ressurgimento de pragas secundárias. Assim, o conhecimento dos efeitos subletais de inseticidas sobre pragas de insetos e seus inimigos naturais, bem como a tolerância potencial de inimigos naturais a inseticidas, pode melhorar as estratégias de manejo integrado de pragas, contribuindo para uma agricultura mais sustentável. Este estudo teve como objetivo analisar o efeito subletal de dois inseticidas com modos de ação diferentes: verificar uma possível alteração sobre a expressão gênica dos testículos de adultos de *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae) expostos ao inseticida piriproxifen durante a fase larval, e avaliar a resposta celular dos ovários e testículos de adultos de *C. claveri* expostos ao inseticida sulfoxaflor, também durante a fase larval. Dados oriundos de RNA-seq de machos expostos à duas doses do inseticida piriproxifen (50 mg i.a. L^{-1}e 100 mg i.a. L^{-1}) durante a fase larval foram analisados para identificar genes diferencialmente expressos relacionados. Por meio de dados de RNA-seq, as leituras foram montadas *de novo* para construir o transcriptoma do testículo, os genes foram anotados por homologia de ORFs e uma matriz de contagem foi construída para obter os genes diferencialmente expressos (GDEs). Quatro DEGs foram selecionados para serem validados por RT-qPCR: BPHL, Large2, MLX e Talin-1. Larvas de primeiro ínstar de *C. claveri* foram alimentadas com ovos de *Diatraea saccharalis* (Lepidoptera: Crambidae) contendo diferentes concentrações de sulfoxaflor (15,02 mg i.a. L^{-1}, 45,05 mg i.a. L^{-1} e 75,10 mg i.a. L^{-1}) por 10 dias. O ciclo de vida e a sobrevivência foram analisados após os insetos emergirem para adultos. Testículos e ovários de adultos de 10 dias de idade de *C. claveri* foram coletados para análises histológicas sob microscopia óptica e eletrônica de transmissão. Nenhuma alteração nos dias de desenvolvimento e sobrevivência foi encontrada quando os insetos tratados com sulfoxaflor foram comparados ao grupo controle. Os testículos e ovários exibiram espaçamentos entre as células, sem danos visíveis às células da linhagem espermatogênica e oogênica. Este trabalho demonstrou que o inseticida piriproxifen modificou a expressão gênica do testículo de *C. claveri*, e que o inseticida sulfoxaflor alterou a morfologia dos órgãos reprodutivos deste inseto, porém sem danos às células da linhagem espermatogênica e oogênica, indicando uma possível tolerância e compatibilidade desses inseticidas associados ao manejo integrado de pragas (MIP).

PALAVRAS-CHAVE: bicho-lixeiro; inseticida; efeitos subletais; RNA-seq; reprodução.

ABSTRACT

Insect pest management often involves optimizing and combining the use of selective insecticides with biological control methods involving natural enemies. Insecticides like pyriproxyfen and sulfoxaflor have various effects on pests (target insects) and non-target insects such as natural predators, with the goal of minimizing toxicity to beneficial arthropods. Tolerance to insecticides in non-target insects is a desirable trait because conserving and maintaining beneficial insects can help control pest populations and prevent the resurgence of secondary pests. Therefore, understanding the sublethal effects of insecticides on both pests and their natural enemies, as well as the potential for natural enemies to tolerate these chemicals, can enhance integrated pest management (IPM) strategies and promote more sustainable agriculture. This study aimed to analyze the late sublethal effects of pyriproxyfen on the testes of adult *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae) exposed during the larval stage, and to evaluate the cellular responses of the ovaries and testes of adults exposed to sulfoxaflor during the same stage. We analyzed RNA-seq data from males exposed to pyriproxyfen during the larval stage (Garcia, 2021) to identify differentially expressed genes (DEGs). Using this data, we assembled the testis transcriptome de novo, annotated genes based on ORF homology, and created a counting matrix to identify DEGs. Four genes were selected for validation via RT-qPCR: BPHL, Large2, MLX, and Talin-1. First-instar *C. claveri* larvae were fed *Diatraea saccharalis* (Lepidoptera: Crambidae) eggs containing different concentrations of sulfoxaflor for 10 days. We then analyzed their life cycle and survival after they emerged as adults. Additionally, tests and ovaries from 10-day-old adults were collected for histological analysis using light and transmission electron microscopy. No significant differences in developmental time or survival rates were observed between the sulfoxaflor-treated insects and the control group. Although spacing between cells was noted in the reproductive organs, no visible damage to spermatogenesis or oogenesis was detected. Overall, this work showed that pyriproxyfen and sulfoxaflor can cause some damage to the reproductive organs of *C. claveri* and induce changes in gene expression (notably with pyriproxyfen). However, these insecticides did not disrupt the processes of spermatogenesis and oogenesis, suggesting a possible tolerance and compatibility with IPM strategies aimed at sustainable pest control.

KEYWORDS: green lacewing; insecticide; sublethal effects; RNA-seq; reproduction.

1. INTRODUCTION

The search for greater productivity in agroecosystems increases the use of new molecules/insecticides aimed at controlling considered pests insects, which raises concerns about the impact of these products on the environment, food production and other species who are at the same environment, including arthropods that are predators of this pests.

The use of synthetic or natural insecticides is necessary, since insect pests are a problem for agribusiness and the economy, directly damaging crops through their feeding habits, or indirectly through contamination by spreading viruses, fungi, and other diseases. Controlling these organisms is undeniable and increasingly challenging, since several species can develop resistance to insecticides (Sparks *et al.*, 2013; Sparks and Nauen, 2015; Chen *et al.*, 2016; Ma *et al.*, 2019a; Ma *et al.*, 2019b, Sohail *et al.*, 2019, Watson *et al.*, 2021).

Insects that are not targeted by insecticides coexist in the field with insects that are targeted to be controlled, being exposed to the same effects of insecticide action. Several studies show the lethal effects caused by insecticides on non-target insects, but few demonstrate the sublethal effects where the insect survives in the field, but its biological parameters are affected at various stages of their life cycle, such as growth and development of the larvae and fecundity and fertility in adults (Desneux *et al.*, 2007; Fogel *et al.*, 2016; Ono *et al.*, 2017; He *et al.*, 2018; Sohail *et al.*, 2019; Garcia *et al.*, 2019; Gastelbondo-Pastrana *et al.*, 2019, 2025; Garcia *et al.*, 2020; Jindra and Bittova, 2020; Scudeler *et al.*, 2022, 2024).

In 2022, Brazil was the world's largest consumer of pesticides (Figure 1.1), with a total of 801 kilotons (kt) applied for agricultural purposes (FAO, 2024). Among these pesticides, insecticides ranked as the third most sold in Brazil (IBAMA, 2024). Insecticides differ in their modes of action, which are based on the best available evidence regarding their target sites. This information can be found on the IRAC – Insecticide Resistance Action Committee website (http://www.irac-online.org). Due to their mode of action, insecticides targeting nerve and muscle functions are the most widely sold, followed by growth regulators (Figure 1.2) (Sparks and Nauen, 2015).

Among these insecticides, there are pyriproxyfen and sulfoxaflor. Pyriproxyfen is a synthetic compound that acts as a mimetic of the juvenile hormone (IRAC, 2025), which

leads to an imbalance of this hormone in the insect, causing damage to embryogenesis, metamorphosis and sterility (Medina *et al.*, 2003; Boina *et al.*, 2010; Fernandez *et al.*, 2012). Sulfoxaflor belongs to the sulfoxamine group (IRAC, 2025), uses the same site of action as neonicotinoid insecticides, which act as agonists of nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects (Chen *et al.*, 2016; Zhen *et al.*, 2018; Sâmia *et al.*, 2019; Ma *et al.*, 2019a; Ma *et al.*, 2019b).

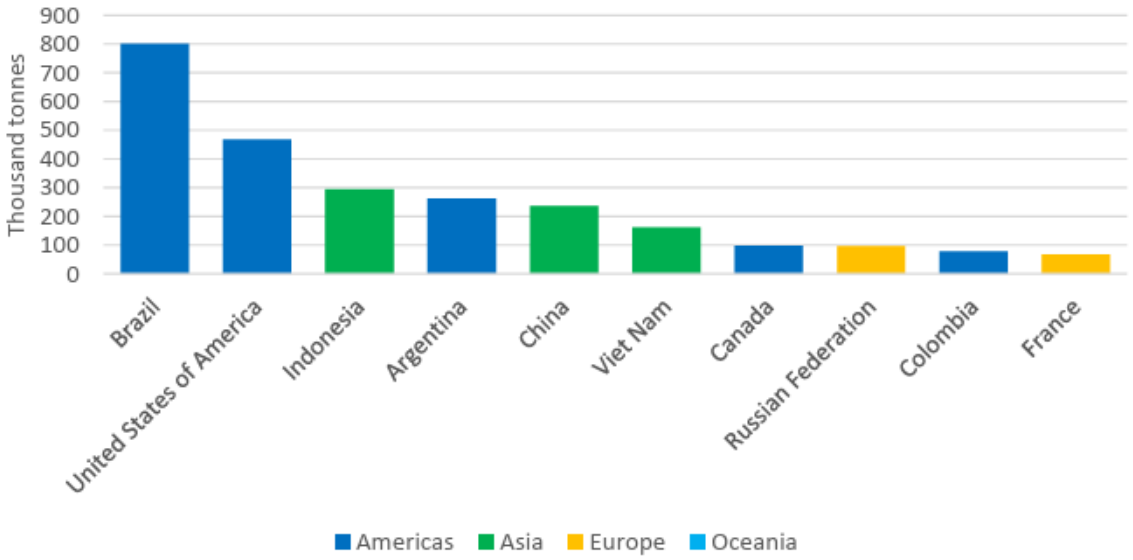

Figure 1.1 – Top countries of pesticides use in 2022. Source: FAO, 2024.

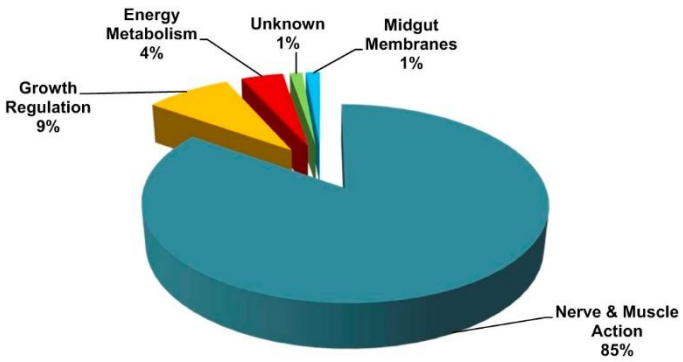

Figure 1.2 – Insecticide sales by mode of action. Source: Sparks and Nauen, 2015.

The compability of lacewings and other natural predators after pyriproxyfen exposure in the field has been reported in the literature (Rugno *et al.*, 2015; 2016), but

some studies have shown that this exposure could be harmful, showing ovicidal and larvicidal effects, reducing female fecundity and egg viability, and pyriproxyfen compatibility with natural predators have been questioned (Chen and Liu, 2002; Cloyd and Dickinson, 2006; Ginjupalli and Baldwin, 2013; Fogel *et al.*, 2016; Ono *et al.*, 2017; He *et al.*, 2018; Sohail *et al.*, 2019). Most of these studies only report the effects on the lifespan and some reproductive aspects of insects such as fecundity and fertility, while morphological and/or ultrastructural aspects of the reproductive organs are not considered.

Sulfoxaflor shows satisfactory results in bioassays about its insecticidal power (Babcock *et al.*, 2011), differing from neonicotinoids due to its different aspect of interactions with nicotinic receptors and chemical structures, increasing its efficiency (Sparks *et al.*, 2013). An insect intoxicated with sulfoxaflor shows changes in the biology and behavior, such as reduced lifespan, development rate, fertility, fecundity and oviposition, tremors, antennal undulations and extension or curling of the legs, paralysis and death (Sparks *et al.*, 2013; Zhen *et al.*, 2018; Dai *et al.*, 2021). In addition to being efficient against insects that have already acquired resistance to insecticides and not being harmful to mammals (Watson *et al.*, 2011; Zhu *et al.*, 2011; Sparks *et al.*, 2013; Lu *et al.*, 2020), sulfoxaflor has been shown to be less aggressive to non-target insects when compared to other insecticides (Tran *et al.*, 2016; Siviter *et al.*, 2019; Tamburini *et al.*, 2021).

Insecticide tolerance is a desirable trait for natural enemies, since the conservation and maintenance of beneficial insects can prevent pest population resistance in surviving insects in the field after chemical control, and/or the resurgence of secondary pests. Non-target insects when exposed to insecticides and do not present major damage to their population (Ghosh *et al.*, 2013; Tran *et al.*, 2016), it is suspected that these organisms may have hyperregulated detoxifying mechanisms against insecticides (Guedes *et al.*, 2016), suggesting a possible tolerance and/or resistance through hormesis (Guedes and Cutler, 2013).

Molecular techniques have indicated resistance mechanisms of insects against insecticides (Dawkar *et al.*, 2013) and with next-generation sequencing (NGS) technology being more and more used for this purpose, it has become a valuable tool to obtain large amounts of genetic information from non-model organisms. Transcriptome analysis is a powerful tool for research, allowing the development of pest control strategies based on the characterization and exploration of genes related to several

important factors, such as insecticide action and development of resistance mechanisms (Oppenheim *et al.*, 2015; Liu *et al.*, 2016).

The use of bioinformatics tools has been widely used in the context of gene expression based on read counts (Anders and Huber, 2010; Anders *et al.*, 2013). These tools associated with gene ontology (GO) analyses allow the understanding of the correlation between altered genes and their respective functions in a global and effective way, enabling a better understanding of the variations present between different types of treatments. In this context, evaluating the possible sublethal effects of the use of insecticides on non-target insects has contributed greatly to knowledge about the possible negative effects on the natural control of pests in an agroecosystem, which can generate harmful effects for beneficial insects, compromising their biological and physiological functions and their maintenance in the environment (Desneux *et al.*, 2007; Cloyd, 2012; Scudeler *et al.*, 2019).

Lacewings (also called green lacewings) is the popular name for insects belonging to the Chrysopidae family, are predators found in many crops of economic interest that play an important role in biological control because of their voracious predator habit during larval phase, being used in integrated pest control (IPM) programs (Pappas *et al.*, 2011). Approximately 7.537 species of chrysopids are described, standing out as the second largest family in terms of number and diversity of species within the Neuroptera order (Oswald, 2025). They are found preying other insects in several cultivated plants, such as pepper, eggplant pea, potato, cotton, cucumber, lettuce and apple orchards (Pappas *et al.*, 2011).

Lacewings are holometabolous insects where larvae differ completely from adults not only in appearance, but also in habits, habitats and niches, which allows these insects a wide exploration capacity and variety of prey. They have three larval instars, and the time (days) within each instar is influenced by temperature, humidity and feeding (Freitas and Penny, 2001; Almeida *et al.*, 2009; Bezerra *et al.*, 2009).

Ceraeochrysa claveri Navás, 1911 (Neuroptera: Chrysopidae) larvae are predators during all the larval phase (from first to third instar). During the third instar they are more voracious and effective against various pests, such as aphids, phytophagous mites, scale insects, leafhoppers, whiteflies, psyllids, thrips, and eggs and larvae of insects of the orders Lepidoptera, Coleoptera, and Diptera (Albuquerque *et al.*, 2012; Pappas *et al.*, 2011). Adults are generally green in color, with filiform antennae and hyaline wings (Machado *et al.*, 2024; Bezerra *et al.*, 2009). Their feeding habits are varied, and they can

feed on pollen, sugary plant exudates, honeydew, and nectar; others can be predators (Albuquerque *et al.*, 2012). In Brazil, *Chrysoperla externa* Hagen, 1861 and *Ceraeochrysa cubana* Hagen, 1861 stand out as two species widely studied for integrated pest control (Bezerra *et al.*, 2009).

Few studies show the histopathological effects of insecticides on *C. claveri* with an emphasis on sublethal doses (Scudeler *et al.*, 2013; 2022, Garcia *et al.*, 2019; 2020). However, evaluating the possible impact of different pesticides (e.g., pyriproxyfen and sulfoxaflor) on predatory species is of great importance for maintaining beneficial species in the environment, contributing to improving integrated pest control, minimizing the negative effect on populations of non-target species.

2. OBJECTIVE

Considering the economic importance of *Ceraeochrysa claveri* in integrated pest management, this study aims to answer the following question: Can the insecticides pyriproxyfen and sulfoxaflor affect the reproductive organs of adult *C. claveri*, when they fed on contaminated food during the larval stage?

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Specific objectives:

• Analyze the late effects on testis of adult *C. claveri* exposed to the insecticide pyriproxyfen during the larval stage using RNA-seq data.

• Investigate under laboratory conditions the sublethal effects on reproductive organs of adult *C. claveri* after sulfoxaflor intake during the larval stage, focusing on the life cycle of the species and the morphology of the testis and ovary.

3. CHAPTERS

This work is organized into two chapters:

Chapter 1 – Intake of pyriproxyfen through contaminated food by the predator *Ceraeochrysa claveri* Navás, 1911 (Neuroptera: Chrysopidae): Evaluation of long-term effects on testes via transcriptome analysis

Chapter 2 – Histomorphological analyses of the testes and ovaries in adult *Ceraeochrysa claveri* Navás, 1911 (Neuroptera: Chrysopidae) following larval exposure to sulfoxaflor

4. CHAPTER 1

Intake of pyriproxyfen through contaminated food by the predator *Ceraeochrysa claveri* Navás, 1911 (Neuroptera: Chrysopidae): Evaluation of long-term effects on testes via transcriptome analysis

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4.1 ABSTRACT

Understanding the sublethal effects of insecticides on non-target insects is essential for integrated pest management (IPM). This study aimed to evaluate the differentially expressed genes (DEGs) in the testes of *Ceraeochrysa claveri* adults exposed to pyriproxyfen during the larval stage. Larvae (0–12 h) were fed *Diatraea saccharalis* eggs treated with pyriproxyfen (50 and 100 mg a.i. L^{-1}) for 10 days. After this exposure, the larvae were fed untreated eggs until pupation. The testes from the adults were extracted for RNA extraction, library construction, and sequencing. The reads were de novo assembled, and the genes annotated based on their ORF homology. A total of 46 DEGs were identified for the 50 mg a.i. L^{-1} vs. control, 47 DEGs for the 100 mg a.i. L^{-1} vs. control, and 64 DEGs for 50 mg vs. 100 mg a.i. L^{-1} treatments. To validate the DEGs through RT-qPCR, the genes BPHL, Large2, MLX, and Talin-1 were selected. The results indicate that the exposure of *C. claveri* larvae to pyriproxyfen could alter the gene expression and lead to delayed effects in adults. This study provided a novel approach for assessing the sublethal effects of pyriproxyfen *C. claveri* and contributed valuable information to enhance IPM strategies.

KEYWORDS: green lacewing, insecticide, sublethal effects, RNA-seq, testis.

4.2. INTRODUCTION

Neuropterans (Neuroptera Linnaeus, 1758) are cosmopolitan insects, with 6697 species described across sixteen extant families. Chrysopidae is the second-largest family within Neuroptera, comprising approximately 1486 species (Vasilikopoulos *et al.*, 2020; Oswald, 2025). These insects play a crucial role in biological control as part of integrated pest management (IPM) programs due to their polyphagous feeding habits; they prey on aphids, phytophagous mites, leafhoppers, whiteflies, psyllids, and thrips, as well as the eggs and larvae of Lepidoptera, Coleoptera, and Diptera (Freitas and Penny, 2001; Pappas *et al.*, 2011; Albuquerque *et al.*, 2012; Machado *et al.*, 2024).

However, non-target insects coexist in the same environment as pests, and both groups are affected by the mode of action of insecticides. While various studies have documented the lethal effects of insecticides on non-target insects, fewer have investigated the sublethal effects on beneficial insects that survive exposure (Fogel *et al.*, 2016; Ono *et al.*, 2017; Sohail *et al.*, 2019). Among the available synthetic insecticides, pyriproxyfen is a juvenile hormone analog (Jindra and Bittova, 2020). It interferes with the natural hormone system in insects, affecting embryonic development, metamorphosis, and causing sterility (Boina *et al.*, 2010; IRAC, 2025). Molecular studies are used to analyze the effects of pyriproxyfen on target insects, helping evaluate its effectiveness in reducing target insects (Xu *et al.*, 2015; Duan *et al.*, 2016; Kancharlapalli and Brelsfoard, 2024). Conversely, molecular approaches are also employed to assess the extent of pyriproxyfen's impact on non-target insects (Qian *et al.*, 2020; Zhao *et al.*, 2020; Guo *et al.*, 2021; Luo *et al.*, 2021; Wang *et al.*, 2023; Litsey and Fine, 2024). In chrysopids, also known as lacewings, pyriproxyfen has been shown to cause cellular damage in the testes, midgut, and fat body (Garcia *et al.*, 2020; Scudeler *et al.*, 2022). Despite these facts, these insects exhibit potential tolerances or resistances to several insecticides (Venkatesan *et al.*, 2009; Pathan *et al.*, 2010; Fátima *et al.*, 2013; Mansoor *et al.*, 2013; Mansoor and Shad, 2020; Pathak *et al.*, 2022), allowing them to reproduce and sustain their populations in the field, which is advantageous for IPM strategies. Regardless of this, little is known about their genetic responses to such exposures.

As a molecular tool, transcriptome analysis (RNA-seq) enables the construction of a comprehensive transcript database, which is essential for understanding the diversity of mRNA (Graveley *et al.*, 2011). Several studies that have focused on non-model insects have been conducted to obtain unbiased transcript profiles, allowing for a holistic analysis of insect physiology (Oppenheim *et al.*, 2015). However, there have been few studies that

have utilized transcriptomic analysis to address unresolved issues in chrysopid taxonomy (Wang *et al.*, 2019) or to apply findings to agricultural contexts. For instance, *Chrysoperla sinica* (Tjeder) has been used to elucidate the molecular mechanisms of flight (Liu *et al.*, 2021) and to identify candidates for olfactory genes through antennal transcriptome analysis (Li *et al.*, 2015). *Chrysopa pallens* (Rambur, 1838) was exposed to the neonicotinoid nitenpyram (NIT) and acute toxicity was assessed by analyzing differentially expressed genes (DEGs) (Du *et al.*, 2024). Additionally, chemoreception genes specific to this species were also identified (Li *et al.*, 2013). Several cytochrome P450 genes in *Chrysoperla zastrowi sillemi* Esben-Petersen were found to be differentially expressed in response to imidacloprid (Pathak *et al.*, 2022). Furthermore, *Chrysoperla carnea* Stephens was studied in relation to a biostable kinin peptide, demonstrating that IPM strategies could effectively integrate chrysopids and bioinsecticides (Shi *et al.*, 2023).

Ceraeochrysa Adams is an important genus of green lacewings used in integrated pest management (IPM) programs in Brazil due to their high reproductive potential, resistance to various insecticides, and the voracious predatory behavior of their larvae (Machado *et al.*, 2024). Among these, *Ceraeochrysa claveri* (Navás, 1911) is a non-model insect employed in ecotoxicological studies to demonstrate the sublethal effects of insecticides on beneficial insects. Previous studies have shown that exposure to pyriproxyfen results in damage to the Malpighian tubules, midgut, fat body, cocoon spinning, and testes of *C. claveri* (Garcia *et al.*, 2020; Scudeler *et al.*, 2022; Scudeler *et al.*, 2024). However, until now, there have been no transcriptomic data available to evaluate the molecular effects of insecticide exposure on this species. Since maintaining this beneficial insect in the field is desirable, developing improved IPM strategies to prevent population decline is essential. Given that previous studies confirmed that pyriproxyfen can cause morphological changes in testicular tissue (Garcia *et al.*, 2020), the aim was to evaluate its sublethal effects on gene expression in adult *C. claveri* using RNA-seq.

4.3. MATERIAL AND METHODS

4.3.1. Insect rearing

C. claveri larvae were reared in the Insect Laboratory at the Biosciences Institute, São Paulo State University (UNESP), Botucatu, SP, Brazil. The insect colony was maintained in a growth chamber under controlled environmental conditions: temperature

of 25 ± 1 °C, relative humidity of 70 ± 10%, and a photoperiod of 12:12 h (light/dark). The larvae were fed ad libitum on *Diatraea saccharalis* (Lepidoptera: Crambidae) eggs until pupation. The adult insects were raised on an artificial diet composed of honey and brewer's yeast (1:1). *D. saccharalis* eggs were supplied by CETMA Comércio de Agentes para Controle Biológico, Lençóis Paulista, SP, Brazil.

4.3.2. Bioassays

Newly hatched *C. claveri* larvae (0–12 h) were fed *ad libitum* on *D. saccharalis* eggs treated with two concentrations of the insecticide pyriproxyfen, which was used in its commercial formulation, Tiger 100 EC® (Sumitomo Chemical do Brasil Representações Ltd., São Paulo, SP, Brazil). The insecticide was diluted in deionized water to concentrations of 50 mg a.i. L^{-1} and 100 mg a.i. L^{-1}, representing 50% and 100% of the maximum field recommended concentrations (MFRCs) of pyriproxyfen for Brazilian crops, like soybean, coffee, tomato, and citrus, where chrysopids are commonly found (MAPA, 2024).

C. claveri larvae were randomly divided into three experimental groups, with each group consisting of three replicates (n = 50 per replicate, totaling 150 insects per group). The larvae were individually placed in polyethylene pots. *D. saccharalis* eggs were treated with two dosages of pyriproxyfen using a dipping method: the egg clusters were immersed in the insecticide solution for 5 s, then dried at room temperature for 1 h. The treated eggs were provided ad libitum and replaced every three days, to prevent dehydration and maintain insecticide efficacy. The control group was fed eggs that had been immersed only in distilled water for 5 s and then dried at room temperature for 1 h (Garcia *et al.*, 2020; Scudeler *et al.*, 2022, 2024). All experimental groups were maintained under the same environmental conditions as the insect rearing.

Newly emerged adults from each experimental group were placed in polyethylene pots and fed on the previously described artificial diet. Afterward, only adult males aged 10 days (when they reach sexual maturity) were selected for testes extraction.

4.3.3. RNA extraction, library preparation, and sequencing

C. claveri adults (10 days old) were dissected, and the testes were collected in pools of 50 testes each. Three replicates were performed for each group (comprising a control group and two treated groups), which resulted in a total of 150 testes per group. The insects were cryoanesthetized and dissected from the dorsal region, then immersed

in an insect saline solution (0.1 M NaCl; 0.1 M Na_2HPO_4; 0.1 M KH_2PO_4). The testes were immediately frozen in liquid nitrogen and stored in a biofreezer at −80 °C. RNA extraction and purification, library construction, and sequencing were conducted by NGS Soluções Genômicas (Piracicaba, SP, Brazil) using the Illumina platform (HiSeq 2000, 2 × 100). The raw sequence data have been deposited in the SRA under the accession code PRJNA1153524.

4.3.4. Reads filtering, *de novo* assembly, and annotation

A read quality analysis and the identification of highly representative sequences were conducted using FastQC v.0.12.0 (Andrews, 2010). The read quality was assessed (≥80%, phred value = 33), and adapter removal was performed using Trimmomatic v.0.39 (Bolger, 2014). Contaminants and rRNA were eliminated through a customized database of microorganisms and rRNA using the software BBduk v.38.90 (Bushnell, 2014).

The reads were assembled de novo with Trinity v.2.15.0 (Grabherr *et al.*, 2011; Haas *et al.*, 2013). Redundant transcripts with ≥98% identity were removed using CD-HIT EST v.4.8.1 (Li *et al.*, 2006; Fu *et al.*, 2012). The final assembled transcripts underwent quality analysis and were subsequently used as a reference for read alignment with HISAT2 v.2.2.1 (Kim *et al.*, 2019). Additionally, a search for orthologous genes was performed using BUSCO v.5.4.7 (Manni *et al.*, 2021a, 2021b) with the *arthropoda odb10* database (accessed on 20 March 2023).

Gene annotation was carried out by scanning the open reading frames (ORFs) for homology against the UniProt insect database and the Pfam database using TransDecoder v.5.7.0 (Haas, B. J., https://github.com/TransDecoder/TransDecoder) and Sma3s (Casimiro-Soriguer *et al.*, 2017), both of which were run with default parameters.

4.3.5. Differentially expressed genes (DEGs) and Gene Ontology

The transcript count matrix was generated using the scripts align_and_estimate_abundance.pl and abundance_estimates_to_matrix.pl from Salmon (Patro *et al.*, 2017). Library analyses, TMM normalization, and the assessment of differentially expressed genes (DEGs) between the groups were conducted using the EdgeR package (Robinson *et al.*, 2010; McCarthy *et al.*, 2012; Chen *et al.*, 2016) in the R/Bioconductor environment v.4.1.2 (R Core Team, 2025). Transcripts with a log2 fold change ≥2, log2 fold change ≤−2, and an FDR ≤ 0.05 were considered differentially expressed (Benjamini and Yekutieli, 2001).

Additionally, a gene ontology analysis was performed for the DEGs against the Gene Ontology database (Carbon *et al.*, 2009). The DEGs data were validated by RT-qPCR, as described below.

4.3.6. RT-qPCR for DEGs validation

Four genes were randomly selected for the validation of differentially expressed genes (DEGs) using RT-qPCR. The analyses were conducted at the Molecular and Reproductive Biology Lab, Biosciences Institute, UNESP, Botucatu, SP, Brazil. Primers (listed in Table 4.1) were selected from the DEGs identified through RNA-seq analysis and were designed using Primer3Plus (Untergasser *et al.*, 2007), PCR Primer Stats (Stothard, 2000), and OligoCalc (Kibbe, 2007). The total RNA used for the sequencing was used to synthesize cDNA with the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA).

Table 4.1 – Primer sequence for RT-qPCR

Gene name	Forward Primer (5'-3')	Reverse Primer (5'-3')
BPHL	CCCCACCACTCCATCCTACT	GTTTTCTTGTTGCCGGGTGC
GAPDH	GAACGGGGTCAAGGTAGTGG	AAGTGGTGAAGACTCCGGTT
Glycosyltransferase -like protein large2	GGACTGTATTTGTCATTAAAAAGG	GTTCGTGACCTTTGGTCCATA
MLX- interacting	TAACATGGCTGCTTTGCTTA	ACCTTTGTCACCCGCTGAAT
Talin-1	AACTTCTCGTCCTGCACCT	AATGAAACCGGTCCACTTTG

RT-qPCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) on a StepOnePlus™ (Applied Biosystems™, Waltham, MA, USA) according to the manufacturer's instructions. The reaction mix consisted of 10 µL total volume: 5 µL of SYBR qPCR premixture, 1 µL of each 10 µM primer, 2.5 µL of cDNA, and 0.5 µL of Milli-Q water. Three cDNA replicates, each with two technical replicates, were used for the samples (control, 50 mg a.i. L^{-1}, and 100 mg a.i. L^{-1} treatments). The qPCR conditions were set as follows: 95 °C for 10 min, followed by 35 cycles of 95 °C for 15 s and 60 °C for 1 min.

To normalize the relative expression, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as the housekeeping gene (Lü *et al.*, 2018) while employing the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The experiment was conducted in accordance with the MIQE guidelines (Bustin *et al.*, 2009). One-way

ANOVA was performed for comparisons between the groups using GraphPad Prism v.8.0.1.244.

4.4 RESULTS

4.4.1. *De novo* assembly and annotation

After sequencing, a total of 476,870,972 raw reads were generated. These reads were assessed for quality using FastQC and subsequently trimmed with Trimommatic and BBduk, which resulted in 448,210,677 filtered reads, which accounted for 94% of the original raw reads. The de novo assembly produced 260,417 transcripts, of which 172,686 were classified as genes. The assembly exhibited a GC content of 30.68%, an N50 length of 914 bp, and a median contig length of 370 bp.

The quality of the assembly demonstrated a 98.38% alignment of the reads against the transcriptome assembly used as a reference. In the BUSCO analysis, 1013 groups were searched for orthologous genes, where 991 groups matched, which represented 97.8% of the data. Among these, 630 groups were classified as complete and a single copy (62.2%), 361 groups as complete and duplicated (35.6%), 15 groups as fragmented (1.5%), and 7 groups were classified as missing (0.7%).

4.4.1.2. DEGs analyses

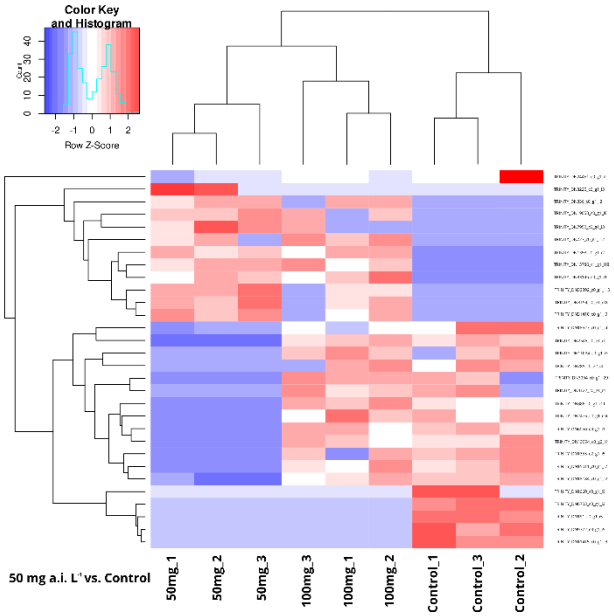
The normalized gene expression data were used to create a heatmap for the differentially expressed genes (DEGs), visually illustrating the expression profiles across all samples, with blue indicating downregulation and red indicating upregulation (Figure 4.1). A total of 46 DEGs were identified for the 50 mg a.i. L^{-1} treatment compared with the control group (15 upregulated and 31 downregulated). For the 100 mg a.i. L^{-1} treatment versus the control, 47 DEGs were found (16 upregulated and 31 downregulated). Additionally, there were 64 DEGs identified when comparing the 50 mg a.i. L^{-1} and 100 mg a.i. L^{-1} groups (33 upregulated and 31 downregulated) (see Appendix: Table S1).

A Venn diagram illustrating the DEGs highlights the three evaluated groups and the percentage of genes common to all comparisons (Figure 4.2). Gene ontology (GO) analysis of the DEGs revealed key terms within the main GO categories under 'Biological Processes' (BP) and 'Cellular Components' (CC) ontologies. The most enriched terms for 'BP' included Transcription (14.6%), Transport (11.7%), and Cell Cycle (4.4%) (see Appendix: Table S2). In the 'CC' category, the top three terms were Membrane (23.5%),

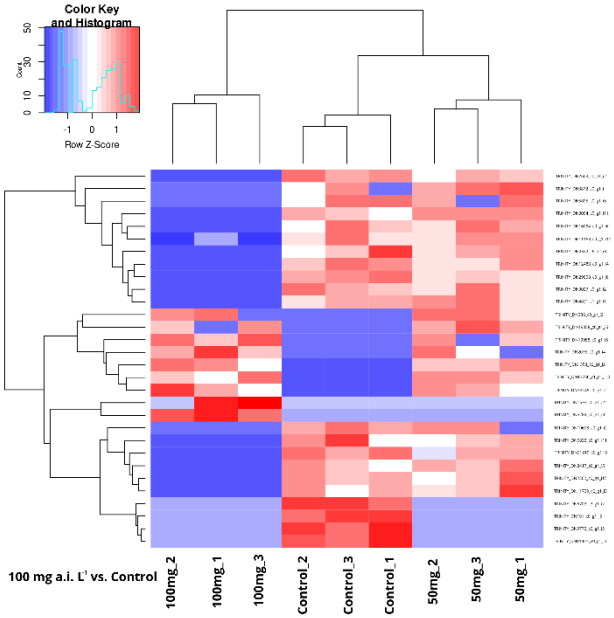
Nucleus (18.7%), and Cytoplasm (17.4%) (see Appendix: Table S2). Interestingly, the term ‘Molecular Function’ (MF) was not included, so a manual search for MF associated with each DEG can be found in the Supplementary Materials (see Appendix: Table S3).

4.4.2. RT-qPCR

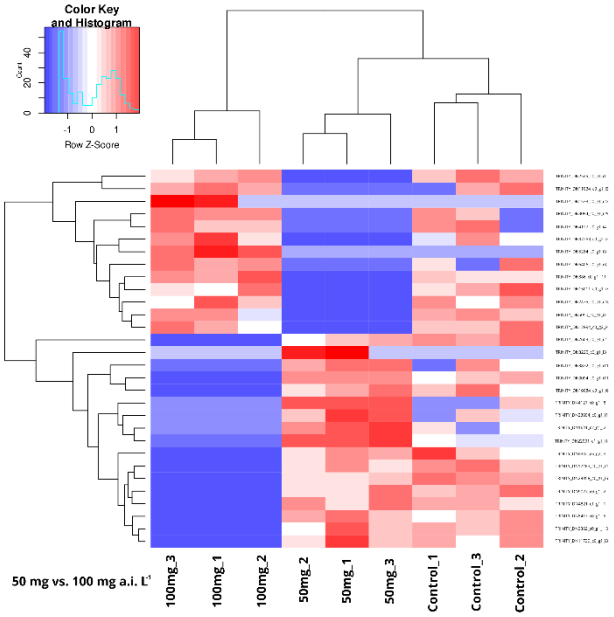
Four genes were randomly chosen and analyzed to validate the RNA-seq data: Glycosyltransferase-like protein large2, BPHL (Byphenil hydrolase-like), MLX (Max-like protein X)-interacting, and Talin-1. The RT-qPCR fold change results were all upregulated as in the RNA-seq results, indicating that the data were reliable (Figure 4.3).



50 mg a.i. L^{-1} vs. Control

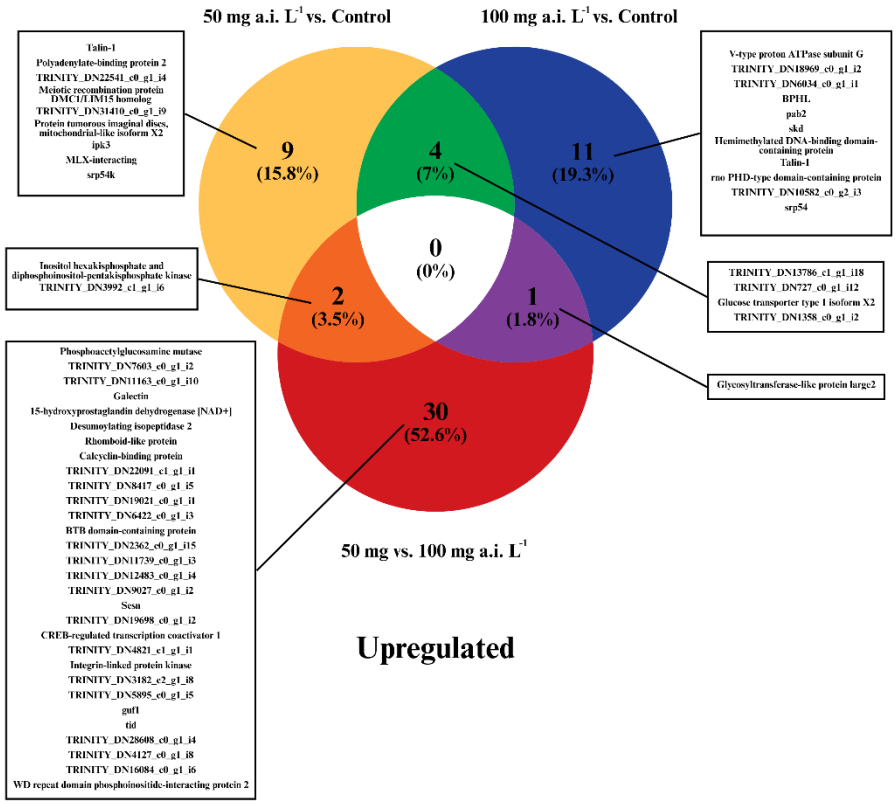


100 mg a.i. L^{-1} vs. Control



50 mg vs. 100 mg a.i. L^{-1}

Figure 4.1 – Heatmap analysis of hierarchical clustering of the differentially expressed genes from the testes of *Ceraeochrysa claveri* adults exposed to pyriproxyfen during the larval stage. The heatmap visually represents the expression profiles of all samples using a color gradient, with blue indicating downregulation and red indicating upregulation.


50 mg a.i. L-1 vs. Control
100 mg a.i. L-1 vs. Control
Talin-1
Polyadenylate-binding protein 2
TRINITY_DN22541_c0_g1_i4
Meiotic recombination protein DMC1/LIM15 homolog
TRINITY_DN31410_c0_g1_i9
Protein tumorous imaginal discs, mitochondrial-like isoform X2
ipk3
MLX-interacting
srp54k
9
(15.8%)
4
(7%)
11
(19.3%)
V-type proton ATPase subunit G
TRINITY_DN18969_c0_g1_i2
TRINITY_DN6034_c0_g1_i1
BPHL
pab2
skd
Hemimethylated DNA-binding domain-containing protein
Talin-1
rno PHD-type domain-containing protein
TRINITY_DN10582_c0_g2_i3
srp54
0
(0%)
Inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase
TRINITY_DN3992_c1_g1_i6
2
(3.5%)
1
(1.8%)
TRINITY_DN13786_c1_g1_i18
TRINITY_DN727_c0_g1_i12
Glucose transporter type 1 isoform X2
TRINITY_DN1358_c0_g1_i2
Glycosyltransferase-like protein large2
Phosphoacetylglucosamine mutase
TRINITY_DN7603_c0_g1_i2
TRINITY_DN11163_c0_g1_i10
Galectin
15-hydroxyprostaglandin dehydrogenase [NAD+]
Desumoylating isopeptidase 2
Rhomboid-like protein
Calcyclin-binding protein
TRINITY_DN22091_c1_g1_i1
TRINITY_DN8417_c0_g1_i5
TRINITY_DN19021_c0_g1_i1
TRINITY_DN6422_c0_g1_i3
BTB domain-containing protein
TRINITY_DN2362_c0_g1_i15
TRINITY_DN11739_c0_g1_i3
TRINITY_DN12483_c0_g1_i4
TRINITY_DN9027_c0_g1_i2
Sesn
TRINITY_DN19698_c0_g1_i2
CREB-regulated transcription coactivator 1
TRINITY_DN4821_c1_g1_i1
Integrin-linked protein kinase
TRINITY_DN3182_c2_g1_i8
TRINITY_DN5895_c0_g1_i5
guf1
tid
TRINITY_DN28608_c0_g1_i4
TRINITY_DN4127_c0_g1_i8
TRINITY_DN16084_c0_g1_i6
WD repeat domain phosphoinositide-interacting protein 2
30
(52.6%)
50 mg vs. 100 mg a.i. L-1
Upregulated

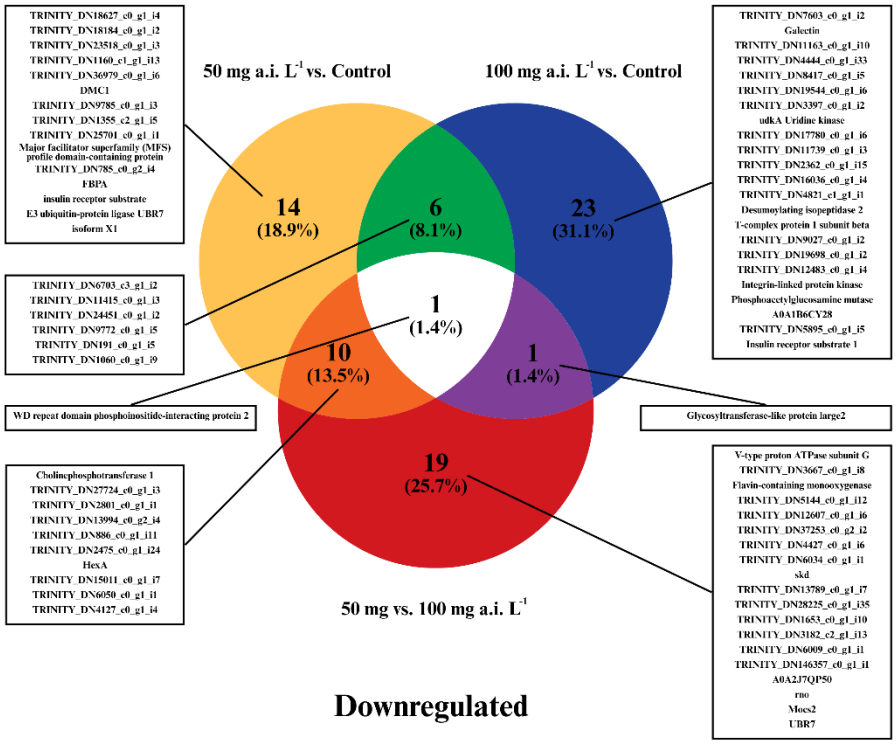

TRINITY_DN18627_c0_g1_i4
TRINITY_DN18184_c0_g1_i2
TRINITY_DN23518_c0_g1_i3
TRINITY_DN1160_c1_g1_i13
TRINITY_DN36979_c0_g1_i6
DMC1
TRINITY_DN9785_c0_g1_i3
TRINITY_DN1355_c2_g1_i5
TRINITY_DN25701_c0_g1_i1
Major facilitator superfamily (MFS) profile domain-containing protein
TRINITY_DN785_c0_g2_i4
FBPA
insulin receptor substrate
E3 ubiquitin-protein ligase UBR7 isoform X1
50 mg a.i. L-1 vs. Control
100 mg a.i. L-1 vs. Control
TRINITY_DN7603_c0_g1_i2
Galectin
TRINITY_DN11163_c0_g1_i10
TRINITY_DN4444_c0_g1_i33
TRINITY_DN8417_c0_g1_i5
TRINITY_DN19544_c0_g1_i6
TRINITY_DN3397_c0_g1_i2
udkA Uridine kinase
TRINITY_DN17780_c0_g1_i6
TRINITY_DN11739_c0_g1_i3
TRINITY_DN2362_c0_g1_i15
TRINITY_DN16036_c0_g1_i4
TRINITY_DN4821_c1_g1_i1
Desumoylating isopeptidase 2
T-complex protein 1 subunit beta
TRINITY_DN9027_c0_g1_i2
TRINITY_DN19698_c0_g1_i2
TRINITY_DN12483_c0_g1_i4
Integrin-linked protein kinase
Phosphoacetylglucosamine mutase
A0A1B6CY28
TRINITY_DN5895_c0_g1_i5
Insulin receptor substrate 1
14
(18.9%)
6
(8.1%)
23
(31.1%)
TRINITY_DN6703_c3_g1_i2
TRINITY_DN11415_c0_g1_i3
TRINITY_DN24451_c0_g1_i2
TRINITY_DN9772_c0_g1_i5
TRINITY_DN191_c0_g1_i5
TRINITY_DN1060_c0_g1_i9
1
(1.4%)
10
(13.5%)
1
(1.4%)
WD repeat domain phosphoinositide-interacting protein 2
Glycosyltransferase-like protein large2
Cholinephosphotransferase 1
TRINITY_DN27724_c0_g1_i3
TRINITY_DN2801_c0_g1_i1
TRINITY_DN13994_c0_g2_i4
TRINITY_DN886_c0_g1_i11
TRINITY_DN2475_c0_g1_i24
HexA
TRINITY_DN15011_c0_g1_i7
TRINITY_DN6050_c0_g1_i1
TRINITY_DN4127_c0_g1_i4
19
(25.7%)
V-type proton ATPase subunit G
TRINITY_DN3667_c0_g1_i8
Flavin-containing monooxygenase
TRINITY_DN5144_c0_g1_i12
TRINITY_DN12607_c0_g1_i6
TRINITY_DN37253_c0_g2_i2
TRINITY_DN4427_c0_g1_i6
TRINITY_DN6034_c0_g1_i1
skd
TRINITY_DN13789_c0_g1_i7
TRINITY_DN28225_c0_g1_i35
TRINITY_DN1653_c0_g1_i10
TRINITY_DN3182_c2_g1_i13
TRINITY_DN6009_c0_g1_i1
TRINITY_DN146357_c0_g1_i1
A0A2J7QP50
rno
Mocs2
UBR7
50 mg vs. 100 mg a.i. L-1
Downregulated

Figure 4.2 – Venn diagrams of the differentially expressed genes (DEGs) of *Ceraeochrysa claveri* testes exposed to pyriproxyfen during the larval stage. The diagrams show the three evaluated groups and the percentages of genes found in all comparisons.

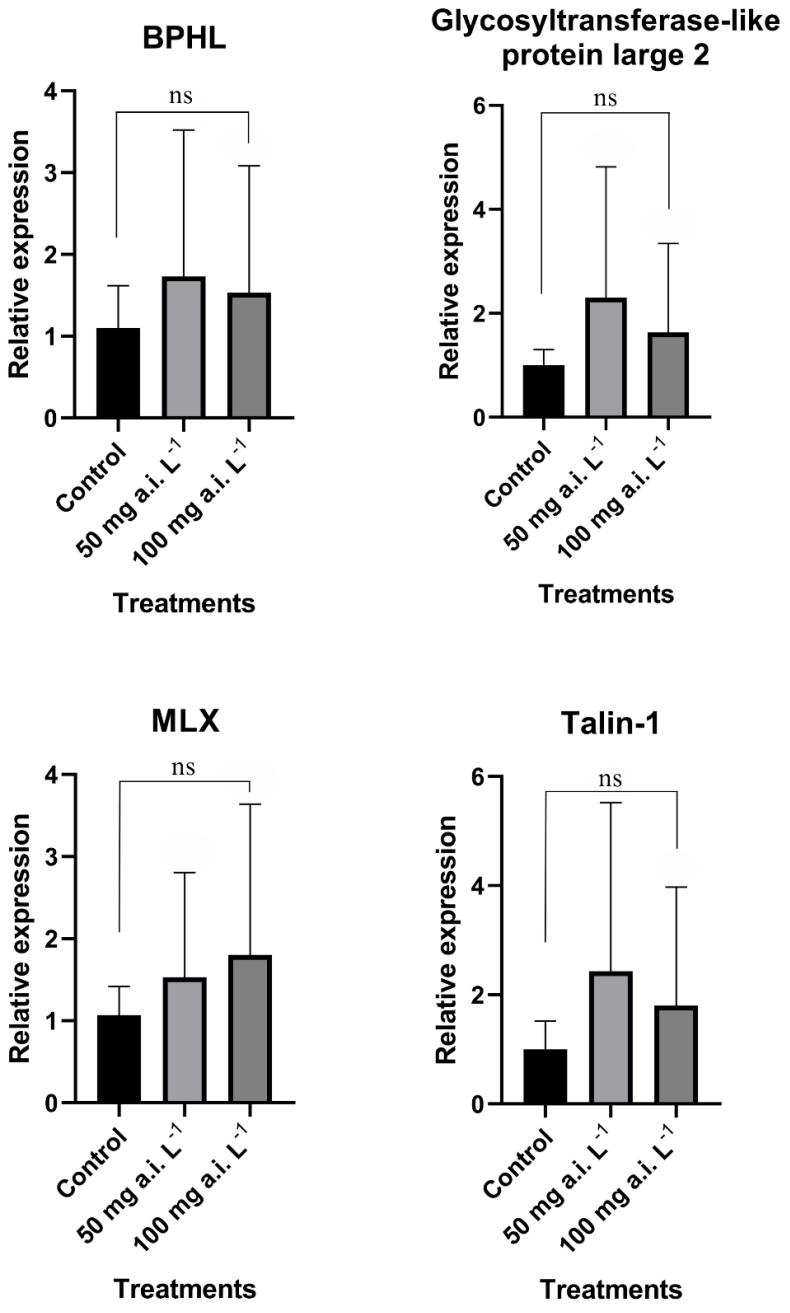


Figure 4.3 – RT-qPCR validation of four differentially expressed genes (DEGs) from the testes of *Ceraeochrysa claveri* adults exposed to two different doses of pyriproxyfen during the larval stage. One-way ANOVA was performed for the comparisons between groups (mean ± SD). ns—not significative.

3.5. DISCUSSION

Despite exhibiting some cellular damage and ultrastructural alterations in testicular cells (Garcia *et al.*, 2020), this species demonstrated tolerance to the insecticide pyriproxyfen, managing to reproduce and sustain its lifespan under laboratory conditions. This resilience may be attributed to the potential detoxification of the insecticide. To date, there have been no transcriptome data available that show the impact of insecticides on the testes of *C. claveri*. The differentially expressed genes discussed here are reported for the first time in this species.

Byphenyl hydrolase-like (BPHL) was initially identified in human breast cancer (Puente and López-Otn, 1995) and is characterized as a serine hydrolase similar to those that degrade biphenyl compounds in prokaryotes (Derewenda and Derewenda, 1991; Cygler *et al.*, 1993). It is highly expressed in the liver and kidneys of mammals, suggesting a role in the detoxification of xenobiotics (Puente and López-Otn, 1995; Zhao *et al.*, 2010). In *Bombyx mori* (Lepidoptera: Bombycidae), BPHL is predominantly expressed in the fat body and hemolymph, with lower expression levels observed in other tissues, including the testes, where it may play roles in detoxification and immune functions (Gao *et al.*, 2016). In our study, BPHL was found to be upregulated in the 100 mg a.i. L^{-1} treatment compared with the control group; however, no significant differences were observed between the 50 mg a.i. L^{-1} treatment and the control or between the 50 mg a.i. L^{-1} and 100 mg a.i. L^{-1} treatments. The testes of *C. claveri* are surrounded by adipose tissue (Garcia *et al.*, 2019), which may facilitate the detoxification of this reproductive organ by enhancing the BPHL expression, thereby ensuring sperm production and viability and supporting offspring production.

LARGE2 is a bifunctional glycosyltransferase (GT) protein that utilizes uridine diphosphate (UDP)–xylose (Xyl) and UDP–glucuronic acid (GlcA) as sugar donors (Grewal *et al.*, 2005; Ashikov *et al.*, 2013). However, it can also accept and donate a variety of sugar ligands, which are implicated in various activities and functionalities, including structural and metabolic functions (Liang *et al.*, 2015; Biswas and Thattai, 2020). The biological processes of GTs in insects are crucial for maintaining homeostasis across several metabolic and physiological processes, such as survival, growth, tissue differentiation, and detoxification (Nagare *et al.*, 2021). Several UDP glycosyltransferases (UGTs) involved in cellular homeostasis have been identified in all insect tissues (Ahn *et al.*, 2012). The expression of GTs related to detoxification may vary according to the developmental stage of the insect, for instance, this has been observed

in *Bombyx mori* during the detoxification of plant allelochemicals (Huang *et al.*, 2008). Additionally, GTs may be expressed for specific purposes, such as the detoxification of insecticides, as demonstrated in studies that involved *Plutella xylostella* (Lepidoptera: Plutellidae) (Li *et al.*, 2017), *Athetis lepigone* (Lepidoptera: Noctuidae) (Zhang *et al.*, 2017), *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) (Kaplanoglu *et al.*, 2017), and *Musca domestica* (Diptera: Muscidae) (Reid *et al.*, 2019). The RNA-seq results indicate that glycosyltransferase expression was both upregulated (TRINITY_DN29058_c0_g1_I7) and downregulated (TRINITY_DN29058_c0_g1_i6) in the 100 mg a.i. L^{-1} treatment group compared with the control group. This discrepancy may be attributed to our analysis detecting two different isoforms (i6 and i7), with each potentially associated with distinct functions in the testis.

Transcription factors (TFs) play a crucial role in coordinating various biological processes by linking networks associated with diverse metabolic responses (Barabási and Oltvai, 2004). They are essential components in regulating key pathways involved in morphological and developmental cellular processes (Alberghina *et al.*, 2009). The basic helix–loop–helix (bHLH) family is a large group of TFs characterized by their DNA-binding and dimerization domains, which are responsible for development, cell proliferation, and cell lineage determination (Atchley and Fitch, 1997; Jones, 2004). MLX protein (Max-like protein X) and Max proteins serve as important bHLH TF regulators of cellular metabolism across several model organisms (Billin *et al.*, 1999, Lüscher, 2001), with their functions being well conserved between these species (Yuan *et al.*, 1998; Steiger *et al.*, 2008; McFerrin and Atchley, 2011). In mice, the MLX–Mondo complex has various functions, including regulating metabolism to prevent apoptosis and ensuring normal spermatogenesis (Carroll *et al.*, 2021). Therefore, the upregulation of MLX may indicate an effort to maintain homeostasis. Similar to BPHL, MLX is also found in reproductive tissues (Carroll *et al.*, 2021), and its upregulation may be aimed at supporting the reproduction of *C. claveri* in the field—a desirable trait for this species that helps preserve normal fecundity and fertility.

Talin is a cytoplasmic protein that regulates the interaction between integrin proteins and their ligands, linking them to the actin cytoskeleton through specific binding sites for actin and vinculin (Tadokoro *et al.*, 2003; Ye *et al.*, 2014). In *Drosophila*, studies have demonstrated that Talin enhances the connection between integrins and the extracellular matrix, either by directly linking integrins to the cytoskeleton or by recruiting other actin-binding proteins to facilitate this connection (Tanentzpf amd

Brown, 2006; Franco-Cea *et al.*, 2010). Talin is also associated with cardiomyocyte remodeling, which is essential for maintaining heart contraction and longevity (Bogatan *et al.*, 2015). Additionally, it plays a role in enhancing forces in developing flight muscles (Lemke *et al.*, 2019). In morphological assays conducted using transmission electron microscopy, testes treated with pyriproxyfen at the same doses used in this study exhibited alterations in the extracellular matrix, including noticeable spacing between cysts (Garcia *et al.*, 2020). Talin-1 was found to be upregulated in both the 50 mg a.i. L^{-1} vs. control and 100 mg a.i. L^{-1} vs. control treatment groups. Given the reduced expression of integrins (Tomacheski *et al.*, 2025b and Table S1), it is possible that Talin-1 was upregulated to help maintain the balance of the cellular ultrastructure.

Over the long term, both the lethal and sublethal effects of insecticides can alter insect diversity in the field, leading to an increase in pest populations and a decline in beneficial insects (Serrão *et al.*, 2022; Ziesche *et al.*, 2024; Quandahor *et al.*, 2024). This underscores the importance of integrated pest management (IPM) with new technologies to explore and develop methodologies that combine pesticides with biological control agents (Stark *et al.*, 2007). Beneficial insects are affected by insecticides not only during application but also while feeding, resulting in secondary intoxication (Quandahor *et al.*, 2024).

Harm effects between insecticides and lacewings have been described. *Chrysoperla genanigra* Freitas (Neuroptera: Chrysopidae) exhibited high larvae mortality when exposed to seven insecticides (azadirachtin, abamectin, cyantraniliprole, thiacloprid, pymetrozine, imidacloprid, and novaluron). Additionally, the surviving adults showed low egg fecundity and fertility (Godoy *et al.*, 2025). High mortality rates in both larvae and adults were observed in *C. carnea* and *Chrysoperla johnsoni* Henry, Wells and Pupedis (Neuroptera: Chrysopidae) upon exposure to various insecticides. The sublethal effects included reduced survival from larvae to adults and shortened male and female lifespans, as well as decreased fecundity, fertility, and egg viability (Amarasekare and Shearer, 2013; Golmohammadi *et al.*, 2021). Similar results—regarding mortality, female fecundity, and egg fertility—were reported when *Chrysoperla externa* Hagen, 1861 (Neuroptera: Chrysopidae) was exposed to acetamiprid + etofenproxy, spinetoram, indoxacarb, and methoxyfenozide (Armas *et al.*, 2023). The exposure of *C. claveri* to pyriproxyfen led to increased larvae mortality (Scudeler *et al.*, 2022, 2024) and caused histomorphological alterations in the testes

(Garcia *et al.*, 2020), which could result in a decline in *C. claveri* populations and negatively impact its beneficial predation of insect pests.

In this study, all chrysopid adults were survivors of pyriproxyfen exposure during their larval stage, and the findings indicate that the effects of such exposure can persist throughout their adult lifespan, manifesting as alterations in morphology (Garcia *et al.*, 2020) and gene expression, as demonstrated in this research. These results were obtained using RNA sequencing (RNA-seq), a relatively uncommon tool in IPM strategies. Molecular analyses can enhance the approach of IPM in managing pest populations in the field (Van Leeuwen *et al.*, 2020), providing genetic data that were previously unavailable for decision-making. Potential discrepancies between the experimental conditions and field applications might end in different results from the same experiment (e.g., insecticide exposure). In the field, direct and indirect contact with insecticides could happen by dermal contact, ingestion, and inhalation (EPA, 2025). Different from lab conditions, environmental variations, such as abiotic (temperature, humidity, and light changes) and biotic (different diet, vegetation, and crowding population) variations, will change the insect life cycle, physiology, and survival (Khaliq *et al.*, 2014), and even with lab results leading to indicators of field performance, further studies involving molecular mechanisms of pyriproxyfen's disruption of the reproductive–survival balance via testicular gene expression regulation are necessary to solve this issue.

To advance the mechanistic clarity, future studies integrating multi-omics datasets (e.g., proteomics and metabolomics) could map interconnected signaling pathways and reconstruct the regulatory network. As for deeper field exploration, studies comparing the three standard environments [lab, semi-field (cages and greenhouses), and field] (Enserink, 2002; Terblanche, 2014; Azpiazu *et al.*, 2019) should be performed to enlighten the effects and check whether they persist or disappear when the tested environment changes. Interdisciplinary studies can improve the effectiveness of IPM by reducing the reliance on insecticides, which not only increases profitability (by lowering expenditure on insecticides) but also minimizes the environmental impact of agriculture (Rejesus and Jones, 2020; Mulungu *et al.*, 2024), thereby helping to preserve beneficial insect populations.

5. CHAPTER 2

Morphological analyses of the testes and ovaries in adult *Ceraeochrysa claveri* Navás, 1911 (Neuroptera: Chrysopidae) following larval exposure to sulfoxaflor

5.1 ABSTRACT

Sulfoxaflor is a worldwide used insecticide, designed for protecting several cultivated plants from insects considered pests. The use of different approaches with both insecticides and natural enemies is tested in order to improve the control of insects that cause damage to crop and preserve the others. *Ceraeochrysa claveri* Navás, 1911 (Neuroptera: Chrysopidae) is an important natural enemy to insect pests, and few information about sublethal exposure to insecticides under this insect is available. This work aims to understand the effects of sublethal doses of sulfoxaflor on the life cycle and on reproductive organs of *C. claveri*. We exposed first instar larvae to three different doses of sulfoxaflor (10%, 30% and 50% of LC_{50}) by ingestion of contaminated food (*Diatraea saccharalis* eggs) for 10 days. When the insects emerged as adults, the life cycle of larvae and pupae was measured. The testes and ovaries were collected and analyzed under light and transmission electron microscopy. The results showed that the sublethal doses of sulfoxaflor did not affect the life cycle and survivorship of larvae and pupae. Morphological analysis of testes presented space among cells and modification on the testis tunic and gaps among cells of the ovarioles. We concluded that sulfoxaflor did not impact on life of larvae and pupae of *C. claveri*, but cellular damages on reproductive organs were found. The application of this insecticide should be done with caution, and further analysis using sulfoxaflor and chrysopids should be done to enlighten this issue.

KEYWORDS: green lacewing; reproduction; sulfoxamine; survivorship.

5.2 INTRODUCTION

The use of synthetic or natural insecticides is essential because insect pests pose a significant problem for agriculture and, consequently, the economy. They directly damage crops through feeding and indirectly spread viruses, fungi, and other diseases. Combating these pests is undeniably necessary and increasingly challenging, as many species develop resistance to commonly used insecticides such as organophosphates, carbamates, and neonicotinoids (Sparks *et al.*, 2013; Sparks and Nauen, 2015; Chen *et al.*, 2016; Ma *et al.*, 2019a; Ma *et al.*, 2019b; Sohail *et al.*, 2019; Watson *et al.*, 2021).

Neonicotinoids are a class of insecticides derived from nicotine that act as agonists of nicotinic acetylcholine receptors (nAChRs) in the central nervous system (Sâmia *et al.*, 2019). Sulfoxamines are another group of insecticides that also target nAChRs, but they differ in their chemical structure and interaction mechanisms, which helps maintain their insecticidal effectiveness (Sparks *et al.*, 2013).

Sulfoxaflor is the first insecticide in the sulfoxamine class to be commercially available. It was developed by Dow AgroSciences in 2010 and is classified by the Insecticide Resistance Action Committee as a group 4C insecticide (IRAC, 2025). Like neonicotinoids, sulfoxaflor acts on the central nervous system of insects (Chen *et al.*, 2016; Zhen *et al.*, 2018; Ma *et al.*, 2019a; Ma *et al.*, 2019b). The symptoms of an insect poisoned with sulfoxaflor are behavioral and can vary widely, including reduced lifespan, slower development, decreased fertility and fecundity, and less oviposition. It can also cause tremors, antennal undulations, extension or curling of the legs, paralysis, and ultimately death (Sparks *et al.*, 2013; Zhen *et al.*, 2018; Dai *et al.*, 2021).

Because of its broad spectrum of effectiveness against pests, sulfoxaflor has become popular among farmers worldwide and can be used on various types of crops. In addition to being effective against insects that have developed resistance to other insecticides, sulfoxaflor is also considered safe for mammals (Watson *et al.*, 2011; Zhu *et al.*, 2011; Sparks *et al.*, 2013; Lu *et al.*, 2020). Moreover, studies have shown that sulfoxaflor is less harmful to beneficial insects compared to other insecticides (Tran *et al.*, 2016; Siviter *et al.*, 2019; Tamburini *et al.*, 2021).

Non-target insects when exposed to insecticides and do not exhibit significant population declines (Ghosh *et al.*, 2013; Tran *et al.*, 2016), it is suspected that these insects may have upregulated detoxifying mechanisms against insecticides (Guedes *et al.*, 2016), indicating a possible tolerance or resistance, potentially through hormesis (Guedes and Cutler, 2013). However, other studies report harmful effects of insecticides on natural

enemies, including ovicidal and larvicidal impacts, which can reduce female fecundity and egg viability (Ono *et al.*, 2017; He *et al.*, 2018; Sohail *et al.*, 2019). Most of these studies focus on effects related to the insect's life cycle and certain reproductive aspects, such as fecundity and fertility, but they do not address morphological or ultrastructural changes in the reproductive organs.

Found in many economically important crops, such as potato, cotton and apple, lacewings (Neuroptera: Chrysopidae) play a vital role in biological control by reducing the population densities of various pest arthropods. Beyond their ecological diversity, they hold significant potential in agricultural systems due to their highly voracious larvae, making them valuable in integrated pest management (IPM) programs (Pappas *et al.*, 2011). *Ceraeochrysa claveri* Navás, 1911 (Neuroptera: Chrysopidae) is a non-target insect and could be used as a model for ecotoxicological studies, showing the sublethal effects of insecticides exposures (Scudeler and Santos, 2013; Scudeler *et al.*, 2017, 2019, 2022, 2024; Caballero-Gallardo *et al.*, 2023) since chrysopids show potential tolerance against insecticides exposures (Venkatesan *et al.*, 2009; Pathan *et al.*, 2010; Fátima *et al.*, 2013; Mansoor *et al.*, 2013; Mansoor and Shad, 2020; Pathak *et al.*, 2022)

In chrysopids, the male reproductive system consists of a pair of testes, vas deferens, seminal vesicles, accessory glands, and an ejaculatory duct. Its primary functions are the production and storage of sperm, as well as their transport in a viable form to the female reproductive tract (Chapman, 2013; Gullan and Cranston, 2014). The testes are composed of testicular follicles. In *C. claveri* larvae, these follicles appear in pairs, with a rounded shape and whitish color, changing to an elongated form during pupation and adopting a spiral shape in adults, with an intense yellow coloration (Garcia *et al.*, 2019). Germ cells within the testes are grouped into cysts, which are at various stages of maturation along the apical-basal axis. Development within each cyst is synchronous, facilitated by cytoplasmic bridges that form during incomplete cytokinesis of germ cells (Gullan and Cranston, 2014; Garcia *et al.*, 2019; Liu *et al.*, 2023).

The female reproductive system includes a pair of ovaries, each containing multiple ovarioles (Büning, 1994). Each ovariole consists of an anterior germarium, which houses the stem cell niche or resting oogonia, and a vitellarium where developing oocytes are arranged in an ontogenic series from anterior to posterior. The ovariole terminates in a connection to a common oviduct. The number of ovarioles varies among species, and the tissue morphology within ovarioles also differs across insects. Based on the presence and position of specialized oocyte-associated cells called nurse cells,

oogenesis has been classified into several modes, including panoistic and meroistic ovaries. The latter is further divided into polytrophic or telotrophic types, depending on the arrangement of nurse cells forming an egg chamber (Büning, 1994; Kubrakiewicz, 1997; Hodin, 2009; Garbiec and Kubrakiewicz, 2012; Vacacela *et al.*, 2017; Dantas *et al.*, 2020; Church *et al.*, 2021).

So far, there is only one study on the insecticide sulfoxaflor and its effects on the green lacewing, *Ceraeochrysa claveri*. Pereira (2022) established the LC50 for *C. claveri* exposed to sulfoxaflor and examined the effects on the midgut and Malpighian tubules. Given the importance of *C. claveri* as a natural enemy of insect pests in agro-environments and the limited information on insecticide exposure for this non-target species, this work aims to evaluate the effects of sublethal doses of sulfoxaflor on the reproductive organs of *C. claveri* throughout its life cycle, using both life-cycle and morphological analyses.

5.3 MATERIALS AND METHODS

5.3.1 Rearing

The chrysopids were reared and maintained in a controlled environment room at the Insect Lab, located at the Institute of Biosciences of Botucatu, São Paulo State University – UNESP. The conditions were set to a temperature of 25±1ºC, relative humidity of 70%±10%, and a photoperiod of 12 hours light and 12 hours dark.

The larvae of *C. claveri* were fed with eggs of *Diatraea saccharalis* (Lepidoptera: Crambidae), while the adults were provided with a diet consisting of equal parts brewer's yeast and honey (1:1). Adult insects were kept in polyethylene cages, with the interior and upper surfaces covered with white sulfite paper, which served as a substrate for oviposition. The eggs were collected during cage cleaning and maintenance, then placed in plastic boxes until hatching.

The larvae were individually separated into plastic pots measuring 6 cm x 2 cm and fed *ad libitum* on *D. saccharalis* eggs. The pupae remained in the same pots used during the larval stage, as the cocoons stayed attached to the surface of the pots. Once the adults emerged, they were removed and paired (one male and one female) in polyethylene cages to facilitate breeding.

To obtain *D. saccharalis* eggs, moths were supplied from the breeding program at CETMA (Comércio de Agentes para Controle Biológico Ltda.), located in Lençóis Paulista – SP. In the Insect Lab, pairs of moths were kept in polyethylene cages lined with

sulfite paper, maintained at the same controlled temperature conditions described above, to ensure a steady supply of eggs.

5.3.2 Bioassays

First instar larvae (0–12 hours old) of *C. claveri* were individually placed in plastic pots measuring 6 cm x 2 cm and fed for 10 days on *D. saccharalis* eggs treated with three different concentrations of sulfoxaflor (Verter® SC) (15.02 mg a.i. L^{-1}, 45.05 mg a.i. L^{-1}, and 75.10 mg a.i. L^{-1}) diluted in distilled water. These concentrations correspond to 10%, 30%, and 50% of the LC_{50}, as established by Pereira (2022). According to Verter® SC datasheet, the minimum and maximum field-recommended concentrations (MFRCs) of sulfoxaflor for Brazilian crops (e.g., cotton, potato, citrus, soybean and wheat) are 12 g/L and 96 g/L, respectively (MAPA, 2025).

The eggs were immersed in either distilled water (control) or distilled water containing sulfoxaflor (treated) for 20 minutes, then dried at room temperature for 2 hours. The eggs were then offered *ad libitum* to the larvae, with the food replaced every three days to prevent excessive dehydration of uneaten food and to maintain consistent insecticide levels. After 10 days, all groups were fed on untreated *D. saccharalis* eggs (control group) (Pereira, 2022).

When the adults emerged, 10-day-old males and females of *C. claveri* were dissected (when they are sexually mature), and their ovaries and testes were removed for histological and ultrastructural analyses using various methodological techniques. The experimental design was completely randomized, with four replicates for each concentration. Each replicate consisted of 15 specimens, totaling 60 individuals per group.

5.3.3 Life cycle data

The longevity and survivorship of the insects involved in the bioassay were evaluated using appropriate statistical methods based on the analysis. The longevity of each instar was measured (including both alive and dead individuals) within each experimental group (control and treatment). These measurements were established through position metrics and data variability, expressed as mean ± standard deviation (SD). Additionally, the longevity of males and females that survived the entire assay was also recorded as mean ± SD (Norman and Streiner, 2014).

To interpret these results, intervals with no overlap indicate a statistically significant difference between the mean responses, while overlapping intervals suggest no significant difference. Both analyses were conducted using 95% confidence intervals for the population mean (Norman and Streiner, 2014) and were performed with R Statistical Software v.4.4.3 (R Core Team, 2025).

Survivorship (%) was also calculated for each experimental group and instar. The data were analyzed using the Goodman association test, which compares binomial populations, with a significance level set at 5% (Goodman, 1964). The graphical representation was created using GraphPad Prism 8 (v.8.0.1.244).

5.3.4 Morphological analyses

5.3.4.1 Histological processing of the ovaries and testes from *C. claveri* adults

Males and females (n = 10 males and 10 females for each experimental groups) were dissected at 10-days-old (as described above). The specimens were cryoanesthetized (4ºC) for 15 seconds, dissected through the dorsal region using saline solution for insects (0.1M NaCl; 0.1M Na_2HPO_4; 0.1M KH_2PO_4) under a stereoscopic microscope. The organs were immediately pre-fixed in place by dripping Karnovsky solution (4% paraformaldehyde and 2.5% glutaraldehyde in 0.1M L^{-1} phosphate buffer, pH 7.3) (Karnovsky, 1965) and then transferred to a container containing the same solution.

5.3.4.2 Light microscopy

After initial fixation for 24 hours, the organs were dehydrated in ethanol solutions (70-95%) then submitted to the methacrylate-glycol embedding technique (Leica HistoResin Embedding Kit, Leica Biosystems, Wetzlar, Germany). Histological sections (3 μm) were cut using a Leica RM2045 microtome, stained with Hematoxylin and Eosin (H.E.) (Pearse, 1972) and the slides were photographed under Leica DM500 photomicroscope.

5.3.4.3 Transmission Electron Microscopy (TEM)

The organs were fragmented and submitted to the routine protocol (CME, 2025) from the Electron Microscopy Center of the Institute of Biosciences of Botucatu, UNESP: post-fixation in 1% osmium tetroxide in the same buffer (2h); washing in distilled water (three times of five minutes); block contrasting in 0.5% uranyl acetate solution (2h); dehydration in increasing series of acetone solutions [acetone 50% for 10 min (2 times);

acetone 70% for 10 min (2 times); acetone 90% for 15 min (3 times); acetone 100% for 15 min (3 times)], embedding in a mixture of resin (Araldite®) and 100% acetone (1:1) (12h); embedding in pure resin in an oven at 37°C (1h) and embedding in pure resin for polymerization in an oven at 60°C (72h). Ultra-thin sections (90 ηm) were contrasted in uranyl acetate and lead citrate and subsequently analyzed and photographed with a Tecnai Spirit transmission electron microscope (FEI Company, Eindhoven, Netherlands).

5.4 RESULTS

5.4.1 Life cycle

We analyzed the duration of each instar for all insects within each group (n=60), regardless of whether they were alive or dead. Once the survivors reached adulthood, we performed the same analysis, including a comparison between males and females. At the end of the experiment, no statistically significant differences (95% confidence interval) were observed in the duration (days) of each instar when comparing the sulfoxaflor treatment groups to the control group (Tables 5.1 and 5.2).

All four groups (control plus three sulfoxaflor treatments) started the experiment with 60 insects each. By the end, the survivorship rates were as follows: 80% (n=48) for the control group, 61.7% (n=37) for the 15.02 mg a.i. L^{-1} group, 70% (n=42) for the 45.05 mg a.i. L^{-1} group, and 90% (n=54) for the 75.10 mg a.i. L^{-1} group. There were no statistically significant differences ($p > 0.05$) among the sulfoxaflor-treated groups compared to the control group (Figure 5.1).

Table 5.1 – Descriptive measurements [days (mean ± SD)*] of each instar of *Ceraeochrysa claveri* treated with three subdoses of sulfoxaflor during the larval stage.

Group	1st instar	2nd instar	3rd instar	Pre-pupae	Pupae
Control	5.13 ±1.00	4.81 ±0.74	5.11 ±0.68	4.39 ±0.53	9.67 ±0.52
15.02 mg a.i. L^{-1}	5.34 ±1.05	5.62 ±1.63	5.32 ±0.96	4.50 ±0.67	9.54 ±0.90
45.05 mg a.i. L^{-1}	5.39 ±0.87	3.98 ±1.31	5.17 ±0.98	4.61 ±0.49	9.14 ±0.61
75.05 mg a.i. L^{-1}	5.29 ±0.73	4.42 ±1.12	4.66 ±0.82	4.56 ±0.50	9.19 ±0.62

*95% confidence interval

Table 5.2 – Descriptive measurements [days (mean ±SD)[*]] of surviving male and female *Ceraeochrysa claveri* that were exposed to three subdoses of sulfoxaflor during the larval stage.

Group	Sex[**]	1st instar	2nd instar	3rd instar	Pre-pupae	Pupae
Control	F	5.15 ±0.91 aA	4.74 ±0.71 aB	4.93 ±0.62 aA	4.30 ±0.47 aA	9.78 ±0.51 aB
	M	5.00 ±1.05 aA	4.81 ±0.68 aB	5.33 ±0.73 aAB	4.33 ±0.48 aA	9.52 ±0.51 aA
15.02mg a.i. L^{-1}	F	5.05 ±0.84 aA	5.00 ±1.20 aB	5.14 ±0.77 aA	4.50 ±0.51 aAB	9.46 ±0.51 aAB
	M	4.87 ±0.83 aA	5.73 ±1.39 aB	5.60 ±1.06 aB	4.27 ±0.59 aA	9.67 ±1.29 aA
45.05mg a.i. L^{-1}	F	5.72 ±1.01 aA	3.44 ±0.71 aA	5.11 ±0.68 aA	4.83 ±0.38 aB	8.94 ±0.64 aA
	M	5.04 ±0.75 aA	4.04 ±0.75 aA	5.29 ±1.20 aAB	4.46 ±0.51 aA	9.29 ±0.55 aA
75.05mg a.i. L^{-1}	F	5.48 ±0.68 aA	4.24 ±1.14 aAB	4.43 ±0.75 aA	4.71 ±0.46 aAB	9.29 ±0.78 aAB
	M	5.15 ±0.76 aA	4.36 ±0.82 aA	4.79 ±0.86 aA	4.49 ±0.51 aA	9.12 ±0.49 aA

[*]95% confidence interval.
[**]F – Female, M – Male.
Lowercase letter – Comparison between male and female fixed group and instar.
Capital letter – Comparison of groups fixed sex and instar.

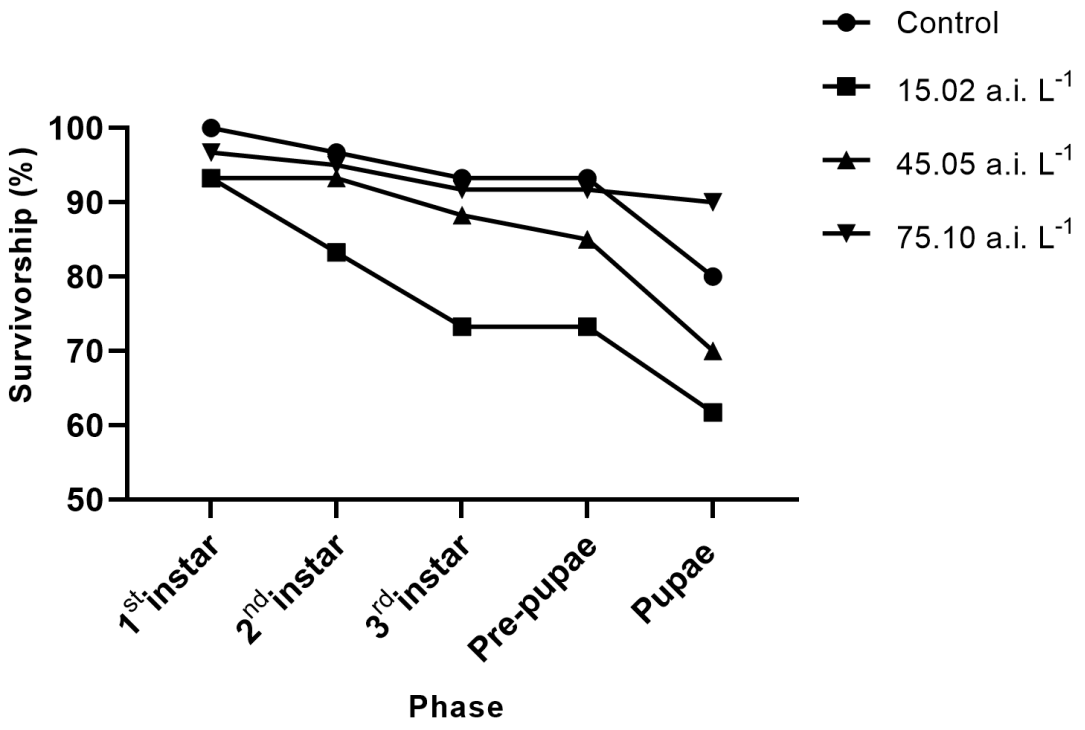


Figure 5.1 – Survivorship (%) of *Ceraeochrysa claveri* exposed to three subdoses of sulfoxaflor during the larval stage.

5.4.2 Testes

The male chrysopid possesses two yellow, twisted testes located below the gut (Figure 5.2). Each testis is covered by a two-layered membrane tissue: the external tunic and the internal tunic. Different zones within the testis are formed along a developmental gradient, starting from stem cells and spermatogonia at the apical end and progressing to mature spermatozoa at the basal end (Figure 5.3).

Spermatogenesis begins at the apical portion of the testes, where the stem cells are situated. Somatic cells enclose the spermatogonia, forming cysts. Within each cyst, spermatogonia continue dividing through incomplete mitosis, maintaining connections via ring canals. After several rounds of incomplete mitotic divisions and growth, spermatogonia develop into spermatocytes. These spermatocytes undergo meiosis to become haploid spermatids. In the final stage of spermatogenesis, called spermiogenesis, each spermatid is encased and individualized from the bundle within its own membrane, resulting in mature spermatozoa (Figure 5.3c, f and l).

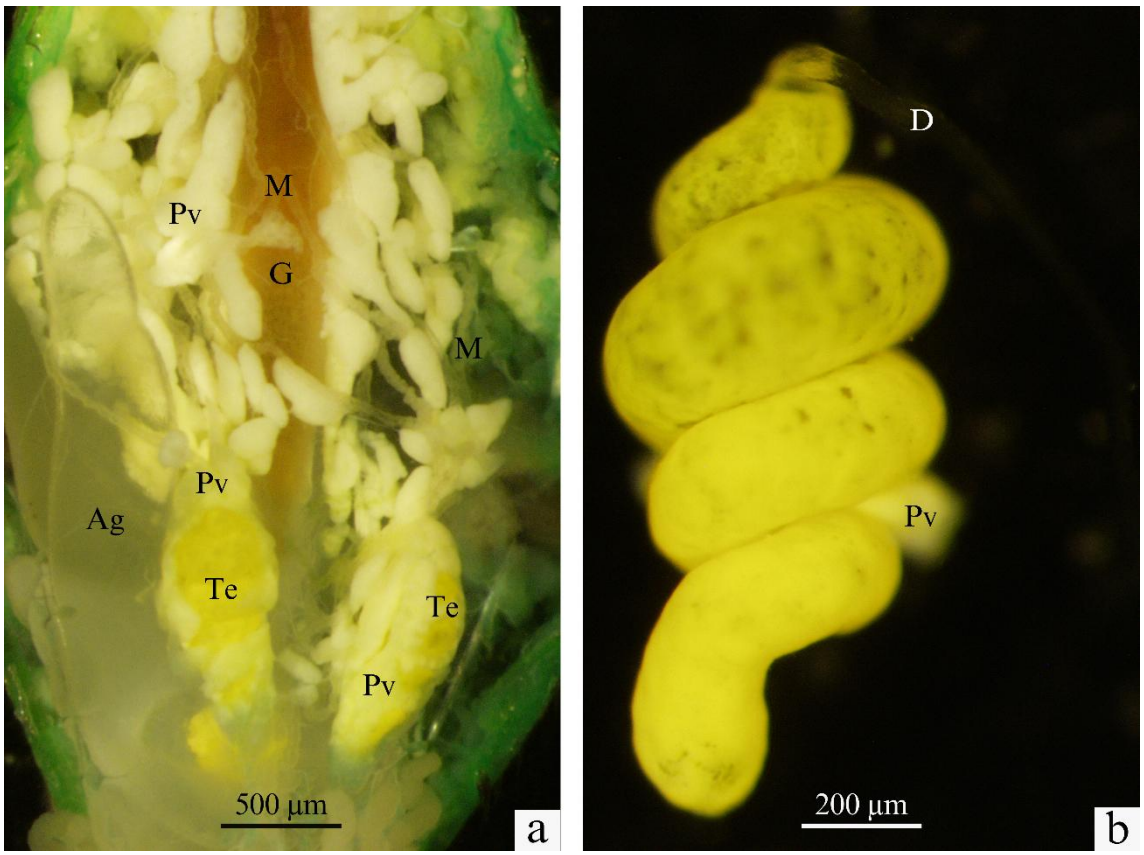


Figure 5.2 – Extraction of the testes from *Ceraeochrysa claveri*. a – Opening of the dorsal region. The testis (Te), gut (G), perivisceral fat (Pv), Malpighian tubules (M), and accessory gland (Ag) are visible. b – Yellow, twisted testis of *C. claveri* with translucent vas deferens (D).

The treated groups exhibited spacing among the cysts and changes in the testis tunic; however, no damage was observed in the spermatogenesis cycle (spermatogonia, spermatocytes, spermatids, and spermatozoa) (Figure 5.3d-l). Transmission electron microscopy revealed the integrity of spermatozoa and spermatids in males exposed to sulfoxaflor (Figure 5.4).

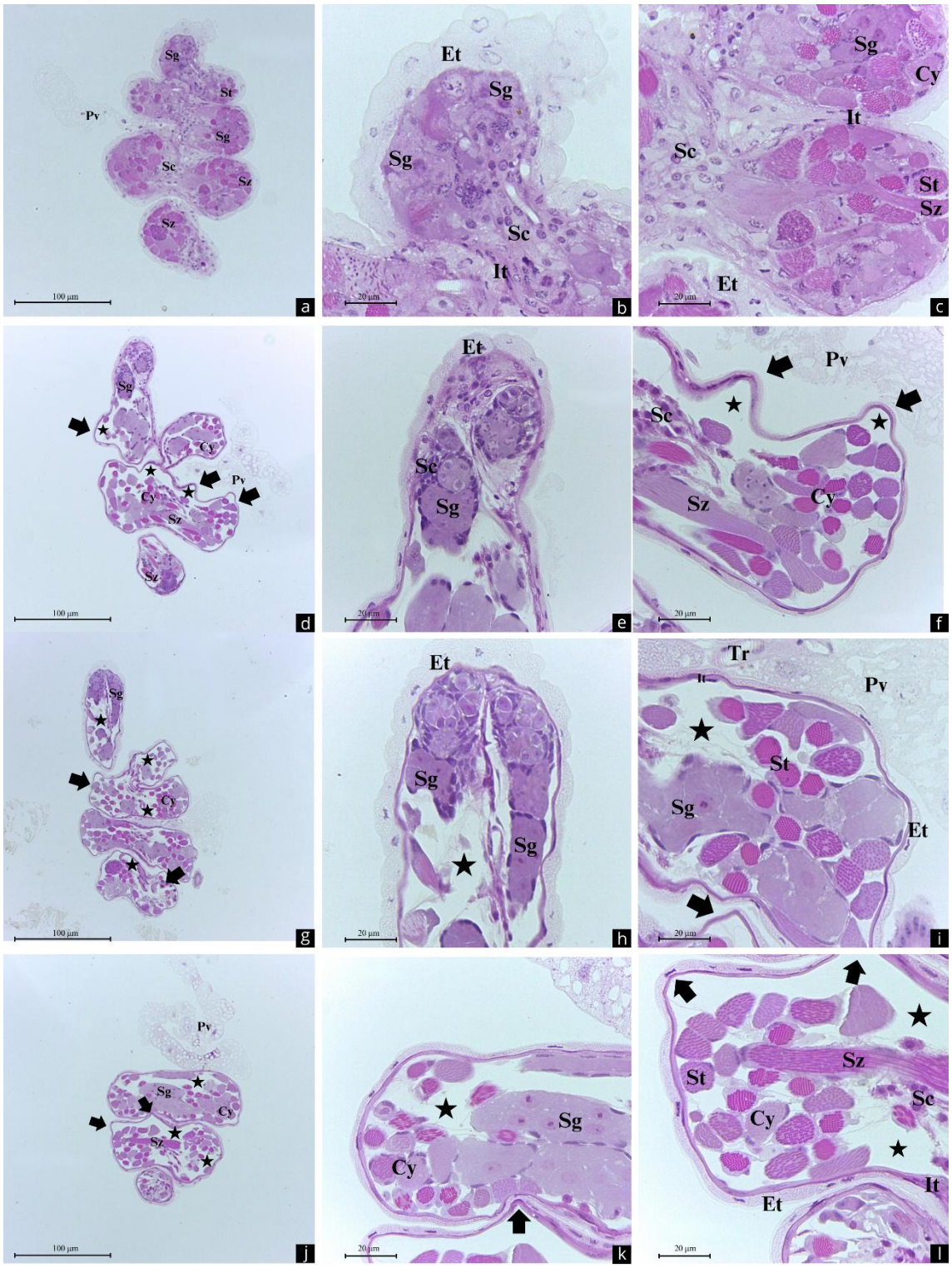


Figure 5.3 – Cross section from the testis of *Ceraeochrysa claveri* exposed to different doses of sulfoxaflor during the larval stage. Control group (a-c). Sulfoxaflor treated groups: d-f (15.02 mg a.i. L^{-1}); g-i (45.05 mg a.i. L^{-1}) and j-l (75.10 mg a.i. L^{-1}). The treated groups showed extracellular spacing (star), and changes in the testis tunic (arrow) but no damages to spermatogenesis and somatic cells (Sc). Spermatogonia (Sg), Cysts

(Cy) containing Spermatocytes (St) and Spermatids (Sp), Spermatozoa (Sz), External tunic (Et), Internal tunic (It), Perivisceral fat (Pv), Trachea (Tr). H.E. staining.

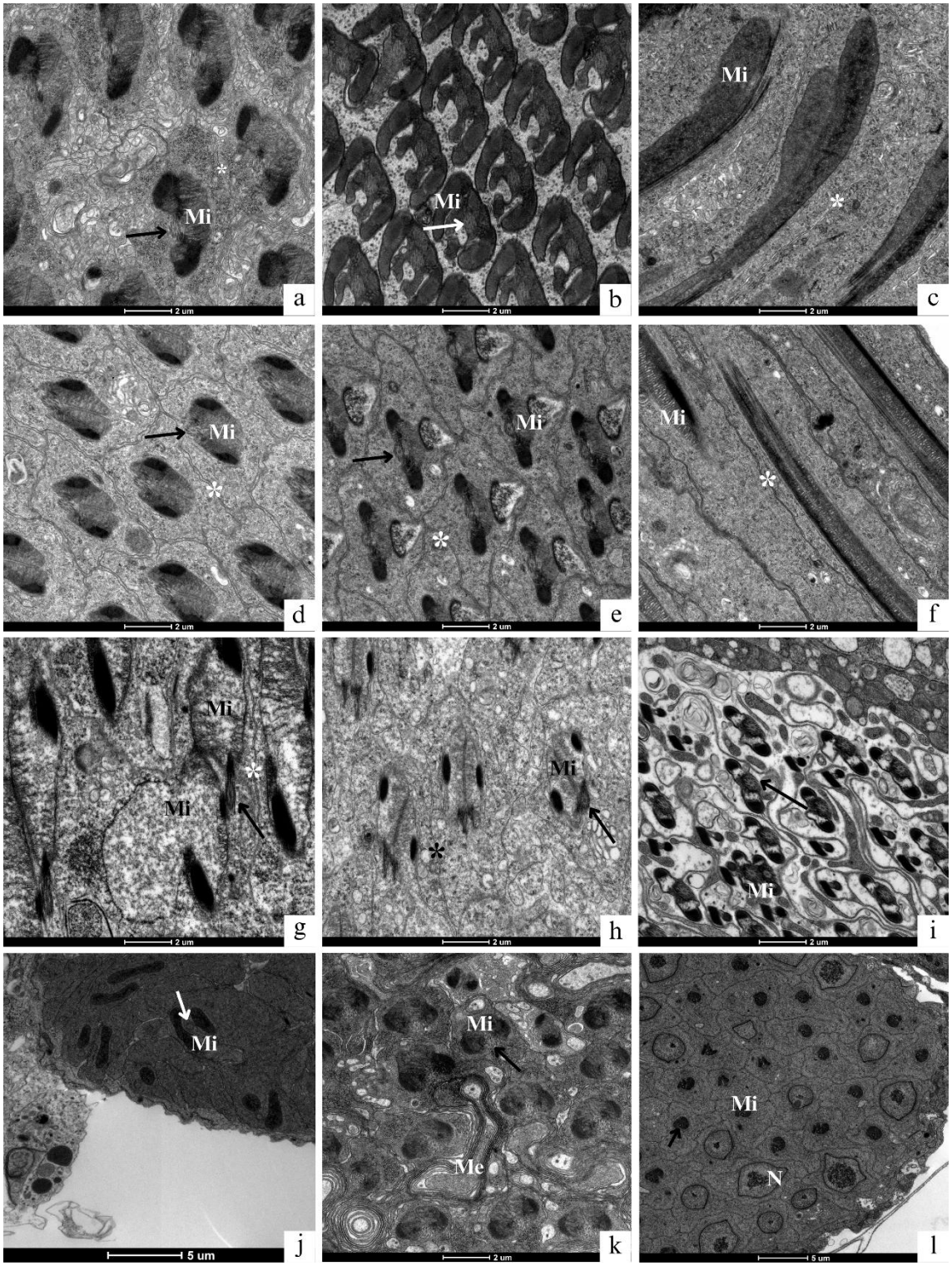


Figure 5.4 – Ultrastructure of adult spermatogenesis of *Ceraeochrysa claveri* under exposition to the insecticide sulfoxaflor during larval phase. TEM – Spermatids (in different stages of development) and spermatozoa (c, f). Control group (a-c). Sulfoxaflor

treatments: d-f (15.02 mg a.i. L^{-1}); g-i (45.05 mg a.i. L^{-1}) and j-l (75.10 mg a.i. L^{-1}). Mitochondria (Mi), membrane (Me), plasmic membrane (*), axoneme (arrow), nucleus (N).

5.4.3 Ovaries

A pair of green polytrophic meroistic ovaries are located below the gut, formed by 10 ovarioles (Figure 5.5). As shown in testes, the ovarioles developmental processes occur from apical to basal part, where the ovarioles are divided into two different development regions: germarium (apical part) and vitellarium, the last one occupying the biggest part of the ovariole (Figure 5.6).

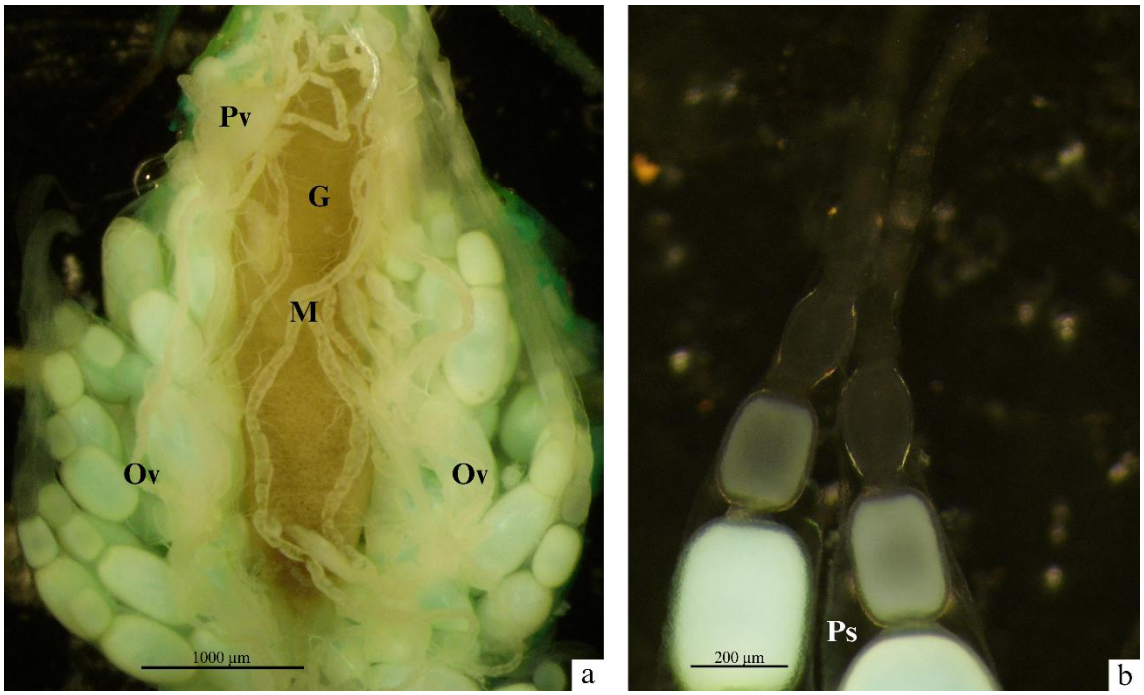


Figure 5.5 – Extraction of the ovaries from *Ceraeochrysa claveri*. a – Opening of dorsal region: Perivisceral fat (Pv), gut (G), Malpighian tubules (M), ovary (Ov). b – Two extracted ovarioles. Ps – Peritoneal sheath.

At the tip of the germarium there is a terminal filament (Figure 5.5e), responsible for attaching the ovarioles to the tegument, and below that there are the germ cells. When a germ cell divides, the closest to the terminal filament cells continue as a germ cell, and the other one becomes a cystoblast, which will divide by unfinished mitosis (incomplete cytokinesis) and turn into cystocytes interconnected by ring canals, forming a germ cell cluster. Within each cluster, the cystocytes will turn into two different cells: the oocyte,

which will become the egg in the oogenesis processes, and the nurse cells, responsible for providing the nourishment of the oocyte (Figure 5.6b).

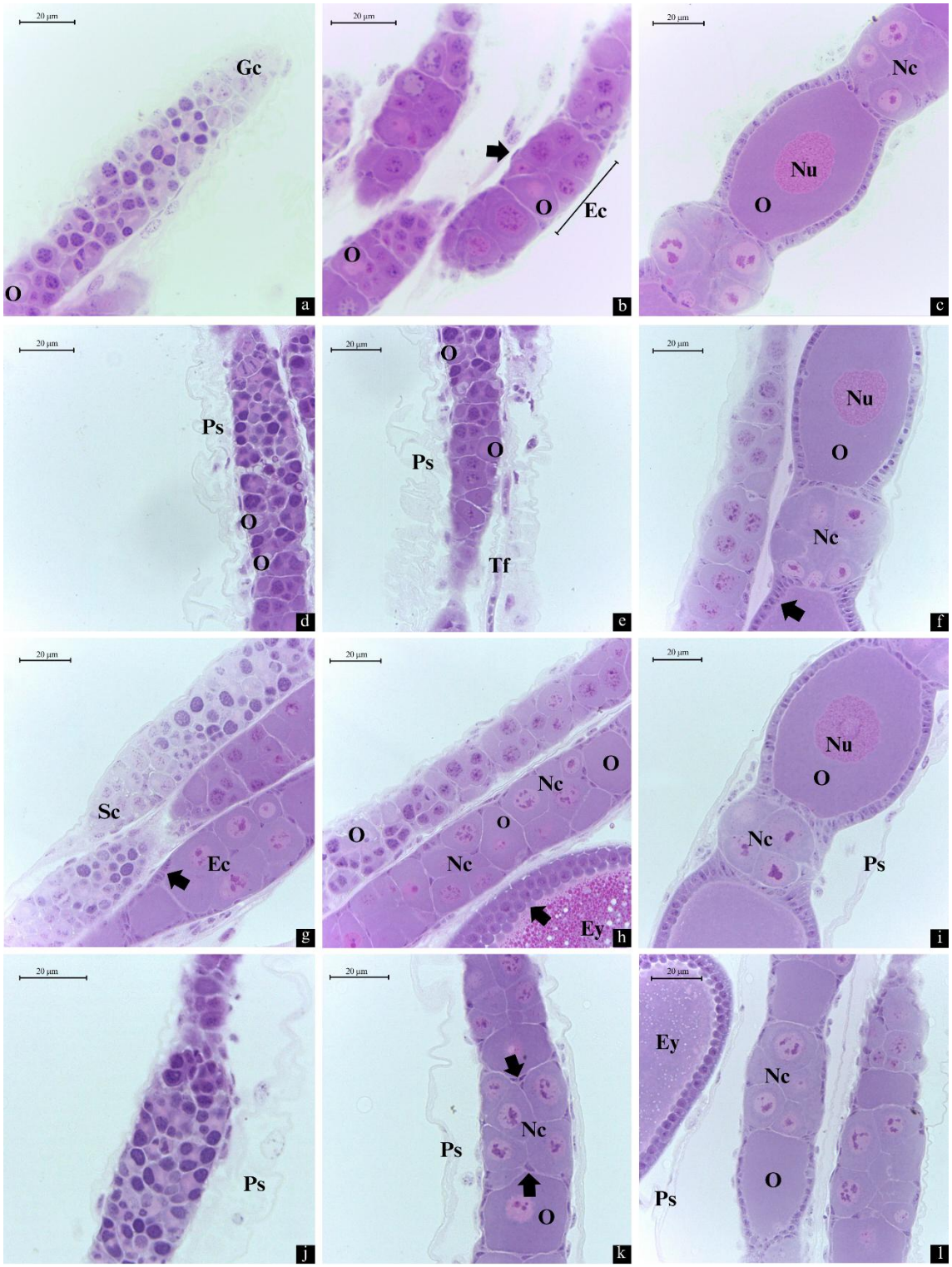


Figure 5.6 – Light microscopy from the ovarioles of adult *Ceraeochrysa claveri* exposed to different doses of sulfoxaflor during the larval stage. Control group – a-c. Sulfoxaflor treated groups: d-f (15.02 mg a.i. L^{-1}); g-i (45.05 mg a.i. L^{-1}) and j-l (75.10 mg a.i. L^{-1}).

Germ cell (Gc), oocyte (O), egg chamber (Ec), nurse cells (Nc), Nucleus (Nu), peritoneal sheath (Ps), egg yolk (Ey). H.E. staining.

No morphological changes were found in ovaries treated with sulfoxaflor compared to the control group when analyzed by light microscopy, but under transmission electron microscopy analysis it was possible to notice some spacing among the cells, with no damage to the cellular compounds (Figure 5.7).

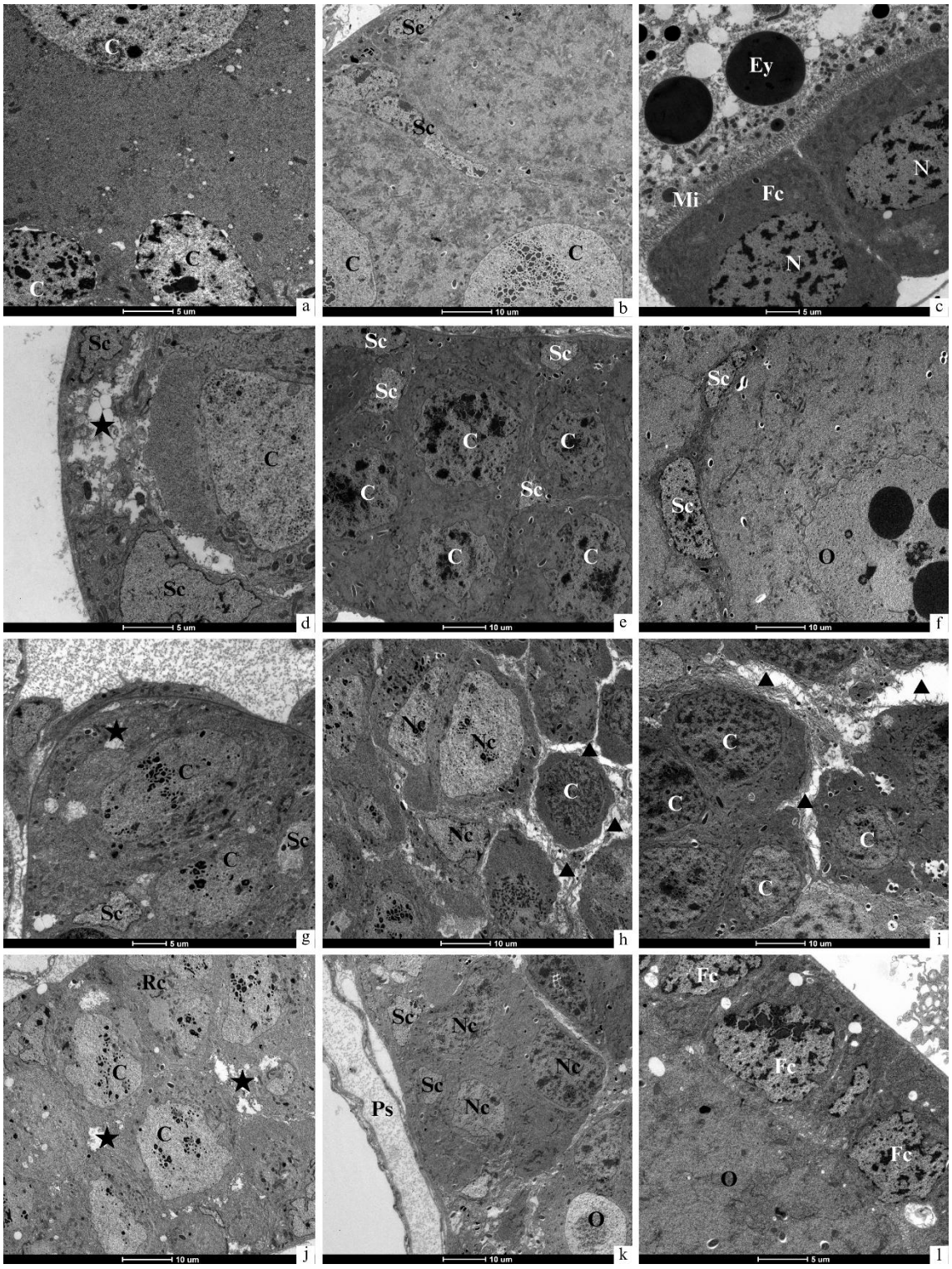


Figure 5.7 – Transmission electron microscopy (TEM) of germ cells from the ovariole of adult *Ceraeochrysa claveri* treated with sulfoxaflor during the larval stage. a – Control group. Sulfoxaflor treatments: (b) 15.02 mg a.i. L^{-1}, (c) 45.05 mg a.i. L^{-1} and (d) 75.10 mg a.i. L^{-1}. Cytoplasmic rarefaction (star) and spacing among cells (triangle). Oocyte (O), somatic cell (Sc), cystocyte (C), ring canal (Rc), follicular cell (Fc), peritoneal sheath (Ps), nucleus (N), microvilli (Mi), egg yolk (Ey).

5.5 DISCUSSION

Neuroptera is the second most important insect order for agricultural purposes due to the predatory behavior of some families within this order. Chrysopids, in particular, are currently widely used insects in integrated pest management (IPM) programs because of their high reproductive capacity, resistance to various insecticides, and voracious larvae (Machado *et al.*, 2024). The genus *Ceraeochrysa* is employed in IPM strategies because of its ease of rearing under laboratory conditions (Machado *et al.*, 2024). Specifically, *Ceraeochrysa claveri* has been used as a model organism in insecticide cytotoxicity assays (Scudeler and Santos, 2013; Scudeler *et al.*, 2014, 2017, 2019, 2022, 2024; Caballero-Gallardo *et al.*, 2023), highlighting its significant ecological importance.

Sulfoxamine insecticide acts as a competitive modulator of nicotinic acetylcholine receptors (4C class), targeting the same site as neonicotinoids (IRAC, 2025). Studies with neonicotinoids have demonstrated that these insecticides can be harmful to chrysopids. For example, doses of clothianidin and imidacloprid used at recommended field levels were classified as harmful to first-instar larvae (0-24 hours) of *Chrysoperla genanigra* (Neuroptera: Chrysopidae). Similarly, newly hatched larvae of *C. externa* exhibited high mortality levels when exposed to Eleitto® (a combination of neonicotinoid and diphenyl ether) by contact on grapevine leaves. In this study, the insecticide was sprayed on the leaves, and after three days, the larvae were exposed to it (Silva *et al.*, 2017; Armas *et al.*, 2023).

Su *et al.* (2022) applied sublethal doses of acetamiprid and dinotefuran via topical drops on the abdomen of second-instar *Chrysopa pallens* larvae, using LD_{30}. The larval and pupal stages were longer than those in the control group. The same delay was observed in the adult preoviposition and total preoviposition periods; however, there were no significant differences in adult longevity, fecundity, or reproductive days. Fecundity (number of eggs laid) of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) was not affected by exposure to sulfoxaflor, although the adult development time was delayed (Aita *et al.*, 2020).

Larvae and adults of *Chrysoperla carnea* responded differently to sulfoxaflor exposure, whether by contact or ingestion. Adults fed on honey contaminated with sulfoxaflor showed more severe effects, including higher mortality rates, than larvae (Barbosa *et al.*, 2017). Additionally, adult *C. carnea* had reduced survivorship after feeding on nectar from flowers treated with imidacloprid (Rogers *et al.*, 2007). Garzón *et al.* (2015), using a sublethal dose of sulfoxaflor (63.6 mg a.i. L^{-1}) on third-instar larvae

and adults of *C. carnea* via residual contact, found no significant difference in pupation time between treated and control larvae. However, adult fertility was affected in the sulfoxaflor group compared to controls.

In our study, *C. claveri* fed on eggs treated with sulfoxaflor showed no significant differences in the developmental times of larvae and pupae for both males and females. Similarly, larval survivorship throughout the experiment did not differ among the three sulfoxaflor treatments compared to the control group ($p > 0.05$) (Figure 5.1). Pereira (2022) found that fecundity and fertility of adult *C. claveri* exposed to the same sulfoxaflor doses used in this study (15.02 mg a.i. L^{-1}, 45.05 mg a.i. L^{-1}, and 75.10 mg a.i. L^{-1}) were lower than those observed in individuals with no insecticide exposure. Interestingly, the lowest dose (15.02 mg a.i. L^{-1}) exhibited the highest mortality rate among the treated groups, which may be due to not reducing food consumption at lower dosages. Similar patterns, where the lowest doses produced great negative effects, have been observed in other studies, such as when *C. claveri* was fed on azadirachtin-contaminated food (Gastelbondo-Pastrana *et al.*, 2025) and sulfoxaflor (Pereira, 2021). In the latter study, *C. claveri* was fed the same sulfoxaflor doses as in the current study.

Regarding the reproductive systems, there is information available about the morphology and histology of the testes and ovaries of chrysopids (Kubrakiewicz, 1997; Garbiec and Kubrakiewicz, 2012; Vacacela *et al.*, 2017; Kuznetsova *et al.*, 2019; Dantas *et al.*, 2020; Church *et al.*, 2021; Liu *et al.*, 2023). However, data focusing on the effects of insecticides on these organs are scarce. Understanding what sublethal doses of insecticides could cause to reproductive organs is important, given the significance of chrysopids to agriculture and the environment.

So far, it is known that sublethal doses of sulfoxaflor may induce undesirable effects on pests (e.g., resurgence) and non-target insects (e.g., reductions in biological parameters) (Siviter *et al.*, 2018, 2020; Lu *et al.*, 2020; Ibraim *et al.*, 2023; Skouras *et al.*, 2023; Wang *et al.*, 2023a, 2023b). For example, *C. claveri* exposed to neem oil showed cellular damage in the testes of larvae, pupae, and adults, along with modifications in spermatogenesis (Garcia *et al.*, 2019). In another study, larvae of *C. claveri* that ingested doses of azadirachtin and pyriproxyfen exhibited ultrastructural changes in the testis tissue, such as spaces among cysts and irregularities in the cellular tunic (Garcia *et al.*, 2020). Additionally, Tomacheski *et al.* (2025) demonstrated changes in gene expression in the testes of adult *C. claveri* following ingestion of pyriproxyfen through contaminated food during the larval stage. Intestinal and ovarian cells from adult *C. claveri* were

evaluated using the comet assay, revealing DNA damage when larvae were fed *D. saccharalis* eggs contaminated with azadirachtin (Gastelbondo-Pastrana *et al.*, 2019). Azadirachtin also affected larval and pupal development times, damaged ovarian ultrastructure, and induced cell death in ovarian follicules (Gastelbondo-Pastrana *et al.*, 2025). Larvae of *C. claveri* fed on *D. saccharalis* eggs contaminated with sulfoxaflor (at the same concentration used in this study) showed deformation of midgut cells, including stretched columnar cells, apical dilatation, and likely cell lysis (Pereira, 2022).

Our findings regarding the reproductive tissues showed that the testes had large spaces among the cysts and some deformation of the tunic, but the cystic cells remained preserved. The ovaries exhibited slight spacing among the germ cells in the ovarioles. Since fecundity and fertility differ between adult *C. claveri* treated and untreated with sulfoxaflor (Pereira, 2022), and despite limited morphological evidence of cell alterations in the testes and ovaries, sulfoxaflor may still potentially impact the lacewing population by reducing the number of offspring.

5.6 CONCLUSION

This study demonstrated that sulfoxaflor did not significantly affect the life cycle of *Ceraeochrysa claveri* larvae. However, it could cause delayed effects on the reproductive organs of adult *C. claveri*, which might threaten their population in the field. More research is needed to better understand the effects of low doses of sulfoxaflor on non-target insects, aiming to support the conservation of these beneficial organisms in agricultural environments. This approach helps preserve the natural ecosystem and reduces the use of agrochemicals that may be incompatible with beneficial insects.

6. CONCLUSION

This study aimed to provide reliable data on the use of the insecticides pyriproxyfen and sulfoxaflor, with a focus on preserving natural enemies. We used *Ceraeochrysa claveri*, a chrysopid known as an effective predator of economically important pest insects, as a model organism. Our findings showed that the insecticides pyriproxyfen and sulfoxaflor can affect the reproductive organs of adult *C. claveri* when they fed on contaminated food during the larval stage. Exposure to pyriproxyfen during the larval stage can have lasting effects throughout the chrysopid's adult life, including changes in gene expression. In contrast, sulfoxaflor did not affect the duration of the larval and pupal development stages of *C. claveri*, but it did cause morphological damages to the testes and ovaries, without affecting the spermatogenic and oogenic lineage. Overall, our data provides valuable insights to support better decision-making in integrated pest management (IPM) strategies, particularly regarding the preservation of chrysopids in the field.

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8. APPENDIX

Table S1 – Pyriproxyfen DEGs

Differentially expressed genes analysis – Pyriproxyfen 50mg vs. Control

Gene-id	Gene-symbol	id	logFC
A0A834Y0Z1	LOC552795	TRINITY_DN2545_c0_g1_i1	-10.3505790191755
A0A7F5R4W8	LOC108734991	TRINITY_DN18627_c0_g1_i4	-3.12654627326808
	unknow	TRINITY_DN18184_c0_g1_i2	-3.99245816810396
	unknow	TRINITY_DN23518_c0_g1_i3	-4.26248960915856
	unknow	TRINITY_DN1160_c1_g1_i13	-6.33701517738979
novel	unknow	TRINITY_DN36979_c0_g1_i6	-6.56221903373465
	unknow	TRINITY_DN6703_c3_g1_i2	-6.63872953928322
	unknow	TRINITY_DN27724_c0_g1_i3	-6.74099482567556
	unknow	TRINITY_DN11415_c0_g1_i3	-6.75205981442829
	unknow	TRINITY_DN2801_c0_g1_i1	-6.89274772906895
T1HJR2	DMC1	TRINITY_DN32392_c0_g1_i4	-7.00411832490832
	unknow	TRINITY_DN24451_c0_g1_i2	-7.03070382822012
	unknow	TRINITY_DN9772_c0_g1_i5	-7.07665641304011
	unknow	TRINITY_DN9785_c0_g1_i3	-7.23080761567204
	unknow	TRINITY_DN191_c0_g1_i5	-7.30430635179891
	unknow	TRINITY_DN1355_c2_g1_i5	-7.40420620197958
	unknow	TRINITY_DN25701_c0_g1_i1	-7.4419593426749
	unknow	TRINITY_DN13994_c0_g2_i4	-7.45933685208057
	unknow	TRINITY_DN886_c0_g1_i11	-7.48252264601387
	unknow	TRINITY_DN2475_c0_g1_i24	-7.52255706691368
A0A834IJA8	Major facilitator superfamily (MFS) profile domain-containing protein	TRINITY_DN11621_c0_g2_i2	-7.55419368314846
A0A084VS76	HexA	TRINITY_DN6406_c0_g1_i3	-7.60333534536872
	unknow	TRINITY_DN15011_c0_g1_i7	-7.86100005480116
A0A2J7PKT1	LOC4814128	TRINITY_DN3094_c0_g1_i29	-7.90737481079492
	unknow	TRINITY_DN6050_c0_g1_i1	-7.9779702773169
A0A8S9X9A4	chloroplast protein-transporting ATPase	TRINITY_DN4127_c0_g1_i4	-8.34793679341058
A0A2J7QP50	unknow	TRINITY_DN785_c0_g2_i4	-8.40815548829055
A0A7M6UVM6	FBPA	TRINITY_DN29009_c0_g1_i2	-8.62430359629696
A0A7J7A4F6	insulin receptor substrate	TRINITY_DN11005_c3_g1_i4	-8.95663413662815
	unknow	TRINITY_DN1060_c0_g1_i9	-9.15384547709813
A0A6J0B309	UBR7	TRINITY_DN11034_c0_g1_i3	-9.34464839126486
A0A7F5RBW5	LOC725179	TRINITY_DN8225_c0_g1_i3	10.2568890521887
A0A7R9GXI9	LOC551272	TRINITY_DN19058_c0_g1_i6	10.2651650495721
A0A1W4WYD2	pab2	TRINITY_DN33145_c0_g1_i1	6.94280498763393
	unknow	TRINITY_DN22541_c0_g1_i4	7.04474969808684
	unknow	TRINITY_DN13786_c1_g1_i18	7.18119772702853
A0A6J1S461	DMC1	TRINITY_DN32392_c0_g1_i15	7.60622597854907
	unknow	TRINITY_DN727_c0_g1_i12	7.81080662854015
A0A6P3Y1T8	LOC409933	TRINITY_DN2075_c0_g1_i4	7.97888168908767
	unknow	TRINITY_DN31410_c0_g1_i9	8.19463208440616
	unknow	TRINITY_DN1358_c0_g1_i2	8.19772448150854
A0A6J0BJE3	LOC107220685	TRINITY_DN5566_c0_g1_i22	8.2685707356042
	unknow	TRINITY_DN3992_c1_g1_i6	8.30528515553383
A0A2J7Q4N6	ipk3	TRINITY_DN8154_c0_g1_i18	8.54492561598094
A0A8K0GJF7	MLX-interacting	TRINITY_DN7952_c2_g1_i3	9.22570239584015
A0A894YZV6	srp54k	TRINITY_DN336_c0_g1_i2	9.95145616739879

blue: down regulated; green: up regulated

Differentially expressed genes analysis – Pyriproxyfen 100 mg vs. Control

Gene-id	Gene-symbol		id	logFC
			TRINITY_DN7603_c0_g1_i2	-11.7036387804545
A0A6J2X3K1	LOC115874684		TRINITY_DN1769_c0_g1_i2	-4.28187419850262
			TRINITY_DN11163_c0_g1_i10	-4.30020247784356
			TRINITY_DN24451_c0_g1_i2	-6.32163045044749
			TRINITY_DN4444_c0_g1_i33	-6.5142603078654
			TRINITY_DN6703_c3_g1_i2	-6.53988829472629
			TRINITY_DN8417_c0_g1_i5	-6.57282249584988
			TRINITY_DN11415_c0_g1_i3	-6.65313871141414
			TRINITY_DN19544_c0_g1_i6	-6.70973053916519
			TRINITY_DN3397_c0_g1_i2	-6.76062987226078
A0A6J0B7K3	udkA Uridine kinase		TRINITY_DN5833_c0_g1_i11	-6.88833788450644
			TRINITY_DN17780_c0_g1_i6	-6.91479728478093
			TRINITY_DN11739_c0_g1_i3	-6.91955000090425
			TRINITY_DN9772_c0_g1_i5	-6.97754286412913
			TRINITY_DN191_c0_g1_i5	-7.20507969181891
			TRINITY_DN2362_c0_g1_i15	-7.21576828110246
			TRINITY_DN16036_c0_g1_i4	-7.24197434651496
			TRINITY_DN4821_c1_g1_i1	-7.27833247445537
A0A6P4J309	LOC108079173		TRINITY_DN21819_c0_g1_i5	-7.42148223347037
A0A7J7A9Y2	cct2		TRINITY_DN124771_c0_g1_i3	-7.42878278003788
			TRINITY_DN9027_c0_g1_i2	-7.75014867168968
A0A7R8VUK5	Glycosyltransferase-like protein large2		TRINITY_DN29058_c0_g1_i6	-7.7685595917068
			TRINITY_DN19698_c0_g1_i2	-7.95072847738175
			TRINITY_DN12483_c0_g1_i4	-8.01606245236611
A0A2J7QNW1	LOC113210784		TRINITY_DN4342_c6_g2_i3	-8.23791447837209
A0A2J7PKT1	LOC4814128		TRINITY_DN3094_c0_g1_i11	-8.35698358697595
D6X3A7	AGM1		TRINITY_DN3872_c0_g1_i11	-8.47768725846543
A0A1B6CY28	Uncharacterized		TRINITY_DN16084_c0_g1_i6	-8.60497901815156
A0A8S3XB87	LOC552612		TRINITY_DN5895_c0_g1_i5	-8.60819620875199
A0A7J7A4F6	Insulin receptor substrate 1		TRINITY_DN11005_c3_g1_i4	-8.85696221617868
			TRINITY_DN1060_c0_g1_i9	-9.0541470141902
E0VSA0		8234317	TRINITY_DN1533_c0_g1_i22	10.318373251825
			TRINITY_DN18969_c0_g1_i2	6.40924479889965
	Glycosyltransferase-like protein large2		TRINITY_DN29058_c0_g1_i7	6.77493782473754
			TRINITY_DN13786_c1_g1_i18	6.91643608553075
			TRINITY_DN6034_c0_g1_i1	7.05635444398142
C1BS44	BPHL		TRINITY_DN6432_c0_g1_i5	7.07913907563457
A0A1W4WYD2	pab2		TRINITY_DN33145_c0_g1_i1	7.2176759267625
Q7KTX8	skd		TRINITY_DN5284_c0_g1_i3	7.31863178165228
			TRINITY_DN1358_c0_g1_i2	8.15667830784418
A0A8K0C9Y2	Hemimethylated DNA-binding domain-containing protein		TRINITY_DN37160_c3_g1_i17	8.48326919112984
A0A7R9GXI9	Talin-1 isoform X1		TRINITY_DN19058_c0_g1_i6	8.76290092906921
			TRINITY_DN727_c0_g1_i12	9.03451554669836
A0A6L2PW18	rno PHD-type domain-containing protein		TRINITY_DN1558_c0_g1_i1	9.05504797520669
A0A6P3Y1T8	LOC409933		TRINITY_DN2075_c0_g1_i4	9.06522085267747
			TRINITY_DN10582_c0_g2_i3	9.36876665581597
A0A894YZV6	srp54		TRINITY_DN336_c0_g1_i2	9.4098558678924

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Gene-id	Gene-symbol	id	logFC
A0A834Y0Z1	LOC552795	TRINITY_DN2545_c0_g1_i1	-10.0435827987525
E0VSA0	8234317	TRINITY_DN1533_c0_g1_i22	-10.3067161658219
		TRINITY_DN3667_c0_g1_i8	-3.36009319708245
W5JX83	AND_000258	TRINITY_DN28_c0_g1_i3	-4.19596058321583
		TRINITY_DN5144_c0_g1_i12	-4.37039533166068
		TRINITY_DN12607_c0_g1_i6	-6.25880485394634
	Glycosyltransferase-like protein large2	TRINITY_DN29058_c0_g1_i7	-6.76337867703349
		TRINITY_DN27724_c0_g1_i3	-6.83105500535991
		TRINITY_DN37253_c0_g2_i2	-6.87042364920458
		TRINITY_DN4427_c0_g1_i6	-6.87874449184146
		TRINITY_DN2801_c0_g1_i1	-6.90428047057225
		TRINITY_DN6034_c0_g1_i1	-7.04477697126592
		TRINITY_DN13994_c0_g2_i4	-7.30360064440451
Q7KTX8	skd	TRINITY_DN5284_c0_g1_i3	-7.30703932673218
		TRINITY_DN15011_c0_g1_i7	-7.46752154969492
		TRINITY_DN13789_c0_g1_i7	-7.50353052022627
		TRINITY_DN28225_c0_g1_i35	-7.55807924295339
		TRINITY_DN1653_c0_g1_i10	-7.59557225818755
		TRINITY_DN2475_c0_g1_i24	-7.61283774869016
		TRINITY_DN3182_c2_g1_i13	-7.74639053397775
		TRINITY_DN6050_c0_g1_i1	-7.75071508114774
		TRINITY_DN6009_c0_g1_i1	-7.91082313917855
A0A1Y1L5D7	Covalently-linked cell wall protein	TRINITY_DN146357_c0_g1_i1	-8.24058623032527
A0A084VS76	HexA	TRINITY_DN6406_c0_g1_i3	-8.27691094497325
A0A8S9X9A4	chloroplast protein-transporting ATPase	TRINITY_DN4127_c0_g1_i4	-8.27746408182977
A0A2J7QP50	Uncharacterized protein	TRINITY_DN785_c0_g2_i4	-8.4670198426473
		TRINITY_DN886_c0_g1_i11	-8.68466221626145
A0A2J7PKT1	LOC4814128	TRINITY_DN3094_c0_g1_i29	-8.96399966084124
A0A6L2PW18	rno	TRINITY_DN1558_c0_g1_i1	-9.04340369707671
Q9VBX2	Mocs2	TRINITY_DN3781_c0_g1_i27	-9.25252822134116
A0A6J0B309	UBR7	TRINITY_DN11034_c0_g1_i3	-9.33684250665681
D6X3A7	AGM1	TRINITY_DN3872_c0_g1_i11	10.03145735073
A0A7F5RBW5	LOC725179	TRINITY_DN8225_c0_g1_i3	10.1454376333364
		TRINITY_DN7603_c0_g1_i2	10.154558849613
		TRINITY_DN11163_c0_g1_i10	4.31437136149309
A0A6J2X3K1	LOC115874684	TRINITY_DN1769_c0_g1_i2	4.32853049019895
A0A140KPR8	hpgd	TRINITY_DN107326_c0_g1_i2	6.40288032198779
A0A6P4J309	LOC108079173	TRINITY_DN21819_c0_g1_i5	6.54660822468493
A0A1B0FHL1	Rhomboid-like protein	TRINITY_DN3789_c1_g1_i2	6.65444197319084
A0A6P7G295	LOC114333481	TRINITY_DN8480_c0_g1_i1	6.8198169034339
A0A7R8VUK5	Glycosyltransferase-like protein large2	TRINITY_DN29058_c0_g1_i6	6.8297760334599
		TRINITY_DN22091_c1_g1_i1	6.88736891405282
		TRINITY_DN8417_c0_g1_i5	7.01543770506659
		TRINITY_DN19021_c0_g1_i1	7.10907915961526
		TRINITY_DN6422_c0_g1_i3	7.31450102638207
A0A921YYL8	LOC111601668	TRINITY_DN1641_c2_g1_i2	7.33155322077195
		TRINITY_DN2362_c0_g1_i15	7.36403978481786
		TRINITY_DN11739_c0_g1_i3	7.37741630845932
		TRINITY_DN12483_c0_g1_i4	7.45358220044231
		TRINITY_DN9027_c0_g1_i2	7.46099515047817
A0A6J2YBX7	Sesn	TRINITY_DN2830_c0_g1_i1	7.46547039390489
		TRINITY_DN19698_c0_g1_i2	7.58948834565742
A0A7J6ZK76	CREB-regulated transcription coactivator 1	TRINITY_DN23694_c0_g1_i1	7.66256597292129
		TRINITY_DN4821_c1_g1_i1	7.66936262232651
A0A2J7QNW1	LOC113210784	TRINITY_DN4342_c6_g2_i3	7.76986794357084
		TRINITY_DN3182_c2_g1_i8	7.79824122682125
A0A8S3XB87	LOC552612	TRINITY_DN5895_c0_g1_i5	8.04078117513373
D2A0T7	guf1	TRINITY_DN11551_c0_g1_i2	8.14613821947302
A0A6J0BJE3	tid	TRINITY_DN5566_c0_g1_i22	8.15740080028473
		TRINITY_DN3992_c1_g1_i6	8.19410533228773
A0A8K0D829	LOC552578	TRINITY_DN28608_c0_g1_i4	8.42542531916994
A0A8S9X9A4	chloroplast protein-transporting ATPase	TRINITY_DN4127_c0_g1_i8	8.4901053464253
A0A1B6CY28	Uncharacterized protein	TRINITY_DN16084_c0_g1_i6	8.87938811696508
A0A2J7PKT1	LOC4814128	TRINITY_DN3094_c0_g1_i11	9.23540912480811

blue: down regulated; green: up regulated

Table S2 – UniProt search

#Annotation summary	
Number of query sequences:	228406
With Annotations	19985
With GENENAME	19312
With DESCRIPTION	19912
With ENZYME	6593
With GO	19290
With KEYWORD	18885
With PATHWAY	1903
Annotator a1	0
Annotator a2	0
Annotator a3	6211
Annotator a12	1626
Annotator a123	12148

#UniProt Pathways	
Protein modification	526
Carbohydrate degradation	186
Lipid metabolism	180
Carbohydrate metabolism	118
Amino-acid biosynthesis	94
Cofactor biosynthesis	88
Amino-acid degradation	88
Purine metabolism	82
Pyrimidine metabolism	79
Nucleotide-sugar biosynthesis	58
Glycolipid biosynthesis	48
Phospholipid metabolism	38
Glycan biosynthesis	37
Carbohydrate biosynthesis	33
tRNA modification	29
Porphyrin-containing compound metabolism	29
Sulfur metabolism	29
Energy metabolism	24
Polyol metabolism	23
Metabolic intermediate biosynthesis	22
One-carbon metabolism	16
Isoprenoid biosynthesis	15
Amine and polyamine biosynthesis	12
Catecholamine biosynthesis	11
Alcohol metabolism	11

Metabolic intermediate metabolism	11
Fermentation	11
Nitrogen metabolism	10
Amino-acid metabolism	10
Genetic information processing	10
Aromatic compound metabolism	9
Cofactor metabolism	9
Glycan metabolism	8
Protein biosynthesis	7
Secondary metabolite metabolism	6
Protein degradation	6
Glycerolipid metabolism	5
Aminoacyl-tRNA biosynthesis	5
Sphingolipid metabolism	4
Steroid biosynthesis	3
Ketone metabolism	2
Pigment biosynthesis	2
Organosulfur biosynthesis	2
Signal transduction	2
Carbohydrate acid metabolism	1
Hormone biosynthesis	1
Quinol/quinone metabolism	1
Steroid metabolism	1
Organosulfur degradation	1
Pheromone biosynthesis	1

#UniProt Keyword categories	
#Category "Biological_process"	
Transcription	1884
Transport	1509
Cell cycle	566
Differentiation	522
Ubl conjugation pathway	487
Neurogenesis	487
Lipid metabolism	444
mRNA processing	443
Protein biosynthesis	432
Chemotaxis	416
Sensory transduction	406
DNA damage	309
Stress response	256
Meiosis	208
Glycolysis	206
DNA recombination	206

Carbohydrate metabolism	179
Cell adhesion	179
Ribosome biogenesis	170
tRNA processing	160
Tricarboxylic acid cycle	155
rRNA processing	154
Behavior	143
Immunity	141
DNA replication	135
Translation regulation	131
Autophagy	128
Apoptosis	124
Wnt signaling pathway	103
DNA integration	100
Biological rhythms	93
RNA-mediated gene silencing	86
Endocytosis	84
Phagocytosis	80
Notch signaling pathway	76
Growth regulation	75
Amino-acid biosynthesis	73
Cilium biogenesis/degradation	68
One-carbon metabolism	67
Exocytosis	62
Nucleotide metabolism	59
ATP synthesis	57
Digestion	46
GPI-anchor biosynthesis	46
Purine biosynthesis	43
Gluconeogenesis	41
Necrosis	38
Chromosome partition	36
cGMP biosynthesis	36
Heme biosynthesis	33
Nonsense-mediated mRNA decay	33
Neurotransmitter degradation	32
Antiviral defense	32
Pyrimidine biosynthesis	32
Ubiquinone biosynthesis	31
Detoxification	29
Porphyrin biosynthesis	28
Hydrogen peroxide	26
Peroxisome biogenesis	26
DNA condensation	25

cAMP biosynthesis	24
Purine salvage	23
Isoprene biosynthesis	22
Phenylalanine catabolism	22
Molybdenum cofactor biosynthesis	21
Nucleotide biosynthesis	21
Tetrahydrobiopterin biosynthesis	20
Pentose shunt	20
Gastrulation	19
Neurotransmitter biosynthesis	19
Pyridine nucleotide biosynthesis	19
Luminescence	18
Steroidogenesis	18
Catecholamine biosynthesis	18
Glutathione biosynthesis	16
Purine metabolism	15
Glyoxylate bypass	15
Melanin biosynthesis	13
Collagen degradation	12
Tyrosine catabolism	12
Myogenesis	12
Polyamine biosynthesis	12
Gluconate utilization	12
Glycogen biosynthesis	11
DNA synthesis	11
Deoxyribonucleotide synthesis	10
Serotonin biosynthesis	10
Catecholamine metabolism	9
Iron storage	9
Fertilization	9
Cell shape	8
Unfolded protein response	7
Glycerol metabolism	7
Insecticide resistance	7
Hypusine biosynthesis	6
Branched-chain amino acid catabolism	6
Sporulation	6
Spermidine biosynthesis	5
DNA excision	5
Cytolysis	5
Urea cycle	4
Flight	4
Antibiotic resistance	4
Proline metabolism	4

Transposition	4
Inositol biosynthesis	4
Pyridoxine biosynthesis	3
Arginine metabolism	3
Carnitine biosynthesis	3
Aromatic hydrocarbons catabolism	3
Diaminopimelate biosynthesis	2
Hemolymph clotting	2
Virulence	2
Host-virus interaction	1
Cell wall biogenesis/degradation	1
Pheromone response	1
Menaquinone biosynthesis	1
Histidine metabolism	1
Photosynthesis	1
Carbon dioxide fixation	1
Inflammatory response	1
Riboflavin biosynthesis	1
Tryptophan catabolism	1
Queuosine biosynthesis	1
Leukotriene biosynthesis	1

#Category "Cellular_component"	
Membrane	3724
Nucleus	2962
Cytoplasm	2753
Mitochondrion	846
Endoplasmic reticulum	836
Cell projection	587
Secreted	480
Golgi apparatus	415
Microtubule	379
Chromosome	346
Endosome	298
Cytoplasmic vesicle	262
Spliceosome	250
Proteasome	198
Cell junction	185
Peroxisome	165
Lysosome	145
Cuticle	137
DNA-directed RNA polymerase	136
Dynein	122
Vacuole	113

Plastid	69
Virion	68
Centromere	65
Glycosome	61
Lipid droplet	40
Sarcoplasmic reticulum	34
Kinetochore	31
Signal recognition particle	25
Signalosome	24
Hydrogenosome	20
Exosome	14
Thick filament	11
Primosome	5
Amyloid	3
Thylakoid	2
Intermediate filament	2
Nematocyst	2
Host nucleus	1
Host cytoplasm	1
#Category "Developmental_stage"	
Merozoite	28
Bradyzoite	1
#Category "Disease"	
Allergen	180
Proto-oncogene	33
Tumor suppressor	9
Malaria	5
Neurodegeneration	1
Oncogene	1

Table S3 – Gene Ontology (GO) of the DEGs

Gene	GO/ UniProt Code	Biological Process (BP); Molecular Function (MF); Cellular Component (CC)
Inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase	GO:0032958 GO:0016310 GO:0006020	BP: inositol phosphate biosynthetic process; phosphorylation; inositol metabolic process.
	GO:0005524 GO:0000827 GO:0052723 GO:0052724 GO:0000829 GO:0000828 GO:0033857 GO:0000832	MF: ATP binding; inositol-1,3,4,5,6-pentakisphosphate kinase activity; inositol hexakisphosphate 1-kinase activity; inositol hexakisphosphate 3-kinase activity; inositol heptakisphosphate kinase activity; inositol hexakisphosphate kinase activity; diphosphoinositol-pentakisphosphate kinase activity; inositol hexakisphosphate 5-kinase activity.
	GO:0005829	CC: cytosol.
Talin 1	GO:0007155 GO:0030182 GO:0009887 GO:0048731	BP: cell adhesion; neuron differentiation; animal organ morphogenesis; system development.
	GO:0004497 GO:0016705 GO:0003779 GO:0005200 GO:0051015	MF: monooxygenase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; actin binding; structural constituent of cytoskeleton; actin filament binding.
	GO:0001726 GO:0005737 GO:0005856 GO:0005925	CC: ruffle; cytoplasm; cytoskeleton; focal adhesion.

Polyadenylate-binding protein 2 Polyadenylate-binding protein 2	GO:0006378	BP: mRNA polyadenylation
	GO:0003723 GO:0005085 GO:0008143	MF: RNA binding; guanyl-nucleotide exchange factor activity; poly(A) binding.
	GO:0005634 GO:0055037	CC: nucleus; recycling endosome.
Meiotic recombination protein DMC1/LIM15 homolog	GO:0006302 GO:0042771 GO:0048477 GO:0006268 GO:0051106 GO:0042148 GO:0006310 GO:0070192 GO:0006281 GO:0044671 GO:0007127 GO:0043066 GO:0007294 GO:0000724 GO:0010569 GO:0010032 GO:0030717 GO:0007143 GO:0009949 GO:0007131 GO:0009792 GO:0030716 GO:0006312	BP: double-strand break repair; intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator; oogenesis; DNA unwinding involved in DNA replication; positive regulation of DNA ligation; strand invasion; DNA recombination; chromosome organization involved in meiotic cell cycle; DNA repair; sorocarp spore cell differentiation; meiosis I; negative regulation of apoptotic process; germarium-derived oocyte fate determination; double-strand break repair via homologous recombination; regulation of double-strand break repair via homologous recombination; meiotic chromosome condensation; oocyte karyosome formation; female meiotic nuclear division; polarity specification of anterior/posterior axis; reciprocal meiotic recombination; embryo development ending in birth or egg hatching; oocyte fate determination; mitotic recombination; polarity specification of dorsal/ventral axis; double-strand break repair via synthesis-dependent strand annealing; cell motility; mitotic recombination-dependent replication fork processing; DNA recombinase assembly; intracellular mRNA localization.

Meiotic recombination protein DMC1/LIM15 homolog	GO:0009951 GO:0045003 GO:0048870 GO:1990426 GO:0000730 GO:0008298	
	GO:0003690 GO:0003697 GO:0000150 GO:0042802 GO:0140664 GO:0017116 GO:0017151 GO:0005524 GO:0008094	MF: double-stranded DNA binding; single-stranded DNA binding; DNA strand exchange activity; identical protein binding; ATP-dependent DNA damage sensor activity; single-stranded DNA helicase activity; DEAD/H-box RNA helicase binding; ATP binding; ATP-dependent activity, acting on DNA.
	GO:0043073 GO:0005634 GO:0000794 GO:0000785 GO:0035861	CC: germ cell nucleus; nucleus; condensed nuclear chromosome; chromatin; site of double-strand break.
ipk3	GO:0032958 GO:0016310	BP: inositol phosphate biosynthetic process; phosphorylation.
	GO:0051765 GO:0016301 GO:0008440	MF: inositol tetrakisphosphate kinase activity; kinase activity; inositol-1,4,5-trisphosphate 3-kinase activity.
	A0A2J7Q4N6	CC: cytoplasm; nucleus.
tid	GO:0002119 GO:0006457 GO:0009792 GO:0045879	BP: nematode larval development; protein folding; embryo development ending in birth or egg hatching; negative regulation of smoothened signaling pathway; response to heat; mitochondrion organization; chaperone-mediated protein folding; negative regulation of apoptotic process; chromatin organization.

tid	GO:0009408 GO:0007005 GO:0061077 GO:0043066 GO:0006325	
	GO:0005113 GO:0005524 GO:0051082 GO:0030544 GO:0031072 GO:0046872	MF: patched binding; ATP binding; unfolded protein binding; Hsp70 protein binding; heat shock protein binding; metal ion binding.
	GO:0005829 GO:0005739 GO:0005694 GO:0005741	CC: cytosol ;mitochondrion; chromosome; mitochondrial outer membrane.
MLX-interacting	GO:0070328 GO:0042593 GO:0009744	BP: triglyceride homeostasis; glucose homeostasis; response to sucrose.
	GO:0046983 A0A8K0GJF7	MF: protein dimerization activity; DNA-binding transcription factor activity, RNA polymerase II-specific; RNA polymerase II cis-regulatory region sequence-specific DNA binding.
	A0A8K0GJF7	CC: cytoplasm; nucleus.
srp54k	GO:0006616 GO:0006614	BP: SRP-dependent cotranslational protein targeting to membrane, translocation; SRP-dependent cotranslational protein targeting to membrane.
	GO:0008312 GO:0003924 GO:0030942	MF: 7S RNA binding; GTPase activity; endoplasmic reticulum signal peptide binding; GTP binding.

	GO:0005525	
srp54k	GO:0005783 GO:0005786 GO:0048500 GO:0016607 GO:0045335	CC: endoplasmic reticulum; signal recognition particle, endoplasmic reticulum targeting; signal recognition particle; nuclear speck; phagocytic vesicle.
Cholinephosphotransferase 1	GO:0008654	BP: Phospholipid biosynthetic process;
	GO:0016780 GO:0016747	MF: phosphotransferase activity, for other substituted phosphate groups; acyltransferase activity, transferring groups other than amino-acyl groups.
	GO:0016020	CC: Membrane.
Meiotic recombination protein dmc1	GO:0009949 GO:0007143 GO:0030717 GO:0010032 GO:0009951 GO:0045003 GO:0006312 GO:0030716 GO:0009792 GO:0007131 GO:0000730 GO:1990426 GO:0048870 GO:0008298 GO:0006302 GO:0006268 GO:0051106 GO:0042771 GO:0048477	BP: polarity specification of anterior/posterior axis; female meiotic nuclear division; oocyte karyosome formation; meiotic chromosome condensation; polarity specification of dorsal/ventral axis; double-strand break repair via synthesis-dependent strand annealing; mitotic recombination; oocyte fate determination; embryo development ending in birth or egg hatching; reciprocal meiotic recombination;DNA recombinase assembly; mitotic recombination-dependent replication fork processing; cell motility; intracellular mRNA localization; double-strand break repair; DNA unwinding involved in DNA replication; positive regulation of DNA ligation; intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator; oogenesis; DNA repair; sorocarp spore cell differentiation; chromosome organization involved in meiotic cell cycle; DNA recombination; strand invasion; regulation of double-strand break repair via homologous recombination; double-strand break repair via homologous recombination; germarium-derived oocyte fate determination; negative regulation of apoptotic process; meiosis I.

Meiotic recombination protein dmc1	GO:0006281 GO:0044671 GO:0070192 GO:0006310 GO:0042148 GO:0010569 GO:0000724 GO:0007294 GO:0043066 GO:0007127	
	GO:0017116 GO:0140664 GO:0005524 GO:0017151 GO:0003677 GO:0008094 GO:0003690 GO:0003697 GO:0042802 GO:0000150	MF: single-stranded DNA helicase activity; ATP-dependent DNA damage sensor activity; ATP binding; DEAD/H-box RNA helicase binding; DNA binding; ATP-dependent activity, acting on DNA; double-stranded DNA binding; single-stranded DNA binding; identical protein binding; DNA strand exchange activity.
	GO:0000785 GO:0035861 GO:0043073 GO:0000794 GO:0005634	CC: chromatin; site of double-strand break; germ cell nucleus; condensed nuclear chromosome; nucleus.
Major facilitator superfamily (MFS) profile domain-containing protein	A0A834IJA8 (UniProt code)	MF: sugar transmembrane transporter activity. CC: plasma membrane.
HexA	GO:0045735 GO:0005615	MF: nutrient reservoir activity. CC: extracellular space; larval serum protein complex.

	GO:0005616	
WD repeat domain phosphoinositide-interacting protein 2	GO:0000422 GO:0044804 GO:0010506 GO:0009267 GO:0061365 GO:0098792 GO:0010508 GO:0036093 GO:0043277 GO:0009792 GO:0061723 GO:0006497 GO:0051607 GO:0040024 GO:0035096 GO:0001778 GO:0034497 GO:0008340 GO:0012501 GO:0006914 GO:0097237 GO:0000045 GO:0042078 GO:0050688 GO:0048598 GO:0014067 GO:0030163	BP: autophagy of mitochondrion; autophagy of nucleus; regulation of autophagy; cellular response to starvation; positive regulation of triglyceride lipase activity; xenophagy; positive regulation of autophagy; germ cell proliferation; apoptotic cell clearance; embryo development ending in birth or egg hatching; glycophagy; protein lipidation; defense response to virus; dauer larval development; larval midgut cell programmed cell death; plasma membrane repair; protein localization to phagophore assembly site; determination of adult lifespan; programmed cell death; autophagy; cellular response to toxic substance; autophagosome assembly; germ-line stem cell division; regulation of defense response to virus; embryonic morphogenesis; negative regulation of phosphatidylinositol 3-kinase signaling; protein catabolic process.

WD repeat domain phosphoinositide-interacting protein 2	GO:0080025 GO:0070273 GO:0010314 GO:0032266	MF: phosphatidylinositol-3,5-bisphosphate binding; phosphatidylinositol-4-phosphate binding; phosphatidylinositol-5-phosphate binding; phosphatidylinositol-3-phosphate binding.
	GO:0019898 GO:0034045 GO:0045335 GO:0005776 GO:0030670	CC: extrinsic component of membrane; phagophore assembly site membrane; phagocytic vesicle; autophagosome; phagocytic vesicle membrane.
Fructose-biphosphate aldolase	GO:0008154 GO:0007498 GO:0006000 GO:0006096 GO:0042593 GO:0051289 GO:0030388	BP: actin polymerization or depolymerization; mesoderm development; fructose metabolic process; glycolytic process; glucose homeostasis; protein homotetramerization; fructose 1,6-bisphosphate metabolic process.
	GO:0042802 GO:0004332 GO:0003779	MF: identical protein binding; fructose-bisphosphate aldolase activity; actin binding.
	GO:0016020 GO:0045335 GO:0055120 GO:0031012 GO:0030018 GO:0005829 GO:0020015 GO:0000792 GO:0005737 GO:0020002 GO:0031430	CC: membrane; phagocytic vesicle; striated muscle dense body; extracellular matrix; Z disc; cytosol; glycosome; heterochromatin; cytoplasm; host cell plasma membrane; M band; sarcomere.

	GO:0030017	
Insulin receptor substrate 1	GO:0010897 GO:0042594 GO:0035264 GO:0007568 GO:0014068 GO:0008286 GO:0060250 GO:0007285 GO:0045793 GO:0009267 GO:0035159 GO:0040018 GO:0008284 GO:0034059 GO:1903688 GO:0060291 GO:0061964 GO:0045927 GO:0046622 GO:0048133 GO:0048477 GO:0008355 GO:0050778 GO:0007296 GO:0007295 GO:0048009 GO:0042593 GO:0055088 GO:0043491	BP: negative regulation of triglyceride catabolic process; response to starvation; multicellular organism growth; aging; positive regulation of phosphatidylinositol 3-kinase signaling; insulin receptor signaling pathway; germ-line stem-cell niche homeostasis; primary spermatocyte growth; positive regulation of cell size; cellular response to starvation; regulation of tube length, open tracheal system; positive regulation of multicellular organism growth; positive regulation of cell population proliferation; response to anoxia; positive regulation of border follicle cell migration; long-term synaptic potentiation; negative regulation of entry into reproductive diapause; positive regulation of growth; positive regulation of organ growth; male germ-line stem cell asymmetric division; oogenesis; olfactory learning; positive regulation of immune response; vitellogenesis; growth of a germarium-derived egg chamber; insulin-like growth factor receptor signaling pathway; glucose homeostasis; lipid homeostasis; protein kinase B signaling; determination of adult lifespan.

	GO:0008340	
	GO:0043548 GO:0005158 GO:0042169 GO:0005159	MF: phosphatidylinositol 3-kinase binding; insulin receptor binding; SH2 domain binding; insulin-like growth factor receptor binding.
	GO:0005938 GO:0005829 GO:0043231	CC: cell cortex; cytosol; intracellular membrane-bounded organelle.
E3 ubiquitin-protein ligase UBR7	GO:0008270 GO:0016874 GO:0061630	MF: ubiquitin protein ligase activity; zinc ion binding; ligase activity.
	A0A6J0B309	CC: cytoplasm.
V-type proton ATPase subunit G	E0VSA0	MF: proton-transporting ATPase activity; rotational mechanism.
		CC: vacuolar proton-transporting V-type ATPase complex.
Glycosyltransferase-like protein large2	A0A7R8VUK5	BP: protein O-linked mannolysation
	GO:0016757 A0A7R8VUK5	MF: glycosyltransferase activity; glucuronosyltransferase activity; xylosyltransferase activity.
	GO:0016020 A0A7R8VUK5	CC: membrane; Golgi apparatus.
BPHL	GO:0016787	MF: hydrolase activity
pab2	GO:0006378	BP: mRNA polyadenylation
	GO:0003723 GO:0005085 GO:0008143	MF: RNA binding; guanyl-nucleotide exchange factor activity; poly(A) binding.
	GO:0005634 GO:0055037	CC: nucleus; recycling endosome.
skd	GO:0006367 GO:0036011 GO:0048749	BP: transcription initiation at RNA polymerase II promoter; imaginal disc-derived leg segmentation; compound eye development; wing disc dorsal/ventral pattern formation; cell fate commitment; sex comb development; regulation of transcription by RNA polymerase II;

skd	GO:0048190 GO:0045165 GO:0045498 GO:0006357 GO:0045944 GO:0007526 GO:0022416 GO:0090263	positive regulation of transcription by RNA polymerase II; larval somatic muscle development; chaeta development; positive regulation of canonical Wnt signaling pathway.
	GO:0003712 GO:0003713	MF: transcription coregulator activity; transcription coactivator activity.
	GO:1990904 GO:0005654 GO:0016592 GO:0070847 GO:0005840	CC: ribonucleoprotein complex; nucleoplasm; mediator complex; core mediator complex; ribosome.
Hemimethylated DNA-binding domain-containing protein	A0A8K0C9Y2	MF: DNA binding
PHD-type domain-containing protein	GO:0006325 GO:0042051 GO:0035194 GO:0034729 GO:0006357	BP: chromatin organization; compound eye photoreceptor development; ncRNA-mediated post-transcriptional gene silencing; histone H3-K79 methylation; regulation of transcription by RNA polymerase II.
	GO:0035064 GO:0003682 GO:0031491 GO:0010698 GO:0046872 GO:0042393	MF: methylated histone binding; chromatin binding; nucleosome binding; acetyltransferase activator activity; metal ion binding; histone binding.
	GO:0000793 GO:0005694	CC: condensed chromosome; chromosome;nucleus; histone methyltransferase complex; MOZ/MORF histone acetyltransferase complex; chromatin.

	GO:0005634 GO:0035097 GO:0070776 GO:0000785	
Glucose transporter type 1	GO:0015749 GO:0090277 GO:0008643 GO:1904659 GO:0042593	BP: monosaccharide transmembrane transport; positive regulation of peptide hormone secretion; carbohydrate transport; glucose transmembrane transport; glucose homeostasis.
	GO:0015149 GO:0022857	MF: hexose transmembrane transporter activity; transmembrane transporter activity.
	GO:0016020 GO:0016323	CC: membrane; basolateral plasma membrane.
Galectin	A0A6J2X3K1	MF: Carbohydrate binding;
udkA Uridine kinase	GO:0044206 GO:0008655 GO:1901564 GO:0044211 GO:0016310 GO:0044238 GO:0009792	BP: UMP salvage; pyrimidine-containing compound salvage; organonitrogen compound metabolic process; CTP salvage; phosphorylation; primary metabolic process; embryo development ending in birth or egg hatching.
	GO:0004849 GO:0016301 GO:0043771 GO:0004845 GO:0005524	MF: uridine kinase activity; kinase activity; cytidine kinase activity; uracil phosphoribosyltransferase activity; ATP binding.
	GO:0022625	CC: cytosolic large ribosomal subunit.
Desumoylating isopeptidase 2	A0A6P4J309	MF: Deubiquitinase activity.
T-complex protein 1 subunit beta	GO:0006457	BP: protein folding

T-complex protein 1 subunit beta	GO:0005524 GO:0140662 GO:0016887 GO:0051082	MF: ATP binding; ATP-dependent protein folding chaperone; ATP hydrolysis activity; unfolded protein binding.
	GO:0045335 GO:0005832	CC: phagocytic vesicle;chaperonin-containing T-complex.
Integrin-linked protein kinase	GO:0006887 GO:0007160 GO:0055002 GO:0034446 GO:0032956 GO:1904951 GO:0040017 GO:1904901 GO:0046716 GO:0032259 GO:0030239 GO:1903829 GO:0007005 GO:0006468 GO:0007229 GO:0009792 GO:0060298	BP: exocytosis; cell-matrix adhesion; striated muscle cell development; substrate adhesion-dependent cell spreading; regulation of actin cytoskeleton organization; positive regulation of establishment of protein localization; positive regulation of locomotion; positive regulation of myosin II filament organization; muscle cell cellular homeostasis; methylation; myofibril assembly; positive regulation of protein localization; mitochondrion organization; protein phosphorylation; integrin-mediated signaling pathway; embryo development ending in birth or egg hatching; positive regulation of sarcomere organization.
	GO:0008168 GO:0004672 GO:0016301 GO:0030674 GO:0005524 GO:0005178	MF: methyltransferase activity; protein kinase activity; kinase activity; protein-macromolecule adaptor activity; ATP binding; integrin binding.

Integrin-linked protein kinase	GO:0008305 GO:0009925 GO:0005912 GO:0055120 GO:0044231 GO:0044218 GO:0005925 GO:0031430	CC: integrin complex; basal plasma membrane; adherens junction; striated muscle dense body; host cell presynaptic membrane; other organism cell membrane; focal adhesion; M band.
Phosphoacetylglucosamine mutase	GO:0006048 GO:0016539 GO:0016540 GO:0048731 GO:0005975 GO:0007267	BP: UDP-N-acetylglucosamine biosynthetic process; intein-mediated protein splicing; protein autoprocessing; system development; carbohydrate metabolic process; cell-cell signaling.
	GO:0000287 GO:0004610 GO:0016868	MF: magnesium ion binding; phosphoacetylglucosamine mutase activity; intramolecular transferase activity, phosphotransferases.
15-hydroxyprostaglandin dehydrogenase [NAD+]	A0A140KPR8	MF: oxidoreductase activity; acting on the CH-OH group of donors; NAD or NADP as acceptor.
		CC: cytoplasm.
Rhomboid-like protein	A0A1B0FHL1	MF: Serine-type endopeptidase activity.
		CC: Membrane
Calcyclin-binding protein	A0A6P7G295	BP: heart development
		MF: S100 protein binding; tubulin binding; ubiquitin protein ligase binding.
		CC: cytoplasm; nucleus.
BTB domain-containing protein	GO:0030431 GO:0071805 GO:0006813 GO:0034220 GO:0034765 GO:0051260	BP: sleep; potassium ion transmembrane transport; potassium ion transport; monoatomic ion transmembrane transport; regulation of monoatomic ion transmembrane transport; protein homooligomerization.

BTB domain-containing protein	GO:0005251 GO:0005249 GO:0022843 GO:0005244	MF: delayed rectifier potassium channel activity; voltage-gated potassium channel activity; voltage-gated monoatomic cation channel activity; voltage-gated monoatomic ion channel activity.
	GO:0032809 GO:0032590 GO:0030424 GO:0008076	CC: neuronal cell body membrane; dendrite membrane; axon; voltage-gated potassium channel complex.
Sesn	GO:1901031 GO:0030308 GO:1904262 GO:0016239 GO:1990253 GO:0000422 GO:0071233 GO:2000377 GO:0010259	BP: regulation of response to reactive oxygen species; negative regulation of cell growth; negative regulation of TORC1 signaling; positive regulation of macroautophagy; cellular response to leucine starvation; autophagy of mitochondrion; cellular response to leucine; regulation of reactive oxygen species metabolic process; multicellular organism aging.
	GO:0070728 GO:0016684 GO:0004601	MF: leucine binding; oxidoreductase activity, acting on peroxide as acceptor; peroxidase activity.
	GO:0005737 GO:0005634	CC: cytoplasm; nucleus.
CREB-regulated transcription coactivator 1	M9PFU2	BP: cellular responde to cAMP; positive regulation of DNA-templated transcription; positive regulation of transcription by RNA polymerase II; protein homotetramerization; responde to oxidative stress; response to starvation.
		MF:cAMP response element binding protein binding.
		CC: cytoplasm; cytosol; nucleus.
guf1	GO:0045727 GO:0006412	BP: positive regulation of translation; translation.
	GO:0043022	MF: ribosome binding; mitochondrial ribosome binding; GTP binding; GTPase activity.

guf1	GO:0097177 GO:0005525 GO:0003924	
	GO:0005739 GO:0005759 GO:0005743	CC: mitochondrion; mitochondrial matrix; mitochondrial inner membrane.
Flavin-containing monooxygenase	GO:0050660 GO:0004499 GO:0050661	MF: Flavin adenine dinucleotide binding; N,N-dimethylaniline monooxygenase activity; NADP binding;
Covalently-linked cell wall protein	A0A1Y1L5D7	BP: fungal-type cell wall organization
Mocs2	GO:0006139 GO:0006338 GO:0006777 GO:0006807 GO:0032324 GO:0043605	BP: nucleobase-containing compound metabolic process; chromatin remodeling; Mo-molybdopterin cofactor biosynthetic process; nitrogen compound metabolic process; molybdopterin cofactor biosynthetic process; amide catabolic process.
	GO:0000166 GO:0016811 GO:0030366 GO:0047710 GO:0110050	MF: nucleotide binding; hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides; molybdopterin synthase activity; bis(5'-adenosyl)-triphosphatase activity; deaminated glutathione amidase activity.
	GO:0005700 GO:0005829 GO:0019008 GO:0140672	CC: polytene chromosome; cytosol; molybdopterin synthase complex; ATAC complex.