Universidade de Brasília- Faculdade de Medicina
Laboratório Multidisciplinar de Pesquisa em Doença de Chagas

Proteoma e transcriptoma comparativo das glândulas salivares de barbeiros das espécies de *Rhodnius brethesi*, *Rhodnius robustus* e *Panstrongylus megistus*

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“É muito mais fácil reconhecer o erro do que encontrar a verdade; aquele está na superfície, de modo que se deixa erradicar facilmente; esta repousa no fundo, e investigá-la não é coisa para qualquer um” **Goethe**

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SUMÁRIO

RESUMO

A doença de Chagas causada pelo protozoário *Trypanosoma cruzi* é transmitida pelo triatomíneo hematófago e provoca mais de 17 milhões de infecções em toda America Latina. Mais de 140 espécies de triatomíneos foram descritas. O gênero *Rhodnius* compreende mais da metade dos triatomíneos encontrados na Amazônia, como o *Rhodnius brethesi* e *Rhodnius robustus*. Normalmente esses insetos não estão em contato com os humanos, porém o desflorestamento e as migrações aproximam o contato entre o inseto infectado e o humano. Diferentemente, o *Panstrongylus megistus* é amplamente distribuído do México até a Argentina e coloniza diferentes ambientes silvestres como ninhos, tocas, arvores e até domicílios humanos. Nesse estudo nos descrevemos o transcriptoma das glândulas salivares de *R. brethesi* e *R. robustus* com 56 e 122 clusters respectivamente. Mais de 30% desses clusters nunca tinham sido descritos antes. Nos seqüenciamos também pela primeira vez 45 transcritos de glândulas salivares de *P. megistus*. Além disso, foi realizada uma análise proteômica por espectrometria de massa do conteúdo das glândulas salivares desses insetos. Encontramos 123, 111 e 159 proteinas nos proteomas de *R. brethesi*, *R. robustus* e *P. megistus* respectivamente. Além da grande prevalência de proteínas de manutenção, nos encontramos lipoclinas, inositol polifosfate 5-fosfatase e proteínas com domínio kazal, essenciais para um repasto sanguíneo com sucesso. Foram encontradas também outras proteínas relacionadas com resistência a inseticidas e defesa como a glutationa S transferase e proteína antigeno-5 respectivamente. *Rhodnius* findings showed their genetic distance with *Triatoma*, in the opposite, data confirmed the closeness between *Triatoma* and *P. megistus*.

SUMMARY

Chagas disease caused by the protozoan *Trypanosoma cruzi* is mostly transmitted by the triatomine bug and causes over 17 million infections in Latin America. More than 140 species of triatomines were described. The *Rhodnius* gender comprises almost half of the triatomines found in the Amazon Basin, as *Rhodnius brethesi* and *Rhodnius robustus*, normally these insects aren't in contact with humans, but deforestation and migration are approaching the infected bugs to human habitats and working areas. Differently, *Panstrongylus megistus* is widely distributed from Mexico to Argentina and are known to colonize different wild life dwellings, such as ground burrows, birds nest, crevices on tree barks and human domiciles. In this study we described the transcriptome of the salivary glands of *R. brethesi* and *R. robustus*, respectively comprising 56 and 122 clusters. Over 30% of these clusters had never been described before. We also sequenced for the first time 45 transcripts from the *P. megistus* salivary gland. Furthermore, we performed a proteomic analysis using LC-MS/MS technology and found 123, 111, 159 proteins in *R. brethesi*, *R. robustus* and *P. megistus* proteome, respectively. Besides the highly redundant housekeeping proteins we also could found lipocalins (biogenic amino binding proteins and salivary platelet aggregation inhibitor), inositol polyphosphate 5-phosphatase, and kazal domain proteins, essential proteins used for the successful blood-feeding habits, and glutathione S transferase, and antigen-5 protein, respectively, involved with resistance to insecticide and defense. *Rhodnius* findings showed their genetic distance with *Triatoma*, in the opposite, data confirmed the closeness between *Triatoma* and *P. megistus*.

INTRODUÇÃO

1. Doença de Chagas

Atualmente, a doença de Chagas (tripanossomíase americana) atinge aproximadamente 17,4 milhões de pessoas nas Américas (WHO 2002), sendo considerada como maior endemia produzida por agente infeccioso no hemisfério ocidental (WHO, 1997). O primeiro relato desta doença data de 1909, quando Carlos Chagas descreveu suas características clínicas, anátomo- patológicas, epidemiológicas, seu agente etiológico *Trypanosoma cruzi* e o inseto vetor do parasito.

O barbeiro transmite o *T. cruzi* durante o repasto de sangue no hospedeiro vertebrado (Figura 1). Ao liberar a excreta no local da picada o barbeiro passa as formas tripomastigotas metacíclicos do parasito que penetram através da abrasão feita na pele do corpo humano. Na corrente sanguínea do organismo vertebrado, o parasito é fagocitado pelos macrófagos. No citoplasma dos macrófagos as formas tripomastigotas metacíclicos se transformam em amastigotas que se multiplicam e diferenciam as tripomastigotas sanguíneas. Essa última forma do parasito é liberada na corrente sanguínea disseminando-o para todo o organismo. Quando outro barbeiro se alimenta de sangue naquele hospedeiro infectado, ele ingere as formas tripomastigotas que se transformam no intestino do triatomíneo em amastigotas e epimastigotas (multiplicação por divisão binária). Na porção final do intestino do barbeiro, as epimastigotas se transformam nas formas infectantes do parasito, as tripomastigotas metacíclicos (Teixeira e cols, 2006).

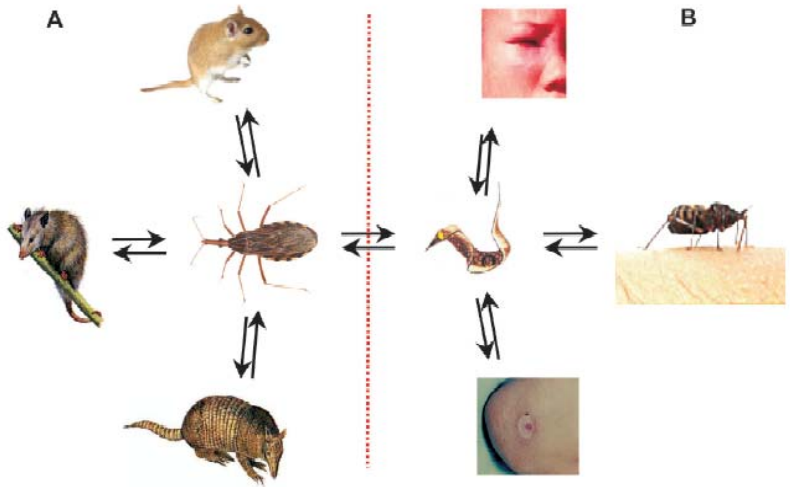


Figura 1: Ciclo de vida do *Trypanosoma cruzi* em hospedeiros mamíferos e no homem. A) ciclo silvestre B) ciclo peri-domiciliar (Fonte: Teixeira e cols, 2006).

O *T. cruzi* está presente em diferentes reservatórios naturais. Os hospedeiros mamíferos mais conhecidos são os gambás (*Didelphis spp.*), tatus (*Dasypus spp.*) e ratos (*Rattus spp*). A principal via de transmissão do *T. cruzi* para o homem é pelo triatomíneo. Os hábitos desses insetos são importantes para o conhecimento da epidemiologia da Doença de Chagas (Gaunt e Miles, 2000).

No começo do século passado, a distribuição observada entre os casos da infecção mostrava que a enfermidade acometia quase exclusivamente a população pobre das áreas rurais do continente Sul Americano. Com o passar do tempo, o homem em atividades predatórias invadiu aqueles ambientes silvestres e peri-domiciliares. Em decorrência houve desequilíbrio ecológico e o desaparecimento gradativo da fauna tem aproximado o homem dos insetos-vetores infectados (Neves, 2004). A doença de Chagas, considerada como uma enfermidade rural e pobre teve sua epidemiologia modificada por causa do êxodo rural.

2. Os triatomíneos

Os insetos triatomíneos, transmissores do *T. cruzi*, pertencem à família *Reduviidae* e subfamília *Triatominae*. Eles são encontrados exclusivamente nas Américas, entre o paralelo 42º ao norte dos Estados Unidos e o paralelo 42º ao sul da Argentina (citado em Teixeira e cols, 2007). Na natureza eles são encontrados em árvores, arbustos, pedras, ninhos, tocas e

podem alimentar-se em mamíferos, aves, anfíbios e répteis. Já existem provas de enzootia com os marsupiais há mais de 90 milhões de anos. Atualmente são conhecidas cinco *tribos* (Alberprosiniini, Bolboderini, Cavernicolini, Rhodiniini e Triatomini) com 16 gêneros (Dias e Macedo, 2005) e 136 espécies de triatomíneos (Diotaiuti, 2007).

Durante a evolução, os diferentes gêneros foram se especializando, de forma que na natureza os *Rhodnius* estão principalmente associados a palmeiras, os *Panstrongylus* são mais encontrados em tocas, cavidades de árvores e no peri-domicílio (Gaunt e Miles, 2000). Eles são hemimetábolos, sofrendo cinco mudas até o estágio de adultos (Figura 2). As fêmeas são relativamente mais ativas, tendo uma maior capacidade de dispersão e de longevidade, sua vida pode durar até dois anos (Dias e Macedo, 2005).

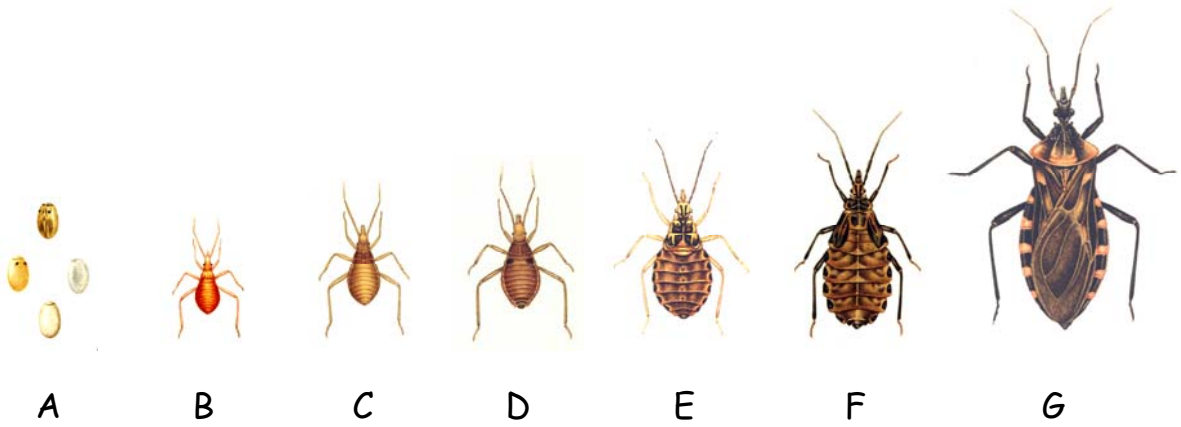


Figura 2: Ciclo de vida do *Panstrongylus megistus*. A) ovos, B - F) ninfas de primeiro a quinto estágio G) fêmea adulta. (Fonte: Diotaiuti, 2007).

Independente do sexo ou da idade, todos são hematófagos obrigatórios e podem ser classificados como ectoparasitas temporários, vez que mantêm contato com o hospedeiro unicamente durante o repasto (Guarneri e cols, 2000). Até o quarto estágio os insetos precisam de apenas um repasto sanguíneo para sofrer a muda, enquanto que os outros estágios necessitam de mais de uma alimentação (Diotaiuti, 2007). Além de precisarem de sangue para sofrer as mudas, os adultos também têm que se alimentar para reprodução. Isso é muito importante para a epidemiologia da infecção, pois quanto mais repastos forem feitos, maior a probabilidade de transmissão da doença. As fêmeas chegam a colocar mais de 600 ovos durante sua vida reprodutiva, isto é, somente na fase adulta (Diotaiuti, 2007). Os ovos demoram entre 15 a 20 dias para eclodirem.

Potencialmente todos os barbeiros são vetores da Doença de Chagas (Brener e cols, 2000). As espécies mais importantes nas Américas são o *Triatoma infestans*, *Rhodnius prolixus* e *Panstrongylus megistus.* Segundo Gaunt e Miles (2000), os *Rhodnius* e *Triatoma* se diferenciaram há aproximadamente 40 milhões de anos, antes do aparecimento da divergência de morcegos e roedores.

2.2 *Panstrongylus megistus*

Segundo Brener e cols, 2000, essa espécie pode ser considerada a mais importante no Brasil por causa de sua ampla distribuição geográfica e elevada susceptibilidade ao *T. cruzi.* Esse barbeiro está presente em grande parte do território brasileiro (Figura 3) preferindo regiões com maior umidade (matas úmidas e matas de galeria em ecossistemas secos).

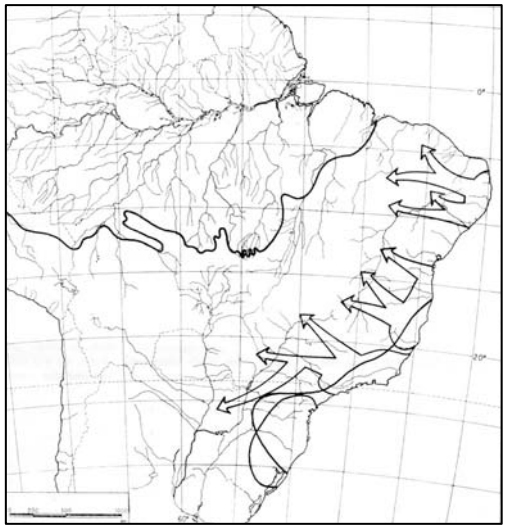


Figura 3: Áreas de dispersão de *Panstrongylus megistus* no Brasil (Fonte: Forattini, 1980).

Sua importância epidemiológica varia conforme a região. No sudeste, por exemplo, essa espécie é uma das responsáveis pelos casos autóctones da Doença de Chagas. Em Minas Gerais, o *P. megistus* possui uma grande importância até em termos históricos, pois, foi nele que Carlos Chagas descreveu primeiramente o *T. cruzi.* Além disso, o *P. megistus* tem uma alta capacidade de recolonizar ambientes que foram pulverizados com inseticidas anteriormente (Falavigna-Guilherme e cols, 2004). Diferentemente, na região sul, o *P.*

megistus não consegue colonizar o ambiente doméstico ficando restrito a ninhos de animais (Diotaiuti, 2007).

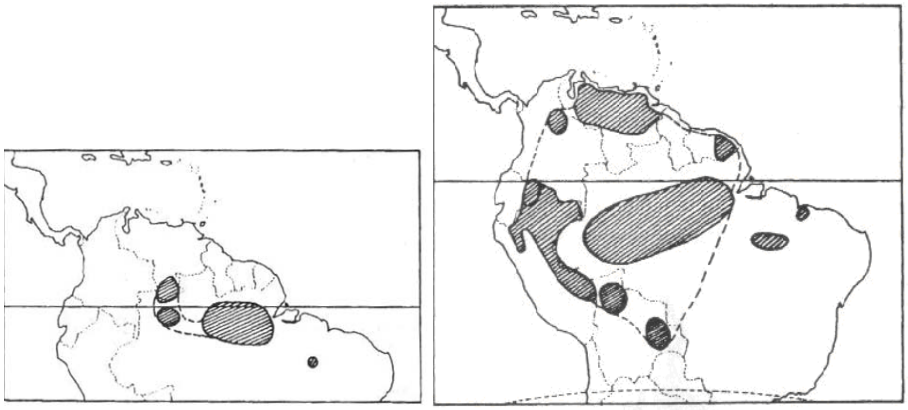
Já foram encontrados *P. megistus* com sangue de diferentes animais como roedores, cães, gatos, morcegos, coelhos, aves, répteis e até hemolinfa de outros barbeiros (Carcavallo e cols, 1998a).

As informações científicas sobre os hábitos alimentares de *P. megistus* mostram que estes são relativamente recentes do ponto de vista evolutivo por causa de sua semelhança com hemípteros predadores de plantas. Além disto, os *P. megistus* mudaram morfologicamente nos últimos dois séculos, e possuem bactérias em seu intestino essenciais para a assimilação de nutrientes importantes (Schofield, 2000; Beard e cols, 2001). Infelizmente, ainda não existem muitos estudos sobre as glândulas salivares de *P. megistus*, apenas alguns sobre as variações nos perfis de saliva de diferentes insetos em diferentes áreas geográficas (Barbosa e cols, 2004).

2.3 *Rhodnius*

O gênero *Rhodnius* compreende 19 espécies divididas em dois grupos que vivem em localizações biogeográficas diferentes. No grupo *picptipes* encontra-se a espécie *R. brethesi* e o grupo *robustus* engloba os *R. robustus* e *R. prolixus* (Abad-Franch e Monteiro, 2007).

Até pouco tempo, a região de floresta tropical úmida que inclui a Amazônia não era considerada uma área endêmica de Doença de Chagas. A infecção era conhecida somente como uma enzootia de animais silvestres (Junqueira e cols, 2005), porém, atualmente têm sido descritos muitos surtos agudos das infecções pelo *T. cruzi*. Naquela grande vastidão Amazônica já foram identificados cerca de 40 espécies de triatomíneos (Teixeira e cols 2001) e, também, verificou-se que *R. brethesi* e *R. robustus* estão presentes em todo território Amazônico (Figura 4).



Rhodnius brethesi *Rhodnius robustus*

Figura 4: Distribuição geográfica dos *Rhodnius brethesi* e *Rhodnius robustus* (Fonte: Carcavallo e cols, 1998b).

A predação antrópica que leva o homem a aproximar-se dos nichos ecológicos dos *Rhodnius* infectados teria sido, provavelmente, fator primordial do desequilíbrio ecológico presente em certas regiões de desmatamento e também por migrações. Segundo Coura e cols (1999), 18 espécies de triatomíneos foram encontradas na Bacia Amazônica sendo que 10 estavam infectados pelo *T. cruzi* (Tabela 1). O gênero *Rhodnius* provavelmente engloba mais de 50 % de todos os triatomíneos existentes na floresta Amazônica (Sherlock, 1999).

Tabela 1: Triatomíneos encontrados por Coura e cols, 1999, na Região Amazônica.

Espécie de Triatomíneos encontrados na Amazônia brasileira	
Belminus herreri	*Rhodnius nasatus*
Cavernicula lenti	*Rhodnius neglectus***
Cavernicula pilosa	*Rhodnius paraensis***
*Eratyrus mucronatus***	*Rhodnius pictipes***
*Microtriatoma trinidadensis***	*Rhodnius prolixus*
*Panstrongylus geniculatus***	*Rhodnius robustus***
*Panstrongylus lignarius***	*Triatoma maculata*
*Panstrongylus rufotuberculatus***	*Triatoma rubrofasciata*
*Rhodnius brethesi***	*Triatoma rubrovaria**

** Encontrado com *T. cruzi* ou outro 'cruzi like'

* Necessita de confirmação, pois é uma espécie nativa do Rio Grande do Sul e Uruguai, pode ter sido introduzido pelas migrações de agricultores do Sul.

R. brethesi e *R. robustus* vivem em palmeiras de piaçava (*Leopoldina piassaba*), sendo que o *R. robustus* se alimenta em aves, roedores, marsupiais, morcegos, lagartos e humanos. O comportamento do *R. brethesi* é mais voraz, atacando os trabalhadores que colhem folhas das piaçabeiras e até invadindo temporariamente as casas quando são trazidos juntos com as palmas ou atraídos pelas luzes (Coura e cols, 2002). Quando as condições de alimentação são favoráveis, pode haver até três gerações por ano de *R. brethesi* (Rocha e cols, 2004). Para chegar até a fase adulta, esses insetos precisam de vários repastos, aproximadamente sete, comprovando que, além da voracidade há grande eficiência no aproveitamento de sangue (Rocha e cols, 2004). Além de se alimentarem com sangue humano, esses insetos também podem sugar roedores, répteis e outros mamíferos (Carcavallo e cols, 1998a).

3. Hemostasia

O sangue é mantido fluido nos vertebrados graças à interação de diferentes fatores celulares (células endoteliais), elementos celulares circulantes (plaquetas) e proteínas plasmáticas (fatores de coagulação e anticoagulação). Quando existe um sangramento, três processos são iniciados: a vasoconstrição, agregação de plaquetas e coagulação sanguínea.

Mesmo uma pequena lesão no sistema vascular como uma picada de inseto desencadeia uma vasoconstrição reflexa (contração do músculo liso) que tem como função diminuir o fluxo sanguíneo local aumentando a concentração dos fatores plasmáticos (Aires, 1999).

Como a parede vascular foi danificada, agora ela expõe partes do tecido conjuntivo como o colágeno onde se fixam as plaquetas. As plaquetas ativas liberam ADP (adenosina difosfato), tromboxano A_2 e ATP, intensificando a reação de interação plaqueta-epitélio. Depois da ativação das plaquetas, elas se agregam, promovem o tampão plaquetário e liberam mediadores de vasoconstrição. As plaquetas ativas vão se agregando e formando o tampão plaquetário para proteger o vaso lesado quando ocorrem pequenas lesões. Mas se a lesão é grande, o tampão plaquetário não poderá conter extravasamentos de sangue, é necessária a formação do coágulo sanguíneo. Durante este processo, a protrombina é convertida em trombina por um ativador de protrombina. A trombina ativada converte o fibrinogênio em fibrina que tem a capacidade de se juntar a outras fibrinas que junto com as plaquetas, hemácias formam o coágulo sanguíneo (Guyton e Hall, 1997).

4. Repasto sanguíneo

O sistema de hemostasia dos vertebrados tem aproximadamente 400 milhões de anos. Já a hematofagia evoluiu independentemente entre 150 e 65 milhões de anos atrás (Mans e Neitz, 2004). Os reduvídeos aderiram à hematofagia por volta do período Cretáceo. Aproximadamente 14000 espécies de insetos são hematófagas, necessitando de sangue para o desenvolvimento dos ovos e sobrevivência (Teixeira e cols, 2006). Alguns triatomíneos, como por exemplo, os do gênero *Psammolestes* só se alimentam de fonte única, vivendo em um micro ambiente específico, já outros, como o *Panstrongylus megistus* podem fazer os repastos em diferentes espécies de animais (Diotaiuti, 2007).

Os vetores hematófagos não são apenas transportadores do parasito, eles também influenciam na instalação de certas infecções já que a saliva é um fluido farmacologicamente ativo. A saliva afeta a resposta hemostática, inflamatória e imune do hospedeiro vertebrado (Andrade e cols, 2005). Os barbeiros localizam suas presas pelo calor (graças a suas antenas que possuem sensores) e a certos agentes químicos (Flores e Lazzari, 1996). A sucção de sangue ocorre após a penetração do aparelho sugador (proboscídeo) na pele do hospedeiro vertebrado.

O proboscídeo penetra na pele do hospedeiro, com vários movimentos circulares até encontrar o vaso capilar. A fase de experimentação (*probing phase*) é iniciada quando o proboscídeo entra em contato com o sangue e o inseto começa a ejetar saliva. A sucção do sangue ocorre com a ativação da bomba cibarial que é controlada por vários músculos, por isso o sangue vem de forma pulsátil. A alimentação cessa quando há estiramento da parede abdominal do inseto (Friend e Smith, 1971). A função anti-hemostática da saliva é importante para o posicionamento do proboscídeo no vaso sanguíneo do hospedeiro (Ribeiro e Garcia, 1981). Durante a ingestão de sangue, o barbeiro também ingere a própria saliva e fatores coagulantes do vertebrado, todos são armazenados no intestino anterior.

5. Glândulas salivares de triatomíneos

As glândulas salivares de triatomíneos têm tamanhos, números, formas e posicionamentos diferentes (Lacombe, 1999). Cada glândula tem uma importância e estrutura diferente. Mesmo assim, todas as glândulas possuem uma camada de células epiteliais com

muitas microvilosidades que aumentam a superfície de secreção/absorção em volta de um grande lúmen. Estas células possuem retículo endoplasmático proeminente e diversas vesículas lipídicas para a produção em larga escala de proteínas que serão secretadas no lúmen das glândulas (Reis e cols, 2003).

Os gêneros *Triatoma* e *Panstrongylus* possuem três pares de glândulas que foram classificados por Barth (1954) como D1 (glândulas principais), D2 (glândulas suplementares) e D3 (glândulas acessórias). Segundo Barth (1954) as glândulas D1 tem propriedades anticoagulantes, a D2 produz uma secreção hemolítica e a D3 é responsável pela produção de uma substância emoliente.

As glândulas D1, reniformes, têm como função produzir substâncias anticoagulantes que são liberadas no lúmen junto com o epitélio que é rapidamente reconstruído (Lacombe, 1999). As glândulas D2 são mais curtas e arredondadas com secreção do tipo apócrina (pequenas gotas que são liberadas no lúmen). Já o par D3 é transparente, redondo e fica localizado mais distante das outras glândulas. Ele possui uma válvula (Lacombe, 1999) e a secreção nunca é armazenada sendo produzida e secretada de imediato. As glândulas têm aparência amarelada em *Panstrongylus* (Figura 5), a glândula D3 não pode ser fotografada, pois ela é muito sensível a manipulação e se rompe facilmente.

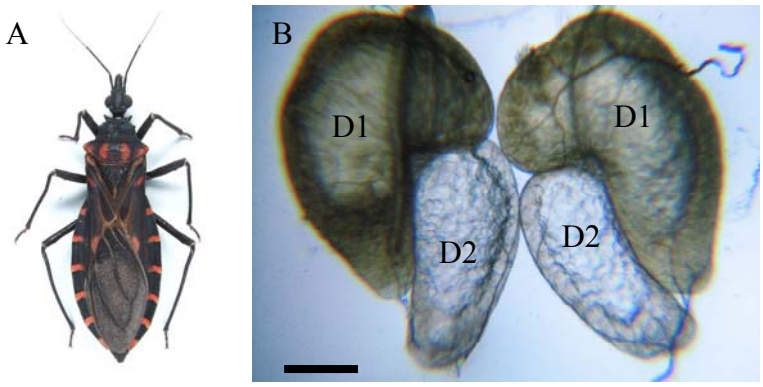


Figura 5: (A) *Panstrongylus megistus* adulto **(B)** Microscopia de contraste de fase 10x de glândulas salivares de adultos não alimentados, os pares de glândulas D1 e D2 estão indicados. A barra representa 0,5 mm.

Os *Rhodnius* apresentam apenas um par de glândulas salivares unilobulares, sem traços de divisão (Figura 6) (Konig e cols, 1993). Estudos mostraram que as proteínas nitroforinas são armazenadas no lúmen das glândulas salivares e que as células epiteliais são

responsáveis pela produção de óxido nítrico (NO) pela enzima NADPH diaforase. As microvilosidades permitem a passagem de NO intracelular para o local do armazenamento das hemoproteínas (Nussenzveig e cols, 1995). A coloração avermelhada das glândulas de *Rhodnius* é devida justamente às proteínas do tipo nitroforina.

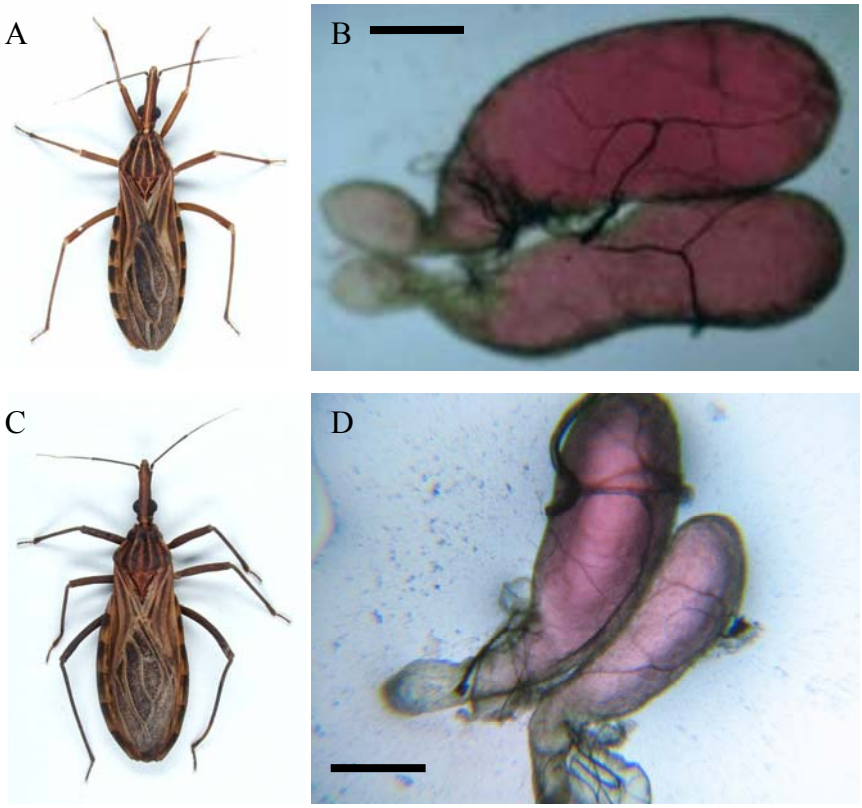


Figura 6: **(A)** Inseto *Rhodnius brethesi* adulto **(B)** Estrutura das glândulas salivares de *Rhodnius brethesi* (Fonte: Lacerda, 2006) em microscopia com contraste de fase 10x. **(C)** Inseto *Rhodnius robustus* adulto **(D)** Estrutura das glândulas salivares de *Rhodnius robustus* em microscopia com contraste de fase 10x. A barra representa 0,5 mm.

6. Propriedades da saliva de insetos hematófagos

A saliva é essencial para o sucesso da alimentação dos triatomíneos (figura 7). Cada espécie tem um padrão salivar próprio, diferindo na composição das proteínas e nas funções realizadas (Pereira e cols, 1996).

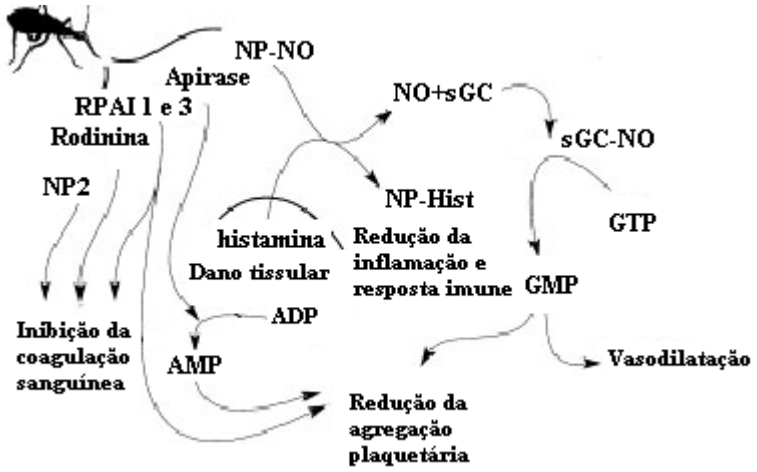


Figura 7: Esquema ilustrando as atividades antihemostásicas encontradas na saliva de *Rhodnius prolixus* e seus efeitos no hospedeiro vertebrado. (Fonte: Montfort e cols, 2000).

A saliva de insetos hematófagos apresenta uma série de substâncias farmacologicamente ativas que afetam o sistema imune de seus hospedeiros, além de combater as respostas inflamatórias e hemostáticas (Ribeiro e cols, 1995). Proteínas com funções imunossupressoras já foram descritas como o IRIS (*Ixodes ricinus* immunosupresor) em ácaros. O IRIS possui a capacidade de modular a atividade de linfócitos e macrófagos (Leboule e cols, 2002). Outra função já conhecida é a atividade anti-complemento que inibe tanto a via clássica como a alternativa do sistema complemento (Valenzuela e cols, 2000, Cavalcante e cols, 2003). Já foram identificadas várias substâncias com efeitos vasodilatador, anti-agregador de plaquetas ou anticoagulantes (Valenzuela, 2002a) que facilitam o repasto. Além disso, os insetos hematófagos também possuem moléculas que anestesiam o local da picada e impedem as reações alérgicas. As picadas de *Triatoma nitida* e de *Panstrongylus geniculatus* costumam ser doloridas e provocam reações alérgicas. Em decorrência disso, esses insetos têm mais dificuldades em obter sangue e têm menos chance de colonizar moradias, porque costumam ser mais combatidos. Em *Rhodnius prolixus,* podemos citar as lipocalinas (Franscischetti e cols, 2000), a apirase (Sarkis e cols, 1996) e as nitroforinas (Moreira e cols, 2003) como exemplos de proteínas com atividade anti-hemostásicas. Além dessas proteínas foram encontradas outras com funções ainda desconhecidas ou não esperadas como uma endonuclease em *Culex quinquefasciatus* (Calvo e Ribeiro, 2006a).

6.1 Anti-agregador de plaquetas

O primeiro mecanismo do hospedeiro para evitar a perda de sangue é a agregação das plaquetas. Essa reação é extremamente rápida, por isso os artrópodes hematófagos têm várias moléculas capazes de coibir a agregação, como por exemplo, a anofelina (Valenzuela e cols, 1999), peptídeo proveniente da saliva de *Anopheles albimanus*.

Em *Rhodnius prolixus* já foi descrito a RPAI-1 (*Rhodnius prolixus* aggregation inhibitor 1) que se liga ao ADP liberado e impede a agregação plaquetária (Francischetti e cols, 2000 e Francischetti e cols, 2002a). A palidipina e triabina, descritas em *Rhodnius pallidipennis* têm a mesma função (Noeske-Jungblut e cols, 1994 e Noeske-Jungblut e cols, 1995).

No Laboratório Multidisciplinar de Pesquisa em Doença de Chagas (LMPDC), Feijó, em 2001, identificou duas proteínas, uma de 14 kDa, a infestilina, com alta identidade com a palidipina e outra denominada triatina com semelhanças com a triabina, de *Triatoma pallidipennis.*

Uma das estratégias mais utilizadas pelos insetos é hidrolisar a molécula de ATP para impedir a sua ação na ativação de plaquetas e subseqüente agregação plaquetária. A apirase (ATP-ADP fosfohidrolase) é uma proteína encontrada na saliva de artrópodes como os triatomíneos que remove o ADP e ATP da circulação sanguínea, prevenindo a agregação das plaquetas (Faudry e cols, 2004). As apirases já foram isoladas e caracterizadas em *Aedes* (Champagne e cols, 1995a), *Anopheles* (Arcà e cols, 1999) e *Triatoma* (Faudry e cols, 2004).

6.2 Vasodilatadores

Vasodilatadores são moléculas que aumentam o fluxo sangüíneo local. Eles agem nas células do músculo liso presente na parede dos vasos, relaxando-o. Em 1991, Lerner e cols descreveram em *Lutzomyia longipalpis* a atividade vasodilatadora de um pequeno peptídeo chamado de maxadilan. Na época o maxadilan tinha 500 vezes mais atividade que o peptídeo mais potente conhecido, a calcitonina. Interessantemente, a grande maioria das moléculas com propriedades vasodilatadoras encontradas até hoje são lipídeos e não proteínas (Valenzuela, 2004a). Em *R. prolixus* foram encontradas várias moléculas com capacidade vasodilatadoras, elas são as nitroforinas (NP1 a NP4) que têm aproximadamente 20 kDa de massa, e três

atividades distintas (Monfort e cols, 2000). O papel mais importante é o armazenamento e transporte de NO que é liberado dependendo do pH do ambiente (Ribeiro e cols, 1993; Champagne e cols, 1995b) e se liga a enzima guanilato ciclase do hospedeiro causando o relaxamento muscular. As nitroforinas também após perderem o NO têm afinidade com a histamina liberada pelos mastócitos. Com a retirada da circulação sanguínea da histamina, a inflamação local e novas respostas imunes são impedidas. A última atividade existe apenas na NP2 que interfere na coagulação sanguínea pelo intermédio do fator X.

6.3 Anticoagulantes

Os anticoagulantes foram descritos na saliva de insetos hematófagos há mais de 80 anos. Cada espécie de inseto foi se adaptando e criando mecanismos para combater a coagulação sanguínea. Os mosquitos, como se alimentam mais rapidamente, não enfrentam tantas dificuldades, pois a coagulação se torna mais evidente depois de 3 a 4 minutos de alimentação. Já os carrapatos passam dias se alimentando e precisam de várias proteínas para aumentar a sua eficiência alimentar. A maioria dos anticoagulantes de saliva tem como alvo a trombina ou o fator Xa (Champagne, 2005). Os anofelinos produzem fatores que agem diretamente com a trombina, como a anofelina em *Anopheles albimanus*, já os culicídeos possuem moléculas contra o fator Xa (Starks e James, 1996).

Estudos mostraram que a saliva de *P. megistus* interfere na via intrínseca da coagulação sanguínea e a saliva de *T. infestans* consegue agir nas duas vias, a intrínseca e extrínseca (Pereira e cols, 1996). Em *R. prolixus* foi encontrada a prolixina-S ou nitroforina 2 que possui uma atividade anti-fator VIII (Ribeiro e cols, 1995).

7. Metodologias para estudo de genes e proteinas das glândulas salivares de triatomíneos

7.1 Biblioteca de cDNA

A metodologia utilizada normalmente para o estudo da saliva de inseto segue os seguintes passos: encontrar uma atividade bioquímica, isolar a proteína, obter alguma informação das seqüências, construir uma sonda de DNA e procurar um clone em uma biblioteca de cDNA.

Com o clone encontrado, a proteína pode ser expressa e a função confirmada. Esse trabalho além de demorado, não conseguia fornecer muitos resultados, e, por isso, foram criados métodos de purificação de proteínas, clonagem de DNA, e seqüenciamentos em grande escala. Bibliotecas de cDNA foram construídas e os clones foram seqüenciados randomicamente, assim os pesquisadores puderam começar a entender a grande diversidade e especialização das proteínas salivares.

O sialoma (RNAm e proteínas encontradas nas glândulas salivares) começou a ser estudado em insetos hematófagos para entender os mecanismos que levaram a adaptação do inseto ao padrão atual de alimentação sangüínea (Ribeiro e cols, 2004a). Para a descrição dos sialomas são utilizadas as bibliotecas de cDNA por causa das vantagens desta metodologia. Esta estratégia permite o uso de pouca quantidade de cDNA. Além disso, não é preciso amplificar a biblioteca, evitando assim a perda de plasmídeos com insertos. Os protocolos mais conhecidos segundo Valenzuela (2002b) não precisam da excisão do vetor, o fago é diretamente amplificado por PCR usando uma seqüência iniciadora. Essas bibliotecas unidirecionais são de alta eficácia, pois a grande maioria das seqüências está completa. Com essa técnica já foram encontradas proteínas com seqüências similares a hialuronidase, adenosina deaminase, família D7, e família antígeno 5.

Os sialomas já descritos mostraram que existem mais proteínas do que era esperado e que muitas ainda não têm funções conhecidas (Valenzuela, 2004a). Já foram feitos sialomas de *Aedes aegypti* (Valenzuela e cols, 2002a, Ribeiro e cols, 2007), *Aedes albopictus* (Arcà e cols, 2007), *Anopheles darlingi* (Calvo e cols, 2004), *Anopheles gambiae* (Franscischetti e cols, 2002b, Arca e cols 2005, Calvo e cols, 2006b), *Anopheles stenphensi* (Valenzuela e cols, 2003), *Culex pipiens quinquefasciatus* (Ribeiro e cols, 2004b), *Culicoides sonorensis* (Campbell e cols, 2005), *Phlebotomus argentipes and P. perniciosus* (Andersen e cols, 2006), *Lutzomyia longipalpis* (Charlab e cols, 1999; Valenzuela e cols, 2004b), *Ixodes scapularis* (Ribeiro e cols, 2006), *Rhodnius prolixus* (Ribeiro e cols, 2004a), *Triatoma brasilienses* (Santos e cols, 2007) e *Triatoma infestans* (Assumpção e cols, 2008).

7.2. Espectrometria de massa

A espectrometria de massa consiste em três etapas: a fonte de íon, o analizador e o detector de massa. As moléculas a serem testadas têm que estar em fase gazosa, ou seja, ionizadas. A ionização pode ocorrer de duas maneiras diferentes: ESI (Electrospray ionization) ou MALDI (Matrix-assisted laser absorption/ionization). Existem vários tipos de analizadores de massa, entre eles encontra-se o *ion trap* onde os íons são "capturados" e submetidos à análise MS ou MS/MS (Aebersold e Mann, 2003).

A espectrometria de massa em larga escala é uma técnica que vem sendo usada juntamente com o seqüenciamento em massa de genomas completos, para correlacionar as seqüências das proteínas. A análise por espectrômetro de massa fornece dados sobre a carga e massa de cada peptídeo ionizado e fragmentado (*m/z*) que serão comparados com dados previamente conhecidos ou serão utilizados para interpretação manual dos espectros por MS/MS (Yates, 1998).

Estudos de espectrometria de massa em larga escala têm sido realizados utilizando a técnica de gel bidimensional e, em seguida a espectrometria de massa. Esse método favorece a separação das proteínas, além de permitir conhecer sobre a massa e PI (ponto isoelétrico) de separação ideal. Mas esta metodologia pode trazer falhas, pois nem todas as proteínas podem ser identificadas em gel (proteínas grandes ou pouco concentradas). O proteoma de glândulas salivares de *T. infestans* foi produzido com ajuda desta metodologia (Charneau e cols, 2007); entre as mais de 200 proteínas apenas 45 foram identificadas, mostrando a exigüidade de conhecimento sobre genoma e transcriptoma desse inseto. A utilidade dos métodos de LC-MS/MS empregados no estudo de glândulas salivares de insetos é muito clara, pois corroboram achados de transcriptomas e ainda apresentam outras proteínas sem função ou descrição conhecida, além daquelas muitas outras para as quais já existe ampla descrição (Assumpção e cols, 2007).

OBJETIVOS

A linha de pesquisa em entomologia molecular do Laboratório Multidisciplinar em Pesquisa em Doença de Chagas tem como objetivo principal conhecer a diversidade molecular e funcional das glândulas salivares de triatomíneos. No escopo desta linha de pesquisa, incluem-se nos objetivos desta Tese de Doutorado:

a) Construção de bibliotecas de cDNA de *Rhodnius brethesi, Rhodnius robustus* e de *Panstrongylus megistus* ;

b) Análises transcriptômicas por banco de dados dos cDNA obtidos nas bibliotecas;

c) Análises proteômicas dos extratos de glândulas salivares por LC-MS/MS.

d) Validação dos achados das bibliotecas de cDNA utilizando a tecnologia de espectrometria de massa em larga escala;

A tese está dividida em dois manuscritos ainda não publicados. O primeiro aborda o transcriptoma e proteoma dos insetos das espécies de *Rhodnius*, como o *Rhodnius brethesi* e o *Rhodnius robustus*. E o segundo manuscrito enfoca as proteínas e transcritos de *Panstrongylus megistus*.

MANUSCRITO 1

Diversity of anti-hemostatic proteins in the salivary glands of *Rhodnius* species

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Abbreviations: DDT: 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane; GST: Glutathione S-transferase; Rb: *Rhodnius brethesi*; Rr: *Rhodnius robustus*;

Keywords: *Rhodnius brethesi*, *Rhodnius robustus*, salivary glands, cDNA library, mass spectrometry.

Abstract:

The Rhodnius gender comprises almost half of the triatomines found in the Amazon Basin. These triatomines well adapted to palm trees in the environment are not capable to colonize human dwellings. Although the importance of theses insects *Trypanosoma cruzi* transmitters are properly recognized, its feeding habits, dependent on its salivary gland apparatus are mostly neglected. In this study we describe the transcriptome of *R. brethesi* and *R. robustus*, respectively comprising 56 and 122 clusters. Over 30% of these clusters had never been described before. We also used LC-MS/MS and found 123 and 111 proteins in *R. brethesi* and *R. robustus* proteome, respectively. Besides the highly redundant housekeeping proteins we also could found lipocalins (biogenic amino binding proteins and salivary platelet aggregation inhibitor), inositol polyphosphate 5-phosphatase, and kazal domain proteins, essential proteins used for the successful blood-feeding habits, and glutathione S transferase, and antigen-5 protein, respectively, involved with resistance to insecticide and defense.

1. Introduction:

The Brazilian Amazon is known to hide countless dwellings of triatomines (Insecta: Hemiptera) transmitters of *Trypanosoma cruzi*. The insects preying upon over 1150 mammalian species generate a huge enzooty [1, 2] and epidemic Chagas disease. In the past five decades increasing deforestation, new population settlements, and demographic growth in the Amazon region have concurred with autochtonous acute cases of human *T. cruzi* infections [3]. Among 137 blood-sucking insects belonging to the Rhodniini genera present in the American and Asian continents there are over 40 *Rhodnius* spp. that have been recognized as true family *Triatominae* dwelling in the Amazon biome [4]. In the *Rhodnius* genera there are 19 species comprising two major groups distributed in separate biogeographic localizations. In the group *pictipes* is included the archetype *Rhodnius brethesi*, and in the group *robustus* is *Rhodnius robustus* and *Rhodnius prolixus* [5].

R. brethesi and *R. robustus* have always been associated with palm trees (*Leopoldinia piassaba*) that serve as dwells of marsupials, rodents, bats and primates. Currently, the closeness of human settlement to palm trees and the predation of local fauna have been

associated with households invasion by *Rhodnius* insects, The strictly hematophagous male and female triatomines attack humans to transmit the *T. cruzi* infections [6]. These triatomines bear sophysticated machinery capable to circumvent difficulties imposed to blood sucking by the host's hemostasis. Several important molecules were described in insect's saliva, such as vasodilators, anticoagulants, platelet aggregation inhibitors and anesthetics [7]. cDNA libraries and mass-spectrometry are considered important tools used to improve the understanding about triatomines saliva pharmacologically active bioamines. The *R. prolixus* transcriptome showed several important bioamines for the blood feeding and revealed the presence of redundant numbers of lipocalins encoded by a possibly growing gene family [8]. Also, salivary transcriptomes from *T. infestans* [9] and *T. brasiliensis* [10] showed prevalence of sequences from lipocalins genes. The mass spectrometry in 2D gels was also used to analyze the *T. infestans* proteome [11], and showed that among 200 proteins, there are 34 platelet aggregation inhibitors belonging to major triabin and apirase families. All those findings showed that the salivary glands of triatomines are replete with anticoagulant proteins, vasodilators, platelet aggregation inhibitors, anesthetics and modulators of vertebrate immune system.

This study aims at to compare the salivary transcriptome and proteome of *Rhodnius* species dwelling in the Amazon. Herein we used cDNA and spectrometry (LC-MS/MS) technologies, providing complementary data and information, which were analyzed and compared. Sequencing of *R. robustus* salivary glands cDNA library yielded 576 ESTs, comprising 122 clusters. The *R. brethesi* cDNA library yielded 427 sequences in 56 clusters. For the LC-MS/MS analysis we found 125 proteins for *R. brethesi* and 111 for *R. robustus* including respectively 86 and 93 salivary gland proteins with recognized pharmacological functions. A diversity of antihemostatic bioamines in the salivary glands of both species is presented here.

2. Materials and Methods:

2.1 Triatomines and salivary gland:

R. brethesi and *R. robustus* were reared in our laboratory with controlled temperature of 28°C, 70% relative humidity and 12:12h light/dark photoperiod. Adults were dissected 3, 5 and 7 days after a blood meal. Usually, 150 pairs of salivary glands were collected in TRIZOL (Invitrogen) for the cDNA library. For the mass spectrometry experiment, we collected 50 pairs of salivary glands, which were punctured with a needle and centrifuged. The saliva in the supernatant was collected and lyophilized for future use.

2.2 cDNA library:

The mRNA was isolated with Micro-fast Track™ mRNA isolation (Invitrogen) and cDNA library was built with SMART cDNA library construction (Clontech) as described in [9, 13]. The PCR were carried on a PTC-100 Programmable thermal controller (MJ Research Inc.). The PCR condition were 95 ºC for 1 minute, 19 cycles (*R. robustus*) or 22 cycles(*R. brethesi*) of 95 ºC for 15 seconds and 68 ºC for 6 minutes. The cDNA double strand obtained was digested with proteinase K (as manufacturer's instructions) and after by *Sfi* I restriction enzyme. The cDNA was then fractionated by size using a Chroma-spin-400 drip column (Clontech) and observed in a 1.1% Agarose/EtBr gel. The samples were collected and ligated into a λTriplEx2 vector (Clontech) and packed using GigaPack Gold III (Stratagen). One µl of *R. robustus* cDNA library and 2 µl of *R. brethesi* cDNA library were platted on LB/MgSO4 plates with X-gal/IPTG. We obtained 90% of recombination clones in both libraries. The white colonies were randomly picked and transferred in 50 µl of deionized water. To amplify the cDNA sample we used 5 µl of the phages as template and the primers PT2F1 and PT2R1 based on the sequence of the vector TriplEx2 (Clontech) [12]. The PCR products were sequenced in unidirectional with PT2F3 primer and DYEnamic ET DyeTerminator Sequencing Kit (Amersham Bioscience, Piscataway, NJ, USA) using MegaBACE 1000 sequencer (Amersham Biosciences, Little Chalfont, UK). The sequence clusters were subject to BLAST algorithm [14], ClustalW [15], CDD [16], and TREEview software [17]. The sequences were translated by Bioedit program. Phylogenetics analyses and statistical neighbor-joining bootstrap tests of the phylogenies were done with de MEGA4 package [18].

2.3 Mass spectrometry LC-MS/MS:

The lyophilized samples of *R. brethesi* and *R. robustus* were diluted in 200 µl of water HPLC grade (Sigma), 10 µl of the saliva's samples were submitted to reduction (DTT 50 mM), alkylation of the cysteine residues (Iodacetamide 100 mM) and digestion with trypsin in NH_4HCO_3 buffer containing urea (Seq grade modified trypsin, Promega) [19]. The samples were desalted with a reverse chromatography reverse phase in-house zip-tip (POROS R2-50 resin, Applied Biosystem) made in a 200 µl pipette tip (Axygen, Union City, CA), with a glass wool layer inside of it to prevent the linking of the resin and sample. We applied on the top of it 30 µl of resin (10-15µm, 300 Å, Vydac, Hesperia, CA), activated the zip tip with methanol and equilibrated with 0.046% TFA. The sample was added and the zip-tip was washed two times with 0.046% TFA, finally, the sample was eluted with 80% ACN/0.046% TFA. After the desalting, the samples were submitted to a strong-cation exchange column. We used the same method of the desalting, replacing the C18 resin with 30 µl of SCX resin (POROS HS50, Applied Biosystems, Framingan, MA). After the column equilibration with 25% ACN / 0.5% FA (equilibration buffer), the sample was loaded and it was washed with 100 µl of equilibration buffer. The peptides were eluted with different gradient of NaCl in equilibration buffer (0, 10, 20, 40, 60, 80, 100, and 150, 200 and 500 mM). The samples were dried (Vacuge ™, Eppendorf, USA), desalted and submitted to liquid chromatography coupled to mass spectrometry (LC-MS/MS) analysis. The samples were dissolved in 10 µl 0.05% FA; the peptides were separated in an Ultimate nanoHPLC system (LC packings, Dionex, Sunnyvale, CA) and analyzed by an eletrospray Linear Ion Trap Mass LTQ XL ™ as described (Rodrigues et al, 2008).

BioWorks version 3.3.1 software (Thermo Electron) converted the raw data obtained in the LC-MS to DTA files. To analyze the MS/MS spectra we first used TurboSequest (Bioworks 3.0 Thermo Electron, San Jose, CA), [19, 20] algorithm with all ncbi database (downloaded on February 14, 2007). The second analysis was made with a database containing: (*i*) ncbi proteins from *Triatoma, Panstrongylus* and *Rhodnius* (downloaded on November 15, 2007); (*ii*) translated transcripts from the cDNA libraries from *R. brethesi, R. robustus* and from *Triatoma infestans* and *Panstrongylus megistus* (unpublished); (*iii)* matches from the first analysis against all ncbi; and (*iv)* random proteins (100K) to be used as false-negative. We calculated the false-positive rate which was 3.73% for *R. brethesi* and

4.2% for *R. robustus*. To obtain those results we used the following filters: distinct peptides (for exclusion of redundant hits); DCn ≥ 0.1; protein probability ≤ $1x10^{-3}$; and Xcorr ≥ 1.5, 2.2, 2.7 and 3.0 for singly, doubly, triply and more than 3 charged peptides, respectively.

3. Results and discussion:

3.1 Salivary glands transcriptome:

We obtained 576 EST grouped in 122 clusters comprising 33 contigs (cluster with more than one sequence) and 89 singlets (only one sequence) for *R. robustus* among the salivary glands cDNA sequences. For the cDNA of *R. brethesi* we found 427 EST in 56 clusters (19 contigs and 37 singlets). The nucleotides sequences were analyzed with blast algorithm (Blastx and Blastn). The sequences were also translated by Bioedit program and the aminoacids were submitted to CDD analysis on the ncbi site and to ClustalW algorithm followed by Mega 4 program.

As expected we found housekeeping function transcripts, 27 % of the *R. robustus* clusters and 12.8% of the clusters of *R. brethesi* (fig. 1). It was observed the presence of 23.5% of housekeeping proteins in *R. prolixus* transcriptome [8], 36.4% in *T. infestans* [9], and 24,4% *T. brasiliensis* [10]. Here we show the *R. robustus* transcriptome with proteins related to cellular (calponin, villin), DNA (helicase), energy (cytochrome c oxidase I, II and III, pyruvate dehydrogenase) and protein metabolisms (ribosomal protein, 5-aminolevulinic acid synthase, histone-lysine n-methyltransferase). Also, we present the *R. brethesi* transcriptome derived proteins related to energy metabolism (cytochrome c oxidase II and III, NADH dehydrogenase subunit 4, truncated ATPase subunit 6) and protein synthesis (mitochondrial ribosomal protein L30).

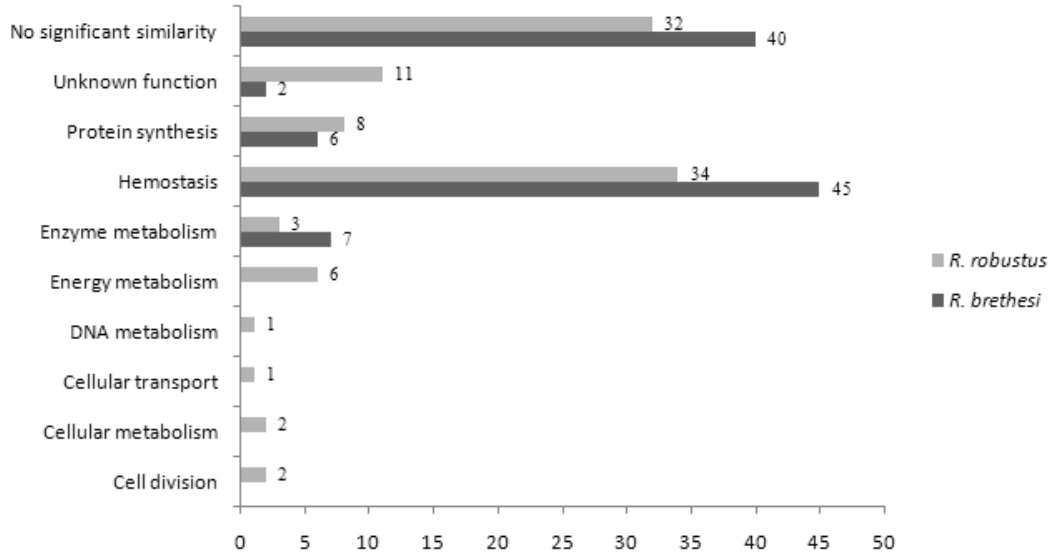

Figure 1: Potential biological function of transcripts expressed in *Rhodnius robustus* and *Rhodnius brethesi* salivary glands.

The important findings in these studies are proteins indispensable to the insect blood-feeding (Table 1). Biogenic amine binding proteins, lipocalins, nitrophorins, pallidipins, salivary platelet aggregation inhibitors and triabins were present in both triatomines species. Some transcripts that were found only in *R. robustus* were brasiliensin, lipocalin AI6, nitrophorin 3B, SPAI2, and inositol polyphosphate 5-phosphatase. Differently, SPAI1 and salivary protein MYS1 precursor were found in *R. brethesi* only.

Several *Rhodnius* transcripts that matched with already sequenced proteins and were posted at ncbi database had unknown function. However, these sequences appeared to be specific of insects: *Drosophila melanogaster*, *Drosophila pseudoobscura*, *Apis mellifera*, *Anopheles gambiae*, *R. prolixus*, *Phlebotomus papatasi*, *Thermobia domestica*, *Nasonia vitripennis.* We detected 40% of *R. brethesi* transcripts and 32% of *R. robustus* transcripts that did not match to any protein sequence in the ncbi database, and, therefore these are undisclosed proteins. Interestingly, the translated transcripts submitted to conserved domain analysis revealed that many hemostasis proteins had triabin domain (Supplementary tables 1 and 2).

Table 1: Transcripts and proteins found in the salivary glands of *Rhodnius brethesi* and *Rhodnius robustus*.

Proteins	Transcriptome		Proteome	
	R. brethesi	*R. robustus*	*R. brethesi*	*R. robustus*
Antigen-5-like protein		1	4	2
Biogenic amine-binding protein	3	1	2	2
Brasiliensin		1		
Heme-binding protein			1	1
Lipocalin	1	1		
Lipocalin AI-3	1	1	1	1
Lipocalin AI-4	1	1	4	4
Lipocalin AI-5	1	3	3	4
Lipocalin AI-6		3	8	9
Lipocalin AI-7	3	4	5	6
Nitrophorin 1	2	4	2	4
Nitrophorin 1A	1	3	4	4
Nitrophorin 2	2	4	6	8
Nitrophorin 3	1	2	7	8
Nitrophorin 3B		2	3	3
Nitrophorin 4			5	6
Nitrophorin 4A			1	2
Nitrophorin 4B	1	2	4	4
Nitrophorin 7			3	2
Pallidipin-like lipocalin 1			2	2
Pallidipin-like lipocalin 2	1	1		
Polylysine protein	1	1		
Salivary inositol polyphosphate 5-phosphatase		1	1	1
Salivary platelet aggregation inhibitor 1	1		1	1
Salivary platelet aggregation inhibitor 2		2	4	4
Salivary protein MYS1 precursor	1		2	2
Triabin-like lipocalin 1	2	1	2	2

Triabin-like lipocalin 2	1	1	7	6
Triabin-like lipocalin 3			1	1
Triabin-like lipocalin 4			2	2

3.2 Salivary glands proteome:

The proteomic analysis of *Rhodnius* sp salivary gland proteins covalidated several findings described in the cDNA library and depicted other non described findings. A high throughput technology approach was used to fabricate these proteomes that were analyzed in two steps. First we matched the whole spectra of data with all ncbi databases, and selected proteins were saved for a second step where a specific database was built aiming at the statistics. The database used retained proteins that matched to the first analysis, translated cDNA transcripts from *R. robustus* and *R. brethesi*, and translated cDNA transcripts library of *P. megistus* and *T. infestans* (unpublished), triatomines proteins deposited in ncbi server (downloaded November 15, 2007), and random proteins. With this ample database we could calculate the false positive rate and attain correct statistical calculations ($p<5\%$).

We obtained 111 proteins from *R. robustus* and 125 from *R. brethesi* (Supplementary table 3 and 4). Among these there were 93 *R. robustus* proteins with pharmacological and enzymatic functions, and 86 of these proteins were present *R. brethesi* proteome (Table 1). This approach allowed us to find several proteins not yet identified in cDNA libraries, such as apolipophorin, heme-binding proteins, nitrophorin 4, 4A and 7, pallidipin-like lipocalin 1, and triabin-like lipocalin 3 and 4.

3.3 Lipocalins and nitrophorins:

The lipocalins comprise a large group of small proteins (160-180 residues) with different functions and tertiary structure. The more recently evolved lipocalins have more substituted residues, more flexible tertiary structure, smaller ligand binding and more efficiency in the binding rate [21]. This protein group has three possible interactions: binding to small hydrophobic proteins, binding to a receptor or forming a macromolecular complex

[21]. Besides the lack of sequence similarity, except some particular domains, lipocalins have in their tertiary structure a cavity to accommodate the ligand. The cavity size and aminoacids sequence are specific to each type of lipocalin and binding ligand [22]. Lipocalins can be described as Kernel proteins or outlier lipocalins [23].

Lipocalins sequences are highly diverse and their alignments are hard to achieve, and consequently a phylogenetic tree cannot be built [22] (data not shown). In this respect, we used some chosen sequences from *T. infestans, T. brasiliensis* and *R. prolixus*, which were divided in lipocalins subtypes 4, 5, 6 and 7 (figure 2A).

Important members of lipocalins family are nitrophorins which are nitric oxide binding proteins. Nitric oxide (NO) is used in the host organism as a signaler that are produced and released by vascular endothelium; it activates the soluble guanylate cyclase, leading to the muscle relaxation [24]. NO appears to be released during transportation by the bug's nitrophorins, depending on the local pH [25]. In low pH of the salivary gland lumen, the NO is connected to the heme protein, whereas in the host organism the increase of the pH releases the NO with induction of vasodilation. Phylogenetic tree of the nitrophorins family shows close relationship between nitrophorins 1 and 4 and proximity of nitrophorins 2 and 3 (data no shown), as expected [26].

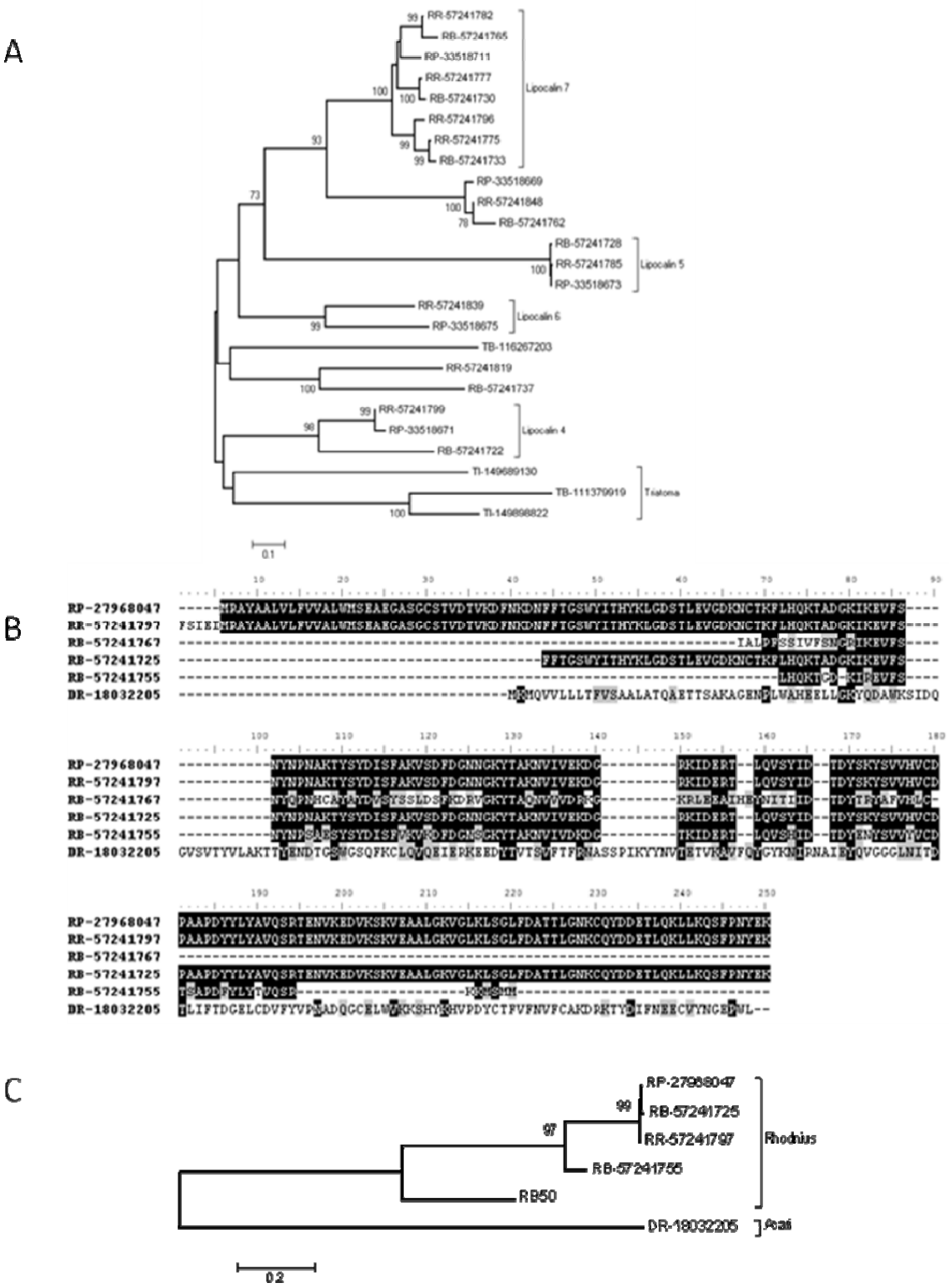

Figure 2: (A) Lipocalins dendogram with lipocalins from *Triatoma infestans* (TI), *Triatoma brasiliensis* (TB), *Rhodnius prolixus* (RP), *Rhodnius brethesi* (RB) and *Rhodnius robustus* (RR). The compared sequences are from nonreduntant protein database of the National Center for

Biotechnology Information (ncbi) and are represented by the first letters of gender and specie followed by the NCBI gi| accession number. The numbers in the dendogram nodes indicate percent bootstrap support for the phylogeny. The bar (bottom) indicates 20% amino acid divergence in the sequences. The graphic was constructed with MEGA4 package [18]; **(B)** ClustalW alignment of biogenic amine binding protein of *R. brethesi* (RB) and *R. robustus* (RR) with similar protein from *Rhodnius prolixus* (RP) and *Dermacentor reticulates* (DR); **(C)** Dendogram of biogenic amine binding protein with related proteins.

3.3.1 Biogenic amine binding proteins:

Biogenic amine binding proteins (BABP) are part of the lipocalin family. Those proteins bind to small biogenic amine such as serotonin, histamine and prevent their dispersion in the blood vessels. In absence of biogenic amines, the host hemostasis is incomplete and that favors insect's blood-sucking a plain meal. A particular BABP from *R. prolixus* that binds to serotonin, epinephrine, norepinephrine, inhibits muscle contractions was already described [27]. As it retained agonists from the platelet activation pathway like biogenic amines serotonin and epinephrine, this protein also had a platelet aggregation inhibitor activity. BABP from *R. prolixus* binding pro-inflammatory molecules could also inhibit inflammatory response. In *Dermacentor reticulatus*, a serotonine-histamine binding protein was described [28] that had function similarity but dissimilar amino acid sequence, suggesting a gene convergence event [27].

We found one transcript of BABP in the cDNA library of *R. robustus* and 3 transcripts in *R. brethesi*; the alignment of those sequences (Figure 2B) showed similarity with BABP from *R. prolixus*. The transcript RR-25 had the same cysteines residues present in *R. prolixus* BABP, where four amino acids formed disulfide-bridge. These cysteines were not detected in *Rhodnius* transcripts, possibly because they appear truncated. Two BABP sequences were also detected in each *Rhodnius* species proteomes. The dendogram of Rhodnius different species clustered in a branch, whereas a BABP protein from *Dermacentor reticulatus* showing considerable phylogenetic distance appears in an independent branch (Fig. 2C).

3.3.2 Salivary platelet aggregation inhibitors:

Several important biochemical activations of proteins appear to be activated immediately after the insect's proboscide enters the host skin. The main purpose of the host is preventing blood loss and repair of damage, and to circumvent this barrier the insect triggers pathways fundamental for inhibiting homeostasis-dependent platelet aggregation, vasoconstriction and blood coagulation.

The host platelets can be activated by collagen, adenosine diphosphate (ADP), tromboxane A2 and thrombin interaction [29]. Upon activation, the platelets release ADP and tromboxane A_2 to amplify the reaction, increasing platelet aggregation and a clot formation. *Rhodnius prolixus* aggregation inhibitor (RPAI) was described with a novel mechanism of action involving ADP in low concentration. Besides binding and cleaving ADP, this molecule prevents further platelet activation [30, 31]. This protein was similar to pallidipin from *T. pallidipennis* triggering the collagen induced platelet aggregation, inhibiting further ADP release [32]. We found two transcripts from *R. robustus* and three from *R. brethesi*, which were classified as transcripts salivary platelet aggregation inhibitor (SPAI) 1 or 2, because they were similar to RPAI1 and RPAI2. The phylogenetic analysis (Figure 3 A) showed the closeness between those sequences and *R. prolixus* transcripts. Using the proteome analysis we found 5 proteins SPAI on each insect, one from SPAI1 and four from SPAI2 (Table 2).

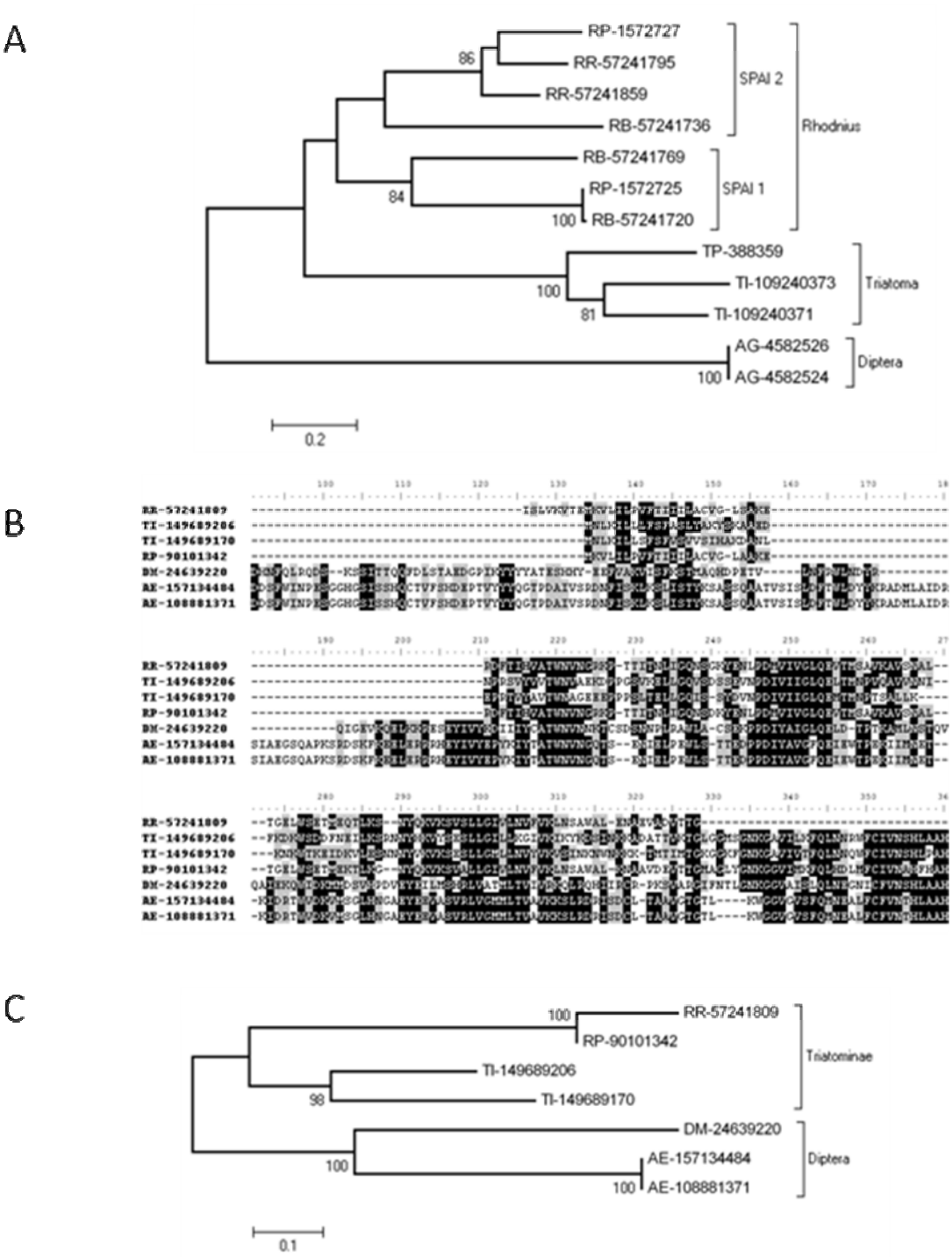

Figure 3: (A) Dendogram of salivary platelet aggregation inhibitor (SPAI) proteins with *Rhodnius prolixus* (RP), *Triatoma infestans* (TI), *Triatoma pallidipennis* (TP), *Anopheles gambiae* (AG), *Rhodnius brethesi* (RB) and *Rhodnius robustus* (RR); **(B)** ClustalW alignment of inositol polyphosphate 5-phosphatase with inositol similar proteins from *Rhodnius prolixus* (RP), *Triatoma infestans* (TI), *Drosophila*

melanogaster (DM), *Aedes aegypti* (AE) and *Rhodnius robustus* (RR) **(C)** Dendogram of inositol polyphosphate 5-phosphatase with the same protein as (B).

3.4 Inositol polyphosphate 5-phosphatase:

Phosphodiesterase enzyme cleaves cyclic nucleotides that regulate some physiological process such as platelet aggregation and muscle contraction. The inositol polyphosphate 5-phosphatase (IPP) are classified in seven different families, each of which showing its specific substrate. The interesting family in insects is the type II family which is composed by enzymes that remove phosphate from the 5-position of the inositol ring [33].

The truncated IPP transcript of *R. robustus* (figure 3B) did not present the conserved motif (FWLGDLNRFI and PSWTDRVLY) of the protein. A polypeptide with similar feature was described in *R. prolixus* [8], which bore inositol or apyrase activity. The *R. prolixus* IPP is composed by a secreted IPP domain with preference for soluble and lipids substrates [34]. Our finding presents the divergence between hemiptera triatomines and other dipteral insects, which is depicted in figure 3C.

Also, the proteome revealed the presence of IPP in *R. robustus* and *R. brethesi* saliva, which matched the Rr37 transcript from the cDNA library of *R. robustus*.

3.5 Kazal domain proteins:

Several kazal proteins were described in hematophagous insects with different functions. Thrombin inhibitor function was described in *R. prolixus* [35]; the rhodiniin protein had 11 kDa and 103 amino acids residues. In *Dipetalogaster maximus* midgut, two dipetalogastins were identified which belonged to non-classical Kazal-type domains family; this protein having two kazal domains [36, 37] was coded by a huge gene, which could express a protein with six Kazal-type domains [38]. Its main function was related to thrombin and trypsin inhibitions [39]. Infestin described in *Triatoma infestans* anterior midgut appears to have a non typical kazal domain protein [40]. In keeping with this finding, the thrombin inhibitory activity was found mostly in the insect midgut, for an inhibitory activity in its

salivary gland could not be detected, and it was suggested that it is not active in the salivary glands. Brasiliensin is the thrombrin inhibitor from *T. brasiliensis* [41] coded by a gene with 8 kazal domains. However, its processed protein has only 2 domains, and the gene knockdown resulted in a lower thrombin activity in the bug's anterior midgut.

We found one sequence of kazal domain protein in *R. robustus*, similar to brasiliensin protein from *T. brasiliensis*. Although its sequence is not complete, the presence of six cysteine residues (Figure 4A) and two other aminoacids (TY) defines the gene family showing kazal domain. The protein alignments allowed the build-up of phylogenetic network (Figure 4B) with a clear divergence between hemiptera triatomines and diptera. It also shows that the thrombin inhibitors are set close in *D. maximus* (dipetalogastin), *T. brasiliensis* (brasiliensin), *R. prolixus* (rhodiniin), *and T. infestans* (infestin and thrombin inhibitor). However, proteins with kazal domain were not present in the proteomes.

3.7 Antigen 5-like protein:

Antigen 5-like protein (Ag5) is part of a protein family named CAP that encloses mostly secreted sequences with similarity in a core of 150 amino acids. Ag5 is described as one of the principal venom of vespid wasp [42] but the function is still unknown. Studies suggested that the high conservative sequence can explain why Ag5 proteins share a common molecular function adapted to diverse physiological functions [43].

We found a single protein similar to Ag5 in the transcriptome of *R. robustus.* Ag5 sequences from *T. infestans* and *R. prolixus* have basic tails replenished of lysines, and it was suggested that this conformation would lead the protein to the platelet activated membrane and could modify their interaction with other protein or modify the releasing of active ligands [9]. Unfortunately the Ag5 sequence found in *R. robustus* appeared to be incomplete. Further study is necessary to understand the real physiological function of this enzyme. Furthermore the proteomes showed four proteins of Ag5 family in *R. brethesi* and three in *R. robustus*.

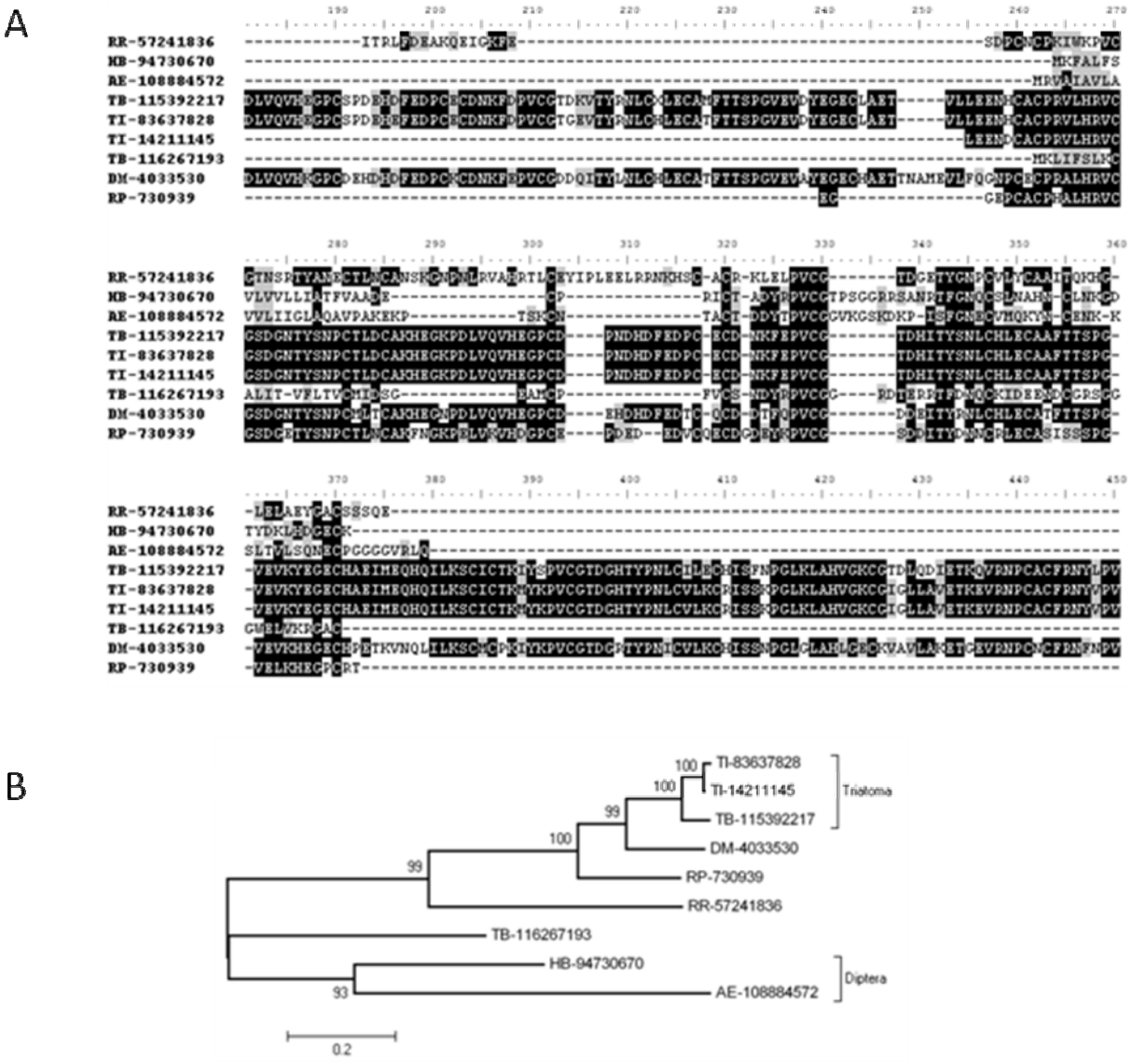

Figure 4: (A) ClustalW alignment of proteins with Kazal domain with proteins from *Rhodnius prolixus* (RP), *Triatoma infestans* (TI), *Triatoma brasiliensis* (TB), *Hybomitra bimaculata* (HB), *Aedes aegypti* (AE), *Dipetalogaster maximus* (DM) and *Rhodnius robustus* (RR); **(B)** Dendogram of Kazal domain proteins from insects;

3.8 Glutathione S- transferase and insect resistance:

Glutathione S-transferase (GST) is part of a diverse enzymatic family involved mostly in detoxication. The enzyme can act in different ways; for example it catalyses the conjugation of eletrophylic compound with thiol group of the reduced glutathione, and after this reaction, the product will becomes highly soluble in water, which makes it easy for excreting [44]. In insects, microsomal and cytosolic GSTs appear to be part of a multigenic family possibly resulting from single gene expression. Detoxication systems in insect depend on the extern stimuli, which can augment the production of protective specialized enzymes as GST [45].

The *R. brethesi* proteome revealed a single protein with similarity to GST from *Anopheles dirus*. Also GST that was described in *T. infestans* was associated to resistance to DTT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] [9, 45], and a similar gene was identified in *R. prolixus* [8].

4.0 Concluding remarks:

The studies concerning the physiology, biochemistry and feeding patterns of triatomines transmitters of the *T. cruzi* infections are definitely important, if scientific knowledge is expected to contribute to control of Chagas disease in the Amazon. Actually, there is not much information on these areas of knowledge concerning the Rhodnius gender, main vectors of the infections in the humid rain-forest major ecosystem. Herein, we used high-throughput molecular transcriptomics and proteomic LC-MS/MS technologies and we describe several unknown features related to blood-feeding physiology of triatomines` insects. For example, we found that lipocalins are farfetched main rhodninin salivary protein in these Amazonian *Rhodnius* species. Also, we detected inositol 5 phosphatase, kazal domain proteins, detoxication protein (GST), antigen5 proteins, which had never been described in these species before. The molecular phylogenetics dendogram separate these Rhodnius sp; *R. brethesi* and *R. robustus* appear to be close relatives to *R. prolixus* sharing several lipocalins, whereas *Triatoma infestans* placed apart, did not. This multidisciplinary study is an attempt to understand triatomines complex salivary gland apparatus that could lead to novel molecules inhibiting metabolic pathways crucial for the successful insect's blood feeding and survival.

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Supplemental table 1: *Rhodnius brethesi* transcriptomics deduced proteins

Clusters	dbEST_Id	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
1	57241719	0.92	ref|XP_001599727.1|	Hypothetical protein	*N. vitripennis*			
2	57241720	2,00E-84	gb|AAB09090.1|	Salivary platelet aggregation inhibitor 1	*R. prolixus*	1,00E-40	pfam03973.8	Triabin
3	57241721	4,00E-90	sp|Q26239|NP1_RHOPR	Nitrophorin 1	*R. prolixus*			
4	57241722	6,00E-57	gb|AAQ20818.1|	Lipocalin AI-4	*R. prolixus*	1,00E-21	pfam03973.8	Triabin
5	57241723	1,00E-54	sp|Q26241|NP2_RHOPR	Nitrophorin 2	*R. prolixus*			
6	57241724	1,00E-08	emb|CAM36311.1|	Hypothetical protein	*T. domestica*			
7	57241725	9,00E-101	gb|AAO25746.1|	Biogenic amine-binding protein	*R. prolixus*			
8	57241726	5,00E-66	ref|NP_068647.1|	Cytochrome c oxidase subunit III	*T. dimidiata*	1,00E-24	cd01665.2	Cyt_c_Oxidase_III
9	57241727	1,00E-23	gb|AAQ20821.1|	Triabin-like lipocalin 1	*R. prolixus*	0.00003	pfam03973.8	Triabin
10	57241728	8,00E-80	gb|AAQ20819.1|	Lipocalin AI-5	*R. prolixus*	1,00E-11	pfam03973.8	Triabin
11	57241729	3,00E-57	sp|Q26239|NP1_RHOPR	Nitrophorin 1	*R. prolixus*			
12	57241730	6,00E-66	gb|AAQ20838.1|	Lipocalin AI-7	*R. prolixus*	6,00E-30	pfam03973.8	Triabin
13	57241731	0.002	pdb|2ASN|X	Nitrophorin 2	*R. prolixus*			
14	57241732	6,00E-92	gb|AAQ20816.1|	Nitrophorin 1A	*R. prolixus*	0.000003	pfam03973.8	Triabin
15	57241733	2,00E-66	gb|AAQ20838.1|	Lipocalin AI-7	*R. prolixus*	7,00E-32	pfam03973.8	Triabin
16	57241734	0.45	gb|AAQ20830.1|	Polylysine protein	*R. prolixus*	0.00001	cd00041.3	CUB
17	57241735	3,00E-102	sp|Q94733|NP3_RHOPR	Nitrophorin 3	*R. prolixus*	0.005	pfam03973.8	Triabin
19	57241736	0.004	gb|AAB09091.1|	Salivary platelet aggregation inhibitor 2	*R. prolixus*			
20	57241737	7,00E-07	gb|ABH09443.1|	Lipocalin	*T. brasiliensis*	7,00E-11	pfam03973.8	Triabin
21	57241738	6.0	ref|NP_172725.2|	Unknown protein	*A. thaliana*			
22	57241739	No significant similarity found.						
23	57241740	No significant similarity found.						
24	57241741	8,00E-39	gb|ABR27887.1|	Cytochrome oxidase subunit 2	*T. infestans*	4,00E-24	pfam00116.12	COX2
25	57241742	No significant similarity found.						
26	57241743	2,00E-34	gb|ABR27945.1|	ATPase subunit 6	*T. infestans*	6,00E-11	pfam00119.12	ATP-synt_A
27	57241744	2,00E-15	ref|NP_068650.1|	NADH dehydrogenase subunit 4	*T. dimidiata*			
28	57241745	No significant similarity found						

Clusters	dbEST_Id	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
29	57241746	2,00E-42	gi|33518687|gb|AAQ20826.1|	Pallidipin-like lipocalin 2	*R. prolixus*	0.008	pfam03973.8	Triabin
30	57241747	1,00E-39	gb|AAQ20822.1|	Triabin-like lipocalin 2	*R. prolixus*	0.000005	pfam03973.8	Triabin
31	57241748	0.008	gb|AAQ20835.1|	MYS1	*R. prolixus*			
32	57241749	No significant similarity found						
33	57241750	No significant similarity found.						
34	57241751	No significant similarity found						
35	57241752	No significant similarity found						
36	57241753	9,00E-69	gb|AAQ20815.1|	Nitrophorin 4B	*R. prolixus*			
37	57241754	No significant similarity found						
38	57241755	1,00E-50	gb|AAO25746.1|	Biogenic amine-binding protein	*R. prolixus*			
39	57241756	No significant similarity found.						
40	57241757	No significant similarity found.						
41	57241758	7,00E-26	ref|NP_525073.1|	Mitochondrial ribosomal protein L30	*D. melanogaster*			
42	57241759	5,00E-94	sp|Q26241|NP2_RHOPR	Nitrophorin 2	*R. prolixus*	0.009	pfam03973.8	Triabin
43	57241760	4,00E-13	gb|AAQ20821.1|	Triabin-like lipocalin 1	*R. prolixus*			
44	57241761	No significant similarity found						
45	57241762	3,00E-68	gb|AAQ20817.1|	Lipocalin AI-3	*R. prolixus*	1,00E-11	pfam03973.8	Triabin
46	57241763	No significant similarity found.						
47	57241764	No significant similarity found.						
48	57241765	4,00E-59	gb|AAQ20838.1|	Lipocalin AI-7	*R. prolixus*	3,00E-26	pfam03973.8	Triabin
49	57241766	No significant similarity found.						
50	57241767	1,00E-20	gb|AAO25746.1|	Biogenic amine-binding protein	*R. prolixus*			
51	57241768	No significant similarity found.						
52	57241769	3,00E-05	gb|AAB09090.1|	Salivary platelet aggregation inhibitor 1	*R. prolixus*	0.0003	pfam03973.8	Triabin
53	57241770	No significant similarity found						
54	57241771	No significant similarity found						
55	57241772	2,00E-08	gb|ABR27925.1|	60S ribosomal protein L26	*T .infestans*			
56	57241773	2,00E-23	ref|NP_524211.1|	Ribosomal protein LP0	*D.melanogaster*			

Supplemental table 2: Proteins identified by transcripts analysis of *Rhodnius robustus*

Clusters	dbEST_Id	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
1	57241774	1,00E-74	sp|Q26241|NP2_RHOPR	Nitrophorin 2	*R. prolixus*			
2	57241775	3,00E-63	gb|AAQ20838.1|	Lipocalin AI-7	*R. prolixus*	2,00E-30	pfam03973.8	Triabin
3	57241776	5,00E-71	gb|AAQ20821.1|	Triabin-like lipocalin 1	*R. prolixus*	2,00E-07	pfam03973.8	Triabin
4	57241777	1,00E-74	gb|AAQ20838.1|	Lipocalin AI-7	*R. prolixus*	8,00E-31	pfam03973.8	Triabin
5	57241778	8,00E-70	gb|AAQ20842.1|	Nitrophorin 3B	*R. prolixus*	1,00E-15	pfam03973.8	Triabin
6	57241779	9,00E-31	gb|AAQ20820.1|	Lipocalin AI-6	*R. prolixus*	1,00E-08	pfam03973.9	Triabin
7	57241780	0.089	gb|AAQ20830.1|	Polylysine	*R. prolixus*			
8	57241781	1,00E-08	emb|CAM36311.1|	Hypothetical protein	*T. domestica*			
9	57241782	3,00E-69	gb|AAQ20838.1|	Lipocalin AI-7	*R. prolixus*	5,00E-30	pfam03973.9	Triabin
10	57241783	1,00E-90	sp|Q26241|NP2_RHOPR	Nitrophorin 2	*R. prolixus*			
12	57241784	1,00E-69	ref|NP_068647.1|	Cytochrome c oxidase subunit III	*T. dimidiata*	1,00E-24	cd01665.2	Cytochrome c oxidase subunit III
13	57241785	6,00E-85	gb|AAQ20819.1|	Lipocalin AI-5	*R. prolixus*	8,00E-13	pfam03973.9	Triabin
14	57241786	3,00E-33	gb|AAC26160.1|	Nitrophorin 3	*R. prolixus*			
15	57241787	4,00E-04	gb|ABV44742.1|	50 kDa midgut protein	*P. papatasi*			
16	57241788	No significant similarity found.						
17	57241789	2,00E-64	gb|AAQ20816.1|	Nitrophorin 1A	*R. prolixus*			
18	57241790	6,00E-64	pdb|1NP1|A	Nitrophorin 1	*R. prolixus*			
19	57241791	7,00E-113	sp|Q26239|NP1_RHOPR	Nitrophorin 1	*R. prolixus*			
21	57241793	2,00E-93	gb|AAQ20816.1|	Nitrophorin 1A	*R. prolixus*	0.000002	pfam03973.9	Triabin
22	57241794	3,00E-112	sp|Q26241|NP2_RHOPR	Nitrophorin 2	*R. prolixus*	0.002	pfam03973.9	Triabin
23	57241795	1,00E-63	gb|AAB09091.1|	Salivary platelet aggregation inhibitor 2	*R. prolixus*	3,00E-42	pfam03973.9	Triabin
24	57241796	5,00E-72	gb|AAQ20838.1|	Lipocalin AI-7	*R. prolixus*	6,00E-31	pfam03973.10	Triabin
25	57241797	5,00E-117	gb|AAO25746.1|	Biogenic amine-binding protein	*R. prolixus*	0.00009	pfam03973.11	Triabin
26	57241798	5,00E-11	ref|XP_001661688.1|	Kkinase C inhibitor	*A. aegypti*			
27	57241799	3,00E-93	gb|AAQ20818.1|	Lipocalin AI-4	*R. prolixus*	5,00E-33	pfam03973.11	Triabin
28	57241800	2,00E-34	gb|AAQ20820.1|	Lipocalin AI-6	*R. prolixus*	8,00E-11	pfam03973.12	Triabin

Clusters	dbEST_Id	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
29	57241801	3,00E-18	gb|AAQ20816.1|	Nitrophorin 1A	*R. prolixus*			
30	57241802	1,00E-55	pdb|1NP1|A	Nitrophorin 1	*R. prolixus*			
31	57241803	3,00E-23	gb|AAQ20826.1|	Pallidipin-like lipocalin 2	*R. prolixus*	0.002	pfam03973.12	Triabin
32	57241804	3,00E-24	sp|Q26239|NP1_RHOPR	Nitrophorin 1	*R. prolixus*			
33	57241805	3,00E-113	sp|Q94733|NP3_RHOPR	Nitrophorin 3	*R. prolixus*	0.004	pfam03973.12	Triabin
34	57241806	2,00E-11	gb|AAQ20822.1|	Triabin-like lipocalin 2	*R. prolixus*	8,00E-09	pfam03973.12	Triabin
35	57241807	1,00E-31	gb|ABR27885.1|	Salivary secreted protein	*T. infestans*			
36	57241808	2.7	gb|AAN71216.1|	GM20929p	*D. melanogaster*			
37	57241809	1,00E-63	gb|AAB08434.2|	Salivary inositol polyphosphate 5-phosphatase	*R. prolixus*	9,00E-22	smart00128.11	Inositol polyphosphate phosphatase
38	57241810	3,00E-16	ref|XP_001664014.1|	Helicase	*A. aegypti*	4,00E-11	COG4581.2	Superfamily II RNA helicase
39	57241811	No significant similarity found.						
40	57241812	No significant similarity found.						
41	57241813	2,00E-06	ref|XP_001655525.1|	Villin	*A. aegypti*	0.0001	smart00153.11	Villin headpiece domain
42	57241814	7,00E-63	ref|XP_312936.3|	AGAP003228-PA	*A. gambiae*			
43	57241815	No significant similarity found.						
44	57241816	6,00E-31	ref|XP_623715.1|	CG6903-PA	*A. mellifera*	0.005	COG4299.2	Uncharacterized protein
45	57241817	7,00E-90	gi|62083451|gb|AAX62450.1|	Ribosomal protein S2	*L. testaceipes*	6,00E-58	cd03302.1	Adenylsuccinate lyase 2
46	57241818	No significant similarity found.						
47	57241819	2,00E-14	gb|ABR27845.1|	Lipocalin	*T. infestans*	1,00E-13	pfam03973.12	Triabin
48	57241820	No significant similarity found.						
49	57241821	1,00E-40	gi|70909937|emb|CAJ17455.1|	Ribosomal protein L36A	*A. mellifera*	5,00E-12	pfam00935.12	Ribosomal protein L44
50	57241822	9,00E-59	ref|XP_001359078.1|	GA17542-PA	*D. pseudoobscura*			
51	57241823	2,00E-86	ref|XP_001654298.1|	40S ribosomal protein S2	*A. aegypti*	2,00E-18	pfam00333.12	Ribosomal protein S5

Clusters	dbEST_Id	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
52	57241824	No significant similarity found.						
53	57241825	2,00E-04	ref|XP_564616.1|	AGAP007621-PB	*A. gambiae*			
54	57241826	No significant similarity found.						
55	57241827	1,00E-29	gb|AAY66891.1|	Ribosomal protein S26	*I. scapularis*	3,00E-23	pfam01283.12	Ribosomal protein S26e
56	57241828	No significant similarity found.						
57	57241829	No significant similarity found.						
58	57241830	4,00E-05	ref|XP_317603.4|	AGAP007888-PA	*A. gambiae*			
59	57241831	No significant similarity found.						
60	57241832	7,00E-43	ref|XP_001652827.1|	60S ribosomal protein L9	*A. aegypti*			
61	57241833	1,00E-50	gb|ABR27860.1|	Calponin	*T. infestans*	3,00E-11	COG5199.2	Calponin
62	57241834	No significant similarity found.						
63	57241835	3,00E-36	ref|XP_313355.3|	AGAP003597-PA	*A. gambiae*	5,00E-21	cd03688.1	eIF2_gamma_II
64	57241836	4,00E-20	gb|ABI96910.1|	Brasiliensin	*T. brasiliensis*	0.000004	cd00104.3	Kazal type serine protease inhibitors and follistatin-like domains
65	57241837	No significant similarity found.						
66	57241838	3,00E-57	gb|AAQ20815.1|	Nitrophorin 4B	*R. prolixus*			
67	57241839	1,00E-38	gb|AAQ20820.1|	Lipocalin AI-6	*R. prolixus*	3,00E-13	pfam03973.12	Triabin
68	57241840	0.19	ref|XP_001358192.1|	GA19525-PA	*D. pseudoobscura*			
69	57241841	No significant similarity found.						
70	57241842	2,00E-38	gb|AAQ20819.1|	Lipocalin AI-5	*R. prolixus*	9,00E-07	pfam03973.12	Triabin
71	57241843	No significant similarity found.						
72	57241844	No significant similarity found.						
73	57241845	0.34	ref|NP_649841.1|	CG8379 CG8379-PA	*D. melanogaster*			
74	57241846	No significant similarity found.						
75	57241847	No significant similarity found.						
76	57241848	2,00E-79	gb|AAQ20817.1|	Lipocalin AI-3	*R. prolixus*	1,00E-19	pfam03973.12	Triabin
77	57241849	4,00E-37	gb|AAQ20835.1|	MYS1	*R. prolixus*			

Clusters	dbEST_Id	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
78	57241850	No significant similarity found.						
79	57241851	No significant similarity found.						
80	57241852	No significant similarity found.						
81	57241853	1,00E-07	ref|XP_001352331.1|	GA20958-PA	*D. pseudoobscura*			
82	57241854	7,00E-46	ref|XP_001658653.1|	5-aminolevulinic acid synthase	*A. aegypti*	1,00E-28	COG0156.2	7-keto-8-aminopelargonate synthetase
83	57241855	1,00E-41	ref|XP_395047.3|	Phosphoglycerate kinase isoform 1	*A. mellifera*	1,00E-42	cd00318.2	Phosphoglycerate kinase
84	57241856	3,00E-37	gb|AAQ20835.1|	MYS1	*R. prolixus*			
85	57241857	6,00E-15	ref|XP_001658906.1|	Histone-lysine n-methyltransferase	*A. aegypti*	0.000001	pfam00856.12	SET domain lysine methyltransferase enzymes
86	57241858	3.6	ref|XP_396862.3|	SMC4	*A.mellifera*			
87	57241859	3,00E-10	gb|AAB09091.1|	Salivary platelet aggregation inhibitor 2	*R. prolixus*	0.0001	pfam03973.12	Triabin
88	57241860	0.085	gb|AAQ20832.1|	Antigen-5-like protein	*R. prolixus*			
89	57241861	No significant similarity found.						
90	57241862	1,00E-23	gb|ABR27901.1|	Hypothetical protein	*T. infestans*			
91	57241863	No significant similarity found.						
92	57241864	6.0	ref|NP_727067.1|	CG32750	*D. melanogaster*			
93	57241865	0.19	ref|XP_624943.2|	Ribosomal protein S7	*A. mellifera*	0.001	pfam01251.12	Ribosomal protein S7e
94	57241866	No significant similarity found.						
95	57241867	4,00E-46	pdb|2ASN|X	Nitrophorin 2	*R. prolixus*			
96	57241868	7.9	ref|XP_001120849.1|	CG5645-PA	*A. mellifera*			
97	57241869	2,00E-23	gb|ABR27887.1|	Cytochrome oxidase subunit 2	*T. infestans*			
98	57241870	1,00E-04	ref|XP_001121125.1|	Hypothetical protein	*A. mellifera*			
99	57241871	No significant similarity found.						
100	57241872	No significant similarity found.						
101	57241894	No significant similarity found.						
102	57241893	No significant similarity found.						

Clusters	dbEST_Id	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
103	57241873	1,00E-66	gb|AAM20928.1|AF449138_1	Cytochrome oxidase 1	*R. prolixus*			
104	57241874	No significant similarity found.						
105	57241875	1,00E-10	ref|XP_001660286.1|	Dehydrogenase	*A. aegypti*	2,00E-08	COG0300.2	dehydrogenases
106	57241876	2,00E-11	gb|ABR27888.1|	Salivary secreted protein	*T. infestans*			
107	57241877	4,00E-48	ref|XP_316550.3|	AGAP006518-PA	*A. gambiae*	2,00E-17	pfam03357.11	SNF7
108	57241878	2,00E-30	ref|XP_001655512.1|	Hypothetical protein AaeL	*A. aegypti*	0.000001	pfam03966.8	Protein of unknown function (DUF343).
109	57241879	No significant similarity found.						
110	57241880	No significant similarity found.						
111	57241881	3,00E-08	gb|AAQ20815.1|	Nitrophorin 4B	*R. prolixus*			
112	57241882	No significant similarity found.						
113	57241883	8,00E-06	ref|XP_001648914.1|	Metalloproteinase	*A. aegypti*	0.000001	cd00203.3	Zinc-dependent metalloprotease
114	57241884	2,00E-18	ref|XP_001653215.1|	Pyruvate dehydrogenase	*A. aegypti*	2,00E-08	cd02000.1	Thiamine pyrophosphate
115	57241885	1,00E-06	gb|AAQ20819.1|	Lipocalin AI-5	*R. prolixus*			
116	57241886	No significant similarity found.						
117	57241887	3,00E-11	gb|AAM20926.1|AF449136_1	Cytochrome oxidase 1	*R. pictipes*			
118	57241888	2,00E-27	gb|AAQ20842.1|	Nitrophorin 3B	*R. prolixus*	0.0009	pfam03973.12	Triabin
119	57241889	No significant similarity found.						
120	57241890	No significant similarity found.						
121	57241891	0.003	ref|XP_001607314.1|	Hypothetical protein	*N. vitripennis*			
122	57241892	No significant similarity found.						

Supplemental table 3: Proteins identified by proteomic analysis of *Rhodnius brethesi*

ncbi accession number	Proteins	*Specie*	P (pro) P (pep)	Score XC	MW Sp	Accession RSp	Peptide (Hits) Ions
108871674	Ubiquitin specific protease	*A. aegypti*	9,66E-04	1,41E+01	398125,90	108871674,0	2 (0 1 1 0 0)
11596154	Glutathione transferase	*A. dirus*	8,80E-07	1,02E+01	23381,60	11596154,0	1 (1 0 0 0 0)
59859664	Actin type 1	*Allogromia sp*	4,59E-04	1,61E+01	38556,55	59859664,0	2 (0 2 0 0 0)
42795457	CblT	*B. cenocepacia*	2,21E-04	1,41E+01	125422,80	42795457,0	3 (0 1 0 1 1)
53715660	Putative outer membrane protein probably involved in nutrient binding	*B. fragilis*	4,80E-05	2,01E+01	117280,20	53715660,0	2 (2 0 0 0 0)
119908843	Aster-associated protein	*B. taurus*	6,98E-04	2,01E+01	82140,97	119908843,0	2 (2 0 0 0 0)
50293929	Hypothetical protein CAGL0M00704g	*C. glabrata*	2,33E-04	1,61E+01	33760,14	50293929,0	2 (1 0 1 0 0)
399861	Hemoglobin subunit beta	*C. japonica*	7,02E-07	4,02E+01	16324,60	399861,0	4 (4 0 0 0 0)
66824749	Hypothetical protein DDBDRAFT_0202780	*D. discoideum*	2,68E-05	2,02E+01	78014,39	66824749,0	2 (2 0 0 0 0)
24581387	ABC transporter	*D. melanogaster*	5,14E-05	2,02E+01	30850,99	24581387,0	2 (2 0 0 0 0)
54642249	Catalase CG6871-PA	*D. pseudoobscura*	7,27E-07	2,02E+01	57137,47	54642249,0	2 (2 0 0 0 0)
9972785	Catalase	*D. rerio*	3,14E-05	1,02E+01	59616,55	9972785,0	1 (1 0 0 0 0)
122618	Hemoglobin subunit beta/beta'	*L. ridibundus*	5,11E-05	1,82E+01	16249,56	122618,0	2 (1 1 0 0 0)
85714328	Hypothetical protein NB311A_15292	*Nitrobacter sp.*	1,07E-04	2,01E+01	7726,88	85714328,0	2 (2 0 0 0 0)
21263573	Elongation factor 1-alpha	*O. latipes*	2,47E-07	1,42E+01	50411,62	21263573,0	2 (1 0 0 1 0)
77554900	Retrotransposon protein	*O. sativa*	2,07E-04	1,82E+01	171175,40	77554900,0	2 (1 1 0 0 0)
84319423	Acyl-CoA synthetases	*P. aeruginosa*	1,58E-05	2,02E+01	68944,05	84319423,0	2 (2 0 0 0 0)
2555187	Vitellogenin	*P. nipponica*	4,84E-06	1,81E+01	203367,90	2555187,0	2 (1 1 0 0 0)
118410966	Photosystem I protein F	*P. tricornutum*	1,31E-04	2,01E+01	20543,86	118410966,0	2 (2 0 0 0 0)
27436375	Glyceraldehyde-3-phosphate dehydrogenase	*P. waltl*	9,68E-05	2,02E+01	33383,05	27436375,0	2 (2 0 0 0 0)
57241721	Nitrophorin-1 precursor	*R. brethesi*	4,44E-15	5,04E+01	20702,25		5 (5 0 0 0 0)
57241727	triabin-like lipocalin 1 precursor	*R. brethesi*	2,15E-08	4,02E+01	11832,97		4 (4 0 0 0 0)
57241765	lipocalin AI-7 precursor	*R. brethesi*	3,36E-10	2,03E+01	16628,16		2 (2 0 0 0 0)
119364424	Lipocalin AI-7 precursor	*R. prolixus*	8,81E-11	1,60E+02	20740,29	119364424,0	16 (16 0 0 0 0)
119364504	Salivary platelet aggregation inhibitor 1	*R. prolixus*	2,85E-10	1,58E+02	21472,95	119364504,0	16 (15 1 0 0 0)
119364488	Nitrophorin-2 precursor	*R. prolixus*	1,00E-30	1,46E+02	23918,89	119364488,0	15 (13 2 0 0 0)
119364130	Lipocalin AI-7 precursor	*R. prolixus*	5,49E-09	1,30E+02	20546,21	119364130,0	13 (13 0 0 0 0)
119364494	Nitrophorin 1A precursor	*R. prolixus*	1,12E-12	1,24E+02	24950,38	119364494,0	13 (11 1 1 0 0)
119364520	Lipocalin AI-7 precursor	*R. prolixus*	1,01E-11	1,20E+02	19754,72	119364520,0	12 (12 0 0 0 0)

ncbi accession number	Proteins	*Specie*	P (pro) P (pep)	Score XC	MW Sp	Accession RSp	Peptide (Hits) Ions
119364516	Nitrophorin-4 precursor	*R. prolixus*	3,84E-11	1,14E+02	12645,37	119364516,0	12 (9 3 0 0 0)
119364060	Zinc metalloproteinase	*R. prolixus*	5,15E-12	1,10E+02	31321,23	119364060,0	11 (11 0 0 0 0)
119364327	Antigen-5-like protein precursor	*R. prolixus*	3,22E-14	1,08E+02	22411,16	119364327,0	11 (10 1 0 0 0)
119364502	Biogenic amine-binding protein	*R. prolixus*	5,65E-13	1,06E+02	24966,40	119364502,0	11 (9 2 0 0 0)
119364442	Nitrophorin-2 precursor	*R. prolixus*	6,75E-11	1,00E+02	24484,02	119364442,0	10 (10 0 0 0 0)
119364180	Triabin-like lipocalin 2 precursor	*R. prolixus*	3,35E-11	9,83E+01	21197,38	119364180,0	10 (9 1 0 0 0)
119364496	Lipocalin AI-5 precursor	*R. prolixus*	2,23E-06	8,43E+01	20449,45	119364496,0	9 (8 0 0 1 0)
159779471	Lipocalin AI-4 precursor	*R. prolixus*	3,83E-08	8,42E+01	20221,79	159779471,0	9 (7 1 1 0 0)
119363735	Triabin-like lipocalin 2 precursor	*R. prolixus*	1,00E-30	8,03E+01	21586,58	119363735,0	8 (8 0 0 0 0)
119364482	Salivary platelet aggregation inhibitor 2	*R. prolixus*	1,07E-12	8,03E+01	20562,99	119364482,0	8 (8 0 0 0 0)
119363688	Lipocalin AI-6 precursor	*R. prolixus*	3,03E-10	8,03E+01	23002,90	119363688,0	8 (8 0 0 0 0)
119364459	Nitrophorin-4 precursor	*R. prolixus*	2,59E-05	7,63E+01	22876,41	119364459,0	8 (6 2 0 0 0)
119363874	Salivary nitrophorin 7	*R. prolixus*	8,41E-08	7,02E+01	19881,93	119363874,0	7 (7 0 0 0 0)
119364268	Nitrophorin 1A precursor	*R. prolixus*	8,83E-12	6,83E+01	24568,18	119364268,0	7 (6 1 0 0 0)
119364493	Salivary protein MYS1 precursor	*R. prolixus*	4,10E-09	6,83E+01	16748,78	119364493,0	7 (6 1 0 0 0)
119364262	Lipocalin AI-4 precursor	*R. prolixus*	1,29E-06	6,83E+01	20970,40	119364262,0	7 (6 1 0 0 0)
119364120	Triabin-like lipocalin 2 precursor	*R. prolixus*	1,10E-11	6,03E+01	20461,78	119364120,0	6 (6 0 0 0 0)
119364334	Triabin-like lipocalin 2 precursor	*R. prolixus*	2,69E-11	6,03E+01	21681,76	119364334,0	7 (5 1 0 0 1)
119364242	Nitrophorin-2 precursor	*R. prolixus*	1,22E-07	6,03E+01	22763,41	119364242,0	6 (6 0 0 0 0)
119364436	Lipocalin AI-6 precursor	*R. prolixus*	2,66E-08	6,03E+01	20936,19	119364436,0	6 (6 0 0 0 0)
119364299	Nitrophorin 4B precursor	*R. prolixus*	5,15E-10	6,02E+01	26241,96	119364299,0	6 (6 0 0 0 0)
119364511	Pallidipin-like lipocalin 1 precursor	*R. prolixus*	3,63E-07	6,02E+01	21744,58	119364511,0	6 (6 0 0 0 0)
119364084	Nitrophorin 3B	*R. prolixus*	5,65E-09	5,83E+01	21138,49	119364084,0	6 (5 1 0 0 0)
119364501	Nitrophorin 4A precursor	*R. prolixus*	3,73E-10	5,82E+01	19547,76	119364501,0	6 (5 1 0 0 0)
119363948	Polylysine protein precursor	*R. prolixus*	8,55E-09	5,82E+01	19591,96	119363948,0	6 (5 1 0 0 0)
119364159	Nitrophorin-3 precursor	*R. prolixus*	4,66E-11	5,03E+01	22603,06	119364159,0	5 (5 0 0 0 0)
119364518	Salivary platelet aggregation inhibitor 2	*R. prolixus*	2,75E-12	5,03E+01	13909,79	119364518,0	5 (5 0 0 0 0)
|119363674	Nitrophorin 4B precursor	*R. prolixus*	3,60E-10	5,03E+01	28460,23	119363674,0	5 (5 0 0 0 0)
119363625	Salivary platelet aggregation inhibitor 2	*R. prolixus*	4,27E-09	5,02E+01	20512,18	119363625,0	5 (5 0 0 0 0)
20136465	Heme-binding protein	*R. prolixus*	1,60E-06	5,02E+01	14770,27	20136465,0	5 (5 0 0 0 0)
15783272	Similar to apolipophorin	*R. prolixus*	3,64E-11	4,82E+01	18463,84	157832727,0	5 (4 1 0 0 0)
119363704	Nitrophorin-3 precursor	*R. prolixus*	2,23E-07	4,82E+01	23158,71	119363704,0	5 (4 1 0 0 0)
119364400	Actin	*R. prolixus*	6,00E-14	4,03E+01	20664,21	119364400,0	4 (4 0 0 0 0)

ncbi accession number	Proteins	*Specie*	P (pro) P (pep)	Score XC	MW Sp	Accession RSp	Peptide (Hits) Ions
119364467	Salivary protein MYS1 precursor	*R. prolixus*	2,22E-15	4,03E+01	18477,20	119364467,0	4 (4 0 0 0 0)
119363843	Lipocalin AI-3 precursor	*R. prolixus*	5,78E-11	4,03E+01	21216,73	119363843,0	4 (4 0 0 0 0)
119364187	Putative arginine kinase	*R. prolixus*	9,86E-10	4,02E+01	41204,52	119364187,0	4 (4 0 0 0 0)
119364133	Nitrophorin-1 precursor	*R. prolixus*	1,31E-07	4,02E+01	23295,72	119364133,0	4 (4 0 0 0 0)
119364119	Nitrophorin-3 precursor	*R. prolixus*	7,11E-06	4,02E+01	15353,08	119364119,0	4 (4 0 0 0 0)
119364415	Biogenic amine-binding protein	*R. prolixus*	6,54E-11	3,83E+01	24656,22	119364415,0	4 (3 1 0 0 0)
119364302	Lipocalin AI-7 precursor	*R. prolixus*	1,12E-08	3,82E+01	12776,17	119364302,0	4 (3 1 0 0 0)
119364281	Triabin-like lipocalin 4 precursor	*R. prolixus*	8,29E-06	3,62E+01	20048,14	119364281,0	4 (2 2 0 0 0)
157832659	Similar to Retinoid- and fatty-acid binding protein	*R. prolixus*	1,44E-05	3,62E+01	17258,58	157832659,0	4 (3 0 1 0 0)
159646560	Peroxiredoxin 5	*R. prolixus*	4,83E-09	3,22E+01	21128,82	159646560,0	4 (3 0 0 0 1)
119363916	Nitrophorin-4 precursor	*R. prolixus*	2,13E-11	3,03E+01	22975,47	119363916,0	3 (3 0 0 0 0)
119364298	Nitrophorin-2 precursor	*R. prolixus*	4,09E-08	3,03E+01	24409,91	119364298,0	3 (3 0 0 0 0)
119364437	Lipocalin AI-6 precursor	*R. prolixus*	7,77E-15	3,02E+01	20861,07	119364437,0	3 (3 0 0 0 0)
119364517	Triabin-like lipocalin 3 precursor	*R. prolixus*	3,65E-05	3,02E+01	18972,67	119364517,0	3 (3 0 0 0 0)
119363768	Lipocalin AI-6 precursor	*R. prolixus*	9,14E-10	3,02E+01	22405,96	119363768,0	3 (3 0 0 0 0)
119363986	Triabin-like lipocalin 2 precursor	*R. prolixus*	2,41E-05	3,02E+01	21408,04	119363986,0	3 (3 0 0 0 0)
119364054	Nitrophorin-3 precursor	*R. prolixus*	1,32E-12	2,82E+01	20178,78	119364054,0	3 (2 1 0 0 0)
119363644	Nitrophorin 4B precursor	*R. prolixus*	7,34E-06	2,82E+01	21211,88	119363644,0	3 (2 1 0 0 0)
49259169	Nitrophorin 4	*R. prolixus*	1,13E-09	2,62E+01	20253,03	49259169,0	3 (1 2 0 0 0)
157832391	Similar to Retinoid- and fatty-acid binding protein	*R. prolixus*	1,48E-11	2,03E+01	19441,72	157832391,0	2 (2 0 0 0 0)
119363784	Nitrophorin 4B precursor	*R. prolixus*	2,29E-10	2,03E+01	22885,32	119363784,0	2 (2 0 0 0 0)
119363807	Triabin-like lipocalin 2 precursor	*R. prolixus*	3,24E-13	2,03E+01	19054,25	119363807,0	2 (2 0 0 0 0)
119364476	Nitrophorin-2 precursor	*R. prolixus*	1,26E-11	2,02E+01	23779,88	119364476,0	2 (2 0 0 0 0)
119364469	Lipocalin AI-4 precursor	*R. prolixus*	4,95E-05	2,02E+01	20488,83	119364469,0	2 (2 0 0 0 0)
119363641	Lipocalin AI-6 precursor	*R. prolixus*	1,11E-09	2,02E+01	21679,79	119363641,0	2 (2 0 0 0 0)
119364331	Lipocalin AI-5 precursor	*R. prolixus*	9,63E-08	2,02E+01	18573,35	119364331,0	2 (2 0 0 0 0)
119364490	Salivary nitrophorin 7	*R. prolixus*	1,88E-08	2,02E+01	20324,34	119364490,0	2 (2 0 0 0 0)
110082866	Transferrin	*R. prolixus*	6,67E-05	2,01E+01	10409,60	110082866,0	2 (2 0 0 0 0)
119364091	Lipocalin AI-5 precursor	*R. prolixus*	7,76E-07	1,83E+01	18640,43	119364091,0	2 (1 1 0 0 0)
119363254	Salivary nitrophorin 7	*R. prolixus*	1,80E-09	1,82E+01	24034,51	119363254,0	2 (1 1 0 0 0)
119364098	Nitrophorin-3	*R. prolixus*	7,72E-09	1,82E+01	9855,89	119364098,0	2 (1 1 0 0 0)

ncbi accession number	Proteins	*Specie*	P (pro) P (pep)	Score XC	MW Sp	Accession RSp	Peptide (Hits) Ions
119364499	Nitrophorin 3B	*R. prolixus*	1,79E-09	1,82E+01	22298,27	119364499,0	2 (1 1 0 0 0)
159740332	Lipocalin AI-4 precursor	*R. prolixus*	1,60E-09	1,82E+01	20025,75	159740332,0	2 (1 1 0 0 0)
119363253	Antigen-5-like protein precursor	*R. prolixus*	2,92E-06	1,82E+01	17442,13	119363253,0	2 (1 1 0 0 0)
119363313	Antigen-5-like protein precursor	*R. prolixus*	1,38E-08	1,62E+01	12412,99	119363313,0	2 (1 0 1 0 0)
119363467	Nitrophorin-4 precursor	*R. prolixus*	1,75E-08	1,21E+01	23705,08	119363467,0	2 (0 0 2 0 0)
119364304	Nitrophorin-2 precursor	*R. prolixus*	2,22E-15	1,04E+01	23276,60	119364304,0	1 (1 0 0 0 0)
119364348	Nitrophorin 1A precursor	*R. prolixus*	1,74E-10	1,03E+01	24341,19	119364348,0	1 (1 0 0 0 0)
119363264	Ribosomal protein P2	*R. prolixus*	1,22E-11	1,03E+01	9693,38	119363264,0	1 (1 0 0 0 0)
119364067	Triabin-like lipocalin 4 precursor	*R. prolixus*	2,58E-10	1,03E+01	21833,82	119364067,0	1 (1 0 0 0 0)
159777189	Peroxiredoxin-like protein	*R. prolixus*	2,14E-11	1,03E+01	10690,42	159777189,0	1 (1 0 0 0 0)
119363622	Cytochrome c-like protein	*R. prolixus*	1,92E-10	1,02E+01	12392,51	119363622,0	1 (1 0 0 0 0)
119363239	Antigen-5-like protein precursor	*R. prolixus*	5,07E-11	1,02E+01	26526,79	119363239,0	1 (1 0 0 0 0)
119364410	Pallidipin-like lipocalin 1 precursor	*R. prolixus*	2,18E-09	1,02E+01	21774,59	119364410,0	1 (1 0 0 0 0)
119363509	Nitrophorin-3	*R. prolixus*	9,32E-10	1,02E+01	10950,38	119363509,0	1 (1 0 0 0 0)
119363819	Disulfide isomerase	*R. prolixus*	5,79E-07	1,02E+01	32817,97	119363819,0	1 (1 0 0 0 0)
57241805	Nitrophorin-3 precursor	*R. robustus*	1,93E-08	6,02E+01	23189,37		6 (6 0 0 0 0)
57241779	lipocalin AI-6 precursor	*R. robustus*	1,15E-11	5,03E+01	15898,82		5 (5 0 0 0 0)
57241839	lipocalin AI-6 precursor	*R. robustus*	1,45E-09	5,02E+01	16072,71		5 (5 0 0 0 0)
57241800	lipocalin AI-6 precursor	*R. robustus*	2,52E-08	4,42E+01	16467,00		5 (3 1 1 0 0)
57241806	triabin-like lipocalin 2 precursor	*R. robustus*	1,07E-10	4,03E+01	14829,57		4 (4 0 0 0 0)
57241778	nitrophorin 3B	*R. robustus*	1,23E-10	2,03E+01	19755,89		2 (2 0 0 0 0)
57241863	Hypothetical protein	*R. robustus*	1,02E-08	2,02E+01	9581,62		2 (2 0 0 0 0)
57241776	Triabin-like lipocalin 1 precursor	*R. robustus*	2,58E-04	2,01E+01	17295,98		2 (2 0 0 0 0)
57241789	Nitrophorin 1A precursor	*R. robustus*	2,27E-05	1,03E+01	18707,29		1 (1 0 0 0 0)
57241809	salivary inositol polyphosphate 5-phosphatase	*R. robustus*	4,36E-11	1,02E+01	15324,22		1 (1 0 0 0 0)
57241859	salivary platelet aggregation inhibitor 2	*R. robustus*	1,55E-08	1,02E+01	4997,48		1 (1 0 0 0 0)
|148468370	Actin D	*T. infestans*	1,85E-13	5,83E+01	30811,19	148468370,0	6 (5 1 0 0 0)
148469312	Actin	*T. infestans*	3,21E-09	4,62E+01	15808,87	148469312,0	5 (3 2 0 0 0)
148468109	Thymosin beta	*T. infestans*	1,88E-08	3,02E+01	19528,13	148468109,0	3 (3 0 0 0 0)
148469020	Nucleoside diphosphate kinase	*T. infestans*	5,17E-06	2,02E+01	18893,69	148469020,0	2 (2 0 0 0 0)
123470631	Hypothetical protein TVAG_475310	*T. vaginalis*	7,30E-05	2,02E+01	79307,16	123470631,0	2 (2 0 0 0 0)

Supplemental table 4: Proteins identified by proteomic analysis of *Rhodnius robustus*

ncbi accession number	Proteins	*Specie*	P (pro) / P (pep)	Score / XC	MW / Sp	Accession / RSp	Peptide (Hits) / Ions
59859664	Actin type 1	*Allogromia sp.*	3,83E-04	1,81E+01	38556,55	59859664,0	2 (1 1 0 0 0)
53715660	Putative outer membrane protein involved in nutrient binding	*B. fragilis*	5,10E-05	2,01E+01	117280,20	53715660,0	2 (2 0 0 0 0)
2499109	Vacuolating cytotoxin precursor	*H. pylori*	1,53E-04	1,22E+01	141901,80	2499109,0	2 (0 0 2 0 0)
90961894	DNA integration/recombination/inversion protein	*L. salivarius*	6,98E-04	1,82E+01	35533,91	90961894,0	2 (1 1 0 0 0)
1703156	Actin	*M. sexta*	2,45E-05	1,81E+01	41749,77	1703156,0	2 (1 1 0 0 0)
118473311	Glycogen debranching enzyme GlgX	*M. smegmatis*	4,21E-04	1,62E+01	79470,32	118473311,0	2 (0 2 0 0 0)
15679800	Hypothetical protein MTH1812	*M. thermautotrophicu*	4,45E-04	1,82E+01	19951,91	15679800,0	2 (1 1 0 0 0)
77554900	Retrotransposon protein	*O. sativa*	2,15E-04	1,82E+01	171175,40	77554900,0	2 (1 1 0 0 0)
57241727	Triabin-like lipocalin 1 precursor	*R. brethesi*	2,38E-08	6,02E+01	11832,97		6 (6 0 0 0 0)
57241721	Nitrophorin-1 precursor	*R. brethesi*	8,88E-15	5,04E+01	20702,25		5 (5 0 0 0 0)
118063180	4-amino-4-deoxy-L-arabinose transferase and related glycosyltransferases of PMT family-like	*R. castenholzii*	2,34E-04	2,02E+01	57676,32	118063180,0	2 (2 0 0 0 0)
119364424	Lipocalin AI-7 precursor	*R. prolixus*	4,73E-11	1,66E+02	20740,29	119364424,0	17 (15 2 0 0 0)
119364504	Salivary platelet aggregation inhibitor 1	*R. prolixus*	1,13E-11	1,58E+02	21472,95	119364504,0	16 (15 1 0 0 0)
119364488	Nitrophorin-2 precursor	*R. prolixus*	1,11E-14	1,56E+02	23918,89	119364488,0	16 (14 2 0 0 0)
119364130	Lipocalin AI-7 precursor	*R. prolixus*	4,80E-09	1,40E+02	20546,21	119364130,0	14 (14 0 0 0 0)
119364502	Biogenic amine-binding protein	*R. prolixus*	5,44E-14	1,28E+02	24966,40	119364502,0	13 (12 1 0 0 0)
119364520	Lipocalin AI-7 precursor	*R. prolixus*	3,78E-07	1,16E+02	19754,72	119364520,0	12 (10 2 0 0 0)
119364494	Nitrophorin 1A precursor	*R. prolixus*	4,00E-12	1,14E+02	24950,38	119364494,0	12 (9 3 0 0 0)
119364496	Lipocalin AI-5 precursor	*R. prolixus*	3,72E-07	1,04E+02	20449,45	119364496,0	11 (10 0 0 1 0)
119363688	Lipocalin AI-6 precursor	*R. prolixus*	3,26E-09	1,00E+02	23002,90	119363688,0	10 (10 0 0 0 0)
159779471	Lipocalin AI-4 precursor	*R. prolixus*	9,83E-08	9,82E+01	20221,79	159779471,0	10 (9 1 0 0 0)
119364327	Antigen-5-like protein precursor	*R. prolixus*	5,76E-12	8,83E+01	22411,16	119364327,0	9 (8 1 0 0 0)
119364516	Nitrophorin-4 precursor	*R. prolixus*	1,97E-11	8,64E+01	12645,37	119364516,0	9 (7 2 0 0 0)
119364268	Nitrophorin 1A precursor	*R. prolixus*	4,22E-12	7,23E+01	24568,18	119364268,0	8 (6 1 0 1 0)
119364459	Nitrophorin-4 precursor	*R. prolixus*	4,89E-06	7,23E+01	22876,41	119364459,0	8 (5 2 1 0 0)

ncbi accession number	Proteins	*Specie*	P (pro) P (pep)	Score XC	MW Sp	Accession RSp	Peptide (Hits) Ions
119364159	Nitrophorin-3 precursor	*R. prolixus*	3,64E-11	7,03E+01	22603,06	119364159,0	7 (7 0 0 0 0)
119363735	Triabin-like lipocalin 2 precursor	*R. prolixus*	2,17E-12	7,03E+01	21586,58	119363735,0	7 (7 0 0 0 0)
119364442	Nitrophorin-2 precursor (NP2)	*R. prolixus*	8,83E-11	7,03E+01	24484,02	119364442,0	7 (7 0 0 0 0)
119364334	Triabin-like lipocalin 2 precursor	*R. prolixus*	6,00E-12	7,03E+01	21681,76	119364334,0	7 (7 0 0 0 0)
119364493	Salivary protein MYS1 precursor	*R. prolixus*	6,99E-09	7,03E+01	16748,78	119364493,0	7 (7 0 0 0 0)
119364482	Salivary platelet aggregation inhibitor 2	*R. prolixus*	3,11E-14	7,03E+01	20562,99	119364482,0	7 (7 0 0 0 0)
119364180	Nitrophorin 4A precursor	*R. prolixus*	1,14E-11	6,83E+01	21197,38	119364180,0	7 (6 1 0 0 0)
119364501	Nitrophorin 4A precursor	*R. prolixus*	1,61E-09	6,63E+01	19547,76	119364501,0	7 (5 2 0 0 0)
119363874	Salivary nitrophorin 7	*R. prolixus*	6,60E-12	6,02E+01	19881,93	119363874,0	6 (6 0 0 0 0)
119364511	Pallidipin-like lipocalin 1 precursor	*R. prolixus*	3,05E-06	6,02E+01	21744,58	119364511,0	6 (6 0 0 0 0)
119363625	Salivary platelet aggregation inhibitor 2	*R. prolixus*	1,35E-08	6,02E+01	20512,18	119363625,0	6 (6 0 0 0 0)
119363948	Polylysine protein precursor	*R. prolixus*	4,04E-08	6,02E+01	19591,96	119363948,0	6 (6 0 0 0 0)
119364120	Triabin-like lipocalin 2 precursor	*R. prolixus*	1,10E-11	5,03E+01	20461,78	119364120,0	5 (5 0 0 0 0)
119364242	Nitrophorin-2 precursor	*R. prolixus*	3,56E-07	5,03E+01	22763,41	119364242,0	5 (5 0 0 0 0)
119364299	Nitrophorin 4B precursor	*R. prolixus*	3,09E-06	5,02E+01	26241,96	119364299,0	5 (5 0 0 0 0)
119363674	Nitrophorin 4B precursor	*R. prolixus*	3,44E-07	5,02E+01	28460,23	119363674,0	5 (5 0 0 0 0)
119364084	Nitrophorin 3B	*R. prolixus*	6,17E-10	4,83E+01	21138,49	119364084,0	5 (4 1 0 0 0)
119364262	Lipocalin AI-4 precursor	*R. prolixus*	1,77E-08	4,82E+01	20970,40	119364262,0	5 (4 1 0 0 0)
119364302	Lipocalin AI-7 precursor	*R. prolixus*	1,09E-08	4,82E+01	12776,17	119364302,0	5 (4 1 0 0 0)
119364400	Nitrophorin-1 precursor	*R. prolixus*	5,35E-12	4,03E+01	20664,21	119364400,0	5 (3 1 0 0 1)
119364060	Zinc metalloproteinase	*R. prolixus*	8,18E-13	4,03E+01	31321,23	119364060,0	4 (4 0 0 0 0)
119364476	Nitrophorin-2 precursor	*R. prolixus*	7,17E-10	4,02E+01	23779,88	119364476,0	4 (4 0 0 0 0)
119364133	Nitrophorin-1 precursor	*R. prolixus*	1,73E-07	4,02E+01	23295,72	119364133,0	4 (4 0 0 0 0)
119363843	Lipocalin AI-3 precursor	*R. prolixus*	1,69E-10	4,02E+01	21216,73	119363843,0	4 (4 0 0 0 0)
119364436	Lipocalin AI-6 precursor	*R. prolixus*	9,85E-08	4,02E+01	20936,19	119364436,0	4 (4 0 0 0 0)
119364119	Nitrophorin-3 precursor	*R. prolixus*	9,56E-05	4,02E+01	15353,08	119364119,0	4 (4 0 0 0 0)
119364472	Lipocalin AI-7 precursor	*R. prolixus*	8,69E-05	4,01E+01	18386,82	119364472,0	4 (4 0 0 0 0)
119363704	Nitrophorin-3 precursor	*R. prolixus*	1,47E-06	3,82E+01	23158,71	119363704,0	4 (3 1 0 0 0)
119363253	Antigen-5-like protein precursor	*R. prolixus*	7,26E-06	3,62E+01	17442,13	119363253,0	4 (2 2 0 0 0)
119364467	Salivary protein MYS1 precursor	*R. prolixus*	1,05E-13	3,03E+01	18477,20	119364467,0	3 (3 0 0 0 0)
119363916	Nitrophorin-4 precursor	*R. prolixus*	1,11E-15	3,03E+01	22975,47	119363916,0	3 (3 0 0 0 0)
119364518	Salivary platelet aggregation inhibitor 2	*R. prolixus*	5,60E-11	3,03E+01	13909,79	119364518,0	3 (3 0 0 0 0)
119363768	Lipocalin AI-6 precursor	*R. prolixus*	1,45E-11	3,02E+01	22405,96	119363768,0	3 (3 0 0 0 0)

ncbi accession number	Proteins	*Specie*	P (pro) P (pep)	Score XC	MW Sp	Accession RSp	Peptide (Hits) Ions
119364517	Triabin-like lipocalin 3 precursor	*R. prolixus*	3,48E-05	3,02E+01	18972,67	119364517,0	3 (3 0 0 0 0)
157832727	Apolipophorins precursor	*R. prolixus*	3,15E-06	3,02E+01	18463,84	157832727,0	3 (3 0 0 0 0)
119364490	Salivary nitrophorin 7	*R. prolixus*	1,90E-06	3,02E+01	20324,34	119364490,0	3 (3 0 0 0 0)
119364415	Biogenic amine-binding protein	*R. prolixus*	3,48E-11	2,83E+01	24656,22	119364415,0	3 (2 1 0 0 0)
119364437	Lipocalin AI-6 precursor	*R. prolixus*	8,64E-08	2,82E+01	20861,07	119364437,0	3 (2 1 0 0 0)
119364499	Nitrophorin 3B	*R. prolixus*	5,47E-08	2,62E+01	22298,27	119364499,0	3 (1 2 0 0 0)
119364091	Lipocalin AI-5 precursor	*R. prolixus*	1,82E-10	2,03E+01	18640,43	119364091,0	2 (2 0 0 0 0)
119364298	Nitrophorin-2 precursor	*R. prolixus*	1,22E-07	2,03E+01	24409,91	119364298,0	2 (2 0 0 0 0)
119364054	Nitrophorin-3 precursor	*R. prolixus*	1,16E-08	2,02E+01	20178,78	119364054,0	2 (2 0 0 0 0)
119363807	Triabin-like lipocalin 2 precursor	*R. prolixus*	4,42E-09	2,02E+01	19054,25	119363807,0	2 (2 0 0 0 0)
119363641	Lipocalin AI-6 precursor	*R. prolixus*	7,98E-10	2,02E+01	21679,79	119363641,0	2 (2 0 0 0 0)
119364469	Lipocalin AI-4 precursor	*R. prolixus*	1,27E-04	2,02E+01	20488,83	119364469,0	2 (2 0 0 0 0)
119364331	Lipocalin AI-5 precursor	*R. prolixus*	4,86E-06	2,02E+01	18573,35	119364331,0	2 (2 0 0 0 0)
119363986	Triabin-like lipocalin 2 precursor	*R. prolixus*	7,26E-06	2,02E+01	21408,04	119363986,0	2 (2 0 0 0 0)
119363784	Nitrophorin 4B precursor	*R. prolixus*	2,25E-06	2,02E+01	22885,32	119363784,0	2 (2 0 0 0 0)
159630236	Unknown	*R. prolixus*	4,67E-06	2,02E+01	16085,61	159630236,0	2 (2 0 0 0 0)
119364302	Lipocalin AI-7 precursor	*R. prolixus*	2,09E-04	2,02E+01	13117,98	119364302,0	2 (2 0 0 0 0)
20136465	Heme-binding protein	*R. prolixus*	5,01E-08	2,02E+01	14770,27	20136465,0	2 (2 0 0 0 0)
119363644	Nitrophorin 4B precursor	*R. prolixus*	3,93E-05	2,02E+01	21211,88	119363644,0	2 (2 0 0 0 0)
119364067	Triabin-like lipocalin 4 precursor	*R. prolixus*	3,77E-11	1,83E+01	21833,82	119364067,0	2 (1 1 0 0 0)
119364281	Triabin-like lipocalin 4 precursor	*R. prolixus*	2,32E-06	1,82E+01	20048,14	119364281,0	2 (1 1 0 0 0)
119364098	Nitrophorin-3	*R. prolixus*	9,93E-06	1,82E+01	9855,89	119364098,0	2 (1 1 0 0 0)
119363313	Nitrophorin-3 precursor	*R. prolixus*	5,58E-08	1,82E+01	12412,99	119363313,0	2 (1 1 0 0 0)
49259169	Nitrophorin 4	*R. prolixus*	1,66E-08	1,81E+01	20253,03	49259169,0	2 (1 1 0 0 0)
119364461	Nitrophorin-4 precursor	*R. prolixus*	4,24E-09	1,64E+01	23859,80	119364461,0	2 (0 2 0 0 0)
159740332|	Lipocalin AI-4 precursor	*R. prolixus*	2,44E-09	1,62E+01	20025,75	159740332,0	2 (1 0 1 0 0)
119364304	Nitrophorin-2 precursor	*R. prolixus*	1,00E-30	1,04E+01	23276,60	119364304,0	1 (1 0 0 0 0)
119364348	Nitrophorin 1A precursor	*R. prolixus*	2,52E-11	1,03E+01	24341,19	119364348,0	1 (1 0 0 0 0)
119364428	Nitrophorin-2 precursor	*R. prolixus*	1,64E-13	1,03E+01	23002,30	119364428,0	1 (1 0 0 0 0)
119364039	Nitrophorin-2 precursor	*R. prolixus*	9,65E-11	1,03E+01	22677,26	119364039,0	1 (1 0 0 0 0)
157832391	Similar to apolipophorin	*R. prolixus*	6,34E-10	1,02E+01	19441,72	157832391,0	1 (1 0 0 0 0)
119364410	Pallidipin-like lipocalin 1 precursor	*R. prolixus*	1,11E-09	1,02E+01	21774,59	119364410,0	1 (1 0 0 0 0)
119363509	Nitrophorin-3	*R. prolixus*	1,15E-11	1,02E+01	10950,38	119363509,0	1 (1 0 0 0 0)

ncbi accession number	Proteins	*Specie*	P (pro) / P (pep)	Score / XC	MW / Sp	Accession / RSp	Peptide (Hits) / Ions
119363797	Nitrophorin-4 precursor	*R. prolixus*	4,21E-04	1,02E+01	23760,72	119363797,0	1 (1 0 0 0 0)
119364218	Lipocalin AI-6 precursor	*R. prolixus*	1,28E-08	1,02E+01	21117,37	119364218,0	1 (1 0 0 0 0)
57241805	Nitrophorin-3 precursor	*R. robustus*	2,55E-08	1,16E+02	23189,37		12 (10 2 0 0 0)
57241806	Triabin-like lipocalin 2 precursor	*R. robustus*	1,00E-08	5,03E+01	14829,57		5 (5 0 0 0 0)
57241779	Lipocalin AI-6 precursor	*R. robustus*	1,31E-07	5,02E+01	15898,82		5 (5 0 0 0 0)
57241800	Lipocalin AI-6 precursor	*R. robustus*	1,73E-08	3,02E+01	16467,00		3 (3 0 0 0 0)
57241839	Lipocalin AI-6 precursor	*R. robustus*	3,93E-05	3,02E+01	16072,71		3 (3 0 0 0 0)
57241776	Triabin-like lipocalin 1 precursor	*R. robustus*	6,27E-04	3,02E+01	17295,98		3 (3 0 0 0 0)
57241789	Nitrophorin 1A precursor	*R. robustus*	1,12E-06	2,03E+01	18707,29		2 (2 0 0 0 0)
57241778	Nitrophorin 3B	*R. robustus*	3,65E-07	2,02E+01	19755,89		2 (2 0 0 0 0)
57241859	Salivary platelet aggregation inhibitor 2	*R. robustus*	1,44E-08	2,02E+01	4997,48		2 (2 0 0 0 0)
57241842	Lipocalin AI-5 precursor	*R. robustus*	1,60E-06	2,02E+01	10561,33		2 (2 0 0 0 0)
57241863	Hypothetical protein	*R. robustus*	1,99E-08	2,02E+01	9581,62		2 (2 0 0 0 0)
57241804	Nitrophorin-1 precursor	*R. robustus*	8,71E-04	2,01E+01	8768,49		2 (2 0 0 0 0)
57241809	salivary inositol polyphosphate 5-phosphatase	*R. robustus*	2,08E-06	1,02E+01	15324,22		1 (1 0 0 0 0)
58430496	UDP-glucose glucosyltransferase	*S. aculeatissimum*	4,73E-04	2,02E+01	55293,39	58430496,0	2 (2 0 0 0 0)
118590509	Prephenate dehydratase	*S. aggregata*	2,25E-04	1,82E+01	30533,41	118590509,0	2 (1 1 0 0 0)
89953743	Beta-actin	*S. fibuligera*	6,22E-07	1,82E+01	31467,09	89953743,0	2 (1 1 0 0 0)
148469312	Actin	*T. infestans*	6,09E-08	1,82E+01	15808,87	148469312,0	2 (1 1 0 0 0)
123470631	Hypothetical protein TVAG_475310	*T. vaginalis*	1,91E-04	1,82E+01	79307,16	123470631,0	2 (1 1 0 0 0)
121913543	ABC transporter family protein	*T. vaginalis*	1,47E-04	1,62E+01	90931,09	121913543,0	2 (0 2 0 0 0)

MANUSCRITO 2

Ubiquitous *Panstrongylus megistus* shares several proteins with *Triatoma infestans*

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Abbreviations:

DDT: 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane; GST: Glutathione S-transferase ; Pm: *Panstrongylus megistus*; Rp: *Rhodnius prolixus*; Tb: *Triatoma brasilienses*; Ti: *Triatoma infestans*; Tp: *Triatoma pallidipennis*; X-Gal: 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside.

Key words: *Panstrongylus megistus*, proteomic, transcriptomic, *Triatoma infestans*, lipocalin

Abstract:

The *Panstrongylus megistus* transmitter of *Trypanosoma cruzi* infections to man is a hematophagous bug widely distributed from Mexico to Argentina. This ubiquitous triatomine is known to colonize different wild life dwellings, such as ground burrows, birds nest, crevices on tree barks and human domiciles. Additionally, *P. megistus* synanthropy habits preying upon mammals, birds, reptiles, and eventually being predators upon insects hemolymph probably increases its ability to survive after prolonged fasting. We believe that among several possible mechanism of adaptation to survival is the *P. megistus* salivary gland complex tool-box, showing diverse pharmacologically active proteins for obtaining blood meals. Herein we present for the first time sequences from 45 transcripts from the *P. megistus* salivary gland. Furthermore, we performed a proteomic analysis of the salivary glands content from this insect, which identified 159 proteins securing a diversity of functions related to blood feeding. We report here that a prevalence of proteins with blood clotting, anti-platelet aggregation and anti-vasoconstriction activities, which correlate with the insect's ability to obtain meals from different sources. These features appear to be compatible with enormous *P. megistus* wild life-changing habitats and habits.

1. Introduction:

The Hemiptera: *Reduviidae* triatomine of subfamily *Triatominae* are transmitters of *T. cruzi* agent of Chagas disease widespread in the South American Continent. For most decades of last century, *Triatoma infestans*, *Rhodnius prolixus* and *Panstrongylus megistus* used to be main transmitters of the *T. cruzi* infections. That situation has changed with the successful insecticide dislodgment of *T. infestans* from human dwellings in dry ecosystems, and now it has been observed that *P. megistus* have invaded those empty niches [1, 2] where they have increasingly become real threats to rural human populations health. Nowadays, ubiquitous *P. megistus* highly adapted to wild life and peri-domestic niches in dry and wet climates, besides its enormous adaptability, for example, to synanthropic blood feeding, which have made currently it the most important vectors of Chagas disease in some ecosystems [3].

Scientific investigations have focused upon *T. infestans* and *R. prolixus*, respectively in dry ecosystems of most countries where insecticide spraying programs did not yield effective elimination of household infestations, and in broad-leaf rain forests where is usually not accessible to insecticides and, therefore they continue to be important vectors of *T. cruzi* infections. Nonetheless, scientific information has shown that *Panstrongylus* bugs blood-sucking habits are relatively recent, and that they share some morphological resemblance with blood-sucking and with plant predators' triatomines. Secondly, it appears that *Panstrongylus* have undergone morphological modifications over last two centuries and, thirdly, they hold in the gut some bacteria providers of important nutrients [4, 5]. Also, other studies have shown that *P. megistus* from different geographical areas have major differences in the contents of salivary glands bioamines [6]. The species mechanisms to preventing blood loss during insects' bite are vasoconstriction, platelet aggregation and clotting. To counteracting imposing difficulties towards acquisition of a blood meal, it has been described that *R. prolixus* [7], *T. infestans* [8], *Triatoma brasiliensis* [9] and *Triatoma pallidipennis* [10] have accumulated several proteins in their salivary glands. To a much lesser extent, it was described some diversity in *P. megistus* saliva proteins [6], which bear anti-complement [11] and anticoagulant functions [12].

Regardless of the highly interesting behavior patterns shown by *P. megistus*, we found a paucity of data and information on its molecular features. In this study we show sequences of 45 transcripts of a cDNA library from *P. megistus* salivary glands, which traduced proteins that were covalidated with LC-MS-MS technique. We also describe 159 proteins in the salivary proteome from *P. megistus*, and identified 28 proteins with known blood-feeding activities, in addition to several enzymes and other proteins with unknown function. Here we show that several pharmacologically active salivary gland proteins of *P. megistus* are shared with other triatomine's saliva proteins.

2. Methods:

2.1 Triatomines and salivary glands collection:

P. megistus were captured in the Cerrado (dry savannah-like ecosystem) were brought to our laboratory and reared with controlled temperature of 28^{o}C, 70% relative humidity and 12:12h

light/dark photoperiod. 50 pairs of salivary glands were dissected in 1 mL of TRIZOL (Invitrogen) in adults after 3, 5 and 7 days post blood-feeding in mice. For the mass spectrometry experiment we collected 50 pairs of salivary glands, punctured, and centrifuged to collect the supernatant that was lyophilized for future use.

2.2 cDNA library construction and transcripts analysis:

To isolated the mRNA and construct the cDNA library we used the technical procedures described on the manufacturer protocol with the adaptations [8] of Micro-fast Track™ mRNA isolation (Invitrogen) and SMART cDNA library construction (Clontech). After obtaining the phages with expected insert cells were platted on LB/MgSO4 plates with X-gal/IPTG with 15 µl cDNA library unamplified. Among 90% of recombination clones we picked the white colonies and transferred them individually in 50 µl of pure water. The cDNA that was amplified with different primers sets [13] were sequenced unidirectionally with PT2F3 primer and DYEnamic ET DyeTerminator Sequencing Kit (Amersham Bioscience, Piscataway, NJ, USA) using MegaBACE 1000 sequencer (Amersham Biosciences, Little Chalfont, UK). The transcripts were subjected to BLAST algorithm [14], ClustalW [15], CDD [16], TREEview software [17], and, after the translation with Bioedit program, phylogenetics analysis and statistical neighbor-joining bootstrap tests of the phylogenies were done with de MEGA4 package [18].

2.3 Mass spectrometry by LC-MS/MS:

The *P. megistus* lyophilized salivary gland content was diluted in 200 µl of water HPLC grade (Sigma), reduced with 50 mM DTT, alkylated with iodacetamide 100 mM and trypsin digested in NH_4HCO_3 buffer (Seq grade modified trypsin, Promega). The samples that were desalted with a chromatography in reverse phase in-house zip tip (POROS R2-50 resin, Applied Biosystem), were then submitted to strong-cation exchange columns (POROS HS50, Applied Biosystems, Framingan, MA) and eluted with NaCl gradient in equilibration buffer (0, 10, 20, 40, 60, 80, 100, and 150, 200 and 500 mM). After desalinization, the sample was ready to liquid chromatography mass spectrometry (LC-MS) analysis. The peptides were dissolved in 10 µl 0.05% formic acid and separated in an Ultimate nanoHPLC system (LC packings, Dionex,

Sunnyvale, CA) and analyzed by an electrospray Linear Ion Trap Mass LTQ XL ™ [19]. At first the raw data were converted to DTA files by BioWorks version 3.3.1 software (Thermo Electron). The DTA files were submitted to analysis using TurboSequest (Bioworks 3.0 Thermo Electron, San Jose, CA), [20] algorithm with al ncbi database (downloaded on February 14, 2007) using different filters. The filters were: distinct peptides (for exclusion of redundant hits); DCn ≥ 0.1; protein probability $\leq 1x10^{-3}$; and Xcorr ≥ 1.5, 2.2, 2.7 and 3.0 for singly, doubly, triply and more than 3 charged peptides, respectively. After this, we made a second analysis with another database which included (*i*) ncbi proteins from *Triatoma, Panstrongylus* and *Rhodnius* (downloaded on November 15, 2007); (*ii*) translated transcripts from the cDNA libraries from *Panstrongylus megistus, Rhodnius brethesi*, *Rhodnius robustus* and *Triatoma infestans* (unpublished data); (*iii)* matches from the first analysis against ncbi; and (*iv)* random proteins (100K) used as false-negative. The false-positive rate is 1.8%.

3 Results and discussion:

3.1 ESTs sequences:

The unidirectional sequencing of 45 transcripts of *P. megistus* cDNA library showed the presence of expected housekeeping transcripts such as elongation factor, ribosomal proteins, and translation initiation factor (Supplemental Table 1). We also found several new transcripts that matched described proteins with unknown function; one of these transcripts (ncbi accession number: 57657798) showing a signal peptide appears to indicate that a putative protein is probably secreted in the salivary gland lumen possibly during the blood feeding. Besides, we found two secreted peptides previously described in *T. brasiliensis* [9] and *T. infestans* [8], and a hypothetical protein was also found with similarity to a *T. infestans* derived peptide. Altogether, the transcripts deducing proteins with functions related to blood-feeding comprised mostly lipocalins, pallidipins, triabins and triatins (Table 1).

Table 1: Hematophagy functions present in *Panstrongylus megistus* salivary glands in the transcriptomics and proteomic analysis.

Protein name	Number of transcripts	Number of proteins
79 kDa salivary apyrase precursor		1
Infestin 1-7 precursor		1
Lipocalin	6	8
Lipocalin 1	1	1
Lipocalin 4	1	1
Lipocalin-like Tin66	2	1
Pallidipin 2	3	3
Pallidipin-like salivary lipocalin	5	2
Secreted kazal-type proteinase inhibitor	1	2
Triabin-like lipocalin 4 a	1	1
Trialysin allele	1	1
Triatin	2	2
Triatin-like salivary lipocalin	3	
Triapsin		4

3.1.1 Lipocalins:

The lipocalins were the most abundant transcripts translating proteins used with blood-feeding purpose, which comprised almost 39% of proteins involved in blood-feeding. Here, we described the presence of 10 lipocalins, including lipocalin 1, 4 and lipocalin-like Tin66. Lipocalins belong to a group of small proteins with different functions, many of which are related to transport of small hydrophobic molecules. Their tertiary structure is highly conserved with 8 antiparallel β-barrels forming a cavity with an intern ligand-binding site [21]. Lipocalins were also predominant proteins in transcriptomes of *R. prolixus* [7] and of *T. infestans* [8], attaining 84% and 55% of the secreted proteins, respectively.

Also belonging to the lipocalin family there were eight sequences of pallidipin, which is an inhibitor of collagen-induced platelet aggregation. Pallidipin that was first described in *T.*

pallidipennis [22] blocks the platelet activation, although differently from the majority of lipocalins it bears no binding site. In the salivary gland of *P. megistus* we found one sequence of triabin, a thrombin inhibitor previously described in *T. pallidipennis* [23]. Interestingly, several sequences matched triatin from *T. infestans* previously described in our laboratory (unpublished).

The lipocalins phylogenetic studies have shown an increasing complexity stemming from a great divergence between primary structures caused by a rapid molecular evolution rate and a possible occurrence of gene duplication [24]. In this study we pursued alignment of the lipocalins, aiming at the construction of a neighbor-joining phylogenetic-tree (Fig. 1).

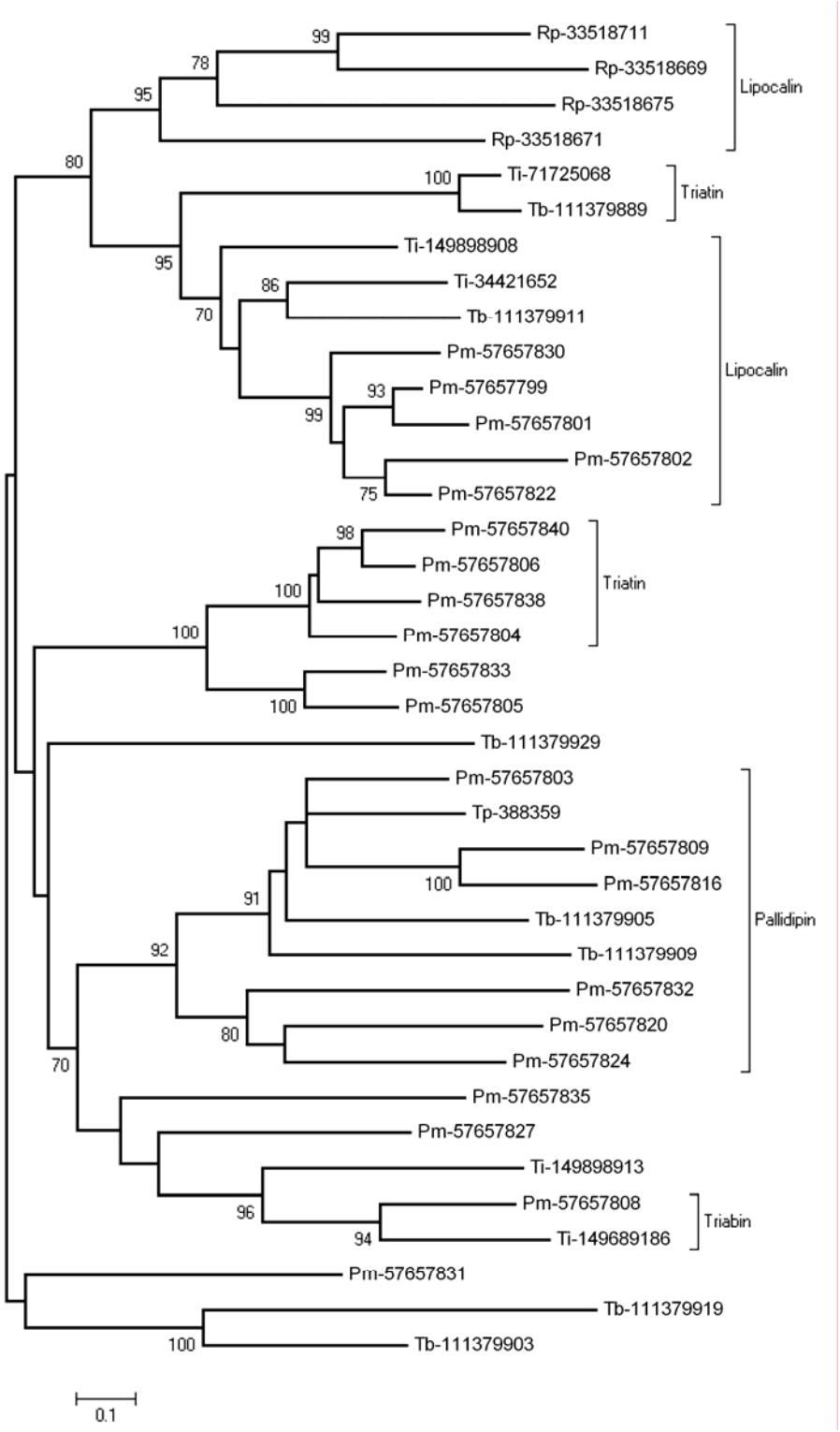

Figure 1: Dendogram showing convergent evolution aspects of several lipocalin family proteins from the saliva of *Panstrongylus megistus* (Pm), *Rhodnius prolixus* (Rp), *Triatoma infestans* (Ti), *Triatoma brasiliensis* (Tb) and *Triatoma pallidipennis* (Tp). The analyses of sequences from nonreduntant protein database of the National Center for Biotechnology Information (NCBI) were made, which are represented by the first letters of

gender and specie followed by the NCBI gi| accession number. The numbers in the dendogram nodes indicate percent bootstrap support for the phylogeny. The bar (bottom) indicates 10% amino acid divergence in the sequences. The dendogram was constructed with MEGA4 package [18].

We have observed that Rhodnius lipocalins are placed apart from those from Triatoma and from Panstrongylus; on the other hand *P. megistus* triabin and pallidipin appear to be closely related to those from *T. infestans*, and from *T. brasiliensis* and *T. pallidipennis*. Contrastingly, we established clusters of *P. megistus* triatin, which appear to be isolated from triatin of *T. infestans* and of *T. brasiliensis*; these features are also confirmed upon alignment (Fig. 2A).

3.1.2 Kazal domain-containing peptides:

It has been described, vasodilatation that increases blood flow and maintains the circulation is essential to insect' feeding success. With this aim, Kazal domain proteins are composed by different important proteins as vasotab from *Hybomitra bimaculata* [25], rhodniin from *R. prolixus* [26] and infestin from *T. infestans* [27]. Additionally, these proteins can assume anticoagulant (rhodniin and infestin 1 and 2) function. This feature appears to depend on a conserved pattern of cysteins [28], and tridimensional conformation with several kazal domains. In this group, there is infestin with two non-classical Kazal-type domains present in six sequences coded by single gene [27]. Regardless of the importance of these observations, its post translation process remains unknown. Furthermore, thrombin specific inhibitor rhodniin protein shows association rate two or three time higher than others kazal domain proteins [26].

The *P. megistus* transcripts sequences revealed a small peptide with similarities with a kazal domain protein showing similarity to *T. infestans* proteinase inhibitors (40% of identity) and to a *T. brasiliensis* secreted peptide (33% of identity). The *T. brasiliensis* secreted peptide bear a vasodilator function similar to vasotab protein from the horse fly *H. bimaculata* [25]. However, we could not find conserved cysteins that were described in a similar protein sequence from *T. infestans.* (Fig. 2B).

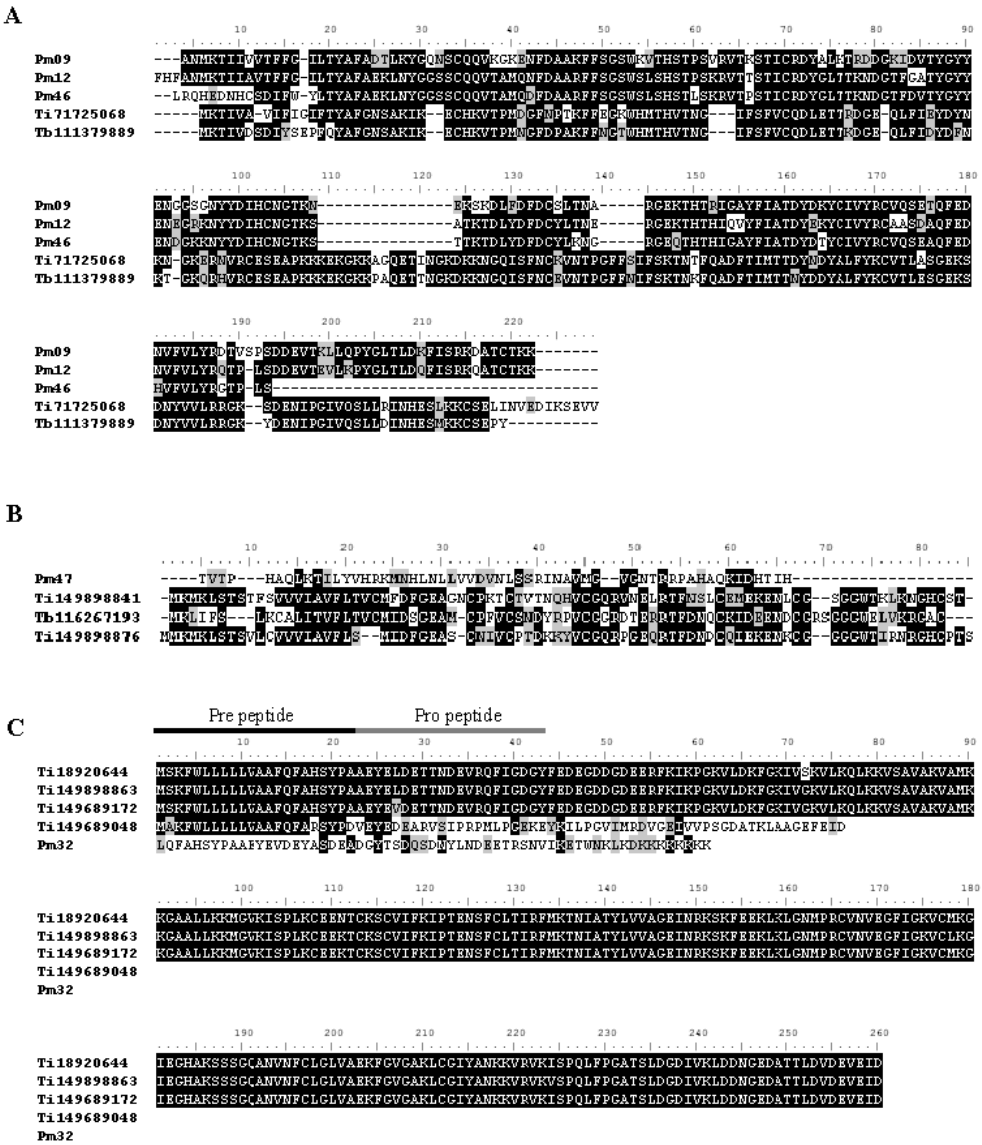

Figure 2: Multiple alignments of sequences from proteins with anticoagulant, vasodilator and microbicide activity (pore formation) present in the saliva of *Panstrongylus megistus*. (A) ClustalW alignment of triatin from *Triatoma infestans* and *Triatoma brasiliensis* (B) ClustalW alignment of Kazal protein of *Triatoma infestans* and *T. brasiliensis* (C) ClustalW alignment of trialysin proteins from *Triatoma infestans*. Pre and pro peptide are shown by black and grey bars respectively.

3.1.3 Trialysin:

Trialysin is a 22kDa protein with the ability to form pores in biological membranes from bacteria to mammalian cells. Interestingly, it has properties similar to those found in other classes of lytic molecules. Trialysin similarities to antimicrobial lytic peptides and to bacterial toxins stem respectively from basic amphypatic lytic motif in the N-terminal region, and to increasing specificity of protein motifs [29].

The alignment of *P. megistus* trialysin cDNA transcript and other species derived trialysin showed 59% identity with trialysin from *T. infestans*, whose gene is the only available in databank. It shows that shared conserved motif comprises a small region in pro and pre peptide (Fig. 2C). *T. infestans* trialysin accumulates in the salivary gland with a propeptide in the N-terminal that prevents its activation, and thus inhibiting its lytic activity. Interestingly, during saliva secretion a proteolytic activity cleaves the propeptide thus forming a mature protein with affinity to cellular membrane. When cleavage takes place, the mature protein conformation changes in the N-terminal portion [30] improves the pore-forming activity.

3.2 LC-MS analysis:

Proteome technology has been used to generate information for understanding the complexity of the salivary secretions that play a role in the insect adaption to blood-feeding. Data on several salivary proteomes are available, which corroborate the findings of the cDNA library sequencing. The *T. infestans* salivary glands proteome was carried out with basis on 2D-gels and mass spectrometry [8, 31]. Finally, procedure of liquid chromatography experiment combined with mass spectrometry has generated complementary knowledge about the insect's salivary gland proteins [32].

The *T. infestans* proteome revealed 200 proteins and 34 of them appeared to be related to lipocalins and apyrases [31]. Herein we show the salivary glands *P. megistus* proteome with 159 proteins (Supplemental Table 2). The majority of these proteins (64.5 %) bear similarities to insects' saliva proteins, mostly hemiptera as expected. Interestingly almost all the salivary proteins spectra have been recognized using the translated cDNA from *P. megistus*, *T. infestans* and *R. robustus* salivary glands. Among 28 sequences 20 were from *P. megistus* translated

cDNA. Additionally, there were housekeeping ribosomes proteins, actin, and metabolism cell enzymes. These intracellular proteins appear in the saliva as apocrine secretions [33]. Consistently, we found 28 proteins with function similarities to those of pallidipin, lipocalins, infestin (non classical kazal type of thrombin inhibitor) and triatin transcripts. Also, novel proteins were present in the *P. megistus* proteome (Table 1).

3.2.1 Trypsin:

Several enzymes in *P. megistus* salivary glands proteome have been detected, which play important roles during the insect's blood-feeding. We found 4 proteins similar to trypsin from *T. infestans* and *T. brasiliensis.* The enzyme similar to *T. infestans* triapsin is a serine protease enzyme stored in the D2 salivary gland. Upon saliva ejection this enzyme with amidolytic function becomes active to participate in the blood-feeding [34].

3.2.2 Glutathione S-transferase:

Belonging to a diversity family of enzymes, glutathione S-transferase (GST) is present in most biological samples. This enzyme can have multiple functions as transporters, oxidative stress protection and, mostly interestingly, a detoxifying role associated with insect's resistance to insecticide. GST already described in *T. infestans* has been associated with resistance against DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane) [35]. Also, here we show that the saliva of *P. megistus* retains a GST with similarity to the protein from *Anopheles dirus*.

3.2.3 Apyrase:

Platelet aggregation is essential for preventing hemostasis in the host, and insect's blood-feeding depends on mechanisms for evading from this important physical barrier. Once activated, the platelet changes its conformation and releases ADP in the blood vessels, which activates more platelets. Apyrase is a nucleoside triphosphate-diphosphohydrolase capable to remove phosphate from ATP and ADP leading consequently to the inhibition of the platelet aggregation. In the *P. megistus* saliva we found a 79 kDa apyrase, enzyme also present in *T. infestans* [8, 31, 36], *T. brasiliensis* [9] and in *R. prolixus* [7] salivary secretion.

4. Concluding remarks:

Among over 130 species of triatomines we found *P. megistus* with its highly interesting ubiquitous habits and its increasing participation in a complex epidemiology chain of transmission of the *T. cruzi* infections in Latin America. To play this role *P. megistus* has possibly gone through a convergent evolution and it has accumulated a great number of pharmacologically active proteins in its salivary glands. In this study we have undertaken transcriptome and proteome techniques to identify the main bioamines related to this insect blood-feeding ability. Due to this species fastidious growth in captivity, obtaining of salivary glands is a bottleneck to molecular and biochemical analyses.

We constructed a cDNA library from wild life *P. megistus* that colonized slowly in the laboratory. A total of 45 transcripts revealed main proteins with vasodilation, anti-clotting, anti-platelet aggregation activities. Additionally, *P. megistus* proteome revealed 159 proteins showing similarities with apyrase, lipocalins, thrombin inhibitor, trialysin, trypsin and glutathione S-transferase.

The main conclusion in this study is the demonstration of a complex salivary gland tool-box with elements capable to perform some functions associated with the insects' blood-feeding and resistance to insecticide. However, further studies are required to demonstrate those proteins related to the insect's behavior and adaptation to different environments making *P. megistus* real threats to human health. The usefulness of complementary techniques used in this study suggests that new tools will certainly bring in further novel information required for new insights necessary for curtailing this insect ubiquitous presence in several ecosystems and Chagas disease transmission.

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Supplemental table 1: Transcripts found in *Panstrongylus megistus* salivary glands

Clusters	dbEST_Id*	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
1	57657797	0.0	AF301594.1	Mitochondrial DNA	*T. dimidiata*	No CD has been identified on this query sequence		
2	57657798	No significant similarity found				No CD has been identified on this query sequence		
3	57657799	7,00E-53	gb|ABH09436.1|	Lipocalin	*T. brasiliensis*	2,00E-19	43890	Triabin
4	57657800	4,00E-08	gb|ABJ96351.1|	Secreted peptide	*T. brasiliensis*	No CD has been identified on this query sequence		
6	57657801	6,00E-45	gb|ABH09436.1|	Lipocalin	*T. brasiliensis*	2,00E-17	43890	Triabin
7	57657802	2,00E-44	gb|ABH09426.1|	Lipocalin 4	*T. brasiliensis*	2,00E-09	43890	Triabin
8	57657803	2,00E-57	gb|AAA30329.1|	Pallidipin 2	*T. pallidipennis*	6,00E-25	43890	Triabin
9	57657804	2,00E-48	gb|ABR27935.1|	Triatin-like salivary lipocalin	*T. infestans*	5,00E-12	43890	Triabin
10	57657805	2,00E-41	gb|ABR27936.1|	Lipocalin	*T. infestans*	1,00E-16	43890	Triabin
12	57657806	1,00E-56	gb|ABR27935.1|	Triatin-like salivary lipocalin	*T. infestans*	5,00E-13	43890	Triabin
13	57657807	1,00E-09	gb|ABJ96351.1|	Secreted peptide	*T. brasiliensis*	No CD has been identified on this query sequence		
14	57657808	5,00E-33	gb|ABR27959.1|	Triabin-like lipocalin 4 a	*T. infestans*	2,00E-07	43890	Triabin
15	57657809	2,00E-22	gb|AAA30329.1|	Pallidipin 2	*T. pallidipennis*	1,00E-09	43890	Triabin
16	57657810	3,00E-27	gb|ABR27956.1|	Ribosomal protein P2	*T. infestans*	5,00E-08	32241	Ribosomal protein
17	57657811	4,00E-06	gb|ABR27901.1|	Hypothetical protein	*T. infestans*	4,00E-19	43890	Triabin
18	57657812	No significant similarity found				No CD has been identified on this query sequence		
19	57657813	No significant similarity found				No CD has been identified on this query sequence		
20	57657814	No significant similarity found				No CD has been identified on this query sequence		
21	57657815	No significant similarity found				No CD has been identified on this query sequence		
22	57657816	5,00E-49	gi|388359|gb|AAA30329.1|	Pallidipin 2	*T. pallidipennis*	2,00E-22	43890	Triabin
23	57657817	2,00E-21	gb|ABR27961.1|	Lipocalin-like Tin66	*T. infestans*	0,002	43890	Triabin
24	57657818	No significant similarity found				No CD has been identified on this query sequence		
25	57657819	2,00E-19	gi|71725068|gb|AAZ38956.1|	Triatin	*T. infestans*	No CD has been identified on this query sequence		
26	57657820	3,00E-19	gb|ABR27944.1|	Pallidipin-like salivary lipocalin	*T. infestans*	7,00E-09	43890	Triabin
27	57657821	No significant similarity found.				No CD has been identified on this query sequence		
28	57657822	1,00E-19	gb|ABH09421.1| s	Salivary lipocalin 1	*T. infestans*	2,00E-07	43890	Triabin
29	57657823	No significant similarity found				No CD has been identified on this query sequence		
30	57657824	7,00E-31	gb|ABR27987.1|	Pallidipin-like lipocalin	*T. infestans*	0,000001	43890	Triabin
31	57657825	3,00E-07	gb|ABR27878.1|	Translation initiation factor 5a	*T. infestans*	No CD has been identified on this query sequence		

Clusters	dbEST_Id*	E-value	NCBI accession number	Protein name	Specie	E-value	Pfam accession number	Domain
32	57657826	4,00E-04	gb|ABR27943.1|	Trialysin allele	*T. infestans*	No CD has been identified on this query sequence		
33	57657827	6,00E-20	gb|ABR27932.1|	Lipocalin	*T. infestans*	2,00E-07	43890	Triabin
34	57657828	7,00E-10	gb|ABR27933.1|	Secreted salivary peptide	*T. infestans*	No CD has been identified on this query sequence		
35	57657829	2,00E-10	gb|ABR27889.1|	40S ribosomal protein S29	*T. infestans*	No CD has been identified on this query sequence		
36	57657830	6,00E-46	gb|ABH09436.1|	Lipocalin	*T. brasiliensis*	4,00E-19	43890	Triabin
37	57657831	2,00E-60	gb|ABR27920.1|	Lipocalin	*T. infestans*	5,00E-16	43890	Triabin
38	57657832	2,00E-19	gb|ABH09429.1|	Pallidipin-like lipocalin	*T. brasiliensis*	5,00E-11	43890	Triabin
39	57657833	7,00E-31	gb|ABR27987.1|	Pallidipin-like lipocalin	*T. infestans*	7,00E-16	43890	Triabin
40	57657834	No significant similarity found				No CD has been identified on this query sequence		
41	57657835	9,00E-15	gb|ABR27987.1|	Pallidipin-like lipocalin	*T. infestans*	2,00E-11	43890	Triabin
42	57657836	No significant similarity found				No CD has been identified on this query sequence		
43	57657837	No significant similarity found				No CD has been identified on this query sequence		
44	57657838	2,00E-46	gb|ABR27936.1|	Lipocalin	*T. infestans*	6,00E-13	43890	Triabin
45	57657839	1,00E-14	gi|71725068|gb|AAZ38956.1|	Triatin	*T. infestans*	0,008	43890	Triabin
46	57657840	4,00E-36	gb|ABR27935.1|	Triatin-like salivary lipocalin	*T. infestans*	2,00E-09	43890	Triabin
47	57657841	1,00E-05	gb|ABR27937.1|	Secreted kazal-type proteinase inhibitor	*T. infestans*	No CD has been identified on this query sequence		

*NCBI access numbers.

Supplemental Table 2: LC-MS/MS of salivary glands proteins from the hematophagous *Panstrongylus megistus*.

ncbi accession number	Proteins	Specie	P (pro) / P (pep)	Score / XC	MW / Sp	Accession / RSp	Peptide (Hits) / Ions
108877641	Hypothetical protein	*A. aegypti*	9,97E-05	2,01E+01	31364,51	108877641,0	2 (2 0 0 0 0)
94468918	Calponin	*A. aegypti*	5,52E-05	2,01E+01	20708,34	94468918,0	2 (2 0 0 0 0)
108875864	Malate dehydrogenase	*A. aegypti*	2,00E-06	1,03E+01	44246,14	108875864,0	1 (1 0 0 0 0)
148469312	Actin 5	*A. aegypti*	2,23E-09	5,63E+01	15808,87	148469312,0	6 (4 2 0 0 0)
88939925	Isocitrate dehydrogenase	*A. cryptum*	1,87E-06	1,02E+01	45739,32	88939925,0	1 (1 0 0 0 0)
11596154	Glutathione transferase GST1-1	*A. dirus*	3,06E-08	1,02E+01	23381,60	11596154,0	1 (1 0 0 0 0)
114321412	S-adenosyl-L-homocysteine hydrolase	*A. ehrlichei*	1,22E-05	2,02E+01	51520,86	114321412,0	2 (2 0 0 0 0)
148469374	AGAP002559-PA	*A. gambiae*	4,46E-10	6,02E+01	29173,04	148469374,0	6 (6 0 0 0 0)
31208299	Calreticulin	*A. gambiae*	2,57E-08	2,82E+01	46329,13	31208299,0	3 (2 1 0 0 0)
57908487	Cofilin/actin-depolymerizing factor homolog	*A. gambiae*	1,34E-06	2,02E+01	16922,44	57908487,0	2 (2 0 0 0 0)
110758129	Heterogeneous nuclear ribonucleoprotein	*A. mellifera*	3,22E-09	1,03E+01	38459,30	110758129,0	1 (1 0 0 0 0)
63333445	Beta-tubulin	*A. spinosa*	6,23E-08	1,82E+01	43144,33	63333445,0	2 (1 1 0 0 0)
18424620	Tubulin beta 2	*A. thaliana*	4,51E-08	5,22E+01	50700,97	18424620,0	6 (3 2 1 0 0)
15226467	Peptidyl-prolyl cis-trans isomerase	*A. thaliana*	5,93E-08	1,01E+01	18452,17	15226467,0	1 (1 0 0 0 0)
24251277	Glutamine synthetase	*B. glabrata*	8,66E-07	1,02E+01	9747,91	24251277,0	1 (1 0 0 0 0)
116242946	Actin	*B. grunniens*	3,40E-07	1,62E+01	41721,74	116242946,0	2 (0 2 0 0 0)
95102548	Actin-depolymerizing factor 4	*B. mori*	2,33E-10	1,02E+01	16998,42	95102548,0	1 (1 0 0 0 0)
95102906	S-adenosyl-L-homocysteine hydrolase	*B. mori*	9,44E-13	1,03E+01	47453,42	95102906,0	1 (1 0 0 0 0)
119909933	ATP-dependent zinc metalloprotease	*B. taurus*	3,05E-05	1,61E+01	81466,56	119909933,0	2 (0 2 0 0 0)
29345439	Putative outer membrane protein	*B. thetaiotaomicron*	4,39E-05	1,02E+01	122007,90	29345439,0	1 (1 0 0 0 0)
78694103	Chemotaxis protein cheA	*Bradyrhizobium sp.*	1,10E-05	2,01E+01	74429,66	78694103,0	2 (2 0 0 0 0)
68467643	Putative S-adenosyl-L-homocysteine hydrolase	*C. albicans*	2,49E-06	1,02E+01	49040,83	68467643,0	1 (1 0 0 0 0)
39596323	Hypothetical protein CBG16354	*C. briggsae*	3,70E-05	1,01E+01	35554,71	39596323,0	1 (1 0 0 0 0)
59894469	Beta-tubulin	*C. deciduum*	5,02E-10	2,03E+01	36429,75	59894469,0	2 (2 0 0 0 0)

ncbi accession number	Proteins	Specie	P (pro) P (pep)	Score XC	MW Sp	Accession RSp	Peptide (Hits) Ions
32564132	Nascent polypeptide-associated complex subunit alpha	*C. elegans*	5,96E-05	1,02E+01	22064,28	32564132,0	1 (1 0 0 0 0)
12230023	Enolase	*C. elegans*	7,44E-06	1,01E+01	46848,84	12230023,0	1 (1 0 0 0 0)
58262484	Heat shock protein	*C. neoformans*	2,77E-07	2,22E+01	88062,52	58262484,0	3 (1 1 0 1 0)
73622733	Elongation factor 1-alpha	*D. californicus*	1,03E-10	2,03E+01	25399,18	73622733,0	3 (1 1 0 0 1)
24645350	Beta-2 tubulin	*D. melanogaster*	2,25E-10	3,02E+01	49837,87	24645350,0	3 (3 0 0 0 0)
17864264	Ribosomal protein L30 CG10652-PA	*D. melanogaster*	4,95E-07	1,02E+01	12226,51	17864264,0	1 (1 0 0 0 0)
17530805	Actin	*D. melanogaster*	1,27E-09	1,03E+01	41794,79	17530805,0	1 (1 0 0 0 0)
24661332	Arginine kinase CG32031-PA	*D. melanogaster*	9,96E-09	8,23E+00	43622,06	24661332,0	1 (0 1 0 0 0)
54637169	Tubulin-beta-3	*D. pseudoobscura*	6,23E-07	8,26E+00	50779,52	54637169,0	1 (0 1 0 0 0)
54638803	Chaperonin	*D. pseudoobscura*	5,41E-08	2,02E+01	59339,38	54638803,0	2 (2 0 0 0 0)
54643520	Acetyl-coa acetyltransferase	*D. pseudoobscura*	2,51E-07	2,02E+01	43433,33	54643520,0	2 (2 0 0 0 0)
54638526	Glutamate dehydrogenase	*D. pseudoobscura*	5,70E-09	4,03E+01	62495,08	54638526,0	4 (4 0 0 0 0)
54642249	Putative catalase	*D. pseudoobscura*	3,06E-07	4,02E+01	57137,47	54642249,0	4 (4 0 0 0 0)
54637612	ATPase coupling factor 6	*D. pseudoobscura*	7,82E-08	2,02E+01	11775,25	54637612,0	2 (2 0 0 0 0)
54638895	Aldolase	*D. pseudoobscura*	5,37E-08	2,02E+01	40043,86	54638895,0	2 (2 0 0 0 0)
54635442	Ribosomal protein L19	*D. pseudoobscura*	2,49E-11	1,03E+01	23912,37	54635442,0	1 (1 0 0 0 0)
54643297	Moesin	*D. pseudoobscura*	5,55E-05	2,01E+01	67359,45	54643297,0	2 (2 0 0 0 0)
54637988	Endoplasmin	*D. pseudoobscura*	5,72E-08	1,82E+01	90456,67	54637988,0	2 (1 1 0 0 0)
68363962	Transcription factor 20	*D. rerio*	7,11E-05	1,01E+01	126098,40	68363962,0	1 (1 0 0 0 0)
19704360	Guanine-hypoxanthine permease	*F. nucleatum*	3,09E-07	1,02E+01	46233,18	19704360,0	1 (1 0 0 0 0)
86143735	Ribosomal protein S6	*Flavobacterium sp.*	2,03E-05	1,81E+01	31670,02	86143735,0	2 (1 1 0 0 0)
9626709	Replicase	*Foxtail mosaic virus*	9,85E-05	1,01E+01	152221,40	9626709,0	1 (1 0 0 0 0)
70909851	Ribosomal protein L32e	*Georissus sp.*	1,61E-08	3,02E+01	15896,81	70909851,0	3 (3 0 0 0 0)
28940	ATP synthase	*H. sapiens*	6,22E-12	2,03E+01	57919,52	28940,0	2 (2 0 0 0 0)
50308047	Unnamed protein product	*K. lactis*	9,95E-05	1,01E+01	77119,17	50308047,0	1 (1 0 0 0 0)
68125115	Oxidoreductase-like protein	*L. major*	6,94E-06	4,02E+01	41950,62	68125115,0	4 (4 0 0 0 0)
93278396	Heat shock protein 90	*L. migratoria*	9,38E-07	3,82E+01	82514,66	93278396,0	4 (3 1 0 0 0)
1703112	Actin	*L. pictus*	5,35E-05	1,62E+01	41846,90	1703112,0	2 (0 2 0 0 0)
51507405	Actin	*L. salmonis*	1,23E-06	1,42E+01	15589,53	51507405,0	2 (0 1 1 0 0)
88603686	Fo 2-phospho-L-lactate transferase	*M. hungatei*	8,78E-06	1,02E+01	32640,81	88603686,0	1 (1 0 0 0 0)

ncbi accession number	Proteins	Specie	P (pro) / P (pep)	Score / XC	MW / Sp	Accession / RSp	Peptide (Hits) / Ions
46206194	Catalase	*M. magnetotacticum*	5,42E-05	1,02E+01	17575,96	46206194,0	1 (1 0 0 0 0)
12585261	Heat shock 70 kDa protein	*M. sexta*	2,97E-06	1,02E+01	71387,38	12585261,0	1 (1 0 0 0 0)
1703156	Actin	*M. sexta*	1,81E-05	2,02E+01	41749,77	1703156,0	2 (2 0 0 0 0)
6606186	Elongation factor-1 alpha	*M. subterraneus*	5,93E-06	1,22E+01	41273,25	6606186,0	2 (0 0 2 0 0)
118747448	Hypothetical protein Mmwyl1_0386	*Marinomonas sp.*	3,86E-05	2,02E+01	24815,70	118747448,0	2 (2 0 0 0 0)
87118702	CRISPR-associated protein	*Marinomonas sp.*	6,85E-06	1,01E+01	38634,42	87118702,0	1 (1 0 0 0 0)
6856315	Elongation factor-1 alpha	*Membracis sp.*	6,00E-05	2,62E+01	33739,55	6856315,0	3 (1 2 0 0 0)
110635547	ATP synthase F1	*Mesorhizobium sp.*	2,44E-07	1,02E+01	54968,69	110635547,0	1 (1 0 0 0 0)
10242170	1 alpha tubulin	*N. coriiceps*	7,51E-05	1,82E+01	21130,27	10242170,0	2 (1 1 0 0 0)
85090447	Eukaryotic initiation factor 4A	*N. crassa*	6,65E-07	2,02E+01	47936,78	85090447,0	2 (2 0 0 0 0)
85109951	Vacuolar ATP synthase subunit B	*N. crassa*	1,74E-13	1,02E+01	56773,25	85109951,0	1 (1 0 0 0 0)
30249732	Respiratory-chain NADH dehydrogenase 51 Kd subunit	*N. europaea*	2,98E-05	2,01E+01	47045,30	30249732,0	2 (2 0 0 0 0)
119500800	GPI-anchored cell surface glycoprotein	*N. fischeri*	5,76E-05	1,02E+01	90080,14	119500800,0	1 (1 0 0 0 0)
82702137	Protein of unknown function predicted ATPase	*N. multiformis*	4,37E-05	2,02E+01	34384,54	82702137,0	2 (2 0 0 0 0)
40549128|	Beta-cyanoalanine synthase	*N. tabacum*	5,17E-05	1,62E+01	13299,55	40549128,0	2 (0 2 0 0 0)
117652169	Elongation factor 1-alpha	*O. hedleyi*	7,20E-12	1,22E+01	18923,69	117652169,0	2 (0 1 0 1 0)
93005886	Cobyrinic acid a,c-diamide synthase	*P. cryohalolentis*	8,54E-05	1,01E+01	27982,67	93005886,0	1 (1 0 0 0 0)
57657833	Pallidipin-like lipocalin	*P. megistus*	3,00E-14	5,03E+01	18245,55		5 (5 0 0 0 0)
57657820	Pallidipin-like salivary lipocalin	*P. megistus*	8,86E-09	5,02E+01	19230,04		5 (5 0 0 0 0)
57657830	Lipocalin	*P. megistus*	7,31E-07	5,02E+01	19703,88		5 (5 0 0 0 0)
57657805	Lipocalin	*P. megistus*	1,33E-10	2,06E+02	22379,77		21 (19 2 0 0 0)
57657806	Triatin-like salivary lipocalin	*P. megistus*	6,20E-11	1,66E+02	22841,93		17 (15 2 0 0 0)
57657809	Pallidipin 2	*P. megistus*	4,09E-13	1,50E+02	12653,03		15 (15 0 0 0 0)
57657799	Lipocalin	*P. megistus*	1,00E-30	1,00E+02	20477,16		10 (10 0 0 0 0)
57657801	Lipocalin	*P. megistus*	1,88E-08	9,03E+01	18761,16		9 (9 0 0 0 0)
57657831	Lipocalin	*P. megistus*	2,49E-08	7,02E+01	21595,96		7 (7 0 0 0 0)
57657840	Triatin-like salivary lipocalin	*P. megistus*	5,65E-11	6,43E+01	19492,98		7 (5 1 1 0 0)
57657827	Lipocalin	*P. megistus*	3,34E-05	4,82E+01	9354,06		5 (4 1 0 0 0)
57657822	Salivary lipocalin 1	*P. megistus*	4,66E-14	4,03E+01	9036,47		4 (4 0 0 0 0)
57657802	Salivary lipocalin 4	*P. megistus*	1,03E-07	4,03E+01	13921,47		4 (4 0 0 0 0)

ncbi accession number	Proteins	Specie	P (pro) P (pep)	Score XC	MW Sp	Accession RSp	Peptide (Hits) Ions
57657817	Lipocalin-like Tin66	*P. megistus*	4,71E-06	4,02E+01	12464,41		4 (4 0 0 0 0)
57657838	Lipocalin	*P. megistus*	2,21E-07	2,03E+01	21011,02		2 (2 0 0 0 0)
57657808	Triabin-like lipocalin 4 a	*P. megistus*	2,11E-06	2,02E+01	14209,55		2 (2 0 0 0 0)
57657810	Ribosomal protein P2	*P. megistus*	1,57E-06	2,02E+01	11813,11		2 (2 0 0 0 0)
57657803	Pallidipin 2	*P. megistus*	2,22E-08	1,83E+01	21305,44		2 (1 1 0 0 0)
57657816	Pallidipin 2	*P. megistus*	5,11E-11	1,02E+01	19939,76		1 (1 0 0 0 0)
57657823	Unknown function	*P. megistus*	1,62E-06	1,01E+01	7987,92		1 (1 0 0 0 0)
135450	Tubulin beta-2 chain	*P. polycephalum*	7,28E-09	8,26E+00	50331,36	135450,0	1 (0 1 0 0 0)
1174593	Tubulin	*P. vulgata*	2,48E-08	4,02E+01	50178,63	1174593,0	4 (4 0 0 0 0)
114769716	S-adenosyl-L-homocysteine hydrolase	*R. bacterium*	1,46E-09	8,20E+00	50882,75	114769716,0	1 (0 1 0 0 0)
4139571	S-Adenosylhomocystein Hydrolase	*R. norvegicus*	4,11E-14	3,83E+01	47376,25	4139571,0	4 (3 1 0 0 0)
66730465	Tubulin	*R. norvegicus*	1,07E-09	8,25E+00	50005,57	66730465,0	1 (0 1 0 0 0)
6981420	Pancreatic trypsin 1	*R. norvegicus*	5,14E-10	8,27E+00	25942,68	6981420,0	1 (0 1 0 0 0)
157832664	Beta1-tubulin	*R. prolixus*	2,74E-05	2,01E+01	11565,27	157832664,0	2 (2 0 0 0 0)
157833103	Putative elongation factor 1-alpha	*R. prolixus*	8,93E-06	2,02E+01	17515,18	157833103,0	2 (2 0 0 0 0)
119364065	Ribosomal protein S4e	*R. prolixus*	5,00E-07	2,02E+01	31199,04	119364065,0	2 (2 0 0 0 0)
119364509	3-hydroxyisobutyrate dehydrogenase	*R. prolixus*	1,68E-11	2,03E+01	32753,80	119364509,0	2 (2 0 0 0 0)
119363881	Heat shock 70 kD protein cognate	*R. prolixus*	5,71E-07	2,02E+01	29104,88	119363881,0	2 (2 0 0 0 0)
119364430	Protein disulfide isomerase	*R. prolixus*	7,83E-12	2,03E+01	30462,70	119364430,0	2 (2 0 0 0 0)
119363891	Calreticulin	*R. prolixus*	7,82E-12	3,03E+01	31701,41	119363891,0	3 (3 0 0 0 0)
119364512	ATP synthase beta subunit	*R. prolixus*	1,17E-09	4,02E+01	36575,59	119364512,0	4 (4 0 0 0 0)
119363356	Beta tubulin	*R. prolixus*	6,66E-15	4,04E+01	21763,83	119363356,0	4 (4 0 0 0 0)
119364187	Putative arginine kinase	*R. prolixus*	9,92E-10	1,00E+02	41204,52	119364187,0	10 (10 0 0 0 0)
119363702	Elongation factor 1 alpha	*R. prolixus*	6,00E-05	1,02E+01	34368,87	119363702,0	1 (1 0 0 0 0)
119363197	Peroxiredoxins	*R. prolixus*	3,65E-09	1,02E+01	25079,40	119363197,0	1 (1 0 0 0 0)
159726785	Heat shock protein	*R. prolixus*	6,62E-09	1,02E+01	12373,70	159726785,0	1 (1 0 0 0 0)
157832962	14-3-3-like protein	*R. prolixus*	4,14E-07	1,03E+01	22394,03	157832962,0	1 (1 0 0 0 0)
157833006	Beta-tubulin	*R. prolixus*	7,36E-10	1,02E+01	11171,05	157833006,0	1 (1 0 0 0 0)
157832299	H+ transporting ATP synthase beta subunit isoform 2	*R. prolixus*	8,38E-07	1,01E+01	13664,13	157832299,0	1 (1 0 0 0 0)
159778315	Nucleoside diphosphate kinase	*R. prolixus*	3,28E-05	1,01E+01	17921,20	159778315,0	1 (1 0 0 0 0)
57241817	Ribosomal protein S2	*R. robustus*	4,32E-06	1,02E+01	15722,25		1 (1 0 0 0 0)

ncbi accession number	Proteins	Specie	P (pro) / P (pep)	Score / XC	MW / Sp	Accession / RSp	Peptide (Hits) / Ions
77462848	ATP synthase subunit A	*R. sphaeroides*	2,84E-08	1,02E+01	55153,02	77462848,0	1 (1 0 0 0 0)
3114952	Heat shock protein 70	*S. domuncula*	3,22E-09	1,02E+01	72010,87	3114952,0	1 (1 0 0 0 0)
89953743	Beta-actin	*S. fibuligera*	3,33E-05	1,62E+01	31467,09	89953743,0	2 (0 2 0 0 0)
37960224	LEAFY-like protein	*S. fuscum*	5,55E-05	1,01E+01	4951,48	37960224,0	1 (1 0 0 0 0)
62912066	Transferrin	*S. invicta*	2,62E-05	1,02E+01	77211,70	62912066,0	1 (1 0 0 0 0)
51988178	Beta tubulin	*S. viridis*	2,40E-10	1,83E+01	50239,84	51988178,0	2 (1 1 0 0 0)
114046005	Peptidase U62	*Shewanella sp.*	3,22E-05	1,62E+01	47830,18	114046005,0	2 (1 0 1 0 0)
33865575	Hypothetical protein SYNW1041	*Synechococcus sp.*	2,51E-05	2,02E+01	10669,57	33865575,0	2 (2 0 0 0 0)
110354212	Thioredoxin-2	*T. brasiliensis*	6,04E-08	2,03E+01	6077,95	110354212,0	2 (2 0 0 0 0)
110354476	Ribosomal protein L23	*T. brasiliensis*	6,77E-14	2,02E+01	19850,30	110354476,0	2 (2 0 0 0 0)
111379925	Secreted salivary trypsin	*T. brasiliensis*	1,09E-08	1,02E+01	21811,26	111379925,0	1 (1 0 0 0 0)
110354070	Kazal-type proteinase inhibitor	*T. brasiliensis*	2,51E-05	1,02E+01	11562,32	110354070,0	1 (1 0 0 0 0)
31541037	Glutamate dehydrogenase	*T. californicus*	7,40E-11	1,02E+01	61335,47	31541037,0	1 (1 0 0 0 0)
52630939	Putative translation elongation factor 2	*T. citricida*	2,29E-05	3,02E+01	94757,20	52630939,0	3 (3 0 0 0 0)
148468977	Heat shock cognate protein	*T. infestans*	1,37E-10	8,43E+01	19590,05	148468977,0	9 (7 1 1 0 0)
148468057	Putative fructose 1,6-bisphosphate aldolase	*T. infestans*	1,92E-13	7,83E+01	35350,07	148468057,0	8 (7 1 0 0 0)
148468370	Actin D	*T. infestans*	9,99E-14	6,03E+01	30811,19	148468370,0	6 (6 0 0 0 0)
148467907	Tubulin alpha chain	*T. infestans*	3,85E-08	4,83E+01	32664,54	148467907,0	5 (4 1 0 0 0)
148468203	60S ribosomal protein L8	*T. infestans*	4,20E-09	4,02E+01	28779,59	148468203,0	4 (4 0 0 0 0)
34481604	Secreted salivary trypsin	*T. infestans*	6,34E-09	3,82E+01	62480,40	34481604,0	4 (3 1 0 0 0)
TiCluster127	Kazal-type proteinase inhibitor	*T. infestans*	2,12E-05	3,02E+01	40838,83		3 (3 0 0 0 0)
148467919	Fumarylacetoacetate hydrolase	*T. infestans*	9,20E-06	3,02E+01	34257,29	148467919,0	3 (3 0 0 0 0)
148469335	Superoxide dismutase	*T. infestans*	9,54E-06	2,82E+01	16621,33	148469335,0	3 (2 1 0 0 0)
148467924	Beta-tubulin	*T. infestans*	7,97E-12	2,03E+01	15805,78	148467924,0	2 (2 0 0 0 0)
148469357	Trialysin allele	*T. infestans*	1,74E-08	2,02E+01	29808,61	148469357,0	2 (2 0 0 0 0)
148468736	Disulfide isomerase-2	*T. infestans*	6,18E-07	2,02E+01	15479,85	148468736,0	2 (2 0 0 0 0)
148468109	Thymosin beta	*T. infestans*	2,28E-06	2,02E+01	19528,13	148468109,0	2 (2 0 0 0 0)
148467924	Beta-tubulin	*T. infestans*	5,28E-08	2,02E+01	17161,64	148467924,0	2 (2 0 0 0 0)
83637828	Infestin 1-7 precursor	*T. infestans*	5,45E-11	1,83E+01	44960,89	83637828,0	2 (1 1 0 0 0)
148467916	79 kDa salivary apyrase precursor	*T. infestans*	8,99E-07	1,62E+01	15993,24	148467916,0	2 (0 2 0 0 0)
148469275	Salivary lipocalin	*T. infestans*	5,63E-09	1,22E+01	66826,88	148469275,0	2 (1 0 0 0 1)

ncbi accession number	Proteins	Specie	P (pro) / P (pep)	Score / XC	MW / Sp	Accession / RSp	Peptide (Hits) / Ions
148468466	Salivary trypsin	*T. infestans*	4,15E-07	1,02E+01	37486,64	148468466,0	1 (1 0 0 0 0)
148468331	Ribosomal protein P1	*T. infestans*	1,52E-05	1,02E+01	12067,17	148468331,0	1 (1 0 0 0 0)
148468634	Ribosomal protein L11	*T. infestans*	6,48E-09	1,02E+01	23289,49	148468634,0	1 (1 0 0 0 0)
148469051	Disulfide isomerase	*T. infestans*	2,21E-10	1,02E+01	19978,96	148469051,0	1 (1 0 0 0 0)
TiCluster114	Trypsin	*T. infestans*	3,38E-07	1,02E+01	20766,36		1 (1 0 0 0 0)
148468275	Calreticulin	*T. infestans*	1,51E-06	1,02E+01	12133,63	148468275,0	1 (1 0 0 0 0)
148468234	Methyltransferase	*T. infestans*	1,83E-11	1,02E+01	22142,52	148468234,0	1 (1 0 0 0 0)
148468549	Heterogeneous nuclear ribonucleoprotein K	*T. infestans*	5,19E-08	1,02E+01	27986,42	148468549,0	1 (1 0 0 0 0)
148468282	Cathepsin D	*T. infestans*	2,95E-05	1,01E+01	17643,01	148468282,0	1 (1 0 0 0 0)
118377813	Hypothetical protein TTHERM_00566830	*T. thermophila*	9,80E-05	2,01E+01	196079,00	118377813,0	2 (2 0 0 0 0)
27363532	Hypothetical protein	*V. vulnificus*	6,30E-05	2,02E+01	25001,57	27363532,0	2 (2 0 0 0 0)
78048276	Putative secreted protein	*X. campestris*	3,69E-05	2,01E+01	11844,23	78048276,0	2 (2 0 0 0 0)
50553694	Chitin synthase	*Y. lipolytica*	2,80E-05	1,81E+01	225494,50	50553694,0	2 (1 1 0 0 0)

CONCLUSÕES

No que diz respeito aos estudos moleculares com os *Rhodnius,* obtivemos duas bibliotecas de cDNA com 56 e 122 clusters em *R. brethesi* e *R. robustus* respectivamente. Quase um terço desses transcritos não foi identificado usando banco de dados específicos. Encontramos também 123 e 111 em *R. brethesi* e *R. robustus* usando LC-MS/MS. Foram encontradas lipocalinas, inositol polyphosphate 5-phosphatase, proteínas com domínio Kazal, glutationa S transferase entre outras.

Em relação ao *P. megistus* nós sequenciamos 45 transcritos a partir de uma biblioteca de cDNA e 159 proteinas no proteoma a partir de espectrometria de massa. Como esperado foram encontradas proteinas como as lipocalinas, palidipina, domínio kazal, triabina, trialisina, triatina, apirase, infestilina, trypsina salivar, GST. Não foram encontradas nitroforinas, proteínas amplamente disseminadas nos insetos da tribo *Rhodnius*. Os triatomíneos *P. megistus*, de acordo com os dendogramas e com as proteinas encontradas se aproximam mais dos *Triatoma* do que dos *Rhodnius*.

Nos últimos anos, os meios de comunicação noticiam frequentes surtos de transmissão de *T. cruzi* em famílias residentes na Amazônia Legal. Traços comuns a essas epidemias são a) predominância de triatomíneos da tribo *Rhodnius*; b) não domiciliação desses insetos, geralmente provenientes de palmeiras nas vizinhanças das moradias, que invadem os domicílios atraídos pela luz e; c) impossibilidade de uso de inseticidas como política pública para controlar as grandes endemias.

Olhando essas ponderações em perspectiva, concordamos que seria melhor estudar mais esses insetos em profundidade nos seus nichos ecológicos, mas não apenas cuidar dos aspectos da pesquisa relacionada ao trabalho de campo. Seria fundamental também juntar aos dados fornecidos pelas pesquisas moleculares para oferecer o combate devido vizando a prevenir as mircroepidemias.

A criação de novas ferramentas eficazes no combate a transmissão das infecções para o homem, depende de conhecimentos que ainda haverão de ser criados nos laboratórios. Oferecemos aqui, até mesmo para evitar especulações, apenas um exemplo desse novo tipo de tecnologia moderna. Talvez, conhecendo melhor a fisiologia e bioquímica dos triatomíneos

surgirá a possibilidade de detectar e sintetizar moléculas repulsivas ao inseto, sem causar danos aos homens.

PERSPECTIVAS

Esse estudo fornece uma série de dados que podem ser aplicados em diferentes novos estudos mais aprofundados. Um ponto muito intrigante são as proteínas que não têm similaridade com nenhuma conhecida atualmente. É importante salientar que algumas dessas proteínas possuem peptídeo sinal, indicando uma possível secreção salivar e também possuem domínios de triabina, corroborando com a possível função durante o repasto sanguineo. Essas proteínas desconhecidas podem ser caracterizadas e estudadas.

Além disso, notamos a presença de outras proteínas com potenciais farmacológicos como, por exemplo, a homocisteina ou a polilisina que não são descritas em salivas de insetos e que devem ter uma importância no hospedeiro, principalmente após o repasto.

Não podendo esquecer as moléculas que são usadas para a detoxicação de inseticida, a GST que não tinha sido descrita nem em *Rhodnius* nem em *Panstrongylus*.

Todas esssas proteínas citadas acima ainda não foram caracterizadas bioquimicamente na saliva desses insetos. Estudos futuros relacionados a elas podem permitir um entendimento melhor em relação à fisiologia e sobrevivência desses hemípteros.

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