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PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

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POTENCIAIS ALVOS NO SINGÂNGLIO PARA CONTROLE DE CARRAPATOS

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Sumário

Lista de abreviaturas, símbolos e unidades

ACP	Peptídeo relacionado a corazonina/hormônio adipocinético
Canais Na^+	Canais de sódio sensíveis a voltagem
Ca^{2+}	Íon cálcio
cAMP	3,5 monofosfato de adenosina cíclico
CCAP	Peptídeo cardioativo de crustáceos
Cl^-	Íon cloreto
EST	Marcador de sequência expressa (*expressed sequence tag*)
GABA-Cl^-	Canais de cloreto mediados por ácido gama-aminobutírico
Glu-Cl^-	Canais de cloreto ativados por glutamato
GPA2	Glicoproteína A2
Gpb	Giga pares de base
GPB5	Glicoproteína B5
GPCR	Receptores acoplados à proteína G
ETH	Hormônio desencadeador da ecdise
IGF	Fator de crescimento do tipo insulina
iPTH	Hormônio da paratireoide de insetos
Kdr	Resistência do tipo *knockdown*
K^+	Íon potássio
NPF	Neuropeptídeo F
RDL	Resistência à dieldrina
RPKM	*Reads* por quilobase por milhão *(reads per kilobase per million*)
SNC	Sistema nervoso central
SNP	Polimorfismo de nucleotídeo único
Super-kdr	Resistência do tipo super *knockdown* (*super-knockdown resistance*)
S. l.	Sensu lato

Resumo

O controle dos carrapatos é realizado, principalmente, pela aplicação de acaricidas sintéticos. No entanto, o seu uso levou a seleção de populações de carrapatos resistentes a esses compostos, havendo a necessidade do desenvolvimento de estratégias alternativas de controle com a identificação de novas proteínas. O singânglio, é o sistema nervoso central desses ectoparasitos, sendo o principal alvo dos acaricidas disponíveis comercialmente. Devido a sua função pleiotrópica, já bem caracterizada em insetos, moléculas sinalizadoras sintetizadas e secretadas pelo singânglio, como os neuropeptídeos têm sido propostas para o desenvolvimento de metodologias alternativas para controle dos carrapatos. Entretanto, o conhecimento funcional dos neuropeptídeos ainda é escasso nesses artrópodes. Nesse sentido, esse trabalho teve como objetivo identificar e caracterizar sequências precursoras de neuropeptídeos, bem como descrever o potencial uso desses peptídeos e de proteínas presentes no singânglio como alvos para controle de carrapatos ixodídeos. Uma análise *in silico* foi realizada a partir de um transcriptoma de singânglio de *Rhipicephalus microplus* e em sequências genômicas públicas. Ao todo, 52 sequências precursoras de neuropeptídeos foram identificadas em *R. microplus*, sendo que maior expressão desses precursores foi observada no singânglio, em comparação a outros órgãos e estádios de *R. microplus* (ovário, glândulas salivares, corpo gorduroso, células digestivas de fêmeas parcialmente e totalmente ingurgitadas, e embrião). Adicionalmente, as sequências precursoras de neuropeptídeos se mostraram conservadas em relação aos neuropeptídeos equivalentes presentes em outras espécies de carrapatos analisadas (de *R. microplus*, *Rhipicephalus sanguineus* sensu lato, *Ixodes persulcatus*, *Dermacentor silvarum*, *Haemaphysalis longicornis*, *Hyalomma asiaticum e Ixodes scapularis)*. Além disso, outros alvos presentes no singânglio, como agonistas e antagonistas de receptores e novas moléculas estão sendo aplicadas no estudo para controle dos carrapatos. Trabalhos adicionais são necessários para caracterizar funcionalmente as proteínas presentes no singânglio na fisiologia desses parasitos e para investigar seu potencial como alvo de drogas.

Abstract

Tick control is mainly performed through the application of synthetic acaricides. However, this led to the selection of resistant tick populations to these compounds, needing the development of alternative control strategies for the identification of new proteins. Synganglion is the tick's central nervous system, and it is the main target of commercially available acaricides. Due to their pleiotropic function, already characterized in insects, signaling molecules synthesized and secreted by the synganglion, such as neuropeptides, have been proposed as potential targets for development of alternative methods for tick control. However, functional knowledge of neuropeptides is still scarce in these arthropods. Thus, this work aimed to identify and characterize neuropeptide precursor sequences, as well as describe the potential use of these peptides and proteins present in synganglion as targets for the control of ixodid ticks. *In silico* analysis was performed from synganglion transcriptome of *Rhipicephalus microplus* and from public genomic sequences. In total, 52 neuropeptide precursor sequences were identified in *R. microplus*, with the highest expression of these precursors being observed in synganglion, in comparison to other *R. microplus* organs and stages (ovary, salivary glands, fat body, digestive cells from partially and fully engorged females, and embryo). In addition, neuropeptide precursor sequences were conserved in relation to equivalent neuropeptides present in the other analyzed tick species (*R. microplus*, *Rhipicephalus sanguineus* sensu lato, *Ixodes persulcatus*, *Dermacentor silvarum*, *Haemaphysalis longicornis*, *Hyalomma asiaticum* and *Ixodes scapularis*). Moreover, other targets present in synganglion, such as receptor agonist and antagonists and new molecules are being applied in the control tick studies. Additional work is needed to functionally characterize neuropeptides in tick physiology and to assess their potential as drug targets.

1. Introdução

Os carrapatos são classificados na subclasse Acari e subdivididos em duas famílias principais, a Ixodidae (carrapatos duros) que apresentam mais de 700 espécies descritas (GUGLIELMONE; PETNEY; ROBBINS, 2020) e a Argasidae (carrapatos moles, não apresentando escudo) com cerca de 200 espécies (ALI *et al*., 2022). Além disso, uma terceira família, denominada de Nuttalliellidae, também é descrita, com somente uma espécie identificada (GUGLIELMONE *et al*., 2010; GUGLIELMONE; PETNEY; ROBBINS, 2020). Esses artrópodes são ectoparasitos hematófagos de mamíferos, anfíbios, répteis, aves, dentre outros, e estão presentes em diferentes locais, entretanto apresentam maior presença em regiões tropicais e subtropicais (ANDERSON; MAGNARELLI, 2005; KEIRANS; DURDEN., 2005).

Juntamente com os mosquitos, os carrapatos são os principais vetores de patógenos que podem afetar a saúde de seres humanos e animais, sendo que estes foram os primeiros artrópodes associados à transmissão de patógenos (DANTAS-TORRES; CHOMEL; OTRANTO, 2012). Durante o processo de hematofagia, os carrapatos podem transmitir bactérias, protozoários e vírus (DANTAS-TORRES; CHOMEL; OTRANTO, 2012; JONGEJAN; UILENBERG, 2004), além de causar alergias, toxicoses e parálises (SONENSHINE; ROE, 2013).

A babesiose foi a primeira doença descrita cujo agente (*Babesia bigemina*) foi transmitido de um artrópode (*Boophilus annulatus*) a um mamífero (bovino) (BOCK *et al*., 2004; SMITH; KILBORNE, 1893). Entretanto, atualmente, há mais de 20 doenças reportadas que são causadas por agentes transmitidos por carrapatos (DANTAS-TORRES; CHOMEL; OTRANTO, 2012; ROCHLIN; TOLEDO, 2020). Dentro da família Ixodidae, que contém a maior gama de espécies descritas, o carrapato *Amblyomma americanum* é considerado uma das espécies mais agressivas nos Estados Unidos, sendo um dos vetores de *Rickettsia rickettsii* (MAVER, 1911), afetando a saúde pública (GODDARD; VARELA-STOKES, 2009; LEVIN *et al*., 2017). Além desse patógeno os parasitos do gênero *Amblyomma* também podem transmitir a *Erlichia* spp. (CAMUS; BARRE, 1995). Já os carrapatos do gênero *Ixodes* , podem transmitir *Borrelia* spp., bactérias causadoras da doença de *Lyme*, importante para saúde humana, sendo considerada a doença mais frequente em que o patógeno é transmitido por carrapatos, especialmente na América do Norte e na Europa (MEAD, 2015; STEERE; COBURN; GLICKSTEIN, 2004). Na Austrália, uma toxina

secretada na saliva do *Ixodes holocyclus* causa toxicose e paralisia em animais e seres humanos (BARKER; WALKER, 2014; HALL-MENDELIN *et al*., 2011).

O parasitismo realizado pelo carrapato pode causar danos extensos ao hospedeiro, levando a reações inflamatórias no local da picada, com consequente prurido e abcessos, perda de sangue e anemia (HURTADO; GIRALDO-RÍOS, 2018; JONGEJAN; UILENBERG, 2004). Isso pode estressar o animal ou levá-lo a morte, afetando os animais de produção, pecuária e consequentemente a economia de um país.

No Brasil, o clima subtropical e tropical favorece a ocorrência de parasitos em bovinos, sendo que os problemas econômicos da pecuária estão fortemente associados a infestações causadas pelo carrapato *Rhipicephalus microplus* (GRISI *et al*., 2014). Apesar das diferentes temperaturas, o *R. microplus* está distribuído por todas as regiões brasileiras, causando danos diretos e indiretos aos bovinos (PEREIRA; SOUZA; BAFFI, 2010). Dados estimam uma perda anual de US$ 3,24 bilhões no Brasil devido a infestações por essa espécie de carrapato (GRISI *et al*., 2014), acarretando redução dos rendimentos pecuários e aumentando custos aos produtores rurais (TABOR *et al*., 2017).

1.1 *Rhipicephalus microplus*

O *R. microplus* é um carrapato originário da Ásia, tendo sido distribuído por diferentes regiões do mundo através das infestações em cervídeos e bovídeos (ESTRADA-PEÑA *et al*., 2006). Em alguns lugares, especificamente na África, recentemente, o *R. microplus* vem substituindo outras espécies de carrapatos, como, por exemplo, o *Rhipicephalus decoloratus*. Uma hipótese é a de que isso venha ocorrendo devido a um ciclo de vida mais rápido do *R. microplus* sobre outras espécies de carrapatos (TØNNESEN *et al*., 2004).

O *R. microplus* completa seu ciclo de vida em um único hospedeiro, sendo denominado de monoxeno, parasitando principalmente *Bos indicus* e *Bos taurus* (MCCOY; LÉGER; DIETRICH, 2013). O *B. indicus* é menos suscetível a infestações do que o *B. taurus* e diferentes respostas imunes podem afetar a resistência desses animais aos parasitos (RIEK, 1962; PIPER *et al*., 2009). Além disso, diferenças de suscetibilidade e resistência entre bovinos pertencentes a mesma raça também foram observadas (WILKINSON, 1955). O ciclo de vida do *R. microplus* compreende duas fases, a parasitária e a não parasitária. No final da fase parasitária, as fêmeas, quando repletas de sangue, se desprendem do hospedeiro,

realizam a postura dos ovos e morrem, apresentando um único ciclo gonadotrófico, dando início a fase de vida livre, ou também denominada não parasitária (SONENSHINE; ROE, 2013). Após a eclosão das larvas, elas necessitam de um período de aproximadamente 20 dias para se tornarem infestantes, posteriormente, se fixam em um hospedeiro vertebrado, marcando o início da fase parasitária (ROCHA, 1998). Em condições ideais de umidade e temperatura as larvas podem permanecer viáveis por mais de seis meses sem se alimentar. Após a fixação, inicia-se o processo de alimentação, as larvas sofrem muda para ninfas, ocorrendo o dimorfismo sexual, e de ninfas chegam ao estádio de adultos. Os carrapatos adultos realizam a cópula, as fêmeas passam de parcialmente a totalmente ingurgitadas, dando início a um novo ciclo (SONENSHINE; ROE, 2013) e os machos podem copular com outras fêmeas, permanecendo mais tempo no bovino (ROCHA, 1998). Para que o sucesso da alimentação ocorra, os carrapatos se evadem das defesas hemostáticas do hospedeiro, reduzindo as respostas inflamatória e imune, e a dor (FRANCISCHETTI, 2009; SONENSHINE; ROE, 2013).

No Brasil, o impacto econômico causado pela infestação por *R. microplus* fica atrás somente de algumas espécies de nematódeos gastrointestinais (ESTRADA-PEÑA *et al*., 2006; GRISI *et al*., 2014). Esse carrapato se alimenta em média de 2 a 3 ml de sangue de seu hospedeiro, causando extensos prejuízos tanto a saúde do animal quanto a produção pecuária (GONZALES, 1995; JONGEJAN; UILENBERG, 2004; JONSSON, 2006). Também, os custos para o controle, aquisição de medicamentos, equipamentos, instalações e mão de obra qualificada têm sido avaliados (CORDOVES CESPEDES, 1997; RODRIGUEZ-VIVAS; JONSSON; BHUSHAN, 2018), com uma perda anual de até US$ 30 bilhões no mundo, sendo estimada devido a infestações pelo carrapato bovino (LEW-TABOR; RODRIGUEZ VALLE, 2016).

1.1.2 Estratégias de controle de carrapatos

A principal forma de controle dos carrapatos é realizada através da aplicação de acaricidas sintéticos, no entanto essa utilização tem levado à seleção de populações resistentes a esses compostos químicos (JONGEJAN; UILENBERG, 2004; OBAID *et al*., 2022). Atualmente, estão disponíveis comercialmente sete classes principais de acaricidas: organofosforados, lactonas macrocíclicas, formamidinas, piretroides sintéticos, benzoilfenil ureias, fenilpirazois e mais recentemente isoxazolinas (GASSEL *et al*., 2014; RECK *et al*.,

2014; RUFENER *et al*., 2017). Entretanto, populações de carrapatos resistentes e multirresistentes aos pesticidas já têm sido descritas (DZEMO; THEKISOE; VUDRIKO, 2022; RECK *et al*., 2014). Devido aos problemas ocasionados pelo uso de parasiticidas, como a contaminação do meio ambiente e consequentemente de alimentos, a qual pode afetar a saúde humana, a seleção de resistência e a aplicação dos pesticidas ser trabalhosa e dispendiosa, estratégias alternativas de controle têm sido exploradas (SAMISH; GINSBERG; GLAZER, 2004).

O controle biológico clássico consiste na introdução de duas espécies competidoras em um mesmo habitat (SAMISH; GINSBERG; GLAZER, 2004). Geralmente, a metodologia de biocontrole não é tóxica ao meio ambiente e trabalhos principalmente envolvendo agentes patogênicos de plantas já mostraram sucesso com essa técnica (OSTFELD *et al*., 2006; SAMISH; GINSBERG; GLAZER, 2004). Em carrapatos suscetíveis e multi-resistentes a acaricidas, testes de imersão foram realizados utilizando o fungo *Metarhizium brunneum* (anteriormente denominado *Metarhizium anisopliae*) (FERNÁNDEZ -SALAS *et al*., 2017). O tratamento levou a 90% de mortalidade de ambas as populações de *R. microplus*, reduzindo também a postura dos ovos. Também, foi demonstrado que isolados resistentes necessitam de menores concentrações de conídios do que quando comparados a isolados sensíveis a acaricidas. Sugere-se que isso ocorra uma vez que isolados suscetíveis são controlados biologicamente em seu ambiente natural, devido a presença de fungos e/ou bactérias presentes no meio em que se encontram, levando a maior adaptação e tolerância desses carrapatos ao biocontrole (ADAMES *et al*., 2011). Isso pode ser devido a um mecanismo de proteção ou aumento da transcrição e/ou expressão de moléculas-chave nesses processos, tais como inibidores de proteases e quitinases (ADAMES *et al*., 2011). Em *Ixodes scapularis*, redução no número de ninfas foi demonstrada após repetidas aplicações de esporos de *M. brunneum*, sendo observado o aparecimento de micoses nos carrapatos coletados (BHARADWAJ; STAFFORD, 2010).

Além disso, o uso de *M. brunneum* associado ao controle químico com a aplicação de acaricidas, tais como clorpirifós e cipermetrina, mostrou aproximadamente 98% de eficácia no tratamento de *R. microplus* resistentes a ectoparasiticidas, enquanto que quando os carrapatos foram expostos somente ao fungo ou aos acaricidas sozinhos a eficácia alcançada foi de 71% e 56%, respectivamente, não havendo diferença significativa entre os tratamentos (WEBSTER *et al*., 2015). Dessa forma, é possível observar um efeito

potencializador quando utilizado fungos integrados aos pesticidas no controle dos carrapatos. Além disso, não foi demonstrada redução na viabilidade do *M. brunneum* devido ao uso conjunto com esses acaricidas. Foi observado que esse fungo é patogênico para diferentes estádios de vida de *Boophilus annulatus*, *Hyalomma excavatum* e *Rhipicephalus sanguineus* s. l., afetando também os ovos dos carrapatos (GINDIN *et al*., 2002). Fungos entomopatogênicos também podem ser transferidos de um espécime infectado para um não infectado, tornando essa uma estratégia útil para o controle de parasitos (GINSBERG *et al*., 2002). Apesar dos resultados promissores com o uso do controle biológico em carrapatos, essa estratégia pode apresentar desvantagens a campo, como por exemplo, espécies não-alvo também serem atacadas pelas espécies predadoras (OSTFELD *et al*., 2006).

O controle imunológico apresenta-se como uma estratégia promissora para a redução da infestação por carrapatos, uma vez que as vacinas podem prover uma imunidade duradoura e reduzir a transmissão de patógenos, além de serem economicamente viáveis e seguras ao meio ambiente (YADAV; UPADHYAY, 2022). Duas vacinas contra o carrapato *R. microplus* foram desenvolvidas e disponibilizadas comercialmente, a primeira na Australia e a outra em Cuba (TickGARD e GAVAC, respectivamente) (CANALES *et al*., 1997; WILLADSEN *et al*., 1995). Ambas se baseiam na teoria dos antígenos ocultos sugerida por WILLADSEN; KEMP (1988), que propõe a utilização de antígenos que não são expostos ao sistema imune do hospedeiro durante a alimentação. Com isso, em uma infestação natural, o hospedeiro não desenvolve imunidade adquirida contra esses antígenos, e o parasito não sofreu pressão evolutiva para um mecanismo de evasão da resposta imunogênica, sendo, portanto, considerados potenciais alvos vacinais. O antígeno utilizado é a Bm86, uma glicoproteína identificada no intestino de fêmeas de carrapatos parcialmente ingurgitados (WILLADSEN; KEMP, 1988). Apesar de induzirem proteção contra outras espécies do gênero *Rhipicephalus* (FRAGOSO *et al*., 1998; HÜE *et al*., 2017; ODONGO *et al*., 2007) e reduzirem a necessidade de aplicação de acaricidas, as vacinas apresentaram eficácia variável contra as diferentes populações de *R. microplus* e somente a GAVAC continua sendo comercializada no continente americano (DE LA FUENTE *et al*., 1999; GUERRERO; MILLER; PÉREZ DE LEÓN, 2012).

Apesar de promissores, os resultados obtidos pelos experimentos com Bm86 mostraram que uma vacina baseada nesse antígeno não protege contra todas as populações de *R. microplus* nos diferentes países testados. Com isto, vem sendo mantida a necessidade

de identificar novos alvos que possam contribuir para o desenvolvimento de uma vacina contra o carrapato com eficácia mais ampla. Neste contexto, proteínas conservadas, com reduzida variação antigênica, são potenciais alvos para o desenvolvimento de estratégias alternativas de controle contra esses ectoparasitos, uma vez que podem induzir reações cruzadas contra diferentes espécies de carrapatos (BHOWMICK; HAN, 2020; DE LA FUENTE; CONTRERAS, 2022; PARIZI *et al*., 2012).

As abordagens ômicas (genômica, transcriptômica e proteômica) têm auxiliado na identificação de novos alvos, possibilitando a predição de estruturas proteicas e potenciais epítopos imunogênicos (VALLE, 2018). Recentemente, foram realizadas análises comparativas de genomas de sete espécies de carrapatos duros (*R. microplus*, *R. sanguineus* s. l., *Ixodes persulcatus*, *Dermacentor silvarum*, *Haemaphysalis longicornis*, *Hyalomma asiaticum* e *I. scapularis*). Taxa de cobertura superior a 90% foi alcançada e a maioria dos genes foram identificados, servindo de base para futuras análises de variabilidade e mutações entre e intraespécies. Além disso, foram observadas composições distintas de perfis de patógenos entre as diferentes espécies de ectoparasitos analisados, sugerindo adaptação dos carrapatos ao meio que habitam (GULIA-NUSS *et al*., 2016; JIA *et al*., 2020; XU *et al*., 2017). Adicionalmente, uma nova análise do genoma de *I. scapularis* foi realizada, levando ao sequenciamento de 2,23 Gpb, e também permitindo a identificação de sequências bacterianas que auxiliam na anotação do genoma de patógenos transmitidos por esses carrapatos (DE *et al*., 2023). Outro estudo transcriptômico e proteômico mostrou que a transcrição e expressão de proteínas é afetada de acordo com o hospedeiro que o carrapato se alimenta (GARCIA *et al*., 2020). Dessa forma, esses trabalhos fornecem dados para a identificação de potenciais alvos relacionados ao controle desses ectoparasitos, bem como aos agentes patogênicos transmitidos por eles.

1.2 Mecanismos de ação de acaricidas

Os impulsos nervosos são coordenados por potenciais de ação que são iniciados e propagados pelos canais de sódio sensíveis a voltagem (canais Na^+) (DONG *et al*., 2014). Esses canais formam um poro, controlados por ativação (despolarização da membrana) e inativação (repouso), permitindo o influxo de Na^+ através da membrana, sendo direcionados para dentro das células (DONG *et al*., 2014). Pesticidas neurotóxicos são frequentemente utilizados para o controle dos carrapatos (ROMA *et al*., 2014). Os piretroides sintéticos estão

entre os principais acaricidas utilizados para controle de ectoparasitos, uma vez que possuem baixa taxa de toxicidade para mamíferos (DONG *et al*., 2014). Essa classe de ectoparasiticidas atua nos canais Na^+ (KUMAR; KLAFKE; MILLER, 2020) prolongando a passagem de corrente iônica (HEMINGWAY *et al*., 2004; LUND; NARAHASHI, 1983; VAIS *et al*., 2001; VIJVERBERG; VAN DER ZALM; VAN DEN BERCKEN, 1982). Entretanto, mutações de ponto na sequência de aminoácidos desses canais, que levam a insensibilidade de sítio-alvo, já foram descritas em carrapatos resistentes a piretroides sintéticos (VUDRIKO *et al*., 2017) e modificam a estrutura dos receptores, causando alterações que afetam o mecanismo de ação dessa classe de acaricidas em seus sítios de interação alostéricos (ZHOROV; DONG, 2022), sendo denominadas de *knockdown resistance (kdr)* ou *super-kdr* (BUSVINE, 1951; ZHOROV; DONG, 2022).

Pesticidas como lindano, ciclodieno, fipronil e isoxazolina (essa última, a mais recente classe de pesticidas) atuam como antagonistas de canais de cloreto mediados por ácido gama-aminobutírico (GABA-Cl^-). Uma vez ligado ao receptor de GABA, o ectoparasiticida bloqueia a ação do neurotransmissor e assim inativa o canal, inibindo o influxo de cloreto (Cl^-) para as células, resultando na hiperexcitação do sistema nervoso central do artrópode (BLOOMQUIST, 1993, 1994, 2001, 2003; OZOE *et al*., 2010). Os genes que codificam os receptores desses canais foram primeiramente associados a resistência a dieldrina (RDL) em diferentes populações de *Drosophila melanogaster*, devido a ocorrência de mutações de ponto em seus domínios (FFRENCH-CONSTANT *et al*., 1993, 1990, 1991). Estudos demonstraram que o fipronil também apresenta seletividade pelos canais de cloreto ativados por glutamato (Glu-Cl^-) (NARAHASHI *et al*., 2010), que são alvos das lactonas macrocíclicas (EL-SABER BATIHA *et al*., 2020). Uma vez que os canais de Glu-Cl^- estão presentes em artrópodes, mas ausentes em mamíferos, representam um potencial alvo e uma alternativa para o desenvolvimento de acaricidas (CLELAND, 1996; RAYMOND; SATTELLE, 2002).

Outra classe de pesticidas, os organofosforados, tem como alvo a acetilcolinesterase, enzima responsável pela hidrólise de acetilcolina no sistema nervoso central (SNC) dos artrópodes (LUSHCHAK *et al*., 2018; TEMEYER, 2018). A ligação desse acaricida à enzima impede que o neurotransmissor seja degradado em colina e acetato, sendo essa uma inibição irreversível, levando ao acúmulo de acetilcolina na fenda sináptica e ao influxo de Ca^{2+} para as células (CASIDA, 1956; CASIDA; DURKIN, 2013; GALLOWAY; HANDY,

2003; LUSHCHAK *et al*., 2018; NOSTRANDT; PADILLA; MOSER, 1997). Uma substituição na sequência dessa enzima foi descrita em *Anopheles gambiae* resistentes a carbamatos e sensíveis a organofosforados (BINYANG *et al*., 2022). Entretanto, mutações do tipo kdr e na sequência que codifica acetilcolinesterase foram identificadas em *Culex* spp. e conferiram resistência a piretróides e organofosforados, respectivamente (WANG *et al*., 2022). Múltiplas mutações na sequência da acetilcolinesterase já foram descritas em carrapatos e estão associadas a resistência a acaricidas (JYOTI *et al*., 2016).

Formamidinas, como amitraz, atuam como agonistas de receptores de octopamina em artrópodes (JONSSON; HOPE, 2007). O pesticida, ao se ligar ao receptor de membrana ativa os canais iônicos voltagem dependentes, levando ao efluxo de potássio (K^+) e reduzido influxo de Ca^{2+}, impedindo a neurotransmissão, ocasionando parálise seguida de morte (BARON *et al*., 2018). Mutações no gene que codifica os receptores β-adrenérgicos foram identificadas em *R. microplus* e foram associadas a resistência a amitraz, entretanto esses polimorfismos não foram identificados em todas as populações, sugerindo que pode haver outros mecanismos envolvidos na resistência a essa classe de acaricidas (CORLEY *et al*., 2013). Em *R. decoloratus*, o gene que codifica para o receptor tiramina/octopamina é similar ao de *R. microplus* e SNP (polimorfismo de nucleotídeo único) foram associados com resistência a amitraz. Além disso, foi observado que as populações de carrapatos das áreas de alta resistência a acaricidas apresentaram diferenças no padrão de mutações quando comparadas aos carrapatos de áreas de baixa pressão (VUDRIKO *et al*., 2022).

1.2.1 Singânglio, neuropeptídeos e receptores acoplados a proteína G

O sistema nervoso é o conjunto de nervos que permite com que o organismo coordene e responda a estímulos oriundos do meio interno e externo (DONG *et al*., 2014). Nos carrapatos, essa massa de nervos é denominada singânglio e representa o SNC desses parasitos (LEES; BOWMAN, 2007). Entretanto, o conhecimento acerca da neurobiologia e dos potenciais alvos para controle, presentes no singânglio, ainda é escasso, em parte devido a quantidade de amostra necessária para realização de experimentos (EGEKWU *et al*., 2014). Apesar disso, os avanços na tecnologia permitiram a realização de trabalhos com menores quantidades de amostra. Nesse sentido, estudos ômicos já foram realizados em *Ixodes ricinus* (RISPE *et al*., 2022), *I. scapularis* (EGEKWU *et al*., 2014), *R. microplus* (GUERRERO *et al*., 2016), *Dermacentor variabilis* (BISSINGER *et al*., 2011) e *R.*

sanguineus s. l. (LEES; WOODS; BOWMAN, 2009), e sugeriram uma relação de genes transcritos no SNC e seus receptores com a regulação da neurotransmissão e influência na fisiologia dos artrópodes.

A regulação fisiológica do organismo, os estímulos e a regulação dos movimentos são influenciados pelo singânglio, através da síntese e secreção de moléculas, como neuropeptídeos (LEES; BOWMAN, 2007; RISPE *et al*., 2022; ŠIMO *et al*., 2013; ŠIMO; PARK, 2014). Em insetos, os neuropeptídeos atuam como reguladores do comportamento e possuem funções pleiotrópicas influenciando no comportamento social, alimentação, reprodução, ritmo circadiano, linguagem e aprendizagem (SCHOOFS; DE LOOF; VAN HIEL, 2017). Esses peptídeos regulatórios são sintetizados como precursores, os preproneuropeptídeos, e se tornam ativos e funcionais após o processamento, com remoção do peptídeo-sinal, e através da clivagem por convertases em sítios proteolíticos, os peptídeos maduros (DONOHUE *et al*., 2010; VEENSTRA, 2000). Essas moléculas sinalizadoras podem atuar como neurotransmissores, neuromoduladores e/ou neuro-hormônios (BURBACH, 2011), tendo atividade intrínseca, em células vizinhas, ou extrínseca, quando são transportados a outros órgãos e tecidos-alvo através da hemolinfa (ŠIMO *et al*., 2013).

A expressão de uma rede de neuropeptídeos foi descrita em *Rhipicephalus appendiculatus* e foi demonstrado que a síntese desses peptídeos pode ocorrer em diferentes locais, por neurônios presentes tanto no SNC quanto no sistema nervoso periférico, e também por células neuroendócrinas (ŠIMO *et al*., 2009). Análises neuropeptidômicas, mostraram que as sequências precursoras de neuropeptídeos de *I. scapularis* são similares a sequências de insetos (NEUPERT *et al*., 2009). Além disso, precursores de neuropeptídeos também foram descritos no singânglio de *D. variabilis* (DONOHUE *et al*., 2010). Segundo CHRISTIE (2008), foram identificados 80 neuropeptídeos em carrapatos ixodídeos.

Os neuropeptídeos se ligam a receptores específicos, atuando em células-alvo (SCHOOFS; DE LOOF; VAN HIEL, 2017). Essas proteínas são denominadas de receptores acoplados a proteína G (GPCR) e possuem domínios transmembrana, sendo responsáveis por regular diferentes estímulos, através de cascatas de reações que envolvem segundos mensageiros (Ca^{2+} ou adenosina 3,5-monofosfato cíclico – cAMP), além de proteínas quinases, tendo atividade na fisiologia do organismo, através da transdução de sinais, a partir da ligação com aminas biogênicas, hormônios peptídicos e neuropeptídeos, dentre outros (HILGER; MASUREEL; KOBILKA, 2018; PIETRANTONIO *et al*., 2018; SCHOOFS; DE

LOOF; VAN HIEL, 2017). Os GPCR necessitam de ligantes para se tornarem ativos, mudando sua conformação e levando à transdução de sinal, entretanto alguns podem ter atividade endógena, sem a necessidade de proteínas ligantes ou possuírem capacidade proteolítica e auto ativadoras (PIETRANTONIO *et al*., 2018). Além disso, são considerados potenciais alvos para fármacos (HILGER; MASUREEL; KOBILKA, 2018).

Em carrapatos, os GPCR já foram identificados no singânglio de *D. variabilis* e *R. microplus* (DONOHUE *et al*., 2010; GUERRERO *et al*., 2016). Diferentemente dos insetos e crustáceos, poucos neuropeptídeos e seus receptores de carrapatos possuem caracterização funcional (DICKINSON; QU; STANHOPE, 2016; SCHOOFS; DE LOOF; VAN HIEL, 2017). Em *R. microplus* foi mostrado que os transcritos que codificam receptores do neuropeptídeo do tipo leucoquinina estão presentes em todos os estádios de vida do carrapato (HOLMES *et al*., 2000). Recentemente, o silenciamento de receptores de quinina foi associado a reduzido ganho de peso, diminuição na eclosão das larvas e aumento da taxa de mortalidade das fêmeas (WULFF *et al*., 2022a).

O estudo de proteínas presentes no singânglio, bem como dos neuropeptídeos e seus receptores, além de contribuir para o aumento do conhecimento sobre a fisiologia dos carrapatos, pode dar suporte ao desenvolvimento de estratégias alternativas baseadas em novos alvos para controle de parasitos (PIETRANTONIO *et al*., 2018). O detalhamento acerca dos neuropeptídeos presentes em *R. microplus* será descrito no capítulo 1, o potencial uso desses peptídeos, bem como de outros alvos para controle, será descrito no capítulo 2 e, por fim, os principais mecanismos de resistência de acaricidas comercialmente disponíveis serão abordados no capítulo 3.

2. Objetivos

2.1 Objetivos gerais

Analisar potenciais alvos presentes no singânglio para controle dos carrapatos ixodídeos.

2.2 Objetivos específicos

- Identificar sequências codificadoras de precursores de neuropeptídeos em um transcriptoma órgão-específico de *R. microplus*.
- Analisar *in silico* e caracterizar as sequências precursoras de neuropeptídeos.
- Identificar e analisar de forma comparativa sequências de neuropeptídeos em outras espécies de carrapatos duros.
- Descrever potenciais alvos de controle no singânglio dos carrapatos e os mecanismos de resistência de acaricidas.

3. Resultados

Esta seção da tese foi dividida em três capítulos. O primeiro capítulo é constituído por artigo científico com resultados que mostram a identificação e caracterização *in silico* de precursores de neuropeptídeos em *R. microplus* e outras espécies de carrapatos. Esse artigo foi publicado na revista *Tick and Tick-borne Diseases*, v.13, p. 101910, 2022. [doi: 10.1016/j.ttbdis.2022.101910]

O segundo capítulo é uma revisão bibliográfica caracterizando o singânglio e os neuropeptídeos como potenciais alvos para controle dos carrapatos. Esta revisão foi publicada na revista *Tick and Tick-borne Diseases*, v. 14, p. 102123, 2023. [doi: 10.1016/j.ttbdis.2023.102123]

O terceiro capítulo é uma revisão bibliográfica sobre os principais mecanismos resistência a acaricidas. Esta revisão foi publicada na revista *Acta Scientiae Veterinariae*, v. 51, p. 1900, 2023. [doi: 10.22456/1679-9216.128913]

3.1. Capítulo 1

Neuropeptides in Rhipicephalus microplus and other hard ticks

Artigo científico publicado na revista *Ticks and Tick-borne Disease*s, v. 13, p. 101910, 2022. [doi: 10.1016/j.ttbdis.2022.101910]

Jéssica Waldman, Marina Amaral Xavier, Larissa Rezende Vieira, Raquel Logullo, Gloria Regina Cardoso Braz, Lucas Tirloni, José Marcos C. Ribeiro, Jan A. Veenstra, Itabajara da Silva Vaz Jr.

Nesse artigo, realizei a análise *in silico* do transcriptoma, análise e anotação dos genes, escrita e revisão crítica do artigo.

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Original article

Neuropeptides in *Rhipicephalus microplus* and other hard ticks

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ABSTRACT

The synganglion is the central nervous system of ticks and, as such, controls tick physiology. It does so through the production and release of signaling molecules, many of which are neuropeptides. These peptides can function as neurotransmitters, neuromodulators and/or neurohormones, although in most cases their functions remain to be established. We identified and performed *in silico* characterization of neuropeptides present in different life stages and organs of *Rhipicephalus microplus*, generating transcriptomes from ovary, salivary glands, fat body, midgut and embryo. Annotation of synganglion transcripts led to the identification of 32 functional categories of proteins, of which the most abundant were: secreted, energetic metabolism and oxidant metabolism/detoxification. Neuropeptide precursors are among the sequences over-represented in *R. microplus* synganglion, with at least 5-fold higher transcription compared with other stages/organs. A total of 52 neuropeptide precursors were identified: AGP, achatin, allatostatins A, CC and CCC, allatotropin, bursicon A/B, calcitonin A and B, CCAP, CCHamide, CCRFamide, CCH/TTP, corazonin, DH31, DH44, eclosion hormone, EFLamide, EFLGGPamide, elevenin, ETH, FMRFamide myosuppressin-like, glycoprotein A2/B5, gonadulin, IGF, inotocin, insulin-like peptides, iPTH, leucokinin, myoinhibitory peptide, NPF 1 and 2, orcokinin, proctolin, pyrokinin/periviscerokinin, relaxin, RYamide, SIFamide, sNPF, sulfakinin, tachykinin and trissin. Several of these neuropeptides have not been previously reported in ticks, as the presence of ETH that was first clearly identified in Parasitiformes, which include ticks and mites. Prediction of the mature neuropeptides from precursor sequences was performed using available information about these peptides from other species, conserved domains and motifs. Almost all neuropeptides identified are also present in other tick species. Characterizing the role of neuropeptides and their respective receptors in tick physiology can aid the evaluation of their potential as drug targets.

1. Introduction

The synganglion is the central nervous system (CNS) of ticks, a highly condensed and fused nerve mass, localized in the anterior ventral region of the body (Sonenshine and Roe, 2013). Among Prostriata, Metastriata and Argasidae ticks, the synganglion presents the same basic arrangement (Lees and Bowman, 2007). In addition, it is not strongly modified by blood acquisition, having nearly the same size in unfed and fed females. On the other hand, the synganglion is impacted by the drop-off from the host, when apoptosis is observed 72 h post-detachment (Freitas et al., 2007; Lees and Bowman, 2007). The synganglion produces numerous neuropeptides that control various internal organs and physiological processes, acting either as hormones or through peripheral innervation (Lees and Bowman, 2007; Šimo et al., 2013b; Sonenshine and Roe, 2013). These neuropeptides act as neurotransmitters, neuromodulators and/or neurohormones (Burbach, 2011).

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In insects, neuropeptides affect social behavior, feeding, reproduction, stress and addiction, circadian rhythms, learning and memory (Schoofs et al., 2017). When axonal delivery of neuropeptides occurs, the same peptide may affect different organs and have distinct effects. For instance, in *Rhodnius prolixus* (Lange et al., 2012), myoinhibitory peptide inhibits hindgut contraction, as well as salivary gland contraction and saliva secretion, thus possibly regulating the digestive process, while a role in the secretion and transport through the ejaculatory duct in males has also been suggested (Lange et al., 2012). Glycoprotein hormone A2/B5 (GPA2/GPB5) is released into the hemolymph after a blood meal in the mosquito *Aedes aegypti* and regulates the activity of V-type H^+-ATPase and P-type Na^+/K^+-ATPase transporters to balance the Na^+/K^+ levels (Paluzzi et al., 2014). Meanwhile, in ticks, knowledge about physiological roles played by neuropeptides is still very limited, since few peptides have been identified or functionally characterized. In *Ixodes scapularis*, myoinhibitory peptide inhibits and SIFamide stimulates hindgut (Šimo and Park, 2014). In addition, both these neuropeptides and elevenin innervate the salivary glands, possibly controlling salivary secretion (Šimo et al., 2013a, 2009; Kim et al., 2018).

Rhipicephalus microplus parasitizes livestock and wild ruminants from subtropical and tropical regions (McCoy et al., 2013). Apart from the damage caused by parasitism, such as anemia and reduced weight gain and milk production, this tick is also a vector of disease-causing *Anaplasma* bacteria and *Babesia* protozoa (Jonsson, 2006). Until now, *R. microplus* control strategies have been based on the use of chemical acaricides. Among the main targets are ion channels, like voltage-gated sodium channel (synthetic pyrethroids), GABA and glutamate-gated chloride channels (phenylpyrazoles and macrocyclic lactones) and acetylcholinesterase (organophosphates) (Baffi et al., 2007; Bloomquist, 2003, 1994, 1993; Kumar et al., 2020; Temeyer et al., 2010). In fact, the synganglion is the target of the majority of the currently used acaricides (Narahashi, 2002; Roma et al., 2014). However, *R. microplus* has become resistant to almost all of the available ectoparasiticide classes (Guerrero et al., 2012; Klafke et al., 2017). At this point, identification of new molecules with acaricidal activity and/or new biochemical targets are required. In arthropods, G protein-coupled receptors (GPCRs) have been suggested as a new target for control (Guerrero et al., 2016; Ngai and McDowell, 2017; Xiong et al., 2020). Neuropeptides are the main ligands of GPCRs, which in ticks are found both in the CNS and the periphery (Guerrero et al., 2016; Veenstra, 2016a). Thus, the understanding of tick physiology and neurobiology may be helpful to find new targets that interfere in salivation, digestion, elimination of sodium after a blood meal, or reproduction. Consequently, these targets could be useful to promote the development of alternative methods or strategies for tick control (Bendena, 2010; Caers et al., 2012; Gough et al., 2017).

This study aimed to identify and characterize neuropeptides in *R. microplus* and other tick species by an *in silico* approach using tick transcriptome and genome databases. Tick neuropeptides were identified based on comparison with coding regions, protein domains and similarity with neuropeptide sequences from other arthropods. Transcripts of 52 neuropeptide precursors were identified: ACP, achatin, allatostatins A, CC and CCC, allatotropin, bursicon A/B, calcitonins A and B, CCAP, CCHamide, CCRFamide, CCH/ITP, corazonin, DH31, DH44, eclosion hormone, EFLamide, EFLGGPamide, elevenin, ETH, FMRFamide myosuppressin-like, glycoprotein A2/B5, gonadulin, IGF, inotocin, two insulin-like peptides, iPTH, leucokinin, myoinhibitory peptide, NPF 1 and 2, orcokinin, proctolin, pyrokinin/periviscerokinin, relaxin, RYamide, SIFamide, sNPF, sulfakinin, tachykinin and trissin. Mature peptides were predicted based on conserved domains, motifs and post-translational modifications, characteristics of each neuropeptide.

2. Materials and methods

2.1. Ethics statement

This work was handled in accordance with the ethic and methodological guidance, in agreement with the International and National Directives and Norms by the Animal Experimentation Ethics Committee of Universidade Federal do Rio Grande do Sul (UFRGS) (project 14403).

2.2. Animals

Rhipicephalus microplus ticks from a laboratory colony (Porto Alegre strain, Porto Alegre, Brazil) were used to infest a Hereford calf, which was brought from a naturally tick-free area. The calf, maintained in an insulated pen, was infested with about 20,000 10-day-old *R. microplus* larvae (Reck et al., 2009). After 21 days, 20 ticks that were manually (partially engorged female) or naturally (fully engorged female) detached from the calf were collected for dissection.

2.3. Synganglion RNA extraction, cDNA library construction and sequencing

Initially, ticks were washed with 70% ethanol and then the synganglion was dissected using a scalpel blade and fine-tipped forceps. Synganglia were washed in ice-cold phosphate-buffered saline pH 7.2 and then immersed in TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was extracted following TRIzol manufacturer's instructions. RNA purity and integrity were checked by PicoGreen® dsDNA Quantitation Reagent and Kits (Invitrogen, Carlsbad, CA, USA). A total of 10 μg of RNA were used to prepare the cDNA library, using the Illumina TruSeq™ RNA Sample Preparation Kit (Illumina, San Diego, USA) according to the manufacturer's recommendations. The current synganglion RNA-seq was performed on Illumina HiSeq 1000, at the same time as other *R. microplus* organs/stages (Tirloni et al., 2020).

2.4. Synganglion transcriptome analysis

Synganglion transcriptome analysis was performed as described previously (Karim et al., 2011; Ribeiro et al., 2014). The raw reads were first quality-filtered by removing Illumina adaptor sequences and low-quality bases and then assembled with Abyss software (*k*-values vary from 50 to 95 at five-fold intervals). Since Abyss software may miss highly expressed contigs, the Trinity assembler was also used on the raw data. The resulting assemblies were merged by an iterative BLAST and CAP3 assembler (Karim et al., 2011).

To extract the coding sequences (CDS), we used an automated pipeline that is based on (i) sequence similarity to known proteins, or (ii) the identification of the larger open reading frame (ORF) containing a signal peptide from each contig. The presence of a signal peptide was evaluated by SignalP software version 5.0 (Almagro Armenteros et al., 2019). These results were combined and redundant sequences were removed. The subsequent CDS and their protein sequences were mapped into a hyperlinked Excel spreadsheet. Other protein features, such as transmembrane domains, furin cleavage and glycosylation sites were determined using the tools from the Center for Biological Sequence Analysis (http://www.cbs.dtu.dk/services/). To automate a functional annotation of proteins, we used the transcripts' matches to several databases, e.g., Gene Ontology, Pfam, Swissprot, KOG, SMART, Refseq-invertebrates and Acari [organism] protein sequences, which were obtained from GenBank. Manual annotation was performed as detailed previously (Karim et al., 2011). In addition, tick genomes (Barrero, et al., 2017; Jia et al., 2020) were used as reference to estimate the assembly quality transcriptome completeness using BUSCO v. 4.1.3 (Seppey et al., 2019). Transcript abundance was estimated by mapping the reads back into the CDS, using BLASTn with a word size of 25 (-W 25

switch); a maximum of two gaps and a minimum score equal to the best score were established as cut-off. From this result we obtained the number of reads for each CDS, considering the synganglion library as well as the other *R. microplus* organs/stages libraries. Additionally, the average, maximum and minimum CDS read coverage were determined for each CDS. The chi-square test was used for statistical analysis (using the number of reads per CDS). Significance was assigned if $p < 0.05$ and the minimal expected read value for the CDS had five or more reads. Reads mapping and RPKM values were included in an Excel spreadsheet. The RPKM values were used to represent relative expression. Values were normalized calculating Z-score and used to generate heatmaps using the heatmap2 function from the ggplot2 library in R.

2.5. Neuropeptide predictions in R. microplus and other tick species

Initially, neuropeptide precursors from other arthropods (sequences from GenBank or FlyBase databases) (Supplementary Table 1) were used to search for putative *R. microplus* neuropeptides in the *R. microplus* transcriptome, including ovary, fat body and synganglion (from partially and fully fed females); salivary glands and digestive cells (from partially fed females); digestive cells (from fully fed females); and embryos. Searches were performed using BLASTx, tBLASTx or tBLASTn tools.

The majority of the *R. microplus* neuropeptide precursors were identified using the following parameters: E-value $\leq 1 \times 10$ e ^{10} and the presence of conserved domains or motifs (analyzed by InterProScan, Prosite and NCBI-CDD) characteristic of neuropeptides. However, for very small neuropeptide sequences, less stringent conditions were used.

In addition, the remaining sequences, i.e. those absent in aforementioned annotated databases, were identified using Sequence Read Archives (SRAs; SRR1186998, SRR1187005, SRR1187007, SRR1187010, SRR1187012, SRR1187013, SRR1187017, SRR7754368, SRR7754369, SRR7754370, SRR7754371, SRR7876048, SRR7876049, SRR13614645, SRR13614646, SRR13614647 and SRR13614648). The SRA files were downloaded using the SRA toolkit package (https://www.ncbi.nlm.nih.gov/sra/docs/toolkitsoft/) and searches were performed via the tBLASTn tool using tick and other arthropod sequences. The carboxyl-terminal amidation at glycine residues was predicted by homology to known arthropod neuropeptide precursors. Sequence alignments and similarity were assessed using the ClustalW tool with default parameters in BioEdit 7.2.5 software (Hall, 1999) and the presence of signal peptide was also verified.

In other arthropods, genes coding CCH/ITP (Dircksen, 2009), calcitonin A and B (Veenstra, 2014), EFLamide and EFLGPPamide (Veenstra et al., 2012) are alternatively spliced. Therefore, we performed manual searches in homologous tick sequences to identify alternative spliced transcripts.

Finally, the neuropeptide sequences identified in the *R. microplus* transcriptome were used to search for the gene sequences in different tick species, namely *R. microplus, Rhipicephalus sanguineus* sensu lato, *Ixodes persulcatus, Dermacentor silvarum, Haemaphysalis longicornis* and *Hyalomma asiaticum* (Jia et al., 2020), as well as *I. scapularis* (Gulia-Nuss et al., 2016). tBLASTn tool (E-value $\leq 1 \times 10$ e ^{5}) was used to search for the transcripts in the genome databases.

2.6. Data availability

Raw reads were deposited in the NCBI Sequence Read Archive (Biosample SAMN02463642 and Bioproject PRJNA232001). Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession GHWJ00000000 (the version described is GHWJ01000000 and TSA Database is SRR1187012). Neuropeptide sequences were deposited in NCBI BankIt, under the accession numbers described :ACP OK001352; Achatin BK059528; Allatostatin-A MT506377; Allatostatin-CC OK001353; Allatostatin-CCC OK001354; Allatotropin MT506374; Bursicon_A MT506355; Bursicon_B MT506364; Calcitonin-A_spliced-variant OK001355; Calcitonin-B_spliced-variant OK001356; CCAP OK001357; CCHamide MT506358; CCRFamide OK001358; Corazonin MT506366; DH31-1 MT506372; DH31-2 OK001371; DH44 MT506356; Eclosion_hormone OK001372; EFLamide_EFLamide_spliced-variant OK001359; EFLamide_EFLGGPamide_spliced-variant OK001373; Elevenin MT506367; ETH OK001374; FMRFamide_Myosuppressin-like OK001360; Glycoprotein_A2 MT506375; Glycoprotein_B5 OK001375; Gonadulin OK001361; I-CHH/ITP MT506363; II-CHH/ITP MT506371; III-CHH/ITP MT506370; II-Insulin-like MT506368; I-Insulin-like MT506369; IGF MT506376; iPTH OK001362; Inotocin/Vasopressin MT506361; IV-CHH/ITP_spliced-variant OK001363; IV-CHH/ITP MT506373; Leucokinin OK001364; Myoinhibitory-peptide MT506354; NPF1 OK001365; NPF2 OK001366; Orcokinin1 OK001367; Orcokinin2 MT506362; Orcokinin3 OK001368; Proctolin OK001369; Pyrokinin/Periviscerokinin OK001376; Relaxin OK001370; RYamide OK001377; SIFamide MT506357; sNPF MT506359; Sulfakinin MT506360; Tachykinin OK001378; Trissin MT506365.

3. Results and discussion

A total of 26,309,385 reads were obtained from *R. microplus* synganglion transcriptome sequencing. The assembly generated 94,813 contigs with a minimum length of 150 bp and an average length of 697 bp. After analyzing the primary sequences of the gene fragments, 18,004 CDS were further functionally annotated (Supplementary Table 2) and reads were mapped back into the assembled transcriptome giving a view of expression level for each gene in terms of RPKM. A BUSCO analysis of the predicted proteome of this tick indicated a 51.8% of completeness.

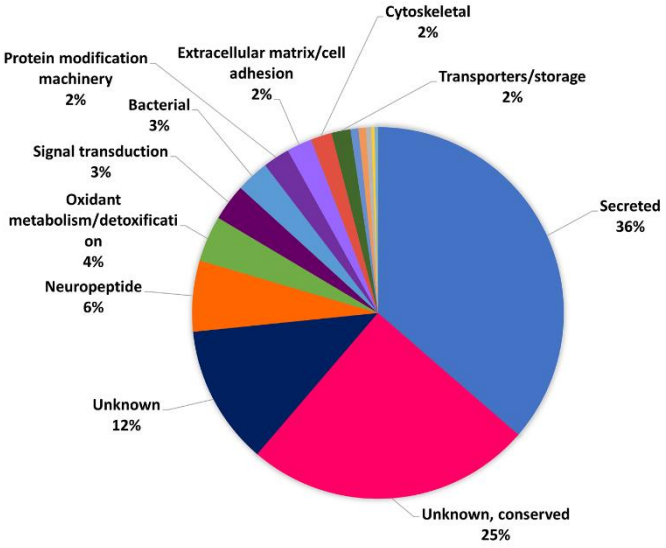
The CDS were annotated based on public databases, as described above, to provide functional information for each sequence. Sequences were classified into 32 categories, of which the most abundant included secreted (29%), unknown (24%), energetic metabolism (8%), followed by unknown conserved, protein synthesis machinery and oxidant metabolism/detoxification (6% each) . Similarly, in the *R. sanguineus* sensu lato synganglion, most CDS were classified as related to cell growth, division and RNA synthesis (27%) and metabolism (15%), unknown CDS corresponding to 34% (Lees et al., 2009).

In a comparison among different *R. microplus* tissues and stages, similar transcription profiles are observed between digestive cells from partially engorged females and fat body and between digestive cells from fully engorged females and salivary glands, while embryo, synganglion and ovary presented distinct profiles (data not shown). Moreover, 166 transcripts were at least five-fold more abundant in the synganglion than in the other organs/stages combined (Fig. 1A). These transcripts highly expressed in synganglion were classified in 17 categories; most of them are related to secreted (36%), unknown conserved (25%) and unknown (12%), followed by neuropeptide (6%) and oxidant metabolism/detoxification (4%). Accordingly, the female *Dermacentor variabilis* synganglion transcriptome showed that, depending on the stage (unfed, partially fed or fully engorged ticks), the transcriptional profile changed, but functional categories remained similar. The main biological functions were related to cellular and metabolic processes, localization and biological regulation (Bissinger et al., 2011).

Similar transcriptional profiles were observed in insect central nervous system. For instance, from the kissing bug (*R. prolixus)*, most transcripts were classified as protein modification, signal transduction, DNA and amino acid metabolic processes, transmembrane transport, ion binding, oxidoreductase and kinase activity (Ons et al., 2016). In the locust *Schistocerca gregaria,* most transcripts were related to primary metabolic process, cellular metabolic process, regulation of cellular process and cellular component organization (Badisco et al., 2011).

Neuronal tissues and endocrine cells are responsible for the synthesis and secretion of signaling molecules such as neuropeptides (Nässel, 1996). In agreement with this conceptualization, data presented here show that neuropeptides represent 0.12% of the total RPKM in *R. microplus* synganglion transcriptome and are a meaningful portion of

A



B

EMB	SG	FB	DIG. P.	DIG. F.	OV	SYN	Neuropeptides
0.22	0.92	0.55	0	0	0	136.68	Allatostatin-A
0.42	0.48	3.22	1.33	3.64	0.55	89.77	Allatotropin
1.49	0.34	0.79	0.21	1.74	0.81	13.31	Bursicon-A
102.37	65.10	144.23	50.76	212.99	70.79	223.64	Calcitonin-B spliced variant
1.14	0.21	0	0.13	0.11	1.40	61.52	CCHamide
0.15	0.92	0.76	0.53	0.99	1.56	35.62	CHH/ITP I
0.77	0.53	0.82	0	0.09	0.17	135.51	CHH/ITP II
1.35	1.31	3.88	0.33	2.47	0.50	30.19	CHH/ITP III
0	0.26	0.53	0	0	0	18.59	CHH/ITP III
0	0.09	0.47	0	0	0	173.30	CHH/ITP IV
10.32	13.65	19.34	7.87	3.79	13.69	307.30	Corazonin
2.67	1.68	3.63	0	0	0.18	373.76	DH31-1
0.68	0.05	0.83	0	0.39	17.61	2.47	Glycoprotein A2
0.75	0.61	0.91	0.09	1.33	1.50	41.60	Inotocin/Vasopressin
0.75	0.17	1.26	0.11	0.60	28.31	4.91	Insulin-like growth factor (aIGF)
0	0.25	0.90	0	0.09	0.89	16.47	Insulin-like I
0.70	0.06	0.34	0	0	0.06	42.26	Insulin-like II
0.22	0.60	0.41	0	0	0.10	73.04	iPTH
4.60	1.70	2.63	0.99	0.57	2.64	183.14	Leucokinin
0	0.20	0.27	0.07	0	0.32	13.92	Myoinhibitory-peptide
1.35	1.31	3.88	0.33	2.47	0.50	30.19	NPF 2
13.67	22.64	22.23	14.43	11.95	33.36	99.12	Orcokinin
0	0.12	0.49	0	0	0.47	240.26	Proctolin
0	0.11	0	0	0	0	3.71	Pyrokinin/Periviscerokinin
2.36	1.07	0.72	0	0	0.77	210.49	SIFamide
0	0.86	0	0	0	0.12	49.29	sNPF
0	0.42	0	0	0	0	42.48	Sulfakinin
0.17	0.62	0.73	9.10	10.65	0.15	71.05	Trissin

Fig. 1. ***Rhipicephalus microplus*** **neuropeptides transcriptome analysis.** A) Pie-chart from CDS that are at least five-fold more abundant in synganglion than in the other organs/stages combined (ovary (OV), synganglion (SYN) and fat body (FB) from partially and fully engorged females; salivary glands (SG) from fully engorged females; embryo (EMB); midgut from partially engorged females (DIG.P) and from fully engorged females (DIG.F)). B) Heatmap comparing neuropeptides transcripts among *R. microplus* organs/stages. The RPKMs of expressed genes in the tissues/stages is shown inside the boxes.

over-represented synganglion CDS (6%), although they are also present in other tissues (Fig. 1B). This work provides an updated list of *R. microplus* neuropeptides. Searches in a previously published transcriptome (Tirloni et al., 2020) allowed to identify the expression levels of 28 neuropeptide precursors in different tick organs. Additionally, a manual search in the respective raw data (SRA files) led to the

Rm_ACP (AKH/CRZ related peptide)
MASVSFKLSAVLLLATLLVMQGAYSQITFSKNWQPGKRSEICSQKEAQAIIKLRHFLLEEAKHLEECRLFYGNEGANED

Rm_Achatin
MSRLERYLVLSALILTNGADHCFAEDAADNQDLFVMNSDPALGQLSANEKENAATAGAYAGGSGGALAEDYDEPSAYERRLLLQGLISKRGFGERR
RRGFGEKKKRGFGEKKRGFGEKRRRTAAAHHSLLAVPSARTPSGARVLLFENPYEGSIP

Rm_Allatostatin-A
MRRFGCSPLQLLRPALAASLALWLLLLLAASEQCRANEAPLGSSVGGGGILPLQQHHPSDKRAGPAPLYSFGLGKRSPLLLMADEPPVDADVDEDEE
DDAMAEAAAASRTGGYLEKRGPREPLRYGFGLGKRRSGHEREYVPYDQEKRERHRFSFGLGKRDKKSKLEDFMKRRYNFGLGKRGIYGDADAGER
WKRSF

Rm_Allatostatin-CC (AstCC)
MAFVLAIIRLSTSLVVTTLLTVCFVPTIGGEPWSDESPGAPAPSSGSSEEDDAVLQSQEEDTLQNLVRLNTILHGDSRAYDLMAPPPSKRSTMLLNKLM
QPLLKAFKSDAEVSYTPPMELRRRKGEGKMFWRCYFNAVSCFRRRK

Rm_Allatostatin-CCC (AstCCC)
MKHIVFISACVLLFLAATGAEVADESGLRGSAVGLGTGGMGGGAMASGGLENLLWNYLMAKQMARQMQREQVDQPPAVPDFQRKRSGWKQCSF
NAVSCFGRRK

Rm_Allatotropin
MAALARAGLLLLLASAVLVGCGGQAADESSAPERQHIRGFQQLRLSTARGFGKRGTLPAIAALLGHIIVALHRLPEPQQPPVIKKGFRNMKISTARGFG
KRADSDTSFLLENDDFDPVDFKNKRDIRRISLSTARGFGKRLSPASSDESYIINAGQPVTTWLAEEMAKGDLNDDGAVYQDTF

Rm_Bursicon_A
MFLTRAVRDRLSRWDSCCGSPTVVHVFLVVVTVMAVPIDCAIGPEESCQLRPVIHVLKQPGCQPKPIPSFACHGSCSSYVQVSGSRYWQVERSCMCC
QEMGEREATKAVFCPKGPGPKFRKLVTRAPVECMCRPGTAPDEASVLPQEFVGL

Rm_Bursicon_B
MTRHSLSSSALTVALWFTTAVVSATWVSASSLDNTGGGVASCRLQETSIRITRDHSDDQGSPVRTCEGTVLVSRCEGTCVSQVQPSITLPHGFLKECN
CCRETYMNRREIQLQDCFDPNGQKLYGADGSMTIFLEEPQDCSCHKCGG

Rm_Calcitonin-A_splice-variant
MVTTTQCKFLAALALVLLCCAMVTTNADYTVDDIRQIVNRRKGLEIIREIVDDLIIRQLTTLIIKRSCIIIDAGLNRGCDYKDIMDAVEENKFWKSRDSP
GRRRRSIEDKATKAATSSQATQDAVVRSVQQAAN

Rm_Calcitonin-B_splice-variant
MVTTTQCKFLAALALVLLCCAMVTTNADYTVDDIRQIVNRRKGLEIIREIVDDLHRQLTTLHKRSDCGPGLLPGQDCIVGNVAGSGQDHDFINGDILP
GRRRRRSTKEA

Rm_CCAP (Crustacean Cardioactive Peptide)
MSTTGLLLFGVLVLSVCLTEAQEKPDDVSEERFEPEQKRPFCNAFTGCGGKRSQYRTRNLMSRLRQRILDASARNHHRSRSQFQGDADSRSALDADE
LRSGAILVNLPSLLRKRRFSMNSP

Rm_CCHamide
MCFQSSLSVLLLCLALLVASAYSAAFGDSVEEGVRDKKIITLLRRNNSCKLYGHSCLGGHGKRSDDGSAPGGAISPDVMQMLRKTSDDEALGPFPPQ
ARFDRVALLEKALIQLLRNRMMV

CCRFamide
MTLTYLCLVSSLLALLLAPSWAGAQPPRCRALCQKDPGEDACERCRLRTFMRFGKRDSSRAIALIREPVLDVQSVLVDDDVDNDRPAGGAAPGGNS
ASREQLVLERPAARPSRDRYGLLLRAIRDVRGRSAD

Rm_I-CHH/ITP
MSAGFLLAPVIICLASALLVCLFMVLTQPLVCVALDIIIIKRSFLELGCRGNFEQSYLAHILERVCEECYQLYQKSEAYNLCRDTCFKNENFDLCAEALL
LKGEMGDLRRMINYLHG

Rm_II-CHH/ITP
MSAGFLVAPVHWLVAALLVCSFMVLTQPSVCAARNLHKRSFHELGCRGNFEQSYLARLERVCEECYQLYQNPEAYNLCRDNCFKNEYFLKCAEALL
LLKDEMDSLKSKVDYLYSR

Rm_III-CHH/ITP
MTASFLMASVRWLAATMVVCSLLLLAQPPVGTASIQIIKRSFLELGCRGNFEQSYLARLERVCEECYQLYQEPKAYNMCRDNCFKNEYFFQCAEALL
LKDEIDSLKSKVDYLYSR

Fig. 2. Predicted structures of neuropeptide precursors. *Rhipicephalus microplus* neuropeptide sequences were predicted and the 52 precursors are presented here alphabetically. Protein identifiers are provided in parenthesis next to the protein name. Signal peptides are shown in yellow, while predicted mature neuropeptides are in dark blue. Processing sites for mature peptides are shaded in red and putative glycine-derived C-terminal amidation sites in turquoise. Cysteines are shaded in pink.

Rm_IV-CHH/ITP
MSASFPVVSARWLAAALLACSLVALTQPGGCAARILHKRSFVELGCRGNFEQSYLARLERVCEECYQLYREPQVYNLCRENCFKNENFLKCAEALLL
KEEMDSLKSKVDYLYSR

Rm_IV-CHH/ITP_splice-variant
MSASFPVVSARWLAAALLACSLVALTQPGGCAARILHKRSFVELGCRGNFEQSYLARLERVCEECYQLYREPQVYNLCRGSCFKNENFDLCADALLL
KDEMTDLRRMINYVYG

Rm_Corazonin
MSRIVATFGLLLFNLVMIVHCQTFQYSRGWTNGKRRDGPIVAVPSRITADHRLLEEFLSKFAPKDRVIVERLGHLLRTLDRNEDEQEY

Rm_DH31-1
MVRSVSVAILVLVAVVLLARASSSAPANFETESWDRDRQNAVYDSSLTPDEDYLLSLLRSRSSNFDVMPKRSRGMLDFGMTRGASGARAAKARLGL
KLANDPFGPGR

Rm_DH31-2
MQLGLPGATWILILAVAAIAASSPARSRPKRDVYYDSSLDAGEEYLLGLLRNRLSHPAVLEQQKRAGGLLDFGVSRGASGAEAAKARLGLKLAHDP
YGPGRR

Rm_DH44
MHPVGPWLVLVLLASEAALGARVGGLDLSDLWKRGPLALRSRPLPSLSMHNRDHMPSLSIVSPLDVLRDKMMQDIIERSIKNKIQANDKILKDLGKR
SDRSTFKESSEELL

Rm_Eclosion hormone
MAWKPQLNWACVTLAVAHLVAARISTRLPEATMICIENCGQCKIMYGEYFNGRECAEECVSTVGYIQPDCDIADTIVKYLRRKP

Rm_EFLamide_EFLamide_splice-variant
MSCLHLLALAVLATCGFFCVVHANMEAKRMGSEFLGKRSPSSRLDASDGLPGGFWEEELVARSPAAALRAARLLRAAAGVSRFGGGAYDNDDDDD
FDLAMAAAAKQKRIGSEFLGKRSSPSDLLPKRMGSEFLGRRKRDAFYSSPDA

Rm_EFLamide_EFLGGPamide_splice-variant
MSCLHLLALAVLATCGFFCVVHANMEDSDGALSRSLQQLYAGDSPFGRSRGYGHDLDWILGHHRARPNKRLSEFLGGPGKRYSEFLGGPGKRYSEF
LGGPGKRYSEFLGGPGKRSVADDVMAAAAERGLLHSAEQQFPGSAASTLRK

Rm_Elevenin
MSRSLFSAQALWSCVCLTVLVGSLLADAELDCRKYPFYYKCRGIGAKRSYVPYSKAEPVSLKELYNDEDEPKNRQKTPEAMLSWIRDRYGDELLDA
YERRQLTDGSFERKPTF

Rm_ETH
MKPGRALRGWLGFEALMKLCCCLALLGVSQGQFFSKTTNTIPRMGRRDLDYAQLEEPRMGLFRAIPARQSASMVDLGYLDGVTEDRAREKQVDGC
HGGRCRAAVLHALQTMDDV

Rm_FMRFamide_Myosuppressin-like (+XP_037272417.1)
MKVVLLLCIVGCYSGSILVCTAEDAKLLSQPEQSTSPPTPLGNSPHVADGARHASDGVSSETPGALNRVSRAARAFDDASGSVDDYRVLRAALNPPR
RHRFLHFGRKRALLAPYYGSPALSSVATASGEEEAEWSGGEDEDDDEVTTSQEGPEAAALQWAKRTLRDGPNSFLHFGEREPEEEGSGGEQLRFKRN
AQEAKSLEAAHMLNTGAEGFEAFNLQAESQDSDNLVPASKTKRSPNHIMHFGKRLQTASTVDENDGVKRGSHNSIMHFGKRNNQQDVDAILDNVY
STMDGTSGKRGANRMMHFGKRDPKYGLTTGLEGEPDLDFVSADKRAPNRILHFGKRPSSSSSSGEGDGDVEEAEKDKRGNRIMHFGKREDEDLSGM
SLSSMNDASTAAVKRSASARSKYSGRPDQLQPFSGIGRQLQRQFQEWKKRNRILHFGKREPQRSVSYYQQKRLGNRIMHFGKRAGYPFNADGASSA
HAAMKDKKLKHSILHFGKREDGIVSVMGDDKRNRIMHFGKRIGNDDQEYQYGQQLPMDKRAGNRILHFGKRLQAPSSPSTWDYGIDAAAETSRTA
ANKRAHRILHFGKRDSGANYGDGGVESNPVVLAQHLDEADASKVSGGHGQPETQNVIRKKRSTPPVESSDYIDDTLAQMMGELMGEDGYPDRITM
GHSGLSGPLHLPHALTQLSSG

Rm_Glycoprotein_A2
MKCSEYWQKMARKHQCATIVLAMLALAVGEANFWERPGCHKVGHTRRVSIPECVEFDITTNACRGFCTSYSIPSPEFTLRMNRNQRVTSFGQCCNIM
DTEDVKVQVRCLDGHRDLVFKSAKSCACFHCKKN

Rm_Glycoprotein_B5
MRTVAVPVLVLCWLLVASAVASTPRHDHENELSSSSMLKGGTSAVRGGDVAVDTLTTIECHRREYSFRATRTDGNGNRCWDDVTAMSCWGRCDS
GEIADWRFPFKKSFHPVCTYGSRKLVRAQLRFCDPDDLDEGDELRAYEYYEALSCSCQVCDSTWTSCEGFRHP

Rm_Gonadulin
MRLVFAIAVFVVAVGPIYAEWIDLHKPVRICSATMLVEVINNVCQTPLTAARRRRRSLRDSDEMIDGVVRSTRFSMEKHAIKLPPNLVRDFANTCCYT
PCRPYRFIDIC

Rm_IGF (Insulin-like growth factor)
MALARKLVLTLSLIGIGPWASVTAVGAGLSDAPAIRLCGRRLADLVWMICMDRGGVHSHMDRRALSTVRRPFHYRPSPMRRDGGTQGRSGDDDGA
TSAEAVATTIAPEPAANSARYHSSQGQYSSGGIVEECCRKPCSFSTLASYCARPSDSSSLDTFNLVASSGSSSSASASSSSSSSESGAQHERQQPSPLIQPP
EPSTTTPPRVVASSRLQTAHEVDREHNEVDNAGPHRRLGAVSRHTTGRALLPSMPFLTSPEDDAPFATRPRIGTFSRHRPYWYVVQAAFNGDADDER

Rm_Inotocin/Vasopressin

Fig. 2. (*continued*).

MERRRYLKMTPLNFLMLGLVGMTSACFITNCPPGGKRSSEPAAVRMCPRCGPGGRGVCYSADVCCTNSACVINDPLLALPCRAESLHSHACQVPGK
RCGAVAQGRCAMRGFCCGADGCRRDESCSNDASTDQFGTSVDMLEYGVSRR

Rm_I-Insulin-like
MTRTTTRLLLPLLLVLPLLADASSPAQTSSQRRCGHVLREFMEFVCPGVIYDPYESAALKRALFGQRFLMVGEKSPSMGFLQPEMAHQLLFKRNSQG
GIVFECCYKACSIVEAQSYCPS

Rm_II-Insulin-like
MASASPSTASVAGCSLLLLLLFALTASSLIEKRTRFCGPFLVETLNALCRGEIFDPAQQKRHIDRQQMALQPLLSPWVTESRLGFLDAKTALQLLRPAS
HRQVRGIIEECCHKPCAVAELLSYCGRVRPTGTDE

Rm_iPTH
MMAGSKLTLLVFLFAVVVLPAVVNCRSLFHRDRRSGISDQRLAELETLAGLKSLRHRLKGITFPVAYGLVDPNKIGKRKRSFESQEETQQPQQQSQSD
RQAGATDASSSSSLYQNIVDDAALEDYLTGLPTPDVTAQRVRSPDQLLRI

Rm_Leucokinin
LHRRGAAGANFTVGESLVLLLVLGVIAFPSDAWAQHVVGSDEARSLLSRGGDTLIRWNISPATLQHMRSESFKRQFSPWGGKRNAAAFESLLDSAGN
QESHRHRLAADASYKVRRLHPVDIAVRAADLFSPWGGKRTDDKKDKDQTFNPWGGKRAGDHFGSWGGKRDTFSAWGGKRQQDSKNAFSPWGGKS
RAVRSPTARNDAARAKQGDGEEDEERSFAPWGGKRGTGEDQAFSPWGGKRGDDGDTSFTPWGGKREDRFNPWGGKRFGPFSPWGGKRDGSNKEG
FFNPWGGKRGADDPFNPWGGKRQDSFNPWGGKKEDGVFRPWGGKKEDNVFRPWGGKKEGNVFGSWGGKREDATPSLSVGRALNYGGPASHDVD
AESLRKKRDSSASEQKGTTLNGEKSGKT

Rm_Myoinhibitory-peptide
MASSSVPSGVWMLALLVLGSLLGVLGEPQAGGDWNSLSGMWGKRASNDWNRLSSMWGKRGPYVPYQALLLRAGESGEIAGHGISARAAGPAALR
ENHWNDLSGYWG

Rm_NPF1
MGAAGRSRMAVTMSLALLVALLVLQMTAVLARADPEEVGGDVVEALRLLGHLDKYYSHVNRPRFGRSVPVRFAGGSTSSSSDEYER

Rm_NPF2
MTPSTLVTMLMVLVASASVSVANMGESQRPNMPHVFQNEKQLSQYLQALDDYYLLLGKPRFGRSFQDSYEVKRWNLPYTELLRTV

Rm_Orcokinin1
MRKLVPGFLLVVVFVFSSLNDARCDESGGGLGPSAASKGAGRLDKLSGRHQLDRLSGGELLRSLVLPYVLRGLQYSPDYVSRRSSAKPGSGEHMRT
AGAFLPGSLPVKRQAPFDSLSGLTFGGDQGGVHKRGYGHGEFDEIDNAGWPGFYKRNFDEIDRSDFGGFYKRNFDEIDRTGFEGFYKRSTSRQ

Fig. 2. (*continued*).

identification of 24 additional neuropeptide sequences (Supplementary Table 3 and Figure 2).

Genome analysis of seven tick species led to the identification of neuropeptide precursors gene sequences in *R. microplus, R. sanguineus* sensu lato, *I. persulcatus, D. silvarum, H. longicornis* and *H. asiaticum* (Jia et al., 2020), as well as those present in *I. scapularis* (Gulia-Nuss et al., 2016) (Supplementary Table 4). The majority of the neuropeptides were identified in all genomes, which indicates that neuropeptides are conserved among the tick species analyzed. However, it was not possible to identify all neuropeptide sequences in the published genome assemblies (Gulia-Nuss et al., 2016; Jia et al., 2020): allatostatin CC and CCC and ACP were not identified in the *R. microplus* assembly, even though transcripts for these peptides were found. Similarly, allatostatin CC and ACP were not identified in *H. asiaticum* and *R. sanguineus* sensu lato, respectively. On the other hand, the absence of sNPF-2 from *R. microplus* transcriptome is not unexpected, since sNPF-2 seems to be a gene duplication specific to a few tick species.

In insects, metamorphosis and reproduction are controlled by juvenile hormones (JH). Synthesis, function and regulation of these hormones, including the participation of neuropeptides, are better understood in these arthropods than in others (Weaver and Audsley, 2009). In some insects, allatostatin A inhibits the biosynthesis and release of JH (Bendena et al., 2020). Although ticks do not synthesize JH (Neese et al., 2000), elements of JH pathway were identified (Zhu et al., 2016). In contrast, allatotropin may be involved in the positive regulation of JH pathway (Egekwu et al., 2016). Moreover, allatostatin A was hypothesized to have a myoinhibitory activity in ticks, like already reported for insects (Šimo and Park, 2014).

In ticks and in many insects, myoinhibitory peptide (MIP), often called allatostatin B (Coast and Schooley, 2011), acts by inhibiting the contraction of hindgut and visceral muscles (Lange et al., 2012; Šimo and Park, 2014), while SIFamide stimulates their motility (Šimo and Park, 2014). Both neuropeptides innervate salivary gland acini (type II and type III) and were proposed to play a role in controlling the secretion of salivary components, which is analogous to the activity on the hindgut (Šimo et al., 2013a, 2009). Elevenin was also suggested to be involved in saliva secretion during rapid engorgement phase in *I. scapularis* females (Kim et al., 2018). In parallel, similar to MIP, a myoinhibitory activity of allatostatin C was described in the moth *Lacanobia oleracea* (Matthews et al., 2007). In arthropods, this somatostatin ortholog went through a gene triplication, codifying for three peptides, allatostatin C, allatostatin CC and allatostatin CCC (Veenstra, 2016b). In ticks, only the last two precursors were found, suggesting the loss of allatostatin C.

Proctolin is known to act as a co-transmitter stimulating the contraction of skeletal, visceral and cardiac muscles in insects (Orchard et al., 2011; Ormerod et al., 2016). The role of proctolin in ticks remains unknown. Myoactivity was also suggested for FMRFamide, since it was detected in muscles of different tick species, like *Ornithodoros parkeri* and *D. variabilis* (Zhu et al., 1995).

Eclosion hormone is a neuropeptide already described in *D. variabilis* (Donohue et al., 2010), and induces the neuropeptide cascade that leads to pre-ecdysis and ecdysis behavior with the release of ETH and CCAP in arthropods (Gammie and Truman, 1997; Park et al., 2002; Žitňan et al., 1996). In a *Drosophila* model, ETH and CCAP knockout insects showed impaired ecdysis behavior and, consequent lethality, mainly in larval and pupal stage, respectively (Park et al., 2003, 2002). ETH has already been described in several arthropod species, such as *A. aegypti* (Dai and Adams, 2009), *Drosophila melanogaster* (Park et al., 1999), *Manduca sexta* (Žitňan et al., 1996), *Anopheles gambiae* (Holt et al., 2002) and *Tribolium castaneum* (Amare and Sweedler, 2007). Although, a genomic DNA fragment has already been identified in *I. scapularis* (Roller et al.,

Rm_Orcokinin2
MASSVIMLLLVSIALCSIIFNEVRGDESGGGAAASSSPSSKATRTLDKINAGEYIRGLSGRHILDKISGGELLRSSDDDAELLRSLMLPYALRGLSSPSLGS
GLRRSLDKIGGGEYIRATGPFPPGARSAKRFDSLSGLTFGGDQSGLHKRGYGHGEFDEIDHAGWPGFYKRNFDEIDRNGFEGFTKRNFDEIDRTGFEGF
YKRSDARKN

Rm_Orcokinin3
MTSFFGVLLLVTASLCSVLMQVRGDEPGGGQASASSSSKGARTLDKLSGGEYIRSLGGRHILDKISGGELLRSADDAELLRSLVLPYALRGLSPSSLGS
GSRRRGLDKIGGGEYIRATGALPSKRFDSLSGLTFGGDHGGLGKRGYGHGEFDEIDHAGWPGFYKRNFDEIDRTGFEGFYKRSAREE

Rm_Proctolin
MVVYRLLSLVALWALVFVVAEGRYLPTRSGDELRGERLRELIRTLFERAELEKAAGLYPYAGDGRSGMLSGQE

Rm_Pyrokinin/Periviscerokinin
MRRAVVWASCIQLALLYVTFAKAGHESFYDDDSWSLAGGRWGELQKRQGLIPFPRVGRSASAHDLNPDDLIPLDTDSAASGGWAFLLLPYKRRSNT
FTPRIGRKRRSVGRPDDGVIIKEDGGSSRSRQAAWADLDWSYSPLSRQLVPVIRNGRGTFVPRLGKRRSLGSGYGDEIDSGWDTSLEVASFMDDPKRG
SFTPRIGRGAFTPRIGRTPFTPRIGRSSGNQEKNSDGDDKSTPRSSSSSSGVQGNAV

Rm_Relaxin
MLAQWTALWLGFLAAGPRAATANGEAPDWNEIFSNRNDDDWARVWHVERHRRCYHELLSHMSWVCEKDIYKMRRRKRDAPLDKDAADLAEVF
LKPEAALSLLSSSATGKQAGGGRRKSWRRQRLGGRGIMDECCDVQTGCSWEEYAEYCPTSRRIRNRRR

Rm_RYamide
MLNARTWAGALLALLVLSVTSAGKGAPQFVPNGRYGRRSVTPPLAGVSRDLTVNFFGDSSISCAYTGFADIYRCSRKTSEDYKDTSFE

Rm_SIFamide
MQSWRAFLVLGTLLLLAVMANMACAAYRKPPFNGSIFGKRSRGDMNNADIKYAMCEAVWDTCTQWFPLPQDGTQ

Rm_sNPF
MPSPAITRCLVLLLLVQAALAFPDYKDIRDLYELMGKGEQEGSGHAKERKAGYTPSLRLRFGRRSDPAWSEARIWDAQRTV

Rm_Sulfakinin
MQFSARFLLFVLVAIIAAASSALGYSASNQVSSQQQQQQQQQRHRINVGRWLKSMLPAAAAAAASAGDTDSRNTADLDTADMIDPVLLASGFAKRQE
DDYGHMRFGRSDDYGHMRFGRK

Rm_Tachykinin
MHHTEMKALALCSLVLLLTLVSAQGASYQSSEASGGGEGGQLGDVLLEEHMGWADGGGPDEEQDAALESKRAFHAMRGKKDDDDAWDSEVKRA
FHAMRGKRLLAPASVDSFIAQLRRAVLQGKRGSGFHGMRGKRQLRNGQKSSRTKFVASRGRRSASGEPAAEAFF

Rm_Trissin
MAKKLGQVLVSASCGMLLLLCALVPRGEASRACNACGPECVTACGTAMFRACCFNYNRKRSGPTTSSSASEIRDMAPSGGGSANLDTTDSATFESRS
EAGDGMAGNGIRQDAWALFWMLPARQRQPGLF

Fig. 2. (*continued*).

2010), this is the first report of a clear presence of ETH in parasitiformes. Furthermore, it is important to highlight that the motif (FFJKXXKXVPRX-NH_2) (Roller et al., 2010) is well conserved among arthropod ETH sequences, where only a few differences in mature peptide sequences were identified (Supplementary Figure 1). Bursicon is a cystine knot glycoprotein responsible for cuticle tanning and slcerotization (post-ecdysis) and is co-localized with CCAP (Dewey et al., 2004; Park et al., 2003). In addition to mimicking aspects of the gregarious phase of migratory locusts (Tawfik et al., 1999), corazonin was also shown to initiate ecdysis behavior in Lepidoptera (Kim et al., 2004). Here, all four neuropeptides (eclosion hormone, ETH, CCAP, bursicon and corazonin) were identified in *R. microplus*. It is interesting to note that bursicon was detected in adult ticks, despite the fact that ticks do not molt again once the adult phase is reached. Although the physiological effects of these neuropeptides have not yet been determined in ticks, it is a reasonable hypothesis that they are associated with cuticle expansion and development (synthesis and sclerotization) during and/or post blood feeding (Bissinger et al., 2011) .

Besides ecdysis, CCAP has also been reported to interact with NPF. Both peptides affected feeding behavior in *Drosophila* and CCAP RNAi induced a reduction in NPF signaling (Williams et al., 2020). On the other hand, in cockroaches, CCAP is produced by enteroendocrine cells and triggers the release of both α-amylase and protease (Sakai et al., 2006, 2004).

Various other peptides and hormones that also affect food intake and energy metabolism in insects act as anorexigenic (inhibits appetite) or orexigenic (stimulates appetite) factors. A relationship between nutritional availability and the peptides sulfakinin, corazonin and periviscerokinin was suggested in *I. scapularis* and *Amblyomma maculatum* (Adamson et al., 2013). Indeed, sulfakinin has been suggested to function as a satiety factor (Meyering-Vos and Müller, 2007). Accordingly, sulfakinin transcription is upregulated in fed *D. variabilis* when compared with the unfed tick (Bissinger et al., 2011). Another neuropeptide involved in feeding regulation is NPF, although its role may be either orexigenic or anorexigenic, depending on the invertebrate species or the type of meal (Fadda et al., 2019). The sNPF neuropeptide is an example of an orexigenic factor. Injection of sNPF in cockroach starved nymphs led to a very significant increase in weight 24 h later, which can only be explained by increased food intake (Zeng et al., 2021). Also, Sudhakar et al. (2020) proposed a positive feedback model between insulin-producing cells and sNPF neurons during short periods of starvation. Whereas ticks have only one CCHamide gene, insects have typically two such genes coding similar peptides, each with its own receptor. CCHamide-2 was described in *D. melanogaster* as a growth regulator depending on nutritional availability (Sano et al., 2015). Feeding induces the release of CCHamide-2 into the circulation, which subsequently modifies feeding behavior (Li et al., 2013) and leads to the release of insulin-like peptides (ILPs) (Sano et al., 2015). Indeed, CCHamide-2 gene knockout led to reduced food intake, locomotion and development, as well as decreased ILP transcription (Ren et al., 2015). Accordingly, transcription of ILP genes is higher in all unfed life stages of *I. scapularis* ticks (Sharma et al., 2019). In addition to its established function as a diuretic peptide (Terhzaz et al., 1999), leucokinin is also related to ILP signaling in *Drosophila*. Increased transcriptional and

protein levels of ILP were observed in flies when the leucokinin precursor gene was knocked out, as well as when the leucokinin receptor gene was absent or inhibited (Zandawala et al., 2018b). As a result, leucokinin mutant flies present a higher resistance to starvation. Furthermore, in *Drosophila*, the inactivation of leucokinin neurons led to an increased abdominal size due to water volume retained in the hemolymph, thus inducing a high frequency of small meals (Al-Anzi et al., 2010; Liu et al., 2015).

Roles in arthropod feeding regulation and digestion were also suggested for RYamide (Mekata et al., 2017; Roller et al., 2016a), and a function in water balance was hypothesized in *Drosophila* (Veenstra and Khammassi, 2017). Trissin is another neuropeptide hypothesized to act regulating gut contractions and food intake in *Bombyx mori* (Roller et al., 2016b). However, its precise role remains to be elucidated. ACP is already known from other arthropods, but is being described for the first time in ticks. In females of the crustacean *Macrobrachium rosenbergii*, the injection of ACP peptide caused an increase in total hemolymph lipid content and a reduction in oocyte proliferation (Suwansa-ard et al., 2016). Interestingly, in the cricket *Gryllus bimaculatus*, researchers observed that AKH and ACP knockdown significantly increased the ingestion of food, but hemolymph lipid level was not affected. Conversely, the administration of ACP induced an increase in lipid and carbohydrates levels (Zhou et al., 2018). More recently, it was shown that ACP is involved in *Locusta migratoria* long distance flight, ACP knockout locusts did not fly as long or as far as the wild type, while the capacity for flight was enhanced by ACP (Hou et al., 2021). Ion balance and water transport are regulated by neurohormones with diuretic and antidiuretic activity. In the mosquito *A. gambiae*, DH44 has a nonspecific role in sodium/potassium transport (diuretic effect), while DH31 stimulates sodium transepithelial transport (natriuretic and diuretic effect) (Coast, 2005). Other effects associated with diuretic hormones were also reported. *Drosophila* DH44 decreases desiccation tolerance, as indicated by reduction of DH44 leading to increased survival during this stress condition (Cannell et al., 2016). DH44 is co-expressed with leucokinin, both neuropeptides modulate diuretic pathways, with effects on fluid secretion. RNAi inactivation of these neuropeptide precursors showed an increase in desiccation and starvation resistance (Zandawala et al., 2018a). In addition, leucokinin was shown to stimulate fluid secretion by the Malpighian tubules (Terhzaz et al., 1999) and a knockdown of this neuropeptide receptor in ticks showed delayed oviposition, egg hatching and reduced egg masses, indicating a role in tick reproduction (Brock et al., 2019). In the green shore crab, DH31 has a myoactive activity, which is related to rhythmic coordination (Alexander et al., 2018). Benguettat et al. (2018) showed that, in the *Drosophila* intestinal lumen, the presence of opportunistic bacteria leads to an increased formation of reactive oxygen species (ROS), which bind to transient receptor potential A1 channel (TRPA) receptors, favoring DH31 release. Then, DH31 binds to receptors in neighboring muscular cells, causing muscular contractions and, consequently, driving a quick expulsion of the bacteria.

GPA2/GPB5 is another cystine knot glycoprotein hormone and regulates hydromineral balance (Paluzzi et al., 2014). Similarly, to what was found in other species, genes coding the two subunits of these neuropeptides are located next to one another in *R. microplus* genome (Hsu et al., 2002; Roller et al., 2008; Sudo et al., 2005). In decapods, the crustacean hyperglycemic hormone (CHH) is best known for increasing hemolymph glucose concentrations, while its insect ortholog, ion transport peptide (ITP), has antidiuretic effects (Chung et al., 2010; Gáliková et al., 2018; Webster et al., 2012). However, CHH has now also been shown to have an indirect role in coordinating ion transport in decapods, since it regulates the expression of Na^+/K^+-ATPase and carbonic anhydrase, enzymes involved in osmotic pressure regulation in *Portunus trituberculatus* gills (Sun et al., 2019). In insects, ITP genes are alternatively spliced into two different forms (Dircksen, 2009). In the *R. microplus* genome, four CHH/ITP genes were identified. Because one of these genes is alternatively spliced, there are in total five different transcripts. Inotocin is a vasopressin ortholog commonly present in insects, but notably absent in flies and bees. This neuropeptide was identified in Y-organs (responsible for ecdysteroid synthesis) during the molting phases of the crab *Carcinus maenas* (Oliphant et al., 2018). In addition, periviscerokinin, the first neurohormone identified in ticks (Neupert et al., 2005), is known to play a role in diuresis, and as a myotropic agent in insects (Wegener et al., 2002).

In insects, a variety of effects have been attributed to orcokinin, e.g. regulation of pigmentation (Wang et al., 2019), vitellogenin transcription (Ons et al., 2015) and circadian rhythmicity (Hofer, 2006; Jiang et al., 2015), but no effects are known in ticks. Orcokinin immunoreactivity was detected in synganglion, hindgut and salivary glands of *I. scapularis*, but LC-MS/MS analysis identified the peptide only in synganglion and hindgut (Roller et al., 2015). In *R. microplus*, orcokinin transcripts were detected not only in synganglion, but also in ovary, salivary glands, fat body, midgut and embryo. Insect tachykinins were initially identified in *L. migratoria* and characterized as neuropeptides that stimulate gut contractions (Schoofs et al., 1990; Siviter et al., 2000). This peptide is expressed in the gut, where in *Drosophila* it regulates lipid biosynthesis (Song et al., 2014). In neurons in the brain, it plays a role in male aggressive behavior (Asahina et al., 2014). Moreover, a tachykinin-like, named natalisin, involved in insect reproduction has been described in *D. melanogaster, T. castaneum* and *B. mori* (Jiang et al., 2013), being observed only in arthropods so far. Both neuropeptide precursors present very similar motif sequences, but only tachykinin was identified in *R. microplus* transcriptome evaluated here (Jiang et al., 2013; Mateos-Hernández et al., 2021). However, these peptides were detected in genome assemblies of tick species (Jia et al., 2020; Gulia-Nuss et al., 2016; Mateos-Hernández et al., 2021), while transcripts of natalisin were not found in the *in silico* analysis of *I. scapularis* embryonic cells (Mateos-Hernández et al., 2021). Also, NPF has many physiological roles and can influence feeding, metabolism, reproduction and stress response (Nässel and Wegener, 2011).

Calcitonin is another neuropeptide gene that produces two spliced variants (named A and B), as previously described in insects and decapods (Veenstra, 2016c, 2014). In *R. microplus*, the calcitonin A transcript is found in the synganglion, while the B transcript is present in digestive cells of partially and fully engorged females, and in fat body. This is similar to the expression of insect calcitonins A and B, that are found in the CNS and the gut, respectively (Veenstra, 2014).

Arthropods have a number of peptides that contain the typical insulin core sequences. Three of these peptides, gonadulin, insulin-growth factor (IGF) and relaxin, seem to have originated from an ancient gene triplication (Veenstra, 2020a), while other insulin-related peptides evolved later from IGF (Veenstra, 2021). Gonadulin is often expressed in the ovary and, at least in *L. migratoria*, silencing it by RNAi strongly diminishes vitellogenesis, while IGF functions as a growth hormone and the function of relaxin is not very clear (Veenstra, 2020a,b, 2021). In *R. microplus*, as in the locust (Veenstra et al., 2021), gonadulin transcripts were more abundant in the ovary than in the synganglion.

We found several *R. microplus* neuropeptides for which the function in arthropods is not known. Achatin was described for the first time in the snail *Achatina fulica* (Kamatani et al., 1989). It contains a d-amino acid residue, which makes it an unusual neuropeptide, but whether this is also the case in arthropod achatins is an open question. Achatin precursors were previously identified in other chelicerates (*Stedodyphus mimosarum, Mesobuthus martensii, Symphilella vulgaris*) (Veenstra, 2016a), as well as in *I. scapularis* ticks (Gulia-Nuss et al., 2016). Another intriguing neuropeptide gene is the EFLamide gene. Initially detected in the spider mite *Tetranychus*, it produces two spliced variants that encode two different peptides, EFLamide and EFLGGPamide (Veenstra et al., 2012). Such alternative transcripts are also produced from the tick EFLamide genes. This gene is abundantly expressed in decapod crustaceans, where only the EFLamide transcript is found (Veenstra, 2016c), but in insects, this gene has either been lost or has a very limited expression. Thus, in *L. migratoria* there are only two EFLamide

expressing neurons, and in *Pyrrhocoris apterus*, null mutants for EFLamide seemed perfectly normal (Kotwica-Rolinska et al., 2020; Veenstra and Šimo, 2020). CCRFamide is a neuropeptide that was identified *in silico* only and little is known about its function, except that it is expressed in the nervous system and hypothesized to act either as a neuromodulator or a neurohormone in the lobster *Homarus americanus* (Hull et al., 2020). Lastly, a novel neuropeptide named iPTH was identified for the first time in ticks. This peptide was recently described in the beetle *T. castaneum* and other arthropods; it is hypothesized to function in the regulation of cuticle formation (Xie et al., 2020).

The characterization of tick metabolic pathways can support the identification of new physiologic targets to develop new methods for tick control. The development of new control strategies is essential, since the continuous use and misuse of acaricides has led to resistance to nearly all drugs used to date. However, most of the active principles act against only a few biological targets, including acetylcholinesterase, GABA-gated chloride channel, sodium channel and octopamine/tyramine (OCT/TYR) receptor, and the development of new molecules against these targets encounters technical and economic challenges (Guerrero et al., 2012; Jonsson, 2018). Therefore, the identification of new biological targets for acaricides is a promising approach to overcome the problem of resistance (Saramago et al., 2018; Yu et al., 2016). In this sense, transcriptome and proteome analysis, together with phylogenetic comparison among tick and insect genes, is a powerful tool to identify and characterize potential targets and to develop novel acaricides. This work describes 52 *R. microplus* neuropeptide precursors that were identified using other arthropods sequences in a transcriptomic approach. For the first time, ACP, allatostatin CCC, calcitonins A and B, CCAP, CCHamide, CCRFamide, EFLGGPamide, ETH, gonadulin, IGF, iPTH, NPF, RYamide and trissin were identified in tick tissues. Virtually all those neuropeptides seem to be ubiquitously present in ticks. The receptor(s) of one or more of these neuropeptides may constitute a good target for a novel generation of acaricides. Clearly, functional studies that characterize the role of these neuropeptides and respective receptors in tick physiology would provide useful information to evaluate and compare their potential as drug targets.

Author contributions

Conceived and designed the experiments: JW, MAX, GRCB, LT, JAV, ISVJ

Performed the experiments: JW, MAX, LR, RL, LT, JAV

Contributed reagents/materials/analysis tools: GRCB, LT, JMCR, JAV, ISVJ

Drafting the article: JW, MAX, JAV, ISVJ

Critical revision of the article: JW, MAX, GRCB, LT, PLO, JMCR, JAV, ISVJ

Declarations of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2022.101910.

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3.2. Capítulo 2

Putative target sites in synganglion for novel ixodid tick control strategies

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Nessa revisão bibliográfica, realizei a revisão da literatura, análise crítica dos dados, redação e revisão final do artigo.

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Review Article

Putative target sites in synganglion for novel ixodid tick control strategies

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ABSTRACT

Acaricide resistance is a global problem that has impacts worldwide. Tick populations with broad resistance to all commercially available acaricides have been reported. Since resistance selection in ticks and their role in pathogen transmission to animals and humans result in important economic and public health burden, it is essential to develop new strategies for their control (i.e., novel chemical compounds, vaccines, biological control). The synganglion is the tick central nervous system and it is responsible for synthesizing and releasing signaling molecules with different physiological functions. Synganglion proteins are the targets of the majority of available acaricides. In this review we provide an overview of the mode-of-action and resistance mechanisms against neurotoxic acaricides in ticks, as well as putative target sites in synganglion, as a supporting tool to identify new target proteins and to develop new strategies for tick control.

1. Introduction

Ticks are arthropods that parasitize several animal classes and present blood feeding behavior in most developmental stages (Mans, 2014; Dantas-Torres et al., 2019). These parasites are distributed worldwide and are the vectors of pathogens to animals and humans. An infected tick feeding on a non-infected host can lead to the transmission of pathogens that are harmful to the host health (United States Department of Health and Human Services, 2020; Dantas-Torres et al, 2012; Perveen, et al., 2021; TBDWG, 2018). Tick-borne diseases include mainly anaplasmosis, babesiosis, and theileriosis in animals, and spotted fever, ehrlichiosis, anaplasmosis, Powassan virus disease and Lyme disease/ borreliosis in humans (Perveen, et al., 2021; Sonenshine and Roe, 2014; Lew-Tabor and Rodriguez Valle, 2016).

Ixodidae is a family of hard ticks occurring in temperate, subtropical, and tropical regions (Apanaskevich and Oliver, 2014). *Ixodes, Haemaphysalis, Hyalomma, Amblyomma* and *Rhipicephalus* are the Ixodidae genera of most importance worldwide regarding transmission of pathogens that cause human or veterinary diseases (Boulanger et al., 2019; TBDWG, 2018).

Ixodes ricinus, Ixodes scapularis, Ixodes persulcatus and *Ixodes pacificus* are the main vectors of *Borrelia burgdorferi* genospecies, the agents of Lyme disease/borreliosis, which is critical to human health being the most common tick-borne disease, especially in the US (United States Department of Health and Human Services, 2020; Mead, 2015; Steere et al., 2004; TBDWG, 2018). Another ixodid tick of medical importance is *Ixodes holocyclus*, endemic in Australia, which causes toxicosis inducing paralysis in humans and several animal species, including birds, dogs, cats, among others (Barker and Walker, 2014; Hall-Mendelin et al., 2011; Raghavan et al., 2021).

Haemaphysalis longicornis is native from Eastern Asia and invasive in Australia, New Zealand, and recently in the United States, in part due to its ability to parasitize different hosts (United States Department of Health and Human Services, 2020; TBDWG, 2018). This tick species is the main vector of *Theileria* protozoa in Asia (Heath, 2016; Irvin, 1987; Tufts et al., 2019). *Theileria sergenti* is transmitted to domestic livestock and can lead to death, being responsible for major economic losses in Asian countries (Liu, et al., 2010; Song and Sang, 2003; Tanaka et al.,

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1993). Also in Asia, mainly in China, *Hyalomma asiaticum* ticks are important vector of pathogens, among them Crimean–Congo hemorrhagic fever virus and the agent of Q-fever, *Coxiella burnetii* (Apanaskevich and Horak, 2010; Chen et al., 2010; Duron et al., 2015; Jia et al., 2022; Wu et al., 2013).

The lone star tick, *Amblyomma americanum*, can parasitize a variety of animals, being one of the most aggressive vector of pathogens and an important tick species that affects public health and the economy in the United States (United States Department of Health and Human Services, 2020; Goddard and Varela-Stokes, 2009; Levin et al., 2017; TBDWG, 2018). All motile stages can feed on humans and spread *Rickettsia rickettsii* (Maver, 1911). Recently, several studies have also associated red meat allergy to galactose-α-1,3-galactose (alpha-gal sugar) inoculated during *A. americanum* tick bite (Commins et al., 2011; Commins and Platts-Mills, 2013; Crispell et al., 2019; Macdougall et al.,2022; Mitchell et al., 2020; Sharma et al., 2021; van Nunen, 2015; TBDWG, 2018). In the Caribbean, Central and South America, other species of the genus *Amblyomma* play roles in the transmission of pathogenic *Ehrlichia* sp. (*Amblyomma variegatum*) and *Rickettsia* spp. (*Amblyomma cajennense* sensu lato., *Amblyomma mixtum*, *Amblyomma sculptum*, and *Amblyomma ovale*) (Camus and Barre, 1995; Estrada-Peña et al, 2019; Nava et al., 2014).

The brown dog tick, *Rhipicephalus sanguineus* s.l., is widely distributed in the world and parasitize humans and animals. This tick species complex is responsible for transmitting multiple pathogens, such as *Babesia* protozoa, and also the bacteria *Ehrlichia* and *Rickettsia* (Dantas-Torres, 2008; Dantas-Torres et al., 2006; Estrada-Peña and Jongejan, 1999; Jongejan and Uilenberg, 2004). The cattle tick species, *Rhipicephalus microplus, Rhipicephalus annulatus* and *Rhipicephalus australis*, are of great economic importance in the world, given its widespread distribution across cattle-producing areas in the tropics and subtropics, and the transmission of *Babesia bovis, Babesia bigemina* and *Anaplasma marginale* (Ali et al., 2016). Cattle tick parasitism leads to host anemia and consequently decrease in meat and milk production, representing one of the main causes of losses in livestock industry (Jongejan and Uilenberg, 2004; Jonsson, 2006; Perveen, et al., 2021). Annual economic losses caused by *R. microplus* infestation reach US$ 3.2 billion in Brazil alone (Grisi et al., 2014).

These different tick species are responsible for the major ixodid tick-borne diseases reported worldwide and their increased abundance and range expansion could be related to many factors, including climatic, ecological and anthropological changes (Medlock et al., 2013). Currently, it is known that the interaction of humans, domestic and wild animals,and vectors is essential to pathogen transmission (Sprong et al., 2018).

Treatment of animal hosts with synthetic chemical pesticides (acaricides) has been the major approach to reduce tick infestations and prevent the transmission of tick-borne pathogens. There are seven chemical classes marketed worldwide for tick control in domestic animals, namely: organophosphates, synthetic pyrethroids, macrocyclic lactones, formamidines, benzoylphenyl ureas, phenylpyrazoles and isoxazolines (Gassel et al., 2014; Reck et al., 2014; Rufener et al., 2017). However, the use of these chemical compounds over the years led to the selection of tick populations resistant to most of these drugs (Jongejan and Uilenberg, 2004). Interestingly, *R. microplus* is the tick species with the highest number of reports of resistance worldwide, having developed resistance to all major acaricide classes marketed for its control (Dzemo et al., 2022; Rodriguez-Vivas et al., 2018; Vilela et al., 2020). Also, field populations with broad resistance to acaricides have been described in Brazil (Klafke et al., 2017; Reck et al., 2014).

Currently, a central concern regarding tick control methods is identifying strategies that are both effective and environmentally friendly (de la Fuente et al., 2007). In this context, biological control (biocontrol) methods have been explored (Samish et al., 2004). The introduction of a competitive species in the same habitat of pest species is a classical control method. However, this tool has disadvantages when both species are non-native to the affected area, or if the predator attacks non-target species (Ostfeld et al., 2006; Stiling, 2004). Therefore, it is important to perform pre-release risk assessment (Reinbacher et al., 2021; Simberloff, 2012). Entomopathogenic fungi, like *Metarhizium brunneum* (formerly *Metarhizium anisopliae*), have proven an effective alternative to reduce *I. scapularis* population, while also indicating to be a safe approach, since it does not affect non-target arthropods communities that may be present at the application site. On the other hand, more than one application is needed to obtain positive results (Bharadwaj and Stafford, 2010; Fischhoff et al., 2017). Application of *M. brunneum* in association with acaricides has been shown to increase effectiveness of the treatment in the control of resistant *R. microplus* strains (Webster et al., 2015), suggesting biocontrol methods in combination with other strategies as an alternative to control tick infestations (Beys-da-Silva et al., 2020).

A sustainable, eco-friendly and economically favorable approach to tick control is the use of vaccines (de la Fuente et al., 2007, 2017; Guerrero et al., 2012b). Therefore, many efforts have been made to develop an efficient vaccine that confer protection against different tick populations (Guerrero et al., 2012b; Parizi et al., 2012). Based on recombinant Bm86 (midgut glycoprotein antigen), two vaccines were developed against *R. microplus* from Australia and Cuba (TickGARD and GAVAC, respectively) (Canales et al., 1997; Willadsen et al., 1995). TickGARD vaccine is not currently available for use, while GAVAC it is still commercialized. However, both vaccines failed to show efficiency worldwide (Guerrero et al., 2012b). On the other hand, different studies have shown that Bm86 and its homologues induce protection against *R. annulatus* (Fragoso et al., 1998), *R. australis* (Hüe et al., 2017) and *Rhipicephalus decoloratus* (Odongo et al., 2007), which can be very useful due to eventual coexistence of *R. microplus* and other tick species across the same area (Parizi et al., 2012). Nevertheless, to date, no effective vaccine against *R. microplus* and other ticks has been brought forward. Thus, the control of tick parasitism and tick-borne diseases in humans and animals is still dependent on acaricide treatment. Serious limitations associated with the application of acaricides have intensified the search for novel tick control methods (Rodriguez-Vivas et al., 2018).

2. Resistance mechanisms to acaricides

Acaricide resistance is defined as a decrease in the susceptibility of a parasite population to a drug, which is a global concern (Devaney, 2013). It was demonstrated that 79% of *R. microplus* from Rio Grande do Sul state (Brazil) present multiple resistance to three or more acaricides tested (cypermethrin, amitraz, chlorpyriphos, ivermectin, and fipronil) (Klafke et al., 2017). Three major mechanisms of resistance to acaricides/insecticides are known: cuticle thickening (reducing chemical penetration) (Schnitzerling et al., 1983), target-site insensitivity (Castro Janer et al., 2019), and detoxification metabolism (Le Gall et al., 2018), with most studies focusing on the last two.

2.1. Target-site insensitivity

In arthropods, similar to other animals, voltage- and ligand-binding gated ion channels are important components of the nervous system, enabling the propagation and processing of cell signaling (Smaragandache-Wellmann, 2016). A common resistance mechanism involves a point mutation causing amino acid sequence modifications in ion channels, which could confer target-site resistance to acaricides (Guerrero et al., 2012a).

Neurotoxic pesticides commonly used for tick control, like synthetic pyrethroids, act on arthropod voltage-sensitive sodium channels (Na^+ channels) (Fig. 1) (Kumar et al., 2020). Voltage-gated ion channels play essential roles in the nervous system, since they are involved in detection and transmission of intracellular chemical signals (Smaragandache-Wellmann, 2016). Interestingly, the central nervous system of ticks, named synganglion, is the main target of several acaricides (Lees

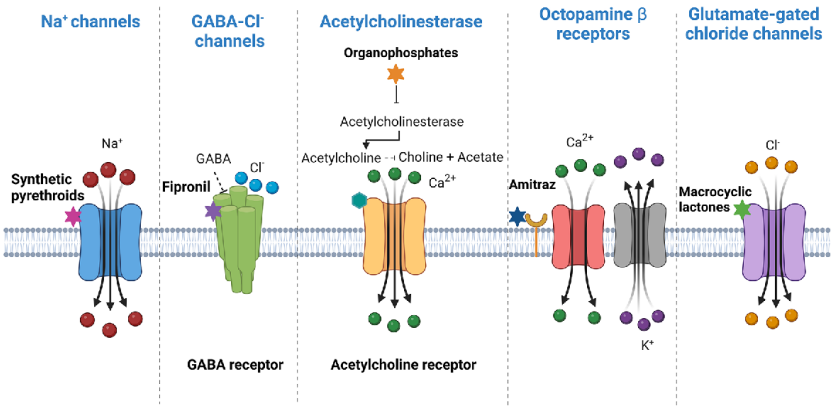

Fig. 1. Targets of commercial acaricides. The main commercial acaricides have neurotoxic activity, interfering in the functions of different channels or receptors in the cells of the central nervous system.

et al., 2010). However, the insensitivity of these channels to drugs has been described in various arthropod species (Du et al., 2016). Specifically, mutations in the ion channels are the one of the factors in the mechanism of acaricide resistance (Klafke et al., 2020; Kumar et al., 2020) and was first described in DTT-resistant house flies (Busvine, 1951). The role of chemical compounds on Na^+ channels is variable, but mainly relate to the extension of activation and inactivation steps (Hemingway et al., 2004; Lund and Narahashi, 1983; Vais et al., 2001).

The most popular acaricides, the synthetic pyrethroids, act on voltage sensitive sodium channels (Na^+ channels) (Kumar et al., 2020). These channels are composed by four homologous domains (DI, DII, DIII and DIV) and six transmembrane helices (S1–S6), while S1 to S4 constitute the voltage-sensing domain, the loop connecting S5 and S6 form a pore and, in response to membrane depolarization, the opening of the channels occurs (Auteri et al., 2018; Catterall, 1995; Oliveira et al., 2013). Mutations in the amino acid sequence of Na^+ channels, known as knockdown resistance (kdr) or super kdr mutations, has also been reported (Du et al., 2016; Kushwah et al., 2020). Kdr mutations in the amino acid sequence from DII has been already related to the increase of pyrethroid resistance in *Aedes aegypti* (Saavedra-Rodriguez et al., 2007; Srisawat et al., 2010). Substitutions from leucine (Leu) to phenylalanine (Phe) in DII of the channel, associated with resistance, were also detected in *Anopheles gambiae* (Martinez et al., 1998). This domain is highly conserved among *Rhipicephalus* spp. ticks (Vudriko et al., 2018) and kdr mutations leading to change in amino acid sequence from Leu to isoleucine (Ile), in DIIS4-5 from Na+ channels, were already identified in *R. microplus* resistant to cypermethrin (Morgan et al., 2009; Vudriko et al., 2018). Also, similar mutations were detected in this tick species from India (Nagar et al., 2018) and in *Rhipicephalus appendiculatus* from Uganda (Vudriko et al., 2018), while super-kdr substitutions were described in *R. decoloratus* (Vudriko et al., 2017). Furthermore, other studies analyzed *R. microplus* pyrethroid-resistant strains from Mexico and United States and identified target-site insensitivity due to the occurrence of kdr and super-kdr mutations in DII and DIII of Na^+ channels amino acid sequence (He et al., 1999; Stone et al., 2014), as well as a point mutation in DIIIS6 nucleotide sequence, causing the substitution from Phe to Leu in amino acid sequence, were also identified in *R. sanguineus* s.l. resistant to pyrethroids phenotype (Klafke et al., 2017).

Gamma-aminobutyric acid-gated chloride channel (GABA-Cl) was also described as a target site for several insecticides (i.e. cyclodienes, lindane and fipronil) which act as antagonists, blocking GABA and causing hyperexcitation of the central nervous system (CNS) (Fig. 1) (Bloomquist, 2003, 2001, 1994; Matsumura and Ghiasuddin, 1983). Besides its activity on GABA-Cl, fipronil (as well as fipronil sulfone, a major metabolite obtained from fipronil oxidation metabolism) has a role in inhibiting glutamate-gated chloride channels (Glu-Cl) in cockroaches (Bobé et al., 1998; Hainzl et al., 1998; Zhao et al., 2005, 2004). In *R. australis*, one amino acid substitution from Thr to Leu on the position 290 of GABA-Cl was associated with resistance to dieldrin (Hope et al., 2010). In fipronil- and lindane-resistant *R. microplus* from Brazil and Uruguay two different substitutions were found on GABA-Cl, A286S and A286L (Castro Janer et al., 2019).

Another target site known to be involved in pesticide resistance is acetylcholinesterase, that degrades acetylcholine (Ach) promoting the neurotransmitter reuptake disrupting the neurotransmission (Colović et al., 2013). However, when organophosphate acaricides bind to this enzyme they inhibit the acetylcholinesterase activity and consequently the hydrolyses of acetylcholine leading to the accumulation of this neurotransmitter in insect CNS (Fig. 1) (Casida, 1956; Casida and Durkin, 2013; Nostrandt et al., 1997).

A partial transcript of acetylcholinestarase-encoded gene (*AchE*) was described in synganglion from *R. sanguineus* s.l. (Lees et al., 2010), while three *AchE* were identified in *R. microplus* (viz. BmAchE1, BmAchE2 and BmAchE3). Interestingly, there was a low similarity among these sequences, also no point mutations were detected in nucleotide sequence from susceptible and resistant isolates to organophosphates, suggesting that other locus or mechanism could be involved (Baxter and Barker, 1998; Hernandez et al., 1999; Temeyer et al., 2004). However, in latest published studies, single nucleotide polymorphisms (SNPs) have already been described at least in two (*AchE1* and *AchE3*) of the three *AchE* from *R. microplus* and associated to organophosphates resistance (Bendele et al., 2015, Temeyer et al., 2010). A substitution from glutamine (Glu) to arginine (Arg) in the BmAchE3 sequence conferred target insensitivity to organophosphates (Temeyer et al., 2007), in addition was found high frequency of this mutation in *R. microplus* resistant strains (Temeyer et al., 2009), besides that it was also present in wild type ticks, thus different mechanisms could act synergistically to provide tick resistance. Indeed, in insects was proposed that several mutations could be occurring at the same time in *AchE* sequences, promoting an increase in the resistance ratio (Mutero et al., 1994). Accordingly, the occurrence of five mutations identified in *D. melanogaster* AchE were related to increase of the organophosphate's insensitivity. In combination, these substitutions presented stronger resistance potential to this pesticide class than when

tested alone, with exception of Gly to Val mutation (G262V position) which led to high resistance ratio (Walsh et al., 2001).

Currently, three arthropod octopamine receptors (AOR) classes have been described: α-adrenergic-like octopamine receptors (αAOR), octopamine/tyramine receptors (OCT/TYR) and β-adrenergic-like octopamine receptors (βAOR) (Corley et al., 2012; Evans and Maqueira, 2005; Han et al., 1998; Nagaya et al., 2002). Formamidines (like amitraz) act as agonists, stimulating AOR and causing CNS toxicity and death (Fig. 1) (Evans and Gee, 1980; Nathanson, 1985). This pesticide is also suggested to have a role in tyramine receptors activation (Gross et al., 2015). Mutations on OCT/TYR, threonine to proline (T8P) and leucine to serine (L22S), have been associated with *R. microplus* resistance to amitraz in Brazil, Philippines, India, Zimbabwe, and South Africa (de La Canal et al., 2021; Alota et al., 2021; Jyoti et al., 2021; Sungirai et al., 2018; Robbertse et al., 2016). However, mutations on βAOR (viz. threonine to proline - T60P; isoleucine to phenylalanine - I61F; isoleucine to threonine - I61T and tyrosine to serine - Y88S) with amitraz resistance in ticks (Jonsson et al., 2018, Takata et al., 2020). Hence, it can be suggested that amitraz resistance in cattle ticks may result from mutations in different octopamine receptors.

2.2. Detoxifying proteins and detoxification pathways

Drug detoxification mechanisms are often associated with enzymes such as esterases, cytochrome P450 (CYP450), and glutathione S-transferases (GSTs) (Koirala et al, 2022; Le Gall et al., 2018), which participate in different phases of pesticide metabolism. To minimize the deleterious effects caused by pesticides, enzymes from phase I (e.g. esterases and CYP450) perform the reduction, oxidation and/or hydrolysis of chemical compounds, with the formed products acting as substrates in the next step. In phase II (conjugation), enzymes such as GSTs render these compounds less toxic and more hydrophilic, facilitating the transport out of the cell (Perry et al., 2011). Moreover, an additional phase, named 0 or III, may also be associated with these processes, controlling the flux of molecules which have not yet reached intracellular compartments, or promoting the excretion of already detoxified drugs by ATP-binding cassette transporters (ABC transporters) (Ishikawa, 1992; Lara et al., 2015; Le Gall et al., 2018; Pohl et al., 2012; Szakács et al., 2008).

Many pesticides, such as organophosphates and synthetic pyrethroids, are esters which possess cyclopropacarboxylic, carbamic and phosphoric acids substituted, thus being subject to degradation by esterases (Devonshire, 1991). However, there are other ways by which these enzymes could promote drug resistance. Mutations in carboxylesterases genes associated with pyrethroid resistance have been described in arthropods (Hemingway and Karunaratne, 1998; Hernandez et al., 2000). Carboxylesterases have a role in pesticide detoxification, and besides sequence mutations, overexpression of these enzymes has also been reported in pyrethroid resistant arthropods, like *Musca domestica* (Feng et al., 2018). In coumaphos-resistant *R. microplus* ticks, an increased hydrolysis capacity of carboxylesterase was detected which is possibly associated to organophosphate resistance (Villarino et al., 2003)

Cytochrome P450 is another superfamily of enzymes involved in endogenous processes, xenobiotics activation and detoxification metabolism, being ubiquitously distributed in many organisms (Bergé et al., 1998; Guzov et al., 1996). In detoxification pathways performed by monooxygenases, two oxygen atoms are involved in the reactions: while one atom is reduced to H_2O, the other is incorporated to the substrate, preparing the substrate to enter phase II metabolism. However, in some cases, these formed products may be more toxic than the initial compounds (Hodgson, 1985). In insects, CYP450 have an important role in resistance to chemical compounds, as indicated by the overexpression of these enzymes and increased activity in resistant organisms (Liu et al., 2015).

After phase I metabolization of toxicants, GSTs play an important role in the final steps of chemical detoxification (Sheehan et al., 2001). These cytosolic enzymes act by catalyzing the conjugation to glutathione (GSH), facilitating that this more hydrophilic substrate be transported out of the cell (Sheehan et al., 2001). In ticks, the GSTmu was detected in different tissues in larvae and adults from *R. annulatus*, suggesting an important physiological role for this enzyme. In addition, a homologous protein was also identified in *Hyalomma dromedarii* and *Rhipicephalus* sp., and interestingly, this protein was not present in the argasidae tick *Ornithodoros moubata* (Shahein et al., 2008).

Lastly, ABC transporters are a broad protein family present in several organisms (Higgins, 2001) and their importance in promoting excretion of endo- and xenobiotics has been reported (Buss and Callaghan, 2008; Lara et al., 2015). These proteins act as active transporters and possess drug-binding sites and two ATP-binding, providing energy for substrate transport. Thereby, once ATP binding and hydrolysis occurs, the cassette is able to transport the hydrophilic substrate to the extracellular space, across the cell membrane (Dermauw and Van Leeuwen, 2014; Nobili et al., 2006). Also, P-glycoproteins (P-gp or MDR1 of the ABCB family), which are related to drug uptake and excretion, have been reported as a protection mechanism against pesticide in mosquitoes, including a multidrug resistance pathway, and may be the first line of defense of cells (Bain and LeBlanc, 1996; Buss et al., 2002; Danø, 1973; Germann and Chambers, 1998; Juliano and Ling, 1976).

3. Tick synganglion

Located in the anterior portion of the tick's body, the synganglion is a fused nerve mass that appears as a single structure representing the entire CNS of these parasites (Šimo et al., 2013). This organ is a major site for neural integration of physiological processes essential to tick survival, reproduction and development, since synganglion neuropeptides control many different bodily processes throughout each life stage of the tick (Rispe et al., 2022) In adult ticks, this organ is approximately 424 μm and 338 μm long in females and males, respectively, with similar basic arrangement: no segmentation, positioned around the esophagous, being subdivided in supraesophageal and subesophageal ganglia. In addition, it has a perigangliónic sheath that supplies this organ with fresh hemolymph, and projections from Haller's organ that originate in the olfactory lobes of the synganglion (Lees and Bowman, 2007; Menezes et al., 2021; Prullage et al., 1992). In *R. sanguineus* s.l., no differences were observed in synganglion morphology among the different life stages, confirming that this tissue remains unchanged from larvae to adults (Roma et al., 2014, 2012). However, 72 h after detachment from the host, *R. microplus* females present a higher level of DNA fragmentation in the synganglion, resulting from apoptosis (Freitas et al., 2007).

Synganglion influences tick physiological regulation, performing several functions as a neurosecretory system that synthesizes and releases signaling molecules which target different organs, like hormones and neuropeptides (Šimo and Park, 2014; Lees and Bowman, 2007; Šimo et al., 2013; Wulff et al.,2022) (Fig. 2). Gene ontology analysis in *Dermacentor variabilis* and *R. sanguineus* s.l. showed that the main functional categories transcribed in the synganglion from unfed, partially fed and fully fed females were cellular process, metabolic process and biological regulation, respectively. In addition, transcripts were differentially regulated according to the feeding stage, including most neuropeptides which were downregulated with the onset of blood feeding (Bissinger et al., 2011; Lees et al., 2010). Neurosecretory cells were detected by immunocytochemistry in *Ornithodoros parkeri* synganglion, confirming this organ as a neurohemal site in ticks (Zhu and Oliver, 1991). Also, in *R. microplus*, a transcriptome of synganglion showed that the most abundant transcripts are related to secretion, energetic metabolism and unknown categories, and that neuropeptides precursors represented around 6% of transcripts (Waldman et al., 2022). Neuropeptides produced by synganglion could act as neurohormones, neurotransmitters or neuromodulators, influencing social behavior,

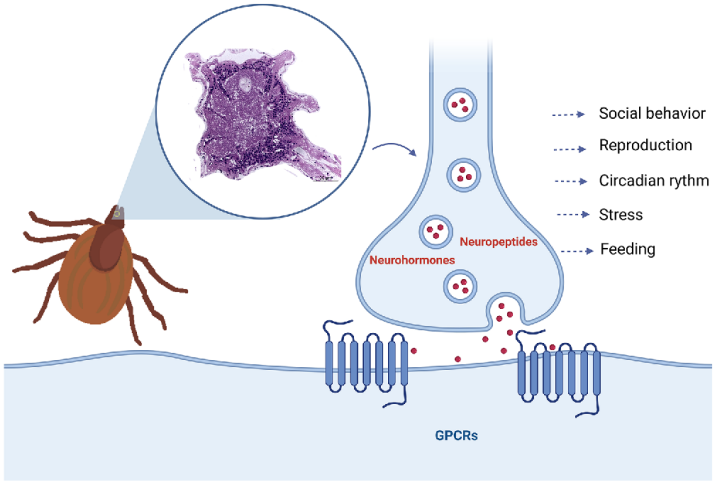

Fig. 2. Schematic view of tick synganglion functions. The neuropeptides and/or neurohormones produced and released by the synganglion bind mainly to G protein-coupled receptors (GPCRs) and have several biological activities that control tick physiology.

learning, circadian rhythm, stress, feeding, reproduction and memory of insects (Burbach, 2011; Schoofs et al., 2017). In the desert locust, *Schistocerca gregaria*, the most comprehensive neuropeptidome assay performed identified 81 neuropeptide precursors using genomic and transcriptomic approach, many of these peptides are mostly expressed in the CNS (Ragionieri et al., 2022). In *A. aegypti*, several neuropeptides were detected in CNS, but also in midgut, showing that these peptides are not restricted to the CNS and are involved in endocrine system of mosquitoes (Predel et al., 2010). In addition, another study has shown that neuropeptides from decapods are similar to those of insects, and although these peptides may not have same role, the sequence conservation facilitates research on neuropeptidomes in different arthropods (Veenstra, 2016). Despite extensive knowledge in insects, little is known about these peptides in ticks. Immunohistochemical staining showed the presence of a complex neuropeptidergic network of molecules produced by endocrine cells and central and peripheral neurons in the *R. appendiculatus* synganglion (Šimo et al., 2009). In *I. scapularis*, 20 neuropeptides were detected by mass spectrometry approach, and this neuropeptidomic study has shown that most of these peptides were similar to *A. americanum* neuropeptides (Neupert et al., 2009). Interestingly, in synganglion transcriptome from *I. scapularis*, 15 putative neuropeptide precursors and 14 receptors were identified, furthermore, transcripts for convertases, that convert precursors into mature sequences, were also detected (Egekwu et al., 2014). Also, the transcription of 14 neuropeptides and five receptors were identified in *D. variabilis* (Donohue et al., 2010), and further research has shown that neuropeptides, as well as their receptors, can be differentially expressed depending on the tick`s developmental stage (unfed, partially or fully fed females) (Bissinger et al., 2011). Currently, an updated list of neuropeptides has identified 52 precursors in *R. microplus* transcriptome and in the genome of hard ticks, such as *R. microplus, R. sanguineus* s.l., *I. scapularis, I. persulcatus, Dermacentor silvarum, H. longicornis, H. asiaticum* (Gulia-Nuss et al., 2016; Jia et al., 2020), showing that these peptides are conserved in different tick species (Waldman et al., 2022). In comparison, only 38 genes and 37 neuropeptide transcripts were identified in the genome and transcriptome from *I. scapularis* cell line, respectively. Interestingly, the level of neuropeptide transcription was influenced by *Anaplasma phagocytophilum* infection, which suggests a neuronal component in tick-pathogen interaction (Mateos-Hernández et al., 2021).

Most acaricides modulate and target synganglion, and although peptide receptors have been identified in different tick species, it is interesting to note that currently none of the neuropeptide receptors are target for acaricides (Roma et al., 2014; Xiong et al., 2020). G-protein coupled receptors (GPCRs) have been linked to effects on several pathways of arthropod physiology, such as development, reproduction, metabolism and ecdysis (Ngai and McDowell, 2017). In *A. aegypti* genome, 135 GPCRs were identified, and a conservation among sequences from this insect and *A. gambiae* and *Drosophila melanogaster* was suggested (Nene et al., 2007). In ticks, the first dataset of *R. microplus* GPCRs identified 112 candidates distributed into the rhodopsin, secretin and glutamate families (Guerrero et al., 2016). In a bioinformatic analysis, novel GPCRs were predicted in the transcriptome of Haller's organ from *R. australis* (Munoz et al., 2017). Moreover, *R. microplus* kinin sequences were reported and had activity on a kinin receptor (an invertebrate-specific GPCR) (Pietrantonio et al., 2018; Xiong et al., 2020). Additionally, the expression of this receptor was detected in *R. microplus* midgut, and a role in reproduction was shown (Brock et al., 2019). In *R. sanguineus* s.l., kinin receptor transcription was identified in the synganglion, salivary glands, gut, Malpighian tubules and oviduct tissues, with more transcripts being present in salivary glands and midgut (Lees et al., 2010). Taken together, these data facilitate the search for new targets for pesticide development.

Neuroendocrine regulation was also shown to act on the oogenesis of *O. parkeri*, since vitellogenesis was inhibited when ovary was separated from the synganglion in tick body, but when synganglion was transplanted again, from unfed or fed females, the complete maturation of the oocytes I (Oliver et al., 1992). Similarly, in *O. moubata* it was demonstrated that the transplantation of synganglion from fed mated females to virgin females induced vitellogenesis, but there was no effect on oviposition, suggesting that this organ has pleotropic functions and also produces stimulating factors for oogenesis (Connat et al., 1986). Thus, the knowledge and understanding of tick physiology and neurobiology, as well as the detection of potential new targets and molecules with acaricidal activity, such as peptides and interacting molecules, can help in the development of alternative methodologies for the tick control,

overcoming the problem of acaricide resistance (Bendena, 2010; Caers et al., 2012; Guerrero et al., 2012b; Saramago et al., 2018).

4. Identification of active molecules and potential targets for tick control

Acaricide application is the most common method for controlling tick infestation, however the continuous use of these compounds increases the selective pressure, favoring the emergence of resistant tick populations. Since the use of acaricides causes an inexorable selection for acaricide-resistant tick populations, there is a continuous need to develop and introduce new commercial products to control ticks. Thereby, there are different strategies to discover new putative active ingredients, mostly focusing on the identification of natural product-based molecules and selection of compounds from complex synthetic chemical libraries. Both approaches have several advantages and disadvantages (Adenubi et al., 2018; Chen et al., 2019; Nyahangare et al., 2015; Stratton et al., 2015). Moreover, the search for novel molecules as potential targets for acaricides is needed, and a better understanding of tick physiology is instrumental to achieving this end, which might lead to the identification of new compounds with potential ectoparasiticide activity (Rufener et al., 2017; Saramago et al., 2018).

Considering that the synganglion represents an important organ in the control of tick physiological processes (as summarized in Fig. 2), metabolic alterations caused by neurotoxic agents could lead to important effects, including functional dysregulation leading to death (Pereira et al., 2017; Roma et al., 2014). A new class of pesticides, named isoxazolines, acts as a non-competitive antagonist of GABA-Cl channels, specifically on arthropod RDL (resistance to dieldrin gene) as main target, and less extensively on Glu-Cl. In addition, this parasiticide presents a stronger inhibition of GABA-Cl than picrotoxinin and dieldrin, and has superior inhibitory and insecticide/acaricide activity than fipronil in the RDL target (Gassel et al., 2014). Similar results were observed in ticks, flies, and sea lice, and no cross-resistance among dieldrin, fipronil and isoxazoline was observed in these arthropods, showing that this pesticide has different binding sites compared to these other known GABA-Cl blockers (Rufener et al., 2017). Also, fluralaner, a molecule from the isoxazoline class, showed potent acaricidal activity against all life stages of *R. sanguineus* s.l. and *O. moubata* nymphs, when used by contact or feeding exposure pathways, respectively (Williams et al., 2015). Okaramine, an alkaloid Glu-Cl activator, was obtained from *Penicillium simplicissimum* and showed a toxic effect against *Bombyx mori* silkworm larvae, but not against human GABA-Cl and glycine-gated chloride, highlighting its use as a putative insecticide (Furutani et al., 2015; Hayashi et al., 1989). Interestingly, this molecule acts on binding sites that are different than those of ivermectin, suggesting that the resistance mutations that affect ivermectin activity could be ineffective against okaramine action (Furutani et al., 2017). In addition to their role in insects, an acaricide activity was also tested for okaramine against *I. scapularis*, showing that, unlike other Glu-Cl blockers such as picrotoxin and fipronil, okaramine activated this channel in a dose-dependent manner. An inhibition of the ivermectin response on Glu-Cl channel by the fungal alkaloid was also observed, confirming that both molecules act on different target sites (Furutani et al., 2018).

Tyramine and octopamine are present in the CNS and act as neurotransmitters that regulate a variety of behavior and physiological processes in arthropods, allowing them to respond to the environment according to external stimuli received. The use of agonists and antagonists of octopamine and tyramine receptors, respectively, results in CNS excitation, leading to similar physiological effects (Hunt, 2007; Roeder et al., 2003). In tick females, octopamine injection was shown to block oviposition, although other β-adrenergic agonists, such as synephrine and apormophine, showed different effects and no inhibitory action on oviposition (Booth, 1989). On the other hand, it was shown that an alteration in tyraminergic pathway (α-adrenergic) inhibited oviposition in *R. microplus* more potently than octopamine. These results indicated that the susceptible strains used could be resistant to amitraz, which acts as an octopamine agonist, and therefore were more tolerant to this neurotransmitter action and did not show the same effect on oviposition (Cossío-Bayúgar et al., 2012).

As other examples of CNS molecules in pest management, neuropeptides have been suggested and investigated. However, due to instability, they could not be applied alone, requiring compound combinations to prevent degradation by peptidases and protect these peptides until reaching their target sites (Nachman et al., 2002). Insect kinins are important peptides that present a variety of functions, including in the excretory system of insects (Coast, 2007). Due to the presence of sites susceptible to peptidase action, the use of kinin analogs, which are more stable and therefore resistant to degradation, has been studied (Nachman et al., 2002). Thus, biostable molecules, such as α-aminoisobutyric kinin analog or polyethylene glycol polymer-conjugated kinin, have been shown to act on mosquito and tick receptors, as well as unconjugated peptides, and may be a useful tool to study the role of kinin in arthropods physiology and subsequent application in tick control.

Due to the difficulty in identifying new acaricides with neurotoxic potential that could serve for ectoparasite control, molecules with different physiological targets have been tested. Triosephosphate isomerase (TIM) is an enzyme that participates in glycolysis and gluconeogenesis metabolism, catalyzing the interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (Knowles, 1991). Structural changes in *Plasmodium falciparum* TIM, due to modification in a cysteine residue, led to the loss of enzymatic activity (Maithal et al., 2002). In ticks, TIM inhibitors were evaluated for their acaricidal efficacy, revealing that, out of the 227 compounds tested, four were able to inhibit enzymatic activity, with a decrease in the percentage of viable cells and reduction in *R. microplus* larvae hatching rate also reported (Saramago et al., 2018). Inhibitors that prevent the degradation of tyrosine, an amino acid obtained from the blood meal, may be a good and safe alternative for parasite control (Sterkel et al., 2016). Other studies have showed that the exposition to anonaine, an alkaloid isolated of plant *Annona crassiflora* (Bezerra et al., 2022) or synthetic molecules (Ozelame et al., 2022) reduce the activity of glutathione-S-transferase and increase tick mortality, suggesting GST as potential target for development of new acaricides mitigating resistance to acaricides (Obaid et al., 2022; Umetsu and Shirai, 2020).Besides inhibitors, natural compounds have also been tested for their pesticidal activity, and present great interest for parasite control, mainly for having reduced environmental impact compared with chemical acaricides (Adenubi et al., 2018). In this context, essential oils extracted from oregano (*Lippia graveolens*), rosemary (*Rosmarinus officinalis*), and garlic (*Allium sativum*) showed high toxicity in a *R. microplus* larval packet test, reaching 100% of mortality at the highest concentrations evaluated (Martinez-Velazquez et al., 2011). Moreover, mortality and reduced oviposition were also observed in engorged ticks exposed to oregano compounds, and these effects may be due to the presence of thymol, carvacrol and p-cymene, which represent the main components of this plant species (Flores-Fernández et al., 2016). Interestingly, sublethal concentrations of acetylcarvacrol affected oocyte development in *R. microplus* engorged females. Despite all of the stages of oocyte development (I, II, III, IV and V) being present, defects in their morphology were detected (Konig et al., 2019). Similar results were also observed in partially engorged females from *R. sanguineus* s.l., where oocyte development was impaired and only stages I and II were present in ticks that were exposed to carvacrol (Lima de Souza et al., 2019). Also, it was observed that *R. microplus* exposed to carvacrol and thymol showed an increase in the activities of glutathione-S-transferase, catalase, superoxide dismutase and glutathione peroxidase, suggesting that the generation of reactive oxygen species is the mechanisms of toxicity of these potential natural acaricides (Tavares et al., 2022). Besides carvacrol, guaiol and bunesol compounds and essential oil from *Bulnesia sarmientoi* also showed larvicidal activity against *R. microplus,*

Rhipicephalus evertsi, Rhipicephalus pulchellus, R. appendiculatus. Interestingly, tolerance to these components was observed in acaricide-resistant *R. microplus* populations when compared with susceptible ticks (Luns et al., 2021). At the same time, phyto-formulations that combine extracts from different plants species, like cumin (*Cuminum cyminum*), cinnamon (*Cinnamomum zeylanicum*) and allspice (*Pimenta dioica*) also showed potent acaricidal activity (Lazcano Díaz et al., 2019), supporting that the application of plant extracts and essential oils or their components could be an addition to the available anti-tick arsenal for the control of ectoparasites, with importantly less residual effect than chemical acaricides (Lara et al., 2015).

An interesting alternative to tick chemical control could be the use of RNA interference (RNAi) or genome editing using CRISPR/Cas9 system to silence genes related to essential physiological functions, including synganglion metabolism. In the last years, these methodologies have emerged as important biological tools for research but, several preliminary studies have been shown the potential applications in pest control (Christiaens et al., 2020; Kaduskar et al., 2022; Lester et al., 2020; Tyagi et al., 2020; Vogel et al., 2019; Yan and Lin, 2022). Genetic engineering tools have been caused controversies, however RNAi and CRISPR methodologies are easier to use and more precise than other DNA-editing tools (Lux and Scharenberg, 2017; Watters et al., 2021). The use in the field of these methodologies has many challenges, including, a better understanding of tick gene expression, methodological security and ethical issues (Christiaens et al., 2020, Hoang et al., 2022; Tirloni et al., 2020; Willow et al., 2021), however different approach could be used reduce and eliminate these obstacles to make these techniques more suitable for pest control (Mehlhorn et al., 2021).

Several potential targets and alternative methodologies have been proposed for tick control, and an increasing knowledge about tick metabolism provides an essential basis for commercial acaricidal development. Nevertheless, the identification of these targets still needs further research and the continuous efforts to understand tick physiology will likely eventually lead to novel control strategies capable of circumventing current acaricide-resistance mechanisms.

CRediT authorship contribution statement

Jéssica Waldman: Writing – original draft, Writing – review & editing. **Guilherme Marcondes Klafke:** Writing – original draft, Writing – review & editing. **Lucas Tirloni:** Writing – original draft, Writing – review & editing. **Carlos Logullo:** Writing – original draft, Writing – review & editing. **Itabajara da Silva Vaz:** Writing – original draft, Writing – review & editing.

Declarations of Competing Interest

None.

Data availability

No data was used for the research described in the article.

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Supplementary materials

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3.3. Capítulo 3

Mechanisms of acaricide resistance in ticks

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Mechanisms of Acaricide Resistance in Ticks

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ABSTRACT

***Background*:** In several countries, including Brazil, the livestock industry plays a key role in the country's economy. Brazil has the second largest bovine herd in the world and the biggest commercial herd. Ticks are an ongoing problem for both large operation cattle producers and small family farmers. *Rhipicephalus microplus* causes expressive losses in cattle breeding, since it occurs in important beef production zones like South America, Africa, and Oceania. Some of the negative consequences of tick infestation to cattle breeding are anemia, loss in milk and beef production, and transmission of Babesia bovis and *B. bigemina*. Significant losses are caused by the cattle tick (*R. microplus*) in several regions of the world, costing around US$ 3.3 billion per year to the Brazilian livestock industry alone. The tick control methods are mainly based on synthetic acaricides. However, the improvement of current tick control requires the identification of new molecular targets in tick physiology and development of molecule compounds to target important physiology pathways. The strategies proposed to address this issue are expand the knowledge about the molecules involved in the detoxification of chemicals to enhance the efficacy of the acaricides as well as to develop new compounds for chemical control.

***Review*:** Tick control is currently based on chemical acaricides; however, effective control and prevention of tick infestation remain distant goals. In recent decades, a progressive decrease in the efficiency of acaricides due to drug resistance has been observed. Acaricide resistance is an evolutionary adaptation, which implies the existence of behavioral and physiological mechanisms that allow the survival of resistant individuals. Four resistance mechanisms are described: behavioral resistance, reduced drug penetration, target site insensitivity and increased drug detoxification. Augmented drug detoxification may be due to increased activity of enzymes or transporters due to increased gene expression or mutations in some genes. Research focus on mechanisms of acaricide resistance in ticks characterized detoxification pathways based on (1) increased activity of enzymes (cytochrome p450, esterase and GST) which play a role in biochemically altering acaricides towards decreased toxicity and, (2) enhanced excretion of the modified less toxic compounds. To bypass the current problems, a better understanding of the biology, physiology, and molecular biology of the mechanisms of resistance to acaricides is fundamental to prolong their efficiency in controlling ticks. Moreover, identifying the genes and proteins associated with resistance can support in the development of more sensitive diagnostic methods to identify acaricide resistance, as well as improving control strategies.

***Discussion*:** In the last years, many researchers have been studying resistance mechanisms and important advances have been made which showed that, in several tick species, ABC transporters, esterases, P-450 cytochromes and glutathione-S-transferases participate in acaricide resistance. The characterization of the alterations in the targets in tick physiology and identification of new drugs with potential to tick control are crucial goals to increase tick control

Keywords: esterases, glutathione S transferases, pyrethroids, organophosphate, acaricide, resistance, parasite, *Rhipicephalus microplus*, bovine.

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I. INTRODUCTION

Ticks are ectoparasites worldwide distributed that infest a variety of vertebrate hosts, presenting a hematophagous behavior and could affect the animal and human health [37,38,126]. The parasitism caused by the cattle tick, *Rhipicephalus microplus*, and the transmission of *Babesia bovis* and *B. bigemina* could lead to host anemia and, consequently, decrease in milk and meat production, with economic annual losses for livestock production reaching US$ 3.2 billion in Brazil [59,72].

Currently, there are 7 classes of commercially available pesticides to control ticks' infestation: organophosphates, synthetic pyrethroids, macrocyclic lactones, formamidines, benzoylphenyl ureas, phenylpyrazoles and isoxazolines [110,118]. The main targets of pesticides are present in the central nervous system of arthropods having neurotoxic activity [99]. Most of them have a role on ion channels, like Gamma-aminobutyric acid gated chloride channel (GABA-Cl), glutamate-gated chloride channel (Glu-Cl) [13-15,91] and voltage-sensitive sodium channels (Na^+) [80], acetylcholinesterase enzyme [19,20] and arthropod octopamine receptors (AOR) [49,50]. However, there is an increasing global concern about tick acaricide resistance, since the application of chemical acaricides, over the years, has led to an increase in the reports of resistant populations to these compounds [71], including multiresistant populations to all commercial acaricides in different countries [52,61,76,107,110,134]. In Brazil, a field population of *R. microplus* has already been identified as resistant to 6 acaricides (cypermethrin, chlorpyriphos, fipronil, amitraz, ivermectin and fluazuron) that belong to different classes [107], also resistance to deltamethrin, fipronil and ivermectin was reported in the brown dog tick, *Rhipicephalus sanguineus* [10].

Until now, 3 main factors have been identified to contribute to resistance selection: cuticle thickening (reducing or delaying the pesticide penetration) [117], target-site insensitivity [22] and detoxification pathways [84], but in ticks the studies are focused on last two. Thus, knowledge and understanding of tick metabolism and the target pathways that contribute resistance selection can help in the identification of new targets, as well as in the development of novel control strategies to overcome increasing resistance to pesticides.

II. TARGET-SITE INSENSITIVITY

1. Voltage channels

Voltage-gated Na+ and K+ channels are responsible for the generation of action potentials in neurons and propagation of electrical signals [140]. In ticks, the synganglion, a mass of fused nerves, is the central nervous system, [108] and is an important target of the current acaricides [111]. However, multiple studies revealed that acaricide resistance occurs in many different species of arthropods, including ticks [25,61,97,139]. Acaricide resistance can be determined by different mechanisms, including the metabolic inactivation or degradation of the active molecule. However, most of the times, acaricide resistance is caused by changes in the drug targets [27,45,113]. The concept of drug resistance was first considered when mosquitoes and housefly became resistant to DDT in Italy in 1946 [62].

Pyrethroids are broad-spectrum acaricides and their major mode of action is via interactions with the voltage-gated sodium channel [125]. Mutation mediated knockdown resistance (kdr) is the most common and a frequent cause of resistance to pyrethroids in ticks [22,31]. Several studies on ticks, especially *R. microplus*, have documented several point mutations in the sodium channel associated with reduce sensitivity to pyrethroids [1,21,31,77,127]. Gamma-aminobutyric acid gated chloride channel (GABA-Cl) and glutamate-gated chloride channels (Glu-Cl) are other essential players in the central nervous system functions [124,138,145]. GABA-Cl are targets for several pesticides, including fipronil, lindane and cyclodienes and the novel acaricide class of isoxazolines [22,142,146].

The hyperexcitation caused by antagonist drugs blocks the GABA current leading to arthropod death [142]. Interestingly, fipronil and fipronil sulfone were reported as inhibitors of both channels, GABA-Cl and Glu-Cl [143,144]. The Glu-Cl channel receptors are part of ion channel protein superfamily detected in invertebrates, but not in vertebrates [26], and appears to be the target of macrocyclic lactones in *Caenorhabditis elegans*, and in *Drosophila melanogaster*, potentiating the glutamate activated current [33,34]. On the other hand, it was not identified cross-resistance between fipronil and ivermectin in *R. microplus*, suggesting that these acaricides do not present the same target-site [23].

Resistance to dieldrin gene (*rdl*) from *Drosophila* was the first member of GABA-Cl channel genes described in invertebrates [53]. In ticks, it was shown that GABA current was blocked when fipronil was administrated in *Xenopus* oocytes that expressed *rdl* gene from *Dermacentor variabilis*, suggesting a role of this pesticide as blocking the opening of GABA-Cl channels and indicate a potential target to tick control [145]. It was identified that ala to glycine (*gly*) substitution in *Anopheles gambiae rdl* locus for GABA receptor conferred resistance to dieldrin [43]. Also, mutations in GABA-Cl gene led to dieldrin and fipronil resistance in *R. microplus* [22,70]. However, a study showed that no significant association was found between presence of rdl mutations and fipronil resistant phenotypes in cattle tick isolates from Uruguay and Argentina [21,115]. Meanwhile, point mutations in Glu-Cl gene which led to the substitution from alanine (ala) to valine (val) and from gly to aspartic acid (asp) were identified in *Plutella xilostella* and *Tetranychus urticae* resistant to abamectin, respectively, both modifications promote a change in the channel conformation interfering with binding of this pesticide to the channel receptor [81,137], but in ticks this resistance mechanism is still unknown.

2. Acetylcholinesterase

The acetylcholinesterase is a serine hydrolase that degrade acetylcholine and terminate neurotransmission [93]. However, tick exposition to organophosphate acaricides result in inhibition of cholinesterase, so acetylcholine accumulates at the cholinergic synapse, keeping the receptors activated causing paralysis and death of tick [54].

In *R. microplus*, single nucleotide polymorphism in 2 acetylcholinesterase genes (*AchE1* and *AchE3*) has been associate to organophosphates resistance [11,133] since the mutation is present in *R. microplus* resistant strains [132]. Interesting, it was observed that in insects and ticks multiple simultaneous mutations can occur in the same acetylcholinesterase gene increasing the level of resistance [54,75,88,92,104].

Amitraz belongs to another class of acaricides that is extensively used for tick control, but resistance to this pesticide have been detected since the 1990's [73]. In the same way of organophosphates, there are still questions about the mechanisms involved in amitraz resistance.

3. Octopamine receptors

Formamidines are a class of acaricides that act as agonists by stimulating the octopamine receptors (AOR), the consequence is a decrease in intracellular Ca^{2+} and activation of K+ efflux leading to interruption of nervous transmission and death [7,49,96]. These pesticides have a role mainly against the βAOR, despite the arthropods have 3 AOR: α-adrenergic-like octopamine receptors (αAOR), octopamine/tyramine receptors (OCT/TYR) and β-adrenergic-like octopamine receptors (βAOR) [29,50,63,95]. In addition, formamidines have also been shown to interfere in octopamine / tyramine receptors activated [60].

Therefore, although OCT/TYR gene has been sequenced in *Rhipicephalus australis* from Australia, mutations in these sequences from susceptible and resistant strains to amitraz were not initially identified in that country, suggesting that other pathways could be involved in this pesticide resistance [9]. However, posteriorly, point mutations in OCT/TYR sequences related to amitraz resistance were detected in resistant ticks from Brazil, Mexico, South Africa, Zimbabwe and India [4,22,24,109,129]. Also, the intragenic recombination of OCT/TYR could be suggested as important in the emergence of resistant populations [8]. Another work associated non-synonymous mutations (from ile to phe) in βAOR sequence from *R. microplus* to amitraz resistance, but not all resistant populations presented this genotype, suggesting the involvement of other mechanisms in resistance pathway [28,73], but this mutation was confirmed to reduce the effect of N2–(2,4-dimethylphenyl)-N1-methyformamidine, an amitraz metabolite, in *Bombyx mori* and their resistance potential needs to be confirmed in ticks [130].

III. DETOXIFYING PROTEINS AND DETOXIFICATION PATHWAYS

The best-known mechanism in acaricide-resistant ticks is the metabolic detoxification [30,39,113]. This pathway is characterized by increased activity of enzymes, such as esterase (phase I), cytochrome P450 (CYP450) (phase I) and glutathione S-transferase (GST) (phase II) [2], playing a role in modifying acaricides toward decreased toxicity, forming more hydrophilic molecules and enhancing excretion of the less toxic compounds out of the cells (phase III) [103].

1. Esterases

Two beta-carboxylesterases (serine hydrolases) sequences were identified in *R. microplus* and a point mutation (G1120A) was found in one of these genes from pyrethroid resistant Mexican strains [64,68]. Carboxylesterases have a role in pesticide detoxification and besides the presence of mutations in their sequence, an overexpression of these enzymes has also been described in other arthropods, like *Musca domestica* [51]. In ticks, an increased hydrolysis capacity of carboxylesterase was detected in *R. microplus* resistant to the organophosphate coumaphos, which is possibly associated to resistance to this pesticide [135]. Increased activity of alpha- and beta-carboxylesterases was also shown in *R. microplus* larvae resistant to fluazuron [56]. Meanwhile, another study analyzed the transcription level of an esterase gene in 2 pyrethroid resistant tick strains. In 1 strain, a higher number of transcripts was observed in larvae and in the hemolymph collected from resistant engorged females, in comparison to pyrethroid-susceptible ticks. However, in the second strain no differences were observed in the expression of the esterase encoding gene in relation to susceptible ticks. Therefore, these results suggest that different pathways can contribute to resistance to the same acaricide [67].

2. Cytochromes P450

Cytochromes P450 metabolize xenobiotics, being a common detoxification mechanism in several arthropod species, and has also been linked to pyrethroid resistance in insects and other arthropods [69]. In several arthropods, CYP450-mediated resistance is characterized by the gene overtranscription, resulting from alterations in the factors that regulate its expression [86,116]. In the red flour beetle, *Tribolium castaneum*, populations resistant to phosphine showed increased susceptibility to this pesticide when a CYP450 inhibitor (piperonyl butoxide - PBO) was used. Also, a significant up-regulation of CYP346B subfamily genes was described in this resistant insect species [136]. In *Drosophila melanogaster* lineages over-expressing *CYP6G1*, *CYP6G2* and *CYP12D1*, increased survival to insecticides DDT, nitenpyram, dicyclanil and diazonin was detected [35]. In *A. gambiae* a CYP450 enzyme was located in oenocytes and it is related to hydrocarbon production, which leads to the thickening of the cuticle of pyrethroids-resistant mosquitoes and consequently insecticide uptake reduction [6]. Also, in *R. microplus*, a proportional increase in transcription of CYP450 was identified in pyrethroids-resistant populations [30]. Similar results were described in *R. sanguineus sensu lato*, since when PBO was used in synergist bioassay, an increase in tick mortality was shown, restoring the permethrin toxicity in pyrethroid-resistant isolates, indicating a role of CYP450 in metabolic detoxification. However, the same pattern has not been described in fipronil-resistant populations, suggesting that another mechanism may be involved in this resistance pathway [46]. In contrast, some CYP450-encoding genes presented decreased transcripts levels, while other CYP450 genes were over transcribed when *R. microplus* resistant to pyrethroids were exposed to deltamethrin. This could be explained by the need of the high transcription of genes necessary for survival post-acaricide treatment [94].

The exposure to PBO slightly increased (1.4-fold) amitraz toxicity in susceptible *R. microplus*, however, the synergistic effect of PBO was significantly increased (2.9-fold) in an amitraz-resistant isolate, suggesting a role of P450s in resistance to the formamidine in cattle ticks [82].

Besides their detoxification role, CYP450 can also act in metabolization and consequent activation and enhancement of the pesticide toxicity, like organophosphates. It was observed that CYP450 activity was reduced in organophosphate-resistant tobacco budworm (*Heliothis virescens*) [78]. The use of PBO decreased the coumaphos toxicity in acaricide-susceptible *R. microplus*, while synergistic effect was shown in resistant isolates, however, the same synergic activity with diazinon was not identified when PBO was used in resistant isolates [85]. On the other hand, it was not detected significantly differences in the CYP450 gene expression levels in organophosphate-resistant and

susceptible *R. microplus*, suggesting a multifactorial resistance mechanism to this acaricide [32].

3. Glutathione S-transferase

The tick GST catalyzes the conjugation of reduced glutathione (GSH) with a wide variety of endogenous and exogenous electrophilic compounds, protecting the cell from oxidative damage [3]. Thus, this enzyme could have a role in the tick resistance to acaricide. While GST enzyme overexpression is frequently associated with drug resistance [10], reports on the participation in acaricide resistant ticks are sparse. However, there is data of increased GST transcription in acaricide resistant tick populations [55,66]. Excitingly, GST-gene RNAi silencing is shown to induce acaricide susceptibility in ticks [44]. Currently, 7 classes of these enzymes are known in mammals, named Alpha, Mu, Pi, Sigma, Theta, Zeta and Omega, according to their chromosomal location [119]. In insects, Delta and Epsilon classes are also present, and are implicated in *Anopheles gambiae* detoxification pathways [105,106]. GST enzymes are subdivided in cytosolic, microsomal, and mitochondrial (Kappa) categories [120], however the last one was not identified in insects so far, but are present in other arthropods, like crustaceans [112]. In insects, cytosolic class are subdivided in Delta, Epsilon, Omega, Theta, Sigma and Zeta [47]. In *Tribolium castaneum*, 36 putative cytosolic GSTs and 5 microsomal GSTs were identified, while in *Bombyx mori*, only 23 cytosolic *GSTs* were observed, it is interesting to highlight that the class which contains the highest number of detected sequences was the *Epsilon* for both insect species [121,141], also in *Drosophila* the main classes are Epsilon followed by Delta [58]. These cytosolic enzymes act by catalyzing the conjugation to glutathione (GSH), facilitating that more hydrophilic substrate to be transported out of the cell [120]. In *Tenebrio molitor*, it was hypothesized that cytosolic GSTs act by sequestration and binding to the pyrethroids, thus allowing the detoxification process. However, no relationship was found between GSH concentration and resistance to insecticides [79]. High transcript levels of Delta *GST* were found in *D. melanogaster* chemosensory organs, after isothiocyanate exposition. This enzyme was over transcribed, suggesting an insecticide protection role [58].

Also, in *Haemaphysalis longicornis*, 2 GSTs-encoding gene transcripts were identified in several organs of larvae, nymph and adults, it is interesting to note that during blood feeding, higher transcript levels were observed, however protein levels decrease after engorgement, moreover location of GST in midgut and salivary glands depends on the feeding, and GST may be related to oxidative stress [66]. The expression of this enzyme is induced by heme, not by iron present in the blood, thereby reducing the cytotoxic effects caused by blood components [100]. Recombinant GSTs (rGSTs) from *H. longicornis* and *Rhipicephalus appendiculatus* had their activity inhibited by acaricides [123]. Similar effects were observed rGST from *R. microplus* using different acaricides. However, coumaphos shown to increase rGST activation, suggesting different interaction mechanisms between acaricide and detoxification enzymes [122]. Furthermore, increasing flumethrin doses led to increase of GST gene transcription in tick males, while GST knockdown decreased the larval survival rate after acaricide treatment [65], accordingly, permethrin has been shown to have more toxic effects on *R. sanguineus sensu lato* knockdown for *GST*, thus suggesting this protein as an alternative control target for ticks [44]. Natural compounds [12,89,131] or synthetic molecules [98] can alter GST activities and can consequently lead to the improvement and development of new acaricides.

4. ABC transporters

Lastly, P-glycoproteins (P-gps) are ABC transporters that influence drug uptake and excretion, interacting with different agents and have been related as a protection mechanism against pesticide in mosquitoes, including a multidrug resistance pathway, may be the first line of defense of cells [5,17,36,57,74]. Nevertheless, ABC transporters are poorly understood in arthropods and only some species present a characterization of putative ABC genes. These were grouped into 8 families (from ABCA to ABCH), in *Tribolium castaneum*, *Tetranychus urticae*, *Bombyx mori*, *Anopheles gambiae*, *Daphnia pulex* and *Drosophila melanogaster* [16,40,42,87,114,128]. In *Anopheles stephensi*, *ABCB* and *ABCG* augmented transcription has been associated with permethrin resistance, since when mosquitoes were exposed to ABC inhibitors and to the insecticide an increase in larval mortality was observed. Also, an over-transcription was detected in ABCG-encoding genes when the mosquitos were exposed to permethrin alone, but similar results were not identified for ABCB-encoding genes [48]. In contrast, a *D. melanogaster* lineage knocked-out to homologous

of mammalian ABCB-encoding genes (*Mdr65*) was more susceptible to several insecticides than other genes tested, showing synergistic activity with ABC transporter inhibitor, whereas flies knocked-out to other Mdr genes were resistant to some pesticides [41].

Although little is known about these transporters in insects, in ticks the knowledge is even scarcer. The first association between ABC transporters and acaricide resistance was demonstrated in *R. microplus* resistant to ivermectin. The exposure of the ticks to compounds that interfere with ABC proteins (cyclosporin-A) in association with the acaricide treatment, lead to the reduction in oviposition and egg viability of treated engorged female ivermectin-resistant ticks. In addition, a decrease in ivermectin lethal concentration was observed [102]. Similar results were observed in *R. sanguineus sensu lato* (exposed to fipronil and ivermectin) [18] and with a multiple acaricide resistant strain of *R. microplus* (exposed to ivermectin, abamectin, moxidectin and chlorpyriphos) [103], suggesting that ABC transporters could act as a multidrug detoxification mechanism, with the ABC transporters inhibition as an approach for tick control. Interestingly, an up-regulation in the transcription of the ABCB-encoding gene was detected for resistant population (Juarez) exposed or not to ivermectin, the same was not identified for ABCC-encoding gene and for susceptible strain (Porto Alegre) [102]. A cell line from *Ixodes ricinus* exposed to 3 acaricides (amitraz, permethrin, and fipronil) showed differences in up- and down-regulation for ABCB and ABCC-encoding genes depending on the pesticide treatment, showing different pathways and cell responses according to the drugs tested [90]. *In vitro* assays were also performed using ivermectin-resistant *R. microplus* embryonic cell line. An increase in ABCB transcriptional level was observed in resistant cells exposed to acaricide, while the resistance decreased after treatment with ABC transporters inhibitor, demonstrating the role of these transporters in resistance to ivermectin, however once again similar results were not observed to ABCC [101]. Moreover, an association between amitraz detoxification and heme transportation in midgut was proposed for *R. microplus* ABCB transporters, suggesting that resistance to acaricides may be a consequence of endogenous compound detoxification pathway [83]. Confirming that several physiological mechanisms can act on pesticide resistance, in *Rhipicephalus microplus*, toxicological assays confirmed that esterases and ABC transporters contribute to ivermectin resistance, followed by GSTs and CYP450 [84].

IV. CONCLUSIONS

Ticks and tick-borne diseases are significant impediments to livestock production. Current tick control methods are mainly based on chemical acaricides; however, effective control and prevention of tick infestation remain distant goals. In recent decades, a progressive decrease in the efficiency of acaricides due to drug resistance has been observed. To bypass the current problems, a better understanding of the physiology and molecular biology of the mechanisms of resistance to acaricides is fundamental to prolong their efficiency in controlling ticks, as well as improving control strategies.

This includes, expand the knowledge about the molecules directly involved in the detoxification of chemicals to enhance the efficacy of the acaricides as well as to develop new compounds for chemical control.

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4. Discussão

O singânglio dos carrapatos é um dos principais alvos de acaricidas, no entanto o seu conhecimento ainda é escasso quando comparado aos insetos (LEES; BOWMAN, 2007; ROMA *et al*., 2014). Por sua vez, a neurobiologia dos carrapatos é essencial para o entendimento da seleção de resistência e para a descoberta de novos alvos para controle desses parasitos (LEES; BOWMAN, 2007). Os avanços nas pesquisas são dependentes do conhecimento acerca do material biológico que está sendo avaliado (DEVANEY, 2013). Anteriormente, entre os fatores limitantes para o estudo do singânglio, estava o tamanho do órgão e, consequentemente, a necessidade de obtenção de um grande número de amostras (CHRISTIE, 2008). Esta dificuldade foi superada por novas metodologias, dentre elas, as abordagens ômicas que vêm sendo utilizadas para entendimento de vários fatores fisiológicos das espécies e como ferramentas para identificar estratégias que contornem a seleção de resistência (DEVANEY, 2013), permitindo análise em grande escala através da utilização de pequenas quantidades de amostra, podendo ser avaliadas em diferentes condições.

Vários estudos transcriptômicos têm sido realizados utilizando o singânglio dos carrapatos ixodídeos. Análises ômicas permitiram a identificação de 603 sequências em *R. sanguineus* s. l. que estão, em sua maioria, relacionadas a crescimento celular, divisão e síntese de RNA, metabolismo e síntese de proteína (LEES; WOODS; BOWMAN, 2009). Em *I. scapularis*, um transcriptoma de singânglio de fêmeas parcialmente, totalmente e não alimentadas, resultou na identificação de mais de 40.000 *contigs*, associados a processos metabólicos e celulares, e regulação biológica (EGEKWU *et al*., 2014). Categorias similares foram descritas em fêmeas adultas de *D. variabilis* (BISSINGER *et al*., 2011). Já em *I. ricinus*¸ mais de 70.000 *contigs* foram classificados em diferentes categorias, sendo as de maior interesse: funções moleculares, processos biológicos e componentes celulares (RISPE *et al*., 2022). No primeiro capítulo desse trabalho, foram gerados dados, a partir de um transcriptoma de singânglio de fêmeas parcialmente e totalmente ingurgitadas de *R. microplus*, e 94.813 *contigs* foram gerados, com 18.004 sequências codificantes anotadas funcionalmente. Trinta e duas categorias funcionais foram descritas, sendo que maior expressão foi observada nas sequências de proteínas secretadas, relacionadas a metabolismo energético e de funções desconhecidas. Adicionalmente, perfis distintos de transcritos foram observados no transcriptoma de singânglio, estudado nesse trabalho, quando comparado a

análise das sequências codificadoras presentes em um transcriptoma órgão/estádio específico de *R. microplus* (TIRLONI *et al*., 2020). O singânglio influencia e participa de diferentes processos fisiológicos, através das interações neurais realizadas (RISPE *et al*., 2022), o que poderia explicar a observação de diferentes perfis de transcritos nesse órgão quando comparado a outros tecidos e estádios.

Informações disponíveis nesse transcriptoma *de novo* foram utilizadas e as sequências precursoras de neuropeptídeos analisadas. Além da anotação automática, procura e anotação manual foram necessárias para identificar as sequências de neuropeptídeos presentes. Os precursores de neuropeptídeos representaram 0,12% do total de transcritos (dados não mostrados), porém quando realizado estudo da análise de sequências codificantes que foram 5x mais abundantes no singânglio do que nos outros órgãos/estádios, as sequências precursoras de neuropeptídeos foram associadas a 6% do total de RPKM. No entanto, o número de neuropeptídeos presentes em carrapatos ainda não está definido (EGEKWU *et al*., 2014).

Sequências neuropeptídicas de carrapatos também já foram descritas através de análises de espectrometria de massas (*I. scapularis*) (NEUPERT *et al*., 2009), de EST (*expressed sequence tag*) (*R. sanguineus* s. l.) (LEES; WOODS; BOWMAN, 2009), de imuno-histoquímica (*R. appendiculatus*) (ŠIMO *et al*., 2009) e de pirossequenciamento (*D. variabilis*) (DONOHUE *et al*., 2010). Uma investigação descreveu a presença de 37 precursores de neuropeptídeos em *I. scapularis, R. microplus* e *A. americanum*, pertencentes a nove grupos (CHRISTIE, 2008). Em contraste com as 29 sequências identificadas em *I. scapularis* anteriormente¸ 15 transcritos foram identificados em uma análise de um transcriptoma de singânglio dessa espécie de carrapato (EGEKWU *et al*., 2014). Resultados similares foram encontrados no transcriptoma de singânglio de *D. variabilis*. O presente trabalho reportou o maior número de neuropeptídeos já identificados em carrapatos, sendo que os grupos identificados são similares aos descritos em insetos, onde o sistema é melhor estudado e caracterizado. Por exemplo, na ordem Coleoptera, análises genômicas e transcriptômicas mostraram a presença de 65 sequências que codificam neuropeptídeos (VEENSTRA, 2019). Já em *Rhodnius prolixus* mais de 20 precursores foram identificados (LEYRIA; ORCHARD; LANGE, 2020), além disso, 21 sequências de precursores de neuropeptídeos em insetos Polyneopteras e 34 em vespas, *Habrobracon hebetor*, também foram reportadas (BLÄSER; PREDEL, 2020; YU *et al*., 2020),

A identificação automática de sequências de neuropeptídeos por programas de bioinformática pode ser difícil, uma vez que essas sequências passam por extensivos processamentos (DE HAES *et al*., 2015), necessitando de algoritmos específicos para esta busca, ou a realização de busca semi-manual baseada em motivos e/ou domínios específicos de cada grupo de neuropeptídeos. Entretanto, a disponibilização de informações em bancos de dados genômicos possibilita e facilita a identificação de sequências precursoras de neuropeptídeos. Adicionalmente, foram realizadas buscas nos dados brutos de genomas de carrapatos duros estudados atualmente para identificação de sequências que codificam para neuropeptídeos. Os precursores identificados no primeiro capítulo desse trabalho foram utilizados para realização de análise comparativa com genomas de carrapatos duros estudados atualmente (GULIA-NUSS *et al*., 2016; JIA *et al*., 2020). Foi demonstrado que esses peptídeos apresentam sequências conservadas entre as diferentes espécies, o que pode ser útil para o estudo de alvos para o desenvolvimento de acaricidas. O mesmo não ocorre em insetos da ordem Coleoptera, uma vez que os estudos mostraram variabilidade na sequência de neuropeptídeos entre diferentes espécies desta ordem. Ausência de neuropeptídeos considerados universais em insetos, como leucoquinina, corazonina e alatostatina A, foi observada entre espécies dessa ordem (Li *et al*., 2008; VEENSTRA, 2019), uma hipótese é de que isso ocorra devido à pressão evolutiva, levando a especialização e/ou perda de função dos peptídeos nos organismos em que essas sequências não foram identificadas.

Conforme visto, estudos prévios já avaliaram a presença de precursores de neuropeptídeos no singânglio de carrapatos (EGEKWU *et al*., 2014; LEES; WOODS; BOWMAN, 2009; RISPE *et al*., 2022), mas não tinha sido possível identificar a maior parte dos neuropeptídeos descritos aqui, nem a presença dessas sequências em diferentes órgãos do carrapato. De maneira geral, a expressão dos neuropeptídeos em diferentes órgãos dos carrapatos é comparável a dos insetos (ŠIMO *et al*., 2009). Nesse trabalho, foi realizada uma análise comparativa das sequências codificadoras de precursores de neuropeptídeos transcritos no singânglio, quando comparado a outros tecidos (ovário, glândulas salivares, corpo gorduroso e células digestivas de fêmeas parcialmente e totalmente ingurgitadas) e estádio (embrião) de *R. microplus*. Esse estudo mostrou que esses precursores apresentam transcritos em todos os órgãos e estádios avaliados, no entanto das 28 sequências identificadas no transcriptoma, 26 apresentam mais transcritos no singânglio, com exceção

do fator de crescimento do tipo insulina (IGF) e da glicoproteína A2 (GPA2) que apresentam maior transcrição no ovário, seguido do singânglio. Entretanto, diferentemente dos dados encontrados no presente estudo, em *Locusta migratória*, IGF foi mais transcrito no cérebro, seguido do ovário, quando avaliado em tecidos de fêmeas imaturas. (VEENSTRA *et al*., 2021). Nos insetos, esse neuropeptídeo está relacionado ao desenvolvimento de órgãos associados à reprodução (WANG *et al*., 2013; VEENSTRA *et al*., 2021). Em *R. prolixus*, o número de ovos postos foi reduzido em fêmeas silenciadas para IGF, sugerindo que esse neuropeptídeo participe da regulação da performance reprodutiva desse artrópode, através da sinalização da via insulina/ToR (LEYRIA; ORCHARD; LANGE, 2021). Esses dados, poderiam explicar o que foi reportado no presente trabalho, associando a expressão desse neuropeptídeo a sua função. Em vertebrados, os neuropeptídeos equivalentes a GPA2/Glicoproteína B5 (GPB5) são denominados de tiroestimulina, a qual também está presente nos ovários, e é expressa de forma constitutiva nesse órgão, sendo um sistema parácrino, controlado pela ação de gonodotrofinas (SUN *et al*., 2010). Diferentemente, em invertebrados, até o momento, não se tem conhecimento se esses neuropeptídeos possuem funções separadas ou atuam como heterodímeros, no entanto sugere-se que possa ter ambas as atividades (ROCCO; PALUZZI, 2016). Em *D. melanogaster*, tanto GPA2 quanto GPB5 apresentam mais transcritos no sistema nervoso central de larvas, enquanto que, em adultos, quando comparadas fêmeas e machos, GPA2 foi mais abundante em gônadas de machos e GPB5 apresentou mais transcritos em órgãos relacionados ao sistema reprodutor das fêmeas (VANDERSMISSEN *et al*., 2014). Aqui, ambos precursores de neuropeptídeos estão presentes, mas somente GPA2 foi analisado quanto a transcrição em diferentes tecidos e contrastou com os dados encontrados no estudo anterior.

Os dados descritos nos capítulos 2 e 3 demonstram que a descoberta de novas moléculas com efeito acaricida e que apresentem sítios de ligação diferentes dos compostos já conhecidos e disponibilizados comercialmente, tem se tornado uma necessidade, tendo em vista a seleção de populações de carrapatos resistentes a pesticidas (HILL; SHARAN; WATTS, 2018). Metodologias como as de RNA de interferência, e de espectrometria de massas e a era pós-genômica trouxeram avanços que auxiliam na identificação de novas proteínas em carrapatos, com a possibilidade de uso delas como alvos para controle. (LEES; BOWMAN, 2007). A predição de genes que codificam para GPCR no singânglio de *R. microplus* foi realizada (GUERRERO *ei al*., 2016), sendo estes potencialmente sugeridos

para o desenvolvimento de novos acaricidas, uma vez que são alvos de pequenas moléculas ou peptídeos ligantes que podem influenciar na fisiologia, afetando o controle dos parasitos (HILL; SHARAN; WATTS, 2018). Em insetos, a presença de mutações nesses genes foi associada com redução de efeitos tóxicos ocasionados por exposição a pesticidas (PANDEY *et al*., 2015). Além disso, uma vacina multiantigênica, baseada em dois neuropeptídeos: SIFamida e peptídeo mioinibitório, já foi avaliada contra *I. ricinus*, mas a vacinação não mostrou redução no número de carrapatos alimentados (ALMAZAN, 2018). Em contrapartida, o silenciamento de um receptor de piroquinina levou a aumento da mortalidade de *R. microplus* e aumento da massa dos ovos (WULFF *et al*., 2022b). Efeitos neurotóxicos, com alterações morfológicas do singânglio de *R. sanguineus* s. l., foram observados quando utilizadas substâncias extraídas de plantas, como os monoterpenos (MATOS *et al*., 2019). No entanto, devido à dificuldade na identificação de moléculas que apresentem efeitos neurotóxicos, outros alvos e metodologias também têm sido estudados. Receptores de vitelogenina bem como outras proteínas associadas a vitelogênese, foram identificados em *R. microplus* (XAVIER *et al.*, 2018) e sugeridos como potenciais alvos de controle, sendo expressos no ovário e estando envolvidos com a maturação desse órgão (MITCHELL III; SONENSHINE; LEÓN, 2019). Inibidores de enzimas envolvidas nas vias gliconeogênicas e glicolíticas também mostraram potente atividade acaricida nos testes de imersão de larvas (SAPORITI *et al*., 2022). Além disso, efeito potencializador de acaricidas (como deltametrina) também tem sido observado, uma vez que uso de nanopartículas de prata revestindo moléculas acaricidas mostrou significativo efeito contra *R. microplus* (AVINASH *et al*., 2017). Apesar de potenciais alvos já terem sido identificados, até o momento, se tem um conhecimento limitado acerca da fisiologia com foco na neurobiologia dos carrapatos, o que vem dificultando o desenvolvimento de metodologias alternativas de controle contra esses ectoparasitos (LEES; BOWMAN, 2007).

Sendo assim, vale destacar que os dados gerados no primeiro capítulo, permitiram reportar pela primeira vez em carrapatos, a presença dos seguintes neuropeptídeos: peptídeo relacionado a corazonina/hormônio adipocinético (ACP), alatostatina CCC, calcitoninas A e B, peptídeo cardioativo de crustáceos (CCAP), CCHamida, CCRFamida, EFLGGPamida, hormônio desencadeador da ecdise (ETH), gonadulina, IGF, hormônio da paratireoide de insetos (iPTH), neuropeptídeo F (NPF), RYamida e trissina. Adicionalmente, conforme descrito nos capítulos 2 e 3, com um objetivo de contornar os mecanismos já conhecidos de

resistências aos acaricidas, diferentes moléculas já foram identificadas e têm sido propostas como potenciais alvos para controle dos carrapatos ixodídeos.

5. Conclusão

Nesse trabalho, foi realizada a identificação de 52 precursores de neuropeptídeos em *R. microplus*, sendo que maior expressão foi observada no singânglio. Dentre as sequências identificadas, 15 foram descritas pela primeira vez em carrapatos. Além disso, esses peptídeos mostraram-se conservados em genomas de diferentes espécies de carrapatos ixodídeos. Adicionalmente, outras moléculas já foram descritas no singânglio e estão sendo estudadas como potenciais alvos para controle dos carrapatos. Entretanto, estudos funcionais ainda são necessários para estabelecer a função desses peptídeos e de seus receptores, bem como de outras proteínas, presentes no sistema nervoso central, na fisiologia desses parasitos para avaliação do seu potencial como alvo de drogas.

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VUDRIKO, P. *et al*. Molecular characterization of octopamine/tyramine receptor gene of amitraz-resistant *Rhipicephalus* (*Boophilus*) *decoloratus* ticks from Uganda. **Microorganisms**, v. 10, n. 12, p. 2384, 2022.

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YADAV, N.; UPADHYAY, R. K. Tick saliva antigen-based vaccines, disease protection and prophylaxis. **European Journal of Biological Research**, v. 12, n. 1, p. 77-101, 2022.

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ZHOROV, B. S.; DONG, K. Pyrethroids in an AlphaFold2 model of the insect sodium channel. **Insects**, v. 13, n. 8, p. 745, 2022.

Anexo

Curriculum Vitae

WALDMAN, J.

1. Dados Pessoais

Nome: Jéssica Waldman

Local de nascimento: Porto Alegre/RS – Brasil

Data de nascimento: 22/09/1992

Endereço eletrônico: jessica.waldman@hotmail.com

2. Formação Acadêmica

2012 - 2015 Graduação em Biotecnologia.
Universidade Federal de Pelotas, UFPEL, Pelotas, Brasil
Título: Produção e caracterização de IgY contra a proteína recombinante LigBrep de *Leptospira interrogans*
Orientador: Éverton Fagonde da Silva
Bolsista: Conselho Nacional de Desenvolvimento Científico e Tecnológico

2017 - 2018 Mestrado em Biologia Celular e Molecular Aplicado à Saúde
Universidade Luterana do Brasil, ULBRA, Canoas, Brasil
Título: Desenvolvimento de método de amplificação isotérmica para detecção de *Salmonella*
Orientador: Vagner Ricardo Lunge
Bolsista: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2018 – atual Doutorado em Biologia Celular e Molecular.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil
Título: Potenciais alvos no singânglio para controle de carrapatos
Orientador: Itabajara da Silva Vaz Júnior
Bolsista: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
Informações adicionais: Prorrogação de seis meses concedida pela CAPES devido a pandemia de COVID19 (prazo de defesa estendido para

maio/2023) e licença maternidade (agosto/2022 a dezembro/2023) com prorrogação da bolsa e prazo de defesa estendido para setembro/2023.

3. Formação complementar

2020-2020: Biologia, importância e controle de carrapatos. Colégio Brasileiro de Parasitologia Veterinária, CBPV, Brasil. (Carga horária: 32h).

2018-2018: Infecções Sexualmente Transmissíveis - Cuidados na execução dos testes rápidos TELELAB, TELELAB, Brasil. (Carga horária: 30h).

2017-2017: Extensão universitária em Curso de Imunohematologia - Teórico-prático - Módulo Básico. Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil. (Carga horária: 12h)

2016-2016: Curso de Extensão em Medicina Legal. Instituto Galeno, GALENO, Brasil. (Carga horária: 10h).

2015-2015: Doença de Chagas - Triagem e Diagnostico Sorológicos. TELELAB, TELELAB, Brasil. (Carga horária: 15h).

2015-2015: IV Curso em Genética e Biologia Molecular Humana. Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. (Carga horária: 44h).

2014-2014: Técnicas moleculares e imunológicas para o diagnóstico. Universidade Federal de Pelotas, UFPEL, Brasil. (Carga horária: 8h).

2014-2014: Extração de DNA e polimorfismos genéticos. Universidade Federal de Pelotas, UFPEL, Brasil. (Carga horária: 4h).

2014-2014: Técnicas de coloração de gram. TELELAB, TELELAB, Brasil. (Carga horária: 15h).

2014-2014: Biossegurança - Laboratórios. TELELAB, TELELAB, Brasil. (Carga horária: 15h).

2013-2013: Cultivo Celular e Engenharia Tecidual. Universidade Federal de Pelotas, UFPEL, Brasil. (Carga horária: 8h).

4. Atuação profissional

2012 – 2012

Universidade Federal de Pelotas - UFPEL

Vínculo: Aluno

Enquadramento funcional: Estagiária

Carga horária: 20h

Regime: Parcial

2013 – 2015

Universidade Federal de Pelotas - UFPEL

Vínculo: Aluno

Enquadramento funcional: Estagiária

Carga horária: 20h

Regime: Parcial

2015 – 2015

Universidade Luterana do Brasil – ULBRA

Estágio Curricular de Final de Curso no Laboratório de células-tronco e engenharia de tecidos, sob orientação da prof. Dra. Nance Nardi

Vínculo: Aluno

Enquadramento funcional: Estagiária

Carga horária: 30h

Regime: Parcial

2017– 2018

Universidade Luterana do Brasil - ULBRA

Vínculo: Aluno

Enquadramento funcional: Aluna de Mestrado
Regime: Dedicação exclusiva

2018 – Atual
Universidade Federal do Rio Grande do Sul – UFRGS
Vínculo: Aluno
Enquadramento funcional: Aluna de Doutorado
Regime: Dedicação exclusiva

5. Prêmios e títulos

2022- 2º lugar - VII Simpósio Brasileiro de Acarologia - Acarologia Médico-Veterinária, VII SIBAC.

2015- Destaque - XXIV Congresso de Iniciação Científica, Universidade Federal de Pelotas.

2015- 3º lugar - XXIV Congresso de Iniciação Científica UFPel - Ciências Agrárias, Universidade Federal de Pelotas.

2014- Destaque - XXIII Congresso de Iniciação Científica, Universidade Federal de Pelotas (coautora).

6. Artigos completos publicados em periódicos

WALDMAN, J.; KLAFKE, G. M.; TIRLONI, L.; LOGULLO, C.; VAZ JÚNIOR, I. S. Putative target sites in synganglion for novel ixodid tick control strategies. Ticks and Tick-Borne Diseases, v. 14, p. 102123, 2023. [doi: 10.1016/j.ttbdis.2023.102123]

WALDMAN, J.; KLAFKE, G. M.; VAZ JÚNIOR, I. S. Mechanisms of acaricide resistance in ticks. ACTA Scientiae Veterinariae, v. 51, p. 1900, 2023. [doi: 10.22456/1679-9216.128913]

WALDMAN, J.; XAVIER, M. A.; VIEIRA, L. R.; LOGULLO, R.; BRAZ, G. R. C.; TIRLONI, L.; RIBEIRO, J. M. C.; VEENSTRA, J. A.; VAZ JÚNIOR, I. S. Neuropeptides

in *Rhipicephalus microplus* and other hard ticks. Ticks and Tick-Borne Diseases, v. 13, p. 101910, 2022. [doi: 10.1016/j.ttbdis.2022.101910]

XAVIER, M. A.; BRUST, F. R.; **WALDMAN, J.**; MACEDO, A. J.; JULIANO, M. A.; VAZ JÚNIOR, I. S.; TERMIGNONI, C. Interfering with cholesterol metabolism impairs tick embryo development and turns eggs susceptible to bacterial colonization. Ticks and Tick-Borne Diseases, v. 12, p. 101790, 2021. [doi: 10.1016/j.ttbdis.2021.101790]

WALDMAN, J.; SOUZA, M. N.; FONSECA, A. S. K.; IKUTA, N.; LUNGE, V. R. Direct detection of *Salmonella* from poultry samples by DNA isothermal amplification. British Poultry Science, v. 61, p. 653-659, 2020. [doi: 10.1080/00071668.2020.1808188]

7. Trabalhos publicados em anais de eventos (resumo)

WALDMAN, J.; XAVIER, M. A.; VIEIRA, L. R.; LOGULLO, R.; BRAZ, G. R. C.; TIRLONI, L.; RIBEIRO, J. M. C.; VEENSTRA, J. A.; VAZ JÚNIOR, I. S. *In silico* characterization of neuropeptides in *Rhipicephalus microplus* and other ticks. In: VII Simpósio Brasileiro de Acarologia, 2022. Anais do VII Simpósio Brasileiro de Acarologia, 2022.

WALDMAN, J.; VAZ JÚNIOR, I. S. Cytocrhome P450 in tick resistance to acaricides. In: XXIII Encontro anual do Grupo Arthromint, 2019, Angra dos Reis. Livro de Resumos XXIII Encontro Anual do Grupo Arthromint, 2019.

WOLF, L. M.; **WALDMAN, J.**; SOUZA, M. N.; WOLF, J. M.; IKUTA, N.; LUNGE, V. R. Detecção dos sorotipos Enteritidis e Heidelberg de *Salmonella* por método de amplificação isotérmica de DNA. In: 4º ENCONTRO ULBRA DE BOLSISTAS CNPq e FAPERGS, 2018, Canoas. 4º ENCONTRO ULBRA DE BOLSISTAS CNPq e FAPERGS, 2018.

WALDMAN, J.; SEIXAS NETO, A.; MACHADO, G. B.; FORTES, T. P.; DEWES, C.; SILVA, E. F. Produção e caracterização de IgY anti-rLigBni de *Leptospira interrogans*. In: XXIV Congresso de Iniciação Científica da Universidade Federal de Pelotas, 2015, Pelotas. XXIV Congresso de Iniciação Científica da Universidade Federal de Pelotas, 2015.

FORTES, T. P.; SEIXAS NETO, A.; DEWES, C.; MACHADO, G. B.; **WALDMAN, J.**; SILVA, E. F. Leptospirose bovina: Produção e caracterização de lipoproteínas recombinantes para uso em vacinas. In: XVII Encontro de Pós-Graduação da Universidade Federal de Pelotas, 2015, Pelotas. XVII Encontro de Pós-Graduação da Universidade Federal de Pelotas, 2015.

BITTENCOURT, N.; SEIXAS NETO, A.; COLONETTI, K.; MEDEIROS, M. A.; **WALDMAN, J.**; SILVA, E. F. Coadministração de proteínas recombinantes conferem proteção parcial contra leptospirose. In: XXIII Congresso de Iniciação Científica da Universidade Federal de Pelotas, 2014, Pelotas. ', 2014.

ANCIUTI, A.; **WALDMAN, J.**; BITTENCOURT, N.; BETTIN, E.; COLONETTI, K.; SILVA, E. F. Produção e caracterização de IgY anti-LigA de *Leptospira interrogans*. In: XXIII Congresso de Iniciação Científica da Universidade Federal de Pelotas, 2014, Pelotas. XXIII Congresso de Iniciação Científica, 2014.

WALDMAN, J.; COLONETTI, K.; BITTENCOURT, N.; SEIXAS NETO, A.; SILVA, E. F. Triagem de proteínas recombinantes como candidatos a uma vacina contra a leptospirose bovina. In: XXII Congresso de Iniciação Científica, 2013, Pelotas. XXII Congresso de Iniciação Científica, 2013.

8. Organização de evento

WALDMAN, J., *et al*. III Simpósio de Biotecnologia - Da Pesquisa à Aplicação. Universidade Federal de Pelotas, Rio Grande do Sul, Brasil. 2015.

9. Participação em banca de comissões julgadoras

19ª Mostra de Ensino, Pesquisa e Extensão do IFRS, 2018. Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul.

10. Licenças

Licença maternidade: 27 de agosto de 2022 a 24 de dezembro de 2022.