**Universidade Federal do Triângulo Mineiro – UFTM**
**Instituto de Ciências Biológicas e Naturais - ICBN**
**Curso de Pós-graduação em Ciências Fisiológicas**

**Tamires Marielem de Carvalho Costa**

# Transcriptomas salivares e intestinais revelam proteínas específicas e expressão gênica diferencial em *Rhodnius neglectus* infectado e não infectado por *Trypanosoma cruzi*

**Uberaba/MG**

**2021**

# **Transcriptomas salivares e intestinais revelam proteínas específicas e expressão gênica diferencial em *Rhodnius neglectus* infectado e não infectado por *Trypanosoma cruzi***

Tese apresentada ao Curso de Pós-Graduação em Ciências Fisiológicas, da Universidade Federal do Triângulo Mineiro, como requisito parcial para obtenção do título de doutora.
Área de concentração: Imunologia, Microbiologia e Parasitologia

Orientador: Prof. Dr. Carlo José Freire de Oliveira

Uberaba/MG

2021

**Catalogação na fonte: Biblioteca da Universidade Federal do Triângulo Mineiro**

C876t

Costa, Tamires Marielem de Carvalho
Transcriptomas salivares e intestinais revelam proteínas específicas e expressão gênica diferencial em *Rhodnius neglectus* infectados e não infectados por *Trypanosoma cruzi* / Tamires Marielem de Carvalho Costa. -- 2021.
154 f. : il., fig., graf., tab.

Tese (Doutorado em Ciências Fisiológicas) -- Universidade Federal do Triângulo Mineiro, Uberaba, MG, 2014
Orientador: Prof. Dr. Carlos José Freire de Oliveira

1. Doença de Chagas. 2. Trypanosoma cruzi. 3. Triatominae. 4.Transcriptoma. 5. Glândulas salivares. 6. Intestino delgado. I. Oliveira, Carlos José Freire de. II. Universidade Federal do Triângulo Mineiro. III. Título.

CDU 616.937

Amanda Franzão R. Silva - CRB-6/3461

**TRANSCRIPTOMAS SALIVARES E INTESTINAIS REVELAM PROTEÍNAS ESPECÍFICAS EEXPRESSÃO GÊNICA DIFERENCIAL EM *Rhodnius neglectus* INFECTADOS E NÃO INFECTADOS POR *T. cruzi***

**Tese apresentada ao Programa de Pós- Graduação em Ciências Fisiológicas, Área de concentração II - Parasitologia, Imunologia e Microbiologia (Linha de Pesquisa: Imunologia e genética da resposta imune na resistência e susceptibilidade às doenças infecciosas humanas), da Universidade Federal do Triângulo Mineiro como requisito parcial para obtenção do título de doutora.**

**Uberaba, 19 de outubro de 2021.**

**Banca Examinadora**

**DR. CARLO JOSÉ FREIRE DE OLIVEIRA - Orientador**
**Universidade Federal do Triângulo Mineiro**

**DR.ª FERNANDA RODRIGUES SOARES**
**Universidade Federal do Triângulo Mineiro**

**DR. DIEGO PANDELÓ JOSÉ**
**Universidade Federal do Triângulo Mineiro-Iturama**

**DR. EDMILSON AMARAL DE SOUZA**
**Universidade Federal de ViçosaCampus Rio Paranaíba**

**DR.ª LARISSA ALMEIDA MARTINS**
**NIAID Rocky Mountain Laboratories: Hamilton, MT, US**

Documento assinado eletronicamente por **CARLO JOSE FREIRE DE OLIVEIRA**, **Professor do Magistério Superior**, em 19/10/2021, às 14:46, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020 e no art. 34 da Portaria Reitoria/UFTM nº 87, de 17 de agosto de 2021.

Documento assinado eletronicamente por **FERNANDA RODRIGUES SOARES**, **Professor do Magistério Superior**, em 19/10/2021, às 16:13, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembrode 2020 e no art. 34 da Portaria Reitoria/UFTM nº 87, de 17 de agosto de 2021.

Documento assinado eletronicamente por **DIEGO PANDELO JOSE**, **Professor do Magistério Superior**, em 19/10/2021, às 16:15, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020 e no art.34 da Portaria Reitoria/UFTM nº 87, de 17 de agosto de 2021.

Documento assinado eletronicamente por **Edmilson Amaral de Souza**, **Usuário Externo**, em 19/10/2021, às 16:54, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020 e no art. 34 da Portaria Reitoria/UFTM nº 87, de 17 de agosto de 2021.

Documento assinado eletronicamente por **Larissa Almeida Martins**, **Usuário Externo**, em 20/10/2021, às 14:59, conforme horário oficial de Brasília, com fundamento no § 3º doart. 4º do Decreto nº 10.543, de 13 de novembro de 2020 e no art. 34 da Portaria Reitoria/UFTM nº 87, de 17 de agosto de 2021.

A autenticidade deste documento pode ser conferida no site http://sei.uftm.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador**0601352** e o código CRC **15A17D1D**.

Dedico este trabalho aos meus pais, José e Zélia, ao meu marido, João, aos meus irmãos Thayson, Tharles e Thiago. Obrigada por acreditarem em mim e pelo amor que nos une. E à minha filha, Olívia, que chegou e mudou minha vida!

## AGRADECIMENTOS

À Deus...

Ao meu orientador Prof. Dr. Carlo José Freire de Oliveira pela oportunidade, incentivo e generosidade em transmitir seus conhecimentos a mim, por sempre me motivar a ir além da "fronteira do conhecimento". Desde o mestrado criando muitas oportunidades de crescimento, por me fazer acreditar na ciência e me mostrar o peso que é ter o título de doutor. Obrigada pela confiança e pelo exemplo. Obrigada por me tornar diferente do que eu era.

Ao Prof. Dr. Virmondes Rodrigues Júnior por ceder a estrutura laboratorial que permitiu a execução de todos os experimentos.

A todos os docentes da Pós-graduação da Universidade Federal do Triângulo Mineiro, pelos ensinamentos transmitidos.

Aos funcionários do laboratório de imunologia e parasitologia pelo apoio técnico prestado. Em especial à Cidinha pelo auxilio com a esterilização dos materiais, e à Mônica e Betânia, pela disponibilidade.

À Bete! A pessoa mais prestativa, eficiente, gentil que eu tive o prazer de encontrar no mestrado e em todo o doutorado! Sempre me salvando com as orientações burocráticas!! Você é um ser de luz! O que seria de mim sem sua ajuda por toda esta estrada!

A todos os colegas do laboratório de imunologia e parasitologia, por contribuírem direta ou indiretamente com este trabalho, pelo acolhimento, amizade e risadas.

A todos os colegas de pós-Graduação (Ciências Fisiológicas), pelo companheirismo nas disciplinas.

Aos colegas e ex-colegas da pós-graduação, César, Djalma, Ton, Paula, Leticia, Jonatas, Guilherme, Jessica, Carol pelos conhecimentos partilhados e bons momentos de convivência.

Ao Rafael Tiveron, que incontáveis vezes me "salvou" em meio a tantos dados e análises! Obrigada pela leitura minuciosa e critica do artigo. Obrigada mesmo, sem sua ajuda eu não teria conseguido!

Aos meus preciosos amigos da Diretoria, Lara, Monique, Marcos e Maria Tays que apesar de longe fisicamente estão sempre presentes! Cada um do seu jeito me impulsiona e me faz melhor, tenho tanta sorte de tê-los na minha vida que agradecer não é o suficiente! Em especial, quero agradecer à Maria Tays! Amiga que o mestrado me deu e levarei sempre no coração! Obrigada por me motivar e inspirar sempre! Sou sua fã! BFF!!

À todos os meus familiares pelo incentivo. Em especial aos meus pais que sempre estão comigo, apoiando minhas investidas, pelo amor que me fortalece, por abdicarem muitos de seus sonhos para realizar os meus. Aos meus irmãos Thayson, Tharles e Thiago, que me motivam a crescer e ser melhor a cada dia. Amo vocês! Às minhas cunhadas, pelo apoio e palavras.

Ao meu marido João, que aguentou firme mais esta etapa, pelo apoio que me motivou a continuar mesmo exausta, pela ajuda e compreensão de todos os momentos de ausência e sobrecarga. Obrigada por estar na minha vida, não foi fácil e seria impossível sem você do meu lado. Obrigada por me dar Olívia e cuidar dela em cada momento que eu precisei escrever.

Aos meus sogros Sr. José e D. Olímpia, aos cunhados William, Ilara e Rhobison, pelas palavras e orações.

À todos que direta ou indiretamente ajudaram na execução desse trabalho.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pela concessão da bolsa de doutorado e pelo apoio financeiro para a realização desta pesquisa. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) - Código de Financiamento 001.

.

"Amarre seu vagão a uma estrela."

Ralph Waldo Emerson

"A excelência pode ser obtida se você se importa mais do que os outros julgam ser necessário; se arrisca mais do que os outros julgam ser seguro, sonha mais do que os outros julgam ser prático, e espera mais do que os outros julgam ser possível."

Vince Lombardi

“Tudo o que você decidir, nós te apoiamos. Vai dar certo.”

José e Zélia (Pais)

“Continue a nadar, continue a nadar...”

Dori

## RESUMO

Carvalho-Costa, T.M. Transcriptomas salivares e intestinais revelam proteínas específicas e expressão gênica diferencial em *Rhodnius neglectus* infectado e não infectado por *T. cruzi*. 156 f. Tese (Doutorado) – Pós-graduação em Ciências Fisiológicas, Universidade Federal do Triângulo Mineiro, Uberaba/MG, 2021.

*Rhodnius neglectus* é um potencial vetor do *Trypanosoma cruzi* (Tc), agente causador da doença de Chagas. Durante a alimentação com sangue, as glândulas salivares (SGs) e o intestino (INT) são ativamente necessários. A saliva dos SGs é injetada no hospedeiro vertebrado, modulando as respostas imunológicas e favorecendo a alimentação para a digestão INT. A infecção por Tc altera significativamente a fisiologia desses tecidos; entretanto, estudos que avaliem isso ainda são escassos. Para melhor compreendê-los, este estudo teve como objetivo avaliar a expressão transcricional global de genes em SGs e INT em jejum (FA), Fed (FE) e Fed na presença de Tc (FE + Tc). Na FA, predominou a expressão de transcritos relacionados às proteínas de manutenção da homeostase durante períodos de estresse. Portanto, os transcritos para as proteínas Tret1-Like e Hsp70Ba foram aumentados. Como esperado, no grupo FE, a presença de sangue pareceu ser responsável pelas alterações encontradas, uma vez que a maioria dos transcritos expressos estava relacionada à digestão, como transcritos para as proteases e Catepsina D. Em FE + Tc, houve diminuição da expressão de genes de processamento de sangue envolvidos no metabolismo do inseto (por exemplo, Antígeno-5 precursor, Pr13a, Obp), desintoxicação (Sult1) em INT e fosfatases ácidas em SG. Também encontramos menor expressão transcricional de lipocalinas e nitroforinas na SG e duas novas proteínas, Pacifastina e Diptericina, no INT. Vários transcritos de proteínas desconhecidas com potencial investigativo foram encontrados em ambos os tecidos. Nossos resultados também mostram que a presença de Tc é capaz de alterar a expressão em ambos os tecidos por um longo ou curto período de tempo. Enquanto no SG a homeostase parece ser restabelecida no dia 9, as alterações no INT ainda são evidentes. As informações geradas neste trabalho podem servir de guia para futuros estudos sobre a interação parasita-vetor e contribuir para o entendimento da fisiologia alimentar e pós-refeição / infecção em triatomíneos.



Palavras-chave: *Trypanosoma cruzi*; triatomíneo; transcriptoma; glândulas salivares; intestino.

## ABSTRACT

Carvalho-Costa, T.M. Salivary and intestinal transcriptomes reveal specific proteins and differential gene expression in *T. cruzi*-infected and non-infected *Rhodnius neglectus*. 156 f. Tese (Doutorado) – Pós-graduação em Ciências Fisiológicas, Universidade Federal do Triângulo Mineiro, Uberaba/MG, 2021.

*Rhodnius neglectus* is a potential vector of *Trypanosoma cruzi* (Tc), the causative agent of Chagas disease. During blood feeding, the salivary glands (SGs) and intestine (INT) are actively needed. The saliva of SGs is injected into the vertebrate host, modulating immune responses and favoring feeding for INT digestion. Tc infection significantly alters the physiology of these tissues; however, studies that assess this are still scarce. To better understand them, this study aimed to evaluate the global transcriptional expression of genes in SGs and INT in fasting (FA), Fed (FE) and Fed in the presence of Tc (FE + Tc). In FA, the expression of transcripts related to homeostasis maintenance proteins during periods of stress was predominant. Therefore, transcripts for Tret1-Like and Hsp70Ba proteins were increased. As expected, in the FE group, the presence of blood seemed to be responsible for the alterations found, since most of the expressed transcripts were related to digestion, such as transcripts for the proteases and Cathepsin D. In FE + Tc, there was decrease expression of blood processing genes for the insect metabolism (e.g. Antigen-5 precursor, Pr13a, Obp), detoxication (Sult1) in INT and acid phosphatases in SG. We also found less transcriptional expression of lipocalins and nitrophorins in SG and two new proteins, Pacifastin and Diptericin, in INT. Several transcripts of unknown proteins with investigative potential were found in both tissues. Our results also show that the presence of Tc is capable of altering the expression in both tissues for a long or short time. While in SG homeostasis seems to be re-established on day 9, changes in INT are still evident. The information generated in this work can serve as a guide for future studies on the parasite-vector interaction and contribute to the understanding of food physiology and post-meal / infection in triatomines.



**Key-words:** *Trypanosoma cruzi*; triatominae; transcriptome; salivary glands; intestine.

## LISTA DE ABREVIATURAS

| | |
|---|---|
| **LPC** | Lisofosfatidilcolina |
| **ATP** | adenosina trifosfato |
| **ADP** | adenosina difosfato |
| **AMP** | adenosina monofosfato |
| **Pi** | fosfato inorgânico |
| **RPAIs ou Ais** | as inibidoras de agregação plaquetária em Rhodnius |
| **Th1** | Linfócito T help 1 |
| **Th2** | Linfócito T help 2 |
| **RNA** | Ácido ribonucleico |
| **DNA** | Ácido desoxirribonucleico |
| **RNAt** | Ácido ribonucleico transportador |
| **RNAm** | Ácido ribonucleico mensageiro |
| **RNAr** | Ácido ribonucleico ribossômico |
| **NGS** | Next Generation Sequencing |
| **NCBI** | National Center for Biotechnology Information |
| **Tc** | *Trypanosoma cruzi* |
| **SG** | Glândula salivar |
| **INT** | Intestino |
| **FA** | Jejum |
| **FE** | Alimentados |
| **FE+Tc** | Alimentados infectados com *Trypanosoma cruzi* |

## LISTA DE ILUSTRAÇÕES



.

**SUMÁRIO**

# 1 INTRODUÇÃO

# 1 INTRODUÇÃO

Descrita pela primeira vez em 1909 por Carlos Chagas, a doença de Chagas atinge entre de 7 – 8 milhões de pessoas em todo o mundo (Who, 2021). O agente etiológico é o protozoário flagelado *Trypanosoma cruzi* (Chagas, 1909) transmitido pelas fezes do triatomíneo contaminado durante o período de repasto sanguíneo no hospedeiro vertebrado (Teixeira *et al.*, 2006; Teixeira *et al.*).

Os triatomíneos (Hemiptera, Reduviidae) são ectoparasitas hematófagos desde as ninfas até à fase adulta e a transmissão do protozoário pode ocorrer em qualquer uma destas fases de desenvolvimento (Lavoipierre *et al.*, 1959; Da Rosa *et al.*, 2010). Cerca de 145 espécies de Triatomíneos são conhecidas atualmente (Goncalves *et al.*, 2013) e todas elas podem ser consideradas vetores potenciais. A espécie *Rhodnius neglectus* faz parte da subfamília Triatominae e do gênero *Rhodnius* que abrange outras espécies importantes como o *Rhodnius prolixus* e o *Rhodnius robustus* (Jurberg, 2015). Este triatomíneo tem grande importância na manutenção da circulação enzoótica do *T. cruzi* na América do Sul (De Oliveira e Da Silva, 2007; Gurgel-Goncalves *et al.*, 2012).

Dotados de aparelho bucal altamente especializado em localizar vasos sanguíneos, os triatomíneos, são capazes de sugar diretamente deles o sangue, enquanto a saliva é depositada no local da picada durante todo o processo de hematofagia garantindo o sucesso da alimentação (Soares *et al.*, 2006). Respostas fisiológicas de reparo, como agregação plaquetária, vasoconstrição, coagulação sanguínea, aumento da permeabilidade vascular e quimiotaxia dos leucócitos devem ser moduladas para que ocorra uma alimentação eficiente e acredita-se que a saliva tenha este importante papel (Fontaine *et al.*, 2011; De Araujo *et al.*, 2012). Além disso, é sabido que moléculas bioativas produzidas pelas glândulas salivares podem contribuir para a melhor transmissão e propagação de patógenos via saliva. É o caso, por exemplo, da LPC – lisofosfatidilcolina, presente na saliva de *R. prolixus*, capaz de aumentar até seis vezes a parasitemia sanguínea em camundongos, demonstrando assim a influência da saliva na transmissão do *T. cruzi* (Mesquita *et al.*, 2008).

Além das glândulas salivares, temos que considerar também o trato digestivo, onde o sangue é armazenado e digerido. É ali que ocorre a absorção dos nutrientes e onde os patógenos se multiplicam e se transformam em formas infectantes. O trato gastrointestinal do triatomíneo é um tubo contínuo relativamente simples que possui partes morfologicamente e funcionalmente diferentes. A estocagem do sangue e o processamento dele por moléculas

responsáveis pela hemaglutinação e anticoagulação são realizados na porção anterior do intestino médio. O sangue se dirige para o intestino médio posterior onde será absorvido e digerido por catepsinas. Neste ponto, alguns hormônios diuréticos são liberados e a água e os íons são transportados através da parede do tubo digestivo, alcaçam a hemolinfa e se dirigem para os túbulos de Malpighi para serem excretados (Kollien e Schaub, 2000).

O intestino é um local de grande relevância para o desenvolvimento do *T. cruzi* pois é neste tecido que o protozoário se transforma em sua forma infectante e consegue, com o ato da alimentação-defecação, infectar novos hospedeiros vertebrados (Garcia *et al.*, 2007). Importantes moléculas presentes no intestino do inseto podem contribuir para o aumento da ingestão sanguínea em menor tempo, o que consequentemente protege o vetor e confere oportunidade de infecção ao parasito (Rossignol *et al.*, 1985; Paim *et al.*, 2011).

O transcriptoma tem sido uma ferramenta importante nos estudos que envolvem a interação de hospedeiro-vetor, com o objetivo de buscar estratégias para identificar alvos que possam controlar o desenvolvimento de insetos e a transmissão do patógeno. O transcriptoma é a gama completa de transcritos de uma célula ou tecido e inclui RNA mensageiro (mRNA), RNA ribossômico (rRNA), RNA transportadores (tRNA) e os microRNAs. O transcriptoma muda ativamente e pode variar dependendo de fatores como a fase de desenvolvimento, condições ambientais, estímulos físicos e estado fisiológico do organismo (Wang *et al.*, 2009; Wolf, 2013).

Transcriptomas de triatomíneos ainda são escassos quando comparamos a outros artrópodes vetores de importância médica como *A. aegypti* (Ribeiro *et al.*, 2007); *Anopheles darlingi* (Calvo *et al.*, 2004); *Ixodes scapularis* (Ribeiro *et al.*, 2006); *Lutzomyia longipalpis* (Charlab *et al.*, 1999). O *Rhodnius prolixus* é o inseto mais estudado do grupo de triatomíneos, muitos trabalhos são encontrados sobre sua biologia, microbiota, parasitismo, transmissão de patógenos, características genéticas e expressão gênica de muitos dos seus tecidos (Medeiros *et al.*, 2011; Buarque *et al.*, 2013). Por outro lado, outros triatomíneos já tiveram pelo menos parte de seus tecidos sequenciados e analisados por bioinformática como é o caso do *T. infestans* (Assumpcao *et al.*, 2008; Buarque *et al.*, 2013); *T. brasiliensis* (Sant'anna *et al.*, 2001); *T. dimidiata* (Kato *et al.*, 2010); *P. megistus* (Bussacos *et al.*, 2011), *R. brethesi* e *R. robustus* (Bussacos *et al.*, 2011) e *T. rubida* (Ribeiro *et al.*, 2012). No caso da espécie *Rhodinus neglectus* não foi encontrado trabalhos com sequenciamento e análise do intestino e estudo da expressão gênica das

glândulas salivares apresenta informação com um foco limitado a descrição de moléculas relacionadas à hematofagia (Santiago *et al.*, 2016).

De modo geral, as glândulas salivares são os tecidos mais estudados nos artrópodes hematófagos, incluindo nos triatomíneos, talvez pela relevância durante a alimentação e o alto interesse nas moléculas bioativas que são ali produzidas, inclusive, o transcriptoma destes tecidos ganhou um nome específico, o sialoma ou sialotranscriptoma. Apesar de ser o local de infecção e proliferação do *T.cruzi*, os estudos envolvendo o intestino são um pouco menos populares (Buarque *et al.*, 2013; Ribeiro *et al.*, 2014), abrindo um espaço relevante para trabalhos que abordem a dinâmica deste tecido. Ainda falando sobre ausências significativas na literatura, nos deparamos com poucos trabalhos que analisaram o transcriptoma de insetos infectados com *T.cruzi*, e menos ainda os que traçam algum paralelo entre insetos que não estão parasitados (Buarque *et al.*, 2013). Logo, nosso trabalho apresenta informações relevantes na construção do conhecimento sobre a interação parasita-hospedeiro através da análise da expressão gênica tanto nas glândulas salivares quanto no intestino de insetos infectados ou não com *T.cruzi*.

# 2 REVISÃO BIBLIOGRÁFICA

# 2 REVISÃO BIBLIOGRÁFICA

## 2.1 DOENÇA DE CHAGAS E O *TRYPANOSOMA CRUZI*

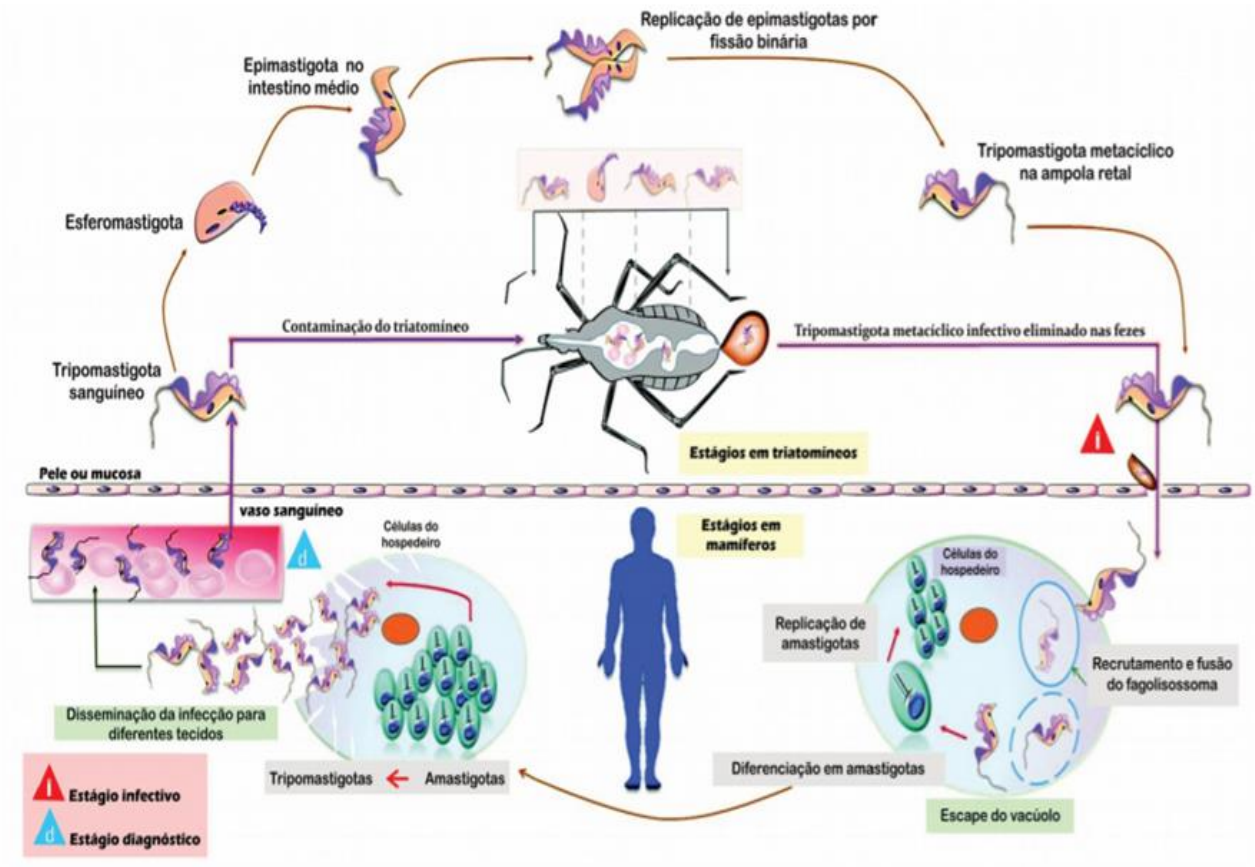
Descrita pela primeira vez em 1909 por Carlos Chagas, a tripanossomíase americana ou, como é mais conhecida, Doença de Chagas, é uma doença que apresenta expressiva morbimortalidade e compõe o grupo de doenças tropicais negligenciadas (Saúde, 2021; Who, 2021). Estima-se que atualmente ainda haja cerca de 7 – 8 milhões de pessoas infectadas em todo o mundo (Who, 2021). Apresenta-se em duas fases clínicas, a fase aguda precede a fase crônica e pode ou não apresentar sintomas, o que culmina com uma doença muitas vezes silenciosa e imperceptível. Já a fase crônica tem a forma clínica indeterminada como a mais comum responsável por cerca de 60% dos casos, manifestações cardíacas, digestivas ou cadiodigestivas também são próprias desta fase (Saúde, 2021).

No Brasil, uma estimativa de 2010 declarou que o número de infectados era aproximadamente de quatro milhões de pessoas (Martins-Melo *et al.*, 2014), o que demonstra que a doença de Chagas ainda é preocupante mesmo com o controle vetorial feito por programas de vigilância entomológica (Mendonca *et al.*, 2009; Santos *et al.*, 2020). Além disso, nas ultimas décadas, esta parasitose tem deixado claramente de ser um problema apenas de países da América Latina para ser um problema mundial. O surgimento na Europa e nos Estados Unidos tem mudado substancialmente o perfil epidemiológico da doença, uma vez que raramente era encontrada nestas regiões (Bern *et al.*, 2019). Este fato se deve à presença de grande número de migrantes, e à transmissão não vetorial, como a transmissão vertical, oral - através da ingestão de alimentos contaminados, através de transfusões sanguíneas ou mesmo por acidentes laboratoriais (Dias, 2009; Gascon *et al.*, 2010; Requena-Mendez *et al.*, 2015; Conners *et al.*, 2016).

O agente etiológico é o protozoário flagelado *Trypanosoma cruzi* (Chagas, 1909), ele é transmitido pelas fezes do triatomíneo contaminado em decorrência do repasto sanguíneo no hospedeiro vertebrado (Figura 1). A forma tripomastigota metacíclica penetra pelo local da picada e encontra a corrente sanguínea onde é fagocitada pelos macrófagos locais. Dentro destas células, as formas metacíclicas escapam da morte e se transformam em amastigotas que se multiplicam e diferenciam em tripomastigotas sanguíneas, estas formas são liberadas e

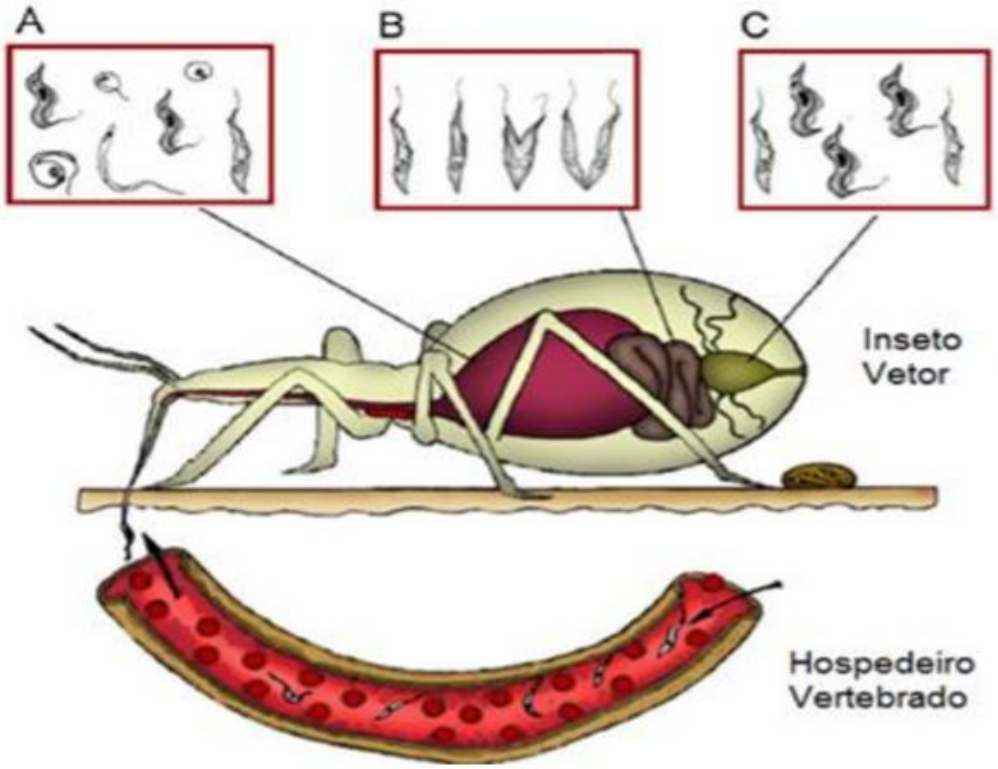
podem então se disseminar pelo organismo do hospedeiro. O triatomíneo, ao se alimentar deste hospedeiro contaminado, adquire a forma tripomastigota sanguínea e se torna infectado (Figura 2). Dentro do hospedeiro invertebrado a forma tripomastigota se transforma em amastigota e epimastigota, no intestino. Na porção final do intestino do inseto as epimastigotas se transformam nas formas infectantes, as tripomastigotas metacíclicas que são transmitidas aos hospedeiros vertebrados dando sequência ao ciclo (Teixeira *et al.*, 2006; Teixeira *et al.*, 2011).

FIGURA 1 – CICLO DE VIDA DO TRYPANOSOMA CRUZI




Fonte: Adaptado de (Lidani *et al.*, 2017)

FIGURA 2 – ESTÁGIOS DO *TRYPANOSOMA CRUZI*




A – Após a ingestão as tripomastigotas ingeridas durante a alimentação se transformam em epimastigotas no intestino medio anterior. B – Elas se dividem e se multiplicam no intestino médio posterior. C - elas se transformam em tripomastigotas metaciclicas que são eliminadas pelas fezes e infectam novo hospedeiro vertebrado. Fonte: Adaptado de Garcia *et. al*, 2007.

A relação do parasito com o hospedeiro invertebrado é extremamente importante. A presença do *T. cruzi* influencia o vetor. Os insetos infectados tendem a picar mais o hospedeiro e apresentam-se mais famintos devido a competição por nutrientes, que é relevante na transmissão do parasita, uma vez que aumenta o estímulo pela busca de novo hospedeiro vertebrado (Schaub, 2006). Por outro lado, o protozoário sofre influência perturbadora direta a todo instante, as moléculas liberadas pelo inseto durante o repasto e para a digestão do conteúdo intestinal interferem no desenvolvimento do parasito. O estado nutricional pode interferir nas formas do *T. cruzi* (Kollien e Schaub, 2000) e na taxa de metaciclogênese (De Lana *et al.*, 1998), causando assim, uma baixa considerável na quantidade de parasitas inicial.

Os parasitos que tem sucesso em adentrar o hospedeiro vertebrado passam por várias adversidades até se estabelecerem. Basicamente, a forma infectante consegue penetrar em quase todos os tipos celulares nucleados do hospedeiro mamífero, o que é extremamente importante, uma vez que a invasão é parte obrigatória do processo de desenvolvimento do parasito (Cardoso *et al.*, 2015; Mattos *et al.*, 2019). Ao mesmo tempo, as barreiras

imunológicas logo são ativadas e trabalham insistentemente no ataque destas formas infectantes tentando eliminá-las. O *T. cruzi*, no entanto, é capaz de interagir também com moléculas na superfície de fagócitos, umas das primeiras células de defesa ativadas, e do mesmo modo que se desenvolve no interior de outras células nucleadas ele é capaz de realizar, nestas células de defesa, parte do seu ciclo. Durante o processo de penetração celular forma-se uma estrutura chamada fagolisossomo como resultado da fusão do parasitóforo, um vacúolo ligado à membrana, com o lisossoma. Nesta estrutura ocorre exposição da forma metacíclica a um ambiente ácido decorrente das enzimas lisossomais, esta variação brusca de pH ativa a diferenciação para a forma amastigota que logo são liberadas para o citoplasma. Alguns parasitas acabam morrendo no fagolisossomo, mas outros conseguem seguir com o ciclo. Ocorre então a reprodução assexuada, podendo formar os pseudocistos nos tecidos infectados. As amastigotas se diferenciam em tripomastigotas que buscam novas células para invadir após a eventual lise da célula parasitada. A infecção pode persistir por toda a vida do hospedeiro vertebrado caso não ocorra nenhum tipo de tratamento (Teixeira *et al.*, 2011; Bern *et al.*, 2019).

A fase aguda, quando sintomática, é marcada por sintomas comuns como febre e mal estar, além de linfadenopatia e hepatoesplenomegalia. Já a fase indeterminada, é assintomática, podendo ter longa duração e fazendo do paciente um potencial transmissor do protozoário. A fase crônica tem sintomas relacionados aos órgãos mais acometidos pelo parasito no paciente, que podem ser o coração, intestino ou esôfago. É comum o desenvolvimento de órgãos gigantes chamados megas, com função extremamente prejudicada trazendo muitos malefícios ao paciente portador, inclusive o óbito (Tarleton *et al.*, 2007; Bern *et al.*, 2019; Guarner, 2019).

O tratamento existente ainda é inadequado e limitado, deixando os pacientes infectados com poucas chances de cura na fase crônica. Os medicamentos utilizados hoje tratam melhor a fase aguda da doença, mas mesmo assim apresentam no máximo cerca de 70% de eficácia (Dias, 2009 2015; Guarner, 2019).

## 2.2 TRIATOMÍNEOS

Os triatomíneos (Hemiptera, Reduviidae), conhecidos popularmente como barbeiros, são ectoparasitas hematófagos durante todos os estádios de vida, desde as ninfas até à fase adulta. São paurometábolos e, portanto, passam pelo ovo e mais cinco fases ninfais até

chegarem a se tornar adultos. Por serem os vetores do *T. cruzi*, estes artrópodes possuem relevância médica e destaque em muitos trabalhos científicos (Lavoipierre *et al.*, 1959). Aproximadamente 145 espécies de triatomíneos são conhecidas nos dias de hoje (Goncalves *et al.*, 2013) e todas elas podem ser consideradas vetores potencias do protozoário sanguíneo causador de Doença de Chagas. São classificados pelas particularidades morfológicas e cromáticas que apresentam, em casos em que as espécies são muito semelhantes, são utilizadas análises mais minuciosas para identificação, como as moleculares (Monteiro *et al.*, 2003).

A transmissão do *T. cruzi* pode acontecer em qualquer estádio de evolução destes insetos, uma vez que o repasto é realizado em todas as fases de desenvolvimento (Da Rosa *et al.*, 2010). Os insetos adultos se alimentam também para a reprodução, e a quantidade de repastos está proporcionalmente relacionada à capacidade de transmissão do parasito, se o inseto estiver infectado. Aproximadamente 600 ovos são postos durante toda a vida de um triatomíneo e eles levam em média 17 dias para eclodirem (Diotaiuti *et al.*, 2000). No Brasil existem pelo menos 62 espécies de triatomíneos já identificados, sendo que a maioria é silvestre.

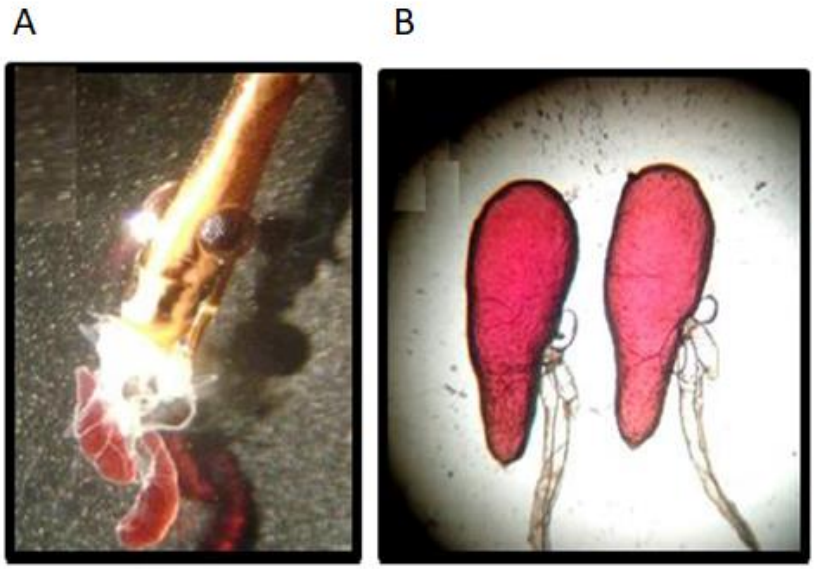
O comportamento alimentar é preferencialmente em animais de sangue quente, porem relatos encontrados na literatura demonstram que pode ocorrer repasto em animais exotérmicos, alimentação a partir de hemolinfa de outros triatomíneos e até mesmo do conteúdo intestinal diretamente (Sandoval *et al.*, 2004; Freitas *et al.*, 2005). Em situações excepcionais, a alimentação com produtos vegetais também já foi relatada (Ribeiro *et al.*, 2014).

Os triatomíneos possuem comportamento noturno e conseguem se guiar pelas taxas de $CO_2$, radiação infravermelha, e outras moléculas que os hospedeiros de sangue quente secretam (Schmitz, 2000; Barrozo e Lazzari, 2006; Fresquet e Lazzari, 2011). Interessante é que o estado fisiológico do inseto pode alterar a maneira como ele é atraído, se ele tiver alimentado recentemente, por exemplo, as substâncias que o atrairiam passam a funcionar como repelente para protegê-lo (Bodin *et al.*, 2009). Estes insetos se alimentam diretamente dos vasos sanguíneos por 20-30 minutos, aumentando seu peso de 6-12 vezes (Sant'anna *et al.*, 2001), possuem o aparelho bucal altamente especializado para localizar os vasos e sugar diretamente deles o sangue, isto é extremamente eficiente e chama atenção para o seu hábito alimentar, uma vez que esta localização não é aleatória (Ferreira *et al.*, 2007). A saliva é

depositada durante todo o processo de hematofagia e é essencial para o sucesso da alimentação (Soares *et al.*, 2006).

A saliva é produzida e armazenada nas glândulas salivares, que dependendo da espécie, possui número, formato, importância e características diferentes (Lacombe, 1999). O gênero *Rhodnius* possui apenas um par de glândulas de coloração avermelhada devido à alta concentração de nitroforinas que compõem a saliva destes insetos (Figura 3) (Nussenzveig *et al.*, 1995). No entanto, três pares de glândulas são comumente encontradas em outros triatomíneos, são chamadas D1, D2 e D3 e possuem funções específicas na alimentação do inseto (Barth, 1954; Lacombe, 1999). As D1 apresentam aspecto leitoso e são relacionadas à produção de anticoagulantes; as D2 possuem coloração mais amarelada e desempenham importante papel na produção de substancias hemolíticas; já as D3 são transparentes e muito frágeis à manipulação, são produtoras de compostos emolientes que são liberados assim que são produzidos (Lacombe, 1999).

FIGURA 3 – GLÂNDULAS SALIVARES

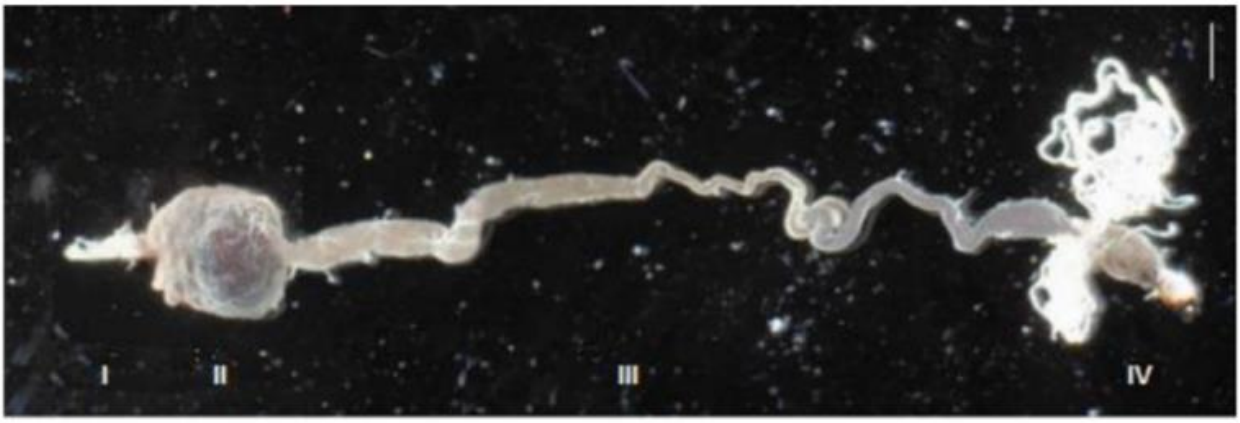



A – Glândulas salivares ainda no inseto durante a dissecação em *Rhodnius prolixus*., semelhante a *Rhodnius neglectus*; *B* - Glândulas salivares retiradas. Fonte: Adaptado de Mendes , 2014.

Além das glândulas salivares, que exercem importante papel durante o processo hematofágico, temos que considerar também o trato digestivo onde o sangue é armazenado e digerido (Figura 4). É ali também que ocorre a absorção dos nutrientes e onde o *T. cruzi* se multiplica e se transforma em formas infectantes. O trato gastrointestinal do triatomíneo é um

tubo contínuo relativamente simples, possui partes morfologicamente e funcionalmente diferentes e é altamente adaptado para uma refeição de sangue. Existe uma divisão em intestino anterior, intestino médio anterior, intestino médio posterior e reto. A estocagem do sangue e o processamento dele por moléculas responsáveis pela hemaglutinação e anticoagulação são realizados na porção anterior do intestino médio. O sangue se dirige para o intestino médio posterior lentamente e ali será absorvido e digerido por proteínas especializadas. No caso dos hemípteras, as catepsinas são as responsáveis por este processo, diferente do que acontece em outros insetos, que utilizam proteínas semelhantes a tripsinas (Kollien e Billingsley, 2002). Além disso, ocorre o deslocamento de água e íons através da parede intestinal, direcionados aos túbulos de Malpighi para serem finalmente excretados, e tudo isso sob o comando de vários hormônios liberados durante a alimentação (Kollien e Schaub, 2000).

FIGURA 4 – TUBO DIGESTIVO DOS TRIATOMÍNEOS



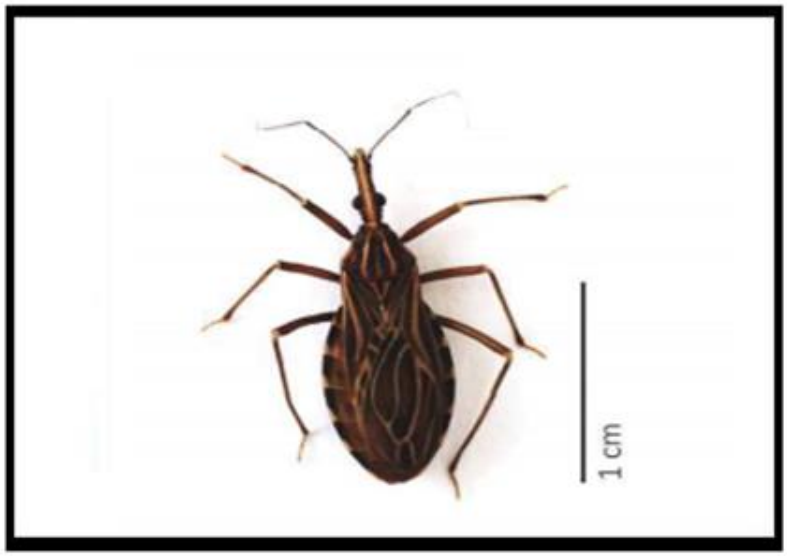

I – Intestino Anterior; II Intestino Médio Anterior; III – Intestino Médio Posterior; IV – Intestino Posterior. Escala: 2 mm.
Fonte: Adaptado de Kollien e Schaub, 2000.

O intestino é um local de grande relevância para o *T. cruzi*. Como comentado anteriormente, é neste local que o protozoário se transforma em sua forma infectante e consegue, com o ato da alimentação-defecação, infectar novos hospedeiros vertebrados (Garcia *et al.*, 2007). Apesar desta importante interação, os estudos com intestino de triatomíneos ainda são escassos, o que confirma a importância de aprofundar os conhecimentos neste órgão tão essencial ao patógeno.

## 2.3 *RHODNIUS NEGLECTUS*

A espécie *R. neglectus* faz parte da subfamília Triatominae e do gênero *Rhodnius* que abrange outras espécies importantes como o *Rhodnius prolixus* e o *Rhodnius robustus* (Figura 4). Os *R. neglectus* (Figura 5) possuem coloração do corpo marrom escura, com pernas sem anéis ou pintas, possuem conexivo claro com manchas escuras em formato retangular. O seu desenvolvimento completo se dá em torno de 340 dias e seu tamanho pode variar entre 17,5 – 20,5 mm (Jurberg, 2015).

FIGURA 5 – RHODNIUS NEGLECTUS




Fonte: Adaptado de Santiago, 2016

Presente principalmente no cerrado, *R. neglectus*, é uma espécie predominantemente silvestre, encontrado em vários estados brasileiros de vegetação aberta e longo período de seca, com habitat preferencial em palmeiras, sendo uma das mais importantes a buriti (*Mauritia flexuosa*), que cresce em veredas, além de ninhos de alguns pássaros e mamíferos (Diotaiuti e Dias, 1984; Gurgel-Goncalves *et al.*, 2003; Gurgel-Goncalves *et al.*).

Este triatomíneo tem grande importância na manutenção da circulação enzoótica do *T. cruzi*, visto que insetos contaminados foram encontrados em Minas Gerais, São Paulo, Piauí, Distrito Federal e Goiás (Gurgel-Goncalves *et al.*, 2004; De Oliveira e Da Silva, 2007; Gurgel-Goncalves *et al.*, 2012). Além disso, *R. neglectus*, já foi encontrado em ambiente peri e intradomiciliar em vários estados brasileiros: Distrito Federal, Minas Gerais e Piauí (Gurgel-Gonçalves *et al.*, 2004); Goiás (De Oliveira e Da Silva, 2007); Mato Grosso do Sul (Mesquita *et al.*, 2008) e Tocantins (Gurgel-Goncalves e Cuba, 2009), o que reforça ainda mais sua importância epidemiológica.

## 2.4 MOLÉCULAS ENVOLVIDAS DURANTE E PÓS REPASTO

### 2.4.1 – Saliva

Respostas fisiológicas de reparo, como agregação plaquetária, vaso constrição, coagulação sanguínea, aumento da permeabilidade vascular e quimiotaxia dos leucócitos devem ser impedidas para que ocorra uma alimentação eficiente e acredita-se que a saliva tenha este importante papel. Muitos trabalhos se dedicam a desvendar e a creditar a complexa mistura de proteínas que compõem a saliva e que estão envolvidas nesta estratégia (Fontaine *et al.*, 2011; De Araujo *et al.*, 2012).

Algumas destas proteínas já foram descritas e estudadas com mais detalhes. Dentre elas temos as moléculas vasodilatadoras. Fisiologicamente, num primeiro momento, ao ser picado, o hospedeiro vertebrado ativa uma cadeia de eventos que promove a constrição dos vasos sanguíneos, como uma maneira de impedir o sucesso do repasto. Em contrapartida, os triatomíneos injetam saliva enquanto sugam o sangue, e com ela, muitas moléculas invadem o local da lesão promovendo um efeito oposto ao mediado pelo hospedeiro. A vasodilatação ocorre especialmente pela ação de nitroforinas, só em *R. prolixus* são quatro diferentes, e todas auxiliam neste processo (Montfort *et al.*, 2000). O mecanismo de ação delas envolve o carreamento do óxido nítrico, importante relaxador da musculatura vascular, promovendo a passagem do sangue livremente enquanto o inseto se alimenta (Montfort *et al.*, 2000).

Além de contrair o vaso lesionado o hospedeiro conta com uma cascata de eventos que culmina na coagulação do sangue e estancamento do sangramento. No entanto, moléculas anticoagulantes também são lançadas no local da picada e a finalidade é garantir a fluidez durante o repasto e depois dele, quando o sangue já tiver no intestino do inseto. Isto é extremamente importante, uma vez que agiliza a alimentação impedindo que o inseto seja percebido pelo hospedeiro e inibe a coagulação durante o processo de digestão (Valenzuela, Charlab, *et al.*, 2002). Triafestinas 1 e 2 (Isawa *et al.*, 2007); nitroforina 2 (Moreira *et al.*, 2003), a nitroforina 1 e 4 (Montfort *et al.*, 2000); rodniina (Friedrich, 1993); triabina (Noeske-Jungblut *et al.*, 1995), são alguns inibidores já descritos na saliva de triatomíneos. Já a infestina (Campos *et al.*, 2002) e a brasiliensina (Santos *et al.*, 2007) são encontradas no intestino e atuam inibindo a trombina, importante componente da cascata de coagulação.

A agregação plaquetária é outra importante defesa do hospedeiro anulada por moléculas do inseto. A estratégia mais comum, mas não a única, usada pelos artrópodes

hematófagos é a hidrólise da adenosina trifosfato (ATP) e da adenosina difosfato (ADP) em adenosina monofosfato (AMP) e fosfato inorgânico (Pi). A família das apirases é eficiente neste sentido, atua na hidrólise do ADP e impede que as plaquetas se agreguem (Valenzuela, Charlab, *et al.*, 2002) e já foi relatada na saliva de muitas espécies de triatomíneos (Calvo *et al.*, 2004; Faudry *et al.*, 2004; Charneau, 2007; Santos *et al.*, 2007; Kato *et al.*, 2010; Bussacos *et al.*, 2011). As lipocalinas são outras proteínas que também atuam nesta etapa da defesa do hospedeiro. Temos as RPAIs (inibidoras de agregação plaquetária em *Rhodnius*), que semelhante às apirases, se ligam ao ADP e bloqueiam a agregação das plaquetas; as triabinas são ligantes de serotonina e epinefrina que atuam como agentes primários na iniciação da agregação plaquetária; as palidipinas e as triplatinas (Morita *et al.*, 2006) inibem a ativação de novas plaquetas através do colágeno e as ABPs (proteínas ligadoras de aminas e nitroforinas) que se ligam à trombina; (Noeske-Jungblut *et al.*, 1995; Francischetti *et al.*, 2000; Francischetti *et al.*, 2002; Calvo *et al.*, 2004; Assumpcao *et al.*, 2008). A interação de duas ou mais moléculas pode potencializar e aprimorar as investidas do inseto. É o caso da agregação plaquetária em *Rhodnius prolixus*, que conta com a ação conjunta das apirases, que degradam as altas doses de ADP, e das RPAI, que degradam baixas doses de adenosina, as que permanecem ainda após a hidrólise. Assim, a agregação plaquetária é inibida com muito mais eficiência (Francischetti *et al.*, 2002).

A habilidade de se tornar "invisível" para o hospedeiro é um recurso impar e permite que o repasto ocorra sem interrupções. Assim, moléculas que anestesiam o local e atuam como anti-hemostásicas favorecem a sobrevivência do inseto. Lipocalinas (Francischetti *et al.*, 2000), nitroforinas (Moreira *et al.*, 2003) e as apirases (Sarkis *et al.*, 1986), já citadas acima com outras funções, atuam também aqui.

A evasão do sistema imune do hospedeiro também é requerida para a sobrevivência. Assim que os antígenos salivares adentram o local da picada, além da hemostasia, o sistema imune é logo ativado na tentativa de interromper o repasto e restaurar a homeostase. Deste modo, um arsenal de compostos antiinflamatórios e imunomoduladores invade o hospedeiro, tanto para neutralizar as respostas inatas quanto adaptativas (Schoeler e Wikel, 2001).

O sistema complemento é uma importante linha de defesa da imunidade inata do hospedeiro, e uma variedade de moléculas com a finalidade de neutraliza-lo já foi relatada em artrópodes hematófagos (Valenzuela *et al.*, 2000; Cavalcante *et al.*, 2003; Mans *et al.*, 2008). Em triatomíneos, o bloqueio de C3b nas vias clássica e alternativa foi evidenciado em glândulas salivares e intestinos de algumas espécies, a hipótese do trabalho é inclusive a

proteção das células intestinais do inseto frente ao sistema complemento enquanto o sangue está no interior do triatomíneo em um momento após o repasto (Barros *et al.*, 2009).

O bloqueio da histamina também faz parte do repertório de atividades dos compostos salivares dos artrópodes hematófagos. Importante na ativação da inflamação e respostas como o prurido, a histamina é um alvo interessante aos insetos durante a alimentação, fazendo com que não sejam percebidos de imediato. Além de outras funções já mencionadas para as nitroforinas, em *R. prolixus* elas também são eficientes ligantes de histamina (Ribeiro e Walker, 1994).

Acredita-se que as substâncias produzidas pelas glândulas salivares de vetores hematófagos possam contribuir para otimizar a infecção dos seus respectivos patógenos. É o caso, por exemplo, da LPC (lisofosfatidilcolina), presente na saliva de *R. prolixus*. Neste caso, a saliva aumentou em cinco vezes a associação de macrófagos com *T. cruzi*, além de aumentar até seis vezes a parasitemia sanguínea em camundongos, demonstrando assim a influência da saliva na transmissão do *T. cruzi* (Mesquita *et al.*, 2008).

Esta apropriação de ambiente imunossuprimido também acontece por outros endoparasitas. É o caso da *Leishmania sp.*, ela se beneficia de moléculas como a maxadilan, presente na saliva de *Lutzomia longipalpis.* Este composto reduziu uma resposta Th1, ruim para o seu desenvolvimento, e induziu uma resposta Th2 bem mais favorável (Brodie *et al.*, 2007). Apesar da relevância, o campo para estudo deste tipo de interação é ainda muito pouco explorado e de grande potencial.

### 2.4.2 – Intestino

Envolvido basicamente nos processos de digestão e absorção de nutrientes, o intestino interage com o sangue retirado do hospedeiro de forma eficiente e precisa. Alguns estudos trazem informações sobre moléculas com atividades anticoagulantes e hemaglutinantes, fundamentais nesta interação. E apontam que estes compostos podem manter uma fluidez sanguínea maior, ocasionando um aumento na taxa de ingestão do sangue em um período de tempo menor, isto é estrategicamente brilhante, uma vez que, diminui o tempo de contato do vetor com o hospedeiro e aumenta as chances do inseto ser bem sucedido (Rossignol *et al.*, 1985; Mendonca *et al.*, 2009; Paim *et al.*, 2011).

Rodinina é um destes compostos com atividade anticoagulante, que foi anteriormente descrita em *R. prolixus* (Friedrich, 1993). Infestina (Campos *et al.*, 2002) e brasiliensina

(Santos *et al.*, 2007; Paim *et al.*, 2011) são outras duas moléculas que auxiliam na manutenção do estado fluido do sangue durante o repasto. Rodinina e brasiliensina se ligam a trombina interferindo na cascata de coagulação. As infestinas interferem na trombina e no fator XIIa da cascata de coagulação e aparenta ter secreção flutuante nos insetos infectados (Buarque *et al.*, 2013). Alguns transcritos do intestino contendo domínios Kazal também atuam na coagulação, e a maioria deles também está relacionada à trombina (Ribeiro *et al.*, 2014; Ouali *et al.*, 2020).

Além disso, a proteômica intestinal envolvida principalmente nos processos digestórios foi explorada recentemente (Vieira *et al.*, 2015; Gumiel *et al.*, 2020; Ouali *et al.*, 2020) tendo como ponto de partida estudos transcriptômicos e genômicos do intestino de *R. prolixus* realizados anteriormente (Ribeiro *et al.*, 2014; Mesquita *et al.*, 2015). As proteínas envolvidas em processos de desintoxicação, degradação de carboidratos, aminoácidos e lipídeos, manutenção da homeostase e envolvidas em processos imunológicos do inseto foram as mais predominantes. Observou-se também a integração e complementação dos estudos, o que é extremamente determinante no processo de construção do conhecimento.

Dentre as proteínas envolvidas no processo de desintoxicação temos as pertencentes à superfamília de proteínas da P450, que também auxiliam na resistência do organismo frente a inseticidas (Mamidala *et al.*, 2011), participam da produção de substancias endógenas, como alguns hormônios (Feyereisen, 2006) e protegem contra reativos de oxigênio (Poupardin *et al.*, 2010). Um provável membro da subfamília CYP6 foi encontrado em intestino de *R. prolixus* alimentados, juntamente à superexpressão de outros transcritos relacionados a desintoxicação e resistência a inseticidas como outros citocromos, sulfotranserases, superóxido dismutase, glutationa transferase e desidrogenases (Ribeiro *et al.*, 2014). Segundo sugerido por Ribeiro e colaboradores, a presença destas moléculas no intestino talvez crie uma rede de proteção contra os reativos de oxigênio que por ventura podem ser produzidos depois do repasto no *R. prolixus*. O gene da Tat (tirosina aminotransferase) é observado em intestino de *R. prolixus* após a alimentação (Ouali *et al.*, 2021). Esta proteína é essencial no processo de desintoxicação da tirosina, processo importante para a sobrevivência no repasto de artrópodes hematófagos (Sterkel *et al.*, 2016). E é requerida na primeira reação para a degradação de aminoácidos, um dos nutrientes mais abundantes no sangue ingerido (Ribeiro *et al.*, 2014). Fato que justifica a presença do transcrito desta proteína em todas as porções do intestino de *R. prolixus*, levemente mais expresso no intestino posterior (Ribeiro *et al.*, 2014).

Ainda sobre a digestão temos as carboxipeptidases, dentre elas, as S10 peptidases, um grupo de enzimas digestivas nos insetos que promove a quebra e remoção de aminoácidos de peptídeos e proteínas pela porção C-terminal (Aviles *et al.*, 1993). As S10 já foram identificadas no intestino de *R. prolixus* e são importantes na membrana intestinal, talvés até sem participar da digestão efetivamente (Ribeiro *et al.*, 2014). Embora raramente descritas em triatomíneos, já foi relatada no intestino anterior e posterior de *R. prolixus* uma serino carboxipeptidase pertencente a esta família após o repasto, o que sugere algum papel no inseto alimentado(Ouali *et al.*, 2021).

A alimentação desencadeia uma série de mudanças fisiológicas e endócrinas no inseto, e os neuropeptídeos são moléculas importantes neste cenário (Orchard, 2009; Ons, 2017). Dentre estas, as miossupressinas já foram encontradas no SNC e nas células endócrinas do intestino de *R. prolixus* (Ons *et al.*; Ons *et al.*, 2011; Lee *et al.*, 2012; Ons, 2017). Em *L. migratória,* por exemplo, suas concentrações são diferentes nas células endócrinas intestinais em diferentes estados de alimentação (Lange, 2001). Em *R. prolixus,* uma miossupressina também contribui para a redução da contração intestinal e do músculo cardíaco (Lee *et al.*, 2012), como papel na manutenção da homeostase fisiológica.

Assim como já mencionado para as moléculas do complemento, outros componentes da defesa do hospedeiro chegam ao intestino do inseto junto do sangue. É o caso das imunoglobulinas, em carrapatos já foram relatados compostos que as neutralizam (Wang e Nuttall, 1994). Além é claro de também possuírem neutralizadores para outros componentes do sistema imune, como citocinas (Sa-Nunes *et al.*, 2007; Sa-Nunes *et al.*, 2009; Carvalho-Costa *et al.*, 2015) e células de defesa (Carvalho-Costa *et al.*, 2015). Espera-se que outros artrópodes hematófagos também possuam um arsenal semelhante. Nitroforinas encontradas no intestino são capazes de se ligar ao óxido nítrico (De Carvalho *et al.*, 2017), impedir a agregação plaquetária (Zhang *et al.*, 1998; Andersen *et al.*, 2005) e se ligar a moléculas de defesa liberadas pelo hospedeiro no sangue, modulando-as (Ribeiro e Walker, 1994) e podem ser as responsáveis por este papel modulador no intestino dos triatomineos.

Os eventos que ocorrem logo após a infecção do parasita no intestino do vetor ainda são pouco explorados. A redução de até 80% do número de parasitos no intestino médio de *R. prolixus* após as primeiras 24 horas é fato sugestivo de que mecanismos são acionados neste local antes que ocorra a epimatogênese (Ferreira *et al.*, 2016).

Com exceção das bactérias que contribuem para o processo digestório e desenvolvimento do barbeiro (Hill *et al.*, 1976; Vallejo *et al.*, 2009), estes microrganismos em

sua maioria, são um problema tanto para os insetos quanto para os parasitas. Elas podem atuar como competidoras de nutrientes; ativar a resposta imune do vetor ou mesmo produzir compostos que sejam nocivos tanto para o inseto, quanto para o parasito (Castro *et al.*, 2007; Weiss e Aksoy, 2011). Um exemplo é a *Serratia marcescens*, após o repasto ela aumenta em grande quantidade no intestino do inseto e produz compostos que possuem atividade tripanolítica (Azambuja *et al.*, 2004; Castro *et al.*, 2012), inclusive, já foi demonstrado que quando esta bactéria está presente, a infecção de *R. prolixus* pela cepa Y de *T. cruzi* não acontece (Azambuja *et al.*, 2004; Vieira *et al.*, 2016). Outros estudos apontam uma mudança na microbiota após ativação da resposta imune do triatomíneo em casos de infecção (Dias *et al.*, 2015). Além da mudança causada pela presença do sangue, é claro. Deste modo, algumas moléculas com atividade antibacteriana são produzidas no intestino, dentre elas as lisozimas e defensinas são as mais conhecidas (Raj e Dentino, 2002; Lopez *et al.*, 2003; Andersen *et al.*, 2005; Araujo *et al.*, 2006). As lisozimas causam lise bacteriana por interferir diretamente nos peptídeoglicanos que compõem a parede celular das bactérias (Kollien, 2003). Essa proteína já foi encontrada superexpressa em insetos infectados com *T. cruzi* e por esta razão acredita-se que possa ter um papel importante durante a infecção (Buarque *et al.*, 2013). As defensinas também já foram encontradas aumentadas nos insetos parasitados (Buarque *et al.*, 2013) e inclusive, na porção posterior do intestino, acredita-se que sofra ação direta do protozoário, uma vez que foi superexpresso neste local e não na porção anterior de insetos infectados (Waniek *et al.*, 2011).

## 2.5 TRANSCRIPTOMA

É evidente a necessidade de aprimoramento dos conhecimentos sobre os triatomíneos e suas relações com o hospedeiro vertebrado e os parasitos. E cada vez mais o investimento em técnicas mais avançadas e precisas, além de ferramentas de bioinformática mais eficientes vem acontecendo. Atualmente contamos, por exemplo, com técnicas de sequenciamento bem mais avançadas que proporcionam à ciência uma considerável quantidade de anotações de transcritos à disposição. Isto é extremamente necessário para que cada vez mais moléculas sejam descritas, além das sequencias depositadas nos bancos públicos poderem se tornar mais completas e assim, a possibilidade de desvendar as tão complexas relações que mencionamos finalmente ser uma possibilidade real. Existe uma variedade de tipos de sequenciamento e estudos, o proteoma e o transcriptoma estão entre os mais realizados (Calvo *et al.*, 2004).

Quando ocorre a identificação de um grupo de proteínas que foram expressas em um determinado momento em um material biológico específico, chamamos de proteoma. O interessante desta técnica é a capacidade de mudança no perfil de proteínas encontrado de acordo com as condições às quais o material biológico é submetido, o que possibilita o estudo das variações dinâmicas que acontecem em um cenário de infecção, por exemplo (Lopez, 2007).

A eletroforese ou a cromatografia líquida separam as proteínas contidas no material analisado, realiza-se um processo de digestão e ionização e em seguida utiliza-se a espectrometria de massa para concluir o proteoma. Todos os dados são finalmente comparados aos bancos de dados disponíveis e assim as proteínas podem ser identificadas com melhor precisão (Aebersold e Mann, 2003).

O transcriptoma é a gama completa de transcritos de uma célula ou tecido e inclui RNA mensageiro, ou mRNA, RNA ribossômico (rRNA), RNA transportadores (tRNA) e os microRNAs. O transcriptoma muda ativamente, ele pode variar dependendo de fatores como a fase de desenvolvimento, condições ambientais, estímulos físicos, estado fisiológico (Wang *et al.*, 2009; Wolf, 2013). Dentre as vantagens deste método estão o uso de pouco material para a realização do sequenciamento; as sequencias são feitas por PCR utilizando um iniciador; as bibliotecas geradas são altamente eficazes permitindo o encontro de sequencias de proteínas já conhecidas e outras ainda não identificadas (Valenzuela, Francischetti, *et al.*, 2002).

O estudo do transcriptoma tem sido uma ferramenta importante nos estudos que envolvem a interação de hospedeiro-vetor, com o objetivo de buscar estratégias para identificar alvos que possam controlar o desenvolvimento de insetos e a transmissão do patógeno. O *R. prolixus* é o inseto mais estudado do grupo de triatomíneos quando nos referimos a sequenciamento, desta forma os estudos estão bastante avançados (Medeiros *et al.*, 2011; Buarque *et al.*, 2013; Leyria *et al.*, 2020a). Dentre os insetos que já tiveram pelo menos parte de seus tecidos sequenciados encontramos *T. infestans* (Charneau, 2007; Assumpcao *et al.*, 2008; Buarque *et al.*, 2013); *T. brasiliensis* (Sant'anna *et al.*, 2001; Santos *et al.*, 2007); *T. dimidiata* (Kato *et al.*, 2010); *P. megistus* (Bussacos *et al.*, 2011), *R. brethesi* e *R. robustus* (Bussacos *et al.*, 2011; Brito *et al.*, 2019), *P. lignarius* (Nevoa *et al.*, 2018); *T. rubida*(Ribeiro *et al.*, 2012); *T. rubrofasciata* (Mizushima *et al.*, 2020) e *R. montegrensis* (Brito *et al.*, 2019). Já para o *R. neglectus* não encontramos trabalhos que envolvam esta técnica para o intestino, apenas utilizando as glândulas salivares (Santiago *et al.*, 2016).

O estudo do transcriptoma de triatomíneos ainda é bem escasso quando comparamos a outros artrópodes vetores de importância médica como *A. aegypti* (Ribeiro *et al.*, 2007); *Anopheles darlingi* (Calvo *et al.*, 2004); *Ixodes scapularis* (Ribeiro *et al.*, 2006); *Lutzomyia longipalpis* (Charlab *et al.*, 1999); entre outros. A maior parte de trabalhos está relacionada à glândula salivar, inclusive este segmento ganhou um nome específico, o sialoma ou sialotrasncriptoma. Estudos envolvendo o intestino já são um pouco menos populares (Buarque *et al.*, 2013; Ribeiro *et al.*, 2014), trabalhos com intestinos infectados também não são muito encontrados ainda.

### 2.5.1 Análise dos transcriptomas

A necessidade de desenvolvimento de novos métodos de sequenciamento, mais baratos e rápidos é crescente. Deste modo o método Sanger, o mais usado por vários anos, tem sido substituído por novos, os chamados Next Generation Sequencing (NGS). A capacidade de geração de dados, mais rápida e mais barata é o grande atrativo destes novos métodos em relação ao tradicional Sanger (Metzker, 2010). O RNA sequencing ou RNA-seq, é uma técnica utilizada para analisar transcriptomas derivados de sequenciamentos destas novas tecnologias. Ela funciona basicamente assim: o RNA de interesse, a amostra, gera fragmentos de DNA complementares (cDNA) aos quais são adicionados adaptadores a uma ou ambas extremidades (3’ ou 5’). Os métodos NGS são utilizados em cada uma destas moléculas para realizar o sequenciamento gerando ao final as sequencias chamadas de reads. Estas sequências são geralmente curtas, variando de 30-400 pares de base de acordo com a plataforma escolhida. E elas podem ser construídas a partir de uma extremidade, o que chamamos de single-end, ou a partir de ambas as extremidades, os paired-ends. Dentre as plataformas que obtem seus resultados através de RNA-seq, temos a Illumina (Wang *et al.*, 2009; Nagalakshmi *et al.*, 2010).

A bioinformática é utilizada após o sequenciamento, nesta etapa as ferramentas são utilizadas para alinhar as reads montadas anteriormente a um genoma disponível no banco de dados. Existem casos onde não há um genoma de referência, é quando o pesquisador está gerando pela primeira vez informações sobre um determinado organismo e tem que construir o transcriptoma com as reads montadas *de novo* em sequencias codificadoras chamadas de contigs (Wang *et al.*, 2009; Nagalakshmi *et al.*, 2010).

A montagem é realizada por softwares que utilizam uma diversidade de maneiras de ler e juntar as informações das reads. Uma das estratégias mais utilizadas é a baseada no gráfico de De Bruijn, onde cada read é representada por um vértice, quando ocorre sobreposição das reads ela é representada por uma seta conectando as duas reads sobrepostas. Assim, o gráfico é formado por uma rede de vértices conectadas. Cada read é dividida em sequencias pequenas chamadas de k-mers, que são utilizadas para formar o gráfico (Compeau *et al.*, 2011). ABySS, Trinity, Oases e SOAPdenovo, programas comumente utilizados na montagem de transcriptomas utilizam desta estratégia. Assim que montado, o transcriptoma passa uma avaliação de qualidade, determinada por softwares estatísticos que analisam variáveis como a cobertura da montagem, a taxa de reads mapeada, o tamanho dos contigs entre outros aspectos (Clarke *et al.*, 2013). As sequencias muito longas não conseguem serem montadas pelos NGS, apontando uma desvantagem (Metzker, 2010). No entanto, varias outras ferramentas podem ser utilizadas para contornar esta incapacidade. Uma delas é a montagem multi k-mer, ela possibilita um maior rendimento, uma vez que consegue identificar contigs menos frequentes e de tamanhos diversos (Gruenheit *et al.*, 2012). Isto é possível graças a softwares que a cada dia são aprimorados e permanecem em contínuo desenvolvimento.

Com os contigs em mãos, eles então são comparados, utilizando outras ferramentas de bioinformáticas, aos bancos de dados disponíveis, tais como NCBI, Gene Ontology e outros. As análises possíveis são diversas e podem ir de simplesmente identificar as sequencias até classifica-las funcionalmente, por exemplo (Metzker, 2010).

# REFERÊNCIAS

AEBERSOLD, R.; MANN, M. Mass spectrometry-based proteomics. **Nature,** v. 422, n. 6928, p. 198-207, Mar 13 2003. ISSN 0028-0836 (Print)

0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed,

AGIRRE, J. et al. Capsid protein identification and analysis of mature Triatoma virus (TrV) virions and naturally occurring empty particles. **Virology,** v. 409, n. 1, p. 91-101, Jan 5 2011. ISSN 1096-0341 (Electronic)

0042-6822 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21030058 >.

AKIMARU, H.; HOU, D. X.; ISHII, S. Drosophila CBP is required for dorsal-dependent twist gene expression. **Nat Genet,** v. 17, n. 2, p. 211-4, Oct 1997. ISSN 1061-4036 (Print)

1061-4036 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9326945 >.

ALMAGRO ARMENTEROS, J. J. et al. Detecting sequence signals in targeting peptides using deep learning. **Life Sci Alliance,** v. 2, n. 5, Oct 2019. ISSN 2575-1077 (Electronic)

2575-1077 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/31570514 >.

ALMAGRO ARMENTEROS, J. J. et al. SignalP 5.0 improves signal peptide predictions using deep neural networks. **Nat Biotechnol,** v. 37, n. 4, p. 420-423, Apr 2019. ISSN 1546-1696 (Electronic)

1087-0156 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/30778233 >.

ANDERSEN, J. F. et al. The role of salivary lipocalins in blood feeding by Rhodnius prolixus. **Arch Insect Biochem Physiol,** v. 58, n. 2, p. 97-105, Feb 2005. ISSN 0739-4462 (Print)

0739-4462 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15660358 >.

ANDERSEN, J. F.; RIBEIRO, J. M. C. Chapter 4 - Salivary Kratagonists: Scavengers of Host Physiological Effectors During Blood Feeding. In: WIKEL, S. K.;AKSOY, S.*, et al* (Ed.). **Arthropod Vector: Controller of Disease Transmission, Volume 2**: Academic Press, 2017. p.51-63. ISBN 978-0-12-805360-7.

ANHE, A. C.; LIMA-OLIVEIRA, A. P.; AZEREDO-OLIVEIRA, M. T. Acid phosphatase activity distribution in salivary glands of triatomines (Heteroptera, Reduviidae, Triatominae). **Genet Mol Res,** v. 6, n. 1, p. 197-205, Mar 29 2007. ISSN 1676-5680 (Electronic)

1676-5680 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17469069 >.

ANKAVAY, M. et al. New insights into the ORF2 capsid protein, a key player of the hepatitis E virus lifecycle. **Sci Rep,** v. 9, n. 1, p. 6243, Apr 18 2019. ISSN 2045-2322 (Electronic)

2045-2322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/31000788 >.

ARAUJO, C. A. et al. Sequence characterization and expression patterns of defensin and lysozyme encoding genes from the gut of the reduviid bug Triatoma brasiliensis. **Insect Biochem Mol Biol,** v. 36, n. 7, p. 547-60, Jul 2006. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16835020 >.

ARAUJO, R. N. et al. RNA-seq analysis of the salivary glands and midgut of the Argasid tick Ornithodoros rostratus. **Sci Rep,** v. 9, n. 1, p. 6764, May 1 2019. ISSN 2045-2322 (Electronic)

2045-2322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/31043627 >.

ASSUMPCAO, T. C. et al. An insight into the sialome of the blood-sucking bug Triatoma infestans, a vector of Chagas' disease. **Insect Biochem Mol Biol,** v. 38, n. 2, p. 213-32, Feb 2008. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18207082 >.

AVILES, F. X. et al. Advances in metallo-procarboxypeptidases. Emerging details on the inhibition mechanism and on the activation process. **Eur J Biochem,** v. 211, n. 3, p. 381-9, Feb 1 1993. ISSN 0014-2956 (Print)

0014-2956 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8436102 >.

AZAMBUJA, P.; FEDER, D.; GARCIA, E. S. Isolation of Serratia marcescens in the midgut of Rhodnius prolixus: impact on the establishment of the parasite Trypanosoma cruzi in the vector. **Exp Parasitol,** v. 107, n. 1-2, p. 89-96, May-Jun 2004. ISSN 0014-4894 (Print)

0014-4894 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15208042 >.

BADISCO, L.; VAN WIELENDAELE, P.; VANDEN BROECK, J. Eat to reproduce: a key role for the insulin signaling pathway in adult insects. **Front Physiol,** v. 4, p. 202, 2013. ISSN 1664-042X (Print)

1664-042X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23966944 >.

BALCZUN, C. et al. Intestinal aspartate proteases TiCatD and TiCatD2 of the haematophagous bug Triatoma infestans (Reduviidae): sequence characterisation, expression pattern and characterisation of proteolytic activity. **Insect Biochem Mol Biol,** v. 42, n. 4, p. 240-50, Apr 2012. ISSN 1879-0240 (Electronic)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22210150 >.

BARROS, V. C. et al. The role of salivary and intestinal complement system inhibitors in the midgut protection of triatomines and mosquitoes. **PLoS One,** v. 4, n. 6, p. e6047, Jun 25 2009. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19557176 >.

BARROZO, R. B.; LAZZARI, C. R. Orientation response of haematophagous bugs to CO2: the effect of the temporal structure of the stimulus. **J Comp Physiol A Neuroethol Sens Neural Behav Physiol,** v. 192, n. 8, p. 827-31, Aug 2006. ISSN 0340-7594 (Print)

0340-7594 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16586085 >.

BARTH, R. Estudos anatômicos e histológicos sôbre a subfamília Triatominae (Heteroptera, Reduviidae): IV. parte: o complexo das glândulas salivares de Triatoma infestans. **Memórias do Instituto Oswaldo Cruz,** v. 52, p. 517-583, 1954. ISSN 0074-0276. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02761954000300003&nrm=iso >.

BEINTEMA, J. J. et al. Evolution of arthropod hemocyanins and insect storage proteins (hexamerins). **Mol Biol Evol,** v. 11, n. 3, p. 493-503, May 1994. ISSN 0737-4038 (Print)

0737-4038 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8015442 >.

BERN, C. et al. Chagas Disease in the United States: a Public Health Approach. **Clin Microbiol Rev,** v. 33, n. 1, Dec 18 2019. ISSN 1098-6618 (Electronic)

0893-8512 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/31776135 >.

BIAN, G.; RAIKHEL, A. S.; ZHU, J. Characterization of a juvenile hormone-regulated chymotrypsin-like serine protease gene in Aedes aegypti mosquito. **Insect Biochem Mol Biol,** v. 38, n. 2, p. 190-200, Feb 2008. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18207080 >.

BODIN, A.; VINAUGER, C.; LAZZARI, C. R. State-dependency of host-seeking in Rhodnius prolixus: the post-ecdysis time. **J Insect Physiol,** v. 55, n. 6, p. 574-9, Jun 2009. ISSN 1879-1611 (Electronic)

0022-1910 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19418597 >.

BOLGER, A. M.; LOHSE, M.; USADEL, B. Trimmomatic: a flexible trimmer for Illumina sequence data. **Bioinformatics,** v. 30, n. 15, p. 2114-20, Aug 1 2014. ISSN 1367-4811 (Electronic)

1367-4803 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24695404 >.

BORGES, E. C. et al. Trypanosoma cruzi: effects of infection on cathepsin D activity in the midgut of Rhodnius prolixus. **Exp Parasitol,** v. 112, n. 2, p. 130-3, Feb 2006. ISSN 0014-4894 (Print)

0014-4894 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16288741 >.

BORK, P.; BECKMANN, G. The CUB domain. A widespread module in developmentally regulated proteins. **J Mol Biol,** v. 231, n. 2, p. 539-45, May 20 1993. ISSN 0022-2836 (Print)

0022-2836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8510165 >.

BRITO, R. N. et al. Transcriptome-based molecular systematics: Rhodnius montenegrensis (Triatominae) and its position within the Rhodnius prolixus-Rhodnius robustus cryptic-species complex. **Parasit Vectors,** v. 12, n. 1, p. 305, Jun 17 2019. ISSN 1756-3305 (Electronic)

1756-3305 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/31208458 >.

BRODIE, T. M. et al. Immunomodulatory effects of the Lutzomyia longipalpis salivary gland protein maxadilan on mouse macrophages. **Infect Immun,** v. 75, n. 5, p. 2359-65, May 2007. ISSN 0019-9567 (Print)

0019-9567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17339357 >.

BUARQUE, D. S. et al. Differential expression profiles in the midgut of Triatoma infestans infected with Trypanosoma cruzi. **PLoS One,** v. 8, n. 5, p. e61203, 2013. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23658688 >.

BUARQUE, D. S. et al. Tigutcystatin, a cysteine protease inhibitor from Triatoma infestans midgut expressed in response to Trypanosoma cruzi. **Biochem Biophys Res Commun,** v. 413, n. 2, p. 241-7, Sep 23 2011. ISSN 1090-2104 (Electronic)

0006-291X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21875578 >.

BUCHFINK, B.; XIE, C.; HUSON, D. H. Fast and sensitive protein alignment using DIAMOND. **Nat Methods,** v. 12, n. 1, p. 59-60, Jan 2015. ISSN 1548-7105 (Electronic)

1548-7091 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25402007 >.

BUSSACOS, A. C. et al. Diversity of anti-haemostatic proteins in the salivary glands of Rhodnius species transmitters of Chagas disease in the greater Amazon. **J Proteomics,** v. 74, n. 9, p. 1664-72, Aug 24 2011. ISSN 1876-7737 (Electronic)

1874-3919 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21742069 >.

CALVO, E. et al. The transcriptome of adult female Anopheles darlingi salivary glands. **Insect Mol Biol,** v. 13, n. 1, p. 73-88, Feb 2004. ISSN 0962-1075 (Print)

0962-1075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14728669 >.

CAMPOS, I. T. et al. Infestin, a thrombin inhibitor presents in Triatoma infestans midgut, a Chagas' disease vector: gene cloning, expression and characterization of the inhibitor. **Insect Biochem Mol Biol,** v. 32, n. 9, p. 991-7, Sep 2002. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12213235 >.

CARDOSO, M. S.; REIS-CUNHA, J. L.; BARTHOLOMEU, D. C. Evasion of the Immune Response by Trypanosoma cruzi during Acute Infection. **Front Immunol,** v. 6, p. 659, 2015. ISSN 1664-3224 (Print)

1664-3224 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26834737 >.

CARVALHO-COSTA, T. M. et al. Immunosuppressive effects of Amblyomma cajennense tick saliva on murine bone marrow-derived dendritic cells. **Parasit Vectors,** v. 8, p. 22, Jan 14 2015. ISSN 1756-3305 (Electronic)

1756-3305 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25586117 >.

CASTRO, D. P. et al. Trypanosoma cruzi immune response modulation decreases microbiota in Rhodnius prolixus gut and is crucial for parasite survival and development. **PLoS One,** v. 7, n. 5, p. e36591, 2012. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22574189 >.

CASTRO, D. P. et al. Trypanosoma cruzi: ultrastructural studies of adhesion, lysis and biofilm formation by Serratia marcescens. **Exp Parasitol,** v. 117, n. 2, p. 201-7, Oct 2007. ISSN 0014-4894 (Print)

0014-4894 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17570364 >.

CAVALCANTE, R. R.; PEREIRA, M. H.; GONTIJO, N. F. Anti-complement activity in the saliva of phlebotomine sand flies and other haematophagous insects. **Parasitology,** v. 127, n. Pt 1, p. 87-93, Jul 2003. ISSN 0031-1820 (Print)

0031-1820 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12885192 >.

CHAGAS, C. Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do Schizotrypanum cruzi n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. **Memórias do Instituto Oswaldo Cruz,** v. 1, p. 159-218, 1909. ISSN 0074-0276. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02761909000200008&nrm=iso >.

CHARLAB, R. et al. Toward an understanding of the biochemical and pharmacological complexity of the saliva of a hematophagous sand fly Lutzomyia longipalpis. **Proc Natl Acad Sci U S A,** v. 96, n. 26, p. 15155-60, Dec 21 1999. ISSN 0027-8424 (Print)

0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10611354 >.

CHARNEAU, S. J., M.; COSTA, C. M.; PIRES, D. L.; FERNANDES, E. S.; BUSSACOS, A. C.; TEIXEIRA, A. R. L. The saliva proteome of the blood-feeding insect Triatoma infestans is rich in platelet-aggregation inhibitors. . **International Journal of Mass Spectrometry,** v. 268(2-3), p. 265–276, 2007.

CHENG, W. et al. DDX5 RNA Helicases: Emerging Roles in Viral Infection. **Int J Mol Sci,** v. 19, n. 4, Apr 9 2018. ISSN 1422-0067 (Electronic)

1422-0067 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29642538 >.

CLARKE, K. et al. Comparative analysis of de novo transcriptome assembly. **Sci China Life Sci,** v. 56, n. 2, p. 156-62, Feb 2013. ISSN 1869-1889 (Electronic)

1674-7305 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23393031 >.

COMINI, M. et al. Trypanothione synthesis in crithidia revisited. **J Biol Chem,** v. 280, n. 8, p. 6850-60, Feb 25 2005. ISSN 0021-9258 (Print)

0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15537651 >.

COMPEAU, P. E.; PEVZNER, P. A.; TESLER, G. How to apply de Bruijn graphs to genome assembly. **Nat Biotechnol,** v. 29, n. 11, p. 987-91, Nov 8 2011. ISSN 1546-1696 (Electronic)

1087-0156 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22068540 >.

CONNERS, E. E. et al. A global systematic review of Chagas disease prevalence among migrants. **Acta Trop,** v. 156, p. 68-78, Apr 2016. ISSN 1873-6254 (Electronic)

0001-706X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26777312 >.

CUDIC, M. et al. Chemical synthesis, antibacterial activity and conformation of diptericin, an 82-mer peptide originally isolated from insects. **Eur J Biochem,** v. 266, n. 2, p. 549-58, Dec 1999. ISSN 0014-2956 (Print)

0014-2956 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10561597 >.

CZIBENER, C. et al. Nucleotide sequence analysis of Triatoma virus shows that it is a member of a novel group of insect RNA viruses. **J Gen Virol,** v. 81, n. Pt 4, p. 1149-54, Apr 2000. ISSN 0022-1317 (Print)

0022-1317 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10725445 >.

DA ROSA, J. A. et al. Characterization of the external female genitalia of six species of Triatominae (Hemiptera: Reduviidade) by scanning electron microscopy. **Mem Inst Oswaldo Cruz,** v. 105, n. 3, p. 286-92, May 2010. ISSN 1678-8060 (Electronic)

0074-0276 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20512241 >.

DANIELSEN, E. T. et al. A Drosophila Genome-Wide Screen Identifies Regulators of Steroid Hormone Production and Developmental Timing. **Dev Cell,** v. 37, n. 6, p. 558-70, Jun 20 2016. ISSN 1878-1551 (Electronic)

1534-5807 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27326933 >.

DE ARAUJO, C. N. et al. Interactome: Smart hematophagous triatomine salivary gland molecules counteract human hemostasis during meal acquisition. **J Proteomics,** v. 75, n. 13, p. 3829-41, Jul 16 2012. ISSN 1876-7737 (Electronic)

1874-3919 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22579750 >.

DE CARVALHO, D. B. et al. Differential transcriptome analysis supports Rhodnius montenegrensis and Rhodnius robustus (Hemiptera, Reduviidae, Triatominae) as distinct species. **PLoS One,** v. 12, n. 4, p. e0174997, 2017. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28406967 >.

DE LANA, M. et al. Trypanosoma cruzi: compared vectorial transmissibility of three major clonal genotypes by Triatoma infestans. **Exp Parasitol,** v. 90, n. 1, p. 20-5, Sep 1998. ISSN 0014-4894 (Print)

0014-4894 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9709026 >.

DE OLIVEIRA, A. W.; DA SILVA, I. G. [Geographical distribution and indicators entomologic of sinantropic triatomines captured in the State of Goias]. **Rev Soc Bras Med Trop,** v. 40, n. 2, p. 204-8, Mar-Apr 2007. ISSN 0037-8682 (Print)

0037-8682 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17568889 >.

DIAS, F. D. A. et al. Monitoring of the Parasite Load in the Digestive Tract of Rhodnius prolixus by Combined qPCR Analysis and Imaging Techniques Provides New Insights into the Trypanosome Life Cycle. **PLoS Negl Trop Dis,** v. 9, n. 10, p. e0004186, 2015. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26496442 >.

DIAS, J. C. Elimination of Chagas disease transmission: perspectives. **Mem Inst Oswaldo Cruz,** v. 104 Suppl 1, p. 41-5, Jul 2009. ISSN 1678-8060 (Electronic)

0074-0276 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19753456 >.

DICK, C. F. et al. Trypanosoma rangeli: differential expression of ecto-phosphatase activities in response to inorganic phosphate starvation. **Exp Parasitol,** v. 124, n. 4, p. 386-93, Apr 2010. ISSN 1090-2449 (Electronic)

0014-4894 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20034491 >.

DICK, C. F. et al. Na+-dependent and Na+-independent mechanisms for inorganic phosphate uptake in Trypanosoma rangeli. **Biochim Biophys Acta,** v. 1820, n. 7, p. 1001-8, Jul 2012. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22456227 >.

DIOTAIUTI, L.; DIAS, J. C. [Occurrence and biology of Rhodnius neglectus Lent, 1954 in palm trees of suburban areas of Belo Horizonte, Minas Gerais]. **Mem Inst Oswaldo Cruz,** v. 79, n. 3, p. 293-301, Jul-Sep 1984. ISSN 0074-0276 (Print)

0074-0276 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/6443015 >.

DIOTAIUTI, L. et al. Aspectos operacionais do controle do Triatoma brasiliensis. **Cadernos de Saúde Pública,** v. 16, p. S61-S67, 2000. ISSN 0102-311X. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-311X2000000800006&nrm=iso >.

DURAI, D. A.; SCHULZ, M. H. Informed kmer selection for de novo transcriptome assembly. **Bioinformatics,** v. 32, n. 11, p. 1670-7, Jun 1 2016. ISSN 1367-4811 (Electronic)

1367-4803 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27153653 >.

ELETR, Z. M.; WILKINSON, K. D. Regulation of proteolysis by human deubiquitinating enzymes. **Biochim Biophys Acta,** v. 1843, n. 1, p. 114-28, Jan 2014. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23845989 >.

EMANUELSSON, O. et al. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. **J Mol Biol,** v. 300, n. 4, p. 1005-16, Jul 21 2000. ISSN 0022-2836 (Print)

0022-2836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10891285 >.

EMMS, D. M.; KELLY, S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. **Genome Biol,** v. 16, p. 157, Aug 6 2015. ISSN 1474-760X (Electronic)

1474-7596 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26243257 >.

FALCONE, R. et al. Differentiation of Rhodnius neglectus and Rhodnius prolixus (Hemiptera: Reduviidae: Triatominae) by multiple parameters. **Rev Soc Bras Med Trop,** v. 53, p. e20190503, 2020. ISSN 1678-9849 (Electronic)

0037-8682 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32267457 >.

FAUDRY, E. et al. Kinetics of expression of the salivary apyrases in Triatoma infestans. **Insect Biochem Mol Biol,** v. 34, n. 10, p. 1051-8, Oct 2004. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15475299 >.

FEDER, M. E.; HOFMANN, G. E. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. **Annu Rev Physiol,** v. 61, p. 243-82, 1999. ISSN 0066-4278 (Print)

0066-4278 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10099689 >.

FELDMEYER, B. et al. Short read Illumina data for the de novo assembly of a non-model snail species transcriptome (Radix balthica, Basommatophora, Pulmonata), and a comparison of assembler performance. **BMC Genomics,** v. 12, p. 317, Jun 16 2011. ISSN 1471-2164 (Electronic)

1471-2164 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21679424 >.

FERGUSON, L. C. et al. Evolution of the insect yellow gene family. **Mol Biol Evol,** v. 28, n. 1, p. 257-72, Jan 2011. ISSN 1537-1719 (Electronic)

0737-4038 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20656794 >.

FERGUSON, M. A.; WILLIAMS, A. F. Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. **Annu Rev Biochem,** v. 57, p. 285-320, 1988. ISSN 0066-4154 (Print)

0066-4154 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/3052274 >.

FERREIRA, R. A. et al. Do haematophagous bugs assess skin surface temperature to detect blood vessels? **PLoS One,** v. 2, n. 9, p. e932, Sep 26 2007. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17895973 >.

FERREIRA, R. C. et al. Colonization of Rhodnius prolixus gut by Trypanosoma cruzi involves an extensive parasite killing. **Parasitology,** v. 143, n. 4, p. 434-443, 2016. ISSN 0031-1820. Disponível em: < https://www.cambridge.org/core/article/colonization-of-rhodnius-prolixus-gut-by-trypanosoma-cruzi-involves-an-extensive-parasite-killing/80D300E86F1A0306E2F087681D5717B0 >.

FEYEREISEN, R. Evolution of insect P450. **Biochem Soc Trans,** v. 34, n. Pt 6, p. 1252-5, Dec 2006. ISSN 0300-5127 (Print)

0300-5127 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17073796 >.

FINKEL, Y.; STERN-GINOSSAR, N.; SCHWARTZ, M. Viral Short ORFs and Their Possible Functions. **Proteomics,** v. 18, n. 10, p. e1700255, May 2018. ISSN 1615-9861 (Electronic)

1615-9853 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29150926 >.

FLO, T. H. et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. **Nature,** v. 432, n. 7019, p. 917-21, Dec 16 2004. ISSN 1476-4687 (Electronic)

0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15531878 >.

FONTAINE, A. et al. Implication of haematophagous arthropod salivary proteins in host-vector interactions. **Parasit Vectors,** v. 4, p. 187, Sep 28 2011. ISSN 1756-3305 (Electronic)

1756-3305 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21951834 >.

FRANCESCHINI, A. et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. **Nucleic Acids Res,** v. 41, n. Database issue, p. D808-15, Jan 2013. ISSN 1362-4962 (Electronic)

0305-1048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23203871 >.

FRANCISCHETTI, I. M.; ANDERSEN, J. F.; RIBEIRO, J. M. Biochemical and functional characterization of recombinant Rhodnius prolixus platelet aggregation inhibitor 1 as a novel lipocalin with high affinity for adenosine diphosphate and other adenine nucleotides. **Biochemistry,** v. 41, n. 11, p. 3810-8, Mar 19 2002. ISSN 0006-2960 (Print)

0006-2960 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11888300 >.

FRANCISCHETTI, I. M. et al. Purification, cloning, expression, and mechanism of action of a novel platelet aggregation inhibitor from the salivary gland of the blood-sucking bug, Rhodnius prolixus. **J Biol Chem,** v. 275, n. 17, p. 12639-50, Apr 28 2000. ISSN 0021-9258 (Print)

0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10777556 >.

FREITAS, S. P. et al. [Feeding patterns of Triatoma pseudomaculata in the state of Ceara, Brazil]. **Rev Saude Publica,** v. 39, n. 1, p. 27-32, Feb 2005. ISSN 0034-8910 (Print)

0034-8910 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15654457 >.

FREJ, A. D. et al. The Inositol-3-Phosphate Synthase Biosynthetic Enzyme Has Distinct Catalytic and Metabolic Roles. **Mol Cell Biol,** v. 36, n. 10, p. 1464-79, May 15 2016. ISSN 1098-5549 (Electronic)

0270-7306 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26951199 >.

FRESQUET, N.; LAZZARI, C. R. Response to heat in Rhodnius prolixus: the role of the thermal background. **J Insect Physiol,** v. 57, n. 10, p. 1446-9, Oct 2011. ISSN 1879-1611 (Electronic)

0022-1910 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21806990 >.

FRIEDRICH, T. K., B., BIALOJAN, S.; LEMAIRE, H.G. ; HÖFFKEN, H.W; REUSCHENBACH, P.; OTTE, M.; DODT, J. . A Kazal-type inhibitor with thrombin specificity from Rhodnius prolixus

**Journal of Biological Chemistry**

v. 1993, p. 16216-16222, 1993. ISSN 0021-9258.

FUENTES-PRIOR, P. et al. Structure of the thrombin complex with triabin, a lipocalin-like exosite-binding inhibitor derived from a triatomine bug. **Proc Natl Acad Sci U S A,** v. 94, n. 22, p. 11845-50, Oct 28 1997. ISSN 0027-8424 (Print)

0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9342325 >.

GANFORNINA, M. D. K., H.; SANCHEZ, D. **Lipocalins in Arthropoda: Diversification and Functional Explorations.** . Madame Curie Bioscience Database Austin (TX): Landes Bioscience 2013.

GARCIA, E. S. et al. Exploring the role of insect host factors in the dynamics of Trypanosoma cruzi-Rhodnius prolixus interactions. **J Insect Physiol,** v. 53, n. 1, p. 11-21, Jan 2007. ISSN 0022-1910 (Print)

0022-1910 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17141801 >.

GASCON, J.; BERN, C.; PINAZO, M. J. Chagas disease in Spain, the United States and other non-endemic countries. **Acta Trop,** v. 115, n. 1-2, p. 22-7, Jul-Aug 2010. ISSN 1873-6254 (Electronic)

0001-706X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19646412 >.

GONCALVES, T. C. et al. Triatoma jatai sp. nov. in the state of Tocantins, Brazil (Hemiptera: Reduviidae: Triatominae). **Mem Inst Oswaldo Cruz,** v. 108, n. 4, p. 429-37, Jun 2013. ISSN 1678-8060 (Electronic)

0074-0276 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23828010 >.

GOODMAN, W. G.; CUSSON, M. 8 - The Juvenile Hormones. In: GILBERT, L. I. (Ed.). **Insect Endocrinology**. San Diego: Academic Press, 2012. p.310-365. ISBN 978-0-12-384749-2.

GRUENHEIT, N. et al. Cutoffs and k-mers: implications from a transcriptome study in allopolyploid plants. **BMC Genomics,** v. 13, p. 92, Mar 14 2012. ISSN 1471-2164 (Electronic)

1471-2164 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22417298 >.

GUARNER, J. Chagas disease as example of a reemerging parasite. **Semin Diagn Pathol,** v. 36, n. 3, p. 164-169, May 2019. ISSN 0740-2570 (Print)

0740-2570 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/31006555 >.

GUMIEL, M. et al. Proteome of the Triatomine Digestive Tract: From Catalytic to Immune Pathways; Focusing on Annexin Expression. **Front Mol Biosci,** v. 7, p. 589435, 2020. ISSN 2296-889X (Print)

2296-889X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/33363206 >.

GURGEL-GONCALVES, R.; CUBA, C. A. Predicting the potential geographical distribution of Rhodnius neglectus (Hemiptera, Reduviidae) based on ecological niche modeling. **J Med Entomol,** v. 46, n. 4, p. 952-60, Jul 2009. ISSN 0022-2585 (Print)

0022-2585 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19645302 >.

GURGEL-GONCALVES, R. et al. [Spatial distribution of Triatominae populations (Hemiptera: Reduviidae) in Mauritia flexuosa palm trees in Federal District of Brazil]. **Rev Soc Bras Med Trop,** v. 37, n. 3, p. 241-7, May-Jun 2004. ISSN 0037-8682 (Print)

0037-8682 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15330065 >.

GURGEL-GONCALVES, R. et al. Geographic distribution of chagas disease vectors in Brazil based on ecological niche modeling. **J Trop Med,** v. 2012, p. 705326, 2012. ISSN 1687-9694 (Electronic)

1687-9686 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22523500 >.

GURGEL-GONCALVES, R. et al. Sampling Rhodnius neglectus in Mauritia flexuosa palm trees: a field study in the Brazilian savanna. **Med Vet Entomol,** v. 17, n. 3, p. 347-50, Sep 2003. ISSN 0269-283X (Print)

0269-283X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12941022 >.

HAAS, B. J. et al. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. **Nat Protoc,** v. 8, n. 8, p. 1494-512, Aug 2013. ISSN 1750-2799 (Electronic)

1750-2799 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23845962 >.

HAJDUSEK, O. et al. Knockdown of proteins involved in iron metabolism limits tick reproduction and development. **Proc Natl Acad Sci U S A,** v. 106, n. 4, p. 1033-8, Jan 27 2009. ISSN 1091-6490 (Electronic)

0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19171899 >.

HATHAWAY, M. et al. Characterization of hexamerin proteins and their mRNAs in the adult lubber grasshopper: The effects of nutrition and juvenile hormone on their levels. **Comp Biochem Physiol A Mol Integr Physiol,** v. 154, n. 3, p. 323-32, Nov 2009. ISSN 1531-4332 (Electronic)

1095-6433 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19563903 >.

HILL, P.; CAMPBELL, J. A.; PETRIE, I. A. Rhodnius prolixus and its symbiotic actinomycete: a microbiological, physiological and behavioural study. **Proc R Soc Lond B Biol Sci,** v. 194, n. 1117, p. 501-25, Nov 12 1976. ISSN 0950-1193 (Print)

0950-1193 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12514 >.

HUANG, X. et al. Drosophila Niemann-Pick type C-2 genes control sterol homeostasis and steroid biosynthesis: a model of human neurodegenerative disease. **Development,** v. 134, n. 20, p. 3733-42, Oct 2007. ISSN 0950-1991 (Print)

0950-1991 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17804599 >.

ISAWA, H. et al. Identification and characterization of plasma kallikrein-kinin system inhibitors from salivary glands of the blood-sucking insect Triatoma infestans. **FEBS J,** v. 274, n. 16, p. 4271-86, Aug 2007. ISSN 1742-464X (Print)

1742-464X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17645545 >.

JURBERG, J. R., JULIANA M. S; MOREIRA, FELIPE F. F.; DALE, CAROLINA ; CORDEIRO, ISABELLE R. S. ; LAMAS JR, VALDIR D. ; GALVÃO, CLEBER; ROCHA, DAYSE S. . **Atlas iconográfico dos triatomíneos do Brasil (vetores da doença de chagas)**. Rio de Janeiro: Instituto Oswaldo Cruz**:** 58 p. 2015.

KANEHISA, M. et al. KEGG for linking genomes to life and the environment. **Nucleic Acids Res,** v. 36, n. Database issue, p. D480-4, Jan 2008. ISSN 1362-4962 (Electronic)

0305-1048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18077471 >.

KANEHISA, M.; SATO, Y.; MORISHIMA, K. BlastKOALA and GhostKOALA: KEGG Tools for Functional Characterization of Genome and Metagenome Sequences. **J Mol Biol,** v. 428, n. 4, p. 726-731, Feb 22 2016. ISSN 1089-8638 (Electronic)

0022-2836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26585406 >.

KARLIN, S.; BROCCHIERI, L. Heat shock protein 70 family: multiple sequence comparisons, function, and evolution. **J Mol Evol,** v. 47, n. 5, p. 565-77, Nov 1998. ISSN 0022-2844 (Print)

0022-2844 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9797407 >.

KATO, H. et al. A repertoire of the dominant transcripts from the salivary glands of the blood-sucking bug, Triatoma dimidiata, a vector of Chagas disease. **Infect Genet Evol,** v. 10, n. 2, p. 184-91, Mar 2010. ISSN 1567-7257 (Electronic)

1567-1348 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19900580 >.

KOLLIEN, A. H.; BILLINGSLEY, P. F. Differential display of mRNAs associated with blood feeding in the midgut of the bloodsucking bug, Triatoma infestans. **Parasitol Res,** v. 88, n. 12, p. 1026-33, Dec 2002. ISSN 0932-0113 (Print)

0932-0113 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12444450 >.

KOLLIEN, A. H.; SCHAUB, G. A. The development of Trypanosoma cruzi in triatominae. **Parasitol Today,** v. 16, n. 9, p. 381-7, Sep 2000. ISSN 0169-4758 (Print)

0169-4758 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10951597 >.

KRAUTH-SIEGEL, R. L.; COMINI, M. A. Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. **Biochim Biophys Acta,** v. 1780, n. 11, p. 1236-48, Nov 2008. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18395526 >.

KROGH, A. et al. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. **J Mol Biol,** v. 305, n. 3, p. 567-80, Jan 19 2001. ISSN 0022-2836 (Print)

0022-2836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11152613 >.

LACOMBE, D. Anatomia e Histologia das Glândulas Salivares nos Triatomíneos. **Memórias do Instituto Oswaldo Cruz,** v. 94, p. 557-564, 1999. ISSN 0074-0276. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02761999000400023&nrm=iso >.

LANGE, A. B. Feeding state influences the content of FMRFamide- and tachykinin-related peptides in endocrine-like cells of the midgut of Locusta migratoria☆. **Peptides,** v. 22, n. 2, p. 229-234, 2001/02/01/ 2001. ISSN 0196-9781. Disponível em: < https://www.sciencedirect.com/science/article/pii/S0196978100003867 >.

LAVOIPIERRE, M. M.; DICKERSON, G.; GORDON, R. M. Studies on the methods of feeding of blood-sucking arthropods. I. The manner in which triatomine bugs obtain their blood-meal, as observed in the tissues of the living rodent, with some remarks on the effects of the bite on human volunteers. **Ann Trop Med Parasitol,** v. 53, p. 235-50, Jun 1959. ISSN 0003-4983 (Print)

0003-4983 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14414675 >.

LEE, D. et al. An unusual myosuppressin from the blood-feeding bug Rhodnius prolixus. **J Exp Biol,** v. 215, n. Pt 12, p. 2088-95, Jun 15 2012. ISSN 1477-9145 (Electronic)

0022-0949 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22623197 >.

LEGRAND, J. M. D. et al. DDX5 plays essential transcriptional and post-transcriptional roles in the maintenance and function of spermatogonia. **Nat Commun,** v. 10, n. 1, p. 2278, May 23 2019. ISSN 2041-1723 (Electronic)

2041-1723 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/31123254 >.

LEYRIA, J.; ORCHARD, I.; LANGE, A. B. Transcriptomic analysis of regulatory pathways involved in female reproductive physiology of Rhodnius prolixus under different nutritional states. **Sci Rep,** v. 10, n. 1, p. 11431, Jul 10 2020a. ISSN 2045-2322 (Electronic)

2045-2322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32651410 >.

_____. What happens after a blood meal? A transcriptome analysis of the main tissues involved in egg production in Rhodnius prolixus, an insect vector of Chagas disease. **PLoS Negl Trop Dis,** v. 14, n. 10, p. e0008516, Oct 2020b. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/33057354 >.

LI, Y. et al. Gene and expression analysis of the hexamerin family proteins from the grasshopper, Locusta migratoria (Orthoptera: Acridoidea). **Biotechnology & Biotechnological Equipment,** v. 31, n. 6, p. 1139-1147, 2017/11/02 2017. ISSN 1310-2818. Disponível em: < https://doi.org/10.1080/13102818.2017.1373601 >.

LIDANI, K. C. F. et al. The Complement System: A Prey of Trypanosoma cruzi. **Front Microbiol,** v. 8, p. 607, 2017. ISSN 1664-302X (Print)

1664-302X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28473804 >.

LIIMATTA, K. et al. A Putative Acetylation System in Vibrio cholerae Modulates Virulence in Arthropod Hosts. **Appl Environ Microbiol,** v. 84, n. 21, Nov 1 2018. ISSN 1098-5336 (Electronic)

0099-2240 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/30143508 >.

LIU, B. et al. The PAR2 signal peptide prevents premature receptor cleavage and activation. **PLoS One,** v. 15, n. 2, p. e0222685, 2020. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32078628 >.

LOPEZ, J. L. Two-dimensional electrophoresis in proteome expression analysis. **J Chromatogr B Analyt Technol Biomed Life Sci,** v. 849, n. 1-2, p. 190-202, Apr 15 2007. ISSN 1570-0232 (Print)

1570-0232 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17188947 >.

LOPEZ, L. et al. Isolation and characterization of a novel insect defensin from Rhodnius prolixus, a vector of Chagas disease. **Insect Biochem Mol Biol,** v. 33, n. 4, p. 439-47, Apr 2003. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12650692 >.

LOVE, M. I.; HUBER, W.; ANDERS, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. **Genome Biol,** v. 15, n. 12, p. 550, 2014. ISSN 1474-760X (Electronic)

1474-7596 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25516281 >.

MACMANES, M. D. The Oyster River Protocol: a multi-assembler and kmer approach for de novo transcriptome assembly. **PeerJ,** v. 6, p. e5428, 2018. ISSN 2167-8359 (Print)

2167-8359 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/30083482 >.

MAJUMDER, A. L.; JOHNSON, M. D.; HENRY, S. A. 1L-myo-inositol-1-phosphate synthase. **Biochim Biophys Acta,** v. 1348, n. 1-2, p. 245-56, Sep 4 1997. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9370339 >.

MAMIDALA, P.; JONES, S. C.; MITTAPALLI, O. Metabolic Resistance in Bed Bugs. **Insects,** v. 2, n. 1, p. 36-48, Mar 18 2011. ISSN 2075-4450 (Print)

2075-4450 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26467498 >.

MANS, B. J. et al. Characterization of anti-hemostatic factors in the argasid, Argas monolakensis: implications for the evolution of blood-feeding in the soft tick family. **Insect Biochem Mol Biol,** v. 38, n. 1, p. 22-41, Jan 2008. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18070663 >.

MARCHANT, A. et al. Under-Expression of Chemosensory Genes in Domiciliary Bugs of the Chagas Disease Vector Triatoma brasiliensis. **PLoS Negl Trop Dis,** v. 10, n. 10, p. e0005067, Oct 2016. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27792774 >.

MARTI, G. A. et al. Exploration for Triatoma virus (TrV) infection in laboratory-reared triatomines of Latin America: a collaborative study*. **International Journal of Tropical Insect Science,** v. 33, n. 4, p. 294-304, 2013. ISSN 1742-7584. Disponível em: < https://www.cambridge.org/core/article/exploration-for-triatoma-virus-trv-infection-in-laboratoryreared-triatomines-of-latin-america-a-collaborative-study/E6C51584C31EA4CCA1F9479DD4B692A0 >.

MARTINEZ-BARNETCHE, J. et al. Adaptations in energy metabolism and gene family expansions revealed by comparative transcriptomics of three Chagas disease triatomine vectors. **BMC Genomics,** v. 19, n. 1, p. 296, Apr 27 2018. ISSN 1471-2164 (Electronic)

1471-2164 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29699489 >.

MARTINI, S. V.; NASCIMENTO, S. B.; MORALES, M. M. Rhodnius prolixus Malpighian tubules and control of diuresis by neurohormones. **An Acad Bras Cienc,** v. 79, n. 1, p. 87-95, Mar 2007. ISSN 0001-3765 (Print)

0001-3765 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17401478 >.

MARTINS-MELO, F. R. et al. Prevalence of Chagas disease in Brazil: a systematic review and meta-analysis. **Acta Trop,** v. 130, p. 167-74, Feb 2014. ISSN 1873-6254 (Electronic)

0001-706X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24139912 >.

MATTOS, E. C. et al. Reprogramming of Trypanosoma cruzi metabolism triggered by parasite interaction with the host cell extracellular matrix. **PLoS Negl Trop Dis,** v. 13, n. 2, p. e0007103, Feb 2019. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/30726203 >.

MEDEIROS, M. N. et al. Transcriptome and gene expression profile of ovarian follicle tissue of the triatomine bug Rhodnius prolixus. **Insect Biochem Mol Biol,** v. 41, n. 10, p. 823-31, Oct 2011. ISSN 1879-0240 (Electronic)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21736942 >.

MENDES, M. T. et al. Effect of the saliva from different triatomine species on the biology and immunity of TLR-4 ligand and Trypanosoma cruzi-stimulated dendritic cells. **Parasit Vectors,** v. 9, n. 1, p. 634, Dec 9 2016. ISSN 1756-3305 (Electronic)

1756-3305 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27938380 >.

MENDONCA, V. J. et al. Phylogeny of Triatoma sherlocki (Hemiptera: Reduviidae: Triatominae) inferred from two mitochondrial genes suggests its location within the Triatoma brasiliensis complex. **Am J Trop Med Hyg,** v. 81, n. 5, p. 858-64, Nov 2009. ISSN 1476-1645 (Electronic)

0002-9637 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19861622 >.

MESQUITA, R. D. et al. Trypanosoma cruzi infection is enhanced by vector saliva through immunosuppressant mechanisms mediated by lysophosphatidylcholine. **Infect Immun,** v. 76, n. 12, p. 5543-52, Dec 2008. ISSN 1098-5522 (Electronic)

0019-9567 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18794282 >.

MESQUITA, R. D. et al. Genome of Rhodnius prolixus, an insect vector of Chagas disease, reveals unique adaptations to hematophagy and parasite infection. **Proc Natl Acad Sci U S A,** v. 112, n. 48, p. 14936-41, Dec 1 2015. ISSN 1091-6490 (Electronic)

0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26627243 >.

METZKER, M. L. Sequencing technologies - the next generation. **Nat Rev Genet,** v. 11, n. 1, p. 31-46, Jan 2010. ISSN 1471-0064 (Electronic)

1471-0056 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19997069 >.

MILLER, J. R.; KOREN, S.; SUTTON, G. Assembly algorithms for next-generation sequencing data. **Genomics,** v. 95, n. 6, p. 315-27, Jun 2010. ISSN 1089-8646 (Electronic)

0888-7543 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20211242 >.

MIZUSHIMA, D. et al. Transcriptome data on salivary lipocalin family of the Asiatic Triatoma rubrofasciata. **Data Brief,** v. 30, p. 105647, Jun 2020. ISSN 2352-3409 (Electronic)

2352-3409 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32420432 >.

MOLLER, S.; CRONING, M. D.; APWEILER, R. Evaluation of methods for the prediction of membrane spanning regions. **Bioinformatics,** v. 17, n. 7, p. 646-53, Jul 2001. ISSN 1367-4803 (Print)

1367-4803 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11448883 >.

MONTEIRO, F. A. et al. Molecular phylogeography of the Amazonian Chagas disease vectors Rhodnius prolixus and R. robustus. **Mol Ecol,** v. 12, n. 4, p. 997-1006, Apr 2003. ISSN 0962-1083 (Print)

0962-1083 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12753218 >.

MONTFORT, W. R.; WEICHSEL, A.; ANDERSEN, J. F. Nitrophorins and related antihemostatic lipocalins from Rhodnius prolixus and other blood-sucking arthropods. **Biochim Biophys Acta,** v. 1482, n. 1-2, p. 110-8, Oct 18 2000. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11058753 >.

MOREIRA, M. F. et al. Changes in salivary nitrophorin profile during the life cycle of the blood-sucking bug Rhodnius prolixus. **Insect Biochem Mol Biol,** v. 33, n. 1, p. 23-8, Jan 2003. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12459197 >.

MORITA, A. et al. Identification and characterization of a collagen-induced platelet aggregation inhibitor, triplatin, from salivary glands of the assassin bug, Triatoma infestans. **FEBS J,** v. 273, n. 13, p. 2955-62, Jul 2006. ISSN 1742-464X (Print)

1742-464X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16759235 >.

MURY, F. B. et al. Alpha-glucosidase promotes hemozoin formation in a blood-sucking bug: an evolutionary history. **PLoS One,** v. 4, n. 9, p. e6966, Sep 9 2009. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19742319 >.

MUSCIO, O. A. et al. Triatoma virus pathogenicity in laboratory colonies of Triatoma infestans (Hemiptera:Reduviidae). **J Med Entomol,** v. 34, n. 3, p. 253-6, May 1997. ISSN 0022-2585 (Print)

0022-2585 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9151486 >.

MUSCIO, O. A.; LA TORRE, J. L.; SCODELLER, E. A. Characterization of Triatoma virus, a picorna-like virus isolated from the triatomine bug Triatoma infestans. **J Gen Virol,** v. 69 ( Pt 11), p. 2929-34, Nov 1988. ISSN 0022-1317 (Print)

0022-1317 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/3053988 >.

NAGALAKSHMI, U.; WAERN, K.; SNYDER, M. RNA-Seq: a method for comprehensive transcriptome analysis. **Curr Protoc Mol Biol,** v. Chapter 4, p. Unit 4 11 1-13, Jan 2010. ISSN 1934-3647 (Electronic)

1934-3647 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20069539 >.

NEVOA, J. C. et al. An insight into the salivary gland and fat body transcriptome of Panstrongylus lignarius (Hemiptera: Heteroptera), the main vector of Chagas disease in Peru. **PLoS Negl Trop Dis,** v. 12, n. 2, p. e0006243, Feb 2018. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29462134 >.

NIELSEN, H. Predicting Secretory Proteins with SignalP. **Methods Mol Biol,** v. 1611, p. 59-73, 2017. ISSN 1940-6029 (Electronic)

1064-3745 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28451972 >.

NOESKE-JUNGBLUT, C. et al. Triabin, a highly potent exosite inhibitor of thrombin. **J Biol Chem,** v. 270, n. 48, p. 28629-34, Dec 1 1995. ISSN 0021-9258 (Print)

0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7499380 >.

NOESKE-JUNGBLUT, C. et al. An inhibitor of collagen-induced platelet aggregation from the saliva of Triatoma pallidipennis. **J Biol Chem,** v. 269, n. 7, p. 5050-3, Feb 18 1994. ISSN 0021-9258 (Print)

0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8106481 >.

NOGUEIRA, N. P. et al. Proliferation and differentiation of Trypanosoma cruzi inside its vector have a new trigger: redox status. **PLoS One,** v. 10, n. 2, p. e0116712, 2015. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25671543 >.

NORIEGA, F. G.; SHAH, D. K.; WELLS, M. A. Juvenile hormone controls early trypsin gene transcription in the midgut of Aedes aegypti. **Insect Mol Biol,** v. 6, n. 1, p. 63-6, Feb 1997. ISSN 0962-1075 (Print)

0962-1075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9013256 >.

NUSSENZVEIG, R. H.; BENTLEY, D. L.; RIBEIRO, J. M. Nitric oxide loading of the salivary nitric-oxide-carrying hemoproteins (nitrophorins) in the blood-sucking bug Rhodnius prolixus. **J Exp Biol,** v. 198, n. Pt 5, p. 1093-8, May 1995. ISSN 0022-0949 (Print)

0022-0949 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8627144 >.

OLIVEIRA, D. S. et al. Functional Characterization of Odorant Binding Protein 27 (RproOBP27) From Rhodnius prolixus Antennae. **Front Physiol,** v. 9, p. 1175, 2018. ISSN 1664-042X (Print)

1664-042X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/30210359 >.

ONS, S. Neuropeptides in the regulation of Rhodnius prolixus physiology. **J Insect Physiol,** v. 97, p. 77-92, Feb - Mar 2017. ISSN 1879-1611 (Electronic)

0022-1910 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27210592 >.

ONS, S. et al. The neuropeptidome of Rhodnius prolixus brain. **Proteomics,** v. 9, n. 3, p. 788-92, Feb 2009. ISSN 1615-9861 (Electronic)

1615-9853 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19137558 >.

ONS, S. et al. Neuropeptide precursor gene discovery in the Chagas disease vector Rhodnius prolixus. **Insect Mol Biol,** v. 20, n. 1, p. 29-44, Feb 2011. ISSN 1365-2583 (Electronic)

0962-1075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20958806 >.

ORCHARD, I. Peptides and serotonin control feeding-related events in Rhodnius prolixus. **Front Biosci (Elite Ed),** v. 1, p. 250-62, Jun 1 2009. ISSN 1945-0508 (Electronic)

1945-0494 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19482642 >.

OUALI, R. et al. High-Throughput Identification of the Rhodnius prolixus Midgut Proteome Unravels a Sophisticated Hematophagic Machinery. **Proteomes,** v. 8, n. 3, Jul 24 2020. ISSN 2227-7382 (Print)

2227-7382 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32722125 >.

OUALI, R. et al. Early Post-Prandial Regulation of Protein Expression in the Midgut of Chagas Disease Vector Rhodnius prolixus Highlights New Potential Targets for Vector Control Strategy. **Microorganisms,** v. 9, n. 4, Apr 11 2021. ISSN 2076-2607 (Print)

2076-2607 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/33920371 >.

PADDOCK, C. D. et al. Identification, cloning, and recombinant expression of procalin, a major triatomine allergen. **J Immunol,** v. 167, n. 5, p. 2694-9, Sep 1 2001. ISSN 0022-1767 (Print)

0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11509613 >.

PAIM, R. M. et al. Influence of the intestinal anticoagulant in the feeding performance of triatomine bugs (Hemiptera; Reduviidae). **Int J Parasitol,** v. 41, n. 7, p. 765-73, Jun 2011. ISSN 1879-0135 (Electronic)

0020-7519 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21447340 >.

PATRO, R. et al. Salmon provides fast and bias-aware quantification of transcript expression. **Nat Methods,** v. 14, n. 4, p. 417-419, Apr 2017. ISSN 1548-7105 (Electronic)

1548-7091 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28263959 >.

POSTBERG, J. et al. The evolutionary history of histone H3 suggests a deep eukaryotic root of chromatin modifying mechanisms. **BMC Evol Biol,** v. 10, p. 259, Aug 25 2010. ISSN 1471-2148 (Electronic)

1471-2148 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20738881 >.

POUPARDIN, R. et al. Transcription profiling of eleven cytochrome P450s potentially involved in xenobiotic metabolism in the mosquito Aedes aegypti. **Insect Mol Biol,** v. 19, n. 2, p. 185-93, Apr 2010. ISSN 1365-2583 (Electronic)

0962-1075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20041961 >.

PUEYO, J. I.; MAGNY, E. G.; COUSO, J. P. New Peptides Under the s(ORF)ace of the Genome. **Trends Biochem Sci,** v. 41, n. 8, p. 665-678, Aug 2016. ISSN 0968-0004 (Print)

0968-0004 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27261332 >.

RAJ, P. A.; DENTINO, A. R. Current status of defensins and their role in innate and adaptive immunity. **FEMS Microbiol Lett,** v. 206, n. 1, p. 9-18, Jan 2 2002. ISSN 0378-1097 (Print)

0378-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11786250 >.

RAWLINGS, N. D.; BARRETT, A. J. Families of cysteine peptidases. **Methods Enzymol,** v. 244, p. 461-86, 1994. ISSN 0076-6879 (Print)

0076-6879 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7845226 >.

REQUENA-MENDEZ, A. et al. Prevalence of Chagas disease in Latin-American migrants living in Europe: a systematic review and meta-analysis. **PLoS Negl Trop Dis,** v. 9, n. 2, p. e0003540, Feb 2015. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25680190 >.

RIBEIRO, A. R. et al. Trypanosoma cruzi isolated from a triatomine found in one of the biggest metropolitan areas of Latin America. **Rev Soc Bras Med Trop,** v. 49, n. 2, p. 183-9, Apr 2016. ISSN 1678-9849 (Electronic)

0037-8682 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27192587 >.

RIBEIRO, J. M. et al. An annotated catalog of salivary gland transcripts from Ixodes scapularis ticks. **Insect Biochem Mol Biol,** v. 36, n. 2, p. 111-29, Feb 2006. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16431279 >.

RIBEIRO, J. M. et al. Exploring the sialome of the blood-sucking bug Rhodnius prolixus. **Insect Biochem Mol Biol,** v. 34, n. 1, p. 61-79, Jan 2004. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14976983 >.

RIBEIRO, J. M. et al. An annotated catalogue of salivary gland transcripts in the adult female mosquito, Aedes aegypti. **BMC Genomics,** v. 8, p. 6, Jan 4 2007. ISSN 1471-2164 (Electronic)

1471-2164 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17204158 >.

RIBEIRO, J. M. et al. An insight into the sialotranscriptome of Triatoma rubida (Hemiptera: Heteroptera). **J Med Entomol,** v. 49, n. 3, p. 563-72, May 2012. ISSN 0022-2585 (Print)

0022-2585 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22679863 >.

RIBEIRO, J. M. et al. An insight into the transcriptome of the digestive tract of the bloodsucking bug, Rhodnius prolixus. **PLoS Negl Trop Dis,** v. 8, n. 1, p. e2594, 2014. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24416461 >.

RIBEIRO, J. M.; SCHWARZ, A.; FRANCISCHETTI, I. M. A Deep Insight Into the Sialotranscriptome of the Chagas Disease Vector, Panstrongylus megistus (Hemiptera: Heteroptera). **J Med Entomol,** v. 52, n. 3, p. 351-8, May 2015. ISSN 0022-2585 (Print)

0022-2585 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26334808 >.

RIBEIRO, J. M.; WALKER, F. A. High affinity histamine-binding and antihistaminic activity of the salivary nitric oxide-carrying heme protein (nitrophorin) of Rhodnius prolixus. **J Exp Med,** v. 180, n. 6, p. 2251-7, Dec 1 1994. ISSN 0022-1007 (Print)

0022-1007 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7964498 >.

RIBEIRO, J. M. C.; ARCÀ, B. Chapter 2 From Sialomes to the Sialoverse: An Insight into Salivary Potion of Blood-Feeding Insects. In: (Ed.). **Advances in Insect Physiology**: Academic Press, v.37, 2009. p.59-118. ISBN 0065-2806.

ROSSIGNOL, P. A. et al. Enhanced mosquito blood-finding success on parasitemic hosts: evidence for vector-parasite mutualism. **Proc Natl Acad Sci U S A,** v. 82, n. 22, p. 7725-7, Nov 1985. ISSN 0027-8424 (Print)

0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/3865192 >.

SA-NUNES, A. et al. The immunomodulatory action of sialostatin L on dendritic cells reveals its potential to interfere with autoimmunity. **J Immunol,** v. 182, n. 12, p. 7422-9, Jun 15 2009. ISSN 1550-6606 (Electronic)

0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19494265 >.

SA-NUNES, A. et al. Prostaglandin E2 is a major inhibitor of dendritic cell maturation and function in Ixodes scapularis saliva. **J Immunol,** v. 179, n. 3, p. 1497-505, Aug 1 2007. ISSN 0022-1767 (Print)

0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17641015 >.

SANDOVAL, C. M. et al. Feeding sources and natural infection of Belminus herreri (Hemiptera, Reduviidae, Triatominae) from dwellings in Cesar, Colombia. **Mem Inst Oswaldo Cruz,** v. 99, n. 2, p. 137-40, Mar 2004. ISSN 0074-0276 (Print)

0074-0276 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15250465 >.

SANT'ANNA, M. R. et al. Feeding behaviour of morphologically similar Rhodnius species: influence of mechanical characteristics and salivary function. **J Insect Physiol,** v. 47, n. 12, p. 1459-1465, Dec 2001. ISSN 1879-1611 (Electronic)

0022-1910 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12770152 >.

SANTIAGO, P. B. et al. A Deep Insight into the Sialome of Rhodnius neglectus, a Vector of Chagas Disease. **PLoS Negl Trop Dis,** v. 10, n. 4, p. e0004581, Apr 2016. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27129103 >.

SANTIAGO, P. B. et al. Proteomic Mapping of Multifunctional Complexes Within Triatomine Saliva. **Front Cell Infect Microbiol,** v. 10, p. 459, 2020. ISSN 2235-2988 (Electronic)

2235-2988 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32984079 >.

SANTIAGO, P. B. et al. Exploring the molecular complexity of Triatoma dimidiata sialome. **J Proteomics,** v. 174, p. 47-60, Mar 1 2018. ISSN 1876-7737 (Electronic)

1874-3919 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29288089 >.

SANTOS, A. et al. The sialotranscriptome of the blood-sucking bug Triatoma brasiliensis (Hemiptera, Triatominae). **Insect Biochem Mol Biol,** v. 37, n. 7, p. 702-12, Jul 2007. ISSN 0965-1748 (Print)

0965-1748 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17550826 >.

SANTOS, E. F. et al. Acute Chagas disease in Brazil from 2001 to 2018: A nationwide spatiotemporal analysis. **PLoS Negl Trop Dis,** v. 14, n. 8, p. e0008445, Aug 2020. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32745113 >.

SARKIS, J. J.; GUIMARAES, J. A.; RIBEIRO, J. M. Salivary apyrase of Rhodnius prolixus. Kinetics and purification. **Biochem J,** v. 233, n. 3, p. 885-91, Feb 1 1986. ISSN 0264-6021 (Print)

0264-6021 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/3010945 >.

SAÚDE, M. D. **Doença de Chagas - 14 de abril | Dia Mundial**

SAÚDE, S. D. V. E. Brasília/ Brasil: Ministério da Saúde. Número Especial | Abril 2021 2021.

SCHAUB, G. A. Parasitogenic alterations of vector behaviour. **Int J Med Microbiol,** v. 296 Suppl 40, p. 37-40, May 2006. ISSN 1438-4221 (Print)

1438-4221 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16530007 >.

SCHMITZ, H. T., S.; HOFMANN, M. H.; BLECKMANN, H. The ability of Rhodnius prolixus (Hemiptera; Reduviidae) to approach a thermal source solely by its infrared radiation,. **Journal of Insect Physiology,** v. 46, n. 5, p. 745-751, 2000. ISSN 0022-1910.

SCHOELER, G. B.; WIKEL, S. K. Modulation of host immunity by haematophagous arthropods. **Ann Trop Med Parasitol,** v. 95, n. 8, p. 755-71, Dec 2001. ISSN 0003-4983 (Print)

0003-4983 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11784430 >.

SCHWARZ, A. et al. An updated insight into the Sialotranscriptome of Triatoma infestans: developmental stage and geographic variations. **PLoS Negl Trop Dis,** v. 8, n. 12, p. e3372, Dec 2014. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25474469 >.

SCHWARZ, A. et al. A systems level analysis reveals transcriptomic and proteomic complexity in Ixodes ricinus midgut and salivary glands during early attachment and feeding. **Mol Cell Proteomics,** v. 13, n. 10, p. 2725-35, Oct 2014. ISSN 1535-9484 (Electronic)

1535-9476 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25048707 >.

SCOTT, J. G.; WEN, Z. Cytochromes P450 of insects: the tip of the iceberg. **Pest Manag Sci,** v. 57, n. 10, p. 958-67, Oct 2001. ISSN 1526-498X (Print)

1526-498X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11695190 >.

SHANNON, P. et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. **Genome Res,** v. 13, n. 11, p. 2498-504, Nov 2003. ISSN 1088-9051 (Print)

1088-9051 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14597658 >.

SIMAO, F. A. et al. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. **Bioinformatics,** v. 31, n. 19, p. 3210-2, Oct 1 2015. ISSN 1367-4811 (Electronic)

1367-4803 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26059717 >.

SMITH-UNNA, R. et al. TransRate: reference-free quality assessment of de novo transcriptome assemblies. **Genome Res,** v. 26, n. 8, p. 1134-44, Aug 2016. ISSN 1549-5469 (Electronic)

1088-9051 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27252236 >.

SOARES, A. C. et al. Salivation pattern of Rhodnius prolixus (Reduviidae; Triatominae) in mouse skin. **J Insect Physiol,** v. 52, n. 5, p. 468-72, May 2006. ISSN 0022-1910 (Print)

0022-1910 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16580013 >.

SOARES, T. S. et al. A Kazal-type inhibitor is modulated by Trypanosoma cruzi to control microbiota inside the anterior midgut of Rhodnius prolixus. **Biochimie,** v. 112, p. 41-8, May 2015. ISSN 1638-6183 (Electronic)

0300-9084 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25731714 >.

SONESON, C.; LOVE, M. I.; ROBINSON, M. D. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. **F1000Res,** v. 4, p. 1521, 2015. ISSN 2046-1402 (Print)

2046-1402 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26925227 >.

SONG, L.; FLOREA, L. Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. **Gigascience,** v. 4, p. 48, 2015. ISSN 2047-217X (Print)

2047-217X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26500767 >.

SPIT, J. et al. Transcriptional Analysis of The Adaptive Digestive System of The Migratory Locust in Response to Plant Defensive Protease Inhibitors. **Sci Rep,** v. 6, p. 32460, Sep 1 2016. ISSN 2045-2322 (Electronic)

2045-2322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27581362 >.

STANKE, M. et al. AUGUSTUS: ab initio prediction of alternative transcripts. **Nucleic Acids Res,** v. 34, n. Web Server issue, p. W435-9, Jul 1 2006. ISSN 1362-4962 (Electronic)

0305-1048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16845043 >.

STERKEL, M. et al. Tyrosine Detoxification Is an Essential Trait in the Life History of Blood-Feeding Arthropods. **Curr Biol,** v. 26, n. 16, p. 2188-93, Aug 22 2016. ISSN 1879-0445 (Electronic)

0960-9822 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27476595 >.

STOKA, A. M. Activity of juvenile hormone and juvenile hormone analogues on the growth of Trypanosoma cruzi. **J Steroid Biochem Mol Biol,** v. 59, n. 5-6, p. 495-500, Dec 1996. ISSN 0960-0760 (Print)

0960-0760 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9010355 >.

SURGET-GROBA, Y.; MONTOYA-BURGOS, J. I. Optimization of de novo transcriptome assembly from next-generation sequencing data. **Genome Res,** v. 20, n. 10, p. 1432-40, Oct 2010. ISSN 1549-5469 (Electronic)

1088-9051 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20693479 >.

TAKANO-LEE, M.; EDMAN, J. D. Lack of manipulation of Rhodnius prolixus (Hemiptera: Reduviidae) vector competence by Trypanosoma cruzi. **J Med Entomol,** v. 39, n. 1, p. 44-51, Jan 2002. ISSN 0022-2585 (Print)

0022-2585 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11931271 >.

TARLETON, R. L. et al. The challenges of Chagas Disease-- grim outlook or glimmer of hope. **PLoS Med,** v. 4, n. 12, p. e332, Dec 2007. ISSN 1549-1676 (Electronic)

1549-1277 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18162039 >.

TASCHUK, F.; CHERRY, S. DEAD-Box Helicases: Sensors, Regulators, and Effectors for Antiviral Defense. **Viruses,** v. 12, n. 2, Feb 5 2020. ISSN 1999-4915 (Electronic)

1999-4915 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32033386 >.

TE BRUGGE, V. A.; SCHOOLEY, D. A.; ORCHARD, I. The biological activity of diuretic factors in Rhodnius prolixus. **Peptides,** v. 23, n. 4, p. 671-681, 2002/04/01/ 2002. ISSN 0196-9781. Disponível em: < https://www.sciencedirect.com/science/article/pii/S0196978101006611 >.

TEIXEIRA, A. R. et al. Pathogenesis of chagas' disease: parasite persistence and autoimmunity. **Clin Microbiol Rev,** v. 24, n. 3, p. 592-630, Jul 2011. ISSN 1098-6618 (Electronic)

0893-8512 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21734249 >.

TEIXEIRA, A. R.; NASCIMENTO, R. J.; STURM, N. R. Evolution and pathology in chagas disease--a review. **Mem Inst Oswaldo Cruz,** v. 101, n. 5, p. 463-91, Aug 2006. ISSN 0074-0276 (Print)

0074-0276 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17072450 >.

TJALSMA, H. et al. Signal peptide-dependent protein transport in Bacillus subtilis: a genome-based survey of the secretome. **Microbiol Mol Biol Rev,** v. 64, n. 3, p. 515-47, Sep 2000. ISSN 1092-2172 (Print)

1092-2172 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10974125 >.

URSIC-BEDOYA, R. J.; LOWENBERGER, C. A. Rhodnius prolixus: identification of immune-related genes up-regulated in response to pathogens and parasites using suppressive subtractive hybridization. **Dev Comp Immunol,** v. 31, n. 2, p. 109-20, 2007. ISSN 0145-305X (Print)

0145-305X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16824597 >.

VALENZUELA, J. G. et al. The D7 family of salivary proteins in blood sucking diptera. **Insect Mol Biol,** v. 11, n. 2, p. 149-55, Apr 2002. ISSN 0962-1075 (Print)

0962-1075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11966880 >.

VALENZUELA, J. G. et al. Purification, cloning, and expression of a novel salivary anticomplement protein from the tick, Ixodes scapularis. **J Biol Chem,** v. 275, n. 25, p. 18717-23, Jun 23 2000. ISSN 0021-9258 (Print)

0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10749868 >.

VALENZUELA, J. G. et al. Exploring the sialome of the tick Ixodes scapularis. **J Exp Biol,** v. 205, n. Pt 18, p. 2843-64, Sep 2002. ISSN 0022-0949 (Print)

0022-0949 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12177149 >.

VALLEJO, G. A.; GUHL, F.; SCHAUB, G. A. Triatominae-Trypanosoma cruzi/T. rangeli: Vector-parasite interactions. **Acta Trop,** v. 110, n. 2-3, p. 137-47, May-Jun 2009. ISSN 1873-6254 (Electronic)

0001-706X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18992212 >.

VERLY, T. et al. Vector competence and feeding-excretion behavior of Triatoma rubrovaria (Blanchard, 1843) (Hemiptera: Reduviidae) infected with Trypanosoma cruzi TcVI. **PLoS Negl Trop Dis,** v. 14, n. 9, p. e0008712, Sep 2020. ISSN 1935-2735 (Electronic)

1935-2727 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/32970687 >.

VIEIRA, C. S. et al. Rhodnius prolixus interaction with Trypanosoma rangeli: modulation of the immune system and microbiota population. **Parasites & Vectors,** v. 8, n. 1, p. 135, 2015/03/01 2015. ISSN 1756-3305. Disponível em: < https://doi.org/10.1186/s13071-015-0736-2 >.

VIEIRA, C. S. et al. Impact of Trypanosoma cruzi on antimicrobial peptide gene expression and activity in the fat body and midgut of Rhodnius prolixus. **Parasit Vectors,** v. 9, p. 119, Mar 1 2016. ISSN 1756-3305 (Electronic)

1756-3305 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26931761 >.

WALKER, A. A. et al. Melt With This Kiss: Paralyzing and Liquefying Venom of The Assassin Bug Pristhesancus plagipennis (Hemiptera: Reduviidae). **Mol Cell Proteomics,** v. 16, n. 4, p. 552-566, Apr 2017. ISSN 1535-9484 (Electronic)

1535-9476 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28130397 >.

WANG, H.; NUTTALL, P. A. Excretion of host immunoglobulin in tick saliva and detection of IgG-binding proteins in tick haemolymph and salivary glands. **Parasitology,** v. 109 ( Pt 4), p. 525-30, Nov 1994. ISSN 0031-1820 (Print)

0031-1820 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7794319 >.

WANG, Z.; GERSTEIN, M.; SNYDER, M. RNA-Seq: a revolutionary tool for transcriptomics. **Nat Rev Genet,** v. 10, n. 1, p. 57-63, Jan 2009. ISSN 1471-0064 (Electronic)

1471-0056 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19015660 >.

WANIEK, P. J.; JANSEN, A. M.; ARAUJO, C. A. Trypanosoma cruzi infection modulates the expression of Triatoma brasiliensis def1 in the midgut. **Vector Borne Zoonotic Dis,** v. 11, n. 7, p. 845-7, Jul 2011. ISSN 1557-7759 (Electronic)

1530-3667 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20925526 >.

WATERHOUSE, R. M. et al. BUSCO Applications from Quality Assessments to Gene Prediction and Phylogenomics. **Mol Biol Evol,** v. 35, n. 3, p. 543-548, Mar 1 2018. ISSN 1537-1719 (Electronic)

0737-4038 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29220515 >.

WATERHOUSE, R. M.; ZDOBNOV, E. M.; KRIVENTSEVA, E. V. Correlating traits of gene retention, sequence divergence, duplicability and essentiality in vertebrates, arthropods, and fungi. **Genome Biol Evol,** v. 3, p. 75-86, 2011. ISSN 1759-6653 (Electronic)

1759-6653 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21148284 >.

WEISS, B.; AKSOY, S. Microbiome influences on insect host vector competence. **Trends Parasitol,** v. 27, n. 11, p. 514-22, Nov 2011. ISSN 1471-5007 (Electronic)

1471-4922 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21697014 >.

WHEELER, D. L. et al. Database resources of the National Center for Biotechnology. **Nucleic Acids Res,** v. 31, n. 1, p. 28-33, Jan 1 2003. ISSN 1362-4962 (Electronic)

0305-1048 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12519941 >.

WHO, W. H. O. E. C. **Chagas disease (American trypanosomiasis).** 2021.

WINGETT, S. W.; ANDREWS, S. FastQ Screen: A tool for multi-genome mapping and quality control. **F1000Res,** v. 7, p. 1338, 2018. ISSN 2046-1402 (Electronic)

2046-1402 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/30254741 >.

WOLF, J. B. Principles of transcriptome analysis and gene expression quantification: an RNA-seq tutorial. **Mol Ecol Resour,** v. 13, n. 4, p. 559-72, Jul 2013. ISSN 1755-0998 (Electronic)

1755-098X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23621713 >.

WOLFE, A. J. The acetate switch. **Microbiol Mol Biol Rev,** v. 69, n. 1, p. 12-50, Mar 2005. ISSN 1092-2172 (Print)

1092-2172 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15755952 >.

WOMMACK, K. E.; BHAVSAR, J.; RAVEL, J. Metagenomics: read length matters. **Appl Environ Microbiol,** v. 74, n. 5, p. 1453-63, Mar 2008. ISSN 1098-5336 (Electronic)

0099-2240 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18192407 >.

WU, M. C.; LU, K. H. Juvenile hormone induction of glutathione S-transferase activity in the larval fat body of the common cutworm, Spodoptera litura (Lepidoptera: Noctuidae). **Arch Insect Biochem Physiol,** v. 68, n. 4, p. 232-40, Aug 2008. ISSN 1520-6327 (Electronic)

0739-4462 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18618763 >.

ZHANG, Y. et al. Nitrophorin-2: a novel mixed-type reversible specific inhibitor of the intrinsic factor-X activating complex. **Biochemistry,** v. 37, n. 30, p. 10681-90, Jul 28 1998. ISSN 0006-2960 (Print)

0006-2960 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9692958 >.

ZHANG, Y. et al. Predicating the Effector Proteins Secreted by Puccinia triticina Through Transcriptomic Analysis and Multiple Prediction Approaches. **Front Microbiol,** v. 11, p. 538032, 2020. ISSN 1664-302X (Print)

1664-302X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/33072007 >.

# APÊNDICES

## APENDICE A - Artigo científico

Artigo científico extraído deste trabalho e submetido à Revista **Frontiers Cellular and Infection Microbiology** como parte das exigências para obtenção do título de Doutor no programa de Ciências Fisiológicas da Universidade Federal do Triângulo Mineiro, Uberaba, 2021. Submetido sob o numero 773357 e intitulado "Salivary and Intestinal Transcriptomes Reveal the Specific Proteins and Differential Gene Expression in *Trypanosoma cruzi*-Infected and Non-Infected *Rhodnius neglectus*"

# Salivary and Intestinal Transcriptomes Reveal the Specific Proteins and Differential Gene Expression in *Trypanosoma cruzi*-Infected and Non-Infected *Rhodnius neglectus*

**Tamires Marielem de Carvalho-Costa[1,†], Rafael Destro Rosa Tiveron [1,†], Maria Tays Mendes [2], Cecília Gomes Barbosa[1], Jessica Coraiola Nevoa[1], Guilherme Augusto Roza[1], Marcos Vinícius Silva[1], Virmondes Rodrigues[1], Siomar de Castro Soares[1], Carlo José Freire Oliveira[1,*]**

[1]Laboratory of Immunology and Bioinformatics, Institute of Biological and Natural Sciences, Federal University of Triangulo Mineiro, Uberaba, MG, Brazil.

[2]Biomedical Research Center, The University of Texas at El Paso, El Paso, Texas USA.

[†]These authors contributed equally to this manuscript and share the first authorship.

*** Correspondence:**
Carlo J. F. Oliveira, Laboratory of Immunology and Bioinformatics, Institute of Biological and Natural Sciences, Federal University of Triangulo Mineiro, Uberaba, MG, Brazil. Rua Frei Paulino, 30 - Nossa Sra. da Abadia, Uberaba, MG 38025-180, Brazil.

Email: carlo.oliveira@uftm.edu.br

**Keywords: *Trypanosoma cruzi*, Triatominae, transcriptome, salivary glands, intestine.**

**Abstract**

*Rhodnius neglectus* is a potential vector of *Trypanosoma cruzi* (Tc), the causative agent of Chagas disease. During blood feeding, the salivary glands (SGs) and intestine (INT) are actively needed. The saliva of SGs is injected into the vertebrate host, modulating immune responses and favoring feeding for INT digestion. Tc infection significantly alters the physiology of these tissues; however, studies that assess this are still scarce. To better understand them, this study aimed to evaluate the global transcriptional expression of genes in SGs and INT in fasting (FA), Fed (FE) and Fed in the presence of Tc (FE + Tc). In FA, the expression of transcripts related to homeostasis maintenance proteins during periods of stress was predominant. Therefore, transcripts for Tret1-Like and Hsp70Ba proteins were increased.

As expected, in the FE group, the presence of blood seemed to be responsible for the alterations found, since most of the expressed transcripts were related to digestion, such as transcripts for the proteases and Cathepsin D. In FE + Tc, there was decrease expression of blood processing genes for the insect metabolism (e.g. Antigen-5 precursor, Pr13a, Obp), detoxication (Sult1) in INT and acid phosphatases in SG. We also found less transcriptional expression of lipocalins and nitrophorins in SG and two new proteins, Pacifastin and Diptericin, in INT. Several transcripts of unknown proteins with investigative potential were found in both tissues. Our results also show that the presence of Tc is capable of altering the expression in both tissues for a long or short time. While in SG homeostasis seems to be re-established on day 9, changes in INT are still evident. The information generated in this work can serve as a guide for future studies on the parasite-vector interaction and contribute to the understanding of food physiology and post-meal / infection in triatomines.

## 1 Introduction

Triatomines, a group of hemimetabolous insects with approximately 140 species, are arthropods that specialize in locating vessels and sucking blood directly from them. In addition to causing direct damage to the host through the insertion of the mouthparts of the triatomine, this food habit can also carry bioactive molecules that facilitate the transmission and propagation of pathogens, such as the flagellate *Trypanosoma cruzi*, the causative agent of Chagas disease (Mesquita et al., 2008; Mendes et al., 2016). Among the many important species of triatomines, it is worth highlighting the species *Rhodnius neglectus*, one of the 20 species of the genus Rhodnius, which is widely distributed in Brazil (Falcone et al., 2020) and plays an important role in the sylvatic maintenance of *T. cruzi* (Tc) in South America (Gurgel-Goncalves et al., 2012).

Triatomine saliva is deposited at the bite site during the entire hematophagy process, and it is essential for successful feeding (Soares et al., 2006); it modulates physiological repair responses, such as platelet aggregation, vasoconstriction, blood coagulation, increased vascular permeability, chemotaxis, and leukocyte immune function (Mesquita et al., 2008; Fontaine et al., 2011; de Araujo et al., 2012; Mendes et al., 2016).

In addition to the salivary glands (SG), the triatomine intestine (INT), where the blood is stored and digested, is of great relevance. Besides the absorption of nutrients and a barrier against aggressor agents, it is in this tissue that Tc transforms itself into the infective form and, with the act of feeding and defecation, can infect new vertebrate hosts (Garcia et al., 2007). Furthermore, essential molecules present in the intestine of triatomines can increase blood intake in a shorter time, protecting the vector and giving the parasite an opportunity for infection (Rossignol et al., 1985; Paim et al., 2011).

Despite the relevance of SGs and INT in triatomine biology and the maintenance and transmission of pathogens, there is still little information about the expression and production of bioactive molecules from these tissues in triatomines infected or not infected with Tc. Studies of transcriptomes in other hematophagous arthropods, including ticks and mosquitoes, have shown that gene expression in these tissues actively changes and can vary with factors

including developmental stage, environmental conditions, physical stimuli, physiological state, presence of infectious agents, and interactions with their hosts (Wang et al., 2009; Wolf, 2013). In other words, understanding the triatomine transcriptome seems to be an essential tool in the search for understanding the feeding dynamics and vector potential, among other host-parasite interactions.

Thus, our study aimed to use improved bioinformatics tools to analyze the transcriptomes of SGs and INT of *R. neglectus*, infected or not infected with Tc, and map the molecules related to the presence of the parasite in the face of the vector's feeding habit.

## 2 Materials and Methods

### 2.1 Insects, feeding, and tissue collection

Adults of both sexes of *R. neglectus*, reared at the University Federal of Triangulo Mineiro Triatomine Insectario (Uberaba, Brazil), were fasted for 30 days. The insects were weighed and separated into the following groups: Fasting Group (FA): insects that remained without feeding; Fed Group (FE): insects fed for 2 h in an artificial feeder with human blood; and Fed and Infected Group (FE + Tc): insects were fed for 2 h in an artificial feeder with human blood infected with trypomastigotes of *T. cruzi* Colombiana strain ($1 \times 10^6$ parasites/mL) previously cultivated for 30 days in MK-2 cells. Artificial feeding success was verified by measuring the weight before and after feeding. In addition to SGs and INTs collected from FA, in FE + Tc, the tissues were collected after 2 and 9 days and in FE after 2 days. These tissues were placed in RNAlater (Qiagen, Valencia, CA), 200 µL and 400 µL respectively, stored at 4 °C for 2 days, and then stored at -80 °C until further analysis.

### 2.2 Quantitative polymerase chain reaction (qPCR)

Tc positivity in the intestinal contents of FE + Tc cells was confirmed by qPCR analyses. The Promega blood-tissue extraction kit (ReliaPrep™ gDNA Tissue Miniprep System) was used according to the manufacturer's guidelines. The primers used were Cruzi 1 (5'-ASTCGGCTGATCGTTTTCGA-3') and Cruzi 2 (5'-AATTCCTCCAAGCAGCGGATA-3'), labeled with 5(6-carboxyfluorescein) and 3BHQ1™ (Black Hole Quencher), which amplify a 166bp Tc satellite DNA fragment (Piron et al. 2007). For amplification control, the primers Bact Fw (5-AGCCATGTACGTAGCCATCCA-3') and Bact Rv (5′-TCTCCGGAGTCCATCACAATG -3'), which amplify an 81 bp fragment of the *Mus musculus* β-actin gene and probe Bact (5'-TGTCCCTGTATGCCTCTGGTCGTACCAC-3') were used. All the FE + Tc samples were positive for Tc.

### 2.3 Extraction of total RNA, construction of library, and transcriptomes sequencing

RNA extraction was performed using the RNAeasy Minikit Kit (50) (Qiagen, Germantown, USA; Cat No/ID:74104) according to the manufacturer's instructions. The material was quantified using Qubit RNA (Thermo Fisher, Eugene, USA), and the quality was verified and validated using TapeStation (Agilent, California, USA). The library construction followed the standard protocol of the TruSeq Exome kit (California, USA)(formerly TruSeq RNA Access

Library Prep Kit, Illumina), except that the second hybridization was not performed. The run was performed on a paired end with 101 base pair (bp) readings.

## 2.4 Bioinformatics analysis

Sequencing quality parameters were evaluated using FastQC (v0.11.7) (https://www.bioinformatics.babraham.ac.uk/publications.html)(Wingett and Andrews, 2018). Reads were trimmed at the ends and stopped at a base ≥ 20 Phred score. Reads with total bp > 30% with < Phred 20 or > 15% with ≤ Phred 15 were removed. Subsequently, barcode sequences were trimmed using Trimmomatic (v0.36) (http://www.usadellab.org/cms/?page=trimmomatic )(Bolger et al., 2014), and random sequencing errors were corrected using Rcorrector (v1.0.4) (https://gigascience.biomedcentral.com/articles/10.1186/s13742-015-0089-y) (Song and Florea, 2015), both through the Oyster River Protocol workflow (ORP v2.2.8) (https://oyster-river-protocol.readthedocs.io/en/latest/strandexamine.html) (MacManes, 2018).

De-novo assembly was performed using a multi-k-mer (multi-assembler) approach in two steps, using Oyster and Orthofuser workflows. In the first stage, Orthofuser was used to determine the unique partial reference formed by deduplicated contigs from each sample assembly. In the second stage, the partial reference was formed by contigs assembled from the junction of the reads of all samples, normalized to a maximum of 1000 reads of identical sequence representation. Finally, both references were merged, the contigs were again deduplicated, and those that were not mapped by salmon (v0.13.1) (https://github.com/COMBINE-lab/salmon) were removed (Patro et al., 2017). Quality assessment of the final assembly was performed using the Oyster River Strand Exam Tool, Transrate (v1.0.3) (https://hibberdlab.com/transrate/index.html)(Smith-Unna et al., 2016), and BUSCO (v4.1.3) (https://gitlab.com/ezlab/busco/-/releases#4.1.4) (Waterhouse et al., 2018).

For transcript annotation, NCBI databases were used (non-redundant proteins – NR complete, including PIR, PDB, and RefSeq), SwissProt, UniProt, SMART, Pfam, KOG, CDD, PRK, TIGR, GO-SeqDB, and MEROPS. Protein matching with the highest bitscore (E-value < 10-4) for each transcript was considered using Diamond (v2.0.5) (https://github.com/bbuchfink/diamond ) (Buchfink et al., 2015) and its standard composition correction. The data were plotted in a spreadsheet with the script in Visual Basic Advanced, EMBLtable, created and provided collaboratively by Dr. José Marcus C. Ribeiro. Other results have also been added.

To predict all putative protein segments of the open reading frame (ORF) translated by the transcripts, three prediction tools were used: TransDecoder (v5.5.0 – workflow Pfam-blastp)( https://www.nature.com/articles/nprot.2013.084) (Haas et al., 2013), Augustus (v3.3.3 – *training set* Rhodnius) (https://github.com/Gaius-Augustus/Augustus) (Stanke et al., 2006), and ORFfinder (v0.4.3)( https://www.ncbi.nlm.nih.gov/orffinder/) (Wheeler et al., 2003). The annotation results were used to precisely determine the coding regions (CDS) and choose among the most extended protein segments. Among the segments predicted with ORFfinder of transcripts that did not have an annotation match, only the four longest transcripts were

selected. Of these segments, due to CDS imprecision, only the longest segment classified was considered to determine whether there was a signal peptide (SP+ or SP).

The SP search in the ORFs was performed using SignalP (v5.1) (http://www.cbs.dtu.dk/services/SignalP/abstract.php#5.0) (Almagro Armenteros et al., 2019a). Using the annotation results, global functional clustering of the transcripts was performed manually. Probable mitochondrial peptides were classified using TargetP (v2.0) (http://www.cbs.dtu.dk/services/TargetP/cite.php) (Almagro Armenteros et al., 2019b), while the prediction of possible transmembrane peptides was performed using TMHMM (v2.0) (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0)(Moller et al., 2001). Detailed functional annotation of transcripts was performed by comparing the coding region against Kegg Orthology's PATHWAY and BRITE databases using the online tool GhostKOALA (v2.2) (https://www.kegg.jp/ghostkoala/)(Kanehisa et al., 2016). The considered annotation had the highest GHOSTscore among matches, corresponding to the predicted ORFs of the same transcript. Finally, the detection of important sites, domains, and protein families was performed on representative ORFs of transcripts without matches or translatable into unknown or hypothetical proteins using complete analysis of InterProScan (v5.51-85.0) (https://www.ebi.ac.uk/interpro/download/), taking into account the lowest E-value for each transcript, provided that < 10-4.

The number of reads aligned to transcripts by salmon was normalized. Differential analysis between the experimental conditions was performed using DESeq2 (v1.28.1) (http://bioconductor.org/packages/release/bioc/html/DESeq2.html) (Love et al., 2014) and tximport (v1.16.1) (https://bioconductor.org/packages/release/bioc/html/tximport.html) (Soneson et al., 2015) packages, both for R. Orthologous transcripts to the biological network of *Rhodnius prolixus* (v11.0) obtained from StringDB (https://pubmed.ncbi.nlm.nih.gov/23203871/) (Franceschini et al., 2013) were identified by comparing deduplicated ORFs against protein sequences of *R. prolixus*, using Orthofinder (v.2.2.1) (https://github.com/davidemms/OrthoFinder ) (Emms and Kelly, 2015). The highest bitscore defined the homologous pair sequences from the ortholog groups by directly aligning the respective transcripts against the network proteins using Diamond. Only the protein-protein interactions (PPI) evidenced experimentally and with a combined score of at least 600 were considered. The final model of the network was set in Cytoscape (v3.8.2) (https://pubmed.ncbi.nlm.nih.gov/14597658/)(Shannon et al., 2003).

## 3 Results

### 3.1 *R. neglectus* INT and SG assembly general description and transcriptome quality

A total of 37.873.676 paired-reads (90.23-98.92% ≥ Q30) were generated, with a Gaussian mean of 100 bp (85-102 bp). After processing the reads, 67.529 transcripts were assembled, with an average of 417 bp. 3.928 transcripts were > 1000 bp aligned with at least 1000 paired-reads (Figure 1A).

Reading assertiveness and transcript completeness were analyzed. The assembly presented a unimodal distribution, right-skewed, with a mean of 0.887 (0.857-1.0) (Figure 1B), as

expected for paired-end assemblies (https://oyster-river-protocol.readthedocs.io/en/latest/strandexamine.html). Combining the two steps with ORP allowed us to exclude uncovered contigs and assemble approximately 5000 contigs with greater coverage than the standard workflow. In the Transrate, the p good mapping was 0.82, p fragment mapping was 0.88, and p good contigs was 0.7, indicating good alignment quality (Smith-Unna et al., 2016). With BUSCO, the contigs were aligned against sequences from the universal arthropod bank. A total of 55.6% (414) were entirely mapped by a single contig, and 2.1% (16) by two or more contigs. 42.3% (315) correspond to "fragmented" or partially recovered sequences (Waterhouse et al., 2018) but did not compromise the annotations performed.

### 3.2 ORFs characterization and taxon-functional annotation

Of *the R. neglectus* transcripts, 37.2% were similar to those from arthropods, 0.8% were similar to other eukaryotes, and 60.9% were not similar to any taxonomic group (Figure 2A). Among the annotated proteins from symbionts and residuals, 681 (1.0%) were similar to bacteria, 77 (0.11%) to viruses, 14 (0.02%) to Tc (FE + Tc), 22 (0.03%) to other protozoa, and 30 (0.04%) to fungi (Figure 2B). The prediction of ORFs is important for indicating the molecular role of the obtained transcripts (Finkel et al., 2016; Finkel et al., 2018). Of the total transcripts (Table 1), 904 produced known secreted isoforms, and 16453 were housekeeping. The proportion of transcripts predicted to produce secreted peptides and housekeeping was higher in SG than in INT. A total of 349 transcripts producing hypothetical or unknown secreted proteins were identified, of which 8930 were SP-. Only 263 transcripts (0.4%) belonged to DNA transposable elements and, among the 60.9% mentioned, 4256 could still generate an SP+ sequence without annotation match.

Only 41.2% have CDS patterns defined by homology or identified/optimized by TransDecoder or Augustus (transcripts with matched CDS group pattern). Of these, 58.7% were housekeeping, followed by 31.5% as Unknown/hypothetical non-secreted peptide generators, which shows that many CDSs are already known, even without a defined function. The total number of ORFs predicted by the tools was 74031 (Figure 3), and only 7.5% of them had SP+. In general, SP-ORFs do not have a transmembrane helix, and their prediction can reach 98% of assertiveness (Krogh et al., 2001). This indicates that these proteins are likely to be present in the intracellular environment (Figure 4). Among the ORFs with only one helix, 8896 are SP-, as expected, only 744 are SP+, and up to 1386 may have mitochondrial targeting (mTP) (Figure 5).

The transcript taxonomic homology showed that most were orthologous to arthropods (37.9%), followed by other eukaryotes (36.9%), trypanosoma (0.1%), and prokaryotes (25.0%) (Figure 6A). However, when evaluating only functionally annotated transcripts, the proportion of orthologs to arthropods increased to 71.2%, while those orthologs to other taxa were smaller. Furthermore, 6.3% of functionally annotated transcripts had undefined taxonomic orthologs (Figure 6B). Most transcripts, both glandular and intestinal, are related to genetic information processing (24%), followed by translation into enzymes (23%) (Figure

7). Functional classes were analyzed considering only arthropod orthologs to avoid asserting unproven findings in arthropods.

Most of the cellular process transcripts (Figure 8) are related to the translation of components involved in transport and catabolism (2658), mainly in INT, which is more diverse than SG. For the processing of genetic information (2205), the diversity of the components in transcription is highlighted, and for the processing of environmental information, those involved in signal transduction (1837) and peptide carriers (1587) are more diverse. Phosphatase transcripts and associated proteins (631), kinase proteins (594), peptidases, and inhibitors (530) participate in these processes that are diversely similar in these tissues. Protein transcripts with endocrine functions (970) and immunological functions (628) are the most diverse among the system components present in these tissues, and transcripts related to carbohydrate metabolism (565) were more diverse than those of other metabolic pathways.

### 3.3 *R. neglectus* salivary and intestinal secretome

Considering the proteins most commonly secreted in arthropods, it is possible to find greater diversity of lipocalin transcripts (70) in *R. neglectus* SG and INT (Supplementary Figure 2), highlighting similarities to the so-called lipocalin precursors 4, 5, 6, and 7, and other precursors of procalins, triabines, and palidipines, followed by unknown transcripts of peptidases and associated inhibitors (54), as well as of unknown secreted proteins (31). On the other hand, the diversity of lipocalins and nitrophorins was greater in SG (Figure 11), consistent with previous observations (Santiago, 2018). Interestingly, there are many protein transcripts with primary membrane or intracellular aspects, but SP+ and without or with only one transmembrane helix (Supplementary Figure 2). The most diverse are related to channels and (co)transport (37) predominantly in the INT (Supplementary Figure 2), the modification and assembly of peptides (27), and adhesion, cytoskeleton, and associated regulators (18).

### 3.4 Differential expression according to feeding status and *T. cruzi* infection

In addition to their high diversity, four transcripts similar to Lipocalin AI-5 precursor were among the 20 most expressed transcripts in any experimental condition in SGs, while those similar to Nitrophorin 1 precursor were among 20 most expressed transcripts in SGs after repast (Supplementary Table 1). In the INT, cathepsin B stood out among the 20 most expressed, which was also transcriptionally elevated in FE + Tc only on the 9th day (Supplementary Table 1). As for lysozyme 1, despite being present in the INT of FE + Tc, it was not observed among 20 most expressed transcripts in 2 days.

Furthermore, transcripts similar to hypothetical proteins were observed among the 20 most expressed transcripts: GE061_03760 (which has no known family/domains) and GE061_06167 (tryptophan aminotransferase-related protein 1 domain) in all groups, except for 9 d FE + Tc, and GE061_01450 (consensual disorder domain) present only in FA. Two transcripts belonging to the unknown family/domain identified in the PANTHER database as PTHR33626 were also observed, but similar to the hypothetical protein GE061_03717, both in SG and INT, regardless of the experimental conditions.

When looking at what changes in the intestinal transcripts' expression when *R. neglectus* performs the feeding (Figure 9A), it is observed a significant reduction in the expression of genes for NADPH P450 reductase proteins, odorant-binding precursor protein (p-Obp), chemosensory-like protein (CSP-like), Tret1-like, DEAD-box helicase 5, DEAD BOX vasa, hexamerin-like protein 1, Hsp70Ba, a member of the C19 peptidase family, and 24 unknown protein transcripts. On the other hand, a significant transcriptional increase occurs for 20 known proteins and around 38 unidentified proteins that are 2-8 times more expressed, highlighting Cyp6a14 (similar to Isoform X3), tyrosine aminotransferase (Tat), three peptidases, one precursor of myosupressin (p-Myossupresin), a precursor of hormone neuroparsin (p-neuroparsin), an Ino1-like (Ino1-like) peptide, a member of the AA peptidases, the precursors of lipocalin AI-5 and nitroporin 3.

However, in the presence of Tc, the gene expression of the proteins Cathepsin D, Defensin C, CREBB (CREB-binding protein), Histone H3v1, the same member of the AA peptidases, lipocalin AI-5, p-myossupresin, and 20 unknown transcripts decreased significantly. At the same time, a significant increase occurred in 27 unknown protein genes, highlighting the sulfotransferase-like peptide Sult1c4 (Sult1c4-like), lipocalin-like 2, and an unassigned T3 peptidase.

The presence of viruses in the intestinal microbiota is possible, and we found transcripts that correspond to viral genes (Figure 2), including the Triatoma virus (TrV) (Supplementary Table 2) pathogenic to triatomines, which inevitably affects wild and colony triatomines (Muscio et al., 1988; Muscio et al., 1997; Marti et al., 2013). Furthermore, our results suggest that the presence of *T. cruzi* in *R. neglectus* lowers the expression of transcripts from ORF 2 (Czibener et al., 2000; Ankavay et al., 2019) and precursor nonstructural protein, both from TrV (Agirre et al., 2011), perhaps due to the competitiveness of these biological agents.

Assessing the global distribution of transcripts at least 2-fold down/upregulated expression between FA, FE, and FE + Tc (Figure 10), similar amounts occurred only in INT (4860) or in SGs (4068) when in FE and only 1552 occurred simultaneously on both. This amount increased by 8% in both tissues when infection occurred (1082), but not all transcripts were the same. It is also interesting to note that the expression profile shared between any experimental condition takes 11443 transcripts, but repast and/or infection cannot alter expression in 10235 (89.4%). Even with less diversity, the most significant proportion of SG transcripts showed no altered expression after the feeding in each tissue, regardless of the infection status, there was an altered expression of 8366 in the INT and 5429 in the SGs. With the parasite presence, such amounts change to 7829 and 7951, respectively. Even though the infected intestine had only 14.2% more transcripts expressed above 2 times than fasting (4436), the number of transcripts expressed below twice increased from 3.8% to 39% (3393).

In general, there was a change in the protein profile expressed in SGs FE + Tc compared to FE. The Kazal domain peptide Pr13a is among the most expressed transcripts in SGs, whose mean expression remains in FE, reduces in FE + Tc in 2$^{nd}$ day, but increases drastically on the 9th day. Among the main triatomine kratagonists (Figure 11), there was a significant increase in the gene expression of the *R. prolixus* lipocalin 4-like protein in SG from *R. neglectus*.

However, the expression remained the same in FE + Tc at 2nd, reaching a higher value only on the 9th day, when the protein expression is similar to the precursor AI-4 of *Pristhesancus plagipennis* unchanged after 2 days, reduces. However, in the infection, the mean transcriptional expression of proteins similar to lipocalins 2 and 3 (*R. prolixus*) and precursor of AI-6 (*P. plagipennis*) changed significantly in 2 days, respectively, to 64 and 16 times less and 4 times more, respectively, compared to fasting, recovering levels similar to this state only on the 9th day.

There was a significant increase in the expression of proteins similar to those of Nitrophorins 7 and 3A and similar Nitrophorins 1A and 4 B precursors, all from R. prolixus, in post-meal SG. The expression behavior of Nitroforin 7 remained the same until the 9th day. However, there was an increase in transcripts of precursors of Nitrophorin 1A and 4 B, which decreased on the 9th day. The mean transcription of Nitrophorin 3A returned to the FA levels during infection. Segments similar to Triabin 4 (from *P. plagipennis*) and the precursors of Triabines 1 and 2 (from *R. prolixus*) have higher gene expression within 2 days after feeding, whereas Triabin 3 (*P. plagipennis*) and the precursors of Triabines 3 and 4 (*R. prolixus*) showed reduced expression. In all these cases, the altered mean levels were discrete, except for the Triabin 3 precursor transcripts, which were up to 16 times smaller.

In the SGs of FE + Tc, the expression of precursors of Triabines 1 and 2 was even higher at 2 days, equaling to uninfected only on the 9th day. In contrast, Triabin 4, 2 days after repast, maintains an average fasting level, increasing only on the 9th day. Similar behavior was observed for the transcript similar to Triabin 1 (from the venom gland of *P. plagipennis*), whose mean increases and decreases are more significant. On the other hand, it is possible to say that the mean gene expression of Triabin 3 and 4 precursors tended to equal the expression of FA on the 9th day, regardless of the presence of parasites. The average expression profile of pallidipine and procaline transcripts found in SGs was the only one that did not change significantly, regardless of the condition. The mean expression of yellow protein transcripts remained the same under all conditions, being lower than that during fasting.

Overall, most of the most transcriptionally expressed *R. neglectus* housekeeping proteins on day 9 of infection had mean levels similar to those of fasting (Supplementary Figure 5). The expression reduction was more significant in FE + Tc than in FE for housekeeping less expressed in 2 days, continuing to be reduced on the 9th day. Among the most expressed transcripts and translatable into unknown/hypothetical non-secretable segments (Supplementary Figure 7), the most expressed after feeding has a domain similar to phospholipase A2. It is also interesting to highlight the sharp decrease in transcriptional levels of three proteins in FE + Tc, one without a known domain (LOC111056117), which also decreases into FE, and two with domains similar to the binding of chitin (LOC106674348) and sodium-coupled monocarboxylate transporter (LOC106677625), whose levels increased on the 9th day. Two secreted proteins among the 50 least expressed, with domains similar to lipopolysaccharide (LPS)- inducedtumor necrosis factor-alpha factor (CAA9997534.1) and a disordered protein region, DPR (BAN20224.1), also showed a reduction only in FE + Tc 2 days and elevation on the 9th day. This expression profile also occurs for 11 unmatched

transcripts among the most expressed non-secreted proteins (Supplementary Figure 9), with only four with identifiable domains and similar to, respectively, in decreasing order of expression, histone linker H1/H5, DPR, tropomyosin, and DPR.

Among the unknown/hypothetical secreted proteins that are more transcriptionally expressed after feeding, the transcript similar to BAN20609.1, with a coil motif, showed greater differential expression in FE and FE + Tc. We can highlight six proteins (e.g., ATU83012.1, ATU83020.1, LOC106661678, GE061_22476), only two with identifiable domains and similar, respectively, to the crystallin family (ATU82838.1) and MBF2 transcription activator (ABR27885.1), which also showed reduced expression on the 2nd d of infection and elevation on the 9th day. On the other hand, among the 50 most expressed and secreted unmatched transcripts, it is possible to notice that most of them have higher expression in FE + Tc than in FE. The 50 less expressed and secreted unmatched transcripts have an inverse profile, except for the two first (Supplementary Figure 8), one with DPR domain, which presents the same profile already mentioned for the unknown/hypothetical secreted proteins in this paragraph.

It is worth noting (Supplementary Figure 10) that lysozymes in SGs present greater gene expression in FE and FE + Tc 9th day, but with a mean reduction in FE + Tc 2 days. There was a slight difference in the transcriptional expression of JH between FA and FE. However, there was a sudden reduction in the presence of *T. cruzi*, which was equal to fasting on the 9th day. The mean gene expression of acid phosphatases increased in FE but decreased in FE + Tc, increasing only on the 9th day, while vitellogenin had a marked increase in SGs on FE + Tc on the 9th day. Cathepsins, defensins, and snake venom cystatin-like cystatin were transcriptionally less expressed in FE and even lower in FE + Tc 2 days, with slightly higher levels on the 9th day for cathepsins.

In INT, p-myossupressin and neuroparsin 1 were among the most expressed transcripts after feeding compared to fasting, with the same expression profile already mentioned in previous sections (Figure 9). Some transcripts among the 50 most expressed in the intestine and not among the 50 most expressed in SG had increased expression in FE and reduced in FE + Tc. Among them are transcripts similar to Venom cub domain protein 2 (from *P. Plagipennis* venom - Walker, 2017) related to developmental processes (Bork and Beckmann, 1993), a transcript similar to the precursor of Antigen-5 described in SGs from *T. brasiliensis* (Santos et al., 2007) and *R. prolixus* (Ribeiro et al., 2004), and transcripts similar to salivary platelet aggregation inhibitors, as was identified for salivary platelet aggregation inhibitor 1 (T1HDI2) in the post-repast *R. prolixus* INT (Ouali et al., 2021). Others were less expressed in FE than in FE + Tc 2 days, such as transcripts similar to the venom glycin rich peptide Pp23a, pacifastin, diptericin, and acetyl CoA synthetase. Among the 50 least expressed in the intestine, transcripts similar to Venom Apolipophorin-like protein 1 and Venom Peptide Pp26a showed a more significant reduction on the 9th day. Similar to p-Obps, they showed a greater reduction only at 2 days and Niemann-Pick C1 (NPC1), a greater reduction after 2 days of infection.

As in SG, we observed a transcriptional increase in nitrophorins and triabines in the INT of uninfected *R. neglectus* compared to the infected group (Figure 11B). Lipocalin 4, which is

transcriptionally higher in SG of FE + Tc 2 days han in FE, was higher in the INT of FE than in FE + Tc 2 days. The same occurs with palidipine 1 in the INT, but more discreetly, but it does not change from fasting to infection and in any condition in SGs. The precursor of lipocalin AI-6, which is higher in the SGs of FE + Tc than FE, is decreased in the INT under the same conditions. The precursors of lipocalins AI-7 and 5 were transcriptionally more expressed only in FE. Lipoca FE + 2 days TC had a higher level in the intestine under the same conditions. Both the most and the least expressed housekeeping protein transcripts, in general, present a profile in the INT very similar to the SGs in all conditions, although they are not the same between the two tissues (Supplementary Figure 11) and, on the 9th day, the overall mean transcriptional expression of secreted or not secreted R. neglectus proteins tended to match that of fasting.

### 3.5 Dynamic transcripts expression in specific pathways of *R. neglectus* SG and INT biological networks

Based on the notes taken, we clustered the subnets of metabolic pathways and systemic components to assess the interactions and expression of nodes. Regardless of the experimental conditions, the metabolic/biosynthesis and systemic pathways had most of the protein transcripts with higher mean expression after the feeding, except for SGs of the secondary metabolites, carbohydrates, immunological, developmental, and regeneration, and endocrine pathways at 2 days after infection (Figure 12). However, the reduced expression affects many nodes in the SGs: all metabolic pathways, the endocrine pathway within 2 days of infection, and the immunological pathway on the 9th day. However, in the INT, it affected the metabolic pathways only on the 9th day. Most components of the immunological pathway also showed a reduction in FE in SGs and on day 9 in FE + Tc in SGs and INT (Supplementary Figures 16 and 17). In SGs, the same occurs in the development and regeneration pathways. In the endocrine pathway, this only occurs in the infection and on the 9th day in the INT. The same also occurs in the environmental adaptation pathway of both tissues, but only on the 9th day.

The hypothetical proteins similar to GE061_15329, which are related to the nervous, aging, and immune system of *R. neglectus*; GE06113134 related to the sensory, nervous, immune, and endocrine system; GE06122676 involved with the nervous and sensory system; and WR2507110 isoform B, which is involved in all systems, except aging, excretion, and development and regeneration, are critical pieces for having a higher degree and deserve greater attention in new studies.

## 4 Discussion

One of the main objectives of this study was to better understand the effects of feeding and Tc infection on the INT and SGs on the physiology of *R. neglectus* by observing the expression of protein transcripts in these tissues. The analysis carried out in this work is the first to evaluate such conditions at different times after a meal. Considering the importance of this knowledge for developing control strategies, we discussed the proteins found according to their involvement in digestion and infection.

## 4.1 Appropriate methodology optimizes results

First, the distribution of the generated transcripts showed that approximately 55% of the sequences were between 201-300 bp, the length from which it is already possible to make reliable annotations without size bias (Wommack et al., 2008). Second, the multi-kmer assembly methodology enables the identification of less frequent and more complete contigs by generating greater sample reads. This is because some regions are adequately assembled with a lower K-mer value, as is the case for genes with a low level of expression (Gruenheit et al., 2012), while other regions are also favored with a higher K-mer value because the distribution of readings is different from other positions within the same analyzed sequence. This number of contigs is greater than the total number of transcripts present in many of the arthropod transcriptomes of medical interest available in current public databases. Thus, the size of the K-mer positively influences the montage significantly (Miller et al., 2010; Feldmeyer et al., 2011; Durai and Schulz, 2016), as well as making it possible to obtain more regions when using larger and smaller k-mers in the same assembly (Miller et al., 2010; Surget-Groba and Montoya-Burgos, 2010; MacManes, 2018). In addition to the multi-kmer approach, ORP uses several bioinformatic processes to obtain high-quality assemblies when compared to the quality of assemblies performed by other methods (MacManes, 2018).

The transrate generates scores based on the coverage and quality of the mapping of reads (Smith-Unna et al., 2016; MacManes, 2018). The following were analyzed: identity of the nucleotides in the contig, size of the assembled transcript, and nucleotide order. Ideally, in a perfect assembly, the contigs obtained are faithful for reading the reads. In this case, the maximum score was 1. However, this scenario is impossible because of interference from assembly errors, even if small (Smith-Unna et al., 2016).

Finally, the BUSCO tool was used to assess quality by searching for orthologs preserved in eukaryotes (Simao et al., 2015), providing quantitative and intuitive metrics of the integrity of the assembled data in terms of gene content. This tool can assess gene duplication, completeness, fragmentation, or absence for a set of contigs by comparing them to the total of possible gene products available in a taxonomic database chosen as a reference (Waterhouse et al., 2018). The group of duplicated sequences tends to be smaller and smaller in more current versions due to the evolution of the tool in providing more unique copies (Waterhouse et al., 2011); in this case, the group covers the complete sequences, which were found more than once. Therefore, the existence of many assembled contigs, but generators of duplicated products, is not desirable. In general, the quality of the assembly proved to be acceptable by BUSCO metrics, considering the differences in interspecific sequences.

The ability to expand the identification of more sequences (known or new) with the multi-kmer approach allowed the identification of a wide variety of transcripts not similar to any taxonomic group, which demonstrates the relevance of applying more comprehensive approaches, or even more than one software in the analysis. For example, this is the case of the prediction of ORFs that had more CDS recognized for translating new protein sequences that are still unknown when more than one tool for this purpose was used.

## 4.2 Correspondence with the literature for *R. neglectus* – validating the methodology

To verify the correspondence between the transcripts generated in this work and the proteins considered as references for *R. neglectus*, the ORFs were also aligned against the proteins and the *R. neglectus* transcripts predicted by Santiago et al. (2016) from SGs (Supplementary Figure 1). Thus, we were able to find only non-redundant proteins that seem to be the only publicly available proteins of this species of triatomines. From these salivary transcripts, it was possible to predict 4080 ORFs using TransDecoder. Of these new ORFs, 3967 (97.2%) were also present among the ORFs generated in our study. Thus, 97% (1711) of the proteins predicted by Santiago et al. matched the ORFs of salivary transcripts sequenced by the same authors, which indicates an excellent predictive match between the use of TransDecoder and the protein prediction methodology defined by them. Even so, the ORFs generated by our group managed to correspond to a more significant number, 98.6% (1739) of the proteins predicted by Santiago et al. With these results, it is possible to say that the sequences were confirmed between the studies because only 25 proteins (1.4%) were not found in our study. On the other hand, it was possible to predict a much more significant amount of protein sequences due to the multi-kmer assembly method, suggesting that the methodology used here was more efficient.

## 4.3 Distribution of generated transcripts

Of the known proteins, the distribution between secreted and housekeeping proteins was similar to that of other triatomines. Proteins secreted in SGs were also found in greater amounts in *T. brasiliensis* (Santos et al., 2007), *R. prolixus* (Ribeiro et al., 2004), *Panstrongylus lignarius* (Nevoa et al., 2018), *T. dimidiata* (Kato et al., 2010), and housekeeping was also found in second place in the number of transcripts found in *T. dimidiata* and *R. prolixus* (Ribeiro et al., 2004; Kato et al., 2010). In *R. neglectus* SGs, more transcripts of proteins classified as housekeeping were also found than in the others analyzed (Santiago et al., 2016), which is very similar to what we found here. This profile is also typical of other hematophages. Among the glandular transcripts of the tick *Ornithodoros rostratus*, those representing secreted proteins were more diverse in SGs than in the INT, while housekeeping was more diverse in the intestine, both in the fed state (Araujo et al., 2019). This was also observed in the evaluation of the transcriptome and proteome of SG and intestine of Ixodes ricinus after repast (Schwarz et al., 2014b).

In addition to the presence or absence of SP, transmembrane helices, which are vital proteins for the cell, as they can initiate various signaling processes and transport various substances across the membrane (Moller et al., 2001). Interestingly, the ORFs with SP+ and the presence of a propeller were interesting. This may be due to differences in accuracy between the software used and the possibility that specific transmembrane proteins present signal peptides but are not secreted in most cases. This occurs with some receptors, for example, and the presence of the signal peptide is believed to assist the hydrophilic N-terminus in crossing the membrane (Liu et al., 2020). However, some of these SP+ proteins also seem to represent specific precursor molecules before secretion, as seen among tissue targets that may be part of the glandular or intestinal secretome (Supplementary Figure 2). Therefore, for ORFs SP- (or

even SP+) and with two or more transmembrane helices, it is suggested that they may be crowded in the cell membrane (Ferguson and Williams, 1988; Nielsen, 2017; Liu et al., 2020). Finally, SP+ and the lack of a transmembrane helix may represent potentially secreted proteins (4529) (Nielsen, 2017).

Once this is done, it is important to assess the addressing of ORFs, and TargetP is considered one of the best available for subcellular location prediction (Emanuelsson et al., 2000; Zhang et al., 2020). With good sensitivity and specificity, this tool classifies transcripts into mTP, SP+, and noTP ORFs (Zhang et al., 2020). Most of the predicted ORFs are wildly noTP precisely because proteins related to indispensable intracellular processes may be responsible for this group being so expressive. However, it is possible to observe many ORFs defined as SP by SignalP, otherwise classified as having SP by TargetP. As it has a recent review, implements deep neural networks in its predictive analyses, and has better sensitivity and specificity rates, we prefer to define the SignalP classification as the most assertive for further analysis ( Armenteros et al., 2019b).

### 4.4 Functional classification - Greater expressiveness of functions is related to hematophagic behavior

After distributing the ORFs according to the above criteria, the GhostKoala tool was used to characterize the functions of genes by associating them with a KO identifier from the KEGG Orthology bank (Kanehisa et al., 2016). This bank of biological systems integrates genomes and transcriptomes with chemical and functional characteristics through the mapping of reference pathways (Kanehisa et al., 2008). The transcripts were classified into functional classes, as shown in Figure 7, and as they are INT and SG, several sequences related to synthesis and metabolism are expected (Ribeiro et al., 2012). INT mainly has intense cell catabolism and transport activity due to the digestive process demonstrated here (Ribeiro et al., 2014; Leyria et al., 2020b). During digestion, the insect has to extract all the nutrients it needs from the ingested blood and then transport them to the hemolymph for use (Leyria et al., 2020b). Therefore, a diversified encounter of transcripts of this type is expected.

It is also observed that the behavior of the triatomine may be related to the expressiveness of a certain functional group. Triatomines are insects that feed once on the host and ingest large amounts of blood. In this way, the insect does not need to be exposed many times to feed; conversely, with the accentuated weight gain resulting from the recent feeding, locomotion is impaired and endangers their lives. To address this issue, triatomines have evolved the ability to process food quickly. For example, *R. prolixus* is capable of removing approximately 50% of the ingested blood within 3 h after a meal (Martini et al., 2007). This is only possible due to the diuretic hormones and serotonin released soon after blood intake, which act to concentrate the blood meal and despise the body's water (Te Brugge et al., 2002), perhaps because of this, the transcripts related to endocrine functions stood out.

In addition to the important endocrine role in the body discussed above, SGs and INT are known to play an important role in the humoral immunity of insects, which involves the production of reactive oxygen species, melanization, and antimicrobial peptides (AMPs)

(Ribeiro et al., 2014). Furthermore, lysozymes, defensins, and antibacterial molecules were found in the intestinal transcriptomes of *R. prolixus* (Ribeiro et al., 2016) and *T. infestans* (Buarque et al., 2013), in the fat body and SG of *P. lignarius* (Nevoa et al., 2018). Justifying the expressive presence of diversity of transcripts related to the immune system.

The proteome of *R. prolixus* (Ouali et al., 2020) shows the carbohydrate transport and metabolism class as one of the functional classes with the most transcripts related to blood ingestion and digestion. α-Glucosidases are enzymes related to glycosidic metabolism and are located in the newly formed perimicrovillar membrane in the intestine of the triatomine after blood ingestion. They have been related to the process of hemozoin formation, which is important for controlling the concentration of free heme that is released after hemoglobin digestion (Mury et al., 2009). However, in *R. neglectus*, the transcriptional expression of INT decreased by approximately half on the 9th day post-repast when compared to FA. At the same time, the expression of the lysosomal isoform increased by 50% by the 2nd d and decreased by half on the 9th day compared to the same previous state. In SG, the increase in transcriptional expression of the lysosomal isoform by approximately 50% was maintained until the 9th day. However, there was an increase in the expression of the secreted isoform on the 9th day by 50%. Suppose that the protein expression levels are similar to those of transcriptional expression. This suggests the importance of investigating whether the presence of these enzymes in the perimicrovillar lipid composition of the insect intestine is primarily due to production and initial secretion in the digestive tract by SGs.

The greater numerical diversity in the groups of enzymes may be related to the high demands of phosphorylation/dephosphorylation in physiological processes, typical of cell maintenance (Ribeiro et al., 2014). As in other hematophagous species, several inhibitors are related to the feeding process of the insect, such as serine protease inhibitor proteins that participate in antagonizing coagulation and the complement system (Nevoa et al., 2018). Despite these larger enzymatic classes, there is still a predominance of several synthesizing enzymes composing the group "other classes" in Figure 8, whose transcripts are also present among those that are part of the intratissue metabolic pathways of *R. neglectus*.

Metabolic changes lead to changes in post-transcriptional control, such as iron metabolism (Hajdusek et al., 2009). The digestion process in insects requires high gene transcription for the synthesis of proteins that participate in blood metabolism and support the probable increase in epithelial cell division that starts after the meal (Ribeiro et al., 2014). In *R. prolixus* INT, there was also a high expression of transcripts related to RNA processing genes after the feeding, related to the metabolic proteins to be generated(Ribeiro et al., 2014). Putative proteins related to transcription processes and protein synthesis were also abundantly found in the midgut of *Triatoma infestans* (Buarque et al., 2013). Notably, the most outstanding global diversity prevailed in the INT, where greater metabolic activity was expected than in the SG after the meal. (Schwarz et al., 2014a).

### 4.5 Protein diversity of the lipocalin family is essential for hematophagy

The secreted proteins set, and all pathways for transport and other cellular activities involved in the secretion process are called secretome (Tjalsma et al., 2000). In other studies, the lipocalin group was also quite numerous. In SG and the fat body of *P. lignarius*, 78 contigs encoding lipocalins were found (Nevoa et al., 2018). In *R. prolixus*, 88 CDSs, including pallidipine and triabin, were found in this family (Ribeiro et al., 2014). In *T. dimidiata*, after 5, 12, and 24 d of fasting, lipocalins corresponded to approximately 90% of the proteins found in the salivary proteome (Santiago et al., 2018). This diversity, even though redundant, is fundamental to the evolution of hematophagy (Santiago et al., 2018). *R. neglectus* SGs have also been described and are related to feeding success (Santiago et al., 2016). The predominance of lipocalins and nitrophorins in SGs, while channels and (co) transporters in INT may be linked to each of these tissue functions. While the SG actively participates in the feeding process, ensuring adequate blood flow efficiency, the INT needs to digest and absorb all the nutrients after the meal (Schwarz et al., 2014a; Araujo et al., 2019).

Furthermore, the fragmentation of a certain amount of nucleotide sequences observed in the analysis results with BUSCO indicates that the SP may not have been identified in them. Therefore, it is possible that some transcripts could not be selected, as shown in our results. Thus, the expected prediction of secreted molecules in each group or class may be greater in the saliva and intestinal lumen of *R. neglectus*.

### 4.6 The 20 most expressed in each fabric

After these evaluations, the intratissue transcripts with greater expression according to the experimental conditions were observed. We selected the first 20 from each group, and one of the interesting facts was that although most of the reference contigs obtained in this study originated from the INT, we observed, in general, that among the most expressed in each tissue, the proportion of known contigs in the total of contigs present was greater in SGs. This may be due to constant advances in invertebrate sialoma studies that add new annotations to public databases.

In SGs, the transcripts for Lipocalin AI-5 precursors and nitrophorin 1A are the best known among the protein transcripts that most appear in general. Both lipocalins and nitrophorins are proteins known to be present in hematophagous saliva expressed close to or during the meal and are involved in insect immunity (Santos et al., 2007; Ribeiro et al., 2014; Santiago et al., 2016; Nevoa et al., 2018; Ouali et al., 2021). Our results show that the high gene expression of the precursors of these molecules may indicate their need even after feeding. In the case of nitrophorin 1A, especially after meals.

Cathepsin B is a lysosomal protease involved in digestive processes (Buarque et al., 2013), and proteases from this family are dominant in terms of expression after blood ingestion (Ouali et al., 2020; Ouali et al.). This protein integrates the 20 most expressed protein transcripts only in the INT FE+Tc on the 9th day, together with lysozyme 1. In *R. prolixus*, there was also no difference in the expression of cathepsin B in the INT with or without Tc infection after 24 h of food (Ursic-Bedoya and Lowenberger, 2007). On the other hand,

lysozyme is transcriptionally expressed in infected T. infestans INT after 24 h of infection, with a suggestive role in Tc modulation (Buarque et al., 2013).

Given the considerable total proportion of unknown/unmatched protein transcripts among the 20 most expressed in both INT (31) and SGs (22), it is essential to further characterize their functions and physiological roles within the context of food and infection with intratissue Tc, thus clarifying the parasite-vector interaction process.

**4.5 Feeding and its transcriptional modification**

Then, a detailed analysis of the behavior of the transcripts was carried out concerning the meal to observe what changes when the insect feeds. First, we examined their expression in FA and FE insects. In fasting, the findings are significant and expected because they are directly related to the feeding process of these arthropods. NADPH P450 reductase is involved in metabolism and detoxification (Scott and Wen, 2001; Feyereisen, 2006; Mamidala et al., 2011), and P-Obp is involved in nutrient transport and contributes to the maintenance of intestinal physiology (Ribeiro et al., 2014). CSP-like is related to the ability to perceive chemical substances necessary for insects to feed, reproduce, or survive, and has already been identified in several tissues of triatomines (Ribeiro et al., 2014; Marchant et al., 2016; Martinez-Barnetche et al., 2018). Tret1-like is a transporter protein that mediates the transfer of trehalose from the fat body to other tissues, controlling the levels of this sugar in the hemolymph. Elevated enzymes that catabolize trehalose have already been detected during the fasting period in *R. prolixus* ovaries (Leyria et al., 2020a), which shows the importance of these molecules for the organic balance of fasting triatomines. The increased expression of the enzyme Ddx5 X1 (DEAD-box helicase 5), also known as p28, is related to RNA processing (Legrand et al., 2019), such as transcription, splicing, miRNA biogenesis, mRNA export, ribosomal biogenesis, and others (Cheng et al., 2018; Taschuk and Cherry, 2020). Here, gene expression was slightly higher in FA than after feeding, and the role of this protein in the elevation of several other transcripts observed during fasting should be better evaluated.

Hexamerins are proteins that are essentially related to the storage of amino acids during the phases of nutrient scarcity and the insect's development until reaching the adult stage. The amino acids stored by this protein are made available in the hemolymph according to the insect's demand (Beintema et al., 1994; Hathaway et al., 2009). It is believed that these proteins also have other functions as they are found in various tissues of the grasshopper *Locusta migratoria* (Li et al., 2017), such as the ability to bind and transport hormones, such as the juvenile hormone (JH) (Spit et al., 2016; Li et al., 2017), related to growth and reproduction, and participate in the control of the production of digestive enzymes (Bian et al., 1997; Bian et al., 2008). The increase in the transcript expression of this protein may be justified by alternative energy during fasting when there is a shortage of nutrients and a lower expression of JH. Thus, it is also possible to investigate whether the greater bioavailability of JH resulting from greater gene expression after blood feeding suggests less need for transport by hexamerin to other tissues, controlling the action of this hormone in the invertebrate organism.

C19 peptidase enzymes are part of the deubiquitinating group; that is, they carry out the ubiquitin deconjugation process, and their transcripts are also overexpressed in FA, as well as Hsp70Ba transcripts. It is known that conjugation with ubiquitin determines the degradation of the protein by the proteasome, and deubiquitinating enzymes act as regulators of this process (Rawlings and Barrett, 1994; Eletr and Wilkinson, 2014). Heat shock proteins are highly conserved among living beings, and HSP70 plays an essential role in protein folding and transport, being present in abundance under conditions of cellular stress (Karlin and Brocchieri, 1998). The finding that this protein is overexpressed in hungry insects is consistent with the stressful situation that fasting can bring to the barber. Furthermore, such relevance can be corroborated by other studies that also found that the gene of this protein was strongly expressed in fasting *T. infestans* (Kollien, 2002) and *R. prolixus* (Leyria et al., 2020b). On the other hand, it is possible that in other species, such as R. prolixus, this enzyme may be related to the body's ability to deal with blood heat after a meal and may have higher transcriptional expression levels in the fed state (Ouali et al.; Ouali et al., 2021), because these proteins function as molecular chaperones, preserving the function of important proteins (Feder and Hofmann, 1999).

When looking at the transcripts overexpressed in the FE it is clear that the vast majority are related to critical biological processes in blood processing. Among these, Cyp6a14, which is related to detoxification and resistance of the body to insecticides (Mamidala et al., 2011), participates in the production of endogenous substances, such as some hormones (Feyereisen, 2006), and protects against reactive oxygen species (Poupardin et al., 2010). According to Ribeiro and collaborators, the presence of these molecules in the INT may create a protective network against reactive oxygen species that may be produced after the meal, as shown for the species *R. prolixus* (Ribeiro et al., 2014). Increased expression of the Tat gene after feeding was similarly observed in a study with the INT of *R. prolixus* (Ouali et al., 2021). This enzyme participates in the first reaction for amino acid degradation and is essential in the tyrosine detoxification process, an important process for the survival of hematophagous arthropods, such as triatomines(Ribeiro et al., 2014; Sterkel et al., 2016). The peptidases identified here are digestive enzymes that act on the intestinal membrane, and their functions in triatomines still need to be better understood (Ribeiro et al., 2014; Ouali et al., 2020; Ouali et al., 2021). The increase in myosuppressin precursor, by triggering physiological and endocrine changes in triatomines (Ons et al., 2009; Ons et al., 2011; Lee et al., 2012; Ons, 2017), suggests that this molecule may be involved in the reduction of intestinal contraction and cardiac muscle (Lee et al., 2012), which plays a role in maintaining the physiological homeostasis of triatomines. The increase in the precursor of the hormone neuroparsin is related to changes in the developmental stages of insects and seems to be also involved in insulin metabolism (Badisco et al., 2013). Thus, the increase in this precursor could explain the need for some metabolic pathways since feeding increases the availability of nutrients in the insect, but this hypothesis still needs to be investigated. As the enzyme inositol-3-phosphate synthase (Ino1) it participates in the production of inositol and compounds with inositol, including phospholipids.The enzyme inositol-3-phosphate synthase (Ino1) participates in the production of inositol and compounds with inositol, including phospholipids (Majumder et al., 1997; Frej et al., 2016). The increase in EF suggests the need

for a greater demand for this enzyme during the probable increase in the lipid metabolic rate after the meal (Figure 9A).

In addition to the previously mentioned involvement in triatomine immunity, lipocalins are involved in vasodilation, anticoagulation, and antiplatelet aggregation (Andersen et al., 2005). During meals, they are abundant in SGs, working as facilitators of this process, as they are injected into the host to optimize the blood flow to the insect. In this study, the transcripts of Lipocalin AI-5 and Nitrophorin 3 precursors were approximately 64 times more expressed in EF, and it is expected that the mature proteins will soon be elevated. Nitrophorins have been reported in several sialotranscriptomes of triatomines, such as *R. prolixus* (Ribeiro et al., 2014; de Carvalho et al., 2017), *T. rubida* (Ribeiro et al., 2012), *P. lignarius* (Nevoa et al., 2018), *and P. megistus* (Ribeiro et al., 2015), and *R. neglectus* (Santiago et al., 2016). In the INT, they can bind to nitric oxide (de Carvalho et al., 2017), prevent platelet aggregation (Andersen et al., 1998; Andersen et al., 2005) and bind to defense molecules released by the host in the blood to modulate them (Ribeiro and Walker, 1994). Nitroforin 3 present in SG is related to the slower release of nitric oxide during the meal, especially at the end of feeding (Andersen et al., 2005). Lipocalin AI-5, on the other hand, probably has a functional difference from other forms of lipocalin already found (Andersen et al., 2005), but studies must still be carried out to determine these activities.

When comparing the FE with the FE + Tc, new transcripts stood out, probably because of the different points of view. However, there is still a predominance of transcripts of proteins related to digestion and the presence of blood. Here, we found that cathepsin D, previously found in SGs from *P. lignarius* (Nevoa et al., 2018) and INT from *T. infestans* (Balczun et al., 2012), is associated with protein metabolism and blood digestion of blood meal. Defensin C, present in *P. lignarius'* SGs (Nevoa et al., 2018), *R. prolixus'* INT (Ribeiro et al., 2014), and *T. infestans* (Buarque et al., 2013), participates in the necessary microbial control both in the ingested blood and at the site of the chopped (Lopez et al., 2003; Ribeiro et al., 2014).

Increased CREBB expression (CREB-binding protein) seems vital in fed insects since, in Drosophila, it plays a critical role in early embryogenesis (Akimaru et al., 1997), but its physiological role remains poorly understood. In *R. neglectus*, a transcript similar to the X4 isoform of this protein was found in the EF. In addition, histone H3v1 is highly conserved across species (Postberg et al., 2010) and is transcriptionally increased in *R. neglectus* FE (Figure 9). Thus, the increase in CREBB and H3v1 suggests high cellular transcriptional activity after feeding.

Some transcripts were overexpressed in EF in the two comparisons mentioned, and this further reinforces the relationship with the digestive process. In the case of these proteins, the presence of Tc seems to maintain the same level of transcriptional expression in FA, possibly harming their optimal levels of activity during the blood digestion of R. neglectus. The overexpression of the transcript, similar to the mature Obp-like protein (represented in this case by a similar Obp-like) in EF is possibly related to the digestive process, as previously mentioned (Ribeiro et al., 2014). However, this expression is lower in the face of Tc infection, so the role of the parasite in this reduction should be further investigated.

The most expressed transcripts in FE+Tc, on the other hand, are more modulating, trying to create a pleasant environment for the parasite. Among them, we have the transcript for Sult1c4-like, a protein previously found in the INT of *R. prolixu*s, which eliminates compounds harmful to the insect (Ribeiro et al., 2014). The Sult family of proteins can metabolize nitro compounds, favoring their removal (Ribeiro et al., 2014). The increase in Sult1c4 may also be related to some process performed by the parasite to eliminate harmful compounds and defend itself and manage to survive in the INT of the insect vector. Similarly, the same may occur in lipocalin-like2. Lipocalin 2 in humans is synthesized by immune cells against bacterial infections and acts by inhibiting bacterial growth by sequestering the iron that the bacteria would use (Flo et al., 2004). Thus, hypothetically in triatomines, local iron reduction probably favors the development of Tc by inhibiting the growth of harmful or competing bacteria. T3 peptidases are essential enzymes in the regulation of metamorphosis (Wu and Lu, 2008), insecticide metabolism (Pikett and Lu, 1989), and in the degradation of glutathione, an essential antioxidant for the cell and for Tc itself (Comini et al., 2005; Krauth-Siegel and Comini, 2008). The fact that a peptidase involved in breaking down a vital insect antioxidant is transcriptionally increased in the infection leads us to think that the triatomine is trying to maintain the oxidizing environment and, therefore, is unfavorable for the development of the parasite. The inorganic phosphate cotransporter, similar to isoform X1, apparently acted on nucleic acid and phospholipid synthesis, signal transduction, and energy metabolism (Dick et al., 2012) and was more expressed in FE+Tc (Figure 9B). The growth of *T. rangeli* strongly depends on the presence of Pi in the culture medium (Dick et al., 2010). Given the physiomorphological similarity between this parasite and Tc and the high identity (~98%) and high similarity (~99%) between its phosphate transporters (Dick et al., 2012), this may justify the need for higher Pi transport when the parasite is present in triatomine cells in order to meet the demands of both organisms.

### 4.5 Food and parasite interference in tissue transcription

After these evaluations, we observed the behavior of proteins in SGs and INT in fed or infected states compared to fasting. The analysis of day 9 was intended to verify which profiles were maintained or not in the presence of the parasite Tc appears to modulate global protein expression in both INT (negative) and SG (positive). In addition, the profile observed after 2 days of infection was different from that observed in uninfected insects or after 9 days of infection in both tissues. After a few hours, the trypomastigote turns into an epimastigote and thus becomes able to colonize the triatomine, which is an important trigger of physiological changes and may be responsible for the changes found here (Nogueira et al., 2015).

Fifty proteins were highlighted in each analyzed group and can be checked as indicated in the results; here, we will discuss some of the findings in each of the tissues starting with the SGs. The Kazal domain peptide Pr13a is one of the proteins that had its transcript overexpressed even after 2 days of feeding and suffered from the presence of the parasite. Proteins from these families are commonly associated with anticoagulants, vasodilators, and antimicrobial activity (Santiago et al., 2016). For other members of this family, contradictorily, in the INT of *R. prolixus*, a Kazal-type inhibitor (RpTI) had gene overexpression 3 h after Tc infection

with blood meal (Soares et al., 2015). In *R. neglectus*, we observed that many protein genes behaved in the same way as the Kazal domain peptide Pr13a, as is the case with the protease inhibitor (I1 unassigned peptidase inhibitor) and a protein kinase (venom protein kinase 1), which could also be explained by the interference of the parasite in the first 2 days after the meal. However, further studies are needed to confirm this hypothesis.

A group of proteins abundantly found in hematophagous saliva is the kratagonist, which acts in different ways to alter the enzymatic function of the protein to which they bind; the term is derived from the Greek Kratos, which means to seize or arrest and essentially function as agonist inhibitors (Ribeiro and Arcà, 2009; Andersen and Ribeiro, 2017). In this group, we have proteins already mentioned above but of unique relevance in the transcriptomic study of triatomines, such as lipocalins and nitrophorins, in addition to triabines, palidipine, and procalin and their respective precursors. For lipocalins, it is interesting to note that, despite the term "precursor" to certain molecules and curiously remembering a negative feedback between these molecules and those without the "precursor" suffix, perhaps there are interaction mechanisms between lipocalins that make a group of kratagonists more expressed transcriptionally at a given moment than the other group, in order to maintain a specific function for an extended period with the alternation of expression of different molecules, and this in the presence or not of the parasite. In general, the presence of nitrophorins and their precursors further increases the transcriptional expression of nitrophorins in *R. neglectus* SGs, while half of the lipocalins show an increase and the other half a reduction. Triabin inhibits thrombin, an important serine protease in the coagulation cascade, leading to the inhibition of platelet aggregation and prolonging clotting time (Fuentes-Prior et al., 1997), which is important during the past. Previously, it was identified in the saliva of *R. neglectus* (Santiago et al., 2016). The expression of several isoforms in a different way after feeding suggests that feeding promotes an unknown modulatory mechanism of protein synthesis during blood processing, as pointed out in samples obtained at different times of tissue collection after feeding and also occurs with cathepsin D in the INT of fed *R. prolixus* (Ouali et al., 2021). Palidipine is related to the inhibition of platelet aggregation and adds to other salivary anticoagulants to maintain blood fluidity during the feeding process (Noeske-Jungblut et al., 1994). Procaline is responsible for saliva allergenic responses, as observed in *Triatoma protracta* (Paddock et al., 2001). However, its role in insects during meals is unknown (Ganfornina, 2013). Redundancy of proteins that perform similar or complementary functions is expected in the saliva of hematophagous arthropods (Santiago et al., 2018), and this may also occur in *R. neglectus* saliva. In addition, our results provide an additional step in the search for the role of proteins such as procaline, which are still poorly studied. Yellow protein is found only in insects and bacteria and has been linked to melanization. However, its functions remain unknown (Ferguson et al., 2011).

Among other relevant proteins, lysozyme, the initial reduction of the transcript of this protein with subsequent re-establishment of expression suggests that the feeding period in *R. neglectus* seems to be the only reason for the increase observed after 9 days in FE+Tc, since the overexpression of lysozymes has already been evidenced in *R. prolixus* even 9 and 12 days after the blood meal (Ribeiro et al., 2014). The JH was also found. This hormone is

related to critical developmental processes, such as metamorphosis, aging, behavioral processes, stress resistance, and reproduction (Goodman and Cusson, 2012). Furthermore, in vitro studies have shown that this hormone inhibits the growth of *T. cruzi* (Stoka, 1996) and the observed reduction in triatomines. JH production may favor the survival of the parasite through modulatory mechanisms that are still unknown. We also have acid phosphatases. These proteins are present in highly metabolic organs, including the SGs. In *T. infestans*, *P. megistus*, *R. neglectus*, and *R. prolixus*, they are present throughout the gland, including the nucleus, demonstrating activity probably related to participation in the secretion of compounds, in addition to phosphatase activity during rRNA transcription (Anhe et al., 2007). Moreover, an increase in vitellogenin expression has already been found in the SGs of *T. infestans* and *R. neglectus*, 21 d after the meal (Santiago et al., 2020). Thus, these proteins seem to be essential not only immediately after feeding.

Other proteins were found to have reduced transcriptional expression. In previous studies, cathepsin D was shown to be transcriptionally upregulated in the INT of infected *T. infestans* (Buarque et al., 2013) and *R. prolixus* (Borges et al., 2006), in contrast to the findings of the present *study*. This expression can then differ depending on the triatomine species. Again, a result different from that found previously is the one observed for cystatin, but in the INT of *T. infestans*, this protein was overexpressed 24 h after infection, and the defensins did not show altered values when the insect was infected. However, the expression of these proteins was not evaluated between 24-48 hours after feeding or even in SG (Buarque et al., 2011; Buarque et al., 2013). The feeding process also favors the secretion of defensins found after feeding in the INT of *R. prolixus* (Ribeiro et al., 2014) and *T. brasiliensis* (Araujo et al., 2006), but according to our results on R. neglectus, there appears to be no significant change in expression in INT (Supplementary Table 1).

In INT, the precursor of myossupressin, neuroparsin 1, venom cub domain protein 2, the precursor of Antigen-5, and transcripts similar to salivary platelet aggregation inhibitors may play a role in the digestive process by presenting increased transcriptional expression after feeding (Ouali et al., 2021). However, the reduction of expression in the presence of the parasite deserves further investigation.

The venom glycin-rich peptide Pp23a, pacifastin, diptericin, and acetyl CoA synthetase were observed with an opposite profile. The increase in acetyl CoA synthetase appears to be influenced by bacterial acetylation (Liimatta et al., 2018). This enzyme converts large quantities of acetate during bacterial growth into acetyl-CoA as an alternative carbon source for these microorganisms (Wolfe, 2005; Liimatta et al., 2018). After the meal, as mentioned, it is common for flora to grow, which can harm the development of Tc. In this sense, the increase in Pp23a and diptericin can help control microbial growth, as seen in serrulin in scorpion, a bioactive peptide rich in glycine with antimicrobial activity (Oliveira et al., 2018). Diptericin, a member of a family of glycine-rich antibacterials (Cudic et al., 1999), is present in the hemolymph of dipterans (Wu, 2018), and Pp23a had not yet been observed in the INT of *R. neglectus*. Thus, the increase in these proteins can be influenced by the presence of the parasite to control the development of the environment. Pacifastin belongs to the serine protease inhibitor family. Although identified in the *R. neglectus*' SGs after the feeding, its

activity has not yet been studied in this organ. However, it has been suggested that it is involved in insect immunity (Santiago et al., 2016). The increase in FE+Tc may indicate an attempt to respond to local infection. The description of this protein in the intestine of *R. neglectus* also occurs for the first time here, probably being modulated by parasitic colonization.

Lipid-carrying apolipophorins were identified in the SG of *R. prolixus* after 21 d of feeding(Santiago et al., 2020). For the first time, we also identified a Venom-like transcript, Apolipophorin-like protein 1, in *R. neglectus*. Present in *Pristhesancus plagipennis* and *Lethocerus differentifemur* (Walker et al., 2017), Pp26a has not yet been reported in *R. neglectus*, and its function is unknown. The NPC1 protein is evolutionarily well conserved among species and is related to the transport of cholesterol into cells, ensuring sufficient amounts for the production of steroid hormones (Huang et al., 2007; Danielsen et al., 2016). However, it is still necessary to define the mechanisms that lead to a reduction in NPC1 transcription in FE+Tc.

It has been observed that protein levels of a triabin-like lipocalin 4a precursor increased significantly in the INT of *R. prolixus* 6h after hematophagy (Ouali et al., 2021). Our results suggest that this increase can be maintained even 2 days after the feeding in triatomines. However, some kratagonists in INT behave slightly differently from what is observed in SGs and diverge from what is found in the literature. Similar to the case of infected *T. infestans* INT analyzed after 24h of feeding, there was overexpression of transcripts for nitrophorins, and lipocalins did not show altered transcriptional expression after infection (Buarque et al., 2013). In general, lipocalins and their precursors demonstrate different expression patterns depending on the tissue, condition, and species evaluated.

### 4.7 Information from networks complements previous findings

Network analysis allows the visualization of information lost within a large volume of data present in a transcriptome. After evaluating the transcripts of metabolic pathways and systemic component subnetworks, we observed that feeding could increase global gene expression, including within SGs, possibly altering saliva production/composition. However, Tc infection contributes more to reducing this expression than to the increase, causing functional damage to the SGs during meals. This effect may reflect the 12% lower intake of these triatomines. However, it should be further investigated, as the parasitic concentration and/or different strains may better explain the volume of blood ingested (Verly et al., 2002; Verly et al., 2020).

INT appears to be more resistant to global gene reduction caused by Tc infection. Therefore, it is important to investigate whether the expression of genes involved in these networks, related to immunity and regeneration, can positively contribute to parasite infection, survival, and development.a

The numerous molecules present in the INT or SGs of hematophagous arthropods work in an integrated manner to promote feeding, adaptation to the environment, aggressive agents, and consequent propagation of the species. Here, we sought to expand our knowledge of the

proteins involved in different scenarios that alter the physiology of *R. neglectus*, such as fasting or blood-feeding in the presence or absence of the *T. cruzi* pathogen. Similar to other hematophagous proteins, lipocalins are among the most common proteins found in SGs and INT under the conditions studied. In contrast, some proteins were first reported in *R. neglectus*. In general, during fasting, many protein genes that participate in insect homeostasis during the hunger period are expressed. The process triggered by blood ingestion causes many changes in the expression of genes in triatomine SGs and INT. However, the presence of *T. cruzi* profoundly modified the gene expression pattern of the two different tissues of *R. neglectus*. Here, we also propose some hypotheses that still need to be explored further in future studies to better understand the different conditions tested, including fasting and blood-feeding in the presence or absence of an arthropod-borne pathogen.

## 5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## 6 Data Availability Statement

The raw sequencing data were deposited at the NCBI Sequence Read Archive under Bioproject No. PRJNA757456.

## 7 Author Contributions

All authors were involved in the design of the study. TMCC, CJFO and RDRT were involved in study design, analyzed the data, and wrote the manuscript. TMCC, CGB, JCN and GAR performed the experiments. MTM, MVS, VR, CJFO, SCS participated in wrote the manuscript. All authors commented on the manuscript, read and approved the final version.

## 8 Funding

This work was supported by the Fundação de Amparo à Pesquisa do Estado Minas Gerais (FAPEMIG), National Council for Scientific and Technological Development (CNPq), and Coordination for the Improvement of Higher Education Personnel (CAPES; finance code 001).

## 9 Ethics

Under the number CAAE 80660417.1.0000.5154, the present work was approved by the Research Ethics Committee (CEP) of the Federal University of Triângulo Mineiro (UFTM).

## 10 Acknowledgments

The authors are especially grateful to Dr. José Marcos Ribeiro (National Institutes of Allergy and Infectious Diseases/National Institutes of Health, USA) for critical and helpful comments and suggestions on the analyzes of the data. And we would like thank Fundação de Amparo à

Pesquisa do Estado Minas Gerais (FAPEMIG), National Council for Scientific and Technological Development (CNPq), and Coordination for the Improvement of Higher Education Personnel (CAPES; finance code 001) for the support.

## 11 References

Agirre, J., Aloria, K., Arizmendi, J.M., Iloro, I., Elortza, F., Sanchez-Eugenia, R., et al. (2011). Capsid protein identification and analysis of mature Triatoma virus (TrV) virions and naturally occurring empty particles. *Virology* 409(1)**,** 91-101. doi: 10.1016/j.virol.2010.09.034.

Akimaru, H., Hou, D.X., and Ishii, S. (1997). Drosophila CBP is required for dorsal-dependent twist gene expression. *Nat Genet* 17(2)**,** 211-214. doi: 10.1038/ng1097-211.

Almagro Armenteros, J.J., Salvatore, M., Emanuelsson, O., Winther, O., von Heijne, G., Elofsson, A., et al. (2019a). Detecting sequence signals in targeting peptides using deep learning. *Life Sci Alliance* 2(5). doi: 10.26508/lsa.201900429.

Almagro Armenteros, J.J., Tsirigos, K.D., Sonderby, C.K., Petersen, T.N., Winther, O., Brunak, S., et al. (2019b). SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat Biotechnol* 37(4)**,** 420-423. doi: 10.1038/s41587-019-0036-z.

Andersen, J.F., Gudderra, N.P., Francischetti, I.M., and Ribeiro, J.M. (2005). The role of salivary lipocalins in blood feeding by Rhodnius prolixus. *Arch Insect Biochem Physiol* 58(2)**,** 97-105. doi: 10.1002/arch.20032.

Andersen, J.F., and Ribeiro, J.M.C. (2017). "Chapter 4 - Salivary Kratagonists: Scavengers of Host Physiological Effectors During Blood Feeding," in *Arthropod Vector: Controller of Disease Transmission, Volume 2,* eds. S.K. Wikel, S. Aksoy & G. Dimopoulos. Academic Press), 51-63.

Anhe, A.C., Lima-Oliveira, A.P., and Azeredo-Oliveira, M.T. (2007). Acid phosphatase activity distribution in salivary glands of triatomines (Heteroptera, Reduviidae, Triatominae). *Genet Mol Res* 6(1)**,** 197-205.

Ankavay, M., Montpellier, C., Sayed, I.M., Saliou, J.M., Wychowski, C., Saas, L., et al. (2019). New insights into the ORF2 capsid protein, a key player of the hepatitis E virus lifecycle. *Sci Rep* 9(1)**,** 6243. doi: 10.1038/s41598-019-42737-2.

Araujo, C.A., Waniek, P.J., Stock, P., Mayer, C., Jansen, A.M., and Schaub, G.A. (2006). Sequence characterization and expression patterns of defensin and lysozyme encoding genes from the gut of the reduviid bug Triatoma brasiliensis. *Insect Biochem Mol Biol* 36(7)**,** 547-560. doi: 10.1016/j.ibmb.2006.04.003.

Araujo, R.N., Silva, N.C.S., Mendes-Sousa, A., Paim, R., Costa, G.C.A., Dias, L.R., et al. (2019). RNA-seq analysis of the salivary glands and midgut of the Argasid tick Ornithodoros rostratus. *Sci Rep* 9(1)**,** 6764. doi: 10.1038/s41598-019-42899-z.

Badisco, L., Van Wielendaele, P., and Vanden Broeck, J. (2013). Eat to reproduce: a key role for the insulin signaling pathway in adult insects. *Front Physiol* 4**,** 202. doi: 10.3389/fphys.2013.00202.

Balczun, C., Siemanowski, J., Pausch, J.K., Helling, S., Marcus, K., Stephan, C., et al. (2012). Intestinal aspartate proteases TiCatD and TiCatD2 of the haematophagous bug Triatoma infestans (Reduviidae): sequence characterisation, expression pattern and characterisation of proteolytic activity. *Insect Biochem Mol Biol* 42(4)**,** 240-250. doi: 10.1016/j.ibmb.2011.12.006.

Beintema, J.J., Stam, W.T., Hazes, B., and Smidt, M.P. (1994). Evolution of arthropod hemocyanins and insect storage proteins (hexamerins). *Mol Biol Evol* 11(3)**,** 493-503. doi: 10.1093/oxfordjournals.molbev.a040129.

Bian, G., Raikhel, A.S., and Zhu, J. (2008). Characterization of a juvenile hormone-regulated chymotrypsin-like serine protease gene in Aedes aegypti mosquito. *Insect Biochem Mol Biol* 38(2)**,** 190-200. doi: 10.1016/j.ibmb.2007.10.008.

Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30(15)**,** 2114-2120. doi: 10.1093/bioinformatics/btu170.

Borges, E.C., Machado, E.M., Garcia, E.S., and Azambuja, P. (2006). Trypanosoma cruzi: effects of infection on cathepsin D activity in the midgut of Rhodnius prolixus. *Exp Parasitol* 112(2)**,** 130-133. doi: 10.1016/j.exppara.2005.09.008.

Bork, P., and Beckmann, G. (1993). The CUB domain. A widespread module in developmentally regulated proteins. *J Mol Biol* 231(2)**,** 539-545. doi: 10.1006/jmbi.1993.1305.

Buarque, D.S., Braz, G.R., Martins, R.M., Tanaka-Azevedo, A.M., Gomes, C.M., Oliveira, F.A., et al. (2013). Differential expression profiles in the midgut of Triatoma infestans infected with Trypanosoma cruzi. *PLoS One* 8(5)**,** e61203. doi: 10.1371/journal.pone.0061203.

Buarque, D.S., Spindola, L.M., Martins, R.M., Braz, G.R., and Tanaka, A.S. (2011). Tigutcystatin, a cysteine protease inhibitor from Triatoma infestans midgut expressed in response to Trypanosoma cruzi. *Biochem Biophys Res Commun* 413(2)**,** 241-247. doi: 10.1016/j.bbrc.2011.08.078.

Buchfink, B., Xie, C., and Huson, D.H. (2015). Fast and sensitive protein alignment using DIAMOND. *Nat Methods* 12(1)**,** 59-60. doi: 10.1038/nmeth.3176.

Cheng, W., Chen, G., Jia, H., He, X., and Jing, Z. (2018). DDX5 RNA Helicases: Emerging Roles in Viral Infection. *Int J Mol Sci* 19(4). doi: 10.3390/ijms19041122.

Comini, M., Menge, U., Wissing, J., and Flohe, L. (2005). Trypanothione synthesis in crithidia revisited. *J Biol Chem* 280(8)**,** 6850-6860. doi: 10.1074/jbc.M404486200.

Cudic, M., Bulet, P., Hoffmann, R., Craik, D.J., and Otvos, L., Jr. (1999). Chemical synthesis, antibacterial activity and conformation of diptericin, an 82-mer peptide originally isolated from insects. *Eur J Biochem* 266(2)**,** 549-558. doi: 10.1046/j.1432-1327.1999.00894.x.

Czibener, C., La Torre, J.L., Muscio, O.A., Ugalde, R.A., and Scodeller, E.A. (2000). Nucleotide sequence analysis of Triatoma virus shows that it is a member of a novel group of insect RNA viruses. *J Gen Virol* 81(Pt 4)**,** 1149-1154. doi: 10.1099/0022-1317-81-4-1149.

Danielsen, E.T., Moeller, M.E., Yamanaka, N., Ou, Q., Laursen, J.M., Soenderholm, C., et al. (2016). A Drosophila Genome-Wide Screen Identifies Regulators of Steroid Hormone Production and Developmental Timing. *Dev Cell* 37(6)**,** 558-570. doi: 10.1016/j.devcel.2016.05.015.

de Araujo, C.N., Bussacos, A.C., Sousa, A.O., Hecht, M.M., and Teixeira, A.R. (2012). Interactome: Smart hematophagous triatomine salivary gland molecules counteract human hemostasis during meal acquisition. *J Proteomics* 75(13)**,** 3829-3841. doi: 10.1016/j.jprot.2012.05.001.

de Carvalho, D.B., Congrains, C., Chahad-Ehlers, S., Pinotti, H., Brito, R.A., and da Rosa, J.A. (2017). Differential transcriptome analysis supports Rhodnius montenegrensis and Rhodnius robustus (Hemiptera, Reduviidae, Triatominae) as distinct species. *PLoS One* 12(4)**,** e0174997. doi: 10.1371/journal.pone.0174997.

Dick, C.F., Dos-Santos, A.L., Fonseca-de-Souza, A.L., Rocha-Ferreira, J., and Meyer-Fernandes, J.R. (2010). Trypanosoma rangeli: differential expression of ecto-phosphatase activities in response to inorganic phosphate starvation. *Exp Parasitol* 124(4)**,** 386-393. doi: 10.1016/j.exppara.2009.12.006.

Dick, C.F., Dos-Santos, A.L., Majerowicz, D., Gondim, K.C., Caruso-Neves, C., Silva, I.V., et al. (2012). Na+-dependent and Na+-independent mechanisms for inorganic phosphate uptake in Trypanosoma rangeli. *Biochim Biophys Acta* 1820(7)**,** 1001-1008. doi: 10.1016/j.bbagen.2012.02.019.

Durai, D.A., and Schulz, M.H. (2016). Informed kmer selection for de novo transcriptome assembly. *Bioinformatics* 32(11)**,** 1670-1677. doi: 10.1093/bioinformatics/btw217.

Eletr, Z.M., and Wilkinson, K.D. (2014). Regulation of proteolysis by human deubiquitinating enzymes. *Biochim Biophys Acta* 1843(1)**,** 114-128. doi: 10.1016/j.bbamcr.2013.06.027.

Emanuelsson, O., Nielsen, H., Brunak, S., and von Heijne, G. (2000). Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *J Mol Biol* 300(4)**,** 1005-1016. doi: 10.1006/jmbi.2000.3903.

Emms, D.M., and Kelly, S. (2015). OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol* 16**,** 157. doi: 10.1186/s13059-015-0721-2.

Falcone, R., Ribeiro, A.R., Oliveira, J., Mendonca, V.J., Graminha, M., and Rosa, J.A.D. (2020). Differentiation of Rhodnius neglectus and Rhodnius prolixus (Hemiptera: Reduviidae: Triatominae) by multiple parameters. *Rev Soc Bras Med Trop* 53**,** e20190503. doi: 10.1590/0037-8682-0503-2019.

Feder, M.E., and Hofmann, G.E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu Rev Physiol* 61**,** 243-282. doi: 10.1146/annurev.physiol.61.1.243.

Feldmeyer, B., Wheat, C.W., Krezdorn, N., Rotter, B., and Pfenninger, M. (2011). Short read Illumina data for the de novo assembly of a non-model snail species transcriptome (Radix balthica, Basommatophora, Pulmonata), and a comparison of assembler performance. *BMC Genomics* 12**,** 317. doi: 10.1186/1471-2164-12-317.

Ferguson, L.C., Green, J., Surridge, A., and Jiggins, C.D. (2011). Evolution of the insect yellow gene family. *Mol Biol Evol* 28(1)**,** 257-272. doi: 10.1093/molbev/msq192.

Ferguson, M.A., and Williams, A.F. (1988). Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. *Annu Rev Biochem* 57**,** 285-320. doi: 10.1146/annurev.bi.57.070188.001441.

Feyereisen, R. (2006). Evolution of insect P450. *Biochem Soc Trans* 34(Pt 6)**,** 1252-1255. doi: 10.1042/BST0341252.

Finkel, Y., Stern-Ginossar, N., and Schwartz, M. (2018). Viral Short ORFs and Their Possible Functions. *Proteomics* 18(10)**,** e1700255. doi: 10.1002/pmic.201700255.

Flo, T.H., Smith, K.D., Sato, S., Rodriguez, D.J., Holmes, M.A., Strong, R.K., et al. (2004). Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. *Nature* 432(7019)**,** 917-921. doi: 10.1038/nature03104.

Fontaine, A., Diouf, I., Bakkali, N., Misse, D., Pages, F., Fusai, T., et al. (2011). Implication of haematophagous arthropod salivary proteins in host-vector interactions. *Parasit Vectors* 4**,** 187. doi: 10.1186/1756-3305-4-187.

Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., et al. (2013). STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res* 41(Database issue)**,** D808-815. doi: 10.1093/nar/gks1094.

Frej, A.D., Clark, J., Le Roy, C.I., Lilla, S., Thomason, P.A., Otto, G.P., et al. (2016). The Inositol-3-Phosphate Synthase Biosynthetic Enzyme Has Distinct Catalytic and Metabolic Roles. *Mol Cell Biol* 36(10)**,** 1464-1479. doi: 10.1128/MCB.00039-16.

Fuentes-Prior, P., Noeske-Jungblut, C., Donner, P., Schleuning, W.D., Huber, R., and Bode, W. (1997). Structure of the thrombin complex with triabin, a lipocalin-like exosite-binding inhibitor derived from a triatomine bug. *Proc Natl Acad Sci U S A* 94(22)**,** 11845-11850. doi: 10.1073/pnas.94.22.11845.

Ganfornina, M.D.K., H.; Sanchez, D. (2013). " Lipocalins in Arthropoda: Diversification and Functional Explorations. ", in: *Madame Curie Bioscience Database* ( Austin (TX): Landes Bioscience).

Garcia, E.S., Ratcliffe, N.A., Whitten, M.M., Gonzalez, M.S., and Azambuja, P. (2007). Exploring the role of insect host factors in the dynamics of Trypanosoma cruzi-Rhodnius prolixus interactions. *J Insect Physiol* 53(1)**,** 11-21. doi: 10.1016/j.jinsphys.2006.10.006.

Goodman, W.G., and Cusson, M. (2012). "8 - The Juvenile Hormones," in *Insect Endocrinology*, ed. L.I. Gilbert. (San Diego: Academic Press), 310-365.

Gruenheit, N., Deusch, O., Esser, C., Becker, M., Voelckel, C., and Lockhart, P. (2012). Cutoffs and k-mers: implications from a transcriptome study in allopolyploid plants. *BMC Genomics* 13**,** 92. doi: 10.1186/1471-2164-13-92.

Gurgel-Goncalves, R., Galvao, C., Costa, J., and Peterson, A.T. (2012). Geographic distribution of chagas disease vectors in Brazil based on ecological niche modeling. *J Trop Med* 2012**,** 705326. doi: 10.1155/2012/705326.

Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., et al. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat Protoc* 8(8)**,** 1494-1512. doi: 10.1038/nprot.2013.084.

Hajdusek, O., Sojka, D., Kopacek, P., Buresova, V., Franta, Z., Sauman, I., et al. (2009). Knockdown of proteins involved in iron metabolism limits tick reproduction and development. *Proc Natl Acad Sci U S A* 106(4)**,** 1033-1038. doi: 10.1073/pnas.0807961106.

Hathaway, M., Hatle, J., Li, S., Ding, X., Barry, T., Hong, F., et al. (2009). Characterization of hexamerin proteins and their mRNAs in the adult lubber grasshopper: The effects of nutrition and juvenile hormone on their levels. *Comp Biochem Physiol A Mol Integr Physiol* 154(3)**,** 323-332. doi: 10.1016/j.cbpa.2009.06.018.

Huang, X., Warren, J.T., Buchanan, J., Gilbert, L.I., and Scott, M.P. (2007). Drosophila Niemann-Pick type C-2 genes control sterol homeostasis and steroid biosynthesis: a model of human neurodegenerative disease. *Development* 134(20)**,** 3733-3742. doi: 10.1242/dev.004572.

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008). KEGG for linking genomes to life and the environment. *Nucleic Acids Res* 36(Database issue)**,** D480-484. doi: 10.1093/nar/gkm882.

Kanehisa, M., Sato, Y., and Morishima, K. (2016). BlastKOALA and GhostKOALA: KEGG Tools for Functional Characterization of Genome and Metagenome Sequences. *J Mol Biol* 428(4)**,** 726-731. doi: 10.1016/j.jmb.2015.11.006.

Karlin, S., and Brocchieri, L. (1998). Heat shock protein 70 family: multiple sequence comparisons, function, and evolution. *J Mol Evol* 47(5)**,** 565-577. doi: 10.1007/pl00006413.

Kato, H., Jochim, R.C., Gomez, E.A., Sakoda, R., Iwata, H., Valenzuela, J.G., et al. (2010). A repertoire of the dominant transcripts from the salivary glands of the blood-sucking bug, Triatoma dimidiata, a vector of Chagas disease. *Infect Genet Evol* 10(2)**,** 184-191. doi: 10.1016/j.meegid.2009.10.012.

Krauth-Siegel, R.L., and Comini, M.A. (2008). Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochim Biophys Acta* 1780(11)**,** 1236-1248. doi: 10.1016/j.bbagen.2008.03.006.

Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E.L. (2001). Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol* 305(3)**,** 567-580. doi: 10.1006/jmbi.2000.4315.

Lee, D., Taufique, H., da Silva, R., and Lange, A.B. (2012). An unusual myosuppressin from the blood-feeding bug Rhodnius prolixus. *J Exp Biol* 215(Pt 12)**,** 2088-2095. doi: 10.1242/jeb.067447.

Legrand, J.M.D., Chan, A.L., La, H.M., Rossello, F.J., Anko, M.L., Fuller-Pace, F.V., et al. (2019). DDX5 plays essential transcriptional and post-transcriptional roles in the maintenance and function of spermatogonia. *Nat Commun* 10(1)**,** 2278. doi: 10.1038/s41467-019-09972-7.

Leyria, J., Orchard, I., and Lange, A.B. (2020a). Transcriptomic analysis of regulatory pathways involved in female reproductive physiology of Rhodnius prolixus under different nutritional states. *Sci Rep* 10(1)**,** 11431. doi: 10.1038/s41598-020-67932-4.

Leyria, J., Orchard, I., and Lange, A.B. (2020b). What happens after a blood meal? A transcriptome analysis of the main tissues involved in egg production in Rhodnius prolixus, an insect vector of Chagas disease. *PLoS Negl Trop Dis* 14(10)**,** e0008516. doi: 10.1371/journal.pntd.0008516.

Li, Y., Zhang, Z., Feng, L., Zhao, X., Zhang, D.C., and Yin, H. (2017). Gene and expression analysis of the hexamerin family proteins from the grasshopper, Locusta migratoria (Orthoptera: Acridoidea). *Biotechnology & Biotechnological Equipment* 31(6)**,** 1139-1147. doi: 10.1080/13102818.2017.1373601.

Liimatta, K., Flaherty, E., Ro, G., Nguyen, D.K., Prado, C., and Purdy, A.E. (2018). A Putative Acetylation System in Vibrio cholerae Modulates Virulence in Arthropod Hosts. *Appl Environ Microbiol* 84(21). doi: 10.1128/AEM.01113-18.

Liu, B., Lee, G., Wu, J., Deming, J., Kuei, C., Harrington, A., et al. (2020). The PAR2 signal peptide prevents premature receptor cleavage and activation. *PLoS One* 15(2)**,** e0222685. doi: 10.1371/journal.pone.0222685.

Lopez, L., Morales, G., Ursic, R., Wolff, M., and Lowenberger, C. (2003). Isolation and characterization of a novel insect defensin from Rhodnius prolixus, a vector of Chagas disease. *Insect Biochem Mol Biol* 33(4)**,** 439-447. doi: 10.1016/s0965-1748(03)00008-0.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12)**,** 550. doi: 10.1186/s13059-014-0550-8.

MacManes, M.D. (2018). The Oyster River Protocol: a multi-assembler and kmer approach for de novo transcriptome assembly. *PeerJ* 6**,** e5428. doi: 10.7717/peerj.5428.

Majumder, A.L., Johnson, M.D., and Henry, S.A. (1997). 1L-myo-inositol-1-phosphate synthase. *Biochim Biophys Acta* 1348(1-2)**,** 245-256. doi: 10.1016/s0005-2760(97)00122-7.

Mamidala, P., Jones, S.C., and Mittapalli, O. (2011). Metabolic Resistance in Bed Bugs. *Insects* 2(1)**,** 36-48. doi: 10.3390/insects2010036.

Marchant, A., Mougel, F., Jacquin-Joly, E., Costa, J., Almeida, C.E., and Harry, M. (2016). Under-Expression of Chemosensory Genes in Domiciliary Bugs of the Chagas Disease Vector Triatoma brasiliensis. *PLoS Negl Trop Dis* 10(10)**,** e0005067. doi: 10.1371/journal.pntd.0005067.

Marti, G.A., Echeverría, M.G., Susevich, M.L., Ceccarelli, S., Balsalobre, A., Rabinovich, J.E., et al. (2013). Exploration for Triatoma virus (TrV) infection in laboratory-reared triatomines of Latin America: a collaborative study*. *International Journal of Tropical Insect Science* 33(4)**,** 294-304. doi: 10.1017/S1742758413000337.

Martinez-Barnetche, J., Lavore, A., Beliera, M., Tellez-Sosa, J., Zumaya-Estrada, F.A., Palacio, V., et al. (2018). Adaptations in energy metabolism and gene family expansions revealed by comparative transcriptomics of three Chagas disease triatomine vectors. *BMC Genomics* 19(1)**,** 296. doi: 10.1186/s12864-018-4696-8.

Martini, S.V., Nascimento, S.B., and Morales, M.M. (2007). Rhodnius prolixus Malpighian tubules and control of diuresis by neurohormones. *An Acad Bras Cienc* 79(1)**,** 87-95. doi: 10.1590/s0001-37652007000100011.

Mendes, M.T., Carvalho-Costa, T.M., da Silva, M.V., Anhe, A.C., Guimaraes, R.M., da Costa, T.A., et al. (2016). Effect of the saliva from different triatomine species on the biology and immunity of TLR-4 ligand and Trypanosoma cruzi-stimulated dendritic cells. *Parasit Vectors* 9(1)**,** 634. doi: 10.1186/s13071-016-1890-x.

Mesquita, R.D., Carneiro, A.B., Bafica, A., Gazos-Lopes, F., Takiya, C.M., Souto-Padron, T., et al. (2008). Trypanosoma cruzi infection is enhanced by vector saliva through

immunosuppressant mechanisms mediated by lysophosphatidylcholine. *Infect Immun* 76(12)**,** 5543-5552. doi: 10.1128/IAI.00683-08.

Miller, J.R., Koren, S., and Sutton, G. (2010). Assembly algorithms for next-generation sequencing data. *Genomics* 95(6)**,** 315-327. doi: 10.1016/j.ygeno.2010.03.001.

Moller, S., Croning, M.D., and Apweiler, R. (2001). Evaluation of methods for the prediction of membrane spanning regions. *Bioinformatics* 17(7)**,** 646-653. doi: 10.1093/bioinformatics/17.7.646.

Mury, F.B., da Silva, J.R., Ferreira, L.S., dos Santos Ferreira, B., de Souza-Filho, G.A., de Souza-Neto, J.A., et al. (2009). Alpha-glucosidase promotes hemozoin formation in a blood-sucking bug: an evolutionary history. *PLoS One* 4(9)**,** e6966. doi: 10.1371/journal.pone.0006966.

Muscio, O.A., La Torre, J., Bonder, M.A., and Scodeller, E.A. (1997). Triatoma virus pathogenicity in laboratory colonies of Triatoma infestans (Hemiptera:Reduviidae). *J Med Entomol* 34(3)**,** 253-256. doi: 10.1093/jmedent/34.3.253.

Muscio, O.A., La Torre, J.L., and Scodeller, E.A. (1988). Characterization of Triatoma virus, a picorna-like virus isolated from the triatomine bug Triatoma infestans. *J Gen Virol* 69 ( Pt 11)**,** 2929-2934. doi: 10.1099/0022-1317-69-11-2929.

Nevoa, J.C., Mendes, M.T., da Silva, M.V., Soares, S.C., Oliveira, C.J.F., and Ribeiro, J.M.C. (2018). An insight into the salivary gland and fat body transcriptome of Panstrongylus lignarius (Hemiptera: Heteroptera), the main vector of Chagas disease in Peru. *PLoS Negl Trop Dis* 12(2)**,** e0006243. doi: 10.1371/journal.pntd.0006243.

Nielsen, H. (2017). Predicting Secretory Proteins with SignalP. *Methods Mol Biol* 1611**,** 59-73. doi: 10.1007/978-1-4939-7015-5_6.

Noeske-Jungblut, C., Kratzschmar, J., Haendler, B., Alagon, A., Possani, L., Verhallen, P., et al. (1994). An inhibitor of collagen-induced platelet aggregation from the saliva of Triatoma pallidipennis. *J Biol Chem* 269(7)**,** 5050-5053.

Nogueira, N.P., Saraiva, F.M., Sultano, P.E., Cunha, P.R., Laranja, G.A., Justo, G.A., et al. (2015). Proliferation and differentiation of Trypanosoma cruzi inside its vector have a new trigger: redox status. *PLoS One* 10(2)**,** e0116712. doi: 10.1371/journal.pone.0116712.

Noriega, F.G., Shah, D.K., and Wells, M.A. (1997). Juvenile hormone controls early trypsin gene transcription in the midgut of Aedes aegypti. *Insect Mol Biol* 6(1)**,** 63-66. doi: 10.1046/j.1365-2583.1997.00154.x.

Oliveira, D.S., Brito, N.F., Franco, T.A., Moreira, M.F., Leal, W.S., and Melo, A.C.A. (2018). Functional Characterization of Odorant Binding Protein 27 (RproOBP27) From Rhodnius prolixus Antennae. *Front Physiol* 9**,** 1175. doi: 10.3389/fphys.2018.01175.

Ons, S. (2017). Neuropeptides in the regulation of Rhodnius prolixus physiology. *J Insect Physiol* 97**,** 77-92. doi: 10.1016/j.jinsphys.2016.05.003.

Ons, S., Richter, F., Urlaub, H., and Pomar, R.R. (2009). The neuropeptidome of Rhodnius prolixus brain. *Proteomics* 9(3)**,** 788-792. doi: 10.1002/pmic.200800499.

Ons, S., Sterkel, M., Diambra, L., Urlaub, H., and Rivera-Pomar, R. (2011). Neuropeptide precursor gene discovery in the Chagas disease vector Rhodnius prolixus. *Insect Mol Biol* 20(1)**,** 29-44. doi: 10.1111/j.1365-2583.2010.01050.x.

Ouali, R., Valentim de Brito, K.C., Salmon, D., and Bousbata, S. (2020). High-Throughput Identification of the Rhodnius prolixus Midgut Proteome Unravels a Sophisticated Hematophagic Machinery. *Proteomes* 8(3). doi: 10.3390/proteomes8030016.

Ouali, R., Vieira, L.R., Salmon, D., and Bousbata, S. (2021). Early Post-Prandial Regulation of Protein Expression in the Midgut of Chagas Disease Vector Rhodnius prolixus Highlights New Potential Targets for Vector Control Strategy. *Microorganisms* 9(4). doi: 10.3390/microorganisms9040804.

Paddock, C.D., McKerrow, J.H., Hansell, E., Foreman, K.W., Hsieh, I., and Marshall, N. (2001). Identification, cloning, and recombinant expression of procalin, a major triatomine allergen. *J Immunol* 167(5)**,** 2694-2699. doi: 10.4049/jimmunol.167.5.2694.

Paim, R.M., Araujo, R.N., Soares, A.C., Lemos, L.C., Tanaka, A.S., Gontijo, N.F., et al. (2011). Influence of the intestinal anticoagulant in the feeding performance of triatomine bugs (Hemiptera; Reduviidae). *Int J Parasitol* 41(7)**,** 765-773. doi: 10.1016/j.ijpara.2011.01.014.

Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., and Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* 14(4)**,** 417-419. doi: 10.1038/nmeth.4197.

Postberg, J., Forcob, S., Chang, W.J., and Lipps, H.J. (2010). The evolutionary history of histone H3 suggests a deep eukaryotic root of chromatin modifying mechanisms. *BMC Evol Biol* 10**,** 259. doi: 10.1186/1471-2148-10-259.

Poupardin, R., Riaz, M.A., Vontas, J., David, J.P., and Reynaud, S. (2010). Transcription profiling of eleven cytochrome P450s potentially involved in xenobiotic metabolism in the mosquito Aedes aegypti. *Insect Mol Biol* 19(2)**,** 185-193. doi: 10.1111/j.1365-2583.2009.00967.x.

Pueyo, J.I., Magny, E.G., and Couso, J.P. (2016). New Peptides Under the s(ORF)ace of the Genome. *Trends Biochem Sci* 41(8)**,** 665-678. doi: 10.1016/j.tibs.2016.05.003.

Rawlings, N.D., and Barrett, A.J. (1994). Families of cysteine peptidases. *Methods Enzymol* 244**,** 461-486. doi: 10.1016/0076-6879(94)44034-4.

Ribeiro, A.R., Oliveira, R.C., Ceretti Junior, W., Lima, L., Almeida, L.A., Nascimento, J.D., et al. (2016). Trypanosoma cruzi isolated from a triatomine found in one of the biggest

metropolitan areas of Latin America. *Rev Soc Bras Med Trop* 49(2)**,** 183-189. doi: 10.1590/0037-8682-0366-2015.

Ribeiro, J.M., Andersen, J., Silva-Neto, M.A., Pham, V.M., Garfield, M.K., and Valenzuela, J.G. (2004). Exploring the sialome of the blood-sucking bug Rhodnius prolixus. *Insect Biochem Mol Biol* 34(1)**,** 61-79. doi: 10.1016/j.ibmb.2003.09.004.

Ribeiro, J.M., Assumpcao, T.C., Pham, V.M., Francischetti, I.M., and Reisenman, C.E. (2012). An insight into the sialotranscriptome of Triatoma rubida (Hemiptera: Heteroptera). *J Med Entomol* 49(3)**,** 563-572. doi: 10.1603/me11243.

Ribeiro, J.M., Genta, F.A., Sorgine, M.H., Logullo, R., Mesquita, R.D., Paiva-Silva, G.O., et al. (2014). An insight into the transcriptome of the digestive tract of the bloodsucking bug, Rhodnius prolixus. *PLoS Negl Trop Dis* 8(1)**,** e2594. doi: 10.1371/journal.pntd.0002594.

Ribeiro, J.M., Schwarz, A., and Francischetti, I.M. (2015). A Deep Insight Into the Sialotranscriptome of the Chagas Disease Vector, Panstrongylus megistus (Hemiptera: Heteroptera). *J Med Entomol* 52(3)**,** 351-358. doi: 10.1093/jme/tjv023.

Ribeiro, J.M., and Walker, F.A. (1994). High affinity histamine-binding and antihistaminic activity of the salivary nitric oxide-carrying heme protein (nitrophorin) of Rhodnius prolixus. *J Exp Med* 180(6)**,** 2251-2257. doi: 10.1084/jem.180.6.2251.

Ribeiro, J.M.C., and Arcà, B. (2009). "Chapter 2 From Sialomes to the Sialoverse: An Insight into Salivary Potion of Blood-Feeding Insects," in *Advances in Insect Physiology*. Academic Press), 59-118.

Rossignol, P.A., Ribeiro, J.M., Jungery, M., Turell, M.J., Spielman, A., and Bailey, C.L. (1985). Enhanced mosquito blood-finding success on parasitemic hosts: evidence for vector-parasite mutualism. *Proc Natl Acad Sci U S A* 82(22)**,** 7725-7727. doi: 10.1073/pnas.82.22.7725.

Santiago, P.B., Assumpcao, T.C., de Araujo, C.N., Bastos, I.M., Neves, D., da Silva, I.G., et al. (2016). A Deep Insight into the Sialome of Rhodnius neglectus, a Vector of Chagas Disease. *PLoS Negl Trop Dis* 10(4)**,** e0004581. doi: 10.1371/journal.pntd.0004581.

Santiago, P.B., Charneau, S., Mandacaru, S.C., Bentes, K., Bastos, I.M.D., de Sousa, M.V., et al. (2020). Proteomic Mapping of Multifunctional Complexes Within Triatomine Saliva. *Front Cell Infect Microbiol* 10**,** 459. doi: 10.3389/fcimb.2020.00459.

Santiago, P.B., de Araujo, C.N., Charneau, S., Bastos, I.M.D., Assumpcao, T.C.F., Queiroz, R.M.L., et al. (2018). Exploring the molecular complexity of Triatoma dimidiata sialome. *J Proteomics* 174**,** 47-60. doi: 10.1016/j.jprot.2017.12.016.

Santos, A., Ribeiro, J.M., Lehane, M.J., Gontijo, N.F., Veloso, A.B., Sant'Anna, M.R., et al. (2007). The sialotranscriptome of the blood-sucking bug Triatoma brasiliensis (Hemiptera, Triatominae). *Insect Biochem Mol Biol* 37(7)**,** 702-712. doi: 10.1016/j.ibmb.2007.04.004.

Schwarz, A., Medrano-Mercado, N., Schaub, G.A., Struchiner, C.J., Bargues, M.D., Levy, M.Z., et al. (2014a). An updated insight into the Sialotranscriptome of Triatoma infestans: developmental stage and geographic variations. *PLoS Negl Trop Dis* 8(12)**,** e3372. doi: 10.1371/journal.pntd.0003372.

Schwarz, A., Tenzer, S., Hackenberg, M., Erhart, J., Gerhold-Ay, A., Mazur, J., et al. (2014b). A systems level analysis reveals transcriptomic and proteomic complexity in Ixodes ricinus midgut and salivary glands during early attachment and feeding. *Mol Cell Proteomics* 13(10)**,** 2725-2735. doi: 10.1074/mcp.M114.039289.

Scott, J.G., and Wen, Z. (2001). Cytochromes P450 of insects: the tip of the iceberg. *Pest Manag Sci* 57(10)**,** 958-967. doi: 10.1002/ps.354.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13(11)**,** 2498-2504. doi: 10.1101/gr.1239303.

Simao, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., and Zdobnov, E.M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31(19)**,** 3210-3212. doi: 10.1093/bioinformatics/btv351.

Smith-Unna, R., Boursnell, C., Patro, R., Hibberd, J.M., and Kelly, S. (2016). TransRate: reference-free quality assessment of de novo transcriptome assemblies. *Genome Res* 26(8)**,** 1134-1144. doi: 10.1101/gr.196469.115.

Soares, A.C., Carvalho-Tavares, J., Gontijo Nde, F., dos Santos, V.C., Teixeira, M.M., and Pereira, M.H. (2006). Salivation pattern of Rhodnius prolixus (Reduviidae; Triatominae) in mouse skin. *J Insect Physiol* 52(5)**,** 468-472. doi: 10.1016/j.jinsphys.2006.01.003.

Soares, T.S., Buarque, D.S., Queiroz, B.R., Gomes, C.M., Braz, G.R., Araujo, R.N., et al. (2015). A Kazal-type inhibitor is modulated by Trypanosoma cruzi to control microbiota inside the anterior midgut of Rhodnius prolixus. *Biochimie* 112**,** 41-48. doi: 10.1016/j.biochi.2015.02.014.

Soneson, C., Love, M.I., and Robinson, M.D. (2015). Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Res* 4**,** 1521. doi: 10.12688/f1000research.7563.2.

Song, L., and Florea, L. (2015). Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. *Gigascience* 4**,** 48. doi: 10.1186/s13742-015-0089-y.

Spit, J., Holtof, M., Badisco, L., Vergauwen, L., Vogel, E., Knapen, D., et al. (2016). Transcriptional Analysis of The Adaptive Digestive System of The Migratory Locust in Response to Plant Defensive Protease Inhibitors. *Sci Rep* 6**,** 32460. doi: 10.1038/srep32460.

Stanke, M., Keller, O., Gunduz, I., Hayes, A., Waack, S., and Morgenstern, B. (2006). AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Res* 34(Web Server issue)**,** W435-439. doi: 10.1093/nar/gkl200.

Sterkel, M., Perdomo, H.D., Guizzo, M.G., Barletta, A.B., Nunes, R.D., Dias, F.A., et al. (2016). Tyrosine Detoxification Is an Essential Trait in the Life History of Blood-Feeding Arthropods. *Curr Biol* 26(16)**,** 2188-2193. doi: 10.1016/j.cub.2016.06.025.

Stoka, A.M. (1996). Activity of juvenile hormone and juvenile hormone analogues on the growth of Trypanosoma cruzi. *J Steroid Biochem Mol Biol* 59(5-6)**,** 495-500. doi: 10.1016/s0960-0760(96)00136-7.

Surget-Groba, Y., and Montoya-Burgos, J.I. (2010). Optimization of de novo transcriptome assembly from next-generation sequencing data. *Genome Res* 20(10)**,** 1432-1440. doi: 10.1101/gr.103846.109.

Takano-Lee, M., and Edman, J.D. (2002). Lack of manipulation of Rhodnius prolixus (Hemiptera: Reduviidae) vector competence by Trypanosoma cruzi. *J Med Entomol* 39(1)**,** 44-51. doi: 10.1603/0022-2585-39.1.44.

Taschuk, F., and Cherry, S. (2020). DEAD-Box Helicases: Sensors, Regulators, and Effectors for Antiviral Defense. *Viruses* 12(2). doi: 10.3390/v12020181.

Te Brugge, V.A., Schooley, D.A., and Orchard, I. (2002). The biological activity of diuretic factors in Rhodnius prolixus. *Peptides* 23(4)**,** 671-681. doi: https://doi.org/10.1016/S0196-9781(01)00661-1.

Tjalsma, H., Bolhuis, A., Jongbloed, J.D., Bron, S., and van Dijl, J.M. (2000). Signal peptide-dependent protein transport in Bacillus subtilis: a genome-based survey of the secretome. *Microbiol Mol Biol Rev* 64(3)**,** 515-547. doi: 10.1128/mmbr.64.3.515-547.2000.

Ursic-Bedoya, R.J., and Lowenberger, C.A. (2007). Rhodnius prolixus: identification of immune-related genes up-regulated in response to pathogens and parasites using suppressive subtractive hybridization. *Dev Comp Immunol* 31(2)**,** 109-120. doi: 10.1016/j.dci.2006.05.008.

Verly, T., Costa, S., Lima, N., Mallet, J., Odencio, F., Pereira, M., et al. (2020). Vector competence and feeding-excretion behavior of Triatoma rubrovaria (Blanchard, 1843) (Hemiptera: Reduviidae) infected with Trypanosoma cruzi TcVI. *PLoS Negl Trop Dis* 14(9)**,** e0008712. doi: 10.1371/journal.pntd.0008712.

Walker, A.A., Madio, B., Jin, J., Undheim, E.A., Fry, B.G., and King, G.F. (2017). Melt With This Kiss: Paralyzing and Liquefying Venom of The Assassin Bug Pristhesancus plagipennis (Hemiptera: Reduviidae). *Mol Cell Proteomics* 16(4)**,** 552-566. doi: 10.1074/mcp.M116.063321.

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10(1)**,** 57-63. doi: 10.1038/nrg2484.

Waterhouse, R.M., Seppey, M., Simao, F.A., Manni, M., Ioannidis, P., Klioutchnikov, G., et al. (2018). BUSCO Applications from Quality Assessments to Gene Prediction and Phylogenomics. *Mol Biol Evol* 35(3)**,** 543-548. doi: 10.1093/molbev/msx319.

Waterhouse, R.M., Zdobnov, E.M., and Kriventseva, E.V. (2011). Correlating traits of gene retention, sequence divergence, duplicability and essentiality in vertebrates, arthropods, and fungi. *Genome Biol Evol* 3**,** 75-86. doi: 10.1093/gbe/evq083.

Wheeler, D.L., Church, D.M., Federhen, S., Lash, A.E., Madden, T.L., Pontius, J.U., et al. (2003). Database resources of the National Center for Biotechnology. *Nucleic Acids Res* 31(1)**,** 28-33. doi: 10.1093/nar/gkg033.

Wingett, S.W., and Andrews, S. (2018). FastQ Screen: A tool for multi-genome mapping and quality control. *F1000Res* 7**,** 1338. doi: 10.12688/f1000research.15931.2.

Wolf, J.B. (2013). Principles of transcriptome analysis and gene expression quantification: an RNA-seq tutorial. *Mol Ecol Resour* 13(4)**,** 559-572. doi: 10.1111/1755-0998.12109.

Wolfe, A.J. (2005). The acetate switch. *Microbiol Mol Biol Rev* 69(1)**,** 12-50. doi: 10.1128/MMBR.69.1.12-50.2005.

Wommack, K.E., Bhavsar, J., and Ravel, J. (2008). Metagenomics: read length matters. *Appl Environ Microbiol* 74(5)**,** 1453-1463. doi: 10.1128/AEM.02181-07.

Wu, M.C., and Lu, K.H. (2008). Juvenile hormone induction of glutathione S-transferase activity in the larval fat body of the common cutworm, Spodoptera litura (Lepidoptera: Noctuidae). *Arch Insect Biochem Physiol* 68(4)**,** 232-240. doi: 10.1002/arch.20257.

Zhang, Y., Ribeiro, J.M., Guimaraes, J.A., and Walsh, P.N. (1998). Nitrophorin-2: a novel mixed-type reversible specific inhibitor of the intrinsic factor-X activating complex. *Biochemistry* 37(30)**,** 10681-10690. doi: 10.1021/bi973050y.

Zhang, Y., Wei, J., Qi, Y., Li, J., Amin, R., Yang, W., et al. (2020). Predicating the Effector Proteins Secreted by Puccinia triticina Through Transcriptomic Analysis and Multiple Prediction Approaches. *Front Microbiol* 11**,** 538032. doi: 10.3389/fmicb.2020.538032.

## 12 Figure Captions

**Figure 1. Distribution of nucleotide sequences considered for global reference.** (**A**) The transcripts were distributed according to the bp. A total of 67.529 transcripts were assembled from 37.873.676 paired-reads of the salivary gland and intestine. The distribution shows that the majority (55% approximately) has between 201-300 bp. Minimum size: 133 bp;

Maximum size: 28.047 bp. (**B**) Distribution of the reads used in the assemblies, according to the reading direction evaluated by the Oyster River Strand Exam Tool. Average: +0.887531.

**Figure 2. Distribution of transcripts according to annotated taxonomic similarity.** (**A**) Annotated distribution of transcripts generated by pairing against sequences from public databases, highlighting arthropod annotations and those not taxonomically defined, constituted by new transcripts identified by the multi k-mer methodology. (**B**) Annotated found that resemble symbiont/residual proteins. The most significant transcripts are similar to bacterial proteins (74.7%), consistent with the salivary gland and intestine findings.

**Figure 3. Total distribution of ORFs predicted by the existence of signal peptide.** Using TransDecoder, Augustus, and ORFfinder, the total ORFs for the transcripts is 74.031. 27.910 (37.9%) with ORFfinder (Matched ORF), in red; 45.670 (61.7%) with ORFfinder (Unmatched ORF), in yellow; 15.278 (20.7%) with TransDecoder, in blue, and 4.668 (6.3%) with Augustus, in green. Only 7.5% of the ORFs have the presence of signal peptide (SP+) against 92.5% with SP-. *___ Corresponding ORFs from the same transcript, but with different amino acid sequences and SP positivity.

**Figure 4. Predicted distribution of ORFs according to the number of transmembrane helices (TMHMM) and the presence of signal peptide (SP+ or SP).** (**A**) TransDecoder 15270 (20.7%). (**B**) Augustus 4668 (6.3%). (**C**) ORFfinder (Matched ORF) 27910 (37.9%). (**D**) ORFfinder (Unmatched ORF) 45670 (61.7%).

**Figure 5. Predicted distribution of ORFs according to the N-terminus predicted by TargetP and by the presence or not of signal peptide (SP+ or SP-) predicted by SignalP.** (**A**) TransDecoder 15270 (20.7%). (**B**) Augustus 4668 (6.3%). (**C**) ORFfinder (Matched ORF) 27910 (37.9%). (**D**) ORFfinder (Unmatched ORF) 45670 (61.7%). ORFs are capable of addressing mitochondria (mTP); with signal peptide (SP), and without signal or mitochondrial targeting peptide (noTP), according to TargetP.

**Figure 6. Taxonomic annotation of transcripts according to the Kegg Orthology GhostKOALA database.** (**A**) Total transcripts according to taxonomy. (**B**) Functionally annotated transcripts according to taxonomy.

**Figure 7. Transcripts distribution according to functional classes.** The genetic information processing (7819) and enzymes (7750) comprise most of the transcripts present in the salivary glands and intestine of *R. neglectus*. However, known to arthropods, functional transcripts of genetic information processing and cellular processes are the most diverse of the triatomine.

**Figure 8. Arthropod orthologous distribution of *R. neglectus* intestine and salivary gland transcripts according to function.** Other cellular coupling proteins: G protein-coupled receptors; Glycosaminoglycan binding proteins; GTP-binding proteins; Nuclear receptors. Peptide carriers: Exosomes; Transporters.

**Figure 9. Differential expression of individual transcripts in the intestine according to blood supply and *T. cruzi* infection.** (**A**) Volcano Plot of transcriptional expression in the

intestine of fasted vs. -fed *R. neglectus*. (**B**) Volcano Plot of transcriptional expression in the intestine of fed uninfected vs. fed infected *R. neglectus*. *Dots not assigned: unknown/hypothetical proteins. *padj < 0.05 (-Log10P < 50-3); Log2 fold change < -2 or > 2. *-like: transcript similar to sequence previously determined as similar to segment translated into a respective protein. Total de 67529 transcritos analisados. *AA peptidase: subfamily AA unassigned peptidase; Acp-1: acid phosphatase-1; C19 peptidase: family C19 unassigned peptidase; Cathepsin D: cathepsin D; Crebbp X4-like: CREB-binding protein isoform X4; Csp-like: chemosensory protein; Cyp6a14 X3-like: cytochrome P450 6a14 isoform X3; Ddx5 X1: ATP-dependent RNA helicase DDX5 isoform X1; Dpys-like: dihydropyrimidinase; Eif4g1X2-like: eukaryotic translation initiation factor 4 gamma 1 isoform X2; Fah: fumarylacetoacetase; H3v1-like: histone H3v1; Hectd2 X2-like: E3 ubiquitin-protein ligase HECTD2 isoform X2; Hgd: homogentisate 1,2-dioxygenase; Hsd17b13-like: 17-beta-hydroxysteroid dehydrogenase 13; Hsp70Ba: major heat shock 70 kDa protein Ba; I1 peptidase inhibitor: family I1 unassigned peptidase inhibitor; Isyna-like: inositol-3-phosphate synthase; Lipocalin-like 2: lipocalin protein 2; Mark2 X10-like: serine/threonine-protein kinase MARK2 isoform X10; Naga-like: Alpha-N-acetylgalactosaminidase; Nudt15 X1: nucleotide triphosphate diphosphatase NUDT15 isoform X1; Obp-like: odorant-binding protein; p-Apy: 79 kDa salivary apyrase precursor; Path X3: proton-coupled amino acid transporter protein pathetic isoform X3; Picot X1: inorganic phosphate cotransporter isoform X1; p-Lipocalin AI-5: lipocalin AI-5 precursor; p-Myosuppressin: myosuppressin precursor; p-Neuroparsin 1: neuroparsin 1 precursor; p-Nitrophorin-3: Nitrophorin-3 precursor; p-NP: nonstructural protein precursor; p-Obp: odorant-binding protein precursor S10 peptidase: family S10 unassigned peptidase; sElp: secreted Esterase/lipase protein; Sno-like: senecionine N-oxygenase; Sult1c4-like: sulfotransferase 1C4; svPF3P-like: secreted venom protein family 3 protein; T3 peptidase: family T3 unassigned peptidase; Tat: tyrosine aminotransferase; Tret1-like: facilitated trehalose transporter Tret1; vCUBDP2-like: venom CUB domain protein 2; vHEX-like 1: venom hexamerin protein 1; vRNase1-like: venom ribonuclease 1; Xfin-like X1: zinc finger protein Xfin-isoform X1.

**Figure 10. *R. neglectus* transcripts distribution per expression level after 2 days fed in the fasted state.** Transcriptional expression comparison in SGs and INT of *R. neglectus*, according to the presence of infection by *T. cruzi*, after 2 days of feeding. *↑: 2 times upregulated; ○: Not changed; ↓: 2 times downregulated; T: Total. SG: Salivary Gland; I: Intestine; Tc: *T. cruzi*.

**Figure 11. Transcripts translatable into lipocalins, nitrophorins, and derivatives.** (**A**) Differential expression of individual transcripts according to the blood supply and infection by *T. cruzi* in the salivary gland of the insect in relation to fasting. (**B**) Differential expression of individual transcripts according to blood feeding and *T. cruzi* infection in the insect's intestine in relation to fasting. *FC: fold change.

**Figure 12. Biological network.** (**A**) Biological network of biosynthesis and intratissue metabolism pathways of *R. neglectus*. (**B**) Biological Network Via Systemic Components. Identified homologous component clusters and with degree > 0. Unmatched transcripts are represented in unnamed nodes

## 13 Tables

**Table 1**. TransDecoder, Augustus, and ORFfinder were used to predict the open reading frames (ORFs). Of the 67529 total transcripts, only 27796 (41.2%) have a CDS pattern defined by homology with the ORFfinder and/or identified/optimized by TransDecoder or Augustus (Transcripts with matched CDS group pattern). Unannotated transcripts are represented as unmatched. *( ): transcripts with secretable and non-secretable signal predicted isoform segments. [1] transcripts with known predicted non-secretable + residual/symbiont-like translatable segments. SG: salivary gland; INT: intestine.

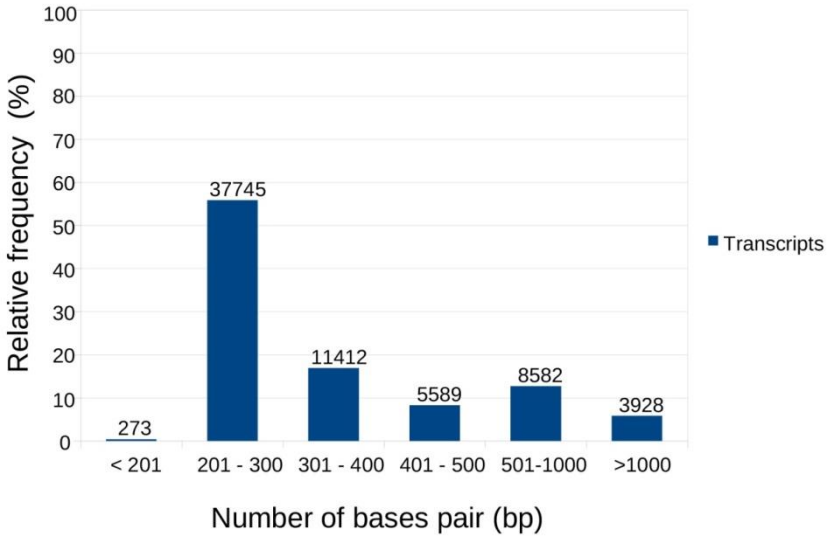
**Table 1 - Classification of assembled and translatable transcripts into coding sequences (CDS)**

| Isoform category | Total transcripts | | | Transcripts with matched CDS group pattern | | |
|---|---|---|---|---|---|---|
| | Total | SG | INT | Total | SG | INT |
| Protein predicted as secreted | 904 (337) 1.3% (0.5%) | 806 (313) 1.6% (0.6%) | 794 (313) 1.2% (0.5%) | 896 (329) 3.2% (1.2%) | 801 (308) 3.5% (1.3%) | 786 (305) 2.9% (1.1%) |
| Housekeeping[1] | 16453 (337) 24.4% (0.5%) | 13673 (313) 27.6% (0.6%) | 16003 (313) 24.5% (0.5%) | 16331 (329) 58.7% (1.2%) | 13590 (308) 59.2% (1.3%) | 15885 (305) 58.9% (1.1%) |
| Unknown/hypothetical protein predicted as secreted | 349 (136) 0.5% (0.2%) | 288 (119) 0.6% (0.2%) | 339 (135) 0.5% (0.2%) | 327 (114) 1.2% (0.4%) | 273 (104) 1.2% (0.5%) | 317 (113) 1 .2% (0.4%) |
| Unknown/hypothetical non-secreted protein | 8930 (136) 13.2% (0.2%) | 7225 (119) 14.6% (0.2%) | 8693 (135) 13.3% (0.2%) | 8742 (114) 31.5% (0.4%) | 7099 (104) 30.9% (0.5%) | 8510 (113) 31 .6% (0.4%) |
| Transposable Elements | 263 (4) 0.4% (0.01%) | 202 (3) 0.4% (0.01%) | 258 (4) 0.4% (0.01%) | 241 (2) 0.9% (0.01%) | 186 (1) 0.8% (0.005%) | 236 (2) 0.9% |
| Unmatched transcript with ORF predicted as secreted | 4256 (4253) 6.3% (6.3%) | 2897 (2897) 5.9% (5.9%) | 4094 (4091) 6.3% (6.2%) | 206 (203) 0.7% (0.7%) | 162 (162) 0.7% (0.7%) | 196 (193) 0 .7% (0.7%) |
| Unmatched transcript with ORF nonsecreted | 41100 (4253) 60.9% (6.3%) | 27695 (2897) 56.0% (5.9%) | 39793 (4091) 60.8% (6.2%) | 1699 (203) 6.1% (0.7%) | 1412 (162) 6.1% (0.7%) | 1627 (193) 6 .0% (0.7%) |
| **Total transcripts** | 67529 | 49457 | 65435 | 27796 | 22949 | 26946 |

# APENDICE B - Figuras

**Figure 1.**

(A)




(B)

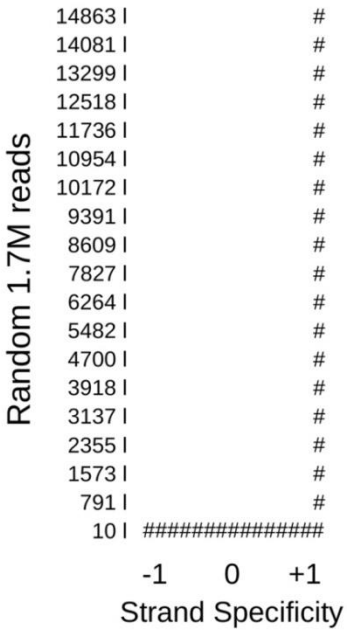
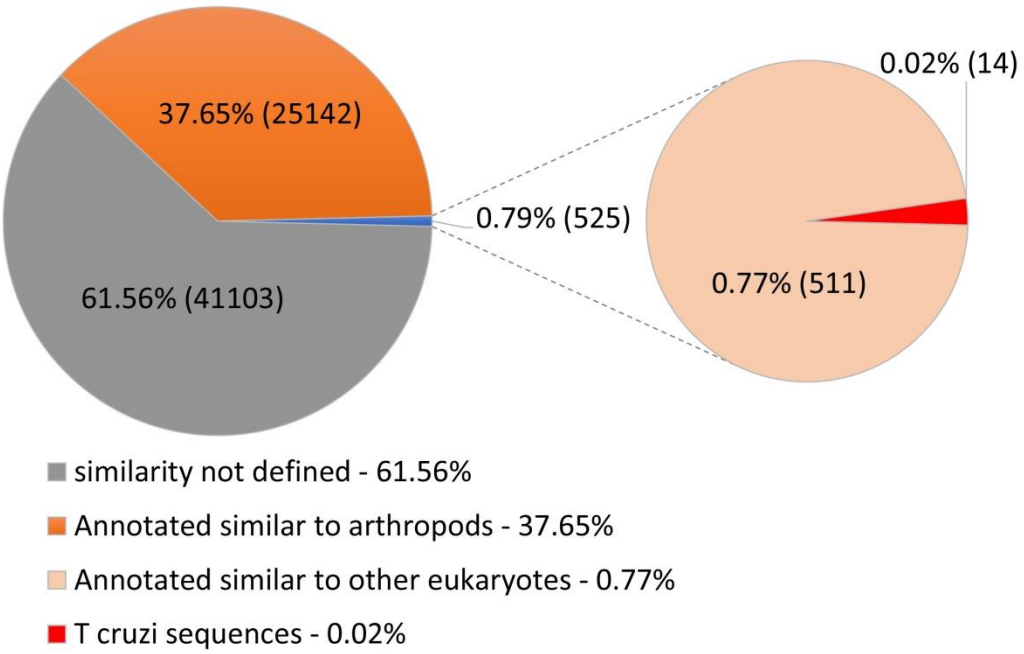


**Figure 2.**

(A)


37.65% (25142)
61.56% (41103)
0.79% (525)
0.02% (14)
0.77% (511)
similarity not defined - 61.56%
Annotated similar to arthropods - 37.65%
Annotated similar to other eukaryotes - 0.77%
T cruzi sequences - 0.02%

(B)

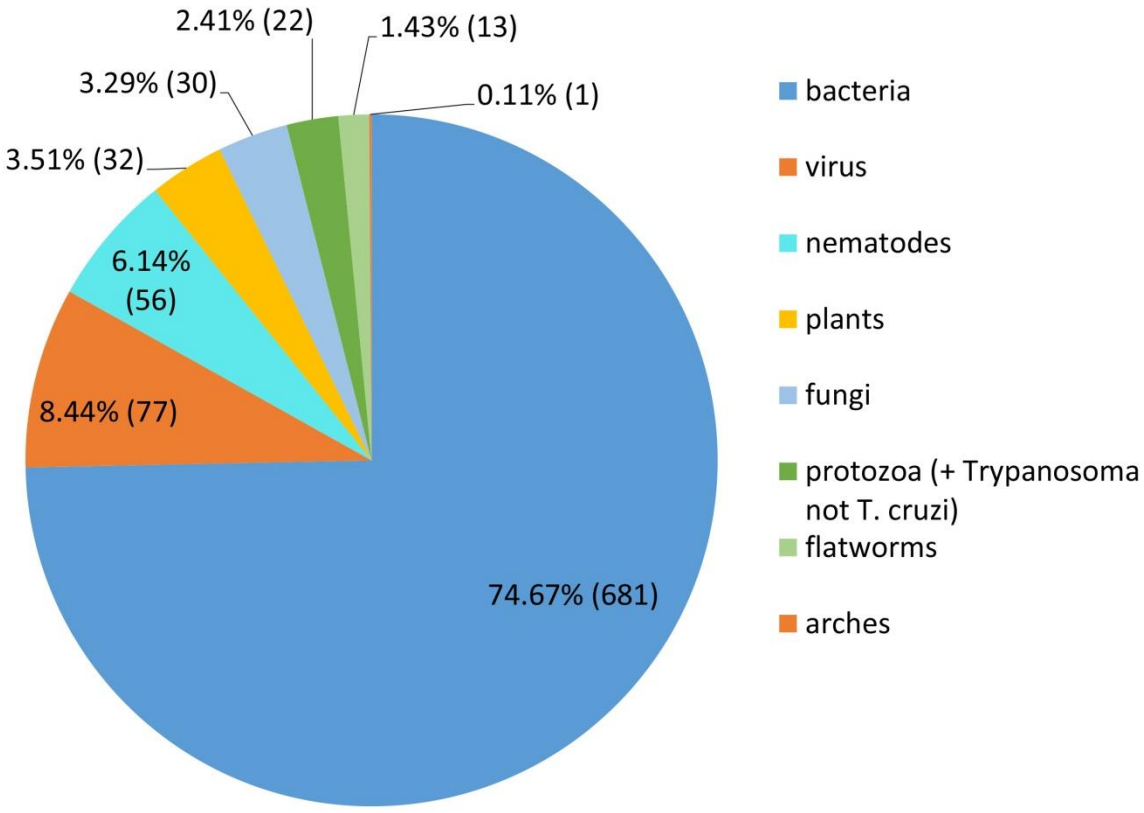

2.41% (22)
1.43% (13)
3.29% (30)
0.11% (1)
3.51% (32)
6.14% (56)
8.44% (77)
74.67% (681)
bacteria
virus
nematodes
plants
fungi
protozoa (+ Trypanosoma not T. cruzi)
flatworms
arches

**Figure 3.**

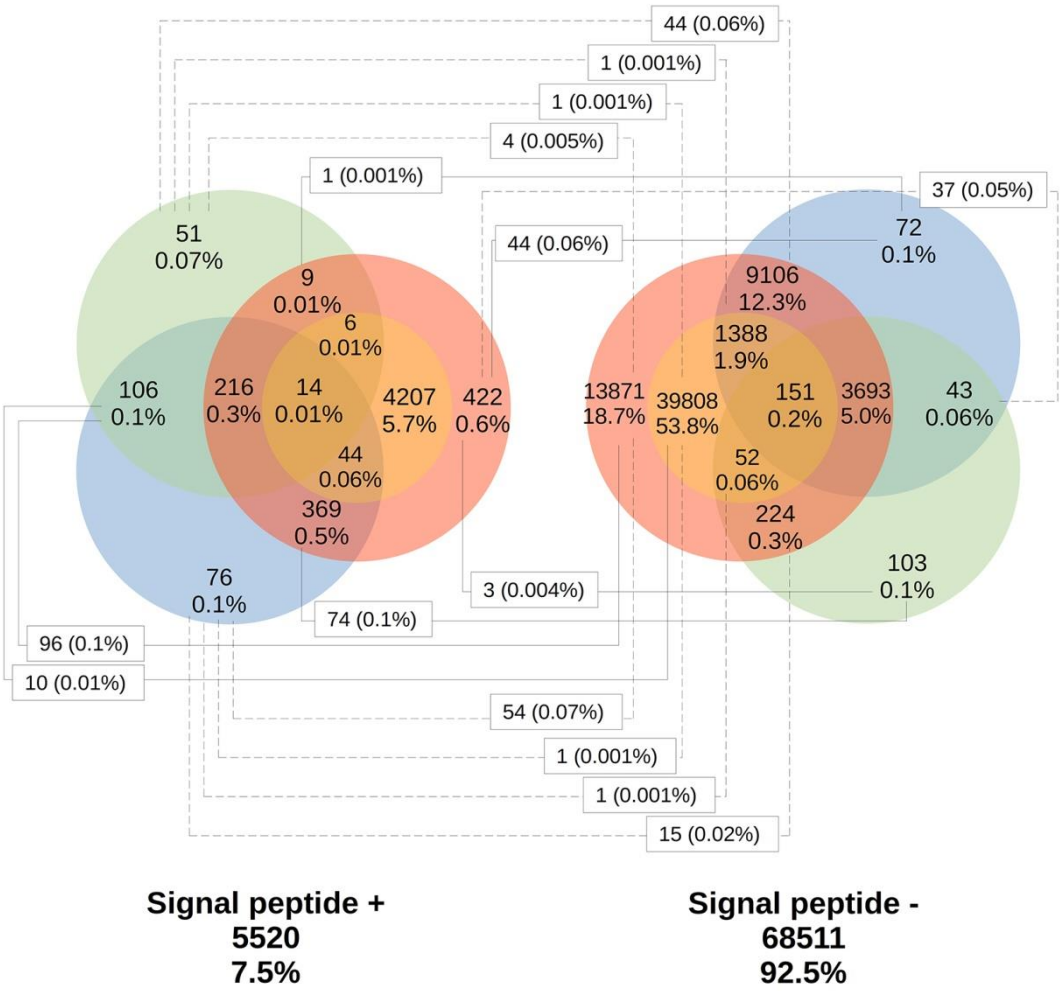
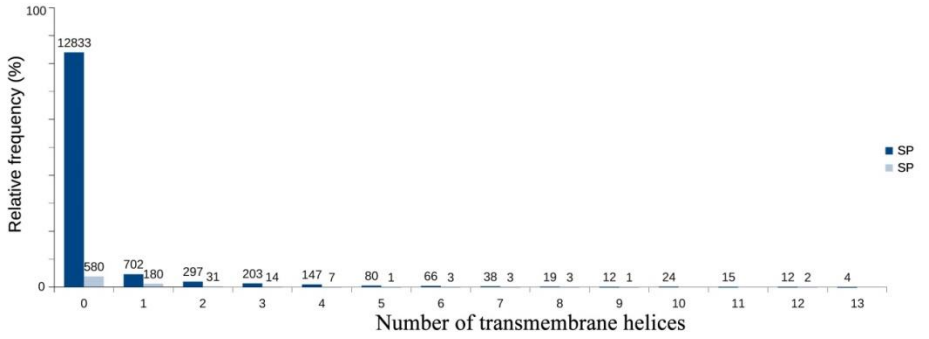
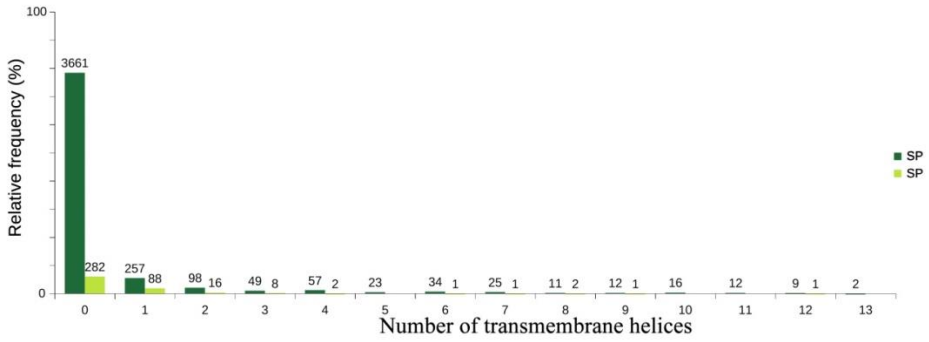

44 (0.06%)
1 (0.001%)
1 (0.001%)
4 (0.005%)
1 (0.001%)
37 (0.05%)
51
0.07%
44 (0.06%)
72
0.1%
9
0.01%
6
0.01%
9106
12.3%
1388
1.9%
106
0.1%
216
0.3%
14
0.01%
4207
5.7%
422
0.6%
13871
18.7%
39808
53.8%
151
0.2%
3693
5.0%
43
0.06%
44
0.06%
52
0.06%
369
0.5%
224
0.3%
76
0.1%
3 (0.004%)
103
0.1%
74 (0.1%)
96 (0.1%)
10 (0.01%)
54 (0.07%)
1 (0.001%)
1 (0.001%)
15 (0.02%)
Signal peptide +
5520
7.5%
Signal peptide -
68511
92.5%

**Figure 4.**

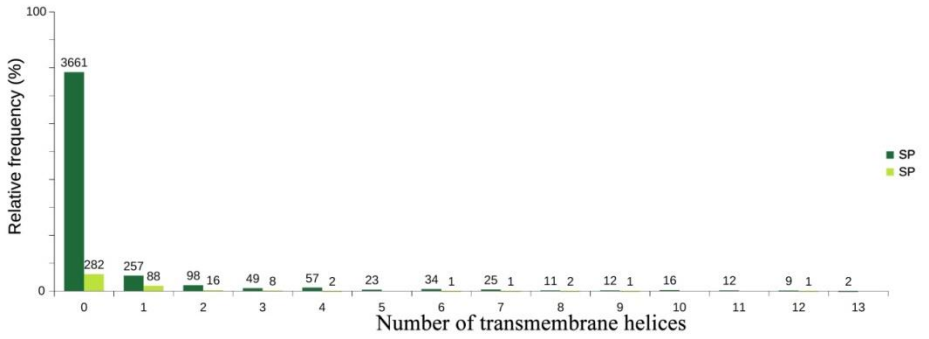
(A)




(B)




(C)




(D)

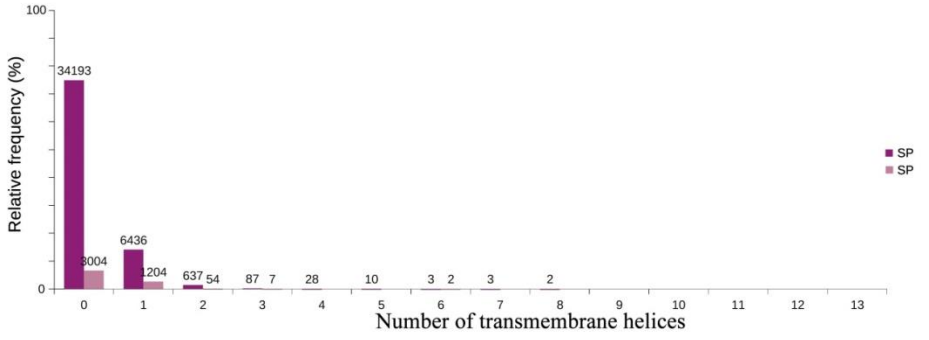
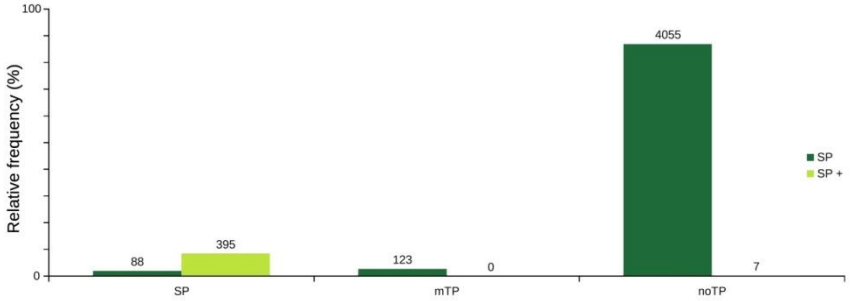
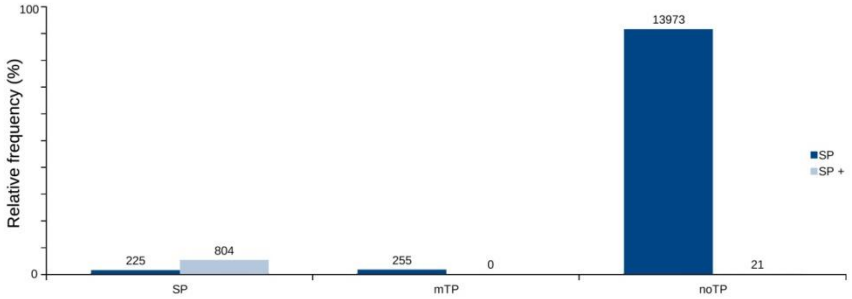


**Figure 5.**

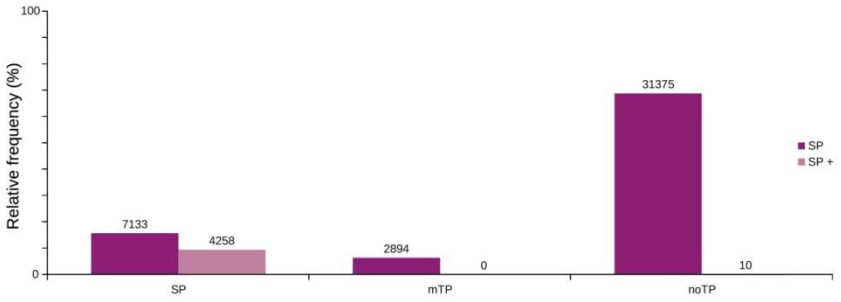
(A)




(B)




(C)




(D)

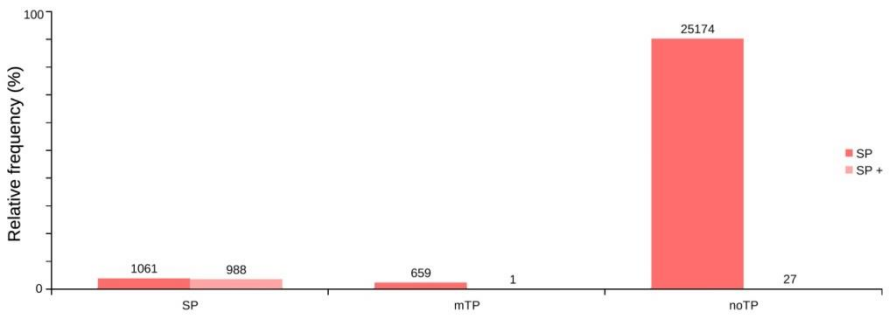
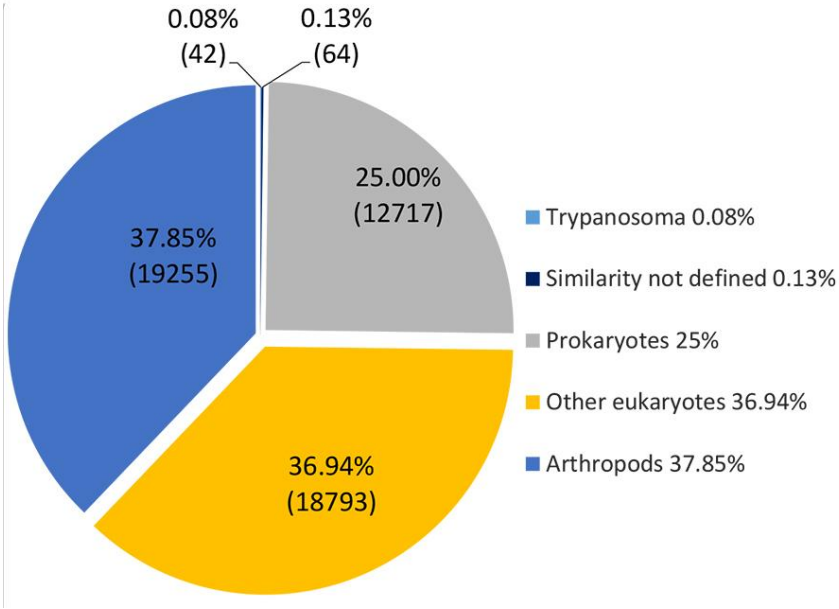


**Figure 6.**

(A)


0.08%
(42)
0.13%
(64)
25.00%
(12717)
37.85%
(19255)
36.94%
(18793)
Trypanosoma 0.08%
Similarity not defined 0.13%
Prokaryotes 25%
Other eukaryotes 36.94%
Arthropods 37.85%

(B)

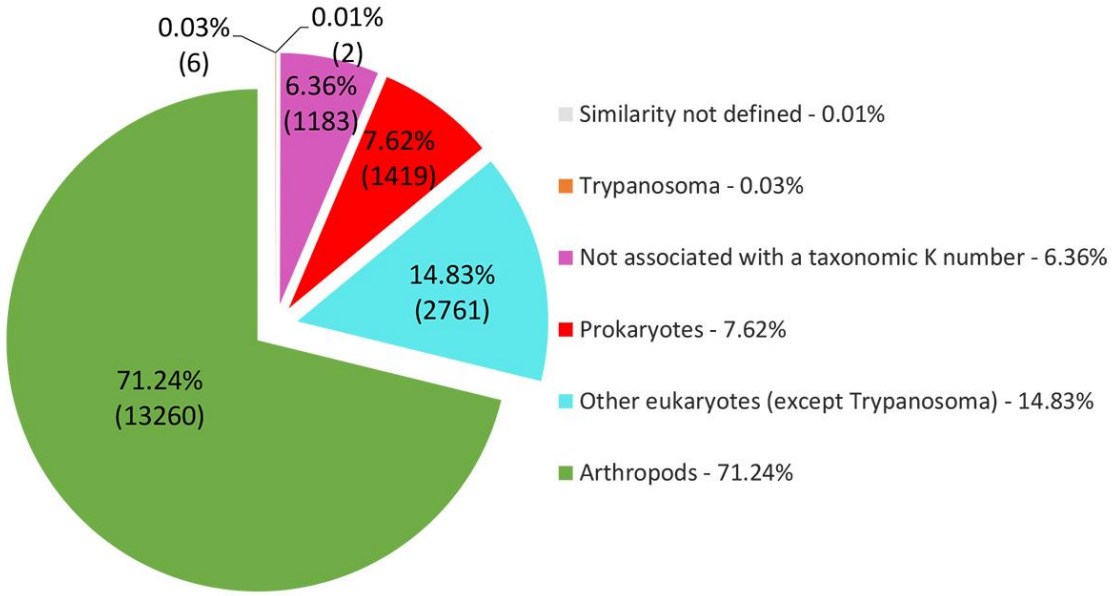

0.03%
(6)
0.01%
(2)
6.36%
(1183)
7.62%
(1419)
14.83%
(2761)
71.24%
(13260)
Similarity not defined - 0.01%
Trypanosoma - 0.03%
Not associated with a taxonomic K number - 6.36%
Prokaryotes - 7.62%
Other eukaryotes (except Trypanosoma) - 14.83%
Arthropods - 71.24%

**Figure 7.**

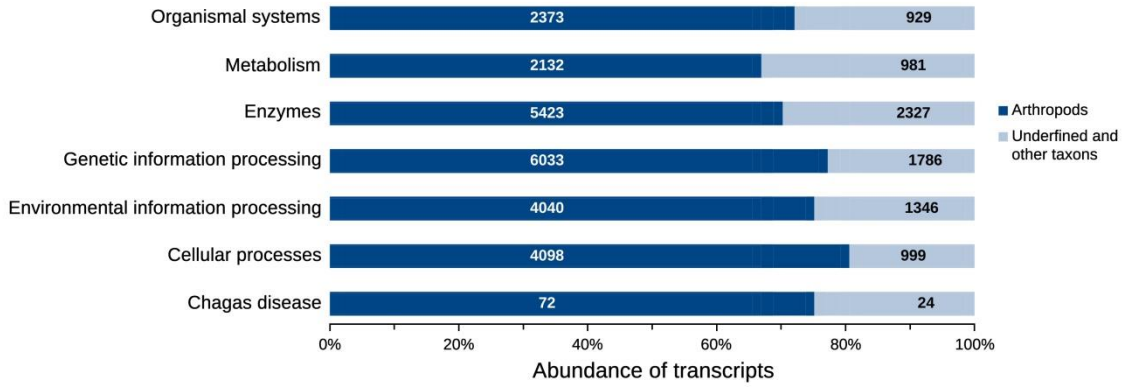

Organismal systems
Metabolism
Enzymes
Genetic information processing
Environmental information processing
Cellular processes
Chagas disease
2373
929
2132
981
5423
2327
6033
1786
4040
1346
4098
999
72
24
0%
20%
40%
60%
80%
100%
Abundance of transcripts
Arthropods
Underfined and other taxons

**Figure 8.**

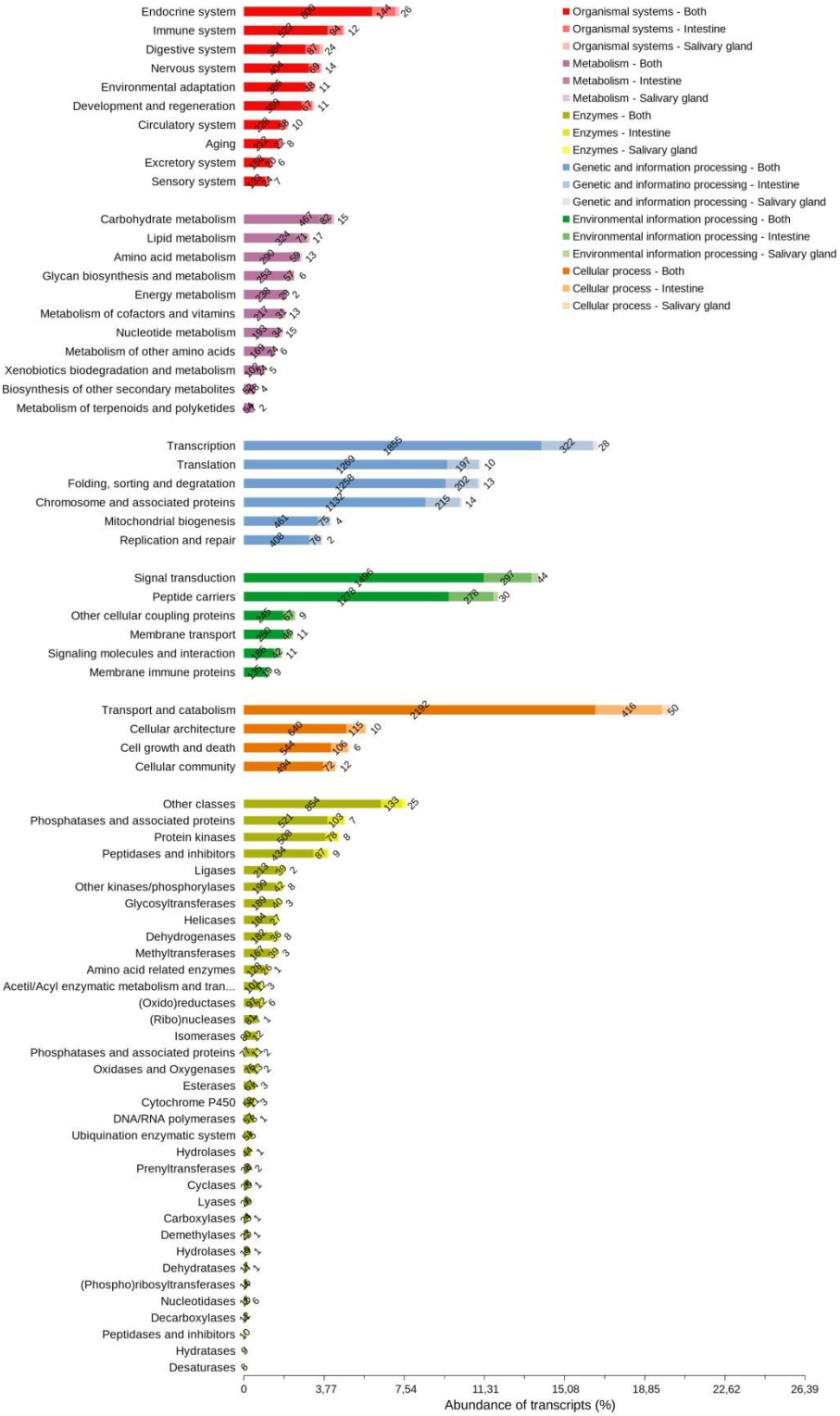
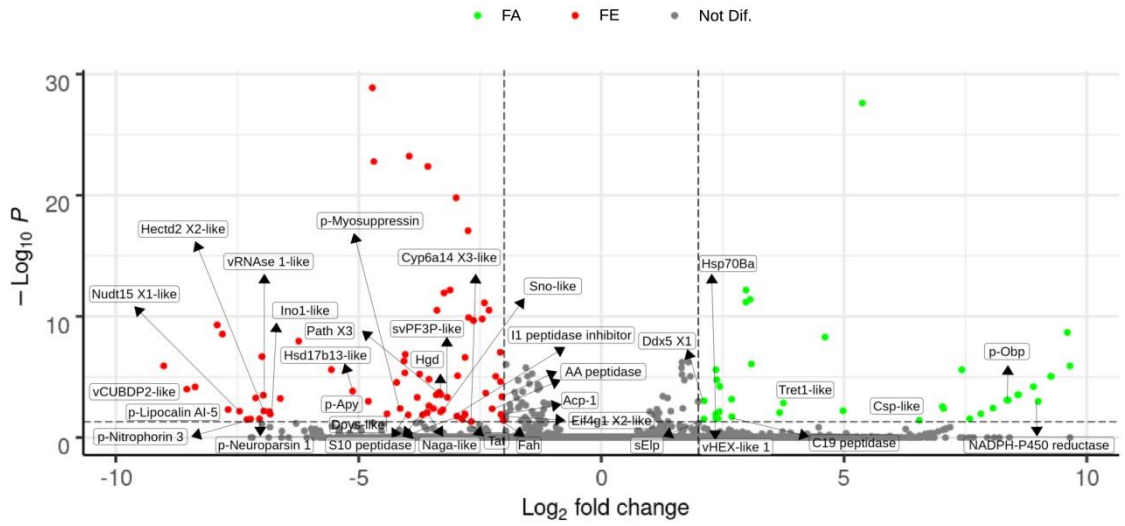

Endocrine system
Immune system
Digestive system
Nervous system
Environmental adaptation
Development and regeneration
Circulatory system
Aging
Excretory system
Sensory system
Carbohydrate metabolism
Lipid metabolism
Amino acid metabolism
Glycan biosynthesis and metabolism
Energy metabolism
Metabolism of cofactors and vitamins
Nucleotide metabolism
Metabolism of other amino acids
Xenobiotics biodegradation and metabolism
Biosynthesis of other secondary metabolites
Metabolism of terpenoids and polyketides
Transcription
Translation
Folding, sorting and degratation
Chromosome and associated proteins
Mitochondrial biogenesis
Replication and repair
Signal transduction
Peptide carriers
Other cellular coupling proteins
Membrane transport
Signaling molecules and interaction
Membrane immune proteins
Transport and catabolism
Cellular architecture
Cell growth and death
Cellular community
Other classes
Phosphatases and associated proteins
Protein kinases
Peptidases and inhibitors
Ligases
Other kinases/phosphorylases
Glycosyltransferases
Helicases
Dehydrogenases
Methyltransferases
Amino acid related enzymes
Acetil/Acyl enzymatic metabolism and tran...
(Oxido)reductases
(Ribo)nucleases
Isomerases
Phosphatases and associated proteins
Oxidases and Oxygenases
Esterases
Cytochrome P450
DNA/RNA polymerases
Ubiquination enzymatic system
Hydrolases
Prenyltransferases
Cyclases
Lyases
Carboxylases
Demethylases
Hydrolases
Dehydratases
(Phospho)ribosyltransferases
Nucleotidases
Decarboxylases
Peptidases and inhibitors
Hydratases
Desaturases
0
3,77
7,54
11,31
15,08
18,85
22,62
26,39
Abundance of transcripts (%)
Organismal systems - Both
Organismal systems - Intestine
Organismal systems - Salivary gland
Metabolism - Both
Metabolism - Intestine
Metabolism - Salivary gland
Enzymes - Both
Enzymes - Intestine
Enzymes - Salivary gland
Genetic and information processing - Both
Genetic and informatino processing - Intestine
Genetic and information processing - Salivary gland
Environmental information processing - Both
Environmental information processing - Intestine
Environmental information processing - Salivary gland
Cellular process - Both
Cellular process - Intestine
Cellular process - Salivary gland

**Figure 9.**

(A)


FA
FE
Not Dif.
30
20
10
0
− Log10 P
Hectd2 X2-like
vRNAse 1-like
p-Myosuppressin
Cyp6a14 X3-like
Hsp70Ba
Nudt15 X1-like
Sno-like
Ino1-like
Path X3
svPF3P-like
Hsd17b13-like
Hgd
l1 peptidase inhibitor
Ddx5 X1
p-Obp
AA peptidase
vCUBDP2-like
Tret1-like
p-Apy
Acp-1
Csp-like
p-Lipocalin AI-5
Dpys-like
Eif4g1 X2-like
p-Nitrophorin 3
p-Neuroparsin 1
S10 peptidase
Naga-like
Fah
sElp
vHEX-like 1
C19 peptidase
NADPH-P450 reductase
-10
-5
0
5
10
Log2 fold change

(B)

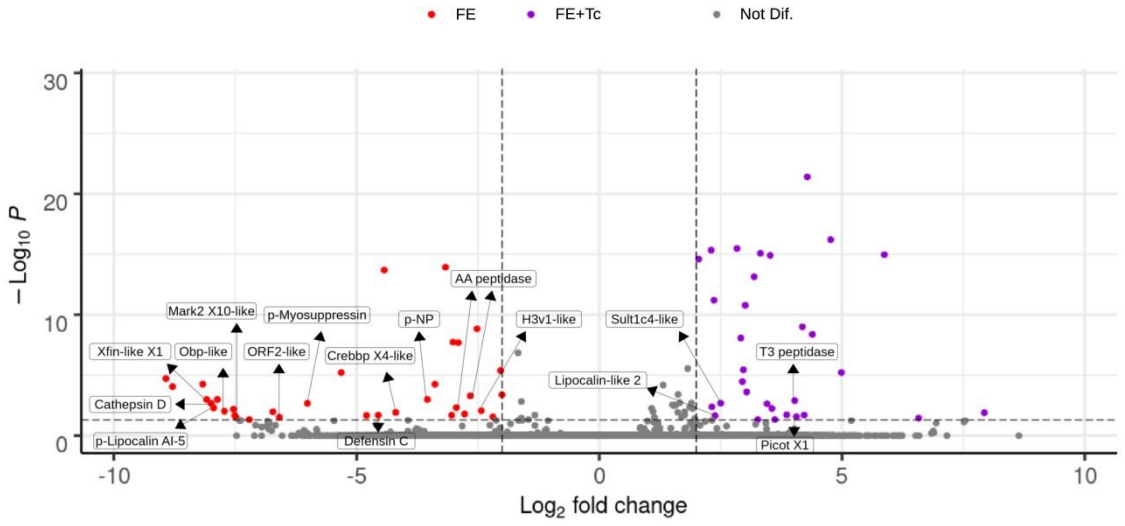

FE
FE+Tc
Not Dif.
30
20
10
0
− Log10 P
AA peptidase
Mark2 X10-like
p-Myosuppressin
p-NP
H3v1-like
Sult1c4-like
Xfin-like X1
Obp-like
ORF2-like
Crebbp X4-like
T3 peptidase
Lipocalin-like 2
Cathepsin D
p-Lipocalin AI-5
Defensin C
Picot X1
-10
-5
0
5
10
Log2 fold change

**Figure 10.**

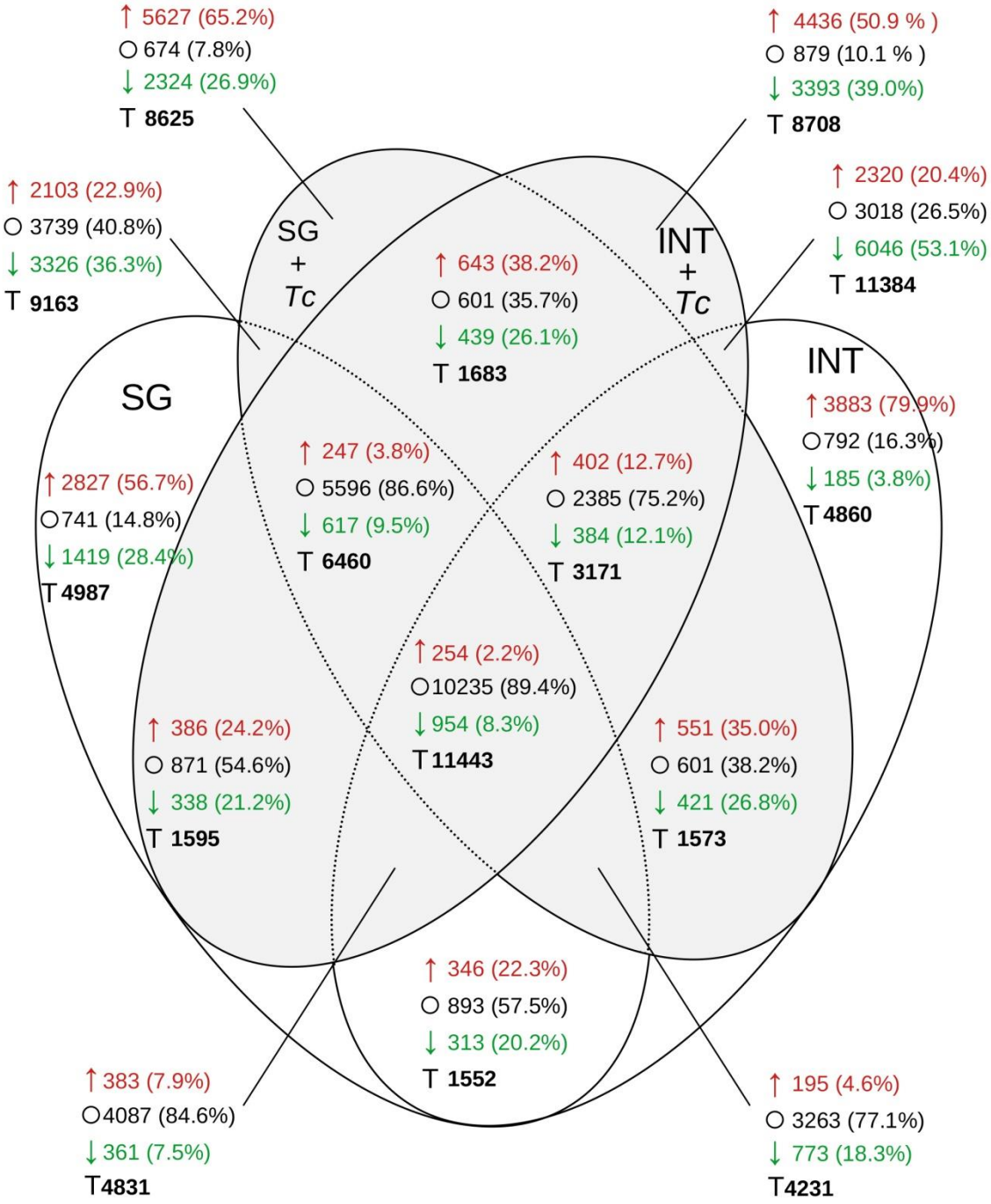
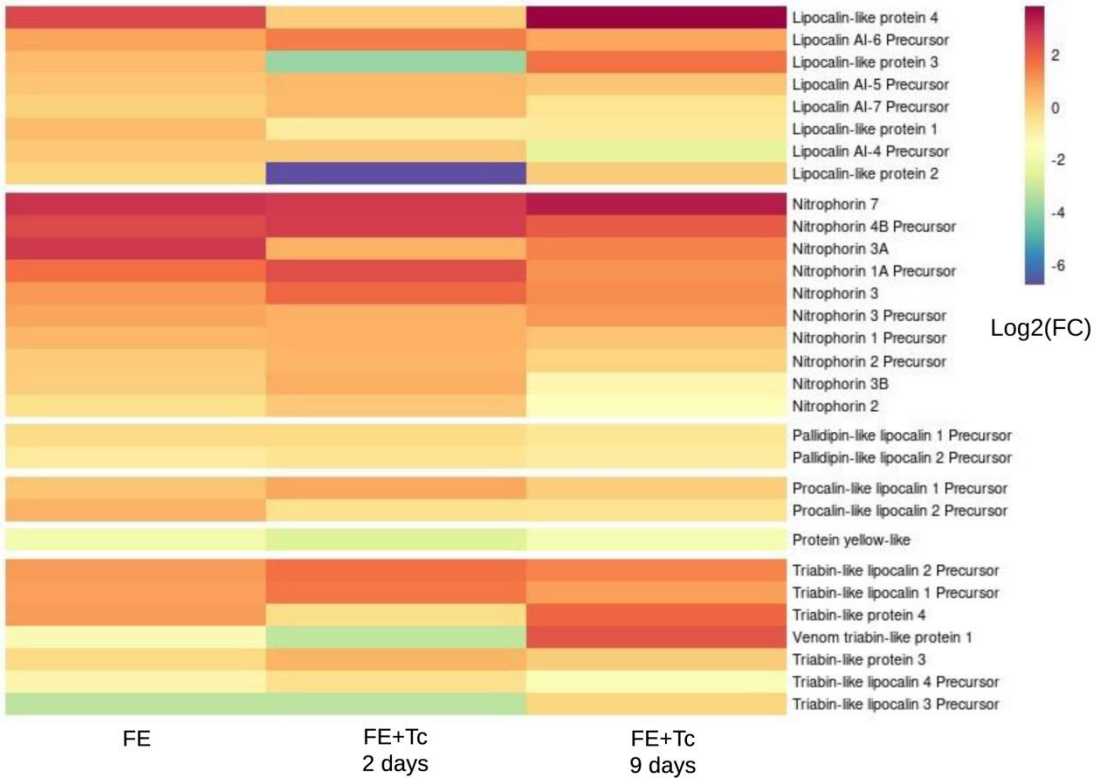

↑ 5627 (65.2%)
○ 674 (7.8%)
↓ 2324 (26.9%)
T 8625
↑ 4436 (50.9 % )
○ 879 (10.1 % )
↓ 3393 (39.0%)
T 8708
↑ 2103 (22.9%)
○ 3739 (40.8%)
↓ 3326 (36.3%)
T 9163
↑ 2320 (20.4%)
○ 3018 (26.5%)
↓ 6046 (53.1%)
T 11384
SG
+
Tc
INT
+
Tc
↑ 643 (38.2%)
○ 601 (35.7%)
↓ 439 (26.1%)
T 1683
SG
INT
↑ 3883 (79.9%)
○ 792 (16.3%)
↓ 185 (3.8%)
T 4860
↑ 247 (3.8%)
○ 5596 (86.6%)
↓ 617 (9.5%)
T 6460
↑ 402 (12.7%)
○ 2385 (75.2%)
↓ 384 (12.1%)
T 3171
↑ 2827 (56.7%)
○ 741 (14.8%)
↓ 1419 (28.4%)
T 4987
↑ 254 (2.2%)
○ 10235 (89.4%)
↓ 954 (8.3%)
T 11443
↑ 386 (24.2%)
○ 871 (54.6%)
↓ 338 (21.2%)
T 1595
↑ 551 (35.0%)
○ 601 (38.2%)
↓ 421 (26.8%)
T 1573
↑ 346 (22.3%)
○ 893 (57.5%)
↓ 313 (20.2%)
T 1552
↑ 383 (7.9%)
○ 4087 (84.6%)
↓ 361 (7.5%)
T 4831
↑ 195 (4.6%)
○ 3263 (77.1%)
↓ 773 (18.3%)
T 4231

**Figure 11.**

(A)


Lipocalin-like protein 4
Lipocalin Al-6 Precursor
Lipocalin-like protein 3
Lipocalin Al-5 Precursor
Lipocalin Al-7 Precursor
Lipocalin-like protein 1
Lipocalin Al-4 Precursor
Lipocalin-like protein 2
Nitrophorin 7
Nitrophorin 4B Precursor
Nitrophorin 3A
Nitrophorin 1A Precursor
Nitrophorin 3
Nitrophorin 3 Precursor
Nitrophorin 1 Precursor
Nitrophorin 2 Precursor
Nitrophorin 3B
Nitrophorin 2
Pallidipin-like lipocalin 1 Precursor
Pallidipin-like lipocalin 2 Precursor
Procalin-like lipocalin 1 Precursor
Procalin-like lipocalin 2 Precursor
Protein yellow-like
Triabin-like lipocalin 2 Precursor
Triabin-like lipocalin 1 Precursor
Triabin-like protein 4
Venom triabin-like protein 1
Triabin-like protein 3
Triabin-like lipocalin 4 Precursor
Triabin-like lipocalin 3 Precursor
2
0
-2
-4
-6
Log2(FC)
FE
FE+Tc
2 days
FE+Tc
9 days

(B)

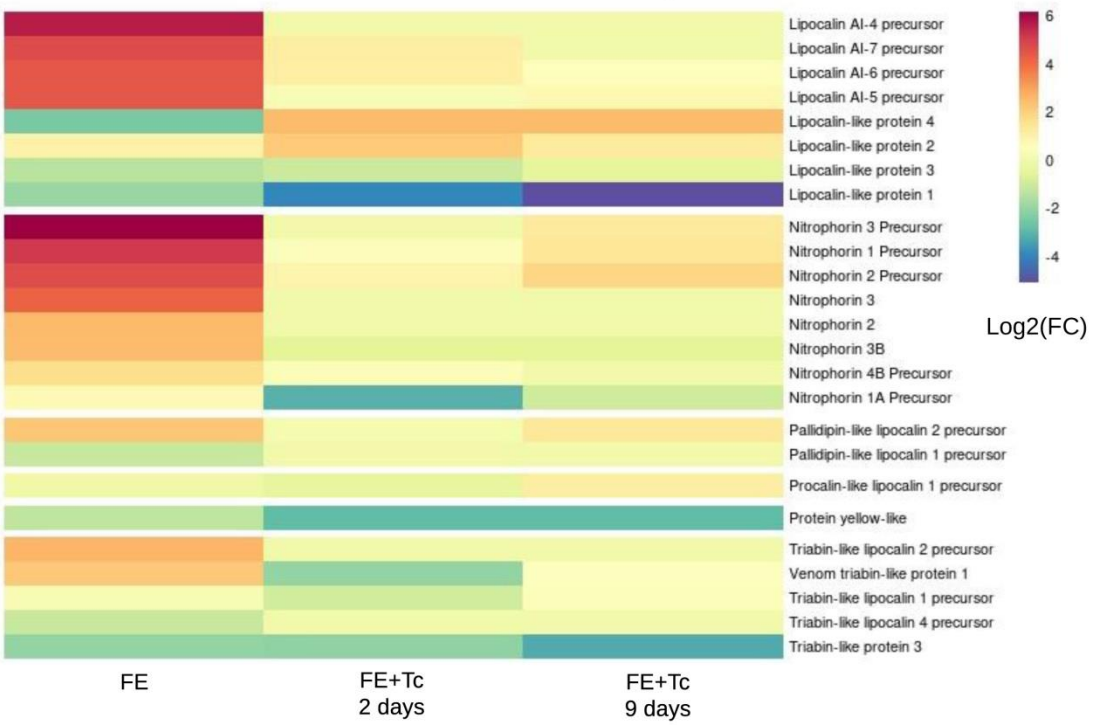
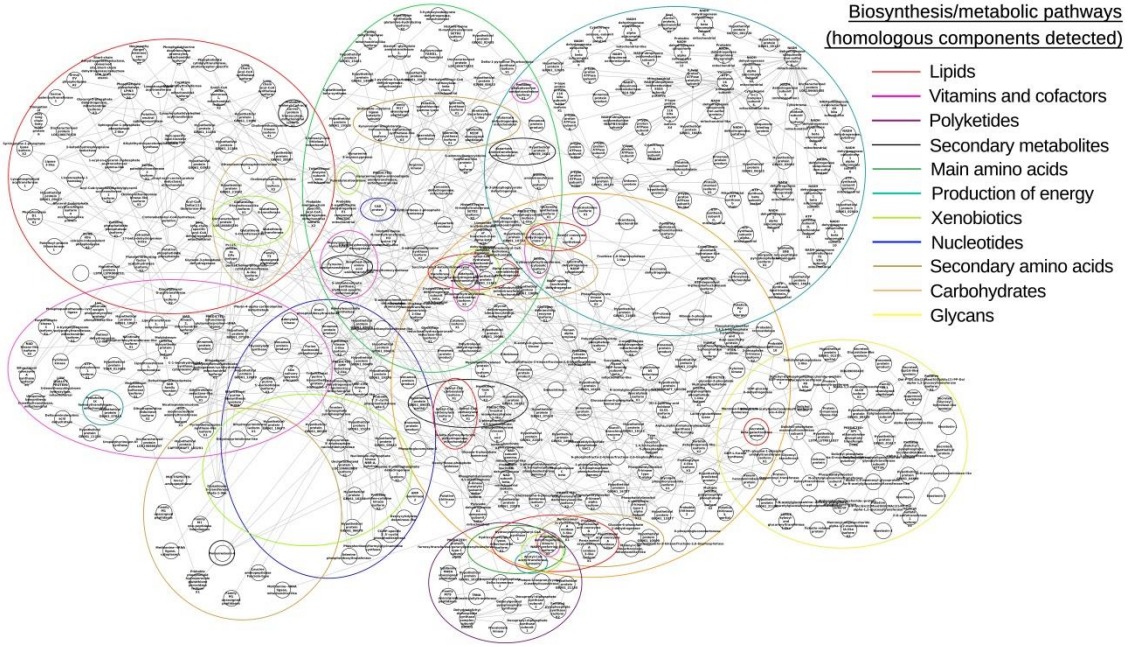
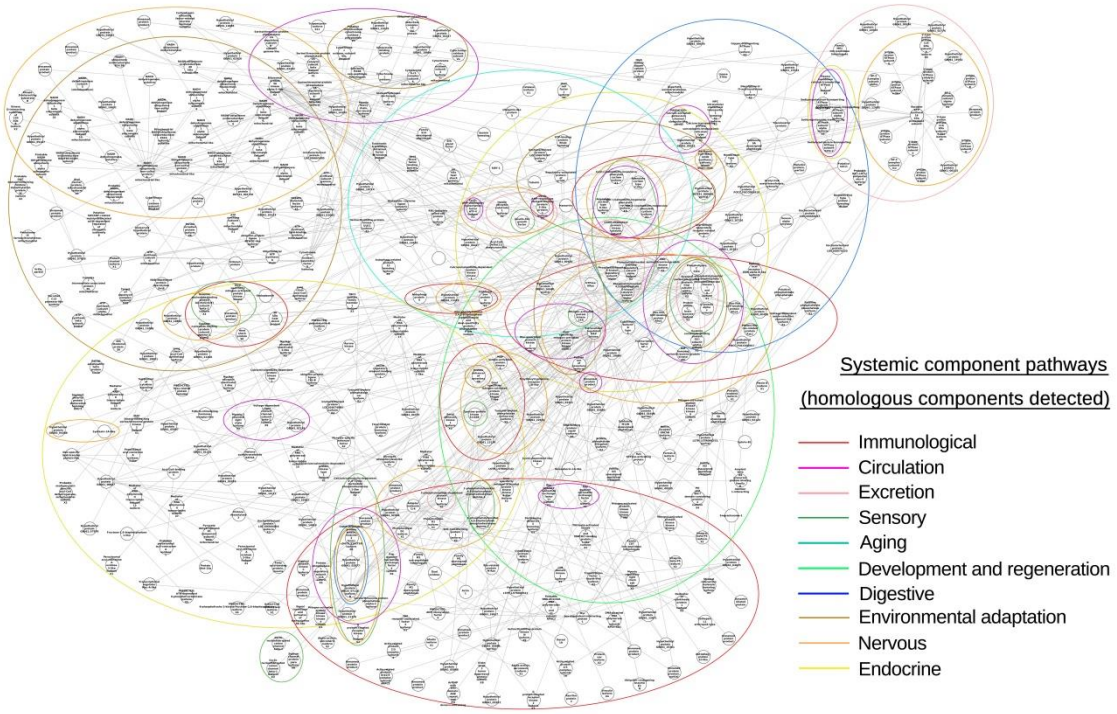

Lipocalin Al-4 precursor
Lipocalin Al-7 precursor
Lipocalin Al-6 precursor
Lipocalin Al-5 precursor
Lipocalin-like protein 4
Lipocalin-like protein 2
Lipocalin-like protein 3
Lipocalin-like protein 1
Nitrophorin 3 Precursor
Nitrophorin 1 Precursor
Nitrophorin 2 Precursor
Nitrophorin 3
Nitrophorin 2
Nitrophorin 3B
Nitrophorin 4B Precursor
Nitrophorin 1A Precursor
Pallidipin-like lipocalin 2 precursor
Pallidipin-like lipocalin 1 precursor
Procalin-like lipocalin 1 precursor
Protein yellow-like
Triabin-like lipocalin 2 precursor
Venom triabin-like protein 1
Triabin-like lipocalin 1 precursor
Triabin-like lipocalin 4 precursor
Triabin-like protein 3
6
4
2
0
-2
-4
Log2(FC)
FE
FE+Tc
2 days
FE+Tc
9 days

**Figure 12.**

(A)


Biosynthesis/metabolic pathways
(homologous components detected)
Lipids
Vitamins and cofactors
Polyketides
Secondary metabolites
Main amino acids
Production of energy
Xenobiotics
Nucleotides
Secondary amino acids
Carbohydrates
Glycans

(B)


Systemic component pathways
(homologous components detected)
Immunological
Circulation
Excretion
Sensory
Aging
Development and regeneration
Digestive
Environmental adaptation
Nervous
Endocrine

**APENDICE C - Comprovante de submissão**

Dear Dr Carvalho-Costa,

We are pleased to inform you that we have received the manuscript "Salivary and Intestinal Transcriptomes Reveal the Specific Proteins and Differential Gene Expression in Trypanosoma cruzi-Infected and Non-Infected Rhodnius neglectus" to be considered for publication in Frontiers in Cellular and Infection Microbiology, section Parasite and Host.

You can access the review forum and track the progress of your manuscript using the following link:
https://www.frontiersin.org//Journal/MySubmission.aspx?stage=100

Your manuscript is now in the initial validation stage to determine its suitability for peer review. Should your manuscript be sent out for peer review, you will receive a notification once we receive the reports from reviewers and the interactive review forum is activated. You will then be able to read the review reports and exchange directly with the reviewers in the interactive review forum as well as submit a revised manuscript, if appropriate. If the required number of reviewers endorse your manuscript in the Independent Review stage, their tabs will be closed and the manuscript will be forwarded to the Review Finalized stage, where you will be able to interact with the handling editor via the Editor tab.

Best regards,

Your Frontiers in Cellular and Infection Microbiology team

Frontiers | Editorial Office - Collaborative Peer Review Team
www.frontiersin.org
Avenue du Tribunal Fédéral 34, 1005 Lausanne, Switzerland
Office T 41 21 510 17 40

For technical issues, please contact our IT Helpdesk (support@frontiersin.org) or visit our Frontiers Help Center (zendesk.frontiersin.org/hc/en-us)

----------MANUSCRIPT DETAILS----------

Manuscript title: Salivary and Intestinal Transcriptomes Reveal the Specific Proteins and Differential Gene Expression in Trypanosoma cruzi-Infected and Non-Infected Rhodnius neglectus

Manuscript ID: 773357

Authors: Tamires Marielem Carvalho-Costa, Rafael Destro Rosa Tiveron, Maria Tays Mendes, Cecília Gomes Barbosa, Jessica Coraiola Nevoa, Guilherme Augusto Roza, Marcos Vinicius Da Silva, Virmondes Rodrigues, Siomar De Castro Soares and Carlo José Freire Oliveira

Journal: Frontiers in Cellular and Infection Microbiology, section Parasite and Host

Article type: Original Research

Submitted on: 09 Sep 2021

Research Topic: The Potential Role of Bioactive Salivary Molecules from Hematophagous Arthropods as Immunopharmacological Tools on Vector-Borne Infections and Noninfectious Diseases

----------ADDITIONAL INFORMATION----------

In order to enable a smooth and efficient review process, please familiarize yourself with the Frontiers review guidelines: https://www.frontiersin.org/Journal/ReviewGuidelines.aspx?s=1861&name=parasite_and_host

To take part in the Resource Identification Initiative please cite antibodies, genetically modified organisms, software tools, data, databases and services using the corresponding catalog number and RRID in the text of your article. Please see here for more information: https://www.frontiersin.org//files/pdf/letter_to_author.pdf

You are receiving this email regarding ongoing activities you have with Frontiers. If you think this was wrongly sent to you, please contact our support team at support@frontiersin.org

# APENDICE D

## Contribuições dos Autores

Todos os autores estiveram envolvidos na concepção do estudo.

Tamires Marielem de Carvalho Costa, Carlo Jose Freire Oliveira e Rafael Destro Rosa Tiveron estiveram envolvidos no desenho do estudo, analisaram os dados e escreveram o manuscrito.

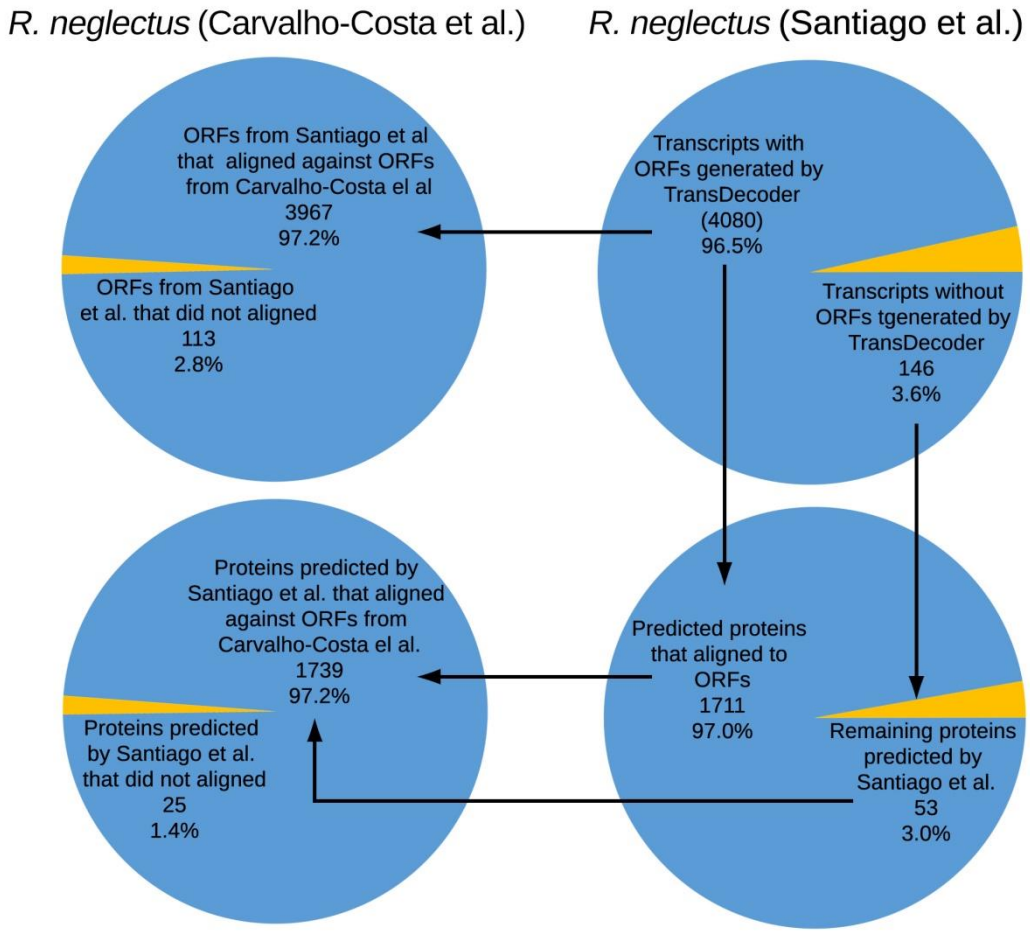
Tamires Marielem de Carvalho Costa, Cecília Gomes Barbosa, Jessica Coraiola Nevoa e Guilherme Augusto Roza realizaram os experimentos.

Maria Tays Mendes, Marcos Vinícius Silva, Virmondes Rodrigues, Carlo Jose Freire Oliveira, Siomar de Castro Soares participaram da redação do manuscrito.

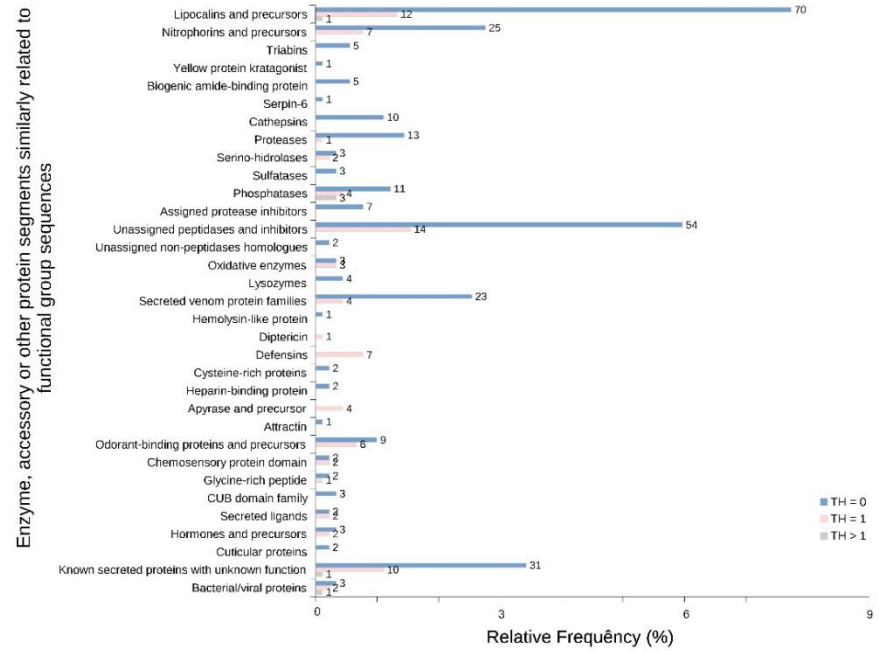
Todos os autores comentaram o manuscrito, leram e aprovaram a versão final.

# APENDICE E - Material suplementar

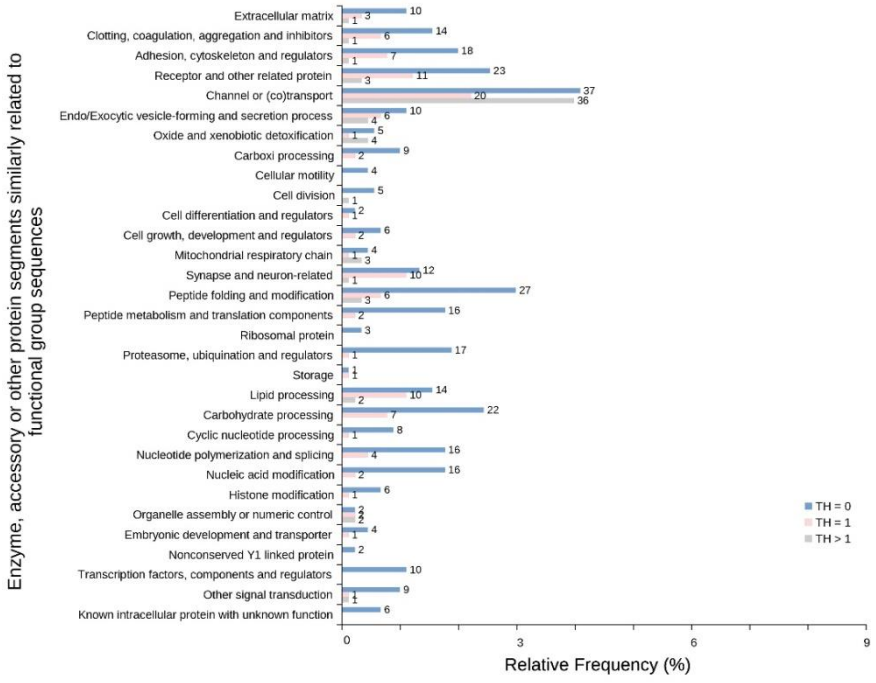
## 1 Supplementary Figures




**Supplementary Figure 1. Alignment between TransDecoder-generated ORFs and predicted protein sequences from *R. neglectus.*** 4080 proteins were predicted by Santiago et al. (2016). Of these, 97.2% were also found in our work.

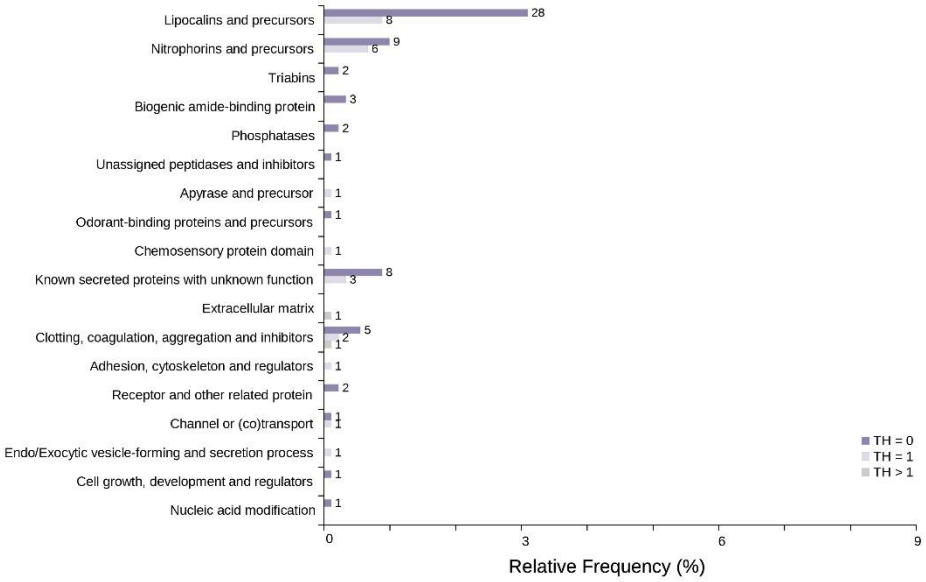



(A)

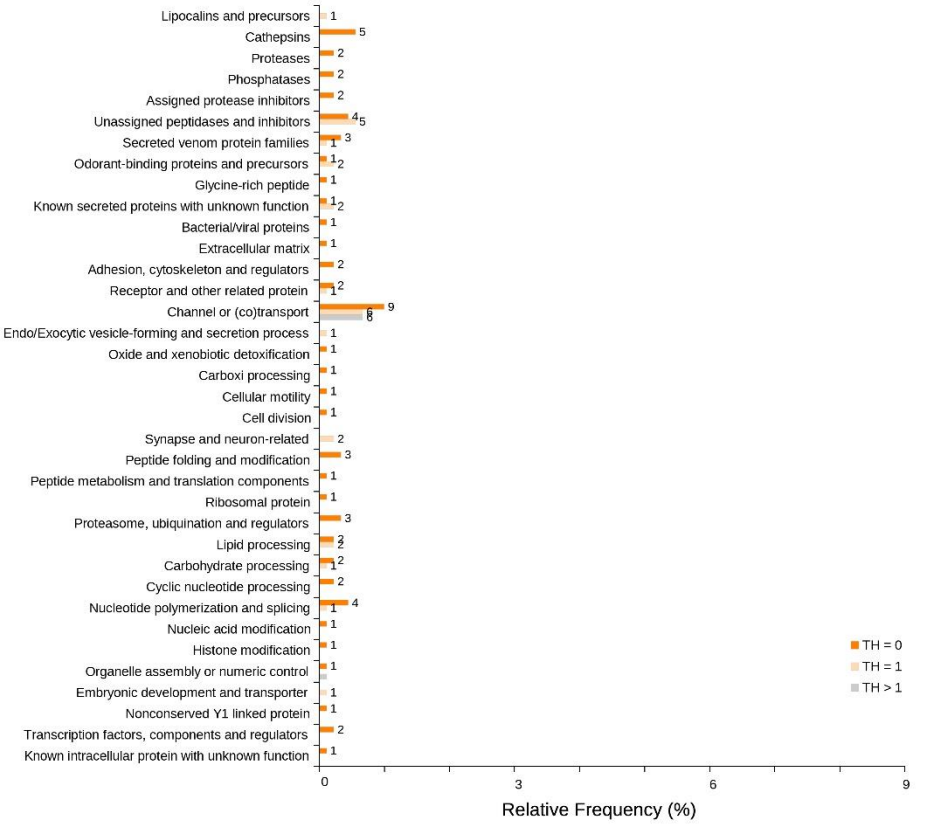



**(B)**

**Supplementary Figure 2. Transcripts of known proteins SP+ in salivary gland secretomes and intestine.** (**A**) Classes of proteins commonly present in arthropod secretomes. (**B**) Functional groups with probable secretable molecules (or of poorly specific prediction). *TH: number of transmembrane helices

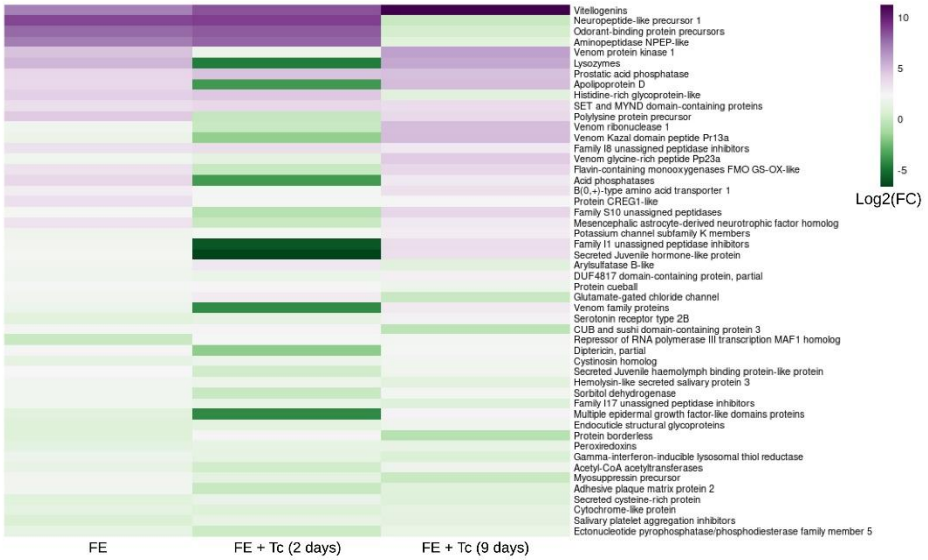



(A)

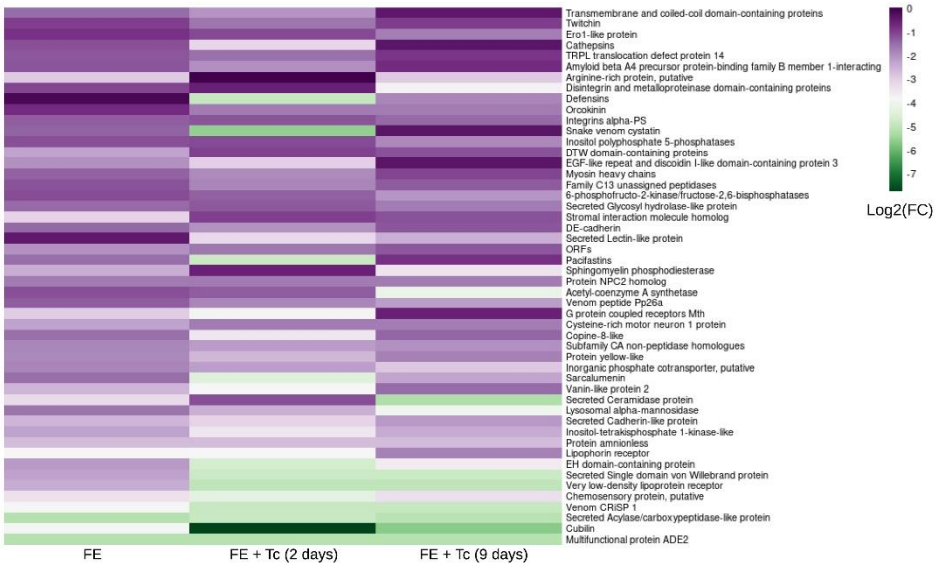



(B)

**Supplementary Figure 3. Transcripts of known proteins SP+ present exclusively in each tissue type.** (**A**) Present in the SGs and not in the INT. (**B**) Present in the INT and not in the SGs. *TH: number of transmembrane helices

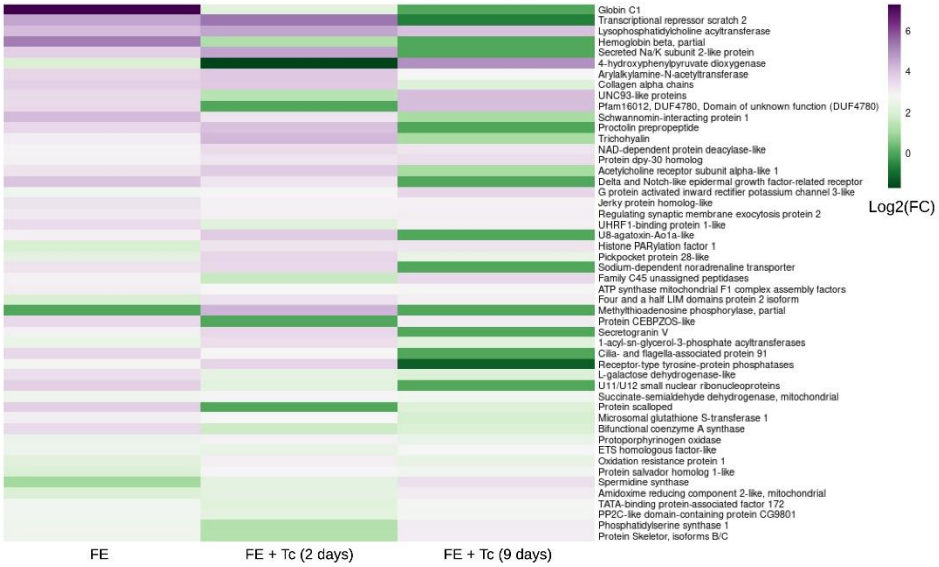



(A)

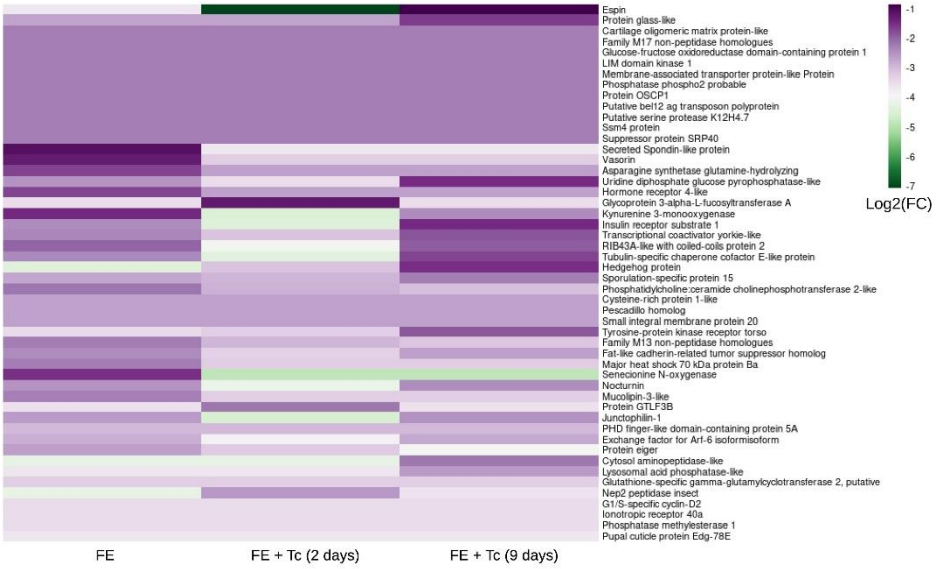



(B)

**Supplementary Figure 4. Transcripts translatable into predicted protein as secreted in the salivary gland.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition regarding fasting. *FC: fold change.

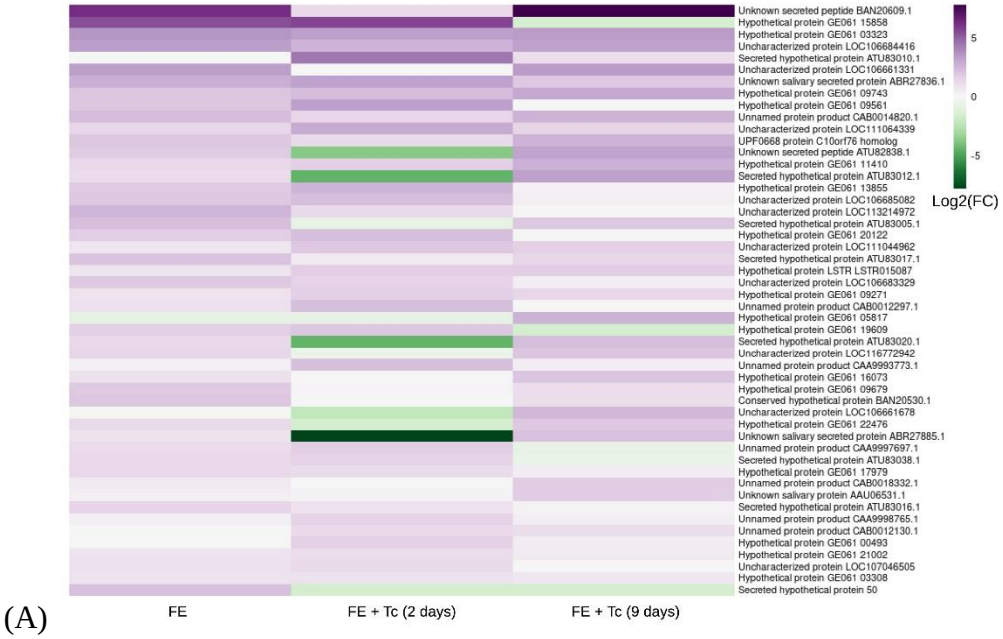



(A)

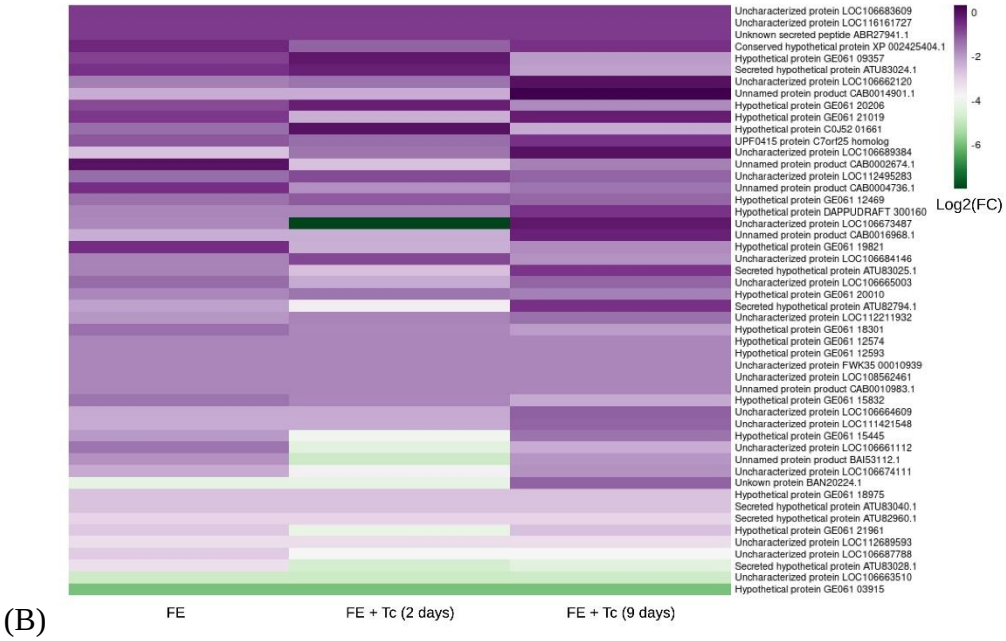



(B)

**Supplementary Figure 5. Transcripts translatable into housekeeping proteins in salivary gland.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

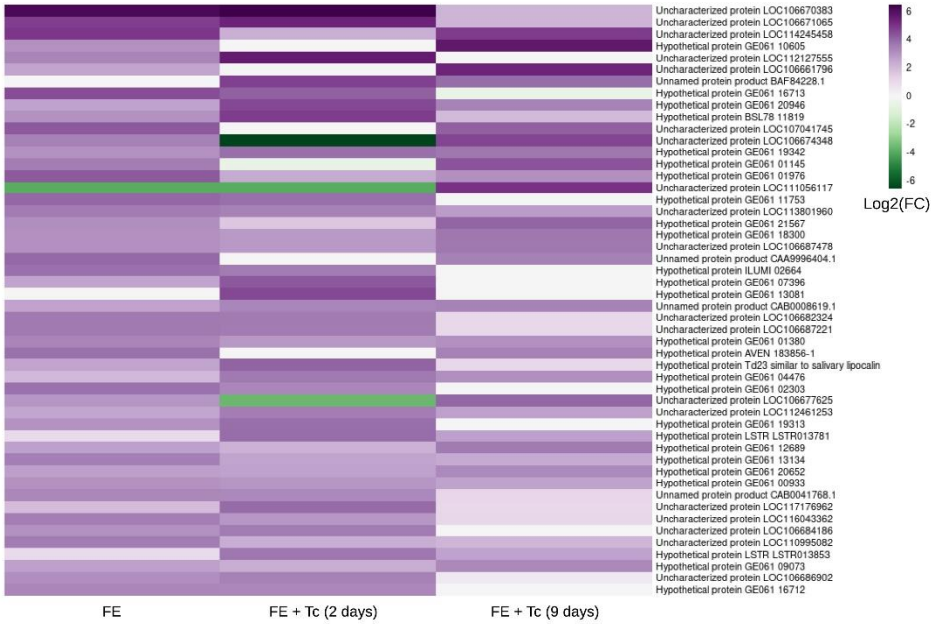



(A)

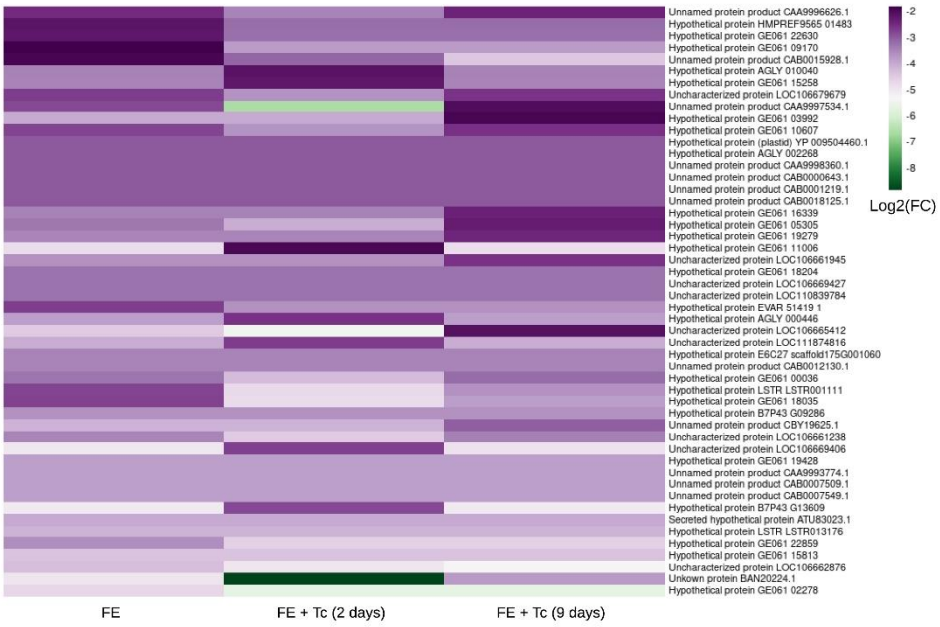



(B)

**Supplementary Figure 6. Transcripts translatable into hypothetical/unknown secretable in the salivary gland.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

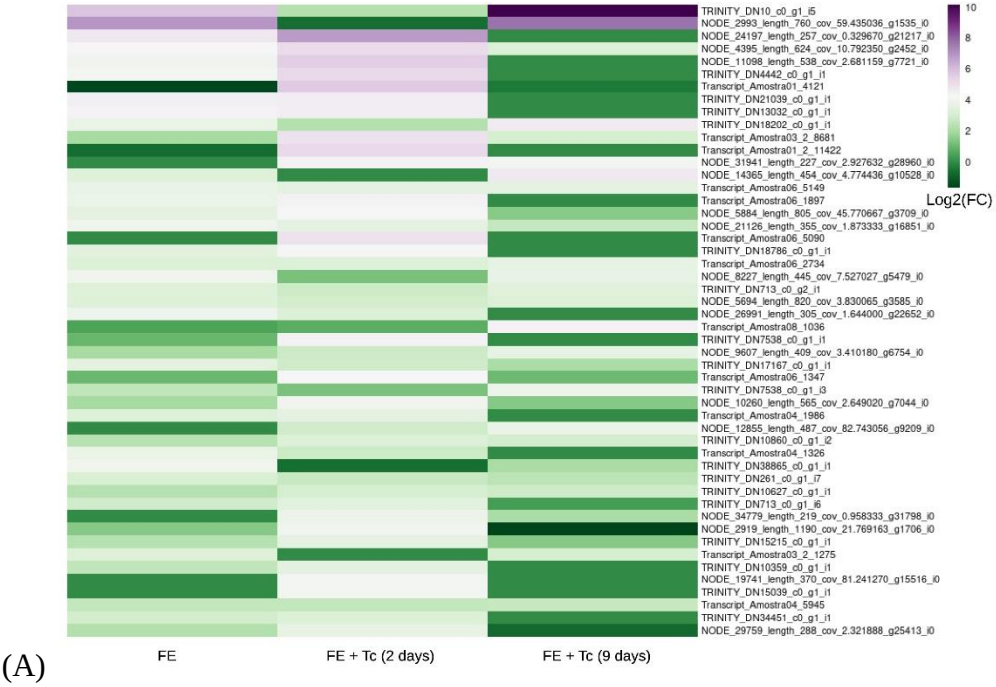



(A)

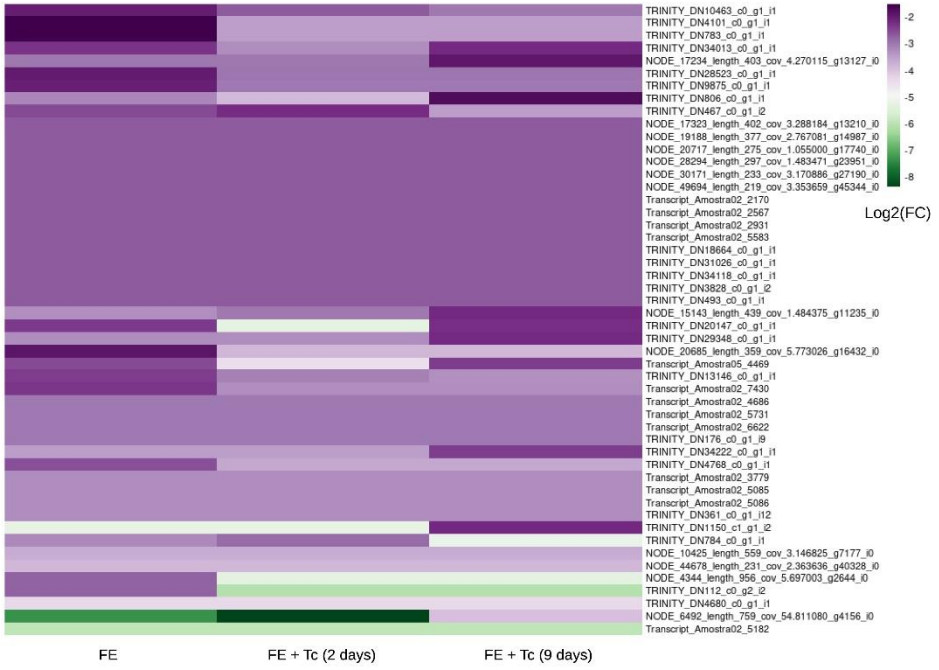



(B)

**Supplementary Figure 7. Transcripts translatable into hypothetical/unknown non-secretable proteins in the salivary gland.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

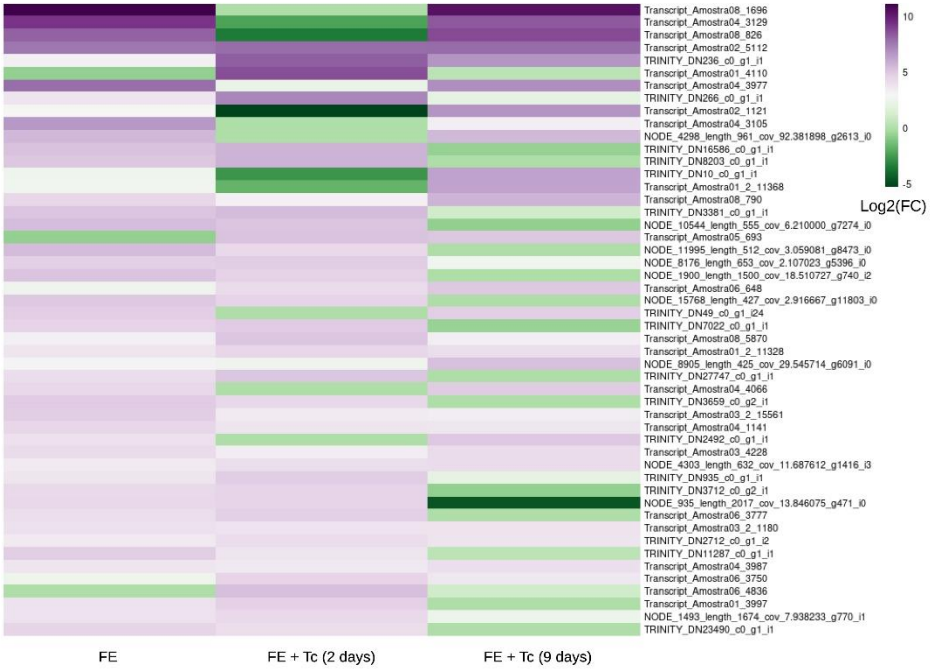



(A)

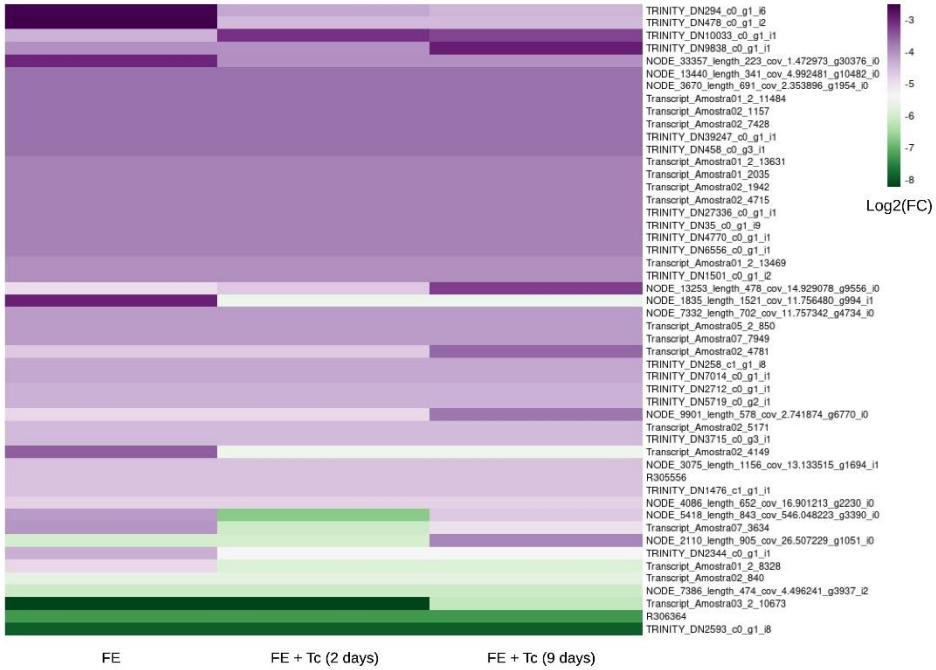



(B)

**Supplementary Figure 8. Unmatched transcripts translatable into secretable proteins in the salivary gland.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

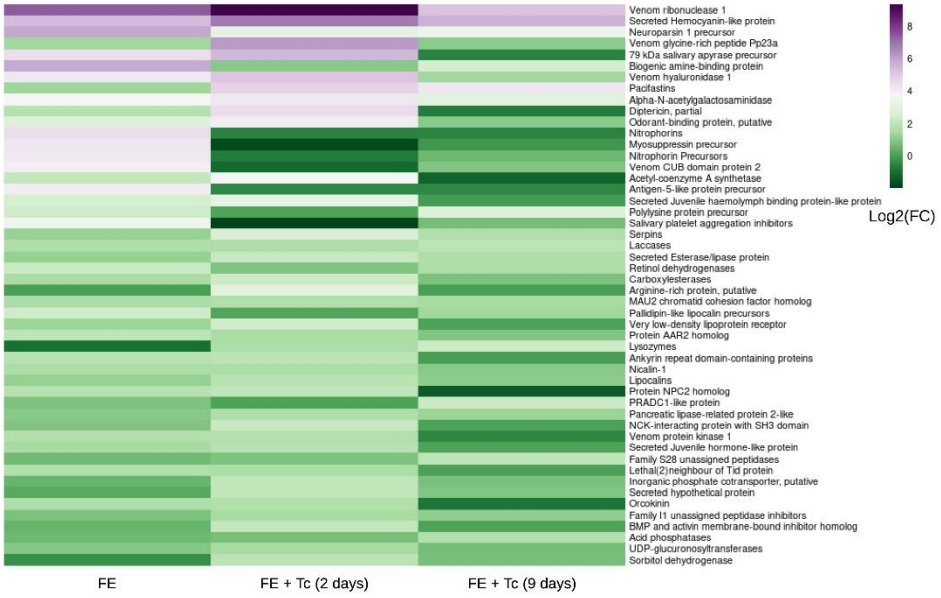



(A)

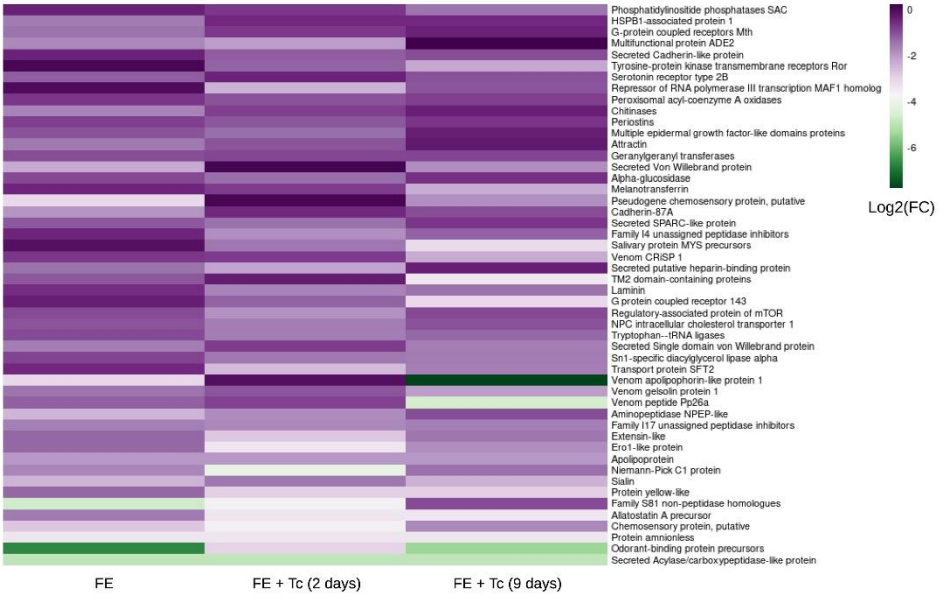



(B)

**Supplementary Figure 9. Unmatched transcripts translatable into non-secretable proteins in the salivary gland.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

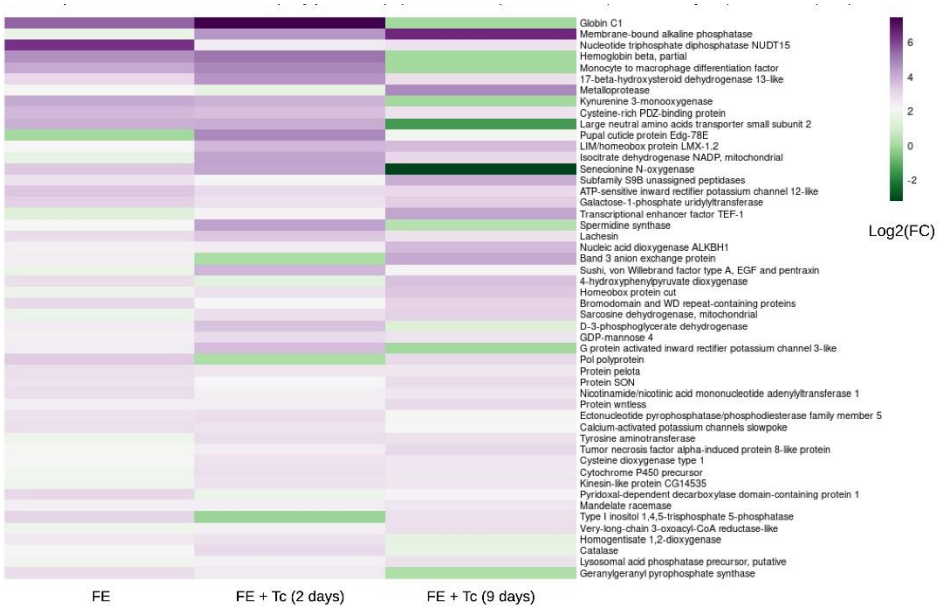



(A)

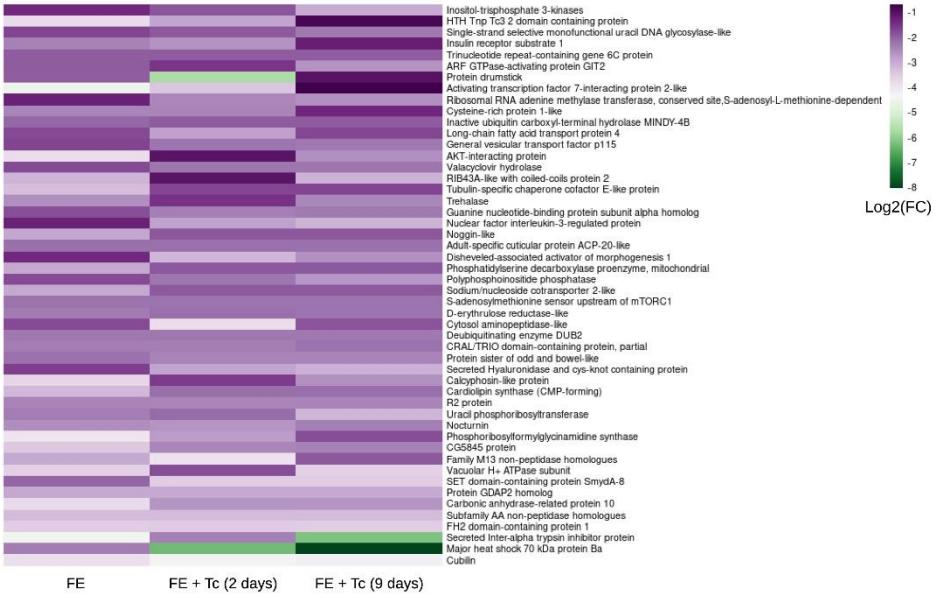



(B)

**Supplementary Figure 10. Transcripts translatable into protein predicted as secreted in the intestine.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

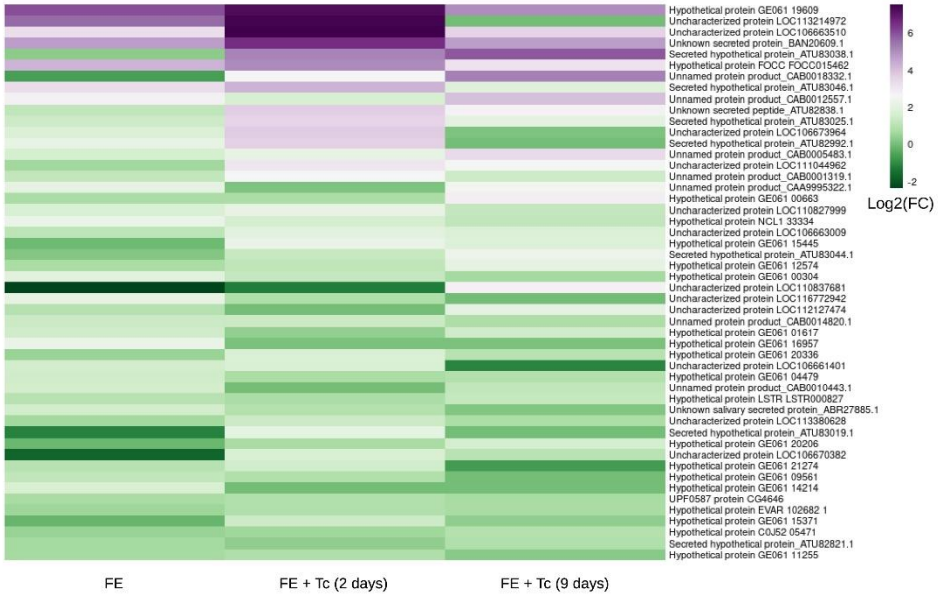



(A)

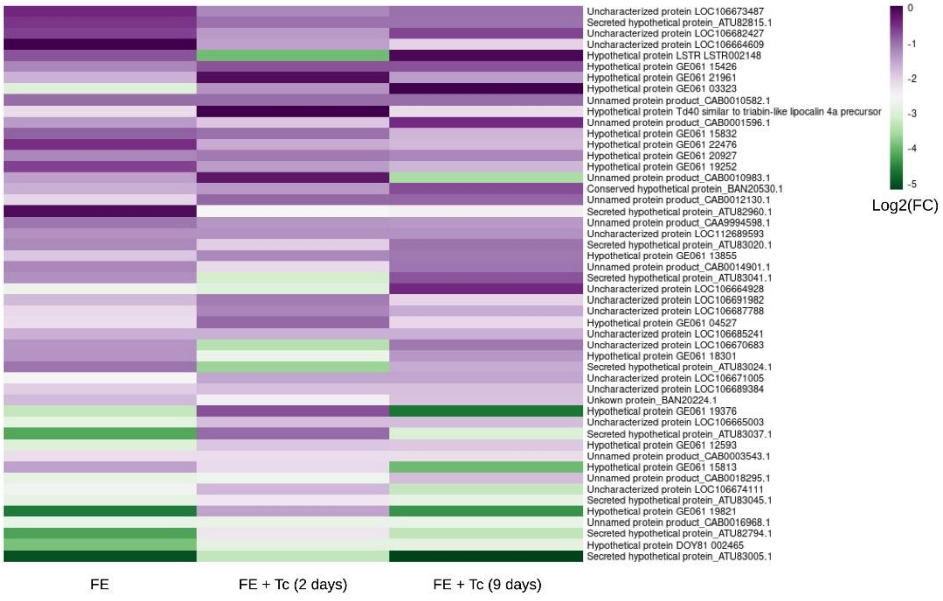



(B)

**Supplementary Figure 11. Transcripts translatable into housekeeping proteins in intestine.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

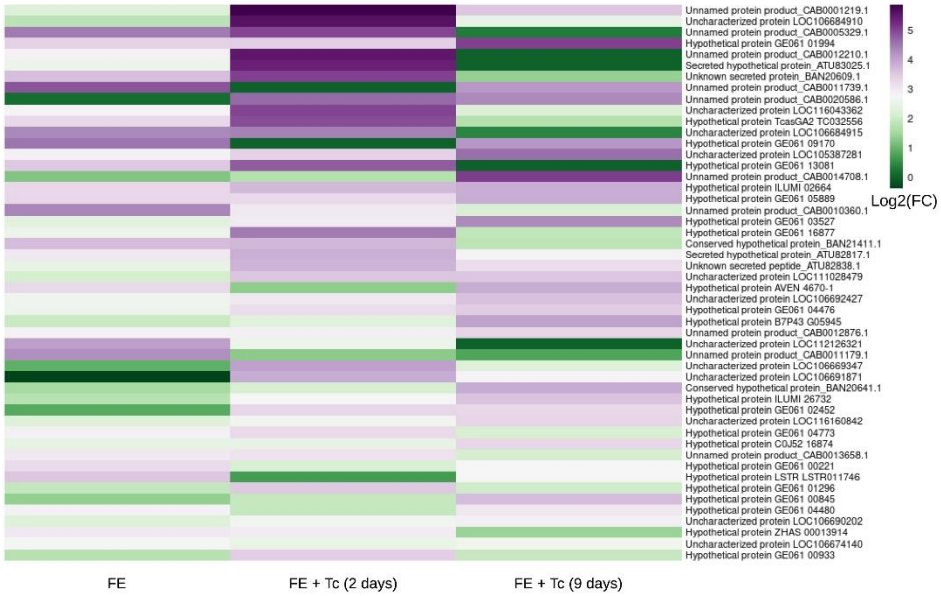



(A)

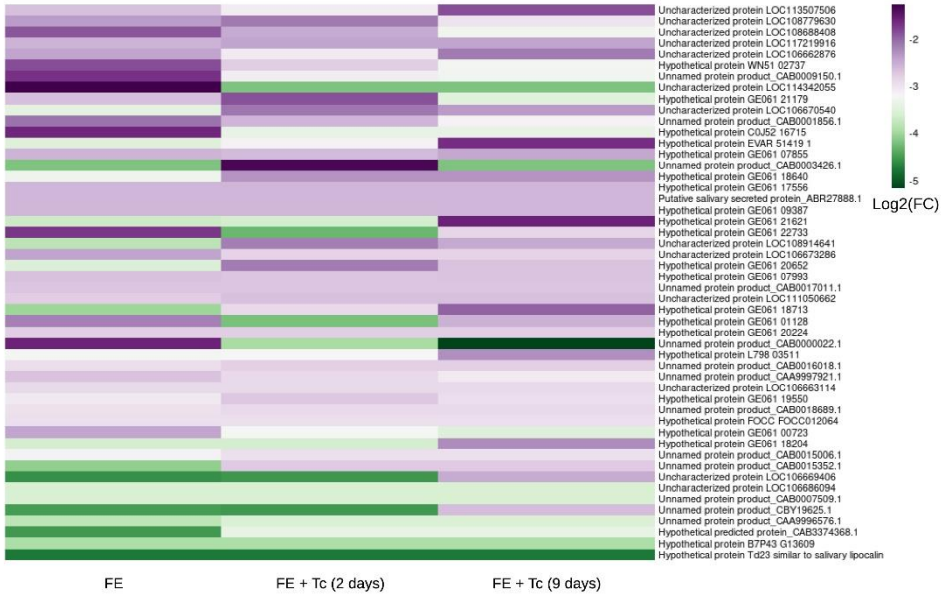



(B)

**Supplementary Figure 12. Transcripts translatable into hypothetical/unknown secretable in the intestine.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

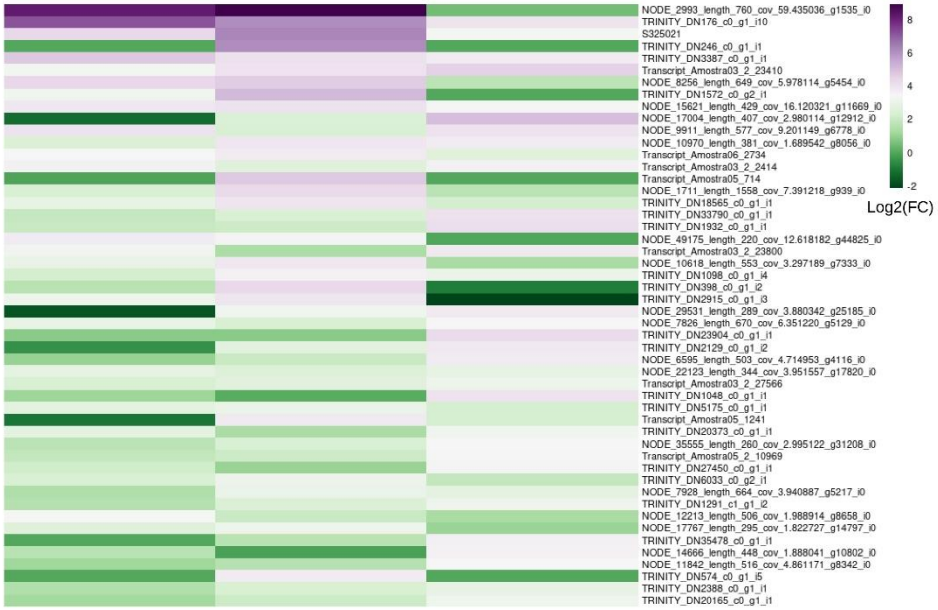



(A)

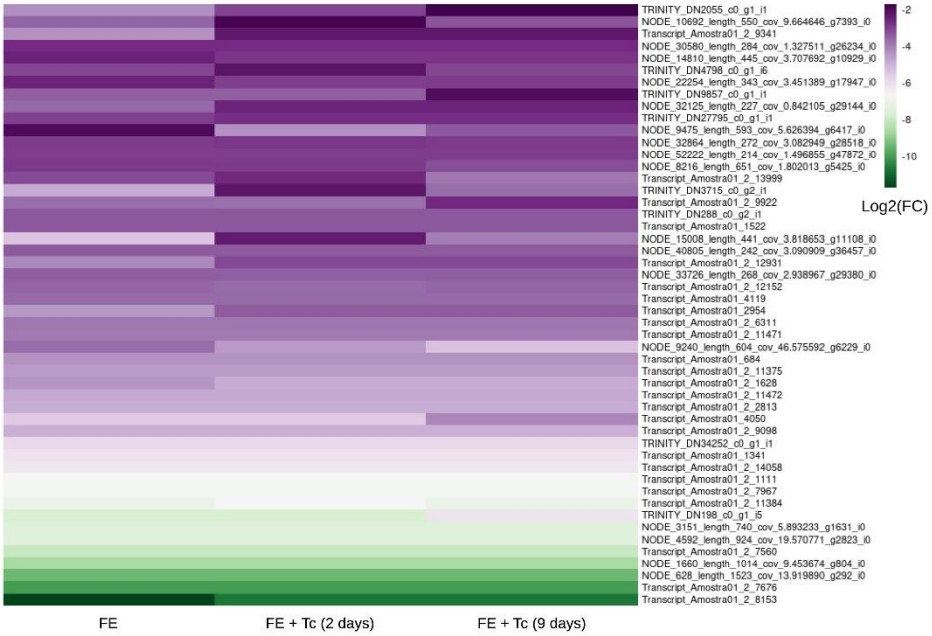



(B)

**Supplementary Figure 13. Transcripts translatable in hypothetical/unknown non-secretable proteins in the intestine.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

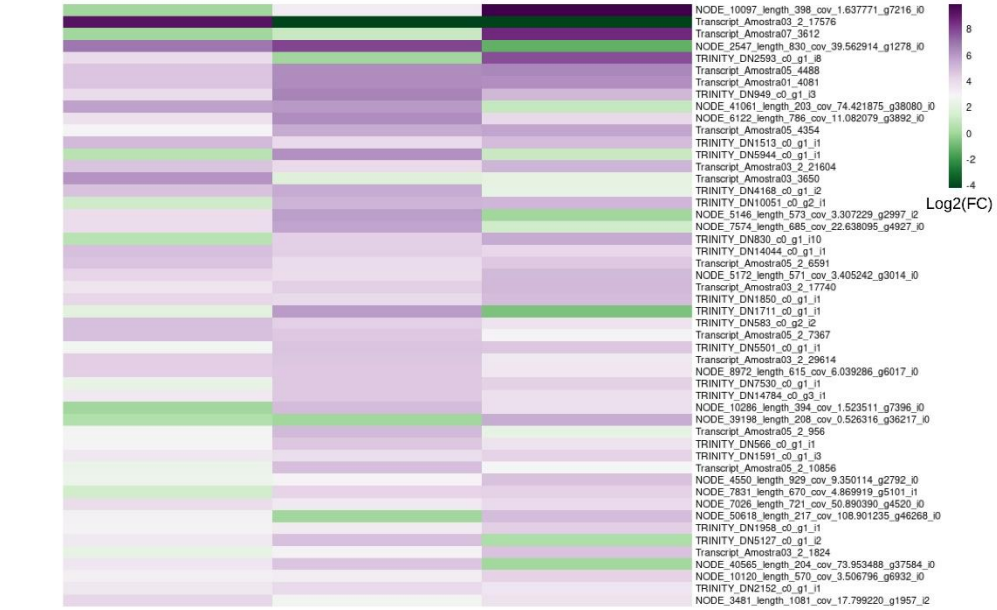



(A)

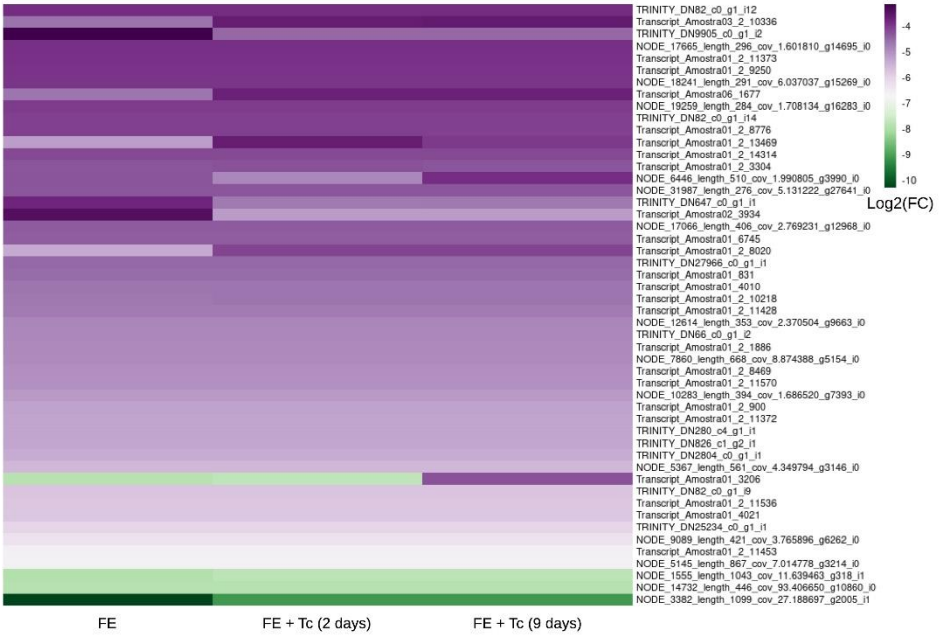



(B)

**Supplementary Figure 14. Unmatched transcripts translatable in secretable proteins in the intestine.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.

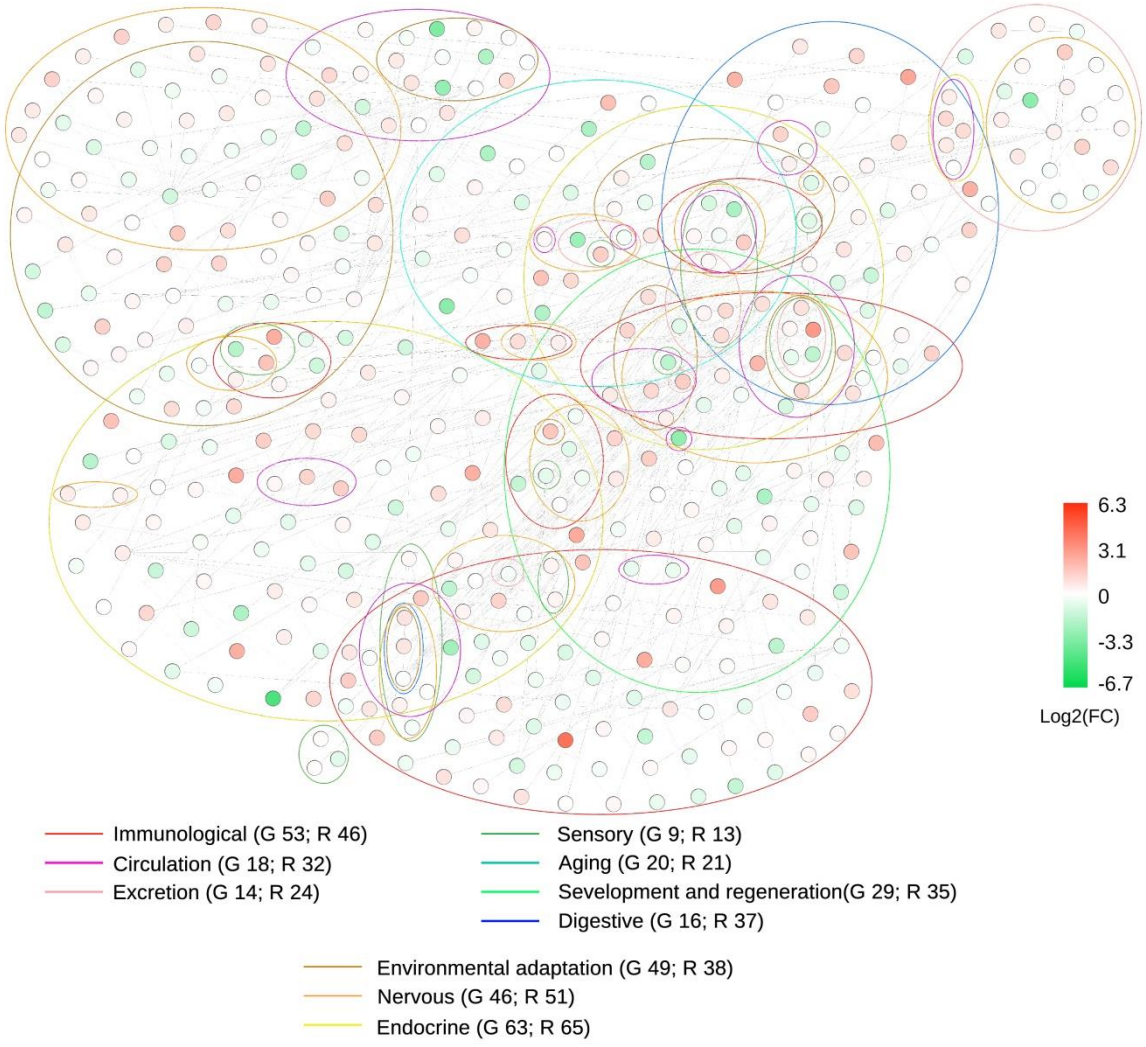



(A)

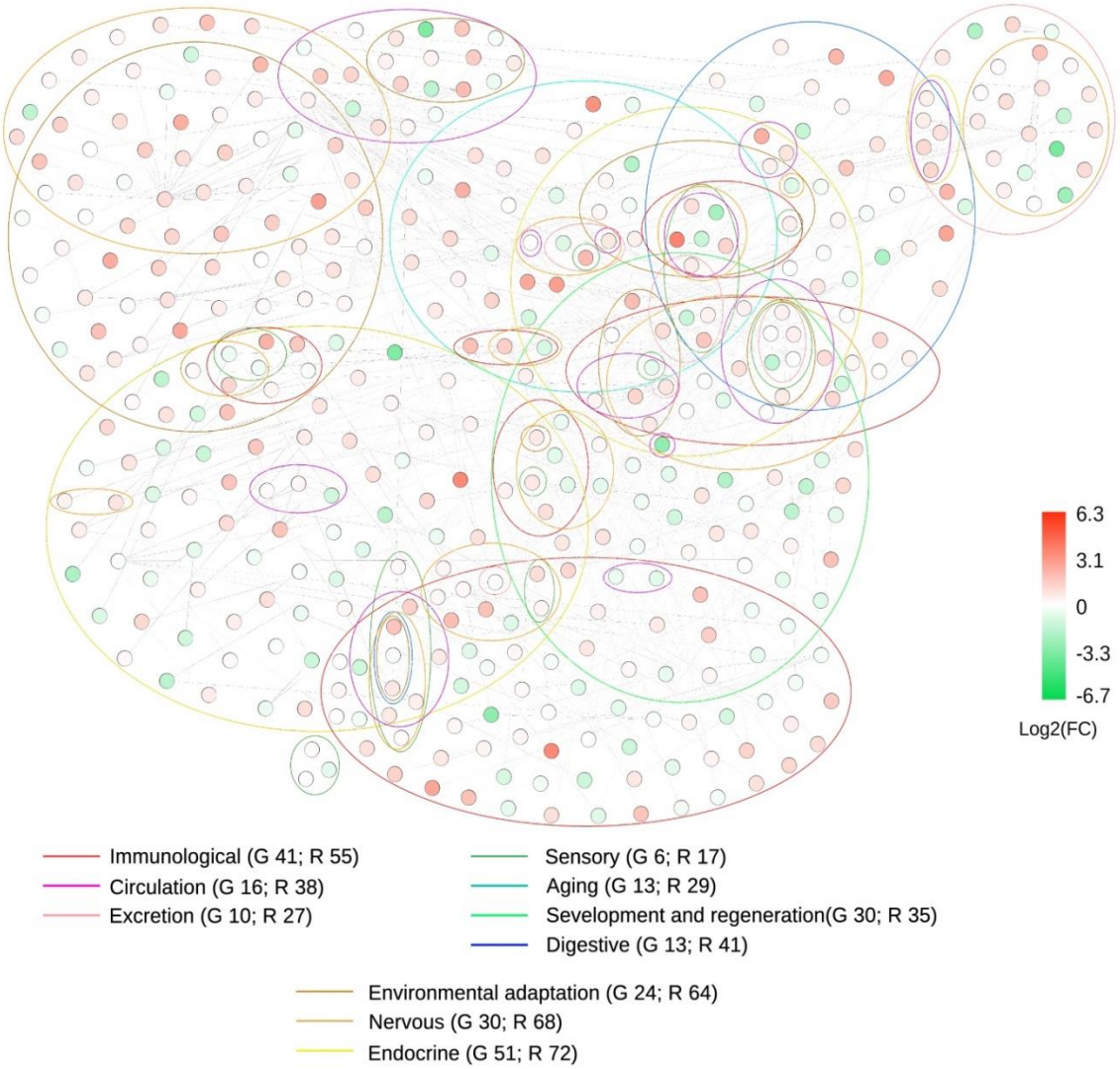



(B)

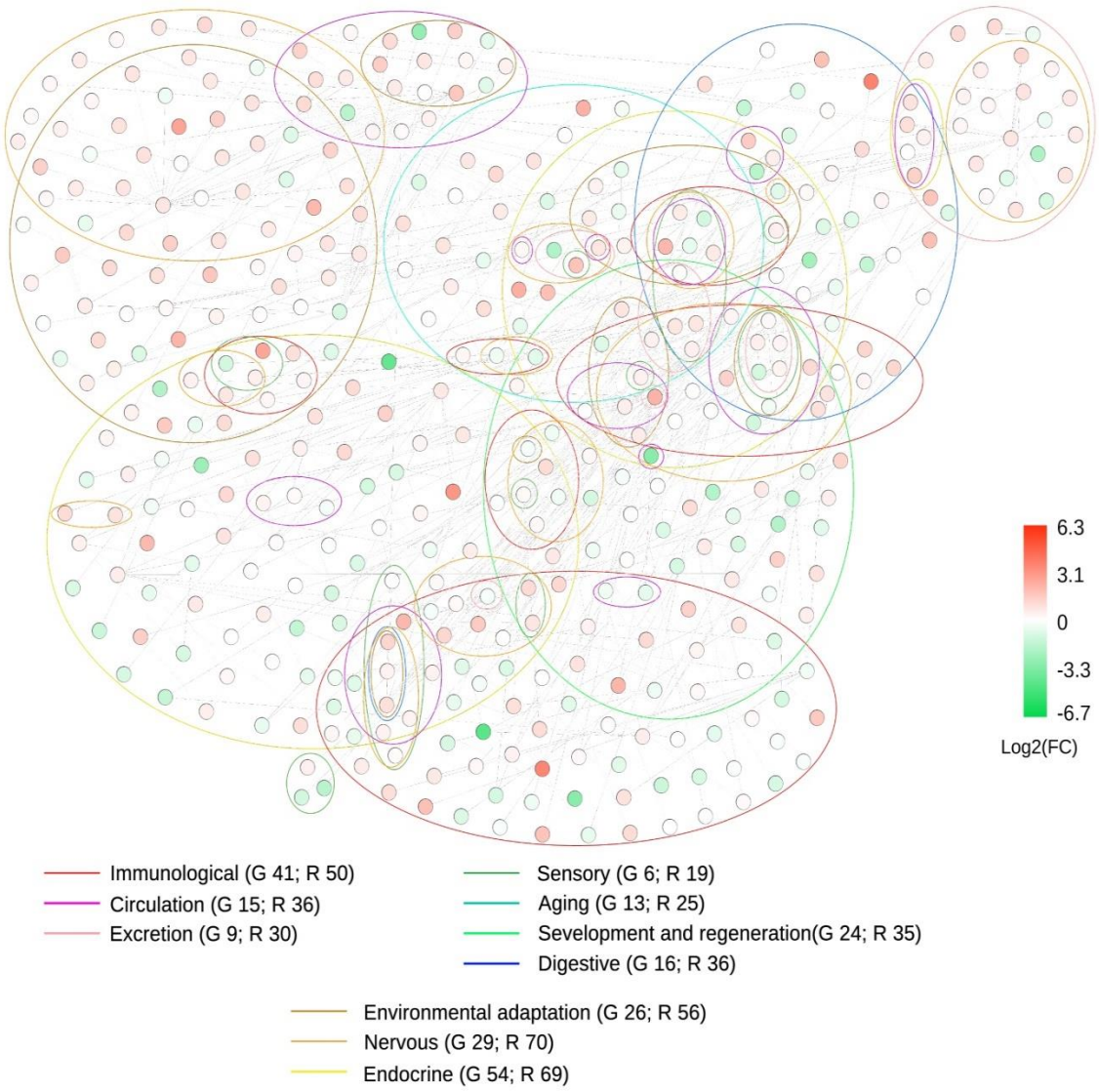
**Supplementary Figure 15. Unmatched transcripts translatable in non-secretable proteins in the intestine.** (**A**) Top 50 upregulated transcript clusters per condition in relation to fasting; (**B**) Top 50 downregulated transcript clusters per condition in relation to fasting. *FC: fold change.


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 53; R 46)
Circulation (G 18; R 32)
Excretion (G 14; R 24)
Sensory (G 9; R 13)
Aging (G 20; R 21)
Sevelopment and regeneration(G 29; R 35)
Digestive (G 16; R 37)
Environmental adaptation (G 49; R 38)
Nervous (G 46; R 51)
Endocrine (G 63; R 65)

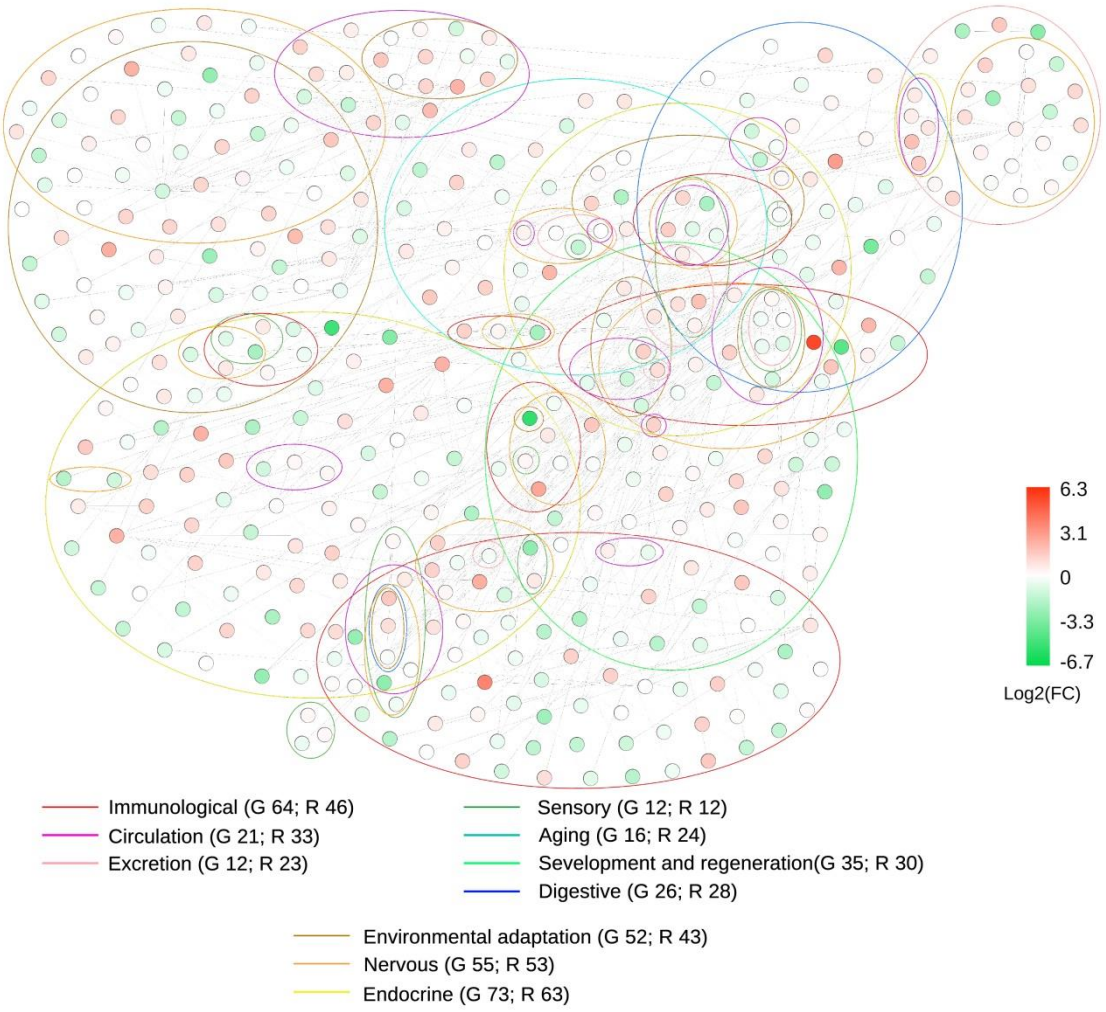
AI


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 41; R 55)
Circulation (G 16; R 38)
Excretion (G 10; R 27)
Sensory (G 6; R 17)
Aging (G 13; R 29)
Sevelopment and regeneration(G 30; R 35)
Digestive (G 13; R 41)
Environmental adaptation (G 24; R 64)
Nervous (G 30; R 68)
Endocrine (G 51; R 72)

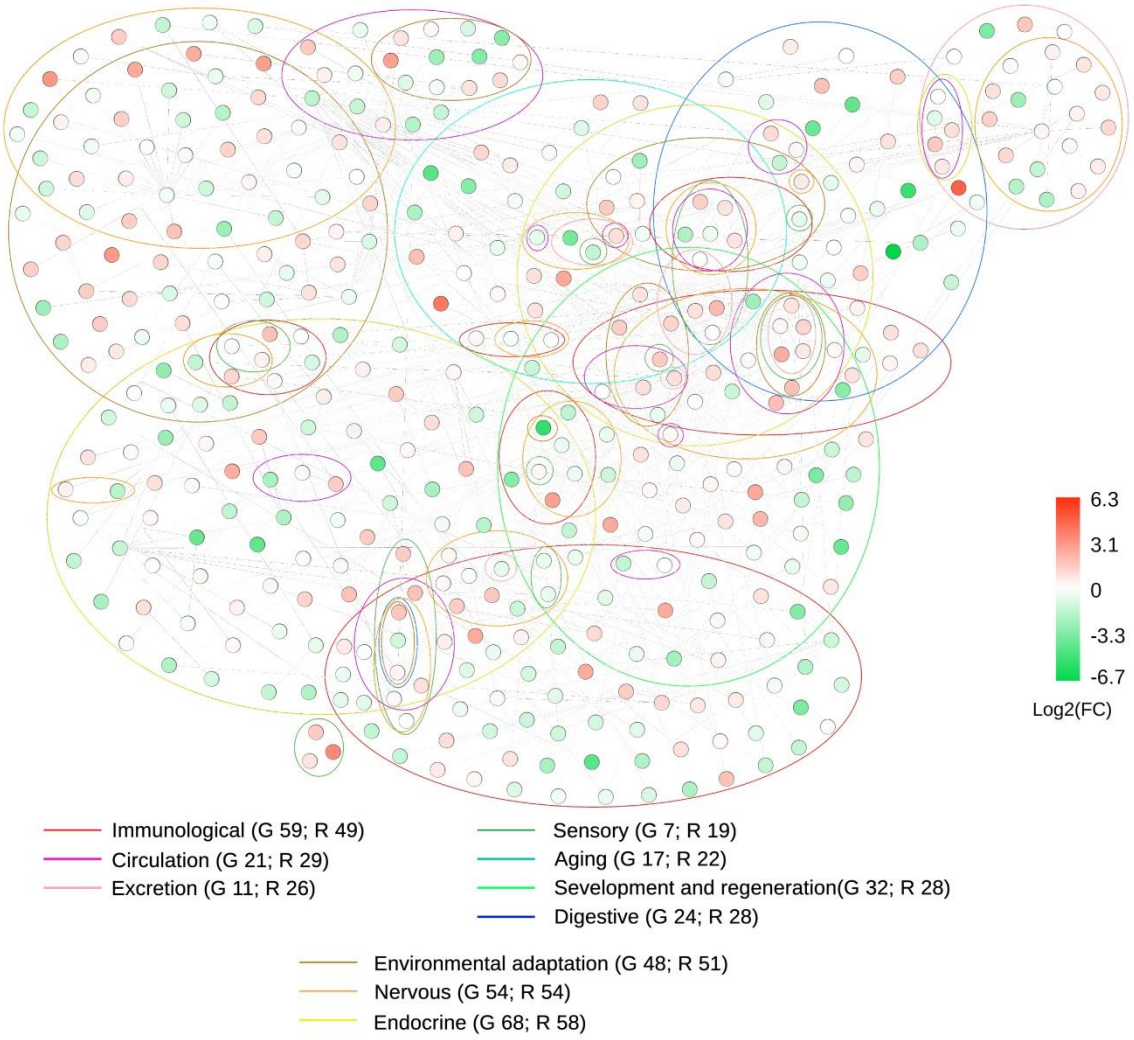
AII


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 41; R 50)
Circulation (G 15; R 36)
Excretion (G 9; R 30)
Sensory (G 6; R 19)
Aging (G 13; R 25)
Sevelopment and regeneration(G 24; R 35)
Digestive (G 16; R 36)
Environmental adaptation (G 26; R 56)
Nervous (G 29; R 70)
Endocrine (G 54; R 69)

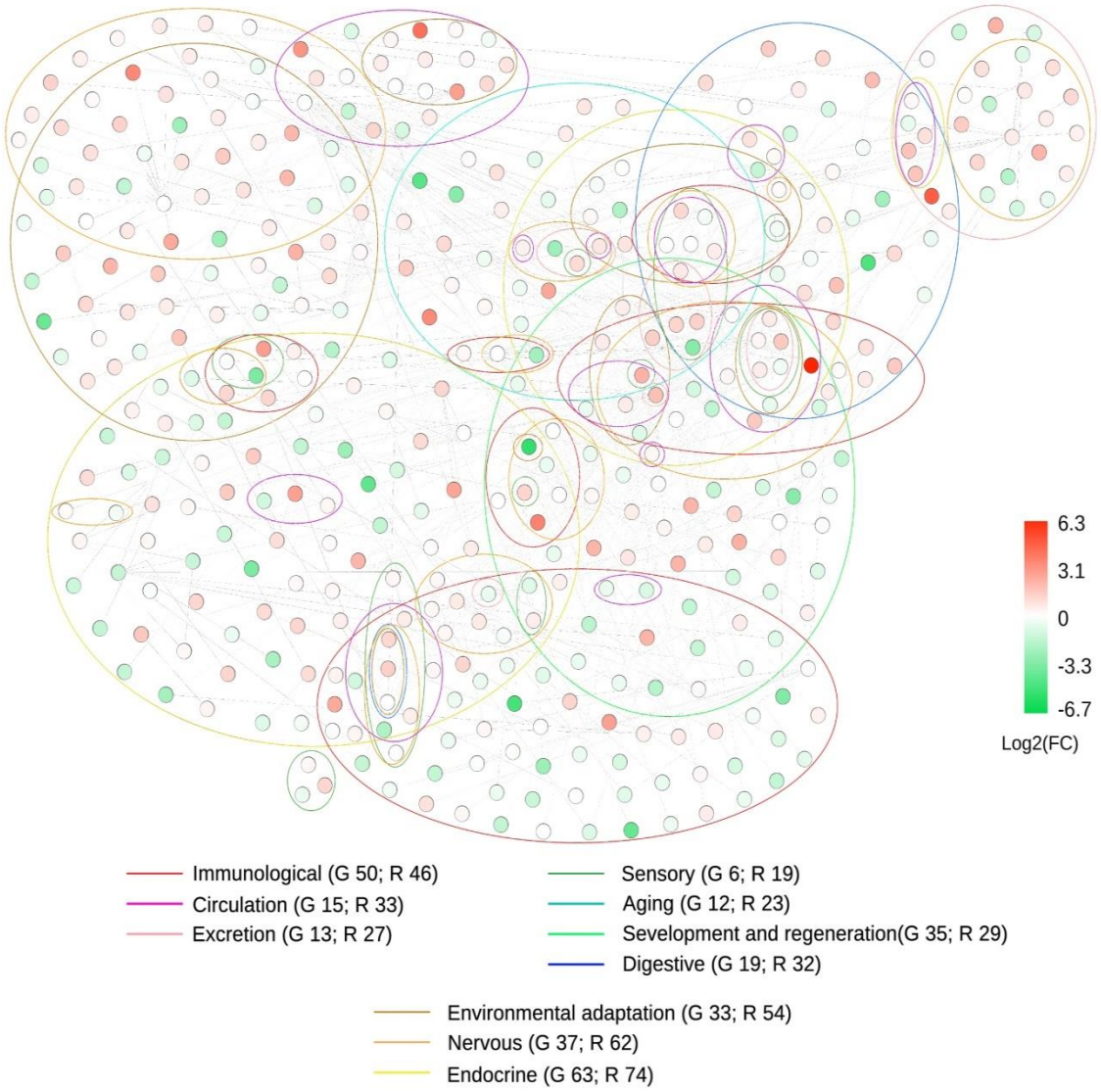
AIII


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 64; R 46)
Circulation (G 21; R 33)
Excretion (G 12; R 23)
Sensory (G 12; R 12)
Aging (G 16; R 24)
Sevelopment and regeneration(G 35; R 30)
Digestive (G 26; R 28)
Environmental adaptation (G 52; R 43)
Nervous (G 55; R 53)
Endocrine (G 73; R 63)

BI

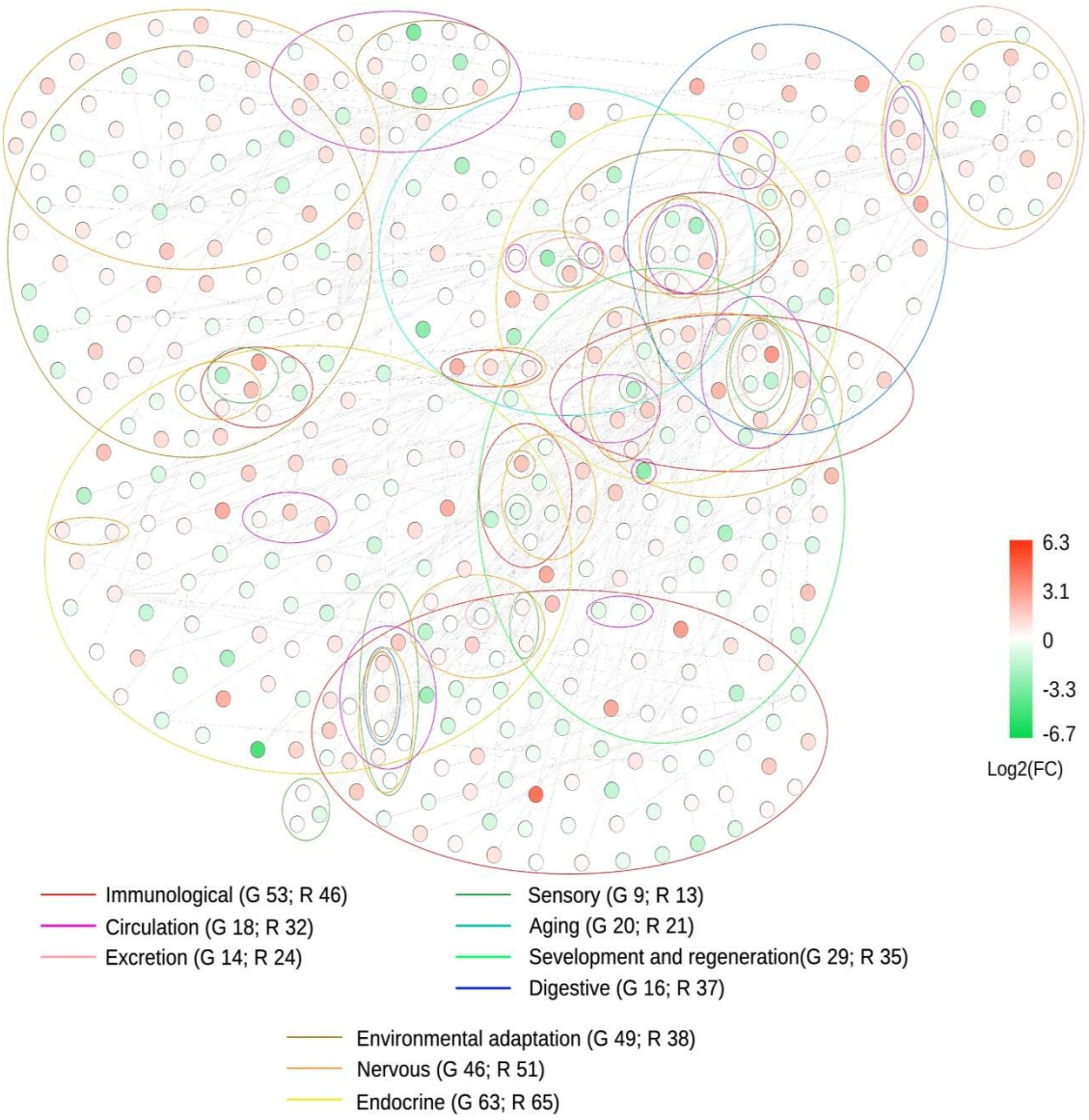

6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 59; R 49)
Circulation (G 21; R 29)
Excretion (G 11; R 26)
Sensory (G 7; R 19)
Aging (G 17; R 22)
Sevelopment and regeneration(G 32; R 28)
Digestive (G 24; R 28)
Environmental adaptation (G 48; R 51)
Nervous (G 54; R 54)
Endocrine (G 68; R 58)

BII

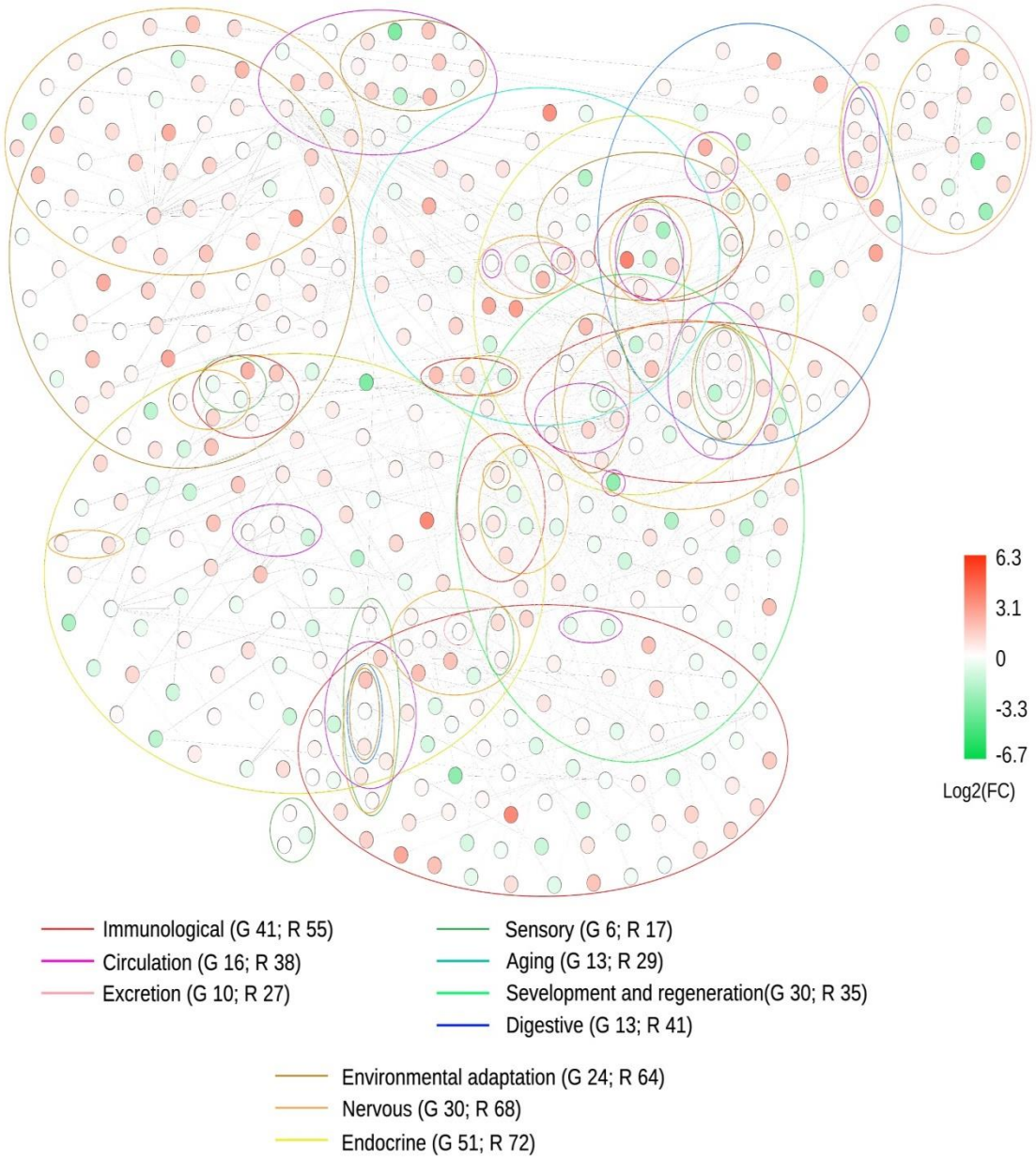



BIII

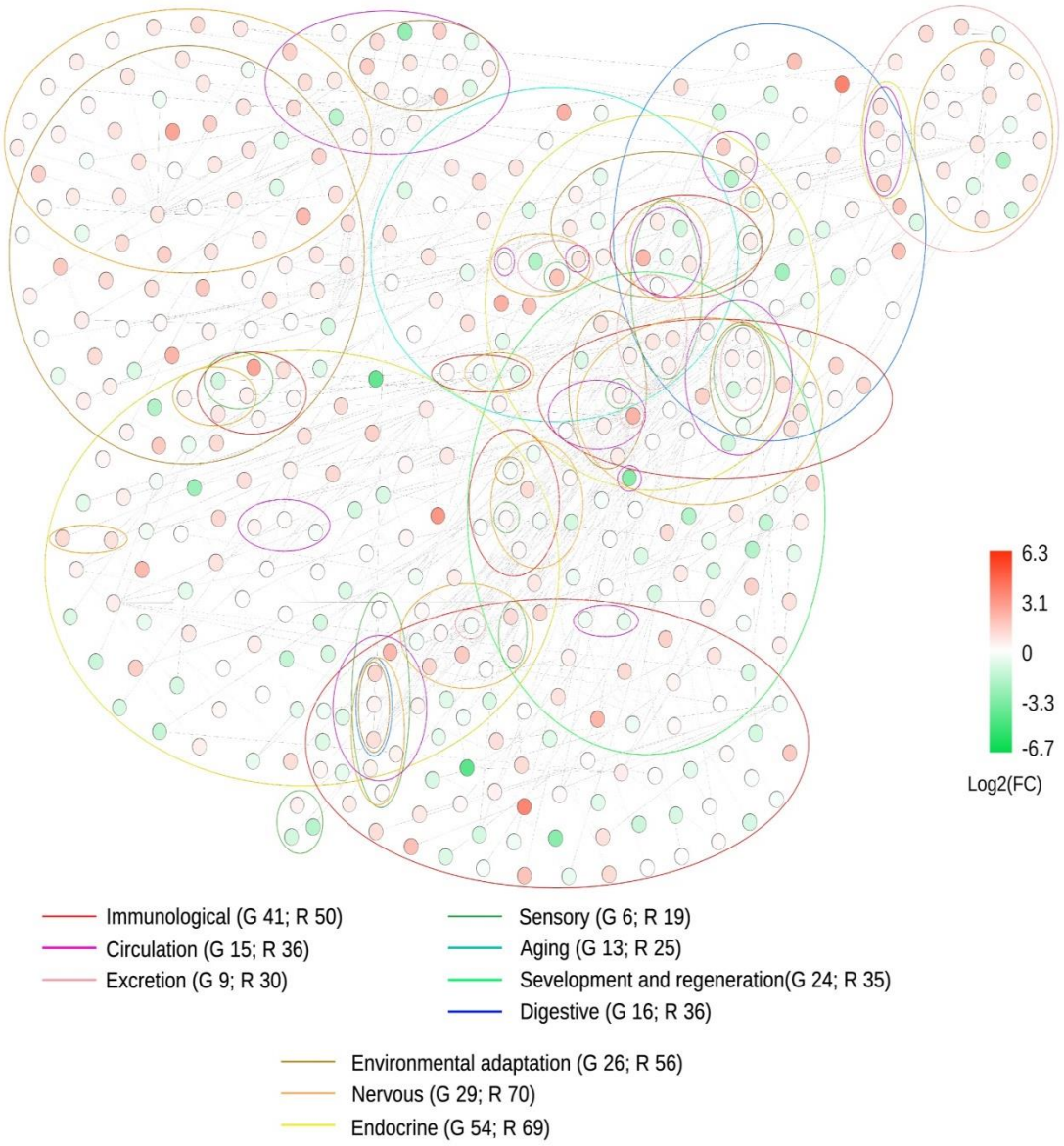
**Supplementary Figure 16. Differential expression of the biosynthesis and metabolism pathways of the biological network components present in analyzed tissues of *R. neglectus*.** Salivary gland: **A-I**. Fed / Fasting. **A-II**. Fed and infected 2 days/Fasting. **A-III.** Fed and infected 9 days/Fasting. Bowel: **B-I**. Fed / Fasting. **B-II**. Fed and infected 2 days/Fasting **B-III**. Fed and infected 9 days/Fasting. *G: green; A: red. FC: fold change. Identified homologous component clusters and with degree > 0.


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 53; R 46)
Circulation (G 18; R 32)
Excretion (G 14; R 24)
Sensory (G 9; R 13)
Aging (G 20; R 21)
Sevelopment and regeneration(G 29; R 35)
Digestive (G 16; R 37)
Environmental adaptation (G 49; R 38)
Nervous (G 46; R 51)
Endocrine (G 63; R 65)

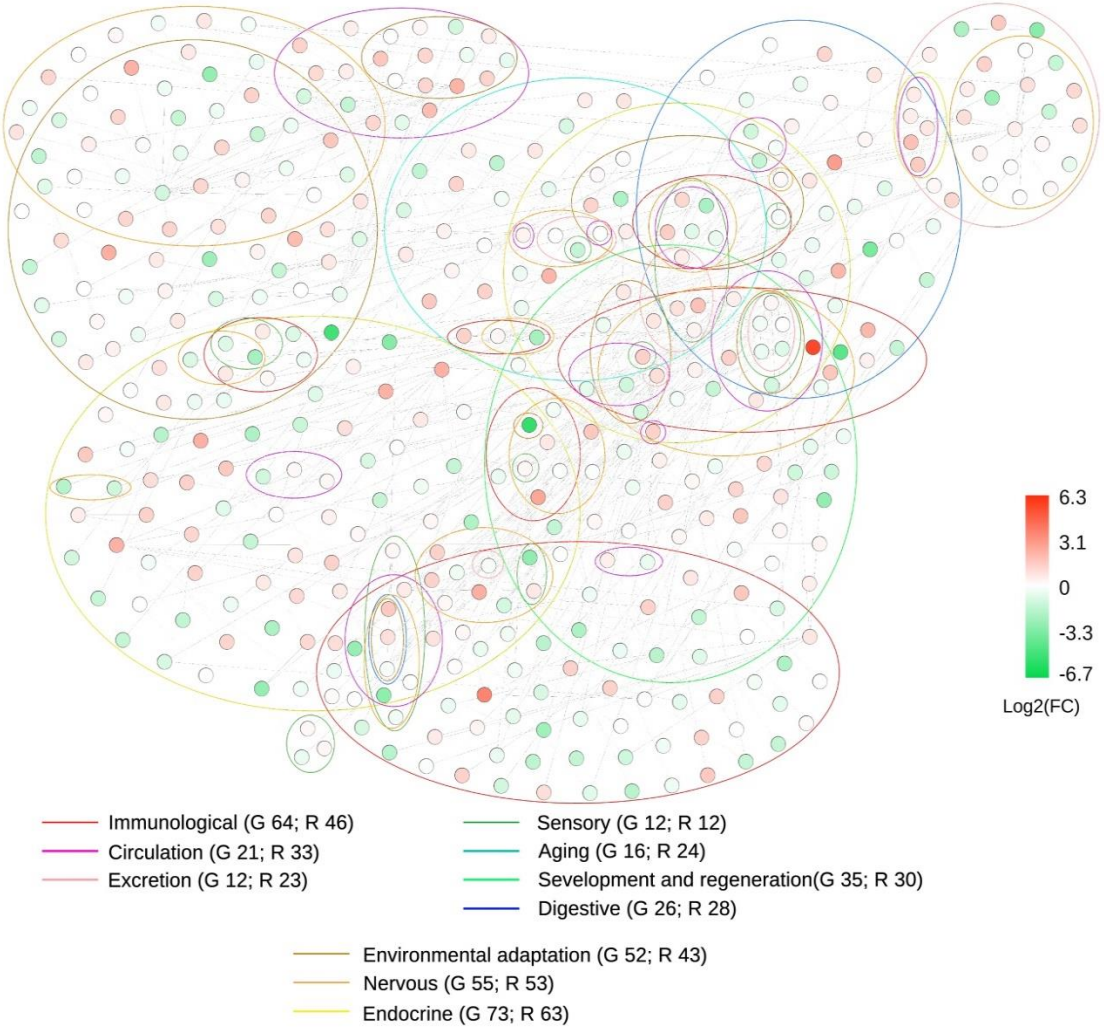
AI


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 41; R 55)
Circulation (G 16; R 38)
Excretion (G 10; R 27)
Sensory (G 6; R 17)
Aging (G 13; R 29)
Sevelopment and regeneration(G 30; R 35)
Digestive (G 13; R 41)
Environmental adaptation (G 24; R 64)
Nervous (G 30; R 68)
Endocrine (G 51; R 72)

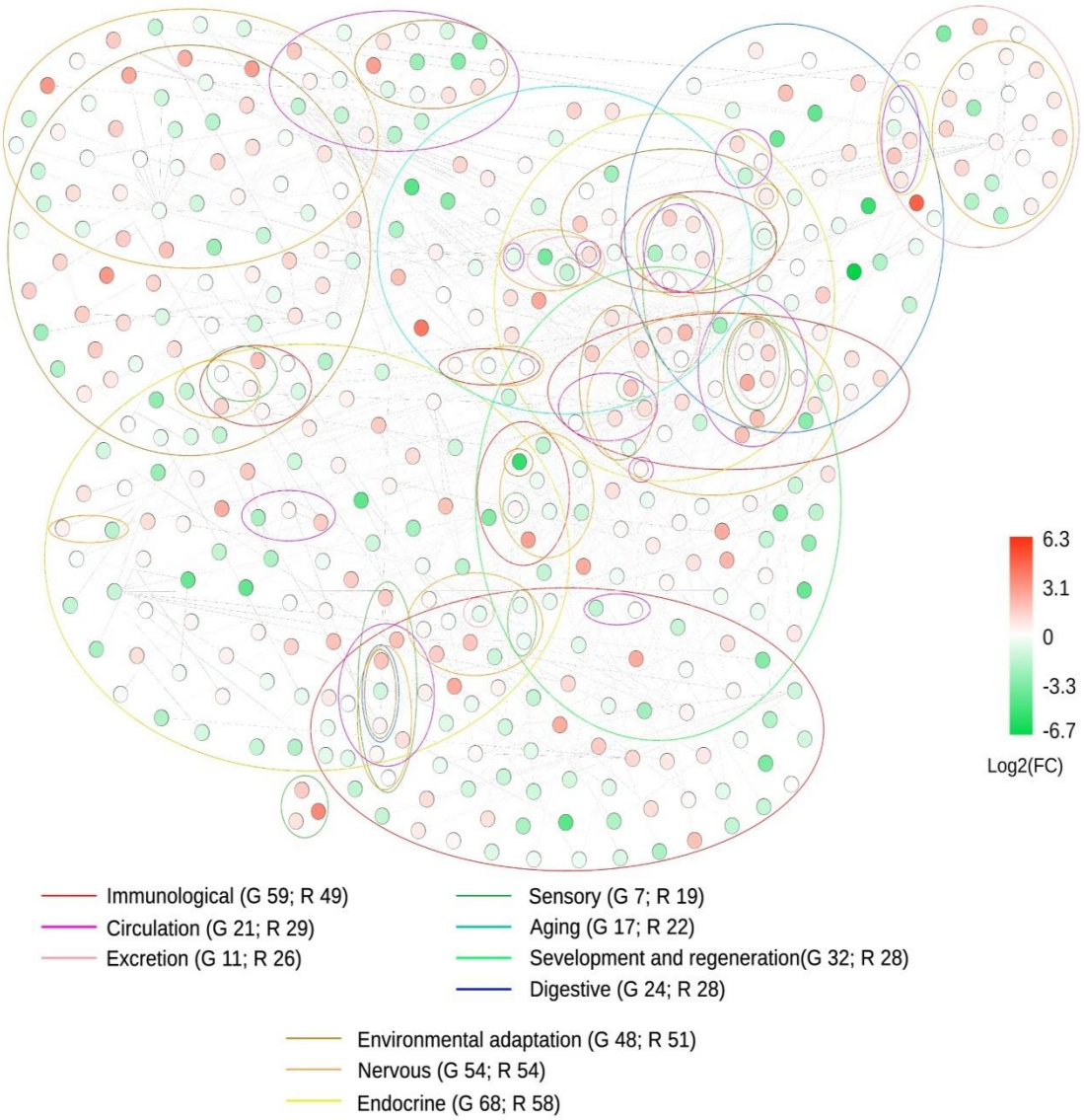
AII


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 41; R 50)
Circulation (G 15; R 36)
Excretion (G 9; R 30)
Sensory (G 6; R 19)
Aging (G 13; R 25)
Sevelopment and regeneration(G 24; R 35)
Digestive (G 16; R 36)
Environmental adaptation (G 26; R 56)
Nervous (G 29; R 70)
Endocrine (G 54; R 69)

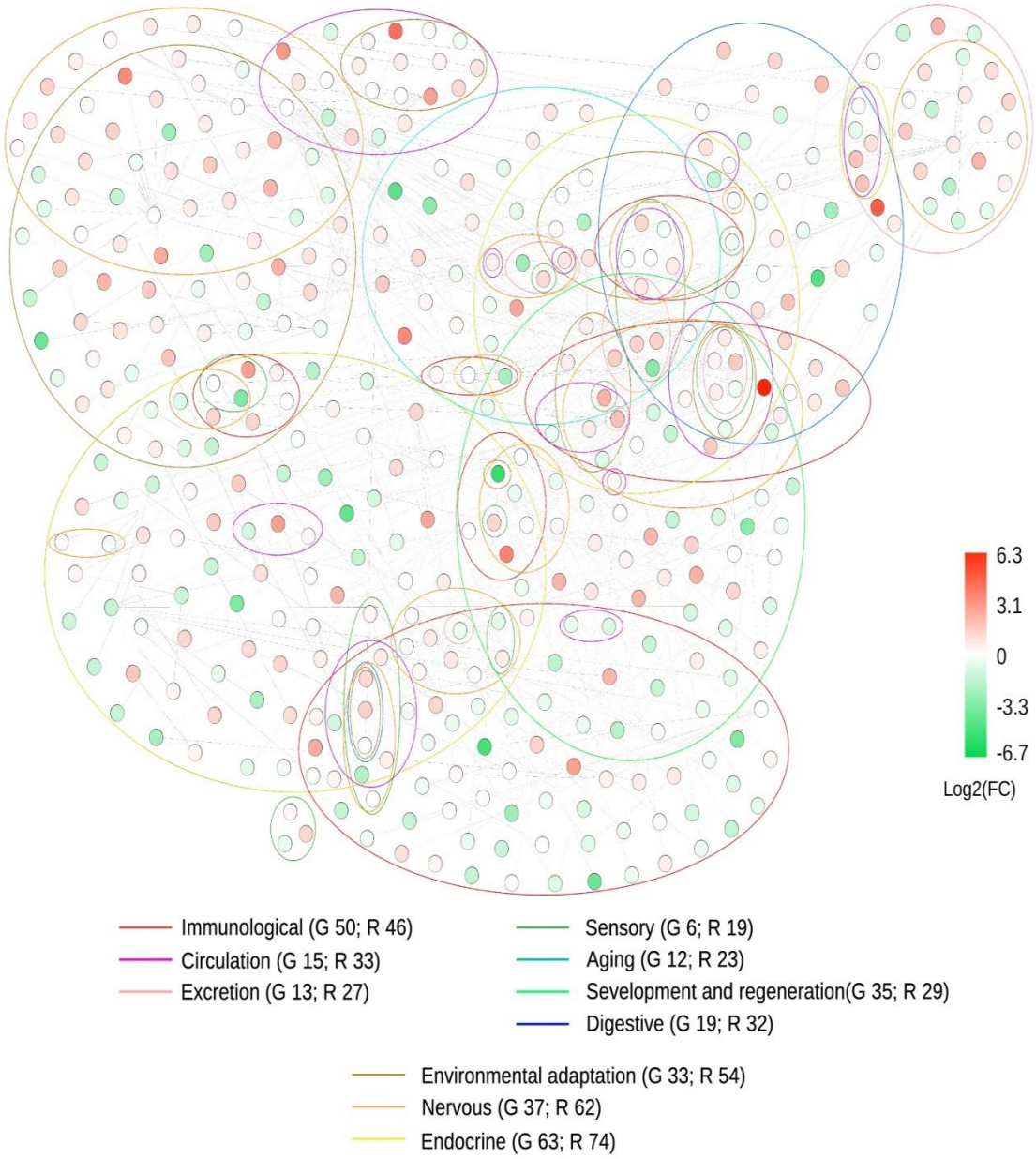
AIII


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 64; R 46)
Circulation (G 21; R 33)
Excretion (G 12; R 23)
Sensory (G 12; R 12)
Aging (G 16; R 24)
Sevelopment and regeneration(G 35; R 30)
Digestive (G 26; R 28)
Environmental adaptation (G 52; R 43)
Nervous (G 55; R 53)
Endocrine (G 73; R 63)

BI


6.3
3.1
0
-3.3
-6.7
Log2(FC)
Immunological (G 59; R 49)
Circulation (G 21; R 29)
Excretion (G 11; R 26)
Sensory (G 7; R 19)
Aging (G 17; R 22)
Sevelopment and regeneration(G 32; R 28)
Digestive (G 24; R 28)
Environmental adaptation (G 48; R 51)
Nervous (G 54; R 54)
Endocrine (G 68; R 58)

BII




BIII

**Supplementary Figure 17. Differential expression of the systemic pathways of components of the biological network present in the analyzed tissues of *R. neglectus*.** Salivary gland: **A-I**. Fed / Fasting. **A-II**. Fed and infected 2 days/Fasting. **A-III**. Fed and infected 9 days/Fasting. Bowel: **B-I**. Fed / Fasting. **B-II**. Fed and infected 2 days/Fasting **B-III**. Fed and infected 9 days/Fasting. *G: green; A: red. FC: fold change. Identified homologous component clusters and with degree > 0.

**Suplemmentary**
**Table 1**

| Salivary glands FA | | | |
|---|---|---|---|
| Transcript | Best match from all databases | Salivary gland – Sample group 02 (Average TPM) | Taxonomy |
| NODE_50_length_5496_cov_1306.112479_g24_i0 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 110425.003412 | Arthropod |
| Transcript_Amostra04_4097 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 101119.291387 | Arthropod |
| Transcript_Amostra05_2_6312 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 58951.4601 | Arthropod |
| NODE_584_length_2437_cov_492.688497_g7_i8 | NADH dehydrogenase subunit I (mitochondrion) | 28958.306546 | Arthropod |
| NODE_600_length_2394_cov_1246.256520_g114_i2 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 20418.435773 | Arthropod |
| Transcript_Amostra03_2_17661 | Lipocalin AI-5 precursor | 10796.516763 | Arthropod |
| Transcript_Amostra01_1350 | Unnamed protein product CAA9995040.1 / - | 9763.627811 | Arthropod |
| Transcript_Amostra03_3652 | - / - | 9198.94282 | Unmatched |
| Transcript_Amostra01_3181 | Uncharacterized protein LOC111255161 / - | 8738.769204 | Arthropod |
| Transcript_Amostra02_5141 | - / - | 8264.482739 | Unmatched |
| Transcript_Amostra04_3976 | - / IPR005819: Linker histone H1/H5 (E-value = 3.4E-5) | 7859.48676 | Unmatched |
| Transcript_Amostra02_3966 | Lipocalin AI-5 precursor | 7369.485974 | Arthropod |
| NODE_8221_length_650_cov_691.573109_g5430_i0 | Lipocalin AI-5 precursor | 7341.16691 | Arthropod |
| TRINITY_DN13_c0_g1_i6 | Lipocalin AI-5 precursor | 6917.810291 | Arthropod |
| Transcript_Amostra07_3570 | - / CDP | 6793.680069 | Unmatched |
| Transcript_Amostra02_3974 | Procalin-like lipocalin 1 precursor | 6007.753317 | Arthropod |
| TRINITY_DN140_c0_g1_i3 | Cytochrome c oxidase subunit I (mitochondrion) | 5955.70184 | Arthropod |
| NODE_11691_length_520_cov_496.483871_g8211_i0 | - / - | 5507.770927 | Unmatched |
| NODE_2868_length_1203_cov_345.717770_g925_i1 | - / - | 5294.889126 | Unmatched |
| TRINITY_DN7_c0_g1_i1 | Proteasome subunit alpha type-4 | 5244.889243 | Arthropod |

Unmatched transcript./ :
InterProScan Annotation
(at right) CDP: Consensus
Disorder Prediction.

| Salivary glands FE(2 days) | | | |
|---|---|---|---|
| Transcript | Best match from all databases | Salivary gland – Sample group 04 (Average TPM) | Taxonomy |
| NODE_50_length_5496_cov_1306.112479_g24_i0 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 122266.086099 | Arthropod |
| Transcript_Amostra04_4097 | Uncharacterized protein LOC112906338 (E-value 1.8E-43) | 107932.865355 | Arthropod |
| Transcript_Amostra05_2_6312 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 60906.812137 | Arthropod |
| NODE_600_length_2394_cov_1246.256520_g114_i2 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 24818.28482 | Arthropod |
| NODE_584_length_2437_cov_492.688497_g7_i8 | NADH dehydrogenase subunit I (mitochondrion) | 20697.375167 | Arthropod |
| Transcript_Amostra03_3652 | - / - | 14576.348444 | Unmatched |
| NODE_11691_length_520_cov_496.483871_g8211_i0 | - / - | 14288.234427 | Unmatched |
| Transcript_Amostra06_2428 | Nitrophorin 1A precursor | 11161.538081 | Arthropod |
| Transcript_Amostra03_2_17661 | Lipocalin AI-5 precursor | 10958.001799 | Arthropod |
| TRINITY_DN13_c0_g1_i6 | Lipocalin AI-5 precursor | 9544.182068 | Arthropod |
| Transcript_Amostra01_3181 | Uncharacterized protein LOC111255161 / - | 8901.096227 | Arthropod |
| Transcript_Amostra04_3976 | - / IPR005819: Linker histone H1/H5 (E-value = 3.4E-5) | 8180.765468 | Unmatched |
| TRINITY_DN7_c0_g1_i1 | Proteasome subunit alpha type-4 | 7820.451775 | Arthropod |
| Transcript_Amostra04_3129 | - / - | 7757.750804 | Unmatched |
| Transcript_Amostra03_2_19232 | - / - | 7281.742619 | Unmatched |
| Transcript_Amostra01_1350 | Unnamed protein product CAA9995040.1 / - | 7211.202009 | Arthropod |
| Transcript_Amostra03_3650 | - / IPR005819: Linker histone H1/H5 (E-value = 9.6E-5) | 6941.139442 | Unmatched |
| Transcript_Amostra08_1696 | - / - | 6639.384202 | Unmatched |
| NODE_3235_length_731_cov_9.541159_g1596_i2 | Biogenic amine-binding protein | 6552.79394 | Arthropod |
| Transcript_Amostra02_3926 | - / - | 6521.625562 | Unmatched |

| Salivary glands FE + Tc (2 days) | | | |
|---|---|---|---|
| Transcript | Best match from all databases | Salivary gland – Sample group 06 (Average TPM) | Taxonomy |
| NODE_50_length_5496_cov_1306.112479_g24_i0 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 120739.983864 | Arthropod |
| Transcript_Amostra04_4097 | Uncharacterized protein LOC112906338 (E-value 1.8E-43) | 107481.178318 | Arthropod |
| Transcript_Amostra05_2_6312 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 60949.823174 | Arthropod |
| Transcript_Amostra02_5141 | - / - | 31296.950409 | Unmatched |
| NODE_600_length_2394_cov_1246.256520_g114_i2 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 28500.815274 | Arthropod |
| NODE_584_length_2437_cov_492.688497_g7_i8 | NADH dehydrogenase subunit I (mitochondrion) | 26753.782806 | Arthropod |
| Transcript_Amostra06_2428 | Nitrophorin 1A precursor | 17827.606489 | Arthropod |
| Transcript_Amostra01_1350 | Unnamed protein product CAA9995040.1 / - | 14049.49275 | Arthropod |
| Transcript_Amostra04_3976 | - / IPR005819: Linker histone H1/H5 (E-value = 3.4E-5) | 12963.310483 | Unmatched |
| Transcript_Amostra03_2_11527 | - / PRINTS01217: Proline rich extensin signature (E-value = 4.4E-9) | 11341.144638 | Unmatched |
| Transcript_Amostra03_3652 | - / - | 10647.515173 | Unmatched |
| TRINITY_DN7_c0_g1_i1 | Proteasome subunit alpha type-4 | 10334.145474 | Arthropod |
| Transcript_Amostra02_3926 | - / - | 9090.527789 | Unmatched |
| Transcript_Amostra04_3074 | Venom CUB domain protein 2 | 9027.432855 | Arthropod |
| Transcript_Amostra02_3974 | Procalin-like lipocalin 1 precursor | 8190.030831 | Arthropod |
| Transcript_Amostra03_3651 | - / CDP | 6939.405917 | Unmatched |
| Transcript_Amostra02_3966 | Lipocalin AI-5 precursor | 6280.033929 | Arthropod |
| NODE_8221_length_650_cov_691.573109_g5430_i0 | Lipocalin AI-5 precursor | 6093.2977 | Arthropod |
| Transcript_Amostra03_2_17661 | Lipocalin AI-5 precursor | 5811.879766 | Arthropod |
| Transcript_Amostra03_2_19230 | - / IPR005819: Linker histone H1/H5 (E-value = 6.0E-5) | 5563.333221 | Unmatched |

| Salivary glands FE +Tc (9 days) | | | |
|---|---|---|---|
| Transcript | Best match from all databases | Salivary gland – Sample group 08 (Average TPM) | Taxonomy |
| NODE_50_length_5496_cov_1306.112479_g24_i0 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 116787.972108 | Arthropod |
| Transcript_Amostra04_4097 | Uncharacterized protein LOC112906338 (E-value 1.8E-43) | 100414.314786 | Arthropod |
| Transcript_Amostra05_2_6312 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 58218.509154 | Arthropod |
| Transcript_Amostra01_1350 | Unnamed protein product CAA9995040.1 / - | 26894.420555 | Arthropod |
| NODE_584_length_2437_cov_492.688497_g7_i8 | NADH dehydrogenase subunit I (mitochondrion) | 24205.068391 | Arthropod |
| NODE_600_length_2394_cov_1246.256520_g114_i2 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 22809.091364 | Arthropod |
| TRINITY_DN13_c0_g1_i6 | Lipocalin AI-5 precursor | 17977.751271 | Arthropod |
| Transcript_Amostra03_3650 | - / IPR005819: Linker histone H1/H5 (E-value = 9.6E-5) | 15872.356584 | Unmatched |
| NODE_3235_length_731_cov_9.541159_g1596_i2 | Biogenic amine-binding protein | 15445.867428 | Arthropod |
| TRINITY_DN7_c0_g1_i1 | Proteasome subunit alpha type-4 | 15319.417967 | Arthropod |
| Transcript_Amostra03_3652 | - / - | 12618.715604 | Unmatched |
| Transcript_Amostra01_3181 | Uncharacterized protein LOC111255161 / - | 9722.253099 | Arthropod |
| Transcript_Amostra03_2_19232 | - / - | 8939.490282 | Unmatched |
| Transcript_Amostra07_3570 | - / CDP | 7972.203397 | Unmatched |
| Transcript_Amostra04_3110 | - / CDP | 7857.059444 | Unmatched |
| NODE_8221_length_650_cov_691.573109_g5430_i0 | Lipocalin AI-5 precursor | 7469.260852 | Arthropod |
| NODE_4754_length_597_cov_14.593870_g2718_i0 | Salivary platelet aggregation inhibitor 2 | 7298.296188 | Arthropod |
| NODE_3702_length_687_cov_11.746732_g1972_i0 | Nitrophorin-1 precursor | 7087.912176 | Arthropod |
| Transcript_Amostra06_2428 | Nitrophorin 1A precursor | 6710.887971 | Arthropod |
| NODE_4088_length_652_cov_8.944541_g2232_i0 | Venom CUB domain protein 2 | 6060.593297 | Arthropod |

| Intestine FA | | | |
|---|---|---|---|
| Transcript | Best match from all databases | Intestine – Sample group 01 (Average TPM) | Taxonomy |
| NODE_50_length_5496_cov_1306.112479_g24_i0 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 129390.295521 | Arthropod |
| TRINITY_DN6118_c0_g1_i1 | Hypothetical protein GE061_03760 / - | 116286.442221 | Arthropod |
| Transcript_Amostra04_4097 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 107709.582187 | Arthropod |
| Transcript_Amostra03_2_25673 | Hypothetical protein GE061_03760 / - | 69902.343849 | Arthropod |
| Transcript_Amostra05_2_6312 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 61103.115789 | Arthropod |
| NODE_584_length_2437_cov_492.688497_g7_i8 | NADH dehydrogenase subunit I (mitochondrion) | 32736.877095 | Arthropod |
| NODE_33576_length_222_cov_3.938776_g30595_i0 | - / CDP | 19706.791783 | Unmatched |
| NODE_600_length_2394_cov_1246.256520_g114_i2 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 19393.245055 | Arthropod |
| TRINITY_DN9118_c0_g1_i2 | Hypothetical protein GE061_06167 / TAR1 (E-value 3.1E-18) | 18949.644756 | Arthropod |
| NODE_58460_length_202_cov_101.367347_g54110_i0 | - / - | 13950.089005 | Unmatched |
| Transcript_Amostra01_1350 | Unnamed protein product CAA9995040.1 / - | 12956.312872 | Arthropod |
| TRINITY_DN7_c0_g1_i1 | Proteasome subunit alpha type-4 | 11002.983491 | Arthropod |
| NODE_21400_length_271_cov_0.704082_g18422_i0 | Conserved hypothetical protein XP_002425519.1 / CDP | 10285.956998 | Arthropod |
| TRINITY_DN140_c0_g1_i3 | Cytochrome c oxidase subunit I (mitochondrion) | 6283.072032 | Arthropod |
| Transcript_Amostra01_3185 | NADH dehydrogenase subunit 4, partial (mitochondrion) | 6049.243602 | Arthropod |
| Transcript_Amostra01_4110 | - / - | 5086.5048 | Unmatched |
| NODE_41058_length_203_cov_74.500000_g38077_i0 | - / - | 4974.283705 | Unmatched |
| Transcript_Amostra08_1142 | - / - | 4849.715305 | Unmatched |
| Transcript_Amostra01_2_11053 | - / CDP | 4677.596155 | Unmatched |
| TRINITY_DN8600_c0_g1_i2 | Hypothetical protein GE061_01450 / CDP | 4344.579011 | Arthropod |

: Unmatched transcript.

/ : InterProScan Annotation (at right).

CDP: Consensus Disorder Prediction.

| Intestine FE | | | |
|---|---|---|---|
| Transcript | Best match from all databases | Intestine – Sample group 03 (Average TPM) | Taxonomy |
| NODE_50_length_5496_cov_1306.112479_g24_i0 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 118067.00112 | Arthropod |
| TRINITY_DN6118_c0_g1_i1 | Hypothetical protein GE061_03760 / - | 106653.250558 | Arthropod |
| Transcript_Amostra04_4097 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 102462.206665 | Arthropod |
| Transcript_Amostra03_2_25673 | Hypothetical protein GE061_03760 / - | 64000.020294 | Arthropod |
| Transcript_Amostra05_2_6312 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 58593.944776 | Arthropod |
| NODE_584_length_2437_cov_492.688497_g7_i8 | NADH dehydrogenase subunit I (mitochondrion) | 26148.224115 | Arthropod |
| Transcript_Amostra01_1350 | Unnamed protein product CAA9995040.1 / - | 22621.22699 | Arthropod |
| TRINITY_DN9118_c0_g1_i2 | Hypothetical protein GE061_06167 / TAR1 (E-value 3.1E-18) | 15659.043863 | Arthropod |
| Transcript_Amostra03_2_17576 | - / - | 15123.399089 | Unmatched |
| TRINITY_DN7_c0_g1_i1 | Proteasome subunit alpha type-4 | 13261.501229 | Arthropod |
| NODE_600_length_2394_cov_1246.256520_g114_i2 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 12527.921014 | Arthropod |
| NODE_33576_length_222_cov_3.938776_g30595_i0 | - / CDP | 11013.159367 | Unmatched |
| NODE_21400_length_271_cov_0.704082_g18422_i0 | Conserved hypothetical protein XP_002425519.1 / CDP | 10847.679057 | Arthropod |
| Transcript_Amostra07_3570 | - / CDP | 10203.814313 | Unmatched |
| Transcript_Amostra01_4081 | - / CDP | 9324.943926 | Unmatched |
| NODE_58460_length_202_cov_101.367347_g54110_i0 | - / - | 9142.882454 | Unmatched |
| Transcript_Amostra02_7427 | NADH dehydrogenase subunit I (mitochondrion) | 7918.066605 | Arthropod |
| TRINITY_DN140_c0_g1_i3 | Cytochrome c oxidase subunit I (mitochondrion) | 6885.924282 | Arthropod |
| Transcript_Amostra01_2_11053 | - / CDP | 6533.577914 | Unmatched |
| NODE_41061_length_203_cov_74.421875_g38080_i0 | - / - | 5314.034893 | Unmatched |

| Intestine FE+ Tc (2 days) | | | |
|---|---|---|---|
| Transcript | Best match from all databases | Intestine – Sample group 05 (Average TPM) | Taxonomy |
| NODE_50_length_5496_cov_1306.112479_g24_i0 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 158603.726733 | Arthropod |
| Transcript_Amostra04_4097 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 128621.031522 | Arthropod |
| TRINITY_DN6118_c0_g1_i1 | Hypothetical protein GE061_03760 / - | 120034.946705 | Arthropod |
| Transcript_Amostra03_2_25673 | Hypothetical protein GE061_03760 / - | 97183.819231 | Arthropod |
| Transcript_Amostra05_2_6312 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 72751.996015 | Arthropod |
| NODE_58460_length_202_cov_101.367347_g54110_i0 | - / - | 34478.505117 | Unmatched |
| NODE_584_length_2437_cov_492.688497_g7_i8 | NADH dehydrogenase subunit I (mitochondrion) | 30376.595009 | Arthropod |
| NODE_600_length_2394_cov_1246.256520_g114_i2 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 23477.64984 | Arthropod |
| Transcript_Amostra01_4081 | - / CDP | 21946.043713 | Unmatched |
| TRINITY_DN24_c0_g1_i1 | Unknown secreted protein BAN20609.1 / - | 18710.556073 | Arthropod |
| NODE_33576_length_222_cov_3.938776_g30595_i0 | - / CDP | 14701.486261 | Unmatched |
| Transcript_Amostra01_1350 | Unnamed protein product CAA9995040.1 / - | 13696.539137 | Arthropod |
| TRINITY_DN7_c0_g1_i1 | Proteasome subunit alpha type-4 | 13029.84376 | Arthropod |
| Transcript_Amostra07_3570 | - / CDP | 10348.611361 | Unmatched |
| TRINITY_DN9118_c0_g1_i2 | Hypothetical protein GE061_06167 / TAR1 (E-value 3.1E-18) | 10009.907843 | Arthropod |
| TRINITY_DN140_c0_g1_i3 | Cytochrome c oxidase subunit I (mitochondrion) | 8555.485653 | Arthropod |
| NODE_53507_length_211_cov_95.365385_g49157_i0 | - / - | 8336.074113 | Unmatched |
| Transcript_Amostra05_2_14442 | Secreted hypothetical protein ATU83025.1 / - | 7768.285258 | Arthropod |
| Transcript_Amostra05_4488 | - / CDP | 7165.130503 | Unmatched |
| Transcript_Amostra01_3181 | Uncharacterized protein LOC111255161 / - | 6836.471386 | Arthropod |

| Intestine FE + Tc (9 days) | | | |
|---|---|---|---|
| Transcript | Best match from all databases | Intestine – Sample group 07 (Average TPM) | Taxonomy |
| NODE_50_length_5496_cov_1306.112479_g24_i0 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 119706.395138 | Arthropod |
| Transcript_Amostra04_4097 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 99991.879039 | Arthropod |
| Transcript_Amostra05_2_6312 | Hypothetical protein GE061_03717 / PTHR33626 (E-value 6.0E-34) | 60502.866184 | Arthropod |
| NODE_600_length_2394_cov_1246.256520_g114_i2 | Uncharacterized protein LOC112906338 / TAR1 (E-value 1.8E-43) | 29922.525386 | Arthropod |
| Transcript_Amostra01_1350 | Unnamed protein product CAA9995040.1 / - | 27766.003088 | Arthropod |
| NODE_584_length_2437_cov_492.688497_g7_i8 | NADH dehydrogenase subunit I (mitochondrion) | 26154.574232 | Arthropod |
| TRINITY_DN7_c0_g1_i1 | Proteasome subunit alpha type-4 | 14915.893917 | Arthropod |
| Transcript_Amostra07_3570 | - / CDP | 11944.686163 | Unmatched |
| Transcript_Amostra02_1121 | - / CDP | 10281.485025 | Unmatched |
| Transcript_Amostra01_4081 | - / CDP | 7137.447989 | Unmatched |
| Transcript_Amostra01_3181 | Uncharacterized protein LOC111255161 / - | 6887.467665 | Arthropod |
| Transcript_Amostra01_2_7526 | - / CDP | 6406.363199 | Unmatched |
| Transcript_Amostra05_4488 | - / CDP | 6215.404311 | Unmatched |
| TRINITY_DN53_c0_g1_i1 | Lysozyme-1 | 6130.0491 | Arthropod |
| Transcript_Amostra08_1142 | - / - | 6025.701255 | Unmatched |
| NODE_4500_length_934_cov_712.780432_g2755_i0 | Family I29 unassigned peptidase inhibitors | 5746.606478 | Arthropod |
| TRINITY_DN128_c0_g1_i1 | Cathepsin B | 5580.846901 | Arthropod |
| TRINITY_DN140_c0_g1_i3 | Cytochrome c oxidase subunit I (mitochondrion) | 4857.867872 | Arthropod |
| TRINITY_DN24_c0_g1_i1 | Unknown secreted protein BAN20609.1 / - | 4563.960492 | Arthropod |
| NODE_2868_length_1203_cov_345.717770_g925_i1 | - / - | 3935.233008 | Unmatched |