UNIVERSIDADE ESTADUAL DE CAMPINAS

Instituto de Biologia

JULIANA DAMIELI NASCIMENTO

ESTUDO DOS ASPECTOS MORFOLÓGICOS, FILOGENÉTICOS E PROTEÔMICO DE *RHODNIUS NEGLECTUS* (HEMIPTERA, REDUVIIDAE)

STUDY OF ASPECTS MORPHOLOGICAL, PHYLOGENETIC AND PROTEOMIC OF *RHODNIUS NEGLECTUS* (HEMIPTERA, REDUVIIDAE)

Campinas

2020

JULIANA DAMIELI NASCIMENTO

ESTUDO DOS ASPECTOS MORFOLÓGICOS, FILOGENÉTICOS E PROTEÔMICO DE *RHODNIUS NEGLECTUS* (HEMIPTERA, REDUVIIDAE)

STUDY OF ASPECTS MORPHOLOGICAL, PHYLOGENETIC AND PROTEOMIC OF RHODNIUS NEGLECTUS (HEMIPTERA, REDUVIIDAE)

Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Biologia Animal, na Área de Relações Antrópicas, Meio Ambiente e Parasitologia

Thesis presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Doctorate in Animal Biology in the field of Anthropic Relations, Environment and Parasitology.

Orientador: Prof. Dr. João Aristeu da Rosa

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA JULIANA DAMIELI NASCIMENTO, E ORIENTADO PELO PROF. DR. JOÃO ARISTEU DA ROSA

Campinas

2020

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

Nascimento, Juliana Damieli, 1990-
N17e Estudo dos aspectos morfológicos, filogenéticos e proteômico de *Rhodnius neglectus* (Hemiptera, Reduviidae) / Juliana Damieli Nascimento. – Campinas, SP : [s.n.], 2020.

Orientador: João Aristeu da Rosa.
Tese (doutorado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. *Rhodnius*. 2. Filogenia. 3. Proteômica. I. Rosa, João Aristeu. II. Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Study of aspects morphological, phylogenetic and proteomic of *Rhodnius neglectus* (Hemiptera, Reduviidae)
Palavras-chave em inglês:
Rhodnius
Phylogeny
Proteomics
Área de concentração: Relações Antrópicas, Meio Ambiente e Parasitologia
Titulação: Doutora em Biologia Animal
Banca examinadora:
João Aristeu da Rosa [Orientador]
Carlos Eduardo Almeida
Mara Cristina Pinto
Vagner José Mendonça
Cléber Galvão Ferreira
Data de defesa: 09-04-2020
Programa de Pós-Graduação: Biologia Animal

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-6892-2453
- Currículo Lattes do autor: http://lattes.cnpq.br/2335413580447528

Campinas, 09/04/2020.

COMISSÃO EXAMINADORA

Prof. Dr. João Aristeu da Rosa (Presidente)

Prof. Dr. Carlos Eduardo de Almeida

Profa. Dra. Mara Cristina Pinto

Prof. Dr. Vagner José Mendonça

Prof. Dr. Cleber Galvão Ferreira

Os membros da Comissão Examinadora acima assinaram a Ata de Defesa, que se encontra no processo de vida acadêmica do aluno.

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa Pós-Graduação em Biologia Animal da Unidade do Instituto de Biologia.

DEDICATÓRIA

A minha família, razão de tudo.

AGRADECIMENTOS

Agradeço a Deus, por minha saúde, paciência e discernimento durante esses quatro anos de estudo e trabalho.

A minha família, em especial à minha mãe Sirlei, meu pai Nelson, meus irmãos Andréia e José Mário, meus sobrinhos Raul e Lívia, meus cunhados Luciana e Luís Fernando, e ao meu namorado, Rafael, que são minha base. Agradeço por compartilharem ao meu lado esta etapa, por todo o apoio moral, amoroso e financeiro durante este período e em todas as minhas decisões. Obrigada por serem meus exemplos, acreditarem em mim, pela compreensão nos momentos de ausência, pelos conselhos e por todas as palavras motivadoras que me consolaram e deram força nos momentos mais difíceis.

Ao Prof. Dr. João Aristeu da Rosa, pela orientação, oportunidade de crescimento profissional e pessoal, principalmente, confiança, incentivo e amizade. Obrigada professor pelo apoio na execução desse projeto, pela convivência e oportunidade de aprendizado.

Ao Prof. Dr. Juan David Ramírez pela orientação durante meu estágio na Universidad del Rosário, Faculdade de Ciências Naturais e Matemáticas, Colômbia. Muito obrigada professor pelo aprendizado e amizade, sobretudo pela confiança depositada, dedicação e carinho.

Aos alunos do grupo de pesquisas em Microbiologia da Universidad del Rosario, Giovani Herrera, Lissa Cruz Saavedra, Marina Muñoz, Adriana Higuera Gelvez e Luz Patiño Blanco por todo companheirismo, paciência e ensinamentos.

A Profa. Dra. Márcia Aparecida da Silva Graminha pela contribuição significativa para o desenvolvimento deste trabalho na Faculdade de Ciências Farmacêuticas de Araraquara, laboratório de Parasitologia clínica. Muito obrigada professora, por toda ajuda e companheirismo nos momentos mais difíceis da pesquisa. Assim como a sua aluna de Pós-Doutorado Ângela Arenas que foi essencial para o desenvolvimento inicial do projeto.

A Profa Dra. Ana Marisa Fusco Almeida por disponibilizar o núcleo de proteômica da Faculdade de Ciências Farmacêuticas de Araraquara para realização do trabalho, assim como ao Dr. Paulo César Gomes, técnico responsável da espectrometria de massas e todos os alunos orientados pela professora.

Aos Drs. Mara Cristina Pinto, Marlene T. Ueta, Regina Maura Bueno Franco, Danilo Ciccone Miguel, pela disponibilidade em compor a banca do exame de qualificação.

Aos demais professores e técnicos do departamento de Parasitologia da Universidade Estadual de Campinas, por toda contribuição para meu aprendizado durante o mestrado e doutorado.

As minhas amigas, Aline Rimoldi Ribeiro, Larissa Aguiar Almeida, Fabrícia Regiane Borges e Luana Elis Sabino, sempre presentes, que conviveram com minha ausência em vários momentos e nunca se afastaram ou deixaram de me apoiar. Obrigada pelos momentos de descontração e imensurável ajuda.

Aos colegas do Laboratório de Parasitologia da Faculdade de Ciências Farmacêuticas de Araraquara em especial Rossana Falcone, Gabriela Kunii, Dennys Sammilian, Flávia Benini e Cintia Oliveira pelo ambiente de trabalho, pelas discussões científicas, momentos de descontração e imensurável ajuda, sem os quais parte do trabalho não seria possível.

A todos os colegas, técnicos, alunos e professores do departamento de Ciências Biológicas da Faculdade de Ciências Farmacêuticas de Araraquara que auxiliaram de algum modo para meu aprendizado acadêmico-profissional e realização das obrigações necessárias para a complementação dessa etapa acadêmica.

A Universidade Estadual de Campinas por prover condições para minha formação acadêmico-profissional e pelos subsídios fornecidos a esta pesquisa.

A Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Ciências Farmacêuticas de Araraquara por prover condições para a realização dos experimentos científicos, pela formação acadêmico-profissional e pelos subsídios fornecidos.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

RESUMO

A doença de Chagas tem por agente etiológico o protozoário *Trypanosoma cruzi* que é transmitido pelas fezes dos triatomíneos. A subfamília Triatominae contém 19 gêneros e 154 espécies, todas potenciais vetoras de *T. cruzi*, exceto três que são fósseis. A diferenciação das espécies de Triatominae é realizada principalmente por caracteres morfológicos e moleculares, diante dessa premissa este trabalho teve como intuito identificar a espécie de *Rhodnius* sp. coletada no município de Taquarussu/MS em 2010. Para tanto foram utilizados caracteres morfológicos e citogenéticos. O desenvolvimento desse estudo resultou na descrição de *R. taquarussuensis* sp. n. Posteriormente por meio de análises das divergências genéticas e delimitações das espécies pelo estudo da variação de dois genes mitocondriais e quatro nucleares, assim como cruzamentos interespecíficos foi demonstrado que *R. taquarussuensis* sp. n. trata-se de uma linhagem fenotípica de *R. neglectus*. Após a publicação de *R. taquarussuensis* sp. n como sinônimo júnior de *R. neglectus*, foi realizado o estudo de espermatecas de fêmeas fecundadas. Para este estudo, foram dissecadas e retiradas 40 espermatecas fecundadas, logo após foram realizadas a extração das proteínas, análise do perfil proteico, digestão tríptica, espectrometria de massas e identificação das proteínas por meio do banco de dados de *R. neglectus* em comparação com os bancos *SwissProt* e *NCBI*. Foram identificadas 34 proteínas pertencentes a quatro classes proteicas distintas, dessas classes destacam-se as musculares, *vacuolar H+-ATPase* (V-ATPases) e Chaperonas com funções correlacionadas na forma como as espermatecas mantém os espermatozoides viáveis e nutridos até a fecundação dos ovócitos, processo necessário para a perpetuação das espécies.

ABSTRACT

Chagas disease is caused by the protozoan *Trypanosoma cruzi*, which is transmitted through the feces of triatomines. The subfamily Triatominae contains 19 genera and 154 species, all potential vectors of *T. cruzi*, except three fossils. The differentiation of the species of Triatominae is carried out mainly by morphological and molecular characters, in view of this premise this study aimed to identify the species of *Rhodnius* sp collected in the municipality of Taquarussu / MS in 2010. For this purpose, morphological and cytogenetic characters were used. The development of this study resulted in the description of *R. taquarussuensis* sp. n. Subsequently, by analyzing genetic divergences and species delimitations by studying two mitochondrial and four nuclear genes, as well as interspecific crosses, it was established that *R. taquarussuensis* sp. n. it is a phenotypic strain of *R. neglectus*. After the publication of *R. taquarussuensis* sp. n as a junior synonym of *R. neglectus*, the study of sperm cells from fertilized females was carried out. For this study, were dissected 40 fertilized spermathecae and removed, shortly after the protein extraction, protein profile analysis, triptych digestion, mass spectrometry and protein identification through the *R. neglectus* database in comparison with *SwissProt* and *NCBI* banks. 34 proteins were identified, belonging to four distinct protein classes. Of these classes, muscle, vacuolar H+-ATPase (V-ATPases) and Chaperones stand out with correlated functions in the way sperm cells keep viable and nourished sperm until fertilization of oocytes, a process necessary for the perpetuation of species of the subfamily Triatominae.

LISTA DE FIGURAS

Capítulo I

Capítulo II

Capítulo III

LISTA DE TABELAS

SUMÁRIO

1 INTRODUÇÃO

1.1 Doença de Chagas e seu perfil epidemiológico

A tripanossomíase americana ou doença de Chagas acomete atualmente 7 milhões de pessoas no mundo. Apesar de ser endêmica em 21 países da América Latina a facilidade de locomoção mundial possibilitou a ocorrência da doença em 22 países (COURA; VIÑAS, 2010). A doença foi descrita por Carlos Chagas em 1909, assim como o seu agente etiológico o protozoário *Trypanosoma cruzi*. Chagas também identificou o vetor *Conorhinus megistus*, atualmente *Panstrongylus megistus* (Burmeister, 1835), assim como a primeira paciente infectada, desse modo descreveu toda a cadeia epidemiológica da antropozoonose. Entretanto, mais de 100 anos se passaram e essa doença ainda é um problema de saúde pública mundial, pois acomete populações negligenciadas e muitos pacientes não procuram auxílio para tratamento ou diagnóstico (CHAGAS, 1909; JR; RASSI; MARIN-NETO, 2009; WHO, 2019).

A compreensão dos aspectos socioculturais é de extrema importância para explicar os aspectos biomédicos atuais, amparar o surgimento, persistência e reemergência da doença. No cenário atual a convivência de diferentes grupos socioeconômicos, étnicos, rurais e urbanos geram diferentes necessidades e torna-se indispensável pesquisas com abordagens sócio estruturais diferentes. Apesar do aumento considerável de pesquisas relacionadas à doença de Chagas poucos estudos são transferidos para a prática no intuito de promover intervenções apropriadas adaptando populações a diferentes contextos e considerando necessidades específicas para cada grupo (VENTURA-GARCIA et al., 2013).

De acordo com o segundo relatório de doenças negligenciadas da Organização Mundial da Saúde essas doenças são sinônimas de pobreza e desvantagem, afeta populações com baixa visibilidade e pouca influência política causando estigma e discriminação (PÉREZ-MOLINA; MOLINA, 2018; WHO, 2010). A doença de Chagas está inserida nesse cenário, assim poucos estudos são realizados em países não endêmicos e devido a mobilidade mundial o continente europeu recebe cada vez mais imigrantes da América Latina.

Em 2017, mais de três milhões de imigrantes de áreas endêmicas viviam na Europa, desses apenas cento e vinte mil pessoas foram diagnosticadas com *T. cruzi*,

provavelmente esse número não representa a realidade sobre o número de pessoas que albergam o protozoário, mas representa a parcela de pessoas que procuraram centros especializados. Com a ausência dos vetores, os mecanismos de transmissão em regiões não endêmicas são a transmissão congênita, transfusão sanguínea, transplante de órgãos e acidentes laboratoriais. Medidas preventivas foram implementadas e auxiliaram no controle da disseminação da doença, mas a transmissão congênita ainda necessita de atenção especial uma vez que os médicos não estão familiarizados e os recém-nascidos assintomáticos podem evoluir para a fase indeterminada da doença. Por isso, políticas de saúde que auxiliem o controle da transmissão congênita estão faltando nas regiões não endêmicas (COURA; VIÑAS, 2010; MONGE-MAILLO; LÓPEZ-VÉLEZ, 2017).

A transmissão sexual do agente etiológico foi confirmada recentemente em estudos com camundongos e humanos e não se pode olvidar essa via transmissão, apesar de não ter relato de transmissão em humanos, é mais uma forma alarmante de dispersão de *T. cruzi* (GOMES et al., 2019).

Os primeiros casos documentados por infecção oral no Brasil ocorreram em 1965 por acometimento de 17 pessoas que resultou em seis óbitos no município de Teutonia/RS, na época acreditou na ingestão de vegetais contaminados com fezes de marsupiais infectados por *T. cruzi* (SILVA et al., 1968), mas atualmente essa hipótese é contraposta pela capacidade de disseminação de tripomastigotas das glândulas odoríferas dos gambas que ao se sentirem ameaçados lançam o conteúdo das glândulas como mecanismos de defesa e consequentemente expulsam as formas tripomastigotas do agente etiológico da doença de Chagas (DEANE; LENZI; JANSEN, 1984; JANSEN; XAVIER; ROQUE, 2020).

Não se pode deixar de referir o surto em 2005 que acometeu 24 pessoas infectadas por meio do suco de cana-de-açúcar no estado de Santa Catarina, esse estudo também revelou gambas infectados com *T. cruzi*. A caracterização de *T. cruzi* encontrado em humanos revelou supremacia do grupo TcII enquanto que em gambas houve predominância do grupo TcI e os triatomíneos infectados revelaram um perfil misto de TcI e TcII (STEINDEL et al., 2008).

Casos de infecção oral mais recentes são notificados com frequência na região Amazônica. O surto da doença em 2007 no estado do Amazonas, período em que houve a infecção de oitenta e oito pessoas por meio do suco de açaí foi um alerta para a

comunidade científica (ALBERTO TOSO; FELIPE VIAL; NORBEL GALANTI, 2011). Em 2015, houve outro surto com dezoitos pessoas infectadas por *T. cruzi* no município de Marcelino Vieira/RN associado principalmente a ingestão de caldo de cana-de-açúcar e no mesmo período foram encontradas espécies como *Triatoma brasiliensis* (Neiva, 1911), *Triatoma pseudomaculata* (Corrêa & Espínola, 1964) e *Panstrongylus lutzi* (Neiva & Pinto, 1923) infectadas com o agente etiológico e próximos as áreas onde eram realizadas as moagens noturnas. Acredita-se que a luz elétrica, atrativa para vários insetos inclusive para triatomíneos, provavelmente atraiu para o local os insetos que caíram nas esteiras de moagens (VARGAS et al., 2018).

O agente etiológico da doença de Chagas é um protozoário generalista capaz de infectar várias células de diferentes mamíferos e invertebrados da subfamília Triatominae, uma vez infectados, os triatomíneos liberam a forma infectante do agente etiológico por toda a vida, nos hospedeiros definitivos o parasita *T. cruzi* também estabelece infecções duradouras e estáveis (JANSEN et al., 2019).

A doença é causada pela transmissão de *T. cruzi* contido nas fezes liberadas pelos triatomíneos no momento do repasto sanguíneo. A forma infectante de *T. cruzi* adentra o hospedeiro vertebrado por meio de esfoliações na pele ou mucosa. Porém, outros métodos de transmissão são determinados, entre os quais: transmissão congênita, transfusão sanguínea, ingestão de alimentos ou bebidas contaminadas como já mencionados anteriormente e ainda por acidentes laboratoriais. Apesar dos diferentes métodos de infecção a transmissão vetorial é a mais comum e eficaz (WHO, 2010).

1.2 A Subfamília Triatominae, vetores da doença de Chagas

Em 1773 ocorreu a descrição da primeira espécie de triatomíneo, *Triatoma rubrofasciata* (De Geer, 1773), que foi descrita inicialmente como *Cimex rubro-fasciatus* (GALVÃO, 2014). Em 1909, o interesse dos estudos dos triatomíneos deixa de ser apenas entomológico e ganha o contexto médico sanitário pela descrição da doença tripanossomíase americana que posteriormente passou a ser denominada doença de Chagas em homenagem ao pesquisador Carlos Chagas (CHAGAS, 1909; KROPF; LACERDA, 2010).

Atualmente são reconhecidas 154 espécies válidas incluindo três espécies fósseis na subfamília Triatominae, agrupadas em cincos tribos com 19 gêneros (DE

OLIVEIRA et al., 2018; DORN et al., 2018; JUSTI; GALVÃO, 2017; LIMA-CORDÓN et al., 2019; NASCIMENTO et al., 2019a; POINAR, 2019). Todas as espécies recentes são potenciais vetoras de *T. cruzi*. Apesar da subfamília de interesse médico sanitário possuir 19 gêneros, apenas três possuem maior importância vetorial por estarem associados ao peri e intra domicílio humano: *Panstrongylus*, *Rhodnius* e *Triatoma* (GALVÃO, 2014; ROSA et al., 2017).

A subfamília Triatominae, é dívidida em cinco tribos, Alberproseniini, Bolboderini, Cavernicolini, Rhodniini e Triatomini (GALVÃO et al., 2003; LENT; WYGODZINSKY, 1979). É necessário ressaltar que o gênero *Meccus* é considerado por alguns autores como *Triatoma*, essa sinonimização foi proposta por JUSTI e GALVÃO 2017, mas ainda é discutida por isso será considerado os 19 gêneros nesse trabalho. As tribos e seus respectivos gêneros são apresentados na Tabela 1, segundo JUSTI e GALVÃO (2017) e GALVÃO (2014).

Tabela 1. Tribos e gêneros da subfamília Triatominae. Fonte: Próprio autor, adaptado de (GALVÃO, 2014; JUSTI; GALVÃO, 2017).

Tribo	**Gênero**
Alberproseniini	*Alberprosenia*
Bolboderini	*Belminus*
	Bolbodera
	Microtriatoma
	Parabelminus
Cavernicolini	*Cavernicola*
Rhodniini	*Rhodnius*
	Pasmmolestes
Triatomini	*Dipetalogaster*
	Eratyrus
	Hermanlentia
	Linshcosteus
	Meccus
	Mepraia
	Nesotriatoma
	Panstrongylus
	Paratriatoma
	Triatoma
	Paleotriaoma

A identificação morfológica das espécies dos gêneros *Panstrongylus* e *Triatoma* é feita facilmente por meio da literatura pertinente, porém quando se trata do gênero *Rhodnius* a sua caracterização específica traz mais dificuldade. Espécies como *R. brethesi* (Matta, 1919) e *R. pictipes* (Stal, 1972) determinam menor dificuldade para identificação fenotípica, porém espécies mais próximas fenotipicamente e filogeneticamente como *R. neglectus* (Lent, 1954), *R. prolixus* (Stal,1959) e *R. robustus* (Larrousse, 1927) exemplo de espécies que compõem o grupo *prolixus* requerem um maior cuidado na identificação por meio de chave entomológica (JUSTI; GALVÃO, 2017; ROSA et al., 2014).

LENT e JUBERG (1969), publicaram um importante estudo que caracterizou a genitália masculina e tornou-se uma metodologia muito utilizada para diferenciar as espécies mais próximas do gênero *Rhodnius*, principalmente pelo processo mediano do pigóforo, estrutura quitinizada que é constituinte do falo. Entretanto, para observar as estruturas do aparelho reprodutor masculino é necessário conhecimento prévio de dissecação. Assim, devido à dificuldade em algumas metodologias para comparação das espécies, novas técnicas que possibilitem a fácil identificação do gênero *Rhodnius* são buscadas constantemente (CARCAVALLO et al., 2000).

Rhodnius neglectus e *R. prolixus* possuem muitas características morfológicas similares, mas acredita-se que *R. prolixus* está presente apenas no norte da Amazônia e os registros realizados pelas secretarias estaduais de saúde na região Centro-Oeste devem ter sido erros de identificação, provavelmente com *R. neglectus* (GALVÃO, 2014).

O estudo das características do exocório e do opérculo de ovos de dez espécies do gênero *Rhodnius* possibilitou o desenvolvimento de uma chave de identificação, inclusive para *R. neglectus* e *R. prolixus* (BARATA, 1981). Outras espécies de *Rhodnius* também foram estudadas por essa metodologia como *R. colombiensis* (Stal, 1859), *R. milesi* (Carcavallo, Rocha, Galvão & Jurberg, 2001) e *R. stali* (Stal, 1872) (SANTOS et al., 2009). Os caracteres morfológicos de ovos de *Rhodnius* também foram utilizados para a descrição da então espécies novas: *R. montenegrensis* (ROSA et al.,2012) e *R. marabaensis* (DOS SANTOS SOUZA et al., 2016).

Outra metodologia utilizada para a diferenciações das espécies são os caracteres da genitália externa feminina, uma metodologia que tem auxiliado a

identificação das espécies *Rhodnius*, por não precisar de processos laboratoriais mais laboriosos que demandam bastante tempo e alto custo (ROSA et al., 2014). Essa metodologia complementa o estudo da genitália masculina de *Rhodnius* que requer trabalho de maior atenção (LENT; JURBERG, 1969).

A morfologia externa é amplamente estudada e utilizada para diferenciação das espécies, porém vale ressaltar a importância da morfologia interna, tanto na compreensão da fisiologia do inseto como na busca de caracteres válidos para diferenciação morfológica. Pesquisadores como Whigglesworth, Pérez, Khalifa, Lacombe, foram importantíssimos entre as décadas de 30 e 60 para auxiliar a compreensão da fisiologia dos triatomíneos (DAVEY, 1959; KHALIFA, 1950; LACOMBE, 1957; PÉREZ, 1969; WIGGLESWORTH, 1931, 1952). Os estudos a respeito de morfologia interna dos triatomíneos se manteve quiescente por um tempo, porém recentemente alguns trabalhos sobre a constituição do espermatóforo e sua morfologia foram publicados, assim como trabalhos a respeito das espermatecas de triatomíneos (NASCIMENTO et al., 2017; PEREIRA-LOURENÇO; SANTOS-MALLET; FREITAS, 2013).

Os triatomíneos machos, diferente de outras espécies de Hemiptera, liberam uma cápsula de mucoproteína denominada espermatóforo isso faz com que não depositem os espermatozoides direto na espermateca da fêmea. O espermatóforo possui morfologia complementar a vagina, semelhante ao processo chave fechadura, fato que impede a fecundação por machos de outras espécies (DAVEY, 1959; GULLAN; CRANSTON, 2010; KHALIFA, 1950).

As glândulas acessórias são responsáveis por produzir substâncias que auxiliam a proteção, armazenamento e ativação dos espermatozoides, essas substâncias são liberadas e compõem o espermatóforo no momento da cópula. O espermatóforo de *Triatoma infestans* (Klug, 1834) é ovoide e possui uma secreção completamente translucida, enquanto que *R. neglectus* tem o espermatóforo em formato de bastão com a região posterior opaca. A morfologia do espermatóforo é completamente diferente nas duas espécies estudadas, fato que demonstra que esse caráter pode ser utilizado como parâmetro de distinção a nível de gênero (PEREIRA-LOURENÇO; SANTOS-MALLET; FREITAS, 2013).

Ainda não é muito clara a forma como os espermatozoides são armazenados nas espermatecas dos Hemíptera, principalmente como resistem a longos períodos de tempo e mantem a viabilidade mesmo depois de anos armazenados (DEN BOER; BOOMSMA; BAER, 2009). Em triatomíneos, as espermatecas são circundadas por ramos de traqueias e delicadas concentrações de corpo adiposo, por isso, a nutrição e manutenção durante longos períodos de armazenamento dos espermatozoides na espermateca estão diretamente relacionados com as traqueias e os corpos adiposos. As traqueias também estão infiltradas nos músculos do oviduto comum, provavelmente para realizar as trocas de oxigênio e suprir a necessidade do tecido (NASCIMENTO et al., 2019).

Os triatomíneos são facilmente diferenciados por meio de algumas características já estabelecidas (ROSA et al., 2014; LENT; JURBERG, 1969; LENT; WYGODZINSKY, 1979), mas alguns táxons requerem análises mais detalhadas, que identifiquem caracteres não perceptíveis por distintas modalidades de microscopia, é o caso das espécies afins, por isso com o desenvolvimento da biologia molecular, os marcadores moleculares se tornaram imprescindíveis, principalmente para auxiliar na resolução sistemática (HYPSA et al., 2002).

Diversos estudos moleculares revolucionaram a forma de associação e relação das espécies dessa subfamília, os marcadores moleculares como COI, Ctyb, COII, 16S, 28S e 18S (MENDONÇA et al., 2009; GARDIM et al., 2014; JUSTI et al., 2014) são alguns do mais utilizados para o estudo desses reduvídeos, além de serem utilizados para validação de novas espécies da subfamília Triatominae (ABAD-FRANCH et al., 2013; GONÇALVES et al., 2013; JURBERG et al., 2013; ROSA et al., 2012).

A origem da subfamília Triatominae ainda é bastante debatida, um dos primeiros estudos que abordam a origem polifilética do grupo foi realizado em 1988, trabalho que trouxe a hipótese de que os triatomíneos do velho mundo teriam evoluído a partir de duas linhagens, a primeira linhagem teria sido derivada de um ancestral comum de *T. rubrofasciata*, uma espécie originada no novo mundo e introduzida posteriormente no velho mundo, e a segunda linhagem representada por espécies do gênero *Linshcosteus* uma linhagem autóctone da Índia (SCHOFIELD, 1988). A teoria polifilética foi testada em 2002 por análise cladística molecular com 57 espécies, o resultado desse estudo mostrou que os gêneros *Triatoma* e *Linshcosteus* são grupos irmãos e consequentemente a origem da subfamília é monofilética (HYPSA et al., 2002).

A partir do impasse, trabalhos com maiores amostragens e diferentes abordagens proporcionaram uma melhor visão da origem da subfamília Triatominae. Em 2014, foi publicado um estudo com 104 espécimes de 54 espécies da tribo Triatomini e 10 espécies da tribo Rhodnini, vale ressaltar a importância do trabalho pois além de uma amostragem robusta também utilizou todos os gêneros da tribo Triatomini. Como resultado esse trabalho evidenciou a origem monofilética da subfamília (JUSTI et al., 2014).

A constatação sobre a origem do grupo ainda traz dificuldades, uma vez que ao se retirar ou introduzir alguns táxons as relações filogenéticas são alteradas. Apesar da dificuldade sabe-se que as mudanças climáticas e geológicas interferem diretamente na relação dos clados como é o caso da diversificação das tribos Rhodnini e Triatomini (JUSTI; GALVÃO, 2017). A concepção sobre a origem da subfamília Triatominae ainda continua em investigação, porém atualmente a origem monofilética é mais aceita, os últimos trabalhos com grandes amostragens sustentam a teoria até a o momento (JUSTI et al., 2014; JUSTI; GALVÃO, 2017; MONTEIRO et al., 2018).

A identificação específica é necessária para auxiliar ações e programas de controle nas regiões onde o ciclo da doença de Chagas é ativo, pois os vetores ainda são os maiores disseminadores da doença pela América Latina (JANSEN et al., 2019).

1.3 *Rhodnius neglectus* Lent, 1954

A tribo Rhodniini que compreende os gêneros *Psammolestes* e *Rhodnius* possuem como característica a adesão dos ovos aos substratos, mecanismo evolutivo que facilita a dispersão passiva das espécies, uma vez que ovos e ninfas podem ser transportados entre as penas dos pássaros e pelos de pequenos mamíferos (GALVÃO, 2014). A espécie *R. neglectus* está geralmente associada as palmeiras dos gêneros *Attalea* (popularmente conhecida como Pindoba), *Acrocomia* (Bocaíuva) e *Mauritia* (Buriti), podem ser encontradas em ninhos de pássaros da família Furnariidae e de mamíferos *Didelphis* (GURGEL-GONÇALVES; CUBA, 2007). Foi relatado a simpatria de *R. neglectus* com *Psammolestes tertius* (Lent & Jurberg, 1965) em palmeiras *M. flexuosa* nos ninhos de *Phacellodomus ruber* (Vieillot, 1817), o popular graveteiro (GURGEL-GONÇALVES et al., 2004).

A hipótese que padrão de coloração dos triatomíneos são influenciados pelo *habitat* foi levantada, principalmente para o gênero *Rhodnius* devido a sua relação com palmeiras (GAUNT; MILES, 2000). A espécie *R. nasutus* (Stal, 1859) com coloração marrom amarelado, ligeiramente alaranjado como descrita inicialmente, tem variação de coloração nos exemplares coletados em *Copernicia prunifera* (Carnaúba), ou seja, essa espécie possui coloração variável de acordo com a espécie de palmeira que habita. Ao comparar os exemplares de *R. nasutus* coletados em cinco espécies de palmeiras foram encontrados seis padrões de variação cromática (DIAS et al., 2008).

A distribuição de *R. nasutus* no nordeste do Brasil é sobreposta em algumas regiões por *R. neglectus*. Alguns exemplares de ambas as espécies provenientes de áreas de transição do cerrado para a caatinga apresentam variação em seu padrão cromático. Acredita-se que houve seleção natural do padrão cromático mais claro para espécimes de *R. nasutus* e *R. neglectus* que habitam a palmeira *C. prunifera* devido ao encontro dos exemplares camuflados no substrato claro das fibras da palmeira (ANTÔNIO, 2016; GURGEL-GONÇALVES, 2008).

O epíteto específico *neglectus* significa negligenciado, devido ao fato da espécie ter permanecida desconhecida por muito tempo provavelmente por sua colonização em palmeiras (BARRETO-SANTANA et al., 2011; GALVÃO, 2014), é a espécie do gênero *Rhodnius* mais distribuída pelo país, está presente em onze estados e no distrito federal e mantém o ciclo reprodutivo contínuo durante o ano, mas possui maior intensidade durante o verão, a estação chuvosa (GURGEL-GONÇALVES; CUBA, 2007).

Apesar de estar associada a ambientes silvestres, as espécies do gênero *Rhodnius* invadem esporadicamente residências a partir de colônias estabelecidas nas palmeiras, fator que aumenta consideravelmente o risco de transmissão vetorial domiciliar sem colonização. Do gênero *Rhodnius* a espécie com maior distribuição geográfica no Brasil é *R. neglectus* que ocorre com maior frequência no cerrado, mas pode ser encontrada em várias outras regiões. Em 2010, foram encontrados 16 espécimes de *R. neglectus* no peri e intradomicílio no Piauí (GURGEL-GONÇALVES et al., 2010).

O encontro de 72 espécimes *R. neglectus* na palmeira *Livistona australis* na cidade de Monte Alto/SP foi um alerta para a comunidade científica, primeiro pelo relato de triatomíneos invadindo domicílios na região central e comercial da cidade e segundo

pela inédita relação do vetor da doença de Chagas com essa palmeira, fato que destaca que o cenário epidemiológico para a doença de Chagas não é estático e a facilidade da dispersão de *R. neglectus* antes estritamente silvestres e agora encontrado em centros urbanos demonstra a migração das espécies ambiente silvestres para urbanos (CARVALHO et al., 2014).

Os vetores disseminados no ambiente silvestre são responsáveis por manter o ciclo epidemiológico silvestre e também estão associados aos surtos por alimentos contaminados como açaí, caldo de cana, suco de goiaba entre outros (SHIKANAI-YASUDA; CARVALHO, 2012). No Brasil, os surtos com infecção oral são preocupantes principalmente na região Norte do país local onde foram registrados 91% dos casos entre 2007 e 2013, entre os meses de agosto a novembro período que coincide com a safra do Açaí no estado do Pará. Esse estado registrou nesse período 75% dos casos de doenças no país (SAÚDE, 2015).

A preocupação com a disseminação da doença na região norte do país é antiga, trabalhos como Lainson; Shaw; Fralha, 1979; Miles et al., 1981 destacam os diferentes grupos de *T. cruzi* no estado no Pará desde a década de 70. Espécies de triatomíneos como *Panstrongylus lignarius*, *P. geniculatus*, *R. pictipes*, *R. paranaensis* e *Eratyrus mucronatus* foram vetores encontrados infectados com *T. cruzi* naquela época como relatado por Miles et al., 1981. No período de 2007 a 2011 a taxa de infecção natural com *T. cruzi* para *R. neglectus* foi de 3,8%, essa porcentagem se torna preocupante quando associamos com a capacidade de dispersão passiva e ampla distribuição geográfica da espécie.

2 JUSTIFICATIVA

A subfamília Triatominae possui importância epidemiológica inegável na cadeia epidemiológica da doença de Chagas, apesar de todo avanço nos estudos desses vetores falta muito para o encontro de soluções que reduzam de forma efetiva a participação dos triatomíneos no ciclo epidemiológico dessa zoonose. Por sua vez o gênero *Rhodnius* que tem ampla importância epidemiológica, a maioria das espécies de tem por *habitat* copas de palmeiras, principalmente aquelas em que as folhas mortas ficam aderidas ao caule por propiciarem o abrigo para roedores, aves e pequenos animais, que se constituem em fontes alimentares para triatomíneos. Em consequência os pequenos animais, entre os quais roedores podem se portadores de *T. cruzi* ou *T. rangeli*. Por sua

vez são conhecidas 20 espécies de *Rhodnius*, que tem como uma de suas características biológicas aderir os ovos ao substrato, de modo que uma das maneiras de dispersão pode ser pela adesão de seus ovos aos pelos de animais e penas de aves.

Na atualidade a transmissão oral da doença de Chagas é comprovada por vários autores, desse modo se constitui na principal via de infecção de *T. cruzi* ao homem e fundamentalmente na região Norte do Brasil, onde o consumo de açaí é um hábito cultural.

Por compreender a complexidade dos triatomíneos e saber da necessidade de estudos que auxiliem a compreensão das características fisiológicas, reprodutivas, relações filogenéticas dos gêneros e espécies, interações parasito-vetor, entre outros, foi desenvolvido o estudo de *Rhodnius taquarussuensis* sinônimo júnior de *R. neglectus* com o intuito de identificar a espécie, esclarecer a filogenia e identificar algumas proteínas relacionadas à reprodução.

Este estudo compreende três trabalhos distintos com a finalidade de auxiliar a melhor compreensão do gênero *Rhodnius*, constituindo-se em tentativa de esclarecer aspectos filogenéticos e proteômico. Devido às distintas características de cada linha do estudo, a tese é apresentada em forma de capítulos.

No primeiro capítulo, foram conduzidos estudos morfológicos, morfométricos e citogenéticos, que resultaram na publicação de uma nova espécie: *Rhodnius taquarussuensis* sp. n. Essa espécie foi descrita a partir de espécimes criados em laboratório e oriundos de uma fêmea de *Rhodnius* sp. coletada em 10/11/2010, no município de Taquarussu, no estado de Mato Grosso do Sul.

No segundo capítulo, consta um artigo publicado que foi resultante de estudos filogenéticos de *R. taquarussuensis*, *R. neglectus* e espécies do grupo *prolixus*, que levaram a invalidação de *R. taquarussuensis* sp. n.

No terceiro capítulo, o propósito foi investigar as proteínas relacionadas às espermatecas fecundadas de *R. neglectus*, com o intuito de identificar proteínas presentes no armazenamento dos espermatozoides, as classes proteicas e as funções que desempenham.

3 OBJETIVOS

3.1 Objetivo geral

Estudar os caracteres morfológicos, citogenéticos e filogenéticos de *Rhodnius* sp., assim como estudar o perfil proteico das espermatecas fecundadas de *R. neglectus*.

3.2 Objetivos específicos

- Realizar estudos morfológicos, morfométricos e citogenéticos de *Rhodnius* sp.
- Verificar a distribuição da variação de dois genes mitocondriais: *Cytochrome b* (CYTB) e *Mitochondrially Encoded NADH Dehydrogenase 4* (ND4) em *Rhodnius* sp.
- Verificar a distribuição da variação de quatro genes nucleares: *Putative chitin binding peritrophin-a* (PCB), *DNA topoisomerase* (TOPO), *Uroporphyrinogen decarboxylase* (URO) e *Toll-Like-2. Transmembrane receptor with TIR domain binding* (ZNFP)em *Rhodnius* sp.
- Identificar proteínas expressas nas espermatecas de fêmeas fecundadas de *R. neglectus.*

4 RESULTADOS

4.1 Capítulo I:

A new species of Rhodnius *from Brazil (Hemiptera, Reduviidae, Triatominae).* **ZOOKEYS.** v 675, pag 1-25 (2017).
doi: 10.3897/zookeys.675.12024

ZooKeys 675: 1–25 (2017)
doi: 10.3897/zookeys.675.12024
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

A new species of *Rhodnius* from Brazil (Hemiptera, Reduviidae, Triatominae)

João Aristeu da Rosa[1], Hernany Henrique Garcia Justino[2], Juliana Damieli Nascimento[3], Vagner José Mendonça[4], Claudia Solano Rocha[1], Danila Blanco de Carvalho[1], Rossana Falcone[1], Maria Tercília Vilela de Azeredo-Oliveira[5], Kaio Cesar Chaboli Alevi[5], Jader de Oliveira[1]

1 *Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Araraquara, SP, Brasil* **2** *Departamento de Vigilância em Saúde, Prefeitura Municipal de Paulínia, SP, Brasil* **3** *Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil* **4** *Departamento de Parasitologia e Imunologia, Universidade Federal do Piauí (UFPI), Teresina, PI, Brasil* **5** *Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), São José do Rio Preto, SP, Brasil*

Corresponding author: *João Aristeu da Rosa* (joaoaristeu@gmail.com)

Academic editor: *G. Zhang* | Received 31 January 2017 | Accepted 30 March 2017 | Published 18 May 2017

http://zoobank.org/73FB6D53-47AC-4FF7-A345-3C19BFF86868

Citation: Rosa JA, Justino HHG, Nascimento JD, Mendonça VJ, Rocha CS, Carvalho DB, Falcone R, Azeredo-Oliveira MTV, Alevi KCC, Oliveira J (2017) A new species of *Rhodnius* from Brazil (Hemiptera, Reduviidae, Triatominae). ZooKeys 675: 1–25. https://doi.org/10.3897/zookeys.675.12024

Abstract

A colony was formed from eggs of a *Rhodnius* sp. female collected in Taquarussu, Mato Grosso do Sul, Brazil, and its specimens were used to describe *R. taquarussuensis* **sp. n.** This species is similar to *R. neglectus*, but distinct characters were observed on the head, thorax, abdomen, female external genitalia and male genitalia. Chromosomal differences between the two species were also established.

Keywords

Brazil, cytotaxonomy, new species, *Rhodnius*, taxonomy, Triatominae

Introduction

In the subfamily Triatominae, the genera *Panstrongylus* (15 species), *Triatoma* (74 species) and *Rhodnius* (20 species) are of particular epidemiological importance, although the other 15 genera (containing 43 species) can also transmit *Trypanosoma cruzi*, which

is the etiological agent of Chagas disease (Poinar 2013, Galvão 2014, Mendonça et al. 2016, Souza et al. 2016). Among the 152 species within the subfamily there are two fossils: *T. dominicana* Poinar, 2005 and *P. hispaniolae* Poinar, 2013.

The first two species identified as belonging to the genus *Rhodnius* were described by Stal (1859): *R. nasutus* and *R. prolixus*. From that year until 1979, a total of 12 species were identified (Lent and Wygodzinsky 1979). In 2003, Galvão et al. considered 16 valid species. The 17th, 18th, 19th and 20th species in that genus were respectively *R. zeledoni* Jurberg et al. 2009; *R. montenegrensis* Rosa et al. 2012; *R. barretti* Abad-Franch et al. 2013, and *R. marabaensis* Souza et al. 2016.

Most *Rhodnius* species live in palm trees, and several cases of transmission of Chagas disease have been associated with the consumption of açaí containing feces of triatomines infected by *T. cruzi* (Ferreira, Branquinho & Leite, 2014; Ministério da Saúde, Brasil, 2017). Apart from such cases, which occur more frequently in the northern region of the country, it is worth noting that *R. neglectus* was found in palm trees (species of *Roystonea*, *Syagrus* and *Acrocomia*] in the city of Araçatuba, São Paulo, in 2009, as well as in palm trees (*Livistona australis*) located in the central square of the city of Monte Alto, São Paulo, in February 2012 (Rodrigues et al. 2014, Carvalho et al. 2013, respectively).

Based on morphological, morphometric and cytogenetic characters, this paper describes *R. taquarussuensis* sp. n., which is similar to *R. neglectus*. The first collected specimen of *R. taquarussuensis* was a female that invaded a domicile and laid eight eggs. The colony formed from those eggs resulted in the specimens used in this description.

Materials and methods

Morphological identification and description

On 10 November 2010 a female of *Rhodnius* sp. invaded a rural dwelling (22°29'07.7"S; 53°21'08.9'W) in the city of Taquarussu, Mato Grosso do Sul, Brazil, and was captured (Fig. 1). That specimen remained alive for a few days and laid eight eggs (Fig. 2). By means of macroscopic identification and subsequent optical microscopy (OM) and using the key of Lent and Wygodzinsky (1979), a clear similarity with *R. neglectus* was noticed. In view of that, all characters observed and documented for *Rhodnius* sp. were checked for *R. neglectus* CTA 229, which is a colony that has been kept since June 27, 2011 at the Triatominae Insectarium of the Faculty of Pharmaceutical Sciences, UNESP, Araraquara (FCFAR/UNESP). The temperature, humidity and light cycle conditions are not controlled due to the insect's biodiversity, but these parameters are measured daily, varying the temperature between 20-35ºC and humidity 50-80%. Insects kept in colonies are fed directly on ducks every 15 days and consists of specimens from the Brazilian National and International Triatominae Taxonomy Reference Laboratory at the Oswaldo Cruz Institute in Rio de Janeiro, Brazil (LNIRTT). The colony was kindly provided by Dr. José Jurberg and Dr. Cleber Galvão, and the specimens that originated it were collected in northern Formoso, Goiás, Brazil.

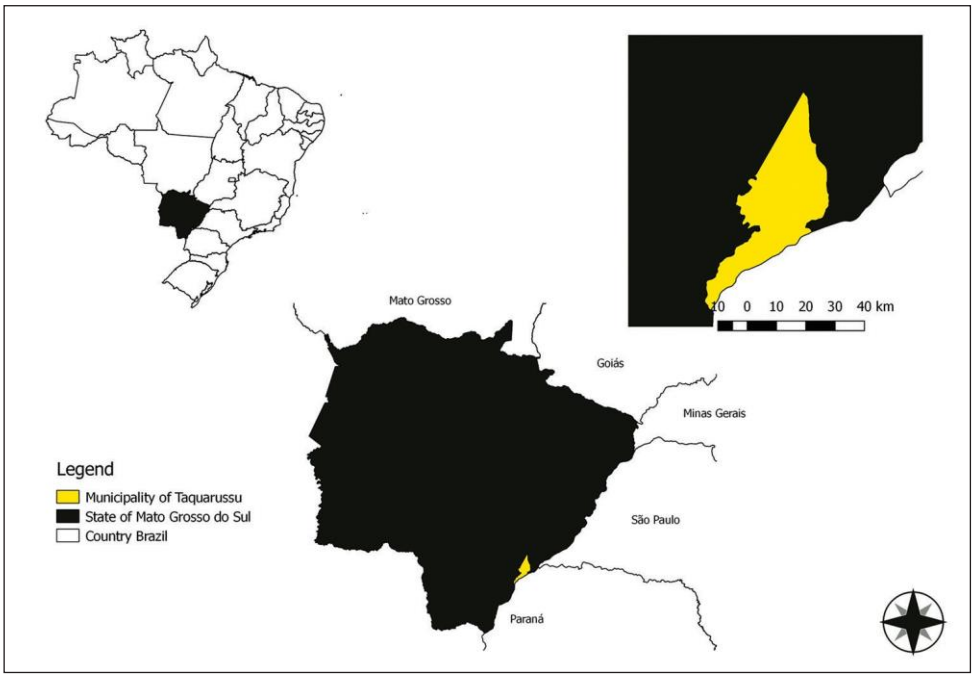


Figure 1. Localization of Taquarussu - MS where female of *R. taquarussuensis* sp. n. is collected (22°29'233"S, 053°21'107"W).

A colony was formed from the eight eggs laid by the *R. taquarussuensis* sp. n. female and identified as Araraquara Triatominae Colony (CTA) 277. The specimens of that colony were used to describe *R. taquarussuensis* sp. n.

Morphological study

The morphological study by OM and scanning electron microscopy (SEM) consisted of the observation of the head, thorax and abdomen of 30 adult females and 30 adult males, as well as 40 eggs of *R. taquarussuensis* sp. n. and the same number of specimens of *R. neglectus*, according to Barata (1981), Quintero (2003), Obara et al. (2007), Rosa et al. (2012), Rosa et al. (2014), Souza et al. (2016) (Figs 3–9).

Female external genitalia were observed from the dorsal, posterior, and ventral sides (Fig. 6) by SEM, according to Rosa et al. (2010). The study of the male genitalia was carried out by OM (Figs 8, 9), following a technique developed by Jader de Oliveira based on Gallati (2016). The denominations used were those defined by Lent and Jurberg (1969).

The Leica MZ APO stereoscope from the Faculty of Pharmaceutical Sciences, UNESP, Araraquara, and the scanning electron microscope Topcon SM-300 located in the Department of Physical Chemistry at the Chemistry Institute, UNESP, Araraquara, were used for observation and capture of images.

Table 1. Mean of measurement (mm) of 15 females and 15 males of *R. taquarussuensis* sp. n. and *R. neglectus*.

	Female		Male	
	R. taquarussuensis	*R.neglectus*	*R. taquarussuensis*	*R. neglectus*
TL	17,25	17,25	**15,24**	**15,96**
MLA	9,86	10,03	**8,41**	**9,05**
MLT	**5,00**	**4,11**	**4,54**	**3,79**
R1	**0,75**	**0,92**	**0,71**	**0,92**
R2	**3,38**	**3,58**	**3,16**	**3,59**
R3	**0,85**	**0,95**	**0,77**	**0,96**
HL	**4.44**	**5,81**	**4.20**	**5,24**
EO	**1.53**	**1,98**	**1.42**	**1,80**
IE	**0.61**	**0,77**	**0.54**	**0,66**
PO	**1.00**	**3,67**	**1.04**	**3,37**
AO	**2.62**	**0,90**	**2.44**	**0,91**
AT	**1.93**	**2,30**	**1.82**	**2,30**
SC	1,96	2,03	1,69	1,86
A1	0,45	0,55	0,29	0,59
A2	3,56	4,13	2,24	4,35
A3	2,03	2,43	1,28	2,50
A4	1,36	1,82	0,87	1,92
Eggs	*R. taquarussuensis*		*R. neglectus*	
TE	**1,72**		**1,62**	
OO	**0,49**		**0,52**	

*30 eggshells were used for each species.

TL, Total length of the triatomine; **MLA**, maximum length of the abdomen; **MLT**, maximum length of the thorax; **R1**, **R2** and **R3**, lengths of first, second, and third rostral segments, respectively; **HL**, head length; **EO**, external distance between ocelli; **IE**, inner distance between eyes; **PO**, postocular distance (excluding neck); **AO**, anteocular distance; **AT**, antenniferous tubercle; **SC**, Scutellum; **A1**, **A2**, **A3** and **A4**, 1st, 2nd, 3rd, and 4th left antennal segments, respectively; **TE**, Total egg length; **OO,** egg opercular opening. The values in bold were significant at $\alpha = 0.05$, using unpaired t-test.

Morphometric study

In the morphometric study by OM, 15 egg shells, 15 females and 15 males from the colony were measured, the same being done for *R. neglectus* CTA 229 (Table 1). The parameters measured were: total length, width of thorax and abdomen, length of the scutellum, three segments of the proboscis and four segments of the antenna, as well as five parameters of the head following Dujardin et al. (1999). Eggs had their length and the diameter of the opercular opening measured. The wings of *R. taquarussuensis* sp. n. and *R. neglectus* were studied by geometric morphometry using seven anatomical landmarks, according to parameters established by Gurgel-Gonçalves et al. (2008), as well as based on Rosa et al. (2012).

The observations and measurements were carried out on a Leica MZ APO stereoscope and the Motic Images Advanced System version 3.2.

Cytogenetic identification

In this study ten male specimens of *R. taquarussuensis* sp. n. were used for C and CMA_3/DAPI–banding analyses and ten male specimens of *R. neglectus* were used for CMA_3/DAPI–banding analyses. After being lacerated and placed on the slide, the seminiferous tubules underwent cytogenetic procedures following the C-banding (Sumner 1972) and CMA_3/DAPI-banding protocols [Schimid 1980, with modifications according to Severi-Aguiar et al. 2006]. C-banding was analyzed under a Jenaval (Zeiss) MO connected to a digital camera and the Axio Vision LE 4.8 image analyzer (Copyright ©2006-2009 Carl Zeiss Imaging Solutions Gmb H), whereas CMA_3/DAPI-banding was analyzed using Zeiss-Axioskop and Olympus BX-FLA fluorescence microscopes (FM).

Taxonomy

Family Reduviidae Latreille, 1807
Subfamily Triatominae Jeannel, 1919
Genus *Rhodnius* Stål, 1859

***Rhodnius taquarussuensis* sp. n.**
http://zoobank.org/16C7EE86-3C36-4BA9-BDFC-E914CC4C2F80
Figure 2

Holotype. BRAZIL: Mato Grosso do Sul: Taquarussu; Residence, 22°29'07.7"S; 53°21'08.9'W, 10 November 2010 H. E. G. Justino. UNESP (♀).

Paratypes. BRAZIL: Colony formed from eggs obtained from the holotype: Araraquara: Triatominae Insectarium of the Faculty of Pharmaceutical Sciences, Araraquara, January 3, 2017, J. A. da Rosa, UNESP (25♂25♀).

Additional paratypes. CTIOC - Collection of Triatomines of the Oswaldo Cruz Institute, Rio de Janeiro - Brazil (2♂2♀). Entomological Reference Collection of the Faculty of Public Health - USP, São Paulo -Brazil (1♂1♀). Collection of the Institute of Entomology of the Metropolitan University of Education Sciences (IEUMCE), Santiago - Chile (2♂2♀).

Etymology. The name *Rhodnius taquarussuensis* sp. n. was chosen because this species was found in the city of Taquarussu, Mato Grosso do Sul, Brazil.

Diagnosis. *Rhodnius taquarussuensis* sp. n. is close to *R. neglectus*, their differences being the color and a variety of morphological, morphometric and cytogenetic characters (Tables 1, 2). The general color of *R. taquarussuensis* sp. n. is brown, whereas *R. neglectus* is dark brown, almost black. This difference is particularly noticeable on the hind wings. The stridulatory sulcus of *R. taquarussuensis* sp. n. is brown at the base and black on the sides, whereas on *R. neglectus* it is completely black.

On the head, differences were noticed on the vertex, genae, antennae and triangular furrow of the first segment of the rostrum. The vertex of the head of *R. taquarussuensis*

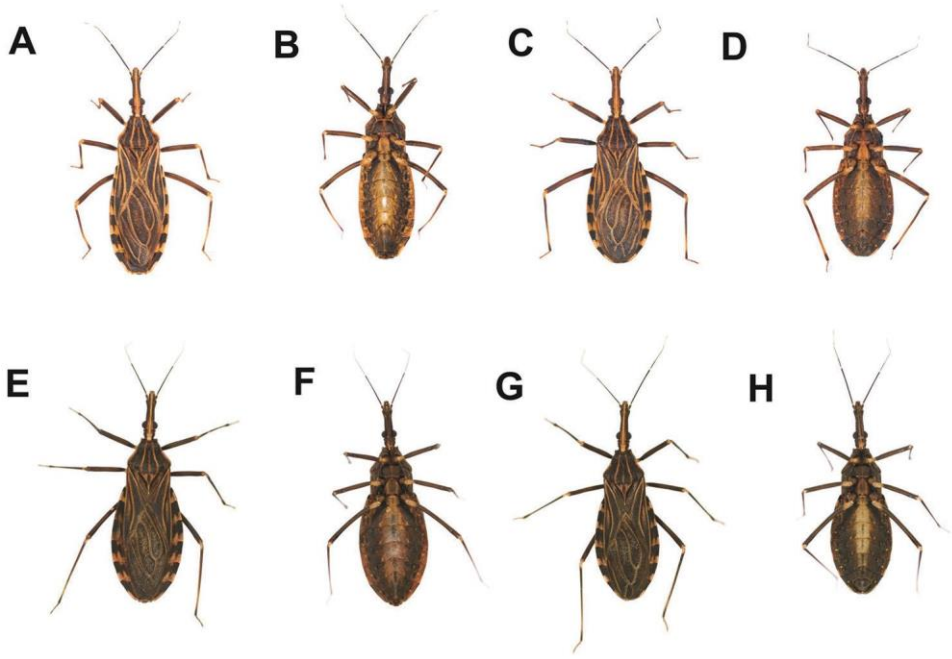


Figure 2. *R. taquarussuensis* sp. n. female **A** dorsal side **B** ventral side, *R. taquarussuensis* sp. n. male **C** dorsal side **D** ventral side, *R. neglectus* female **E** dorsal side **F** ventral side), *R. neglectus* male **G** dorsal side **H** ventral side.

sp. n. is quite visible, whereas on *R. neglectus* it is not (Fig. 3A, B, D, E). The genae of *R. taquarussuensis* sp. n. are longer than those of *R. neglectus* (Fig. 3A, D). On *R. taquarussuensis* sp. n. the 10th part of the second segment of the antenna is brown; on *R. neglectus*, though, only the basis has that color. The triangular furrow of the first segment of the rostrum, towards the second segment, ends in a filamentous way on *R. taquarussuensis* sp. n. and in a rounded way on *R. neglectus* (Fig. 3C, F). On the thorax, differences can be found on the pronotum, wings, scutellum, prosternum, mesosternum and metasternum (Figs 4, 5). The membranous portion of the hind wings is brown on *R. taquarussuensis* sp. n. and dark brown on *R. neglectus*. The scutellum ends in a rounded apex on *R. taquarussuensis* sp. n. and in a filamentous apex on *R. neglectus* (Fig. 4A, B). On *R. taquarussuensis* sp. n. the apex of the scutellum covers the final portion of the urotergite I process, while on *R. neglectus* the apex of the process of the urotergite I is perfectly visible (Fig. 4A, B). The lines limiting the stridulatory sulcus are straight on *R. taquarussuensis* sp. n. and narrowed in the anterior third on *R. neglectus* (Fig. 5A, B). On *R. taquarussuensis* sp. n. the basis of the stridulatory sulcus is brown and the sides are black, whereas on *R. neglectus* the entire stridulatory sulcus is black. The central region of the limit between the mesosternum and the metasternum is regular and half-moon shaped on *R. taquarussuensis* sp. n., while on *R. neglectus* it is pronounced and slightly irregular (Fig. 5C, D). The beginning of the metasternum is narrow on *R. taquarussuensis* sp. n.

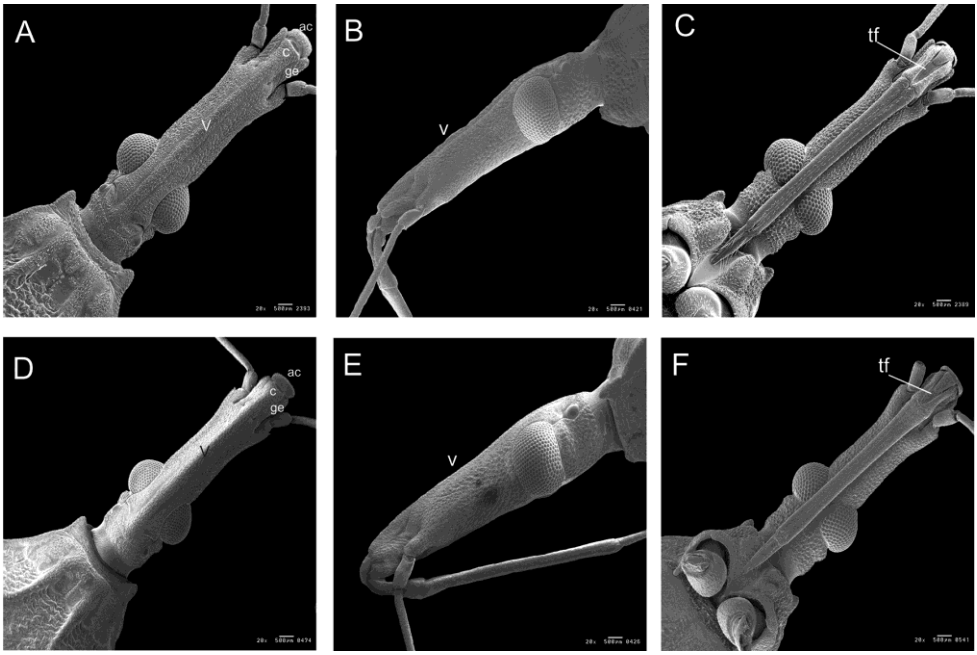


Figure 3. Head by SEM of *R. taquarussuensis* sp. n. **A** dorsal view **B** lateral view, **C** ventral view, *R. neglectus* **D** dorsal view **E** lateral view **F** ventral view. v: vertice, ge: gena, c: clypeus, ac: anteclypeus, tf: triangular furrow.

Table 2. Main distinguishing characters between *R.taquarussuensis* sp. n. and *R. neglectus*.

Distinguishing characters		**Species**	
		R.taquarussuensis	***R. neglectus***
Overall color		Brown	Dark brown
Genae		Lengthier longer	Longer
Vertex		Quite visible	Not visible
Ventral triangular furrow		Filamentous way	Rounded way
Scutellum		Covers the final portion of the urotergite I process	The apex of the process of the urotergite I is perfectly visible
Stridulatory sulcus		Straight	Waisted
Mesothorax		Half-moon shaped and regular	Pronounced and slightly irregular
Female external genitalia	Dorsal side	10^{th} segment presents a concavity in the middle portion	10^{th} segment is straight
	Posterior side	The limits of the 9^{th} segment with gonocoxite VIII are curve	The limits of the 9^{th} segment with gonocoxite VIII are straight
	Ventral side	There is a concavity in the external limit with the 10^{th} segment	There is a straight line in the external limit with the 10^{th} segment
Male genitalia	Phallothecal sclerite	Trapezoidal shape	Rounded shape
	Tip of parameres	Thinner	Thin
Heterochromatin in the autosomes		Present	Absent
CMA^+ in autosomes		Present	Absent

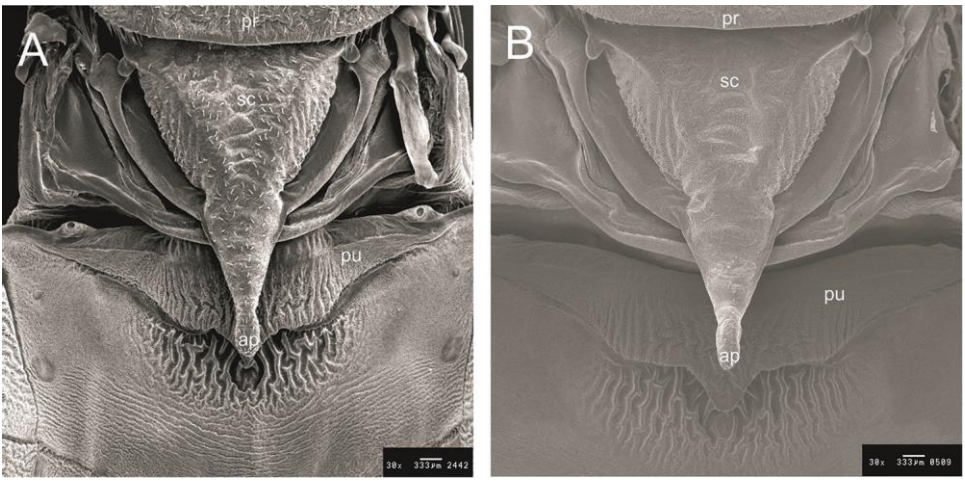


Figure 4. Escutellum and process of I urotergit by SEM. **A** *R. taquarussuensis* sp. n. **B** *R. neglectus*. pr: pronotum, sc: escutelum, pu: process of I urotergit, ap: apex of escutelum.

and wide on *R. neglectus* (Fig. 5C, D). The ventral abdomen of *R. taquarussuensis* sp. n. is light brown, and that of *R. neglectus* is dark brown (Fig. 2). The terminal portion of the paramere of the male genitalia of *R. taquarussuensis* sp. n. is thinner than that of *R. neglectus* (Fig. 9A, C). The dorsal phallothecal sclerite has a trapezoidal shape on *R. taquarussuensis* sp. n. and is rounded on *R. neglectus* (Fig. 8C, D). The external limit of the 10th segment of the dorsal side of the female external genitalia of *R. taquarussuensis* sp. n. presents a concavity in the middle portion, whereas on *R. neglectus* that limit is straight (Fig. 6A, B). From posterior view, the limits of the 9th segment with gonocoxite VIII are curve on *R. taquarussuensis* sp. n. and straight on *R. neglectus*, and the superior line limiting the 10th and 9th segments is straight on *R. taquarussuensis* sp. n. and curve on *R. neglectus* (Fig. 6C, D). In the ventral side of the female external genitalia of *R. taquarussuensis* sp. n. there is a concavity in the external limit with the 10th segment that is also noticed from dorsal view; on *R. neglectus* that limit is a straight line. From ventral view, the external limits of the 9th segment of the female external genitalia are curve on *R. taquarussuensis* sp. n. and straight on *R. neglectus* (Fig. 6E, F).

Among the 19 characters measured, 12 showed significant differences between *R. taquarussuensis* sp. n. and *R. neglectus* in both sexes and also the eggs of both species. Two characters showed differences only between males, and five characters did not show significant differences (Tables1, 2).

Description. A total of 15 adult females and 15 adult males of *R. taquarussuensis* sp. n. and *R. neglectus* were measured, as well as 30 eggs shells of both species. Such measurements are detailed in Table 1.

The head of *R. taquarussuensis* sp. n. has a prominent brown vertex contrasting with the black sides. The clypeus is well defined. The genae are large, visible and dark brown, moving towards the anteclypeus (Figs 2A, C, 3 A, B). The limits between the genae and the clypeus are brown.

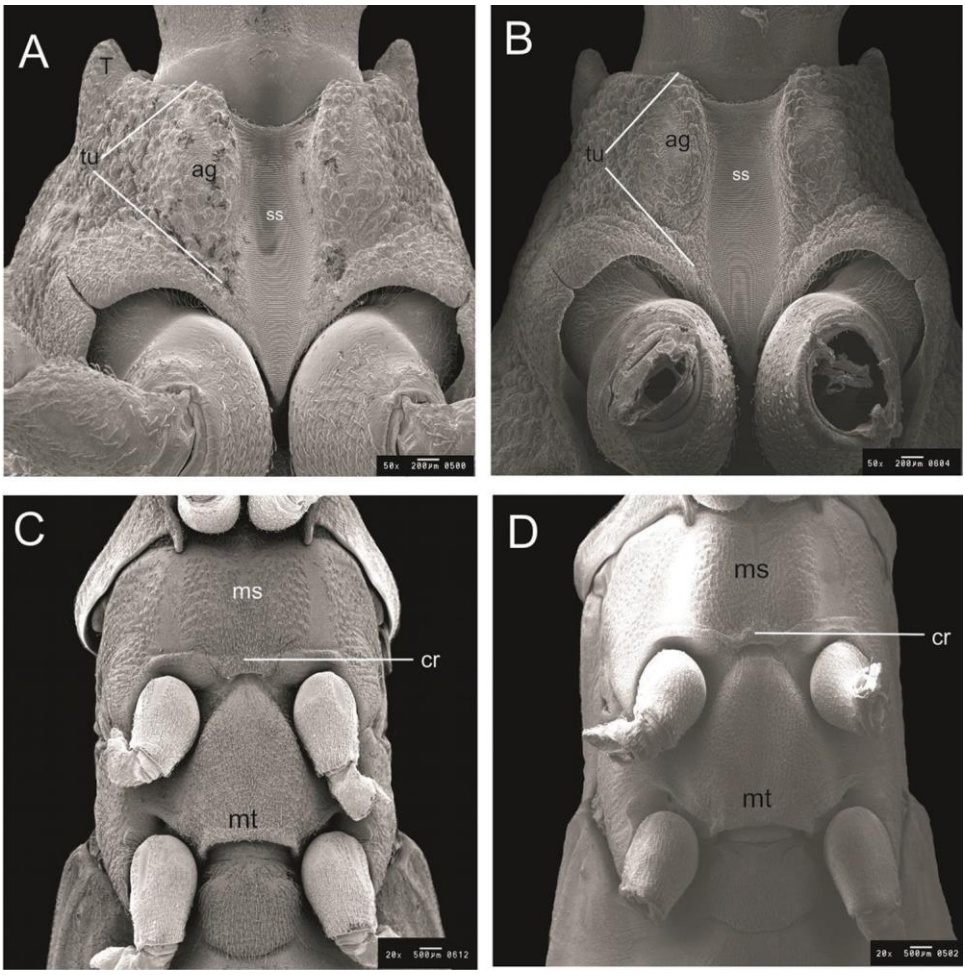


Figure 5. Thorax ventral by SEM. **A, C** *R. taquarussuensis* sp. n. **B, D** *R. neglectus*. ss: stridulatory sulcus, ms: mesosternum, mt: metasternum, tu: tubercle, ga: glabrous area, cr: central region.

The first segment of the antennae is black with mixes of brown. The articulation between the first and second segment of the antennae is brown. Roughly all the 10th part of the beginning of the second antennal segment is brown. The second segment is mostly black. In the articulation between the second and third antennal segment there is a black ring followed by a brown one. The beginning of the third segment (around 1/3) is black and the remaining portions (2/3) are brown. The articulation between the third and fourth antennal segment is brown. The beginning of the fourth segment is black and the remaining portions are brown with mixes of black (Fig. 2A, C).

The eyes are black and the ocelli are brown. The neck has a brown central dorsal strip flanked by two (1+1) black, narrower strips. The ventral portion of the neck between the ocelli is dark brown (Fig. 2A, B, C, D).

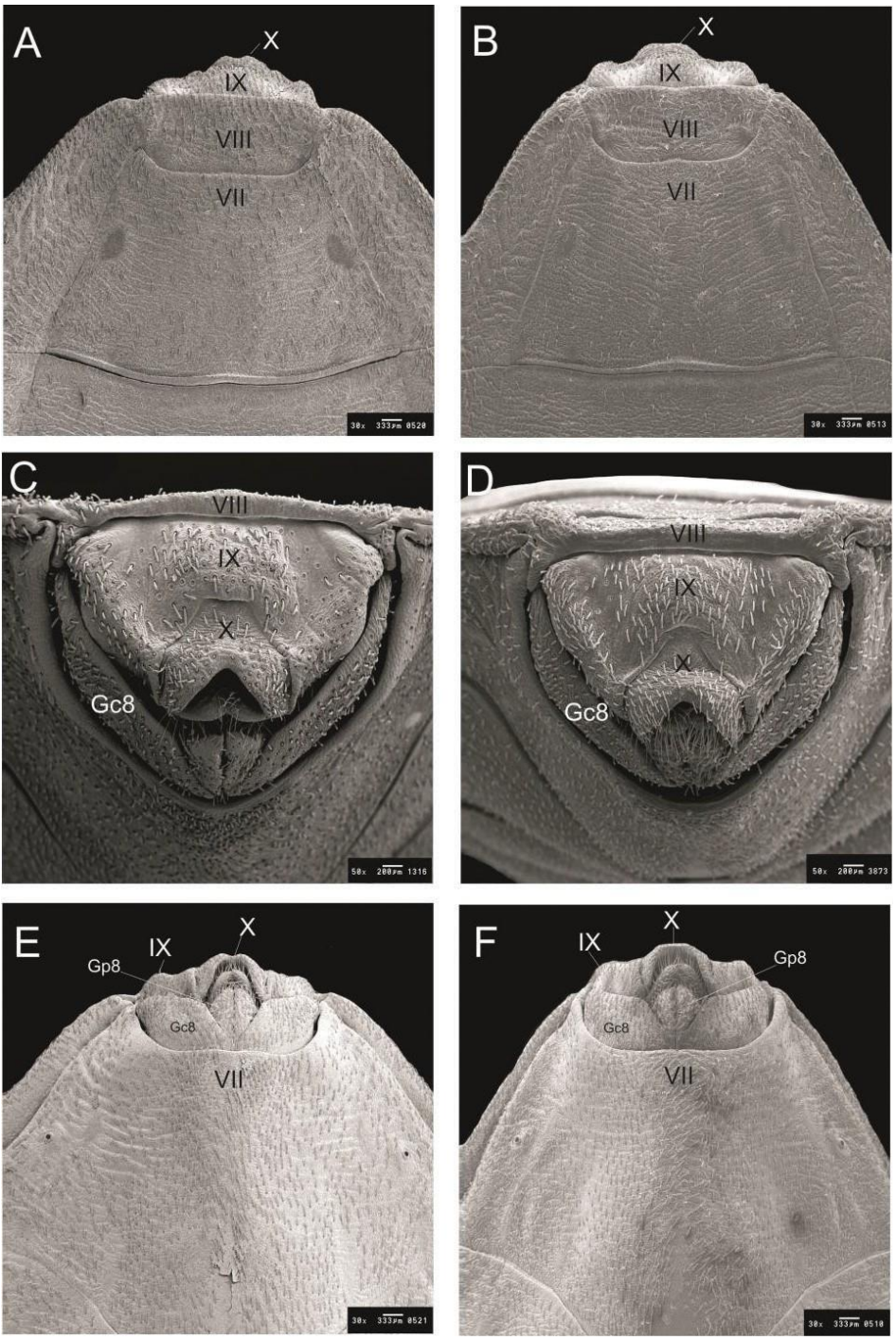


Figure 6. Female external genitalia by SEM *R. taquarussuensis* sp. n. **A** dorsal view, **C** posterior view **E** ventral view, *R. neglectus* **B** dorsal view **D** posterior view **F** ventral view. Gc 8: gonocoxite VIII; Gc 9: gonapophyse IX; Gp 8: gonapophyse VIII; VII, IX: tergites; X: segment.

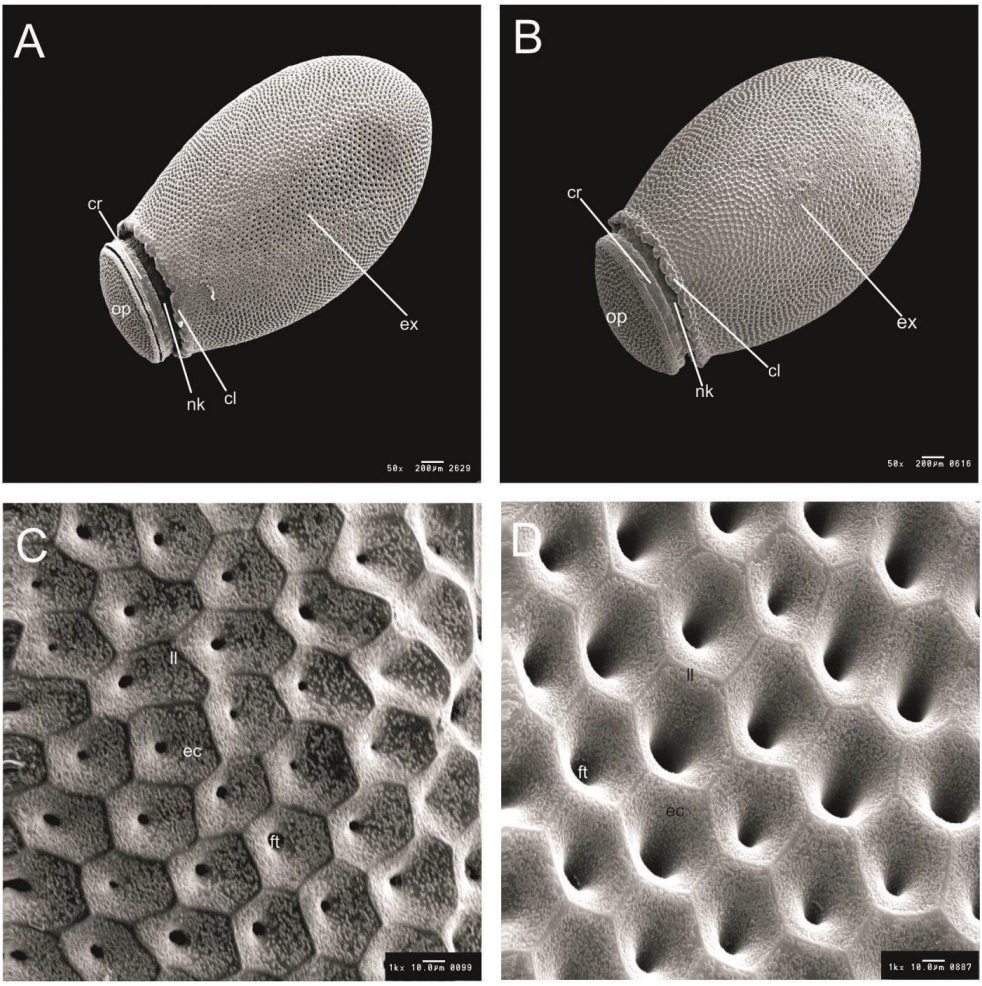


Figure 7. Eggs general vision and egg exochorium detail of *R. taquarussuensis* sp. n. (**A, C**), *R. neglectus* (**B, D**). cl: colar, cr: chorial rim, ex: exochorium, nk: neck, op: operculum, ec: exochorium cell, ft: follicular tubes, ll: limiting line.

The pronotum of the thorax of *R. taquarussuensis* sp. n. has a trapezoidal shape and is limited by a brown carina. In the antero posterior direction the pronotum has other two brown carina in the middle portion and six black strips. The three carina and the three brown strips are interspersed with the six black strips, which are larger. The collar (first portion of the pronotum) in the central part is brown and is followed by two (1+1) black glabrous areas and the two (1+1) antero lateral angles. The anterior portion of the pronotum consists of three anterior lobes which are clearly distinct from the posterior portion (hindlobe). Those three anterior lobes are limited by the carina and on each of them there are two black glabrous areas with a lengthy and irregular outline (Fig. 2A, C).

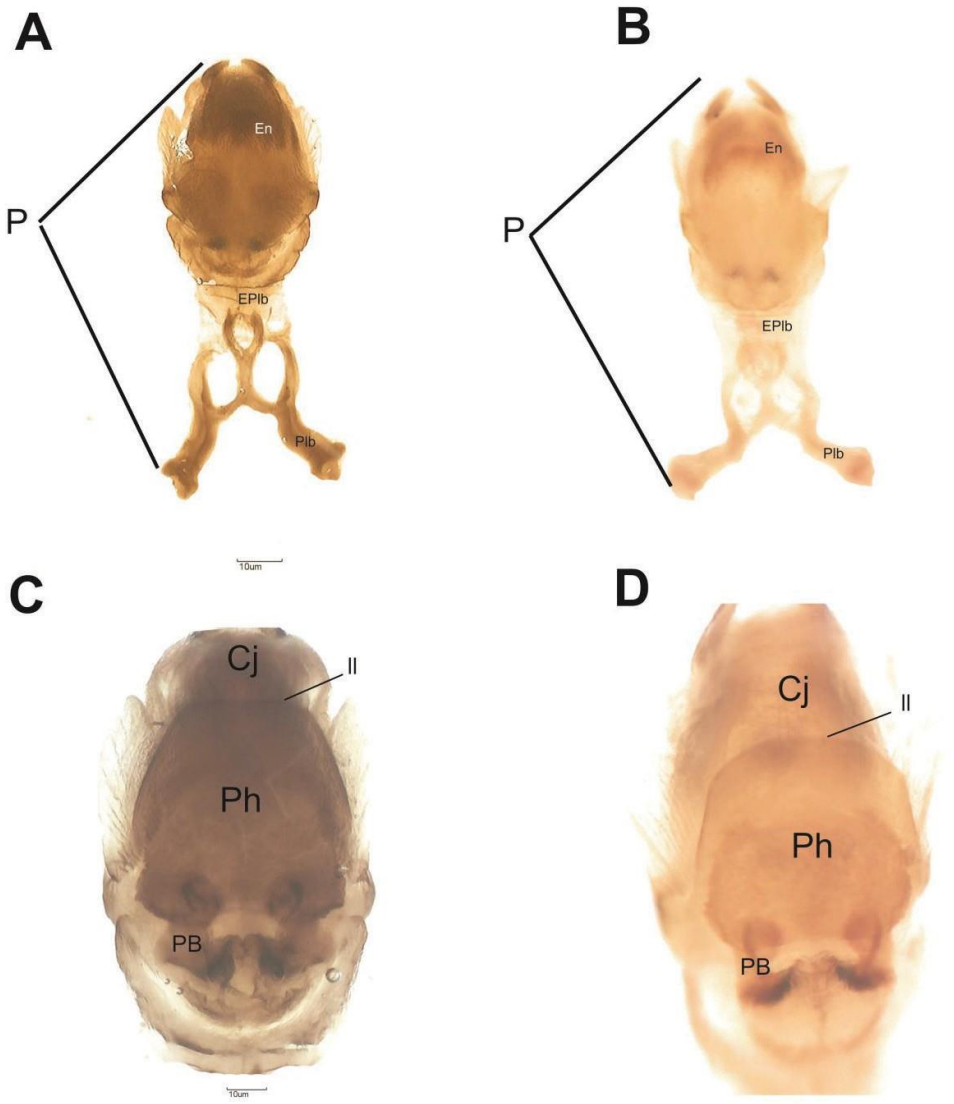


Figure 8. Phallus of *R. taquarussuensis* sp. n. **A** dorsal view **C** ventral view, *R. neglectus* **B** dorsal view **D** ventral view. Cj: conjunctive, En: endosome, EPlb: median extencion of basal plate, P: phallus, Plb: basal plate, PrG: gonopore process, PrPh: phallossoma process, Ph: phallosoma, PrCj: conjunctive process, ll: line limit.

The cuticle involving the veins of the hemelytron is light brown. The corium between the veins of the coriaceous region is dark brown, whereas that of the membrane is brown (Fig. 2A, C).

The prosternum contains the stridulatory sulcus, which moves along that segment in an antero-posterior direction, having a brown color in the background and black on

the sides. Two elongated tubercles limit the anterior half of the stridulatory sulcus. In the superior portion and in diagonal direction from the tubercles there are two black glabrous areas surrounded by a set of brown sensilla (Fig. 5A).

The mesosternum is limited anteriorly by the prosternum and posteriorly by the metasternum, both limits being brown. The central line dividing two dark brown elevations is also brown. Those two elevations are limited by two (1+ 1) black side glabrous areas diagonally placed. The central region of the posterior limit of the mesosternum has a half-moon shape. The metasternum is brown and resembles an isosceles triangle. Its anterior portion, i.e., its limit with the mesosternum, corresponds to the vertex of the triangle and is narrow, whereas its posterior portion, i.e., its limit with the first abdominal segment, corresponds to the basis of the triangle (Fig. 5C).

The three pairs of coxae are brown, except for the black glabrous areas. The trochanters of the anterior pair of legs are brown, but mixed with black glabrous areas. The middle and posterior pairs of trochanters are brown and have no glabrous areas. The three pairs of femora are black and the same color prevails in the three pairs of tibiae, except in the articulations with the femur and the spongy fossula, which are brown. The spongy fossulae are located in the first and second pairs of legs in the final portion of the tibia, alongside the articulations with the tarsi (Fig. 2A, B, C, D).

The abdomen of *R. taquarussuensis* sp. n. presents a brown color in the longitudinal central portion. On the sides of each segment there are (3+3) black glabrous areas, which are mixed with brown and black areas. The connexivum of the dorsal portion lies between the second and seventh segment. For each of those segments the anterior half is black and the posterior one is brown. The dorsal connexivum, also lying between the second and seventh segment, has a black color in 2/3 of the anterior portion, but that black color ends in an irregular way over the remaining 1/3, which is brown. Therefore, the black portion of the connexivum presents two edges moving towards the brown portion: one in the internal limit of the connexivum and the other in the middle portion. However, the connexivum of the second dorsal segment is black in the anterior half and brown in the posterior one, the limit between the portions having a diagonal shape. The seventh segment, on the other hand, is practically all black, except for a small brown strip located in the external posterior half. Type 1 sensilla, which prevail on the head, thorax and abdomen, have a brown color (Fig. 2B, D).

Male genitalia have the typical aspect of the genus *Rhodnius*. The median process of the pygophore (PrP) is short and triangular, but the base is broad and the sides are elongated with a thin edge. Parameres are hairy with a thin edge. From ventral view, the phallosome (Ph) has a broad plate whose superior region has a trapezoidal shape and occupies the middle region of the aedeagus. The support of the phallosome plate (PrPh) is broad. Conjunctival process I (PrcjI) is present and II (PrcjII) is absent. Endosomal process (En) is well-developed when seen from dorsal and ventral view (Figs 8A, C, 9B).

The dorsal side of the female external genitalia presents a concavity in the middle portion of the 10th segment. Seen from posterior view, the limits (1+1) of the 9th segment with gonocoxite VIII are curve, whereas the superior line limiting the 10th and 9th segments is straight. In the central portion of the 10th segment of the ventral side of

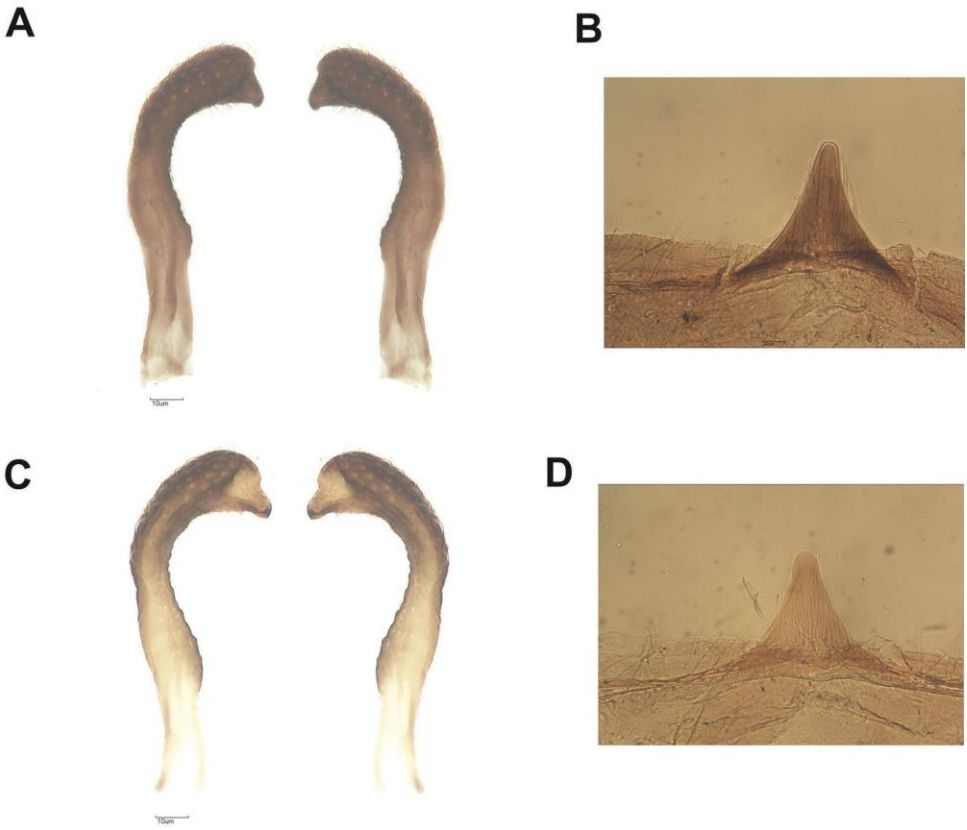


Figure 9. Parameres dorsal view of *R. taquarussuensis* sp. n. (**A**), Median process of the pygophore of *R. taquarussuensis* sp. n. (**B**) Parameres dorsal view of *R. neglectus* (**C**) Median process of the pygophore of *R. neglectus* (**D**).

the female external genitalia there is another concavity that can be noticed from dorsal view. The external limits (1+1) of the 9th segment of the female external genitalia are curve when seen from ventral view (Fig. 6A, C, E).

Egg shells of *R. taquarussuensis* sp. n. have a length of 1.72 mm and an opercular opening of 0.49 mm. They present lateral flattening, collar and exochorion cells, most with pentagonal or hexagonal shape (Fig. 7A, C).

Finally, although *R. taquarussuensis* sp. n. showed the same number of chromosomes as *R. neglectus* and all the tribe Rhodniini, i.e., 2n = 22 (Figure 11B), the constitutive heterochromatin pattern and the composition of the pairs of bases of DNA rich in AT and CG were completely different from *R. neglectus*, as the analysis of the nuclei of the initial prophases of *R. taquarussuensis* sp. n. has revealed a chromocenter consisting of sex chromosomes (arrow) and several heterochromatic blocks dispersed in the nucleus (Fig. 11A). The analysis of metaphase I of *R. taquarussuensis* sp. n. has demonstrated that this triatomine has heterochromatic blocks in both extremities of

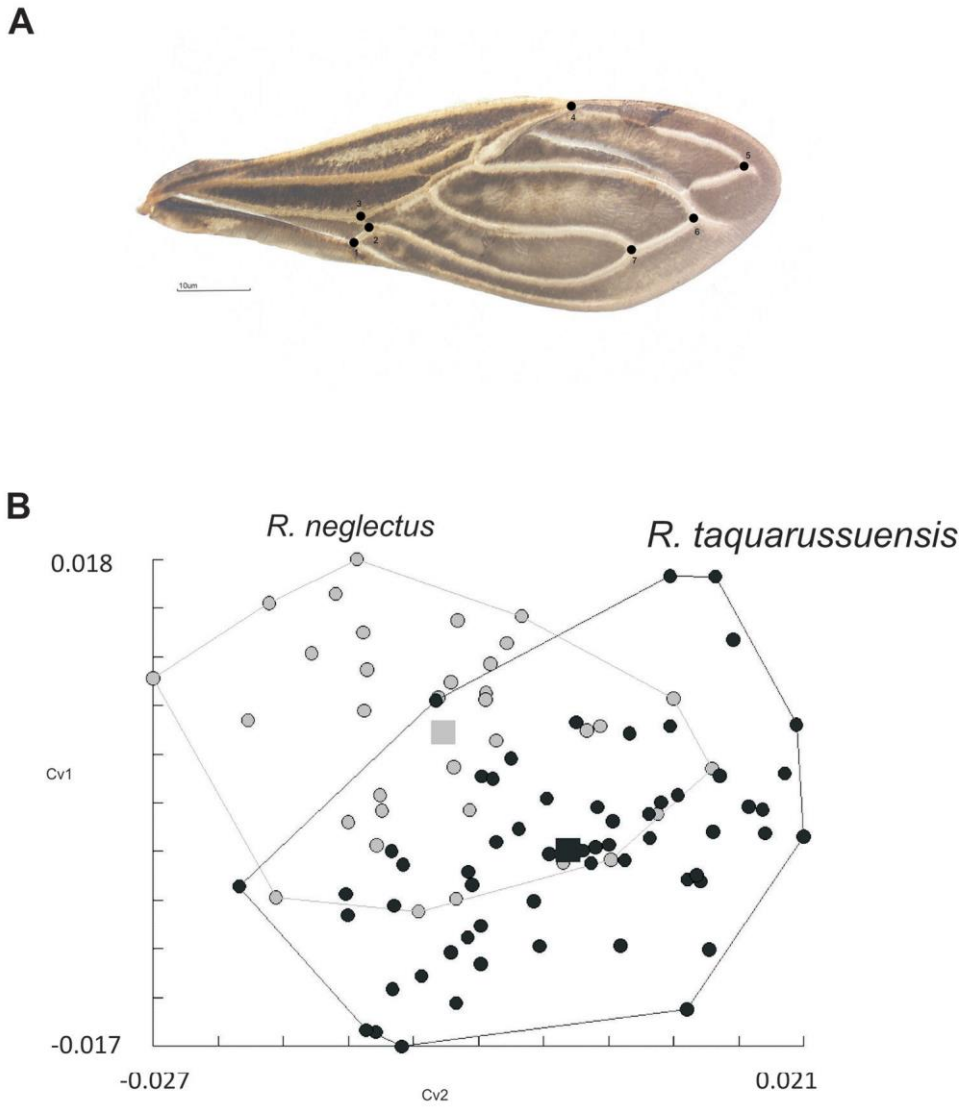


Figure 10. A Right wing of *R. taquarussuensis* sp. n. with the seven landmarks used in morphometric analysis. Following Bookstein (1990), all points correspond to type I landmarks (venation intersections) **B** Factorial maps in the plane of the two discriminant factors of wing shape variation (canonical variables 1 and 2, or CV1 and CV2) presenting the distribution of specimens of *R. taquarussuensis* sp. n. (Rta, black cicle) and *R. neglectus* (Rne, silver cicle).

practically all the autosomes and in the Y sex chromosome (Fig. 11B), unlike what has been recently stated for many populations of *R. neglectus* that do not present heterochromatin in autosomes (Alevi et al. 2015a). Furthermore, *R. taquarussuensis* sp. n. has the X sex chromosome rich in CG (Fig. 12C), the Y rich in AT (Fig. 12D) and various

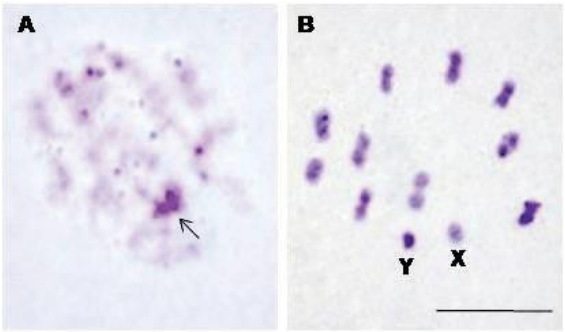


Figure 11. Constitutive heterochromatin pattern in *R. taquarussuensis*. **A** Initial prophases with a chromocenter heterochromatic consisting of sex chromosomes (arrow) and several heterochromatic blocks dispersed in the nucleus **B** Metaphase I with heterochromatic blocks in both extremities of practically all the autosomes and in the Y sex chromosome. X: X sex chromosome, Y: Y sex chromosome. Bar: 10 µm.

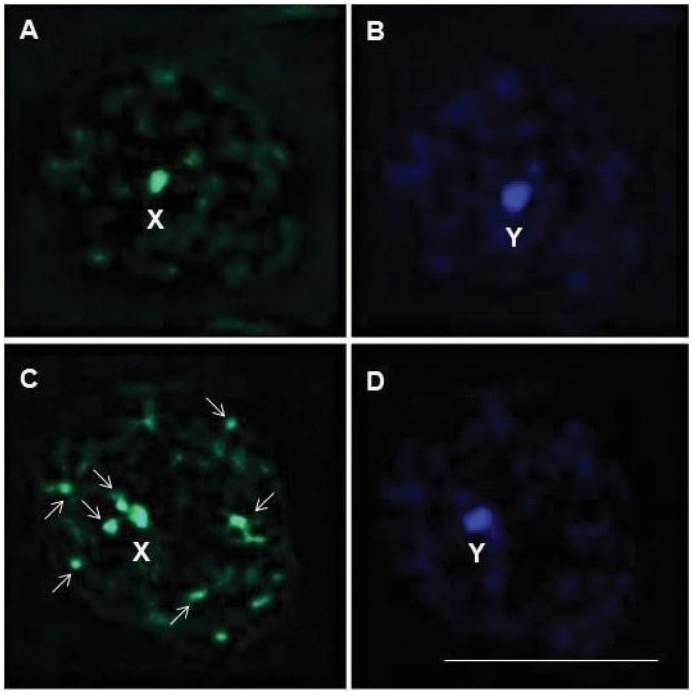


Figure 12. Composition of the pairs of bases of DNA rich in AT and CG in *R. neglectus* (**A, B**) and *R. taquarussuensis* (**C, D**). **A** X sex chromosome rich in CG **B** Y sex chromosome rich in AT **C** X sex chromosome and various blocks dispersed in the prophase nucleus (arrows) rich in CG **D** Y sex chromosome rich in AT. X: X sex chromosome, Y: Y sex chromosome. Bar: 10µm.

blocks rich in CG dispersed in the prophase nucleus (Fig. 12C), while *R. neglectus* only has the X sex chromosome rich in CG (Fig. 12A) and the Y rich in AT (Fig. 12B), which proves the genetic differences between the two *Rhodnius* species.

Discussion

The subfamily Triatominae include 18 genera comprising 152 species, 20 of which belong to the genus *Rhodnius* (Galvão 2014, Mendonça et al. 2016, Souza et al. 2016). The difficulties involved in the specific identification of *Rhodnius* have already been noted by Neiva and Pinto (1923), as well as by Rosa et al. (2012) and Souza et al. (2016). However, even though it is difficult to specify the distinctions among the species of that genus, in the last eight years four species were described: *R. zeledoni*, *R. montenegrensis*, *R. barretti*, and *R. marabaensis*. Therefore, *R. taquarussuensis* sp. n. is the 21st species of the genus and the 5th described in the last eight years. Its similarity with *R. neglectus* was noticed after the capture of the first female specimen, as the most evident macroscopic characters, such as size, general aspect and connexivum, showed no differences. As a result, it was decided to base its description on the differences from *R. neglectus*.

In addition to the macroscopic characters, *R. taquarussuensis* sp. n. and *R. neglectus* were considered "close to" because the OM study indicated similar characters between them, including: placement of black and brown spots on the dorsal and ventral connexivum, length of the four segments of the antenna, pronotum, antero lateral angles, urotergite I process, geometric morphometry of the hind wings, median process of the pygophore and morphological characters of the eggs.

Rhodnius taquarussuensis sp. n. was considered distinct from *R. neglectus* on account of the observation of color, eleven morphological characters, twelve morphometric characters and cytogenetic features (Tables 1, 2). All those differences are consistent with Lent and Wygodzinsky (1979), whose descriptive key to 11 species of *Rhodnius* lists 13 morphological characters for specific distinction: color, tibia, legs, pronotum, head, posterior lobe of the pronotum, anterolateral angles, median process of the pygophore, connexivum, scutellum, eyes and antennae.

Regarding the color, the distinction between *R. taquarussuensis* sp. n. and *R. neglectus* was based on the general aspect, segments of the antenna, hind wings and stridulatory sulcus. The general color of *R. taquarussuensis* sp. n. is brown, whereas *R. neglectus* is dark brown, almost black, or "brown dark", as referred to by Lent and Wygodzinsky (1979).

Out of the eleven morphological characters that distinguish *R. taquarussuensis* sp. n. from *R. neglectus*, three are located on the head: dorsal vertex, genae and triangular furrow of the first segment of the proboscis. The difference related to the vertex was one of the characters used by Souza et al. (2016) to distinguish *R. marabaensis* from *R. prolixus* and *R. robustus*. The differences between the genae for specific characterization are being reported for the first time in this description. The ventral triangular furrow was mentioned by Rosa et al. (1999) in their study of *T. rubrovaria* and it was mapped by Lent and Wygodzinsky (1979), but it was not named and it is being used for the first time as a distinctive character.

In what refers to the thorax, differences on the scutellum, protothorax, mesothorax, and metathorax were noticed. The scutellum of *R. taquarussuensis* sp. n. and *R. neglectus* differs in the shape of the apex and also the position on which that apex reaches uroter-

gite I, since there is no significant difference in terms of length (Table 1). The taxonomic importance of the scutellum was tackled by Obara et al. (2007), who verified the differences of that character in eight species of *Triatoma*. Rosa et al. (2012) and Souza et al. (2016) also used it to describe *R. montenegrensis* and *R. marabaensis*, respectively.

The differentiation of seven genera of triatomines based on the shape of the prosternal stridulatory sulcus was carried out by Lent and Wygodzinsky (1979) and also by Souza et al. (2016) in the description of *R. marabaensis*. The description presented herein points out color and morphological differences between *R. taquarussuensis* sp. n. and *R. neglectus*. The mesothorax and metathorax, which have been used by Souza et al. (2016) to describe a new species, were found to be different in *R. taquarussuensis* sp. n. and *R. neglectus*.

Lent and Jurberg (1969) observed specific features in the characters of the male genitalia of 10 species of *Rhodnius* and since then that structure has been used to describe species of other genera of Triatominae, e.g., *Mepraia parapatrica* Frias, 2010, *P. mitarakaensis* Bérenger & Blanchet, 2007 and *T. jatai* Gonçalves et al., 2013. In the case of *R. taquarussuensis* sp. n. and *R. neglectus*, the difference in the male genitalia is the shape of the phallosome. With respect to the female external genitalia, differences between *R. taquarussuensis* sp. n. and *R. neglectus* could be observed on the dorsal, posterior and ventral sides, which differ from other 13 *Rhodnius* species, according to Rosa et al. (2014).

The eggs of *R. taquarussuensis* sp. n. and *R. neglectus* showed differences in the measurement of their length and opercular opening, the same as *R. montenegrensis* and *R. marabaensis* on the occasion of their description. As for the morphological characters, no differences were recorded. On the other hand, it should be noted that morphological differences were found by Barata (1981) in eggs of 10 *Rhodnius* species, by Santos et al. (2009) in three species, by Rosa et al. (2012) in the description of *R. montenegrensis* and by Santos et al. (2016) when describing *R. marabaensis*.

Dujardin et al. (1999) established the geometric morphometry of the hind wings as a distinctive character among Triatominae while studying the sexual dimorphism of *R. domesticus* and *T. infestans*. The technique proved valid, for instance, to indicate the distinction between *Mepraia spinolai* and *M. gajardoi*; *T. bahiensis* and *T. lenti*; *R. colombiensis*, *R. ecuadoriensis* and *R. pallescens*; five populations of *T. patagonica* (Campos et al. 2011, Díaz et al. 2014, Nattero et al. 2016). However, even though that technique has contributed to distinguish even very close species, it showed no significant results to distinguish *R. taquarussuensis* sp. n. from *R. neglectus*.

According to Justi and Galvão (2016) the group *R. prolixus* comprise the following species: *R. barretti*, *R. dalessandroi*, *R. domesticus*, *R. marabaensis*, *R. milesi*, *R. montenegrensis*, *R. nasutus*, *R. neglectus*, *R. neivai*, *R. prolixus* and *R. robustus*. Since *R. taquarussuensis* sp. n. is close to *R. neglectus* we suggest the inclusion of *R. taquarussuensis* sp. n. in the *R. prolixus* group and we present the main differences between the twelve species (Table 3).

Cytogenetic analyses of *R. taquarussuensis* sp. n. made it possible to describe the karyotype (2n = 22) and observe the constitutive heterochromatin pattern in the chro-

Table 3. Distinguishing characters among twelve species of the group *Rhodnius prolixus*.

Species	Distinctive characters	References
R. barretti	The third antennal segment appears to be relatively shorter. The scutellar process is narrowly pointed.	Abad-Franch et al. 2013
R. dalessandroi	Antenniferous tubercle slightly pilose and with triangular glabrous depression in the upper region. Semicircular spot on the posterior end of the neck.	Carcavallo and Barreto 1976
R. domesticus	Head comparatively long, distinctly longer that pronotum. Process of pygophore rectangular.	Lent and Wygodzinsky 1979
R. marabaensis	The second antennal segment is 10.3 times larger than the first. The scutellum is larger and includes two prominent internal lateral carinea.	Souza et al. 2016
R. milesi	The male genitalia presents a second process of the phallosoma. Divergent antennal tubercle with an apical denticle.	Valente et al. 2001
R. montenegrensis	Anterior wings with well-demarcated veins, notable the Sc by a yellow tonality. Abdomen presents yellow spots interposed with dark ones over the ventral abdomen lengthwise.	Rosa et al. 2012
R. nasutus	Overall color light reddish brown, trochantera not contrasting conspicuously with femora. Median process of pygophore wide at base.	Lent and Wygodzinsky 1979
R. neglectus	Overall color dark brown, trochantera very light colored. Median process of pygophore narrow at base.	Lent and Wygodzinsky 1979
R. neivai	Pronotum entirely dark brown or black, including the carine. Connexivum blackish, with very small reddish spots.	Lent and Wygodzinsky 1979
R. prolixus	Anteocular region slightly over three times as long as postocular. Distance between eyes dorsally larger than width of eyes in dorsal view.	Lent and Wygodzinsky 1979
R.robustus	Anteocular region about four times as long as postocular. Specimens distance between eyes dorsally smaller than, or equal to, width of eye in dorsal view.	Lent and Wygodzinsky 1979
R. taquarussuensis sp. n.	Head with a prominent brown vertex contrasting with the black sides. The phallosome (Ph) has a broad plate whose superior region has a trapezoidal shape and occupies the middle region of the aedeagus.	This work

*group *R. prolixus* according to Justi and Galvão 2016.

mosomes (extremities of most autosomes), which are rich in CG. All the species in the tribe Rhodniini have 22 chromosomes (Alevi et al. 2013, 2015b). On the other hand, out of the 14 species of the genus *Rhodnius* whose chromosomes have been studied in the literature, only four show heterochromatic blocks in the autosomes, namely *R. colombiensis*, *R. nasutus*, *R. pallescens* and *R. pictipes* (Dujardin et al. 2002). *R. neglectus*, which is a similar species for *R. taquarussuensis* sp. n., does not show heterochromatic blocks in the autosomes (Dujardin et al. 2002; Panzera et al. 2012; Alevi et al. 2015a).

Although the evolutionary process in triatomine is disruptive (Dujardin et al. 2009) and intraspecific chromosome variation has been described for *R. ecuadoriensis* (Pita et al. 2013), *R. pallescens* (Gómez-Palacio et al. 2008), *P. geniculatus* (Crossa-Pérez

et al. 2002), *T. dimidiata* (Panzera et al. 2006) *T. infestans* (Panzera et al. 2004, 2012) and *T. sordida* (Panzera et al. 1997), generally the distribution of species is associated with different countries [for exemple, *R. ecuadoriensis* from Peru and Ecuador (Pita et al. 2013) and *T. sordida* from Brazil and Argentina (Panzera et al. 1997)] or different regions [for example, *R. pallescens* from North and West regions from Colombia (Gómez-Palacio et al. 2008) and *T. infestans* from Andean group and Non-Andean group (Panzera et al. 2004, 2012)]. However, a population study was previously performed with *R. neglectus* (endemic species of Brazil) coming from different Brazilian states (Alevi et al. 2015a) and the authors pointed out that there is no intraspecific chromosome variation for this species. This fact and the morphological data described sustain the specific status of *R. taquarussuensis* sp. n., since the gain and loss of heterochromatin in the autosomes of *Rhodnius* are adaptive processes that can be linked to speciation processes, as recently noted for the group *pallescens* (Alevi et al. 2015c).

Authors' contributions

Conceived the study: JAR, HHGJ, JO and KCCA.Colected the bugs:HHGJ.

Prepared samples: JAR, JDN, JO, DBC and JDN. Analysed data: JAR, JO, VJM, RF, CSR and MTAO. Interpreted data: JAR, JO, KCCA, DBC. Wrote the manuscript: JAR, JO and KCCA.

All authors read and approved the final version of the manuscript.

Acknowledgents

Marcelo Ornaghi Orlandi, Mario Cilense e Sebastião Dametto from the Institute of Chemistry at São Paulo State University (UNESP) by the support and help regarding the use of the scanning electron microscope. Heloisa Pinotti, Eder dos Santos Souza, Lucas Abrantes da Silva, Tiago Belintani, Fábio Regis Garcia postgraduates of the Bioscience and Biotechnology Applied to Pharmacy program (FCF / UNESP) by the constant help in the maintenance of triatomine colonies. Nelson Papavero from the Zoological Museum of USP and Hélcio Reinaldo Gil-Santana of Diptera Laboratory, Oswaldo Cruz Institute for suggesting the scientific name of the new species, to Dennys Ortiz postgraduate of Animal Biology (UNICAMP) for the support with maps, to Davi Antonio da Rosa for the photos. The authors are also very grateful to Dr. Guanyang Zhang (Editor of Zookeys), Dr. Daniel A. Frias Lasserre (Laboratory of Entomology, Metropolitan University of Education Sciences, Santiago, Chile), Jean-Michel Bérenger (Laboratory Diagnostic Insect, Bouc-Bel-Air, France) and an anonymous reviewer for their valuable comments and suggestions. Financial Support: Scientific Support and Development Program of School of Pharmaceutical Sciences (UNESP);São Paulo Research Foundation (FAPESP) grant process number 2009/52236-2 and 2013/19764-0; Brazilian Federal Agency for the

Support and Evaluation of Graduate Education (CAPES), grant process number 23038-005285/2011-2012; Brazilian National Council for Scientific Technological Development (CNPq process number 142284/2015-7).

References

Abad-Franch F, Pavan MG, Jaramillo O, Palomeque FS, Dale C, Chaverra D, Monteiro FA (2013) *Rhodnius barretti*, a new species of Triatominae (Hemiptera: Reduviidae) from western Amazonia. Memórias Instituto Oswaldo Cruz 108: 92–99. http://dx.doi.org/10.1590/0074-0276130434

Alevi KCC, Rodas LAC, Tartarotti E, Azeredo-Oliveira MTV, Guirado MM (2015a) Entoepidemiology of Chagas disease in the Western region of the State of São Paulo from 2004 to 2008, and cytogenetic analysis in *Rhodnius neglectus* (Hemiptera, Triatominae). Genetics and Molecular Research 14(2): 5775–5784. http://dx.doi.org/10.4238/2015.May.29.9

Alevi KCC, Ravazi A, Mendonça VJ, Rosa JA, Azeredo-Oliveira MTV (2015b) Karyotype of *Rhodnius montenegrensis* (Hemiptera, Triatominae). Genetics and Molecular Research 12: 222–226. http://dx.doi.org/10.4238/2015.January.16.5

Alevi KCC, Ravazi A, Franco-Bernardes MF, Rosa JA, Azeredo-Oliveira MTV (2015c) Chromosomal evolution in the *pallescens* group (Hemiptera, Triatominae). Genetics and Molecular Research 14: 12654–12659. http://dx.doi.org/10.4238/2015.October.19.9

Alevi KCC, Rosa JA, Oliveira MTVA (2013) Mini Review: Karyotypic Survey in Triatominae Subfamily (Hemiptera, Heteroptera). Entomology, Ornithology & Herpetology: Current Research 2: 2. http://dx.doi.org/10.4172/2161-0983.1000106

Barata JM (1981) Aspectos morfológicos de ovos de triatominae: II – Características macroscópicas e exocoriais de dez espécies do gênero *Rhodnius* Stal, 1859 (Hemiptera – Reduviidae). Revista Saúde Pública 15: 490–542. https://doi.org/10.1590/S0034-89101981000500006

Bérenger JM, Blanchet D (2007) A new species of the genus *Panstrongylus* from French Guiana (Heteroptera; Reduviidae; Triatominae). Memórias do Instituto Oswaldo Cruz 102: 733–736. http://dx.doi.org/10.1590/S0074-02762009000800007

Campos R, Botto-Mahan C, Coronado X, Jaramillo N, Panzera F, Solari A (2011) Wing shape differentiation of *Mepraia* species (Hemiptera: Reduviidae). Infection, Genetics and Evolution 11: 329–333. https://doi.org/10.1016/j.meegid.2010.11.002

Carvalho DB, Almeida CE, Rocha CS, Gardim S, Mendonça VJ, Ribeiro AR, Alves ZC, Ruellas KT, Vedoveli A, da Rosa JA (2013) A novel association between *Rhodnius neglectus* and the *Livistona australis* palm tree in an urban center foreshadowing the risk of Chagas disease transmission by vectorial invasions in Monte Alto City, São Paulo, Brazil. Acta Tropica 130: 35–38. http://dx.doi.org/10.1016/j.actatropica.2013.10.009

Crossa-Pérez R, Hernández M, Caraccio M, Rose V, Valente A, Valente V, Moreno J, Angulo V, Sandoval M, Roldán J, Vargas F, Wolff M, Panzera F (2002) Chromosomal evolution trends of the genus *Panstrongylus* (Hemiptera, Reduviidae), vectors of Chagas Disease. Infection Genetics and Evolution 2: 47–56. http://dx.doi.org/10.1016/S1567-1348(02)00063-1

Díaz S, Panzera F, Jaramillo-Ocampo N, Pérez R, Fernández R, Vallejo G, Saldaña A, Calzada JE, Triana O, Gómez-Palacio A (2014) Genetic, cytogenetic and morphological trends in the evolution of the *Rhodnius* (Triatominae: Rhodniini) trans-Andean group. PLoS One 9: 87493. http://dx.doi.org/10.1371/journal.pone.0087493

Dujardin JP, Costa J, Bustamante D, Jaramillo N, Catala S (2009) Deciphering morphology in Triatominae: the evolutionary signals. Acta Tropica 110: 101–111.https://doi.org/10.1016/j.actatropica.2008.09.026

Dujardin JP, Schofield CJ, Panzera F (2002) Los vectores de la enfermedad de Chagas. Bruxelles : Académie Royale des Sciences d'Outre-Mer 25(3): 1–189.

Dujardin J, Steinden M, Chavez T, Machane M, Schofeld C (1999) Changes in the Sexual Dimorphism of Triatominae in the Transition From Natural to Artifcial Habitats. Memórias Instituto Oswaldo Cruz 94: 565–569. http://dx.doi.org/10.1590/S0074-02761999000400024

Ferreira RTB, Branquinho MR, Leite PC (2014) Transmissão oral da doença de Chagas pelo consumo de açaí: um desafio para a Vigilância Sanitária. Vigilância Sanitária em Debate, Rio de Janeiro 2: 4–11. https://doi.org/10.3395/VD.V2I4.358

Frias-Lasserre D (2010) A new species and karyotype variation in the bordering distribution of *Mepraia spinolai* (Porter) and *Mepraia gajardoi* Frías et al. (Hemiptera: Reduviidae: Triatominae) in chile and its parapatric model of speciation. Neotropical Entomology, Londrina 39(4): 572–583. http://dx.doi.org/10.1590/S1519-566X2010000400017

Galati EAB (2016) Morfologia e Taxonomia: 2.1, Classificação de Phlebotominae, and 2.2. Morfologia, Terminologia de Adultos e Identificação dos táxons da América. In: Rangel EF, Lainson R (Eds) Flebotomíneos do Brasil, Rio de Janeiro, FIOCRUZ, 2003, 23–51[2.1]; 53–75[2.2].

Galvão C (2014) Vetores da doença de chagas no Brasil [online]. Sociedade Brasileira de Zoologia, Curitiba, 289 pp. https://doi.org/10.7476/9788598203096

Galvão C, Carcavallo R, Rocha DS, Juberg JA (2003) Checklist of the current valid species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their geographical distribuition, with nomenclatural and taxonomic note. Zootaxa 202: 1–36. http://dx.doi.org/10.11646/zootaxa.202.1.1

Gómez-Palacio A, Jaramillo-Ocampo N, Triana-Chávez O, Saldaña A, Calzada J, Pérez, R, Panzera F (2008) Chromosome variability in the Chagas disease vector *Rhodnius pallescens* (Hemiptera, Reduviidae, Rhodniini). Memórias do Instituto Oswaldo Cruz 103: 160–164. http://dx.doi.org/10.1590/S0074-02762008000200006

Gonçalves TC, Teves-Neves SC, Santos-Mallet JR, Carbajal-de-la-Fuente AL, Lopes CM (2013) *Triatoma jatai* sp. nov. in the state of Tocantins, Brazil (Hemiptera: Reduviidae: Triatominae). Memórias do Instituto Oswaldo Cruz 108: 429-37. https://doi.org/10.1590/0074-0276108042013006

Gurgel-Gonçalves R, Abad-Franch F, Ferreira JB, Santana DB, Cuba CAR (2008) Is *Rhodnius prolixus* (Triatominae) invading houses in central Brazil? Acta Tropica 107: 90-8. https://doi.org/10.1016/j.actatropica.2008.04.020

Jurberg J, Rocha DS, Galvão C (2009) *Rhodnius zeledoni* sp. nov. afm de *Rhodnius paraenses* Sherlock, Guitton e Milles, 1977 (Hemíptera, Reduviidae, Triatominae). Biota Neotropica 9: 123–128. https://doi.org/10.1590/S1676-06032009000100014

Justi AS, Galvão C (2017) The Evolutionary Origin of Diversity in Chagas Disease Vectors. Trends Parasitology 33(1): 42–52. http://dx.doi.org/10.1016/j.pt.2016.11.002

Lent H, Jurberg J (1969) O gênero *Rhodnius* Stål, 1859, com um estudo sobre a genitália das especies (Hemiptera, Reduviidae, Triatominae). Revista Brasileira Biologia 29: 487–560.

Lent H, Wygodzinsky P (1979) Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. Bulletin of the American Museum of Natural History 163: 123–520.

Mendonça VJ, Alevi KC, Pinotti H, Gurgel-Gonçalves R, Pita S, Guerra AL, Panzera F, De Araújo RF, Azeredo-Oliveira MT, Rosa JA (2016) Revalidation of *Triatoma bahiensis* Sherlock & Serafim, 1967 (Hemiptera: Reduviidae) and phylogeny of the *T. brasiliensis* species complex. Zootaxa 4107: 239–254. https://doi.org/10.4269/ajtmh.2009.08-0664

Ministério da Saúde Brazil (2017) Doença de Chagas. http://portalsaude.saude.gov.br/index.php/ominsterio/principal/secretarias/svs/doencadechagas

Nattero J, Pita S, Calleros L, Crocco L, Panzera Y, Rodríguez CS, Panzera F (2016) Morphological and Genetic Differentiation within the Southernmost Vector of Chagas Disease: *Triatoma patagonica* (Hemiptera - Reduviidae). PLoS ONE 11 (12): e0168853. http://dx.doi.org/10.1371/journal.pone.0168853

Neiva A, Pinto C (1923) Dos hemípteros hematophagos do Norte do Brasil com descrição de duas novas espécies. Brasil Médicina 37: 73–76.

Obara MT, da Rosa JA, Ceretti W, Urbinatti PR, Quintero LO, Barata JM, Galvão C, Jurberg J (2007) A study of the scutellum in eight Chagas disease vector species from genus *Triatoma* (Hemiptera, Reduviidae) using optical and scanning electron microscopy. Memórias do Instituto Oswaldo Cruz 102: 463–468. https://doi.org/10.1590/S0074-02762007005000027

Panzera F, Dujardin JP, Nicolini P, Caraccio MN, Rose V, Tllez T, Bermúdez H, Bargues MD, Mas-Coma S, Connor JH, Perez R (2004) Genomic changes of Chagas disease vector, South America. Emerging Infectious Diseases 10: 438–46. doi: 10.3201/eid1003.020812

Panzera F, Fernandis I, Ramsey J, Ordòñez R, Salazar-Schettino PM, Cabrera M, Monroy MC, Bargues MD, Mas-Coma S, Connor EO, Ângulo VM, Jaramillo N, Cordon-Rosales C, Gómez D, Perez R (2006) Chromosomal variation and genome size support existence of cryptic species of *Triatoma dimidiata* with different epidemiological importance as Chagas disease vectors. Tropical Medicine & International Health 11: 1092–1103. doi:10.1111/j.1365-3156.2006.01656.x

Panzera F, Hornos S, Pereira J, Cestau R, Canale D, Diotaiuti L, Dujardin JP, Perez R (1997) Genetic variability and geographic differentiation among three species of triatomine bugs (Hemiptera-Reduviidae). The American Journal of Tropical Medicine & Hygiene 57: 732–739. https://doi.org/10.4269/ajtmh.1997.57.732

Panzera Y, Pita S, Ferreiro MJ, Ferrandis I, Lages C, Pérez R, Silva AE, Guerra M, Panzera F (2012) High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (Triatominae, Heteroptera). Cytogenetic and Genome Research 138: 56–67. https://doi.org/10.1159/000341888

Pita S, Panzera F, Ferrandis I, Galvão C, Gómez-Palacio A, Panzera Y (2013) Chromosomal divergence and evolutionary inferences in Rhodniini based on the chromosomal loca-

tion of ribosomal genes. Memorias do Instituto Oswaldo Cruz 108: 376–82. https://doi.org/10.1590/S0074-02762013000300017

Poinar G Jr (2005) *Triatoma dominicana* sp. n. (Hemiptera: Reduviidae: Triatominae), and *Trypanosoma antiquus* sp. n. (Stercoraria: Trypanosomatidae), the first fossil evidence of a triatomine-trypanosomatid vector association. Vector Borne Zoonotic Diseases 5(1): 72–81. https://doi.org/10.1089/vbz.2005.5.72

Poinar G Jr (2013) *Panstrongylus hispaniolae* sp. n. (Hemiptera: Reduviidae: Triatominae), a new fossil triatomine in Dominican amber, with evidence of gut flagellates. Palaeodiversity 6: 1–8.

Quintero LO (2002) Avaliação do valor sistemático do processo do I urotergito em machos de onze espécies de importância em saúde pública da subfamília Triatominae (Hemiptera, Reduviidae). PhD thesis, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, 85 pp.

Rodrigues VL, Pauliquevis Junior C, da Silva RA, Wanderley DM, Guirardo MM, Rodas LA, Casanova C, Pachioni ML, Souza WA, Costa AJ, Baitelo D, Tonietti VL (2014) Colonization of palm trees by *Rhodnius neglectus* and household and invasion in an urban area, Araçatuba, São Paulo State, Brazil. Revista do Instituto de Medicina Tropical de São Paulo 56: 213–218. http://dx.doi.org/10.1590/S0036-46652014000300006

Rosa JA, Mendonça VJ, Gardim S, Carvalho DB, Oliveira J, Nascimento JD, Pinotti H, Pinto MC, Cilense M, Galvão C, Barata JM (2014) Study of the external female genitalia of 14 *Rhodnius* species (Hemiptera, Reduviidae, Triatominae) using scanning electron microscopy. Parasites & Vectors 7: 1–17. https://doi.org/10.1186/1756-3305-7-17

Rosa JA, Rocha CS, Gardim S, Pinto MC, Mendonça VJ, Ferreira Filho JCR, Carvalho EOC, Camargo LMA, Oliveira J, Nascimento JD, Cilense M, Almeida CE (2012) Description of *Rhodnius montenegrensis* n. sp. (Hemiptera: Reduviidae: Triatominae) from the state of Rondônia, Brazil. Zootaxa 3478: 62–76.

Rosa JA, Mendonça VJ, Rocha CS, Gardim S, Cilense M (2010) Characterization of the external female genitalia of six species of Triatominae (Hemiptera: Reduviidade) by scanning electron microscopy. Memórias do Instituto Oswaldo Cruz 105: 286–292. https://doi.org/10.1590/S0074-02762010000300007

Rosa JA, Barata JMS, Cilence M, Belda Neto FM (1999) Head morphology of 1st and 5th instar nymphs of *Triatoma circummaculata* and *Triatoma rubrovaria* (Hemiptera, Reduviidae). International Journal of Insect Morphology and Embryology 28: 363–375.

Santos CM, Jurberg J, Galvão C, Rosa JA, Júnior WC, Barata JM, Obara MT (2009) Comparative descriptions of eggs from three species of *Rhodnius* (Hemiptera: Reduviidae: Triatominae). Memórias do Instituto Oswaldo Cruz 104: 1012–1018. https://doi.org/10.1590/S0074-02762009000700013

Schimid M (1980) Chromosoma banding an amphibia IV. Differentiation of GC and AT rich regions in Anura. Chromosoma 77: 83–103.

Severi-Aguiar GD, Lourenco LB, Bicudo HE, Azeredo-Oliveira, MTV (2006) Meiosis aspects and nucleolar activity in *Triatoma vitticeps* (Triatominae, Heteroptera). Genetica 126: 141–151. https://doi.org/10.1007/s10709-005-1443-2

Souza ES, Von Atzingen NCB, Furtado MB, Oliveira J, Nascimento JD, Vendrami DP, Gardim S, da Rosa JA (2016) Description of *Rhodnius marabaensis* sp. n. (Hemiptera, Reduviidae, Triatominae) from Pará State, Brazil. ZooKeys 621: 45–62. https://doi.org/10.3897/zookeys.621.9662

Stal C (1859) Monographie der Gattung Conorhinus und Verwandten. Berliner Entomologische Zeitschrift 3: 99–117. http://biostor.org/reference/61560

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research75: 304–306. https://doi.org/10.1016/0014-4827(72)90558-7

4.2 Capítulo II:

Taxonomical over splitting in the Rhodnius prolixus *(Insecta: Hemiptera: Reduviidae) clade: Are* R. taquarussuensis *(da Rosa et al., 2017) and* R. neglectus *(Lent, 1954) the same species?*

PLOS ONE. 14 (2): e0211285 (2019).

https://doi.org/10.1371/journal.pone.0211285

PLOS ONE

RESEARCH ARTICLE

Taxonomical over splitting in the *Rhodnius prolixus* (Insecta: Hemiptera: Reduviidae) clade: Are *R. taquarussuensis* (da Rosa et al., 2017) and *R. neglectus* (Lent, 1954) the same species?

**Juliana Damieli Nascimento[1], João Aristeu da Rosa[2], Fabian C. Salgado-Roa[3,4], Carolina Hernández[5], Carolina Pardo-Diaz[3], Kaio Cesar Chaboli Alevi[2,6], Amanda Ravazi[6], Jader de Oliveira[2], Maria Terc´ılia Vilela de Azeredo Oliveira[6], Camilo Salazar[3], Juan David [5]*

1 Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil, **2** Laboratório de Parasitologia, Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Araraquara, SP, Brasil, **3** Grupo de Genética Evolutiva, Filogeografía y Ecología de Biodiversidad Neotropical, Programa de Biología, Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá, Colombia, **4** Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, Colombia, **5** Grupo de Investigaciones Microbiológicas-UR (GIMUR), Programa de Biología, Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá, Colombia, **6** Laboratório de Biologia Celular, Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), São José do Rio Preto, SP, Brasil

* juand.ramirez@urosario.edu.co

Abstract

The use of subtle features as species diagnostic traits in taxa with high morphological similarity sometimes fails in discriminating intraspecific variation from interspecific differences, leading to an incorrect species delimitation. A clear assessment of species boundaries is particularly relevant in disease vector organisms in order to understand epidemiological and evolutionary processes that affect transmission capacity. Here, we assess the validity of the recently described *Rhodnius taquarussuensis* (da Rosa et al., 2017) using interspecific crosses and molecular markers. We did not detect differences in hatching rates in interspecific crosses between *R. taquarussuensis* and *R. neglectus* (Lent, 1954). Furthermore, genetic divergence and species delimitation analyses show that *R. taquarussuensis* is not an independent lineage in the *R. prolixus* group. These results suggest that *R. taquarussuensis* is a phenotypic form of *R. neglectus* instead of a distinct species. We would like to stress that different sources of evidence are needed to correctly delimit species. We consider this is an important step in understanding vectorial Chagas disease spread and transmission.

OPEN ACCESS

Citation: Nascimento JD, da Rosa JA, Salgado-Roa FC, Hernández C, Pardo-Diaz C, Alevi KCC, et al. (2019) Taxonomical over splitting in the *Rhodnius prolixus* (Insecta: Hemiptera: Reduviidae) clade: Are *R. taquarussuensis* (da Rosa et al., 2017) and *R. neglectus* (Lent, 1954) the same species? PLoS ONE 14(2): e0211285. https://doi.org/10.1371/journal.pone.0211285

Editor: Ben J. Mans, Onderstepoort Veterinary Institute, SOUTH AFRICA

Received: October 4, 2018

Accepted: January 10, 2019

Published: February 7, 2019

Data Availability Statement: All relevant data are within the manuscript.

Funding: This work was funded by DIRECCION DE INVESTIGACION UNIVERSIDAD DEL ROSARIO. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

The study of the speciation process requires a complete understanding of the phenotypic variation present across the range of the study taxa. This is particularly challenging in organisms where morphological differences are subtle or not obvious, and where other aspects of their biology such as reproduction, ecology, phenology and life traits are also unknown. An increasing number of studies have documented "cryptic" speciation throughout the tree of life (i.e. taxa that cannot readily be distinguished morphologically, yet evidence indicates they are on different evolutionary trajectories). However, such descriptions have been done in absence of a clear definition of what a cryptic species is, and often using alpha taxonomy as the sole approach for detecting and classifying new species [1–4]. This can lead to false species diagnosis when unreliable traits (those lacking discontinuous, nonoverlapping patterns of variation) are used [5], which is particularly important when delimiting vector species with medical relevance, as this directly impacts the control of the diseases transmitted by them.

The subfamily Triatominae has 18 genera, with *Panstrongylus* (Berg, 1879), *Rhodnius* (Stål, 1859) and *Triatoma* (Laporte, 1832) being the most epidemiologically important genera, since they are the main species responsible for the transmission of *Trypanosoma cruzi* (Chagas, 1909), the etiologic agent of Chagas disease [6, 7]. The identification of these three genera is straightforward and is based on the insertion of the antennae on the head, which is macroscopically perceptible: in *Panstrongylus* the antennae are inserted near the eyes, in *Rhodnius* these appendages are on the anterior portion of the head, and in *Triatoma* they are located on the middle portion of the head [8, 9]. Nonetheless, the most recent Triatominae phylogeny showed that the only monophyletic genus is *Rhodnius* [9–11]. Also, species delimitation within these genera remains problematic [12]. In particular, species of *Rhodnius* show low morphological variation and their complex identification relies on few morphological traits and/or mtDNA divergence [11, 13–16]. For example, it is difficult to differentiate between *R. neglectus* and *R. prolixus* (Stål, 1859) [17], *R. robustus* (Larrousse, 1827) and *R. montenegrensis* (da Rosa et al., 2012) [18], *R. amazonicus* (Almeida, Santos and Sposina, 1973) and *R. pictipes* (Stål, 1872) [19], *R. pictipes* and *R. stali* (Lent, Jurberg and Galvão) [20], among many other examples.

Moreover, the classic division of *Rhodnius* presents additional challenges. The genus is divided into three groups: *prolixus*, *pictipes* and *pallescens*. The first two are found east of the Andes (*cis*-Andean), while the third is distributed west of the Andes (*trans*-Andean) [21–23]. The phylogenetic relationships among these groups are still under debate, especially the position of the *pictipes* group that was initially considered closer to the *pallescens* group, but recent evidence found it as sister to the *prolixus* group [23–26].

Because *Rhodnius* has an intrinsic relation with the propagation of *T. cruzi* and *T. rangeli* (Tejera, 1920), resolving its phylogenetic relationships and accurately differentiating its species is a first step to determine the epidemiological threat associated to each species, as well as to understand their ecology and population dynamics [8, 23, 27].

Recently, a new species of the genus *Rhodnius*, *R. taquarussuensis*, was described based on phenotypic and cytogenetic traits [22]. This is the only species of the *prolixus* group that has dispersed heterochromatin throughout the nucleus and autosomes, and it is morphologically similar to *R. neglectus* [22, 28]. However, the specific status of *R. taquarussuensis* requires a more rigorous confirmation that implements both genetic data and tests of reproductive isolation. Here, we used six molecular markers and performed crosses between *R. taquarussuensis* and *R. neglectus* in order to address whether the former is a valid species.

Methods

Sampling and DNA extraction

Individuals of *R. taquarussuensis* were collected in Taquarussu, Mato Grosso do Sul, Brazil (-22.48 Lat, -53.35 Long; Table 1) and those of *R. neglectus* were collected in Formoso, Goiás, Brazil (-13.65 Lat, -48.88 Long; Table 1) and maintained in the Triatominae insectary of the School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil. *Rhodnius prolixus* were collected in Arauca (7.08 Lat, -70.75 Long), Fortul (6.78 Lat, -71.76 Long), Puerto Rondón (6.28 Lat, -71.10 Long) and Saravena (6.95 Lat, -71.87 Long) in Colombia (Table 1). UNIVERSIDAD DEL ROSARIO provided the field permit from ANLA (Autoridad Nacional de Licensias ambientales) 63257–2014. DNA was extracted from the head, legs and intestine using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's protocol. The DNA concentration was determined using a NanoDrop 1000 Spectrophotometer V3.7 (Thermo Fisher Scientific, Wilmington, DE, USA) and stored at −20˚C.

Loci amplification and sequencing

We amplified and sequenced two mitochondrial gene fragments, Cytochrome b (CYTB) and Mitochondrially Encoded NADH Dehydrogenase 4 (ND4) using the conditions reported elsewhere [29]. We also designed primers to develop new coding nuclear markers in *Rhodnius*. In order to do this, we used the *R. prolixus* genome available in VectorBase (https://www.vectorbase.org/organisms/rhodnius-prolixus) and, from the GFF file, we selected four large exon markers (≀700 bp) using a custom script. We then used BLASTn to compare these

Table 1. Genes, primer information and accession numbers.

Symbol	Gene name	*Rn*	*Rp*	*Rt*	Primers (5'-3')	Tm (˚C)	Fragment size (pb)	Accession numbers
CYTB	Cytochrome b≀≀	6	5	8	R: GCW CCA ATT CAR GTT ART AA F: GGA CGW GGW ATT TAT TAT GGA TC	50	659	MH704746—MH704764
ND4	NADH dehydrogenase 4≀≀	5	5	15	F: TAA TTC GTT GTC ATG GTA ATG F: TCA ACA TGA GCC CTT GGA AG	53	560	MH704765—MH704779
PCB	Putative chitin binding peritrophin-a domain protein	8	5	5	R: CAC TAC GGG TCG TGA AGG TT F: ACA TCC TTG GCC ACA AGA AC	55	757	MH704780—MH704797
TOPO	DNA topoisomerase	5	6	5	F: CAA CAC TTG TAA CCC GAG CA F: ATC ATT GGC CGC ATC TTT AG	56	604	MH704798—MH704813
URO	Uroporphyrinogen decarboxylase	11	6	6	R: TTA AGG GCA GCA AGA GGA GA F: AAC ACA TTT CCT GGC CAA AG	54	563	MH704814—MH704828
ZNFP	Toll-like-2. Transmembrane receptor with TIR domain binding	5	5	5	F: TCC TTG CGG TAA TGA TGT GA F: CTC GAA TGG TGT ACG TGG TG	54	588	MH704829—MH704852

Gene IDs correspond to those in the *Rhodnius* genome GFF file annotation.

≀≀Published before. *Rn*: *R. neglectus; Rp*: *R. prolixus; Rt*: *R. taquarussuensis*

https://doi.org/10.1371/journal.pone.0211285.t001

to the *R. prolixus* transcriptome and thus confirm they were single copy markers. Then, we verified the identity of the selected exons in Uniprot with the ID codes registered in the genome. Finally, we designed primers for these loci using Primer 3 [30]. The resulting nuclear markers are Putative chitin binding peritrophin-a (PCB), DNA topoisomerase (TOPO), Uroporphyrinogen decarboxylase (URO) and Toll-Like-2. Transmembrane receptor with TIR domain binding (ZNFP) (Table 1 and Table 2).

PCR reactions had a final volume of 25 μl, consisting of 12.5 μl of GoTaq Green Master Mix (Promega, Madison, WI, USA), 1.25 μL (10 μM) of each primer and, 5.0 μl of DNA (20 ng) and 5μL of H_2O. Amplification was conducted in a Thermal Cycler 4000 (Bio-Rad La-boratories, Inc., Hercules, CA, USA). The following PCR cycling conditions were used: 94˚C for 5 min; 40 cycles of 94˚C for 1 min, 50–56˚C for 1 min (Table 1), and a final extension at 72˚C for 10 min. PCR success was verified by electrophoresis on agarose gel stained with Fast SYBR Green (Applied Biosystems, Foster City, CA, USA) and a molecular weight marker (Promega) adding 2μl of each PCR product. The samples were purified using the PCR kit ExoSAP-IT Product Cleanup (Affymetrix, Santa Clara, CA, USA) and sequenced at Macrogen Inc. (Seoul, Korea).

Sequence analyses

Gene sequences were read, edited and aligned with CLC Main Workbench (Qiagen). For nuclear loci, haplotype inference for heterozygous calls was conducted using the PHASE algorithm implemented in DnaSP v5 [31], accepting haplotypes with a confidence higher than 90% after running 5,000 interactions per simulation. Then, we created alignments for each locus using MUSCLE [32] with the default parameters. These alignments were visualized and corrected by hand in MEGA X [33]. Finally, we translated the sequences to proteins in order to verify for stop codons using MESQUITE 3.04 [34].

Molecular phylogenetics and species delimitation

In order to assess the position of *R. taquarussuensis* within the group *prolixus*, we downloaded from the Genbank all CYTB sequences available for this group and one from *Triatoma infestans* (outgroup; S1 Table) using the following Entrez line: "esearch -db nucleotide -query "<organism> CYTB"| efetch -format fasta" [35]. We combined these data with our sequences and estimated a phylogenetic tree for the group *prolixus* using a Maximum likelihood (ML) optimization in IQ-TREE [36]. The substitution model for CYTB was established in the same software, selecting the model with the lowest BIC score. Node support was calculated with 1,000 ultrafast bootstrap replicates.

Table 2. Nuclear markers (single copy exons) designed in this study.

Gen	Annotation in the *R. prolixus* genome							Region amplified		
	Gene ID		Scaffold	Strand	Start	End	Size (bp)	Location	Start	End
ZNFP	RPRC009262-RA	Tl-like-2: Toll-like-2. Transmembrane receptor with TIR domain binding	KQ034161	+	481476	486977	5501	Exon 1	481599	482146
URO	RPRC013534-RA	UROD: Uroporphyrinogen decarboxylase	KQ034105	-	970351	971418	1067	Exon 1	970699	971261
TOPO	RPRC012703-RA	DNA topoisomerase	KQ034259	+	391034	406927	15893	Exon 3	404730	405333
PCB	RPRC001863-RA	Putative chitin binding peritrophin-a	KQ034056	+	8334541	8342490	7949	Exon 3	8335296	8336052

Gene IDs correspond to those in the *Rhodnius* genome GFF file annotation.

https://doi.org/10.1371/journal.pone.0211285.t002

We also explored the phylogenetic relationships between *R. prolixus*, *R. neglectus* and *R. taquarussuensis*, concatenating all loci (nuclear and mitochondrial; 3731 bp long alignment) in Mesquite 3.04 [34] and estimating a ML phylogenetic tree with in IQ-TREE [36]. We allowed each locus to have its own substitution model, and node support was accessed as above. We also conducted a Bayesian analysis independently for each locus using BEAST 2.5, implementing linked and unlinked tree models [37]. We inferred the nucleotide substitution model, range of the rate of heterogeneity, and proportion of invariant positions during the MCMC analysis with the bModelTest package [38], with transition-transversion split option and empirical frequencies. We ran 10'000,000 generations sampling every 1,000 generations and used TRACER [39] to confirm the coverage of the chain (i.e. effective sample size >200). TreeAnnotator [37] was used to construct a consensus tree per locus and the initial 10% trees were discarded as burn-in. We superimposed and plotted consensus gene trees constructing a Multiphylo object with the densiTree function in R [40].

As the resulting ML and Bayesian topologies were identical, we used the ML tree as input for a species delimitation analysis intended to determine the species boundaries between *R. taquarussuensis*, *R. neglectus* and *R. prolixus*. This analysis was carried out under a phylogenetic species concept using the Bayesian and ML version of PTP with 500,000 MCMC generations, thinning = 100 and burn-in = 0.1 [41]. PTP implements a non-ultrametric phylogeny to model speciation rate as the number of substitutions reflected as branch lengths, assuming that the number of substitutions between species are significantly higher than the number of substitutions within species.

Genetic differentiation analysis and haplotype networks

We calculated segregating sites (SS), nucleotide diversity (π), haplotype diversity (Hd), number of synonymous and non-synonymous substitutions, singletons and Tajima's D with DnaSP v5 [31]. We did not calculate relative genetic differentiation (F_{ST}) as it has been shown to be overestimated when low nucleotide diversities are obtained [42], as in our dataset (Table 3). Instead, we calculated an absolute divergence measure (D_{XY}) and its nucleotide diversity corrected version (Da) with DnaSP v5. D_{XY} was visualized as a heatmap drawn with the R package *"fields"*. We also calculated Kimura 2 parameter distance (K2P) which has been previously used in triatomines to validate different species [43].

Genetic clustering between *R. neglectus* and *R. taquarussuensis* was validated with a discriminant analysis of principal components (DAPC) performed with both nDNA and mtDNA using the '*adegenet*' R package [44]. We did this by transforming fasta sequences into a genind object that contains individual genotypes and loading it into '*adegenet*' [44]. We performed a principal component analysis (PCA) on these data and retained the first two components (that accounted for >90% of the total variation in both mtDNA and nDNA). We then applied a discriminant analysis using the dapc function and assuming two prior groups (i.e. two species). This produced a single canonical function that summarizes the individual genetic variability, which was then visualized with a density plot. Finally, we constructed haplotype median-joining networks per locus with POPART [45].

Interspecific crosses

As a first attempt to determine the presence of reproductive isolation between *R. taquarussuensis* and *R. neglectus*, we performed interspecific (direct and reciprocal) and conspecific crosses. These were conducted in the Triatominae insectary of the School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil, following the methodology established by Costa et al. [46] and Mendonça et al. [47]. Each cross was

Table 3. Summary statistics for each locus.

Species	Gene	Pi (π)	SS	Tajima's D[b]	Hd	Synonymous sites	Non- synonymous sites	Singletons
R. neglectus	CYTB	0	0	0	0	0	0	0
	ND4	0.00089	1	-0.61	0.5	0	1	1
	PCB	0.0012	2	1.085	0.49	1	1	0
	TOPO	0	0	0	0	0	0	0
	URO	0.00015	1	-1.15	0.083	0	1	1
	ZNFP	0	0	0	0	0	0	0
R. taquarussuensis	CYTB	1.00E-07	1	-1.05	0.25	1	0	1
	ND4	0	0	0	0	0	0	0
	PCB	0.00074	1	1.38	0.53	0	1	0
	TOPO	0.00353	6	0.02	0.62	3	3	0
	URO	0.00143	4	-1.38	0.56	0	4	3
	ZNFP	0.00091	1	0.85	0.81	0	1	0
R. prolixus	CYTB	0.00965	13	-1.1	1	2	12	12
	ND4	0.00714	4	0	1	1	3	4
	PCB	0.00141	3	0.021	0.35	1	2	0
	TOPO	0.00028	1	-1.14	0.17	1	0	1
	URO	0.00328	4	1.39	0.77	1	3	0
	ZNFP	0.00181	2	1.031	0.53	0	2	0

[b]None of the Tajima's D were significant.

https://doi.org/10.1371/journal.pone.0211285.t003

replicated three times for a total of 12 matings. First, insects were sexed as 5th instar nymphs [48], and males and females were kept separately until they reached the adult stage [49]. Then, a virgin female was placed with a male inside a plastic box (5cm diameter × 10cm height) for a maximum period of 120 days and kept at room temperature. The success or failure of mating was recorded by direct observation. After seven days, we collected the eggs of each cross weekly throughout the females' oviposition period (120 days). The eggs collected were placed inside a plastic box (5cm diameter × 10 cm height) and their hatching was recorded weekly.

We calculated hatching success of the interspecific crosses as a measure of egg viability relative to conspecific crosses. A likelihood approximation was implemented in Betabino 1.1 [50] to analyze these data. Because using a binomial model alone does not account for the variation in hatching rate among families in each type of cross, Betabino fits a beta-binomial distribution to count data (in our case, number of eggs that hatched), thus solving this issue. Four alternative models that contrast the number of parameters in the data (i.e. mean and variance in the hatching rate) were tested. For details see http://www.ucl.ac.uk/~ucbhdjm/bin/betabino/betabino.pdf and the appendix section in [50].

Results

Molecular phylogenetics and species delimitation

All sequences obtained for this study were deposited in the Genbank and their accession numbers are found in Table 1. Our dataset for the CYTB gene consisted of 162 sequences corresponding to six species and confirmed the phylogenetic relationships previously shown by Monteiro et al. [11]. Briefly, the ML topology obtained with this gene (evolution model TN+F+I; BIC score 4339.957) revealed that the *prolixus* group is subdivided into two clades, one exclusively formed by *R. barreti* (Abad-Franch, Palomeque and Monteiro, 2013), and the second consisting of *R. robustus*, *R. montenegrensis*, *R. prolixus*, *R. neglectus*, *R. nasutus* (Stål,

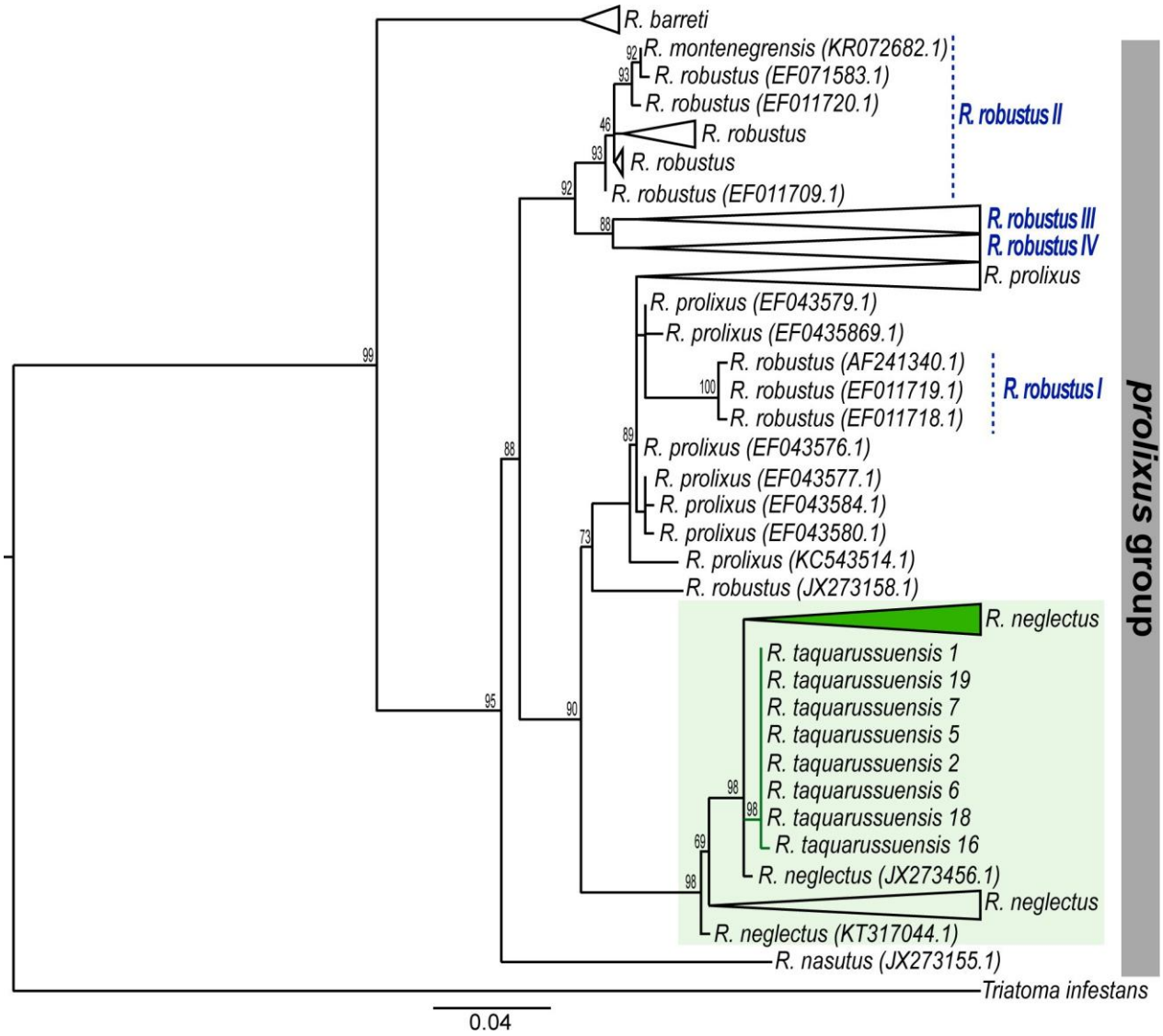


Fig 1. Maximum Likelihood tree for *Rhodnius* based on CYTB. Numbers on the nodes are bootstrap supports. The vertical bar on the right highlight the *prolixus* group. The focal species, namely, *R. taquarussuensis* and *R. neglectus*, are highlighted in the green square. Green branches and the collapsed clade (green triangle) correspond to the sequences obtained here for *R. taquarussuensis* and *R. neglectus* respectively.

https://doi.org/10.1371/journal.pone.0211285.g001

1859), and *R. taquarussuensis*. The relations within this latter clade are complicated. For example, we recovered the four groups previously described for *R. robustus* [11], where *R. robustus*-I falls inside the *R. prolixus* clade, and *R. montenegrensis* is part of *R. robustus*-II (Fig 1 and S1 Fig). Additionally, the species *R. neglectus* is recovered as sister to *R. prolixus* and contains all individuals from the newly described species *R. taquarussuensis*, which although monophyletic, has virtually no differentiation from *R. neglectus* (Fig 1 and S1 Fig).

To better explore this unexpected pattern, we constructed haplotype networks of the gene fragments studied with *R. neglectus*, *R. taquarussuensis* and *R. prolixus* (Fig 2). In the case of CYTB, we found *R. prolixus* separated from the other two species by 15 mutational steps. In contrast, *R. taquarussuensis* haplotypes were less distant to *R. neglectus* (only two mutational steps). In fact, the divergence of *R. taquarussuensis* from *R. neglectus* (H.1 and H.2) is less than the divergence between such haplotypes and others from the same species (i.e. H.3 to H.8).

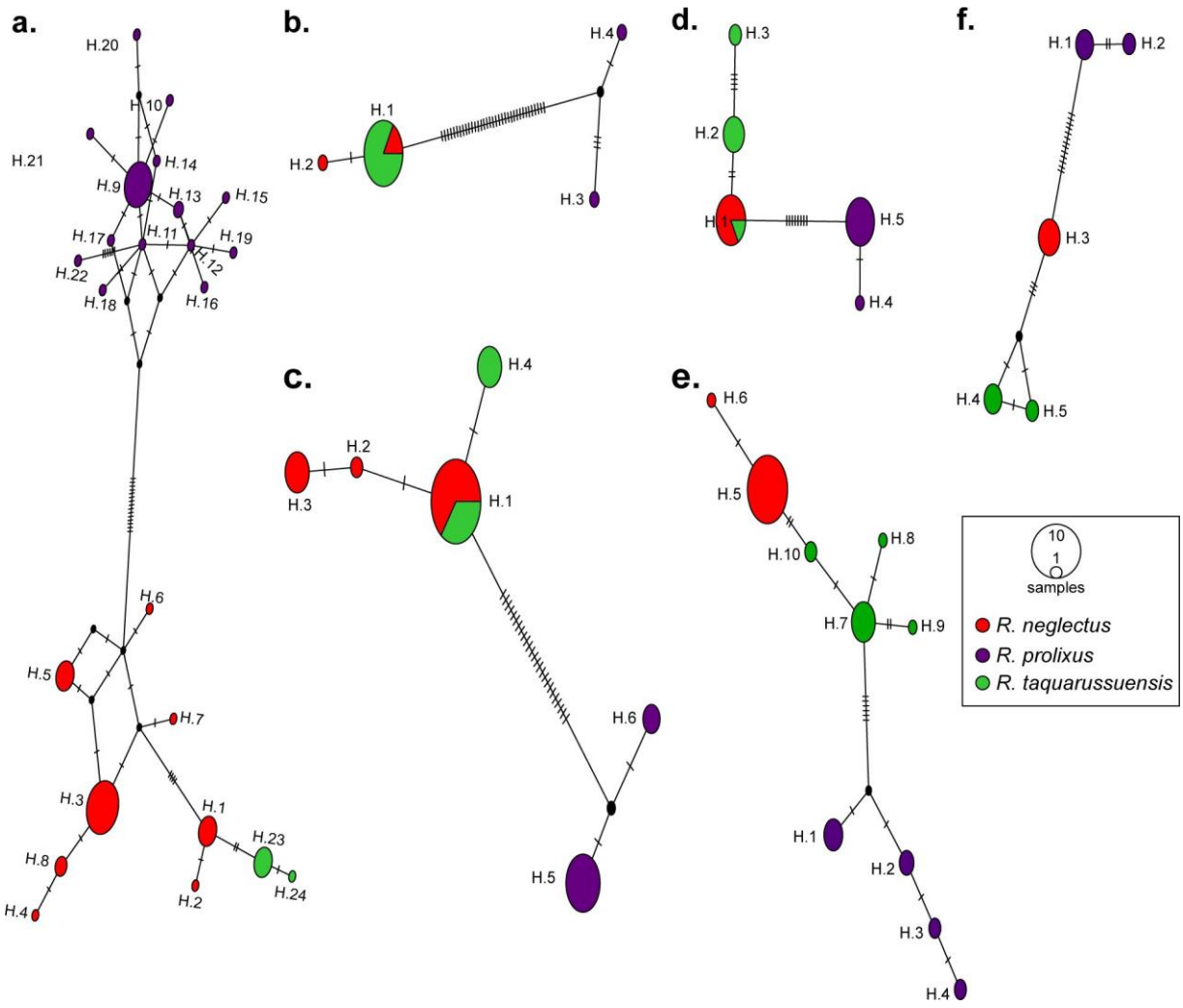


Fig 2. Haplotype networks. (a) CYTB; (b) ND4; (c) PCB; (d) TOPO; (e) URO; (f) ZNFP. Ticks on branches indicate mutational steps between haplotypes. Circle size is proportional to the number of individuals having a haplotype.

https://doi.org/10.1371/journal.pone.0211285.g002

Consistently, nucleotide diversity of *R. prolixus* and *R. neglectus* is higher than that of *R. taquarussuensis* (Table 3).

We recovered the same multilocus phylogeny for *R. prolixus*, *R. neglectus* and *R. taquarussuensis* with ML and Bayesian approaches (ML substitution models were CYTB: HKY+F+I; ND4: HKY+F; PCB: F81+I; TOPO: F81+I; URO: HKY+F; ZNFP: TPM2+F+I). The three species were monophyletic and all of them with posterior probabilities of 100 (Fig 3A) Bootstrap support values were > 90 for *R. prolixus* and *R. neglectus*, while *R. taquarussuensis* has a bootstrap support of 78. Also, the branch length of *R. taquarussuensis* is less than one in a thousand changes. The unlinked and superimposed Bayesian gene trees consistently recovered two main clades: one exclusively composed of *R. prolixus*, and the second where *R. neglectus* and *R. taquarussuensis* show incomplete coalescence (Fig 3B). Consistently, in the analysis of species delimitation (PTP), both the Maximum Likelihood and Bayesian inference found two species as the most probable partition (Fig 4). These two partitions correspond to *R. prolixus* and *R. neglectus*. All other internal nodes had probabilities lower than 0.1 (Fig 4).

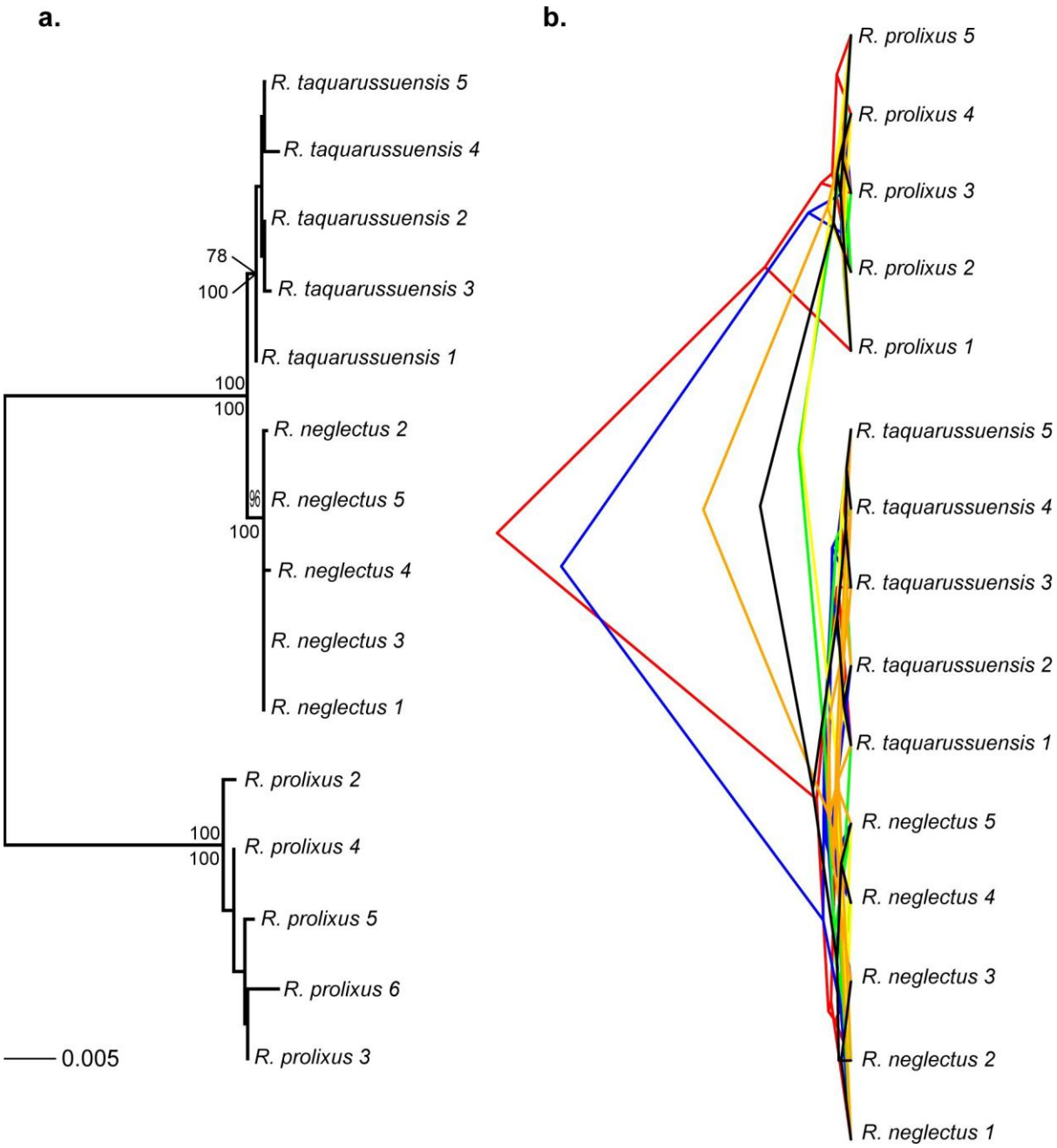


Fig 3. Phylogenetic trees for *R. prolixus*, *R. neglectus* and *R. taquarussuensis* based on all molecular markers. A. Multilocus phylogeny where node support is indicated on each branch: bootstrap (above) and posterior probability (below). B. Bayesian superimposed gene trees: red (CYTB), blue (ND4), green (TOPO), yellow (URO), orange (PCB) and black (ZNFP). The alignment consisted of 3731 bp.

https://doi.org/10.1371/journal.pone.0211285.g003

Genetic differentiation

Overall, all markers showed low genetic diversity for the three taxa, *R. prolixus*, *R. neglectus* and *R. taquarussuensis*. In particular, the loci PCB and ND4 showed the same pattern as CYTB, where *R. taquarussuensis* is less diverse than the other two species (Table 4). The remaining loci showed *R. taquarussuensis* less diverse than *R. prolixus* and the diversity of *R. neglectus* was zero. This is consistent with the low number of haplotypes observed in the

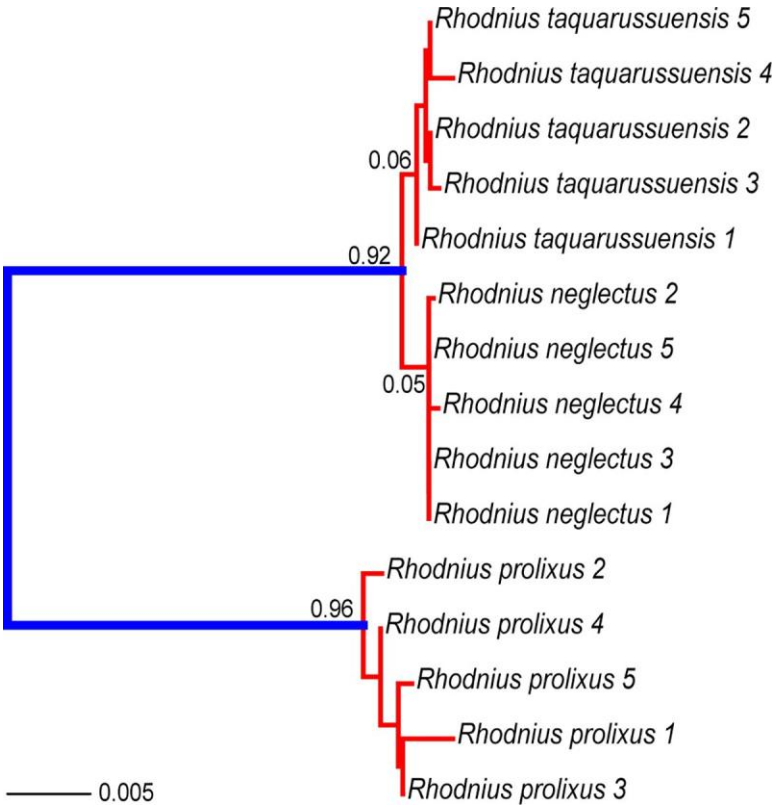


Fig 4. Species delimitation based on the Poisson Tree Process (PTP). Maximum Likelihood and Bayesian inference yielded identical results. Numbers on each node are posterior probabilities of the inner taxa forming one species. Thus, red branches indicate taxa that should be considered as part of the same lineage.

https://doi.org/10.1371/journal.pone.0211285.g004

haplotype networks (Fig 2), where *R. prolixus* has private haplotypes that clearly differentiate it from the other two species (Fig 2B–2F), while *R. taquarussuensis* and *R. neglectus* exhibit substantial haplotype sharing (Fig 2).

Consistent with these findings, D_{XY} shows *R. prolixus* highly differentiated from *R. neglectus* and *R. taquarussuensis* in all loci whilst the latter two taxa do not differentiate between them (S2 Fig). When correcting for the nucleotide diversity, the same pattern is observed (Table 4). The genetic distance (K2P) between *R. neglectus* and *R. taquarussuensis* in all loci was less than 7.5%, a value previously used to define species in triatomines using CYTB [43]. Also, the discriminant analysis = of genetic variation for both mtDNA and nDNA fails to separate the taxa *R. neglectus* and *R. taquarussuensis*, which is reflected by the overlap of their densities on the canonical function (S3 Fig).

Interspecific crosses

All interspecific matings attempted were successful (n = 6), suggesting that there are no mechanical and/or gametic mechanisms that act against hybridization between *R. neglectus* and *R. taquarussuensis*. When we tested homogeneity across categories in the hatching rate, we did not observe differences between interspecific crosses (direct or reciprocal) and controls (Table 5; $G_6 = 7.06$, $P = 0.3152$). Models that have multiple means ($G_3 = 1.243$, $P = 0.7428$) or variances ($G_3 = 2.097$, $P = 0.5525$) for the hatching rate were not supported by the data, indicating the absence of maternal or cytoplasmic effects.

Table 4. Absolute genetic divergence corrected by nucleotide diversity (Da) and Kimura 2 Parameter distance (K2P) between *R. prolixus*, *R. taquarussuensis* and *R. neglectus*.

Gene	Species pair	Da	K2P
CYTB	*R. neglectus–R. taquarussuensis*	0.003	0.003
	R. neglectus–R. prolixus	0.06639	0.082
	R. taquarussuensis–R. prolixus	0.06939	0.086
ND4	*R. neglectus–R. taquarussuensis*	0	0
	R. neglectus–R. prolixus	0.0625	0.075
	R. taquarussuensis–R. prolixus	0.0625	0.075
PCB	*R. neglectus–R. taquarussuensis*	0.00037	0.001
	R. neglectus–R. prolixus	0.0359	0.038
	R. taquarussuensis–R.prolixus	0.0359	0.039
TOPO	*R. neglectus–R. taquarussuensis*	0.00221	0.004
	R. neglectus–R. prolixus	0.01325	0.014
	R.taquarussuensis–R. prolixus	0.01545	0.018
URO	*R. neglectus–R. taquarussuensis*	0.00476	0.005
	R. neglectus–R. prolixus	0.01701	0.017
	R. taquarussuensis–R. prolixus	0.01701	0.012
ZNFP	*R. neglectus–R. taquarussuensis*	0.00635	0.007
	R. neglectus–R. prolixus	0.02234	0.024
	R. taquarussuensis–R. prolixus	0.02585	0.028

https://doi.org/10.1371/journal.pone.0211285.t004

Discussion

Rhodnius exhibits morphological traits that facilitate its identification at the genus level [18, 51], but the low morphological variation within the genus precludes an easy species identification based on morphology alone [23]. This has led to suggest the existence of cryptic species in *Rhodnius*, where multiple look-alike lineages should be considered as different species based on their genetic differentiation [11, 16, 23, 51]. However, morphological species identification in *Rhodnius* relies on intraspecifically variable traits, which can lead to over-estimate the number of species [5]. Therefore, it is necessary to validate the status of the currently described species in the genus implementing a comprehensive approach that uses morphology, genetics, and measures of reproductive isolation.

R. taquarussuensis is the most recently described species in *Rhodnius*, based on morphological, morphometric and cytogenetic evidence [22]. However, the description of this species lacked other crucial evidence. Here, we tested the species status of *R. taquarussuensis* sequencing six molecular markers and performing interspecific crosses. Our results suggest that, despite the morphological differences between *R. taquarussuensis* and *R. neglectus* [22], these taxa constitute a single species.

Firstly, the known distribution range of *R. taquarussuensis* overlaps that of *R. neglectus* (Fig 5). Thus, for them to be different species it would be necessary to evolve strong intrinsic and/

Table 5. Results for interspecific and conspecific crosses. R denotates replicate number for each cross. SE = standard error.

Type of cross		Laid eggs (hatched)				Proportion of viable eggs (SE)	Variance (SE)
		R1	R2	R3	Total		
Interspecific	*R. taquarussuensis* ♀ x *R. neglectus* ♂	230 (198)	86 (80)	230 (193)	510 (471)	0.83 (0.03)	0.0016 (0.002)
	R. neglectus ♀ x *R. taquarussuensis* ♂	300 (275)	181 (105)	256 (244)	708 (624)	0.88 (0.02)	0.0006 (0.0007)
Conspecific	*R. neglectus* ♀ x *R. neglectus* ♂	337 (308)	409 (346)	174 (155)	901 (809)	0.86 (0.02)	0.0001 (0.0016)
	R. taquarussuensis ♀ x *R. taquarussuensis* ♂	151 (127)	168 (150)	201 (156)	501 (433)	0.78 (0.14)	0.034 (0.046)

https://doi.org/10.1371/journal.pone.0211285.t005

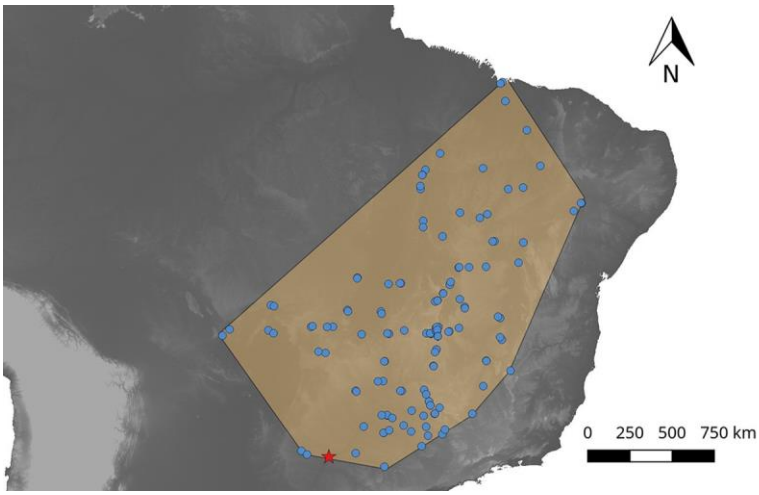


Fig 5. Geographical distribution of *R. neglectus* (blue) and *R. taquarussuensis* (red). Distribution of *R. neglectus* is based on records available on DataTri [55] whilst that of *R. taquarussuensis* is based on collections made by the authors.

https://doi.org/10.1371/journal.pone.0211285.g005

or extrinsic isolation barriers that restrict gene flow. In contrast, we found that *R. taquarussuensis* and *R. neglectus* successfully cross and there are no maternal or cytoplasmic effects that affect offspring viability, as reflected by the high hatching rates we obtained. This also suggests the absence of mechanical or gametic mechanisms acting against their hybridization. Although we did not test the fertility of the "hybrid" offspring, the egg viability observed in our crosses is higher than that reported for other interspecific crosses between different species in the subfamily Triatominae, where hybrid disfunction has been detected [47, 52–54]. However, the role of other pre-zygotic barriers such as temporal asynchrony, mate choice and/or habitat differences, among others, remains to be tested.

Secondly, our phylogenies and haplotype networks showed *R. taquarussuensis* nested within *R. neglectus*, with no differentiation from this species. Consequently, the species delimitation analysis collapsed these two taxa as a single one. Additionally, genetic differentiation measures as well as the discriminant analysis failed to show genetic structure between these lineages. Recent genomic analysis in animals have established that 'good-species' usually have a genetic divergence (Da) > 2%, although there is a "grey zone" of speciation (in which taxonomy is often controversial), that spans from 0.5% to 2% of Da. However, any Da < 0.5% undoubtedly corresponds to populations of the same species [56]. Therefore, our Da values are consistent with a scenario of *R. taquarussuensis* being *R. neglectus* rather than a different species. Furthermore, our genetic distance (K2P) estimates between *R. neglectus* and *R. taquarussuensis* were lower than those between *R. neglectus* and *R. prolixus*, and between *R. taquarussuensis* and *R. prolixus*. This genetic similarity between *R. taquarussuensis* and *R. neglectus* in all our analyses contrast with the clear differentiation observed between *R. neglectus* and *R. prolixus*, which are known to be distinct yet closely related species. In agreement with these findings, recent studies have suggested that *R. milesi* (Carcavalho et al., 2001), another species described based on cytogenetic differences [57, 58], shows high genetic similarity with *R. neglectus* thus questioning its validity as a true species [11]. This further suggests that *R. neglectus* may be a species that shows important polymorphism in cytogenetic patterns, which should not be used for species diagnosis.

The original description of *R. taquarussuensis* reported differences in the constitutive heterochromatin pattern and nanocomposition of TA and CG rich DNA base pairs between *R.*

taquarussuensis and *R. neglectus*, mainly because *R. taquarussuensis* shows more heterochromatic blocks in the autosomes and the Y chromosome compared to the other *Rhodnius* species. Although gain and/or loss of constitutive heterochromatin has been previously used as evidence of species differentiation in the *R. pallescens* group [59], the *T. sordida* subcomplex [60, 61], and *T. dimidiata* (Latreille, 1811) [62], such heterochromatin differences between *R. neglectus* and *R. taquarussuensis* are likely just intraspecific polymorphism of *R. neglectus*. The presence of intraspecific heterochromatin variation with no apparent consequences on speciation is not new in Triatominae and has been observed in *T. infestans* (Klug, 1834) [63–65], *P. geniculatus* (Latreille, 1811) [66], and *R. pallescens* [67]. Therefore, although cytogenetics is a valuable methodology for taxonomic studies [68], heterochromatin variation between populations (i.e. the existence of cytotypes) is not a reliable trait to delimit species when evaluated alone. This agrees with the fact that cytogenetics is known to have a 20% failure rate in delimiting arthropods' species [69]. In conclusion, after performing a comprehensive analysis using mitochondrial and newly developed nuclear markers, as well as crosses between *R. taquarussuensis* and *R. neglectus*, we can confidently suggest that *R. taquarussuensis* is not a separate species and must be considered a synonym of *R. neglectus*. Our study highlights the importance of revising carefully the current taxonomy of *Rhodnius*, because only a confident species delimitation will permit to study the processes and mechanisms involved in their diversification, as well as to unveil vector/parasite associations with epidemiological relevance.

Supporting information

S1 Table. CYTB accession number for individuals downloaded from GenBank.
(DOCX)

S1 Fig. CYTB Maximum likelihood phylogeny.
(DOCX)

S2 Fig. Absolute genetic divergence (DXY) between *R. prolixus*, *R. neglectus* and *R. taquarussuensis*. (a) CYTB; (b) ND4; (c) PCB; (d) TOPO; (e) URO; (f) ZNFP. Note that DXY scale for all genes is not the same.
(DOCX)

S3 Fig. Discriminant analysis based on mtDNA (a) and nDNA (b). Densities for a single discriminant function are shown, with red being *R. taquarussuensis* and blue being *R. neglectus*.
(DOCX)

Author Contributions

Conceptualization: Juliana Damieli Nascimento, João Aristeu da Rosa, Carolina Hernández, Camilo Salazar, Juan David Ram´ırez.

Data curation: Juliana Damieli Nascimento, João Aristeu da Rosa, Fabian C. Salgado-Roa, Carolina Hernández, Kaio Cesar Chaboli Alevi, Camilo Salazar, Juan David Ramírez.

Formal analysis: Juliana Damieli Nascimento, Fabian C. Salgado-Roa, Carolina Hernández, Carolina Pardo-Diaz, Kaio Cesar Chaboli Alevi, Camilo Salazar, Juan David Ram´ırez.

Funding acquisition: Juliana Damieli Nascimento, Camilo Salazar, Juan David Ram´ırez.

Investigation: Fabian C. Salgado-Roa, Carolina Hernández, Carolina Pardo-Diaz, Amanda Ravazi, Jader de Oliveira, Maria Terc´ılia Vilela de Azeredo Oliveira, Camilo Salazar, Juan David Ram´ırez.

Methodology: Fabian C. Salgado-Roa, Carolina Hernández, Carolina Pardo-Diaz, Kaio Cesar Chaboli Alevi, Amanda Ravazi, Jader de Oliveira, Maria Terc´ılia Vilela de Azeredo Oliveira, Camilo Salazar, Juan David Ram´ırez.

Project administration: Camilo Salazar, Juan David Ram´ırez.

Resources: Juan David Ram´ırez.

Software: Fabian C. Salgado-Roa, Juan David Ram´ırez.

Supervision: Camilo Salazar, Juan David Ram´ırez.

Validation: Fabian C. Salgado-Roa, Amanda Ravazi, Camilo Salazar, Juan David Ram´ırez.

Visualization: Fabian C. Salgado-Roa, Amanda Ravazi, Juan David Ram´ırez.

Writing – original draft: Juliana Damieli Nascimento, Camilo Salazar, Juan David Ram´ırez.

Writing – review & editing: Juliana Damieli Nascimento, João Aristeu da Rosa, Fabian C. Salgado-Roa, Carolina Hernández, Carolina Pardo-Diaz, Kaio Cesar Chaboli Alevi, Amanda Ravazi, Jader de Oliveira, Maria Terc´ılia Vilela de Azeredo Oliveira, Camilo Salazar, Juan David Ram´ırez.

References

1. Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, et al. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution. 2007; 22(3):148–55. https://doi.org/10.1016/j.tree.2006.11.004.
2. Minard G, Tran Van V, Tran FH, Melaun C, Klimpel S, Koch LK, et al. Identification of sympatric cryptic species of *Aedes albopictus* subgroup in Vietnam: new perspectives in phylosymbiosis of insect vector. Parasites & Vectors. 2017; 10(1):276. https://doi.org/10.1186/s13071-017-2202-9 PMID: 28577575
3. Noireau F, Gutierrez T, Zegarra M, Flores R, Brenière F, Cardozo L, et al. Cryptic speciation in *Triatoma sordida* (Hemiptera: Reduviidae) from the Bolivian Chaco. Tropical medicine & international health: TM & IH. 1998; 3(5):364–72.
4. Skoracka A, Magalhães S, Rector BG, Kuczyński L. Cryptic speciation in the Acari: a function of species lifestyles or our ability to separate species? Experimental and Applied Acarology. 2015; 67(2):165–82. https://doi.org/10.1007/s10493-015-9954-8 PMID: 26209969
5. Zapata F, Jimenez I. Species delimitation: inferring gaps in morphology across geography. Systematic biology. 2012; 61(2):179–94. Epub 2011/08/16. https://doi.org/10.1093/sysbio/syr084 PMID: 21840841.
6. Galvão C. Vetores da doença de chagas no Brasil: SciELO-Sociedade Brasileira de Zoologia; 2014.
7. WHO. Chagas Disease (American trypanosomiasis). Fact sheet 340. 2018; http://www.who.int/mediacentre/factsheets/fs340/en/ 2018(cited 2018 October 3).
8. Lent H, Wygodzinsky P. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bulletin of the American museum of Natural History. 1979; 163(3):123–520.
9. Pinto C. Valor do rostro e antenas na caracterização dos gêneros de Triatomídeos. Hemiptera, Reduvidioidea. Bol Biol. 1931; 19:45–136.
10. Justi SA, Galvão C, Schrago CG. Geological changes of the Americas and their influence on the diversification of the Neotropical kissing bugs (Hemiptera: Reduviidae: Triatominae). PLoS neglected tropical diseases. 2016; 10(4):e0004527. https://doi.org/10.1371/journal.pntd.0004527 PMID: 27058599
11. Monteiro FA, Weirauch C, Felix M, Lazoski C, Abad-Franch F. Evolution, Systematics, and Biogeography of the Triatominae, Vectors of Chagas Disease. Advances in parasitology. 2018; 99:265–344. https://doi.org/10.1016/bs.apar.2017.12.002 PMID: 29530308
12. Monteiro FA, Barrett TV, Fitzpatrick S, Cordon-Rosales C, Feliciangeli D, Beard CB. Molecular phylogeography of the Amazonian Chagas disease vectors *Rhodnius prolixus* and *R. robustus*. Molecular Ecology. 2003; 12(4):997–1006. PMID: 12753218
13. Rosa JA, Mendonça VJ, Gardim S, de Carvalho DB, de Oliveira J, Nascimento JD, et al. Study of the external female genitalia of 14 *Rhodnius* species (Hemiptera, Reduviidae, Triatominae) using scanning electron microscopy. Parasites & vectors. 2014; 7(1):17.

14. Neiva A, Pinto C. Estado actual dos conhecimentos sobre o gênero *Rhodnius* Stål, com a descrição de uma nova espécie. Bras Med. 1923; 37:20–4.

15. Pavan M, Monteiro F. A multiplex PCR assay that separates *Rhodnius prolixus* from members of the *Rhodnius robustus* cryptic species complex (Hemiptera: Reduviidae). Tropical Medicine & International Health. 2007; 12(6):751–8.

16. Pavan MG, Mesquita RD, Lawrence GG, Lazoski C, Dotson EM, Abubucker S, et al. A nuclear single-nucleotide polymorphism (SNP) potentially useful for the separation of *Rhodnius prolixus* from members of the *Rhodnius robustus* cryptic species complex (Hemiptera: Reduviidae). Infection, Genetics and Evolution. 2013; 14:426–33. https://doi.org/10.1016/j.meegid.2012.10.018. PMID: 23219914

17. Barata JMS. Aspectos morfológicos de ovos de triatominae: II-Características macroscópicas e exocoriais de dez espécies do gênero *Rhodnius* Stal, 1859 (Hemiptera-Reduviidae). Revista de Saúde pública. 1981; 15:490–542. PMID: 7048506

18. Rosa JA, Rocha CS, Gardim S, Pinto MC, Mendonca VJ, Ferreira Filho J, et al. Description of *Rhodnius montenegrensis* n. sp.(Hemiptera: Reduviidae: Triatominae) from the state of Rondônia, Brazil. Zootaxa. 2012; 3478:62–76.

19. Almeida FBd, Santos EI, Sposina G. Triatomíneos da Amazônia III.(). Acta Amazônica. 1973; 3(2):43–6.

20. Lent H, Jurberg J, Galvão C. Rhodnius stali n. sp. related to *Rhodnius pictipes* Stal, 1872 (Hemiptera, Reduviidae, Triatominae). Memórias do Instituto Oswaldo Cruz. 1993; 88(4):605–14.

21. Abad-Franch F, Monteiro FA, Jaramillo N, Gurgel-Gonçalves R, Dias FBS, Diotaiuti L. Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: a multi-scale appraisal of the tribe Rhodniini (Triatominae). Acta Tropica. 2009; 110(2–3):159–77. https://doi.org/10.1016/j.actatropica.2008.06.005 PMID: 18619938

22. Rosa JA, Justino HHG, Nascimento JD, Mendonça VJ, Rocha CS, de Carvalho DB, et al. A new species of *Rhodnius* from Brazil (Hemiptera, Reduviidae, Triatominae). ZooKeys. 2017;(675):1. https://doi.org/10.3897/zookeys.675.12024 PMID: 28769676

23. Justi SA, Galvão C. The evolutionary origin of diversity in Chagas disease vectors. Trends in parasitology. 2017; 33(1):42–52. https://doi.org/10.1016/j.pt.2016.11.002 PMID: 27986547

24. Hypša V, Tietz DF, Zrzavý J, Rego RO, Galvao C, Jurberg J. Phylogeny and biogeography of Triatominae (Hemiptera: Reduviidae): molecular evidence of a New World origin of the Asiatic clade. Molecular phylogenetics and evolution. 2002; 23(3):447–57. PMID: 12099798

25. Justi SA, Russo CA, dos Santos Mallet JR, Obara MT, Galvão C. Molecular phylogeny of Triatomini (Hemiptera: Reduviidae: Triatominae). Parasites & vectors. 2014; 7(1):149.

26. Lyman DF, Monteiro FA, Escalante AA, Cordon-Rosales C, Wesson DM, Dujardin J-P, et al. Mitochondrial DNA sequence variation among triatomine vectors of Chagas' disease. The American journal of tropical medicine and hygiene. 1999; 60(3):377–86. PMID: 10466963

27. Meneguetti DUdO Soares EB, Campaner M Camargo LMA. First report of *Rhodnius montenegrensis* (Hemiptera: Reduviidae: Triatominae) infection by Trypanosoma rangeli. Revista da Sociedade Brasileira de Medicina Tropical. 2014; 47(3):374–6. PMID: 24728471

28. Ravazi A, Alevi K, Oliveira J, Rosa J, Azeredo-Oliveira M. Cytogenetic analysis in different populations of *Rhodnius prolixus* and *R. nasutus* from different countries of South America. Brazilian Journal of Biology. 2018; 78(1):183–5.

29. Díaz S, Panzera F, Jaramillo-O N, Pérez R, Fernández R, Vallejo G, et al. Genetic, cytogenetic and morphological trends in the evolution of the *Rhodnius* (Triatominae: Rhodniini) trans-Andean group. PLoS One. 2014; 9(2):e87493. https://doi.org/10.1371/journal.pone.0087493 PMID: 24498330

30. Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. Bioinformatics (Oxford, England). 2007; 23(10):1289–91. Epub 2007/03/24. https://doi.org/10.1093/bioinformatics/btm091 PMID: 17379693.

31. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinforma. 2009; 25. https://doi.org/10.1093/bioinformatics/btp187 PMID: 19346325

32. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004; 32. https://doi.org/10.1093/nar/gkh340 PMID: 15034147

33. Kumar V, Reinartz W. Customer relationship management: Concept, strategy, and tools: Springer; 2018.

34. Maddison WPMaddison D. Mesquite: a modular system for evolutionary analysis. Version 3.04. 2015.

35. Maglott D, Ostell J, Pruitt KD, Tatusova T. Entrez Gene: gene-centered information at NCBI. Nucleic acids research. 2010; 39(suppl_1):D52–D7.

36. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular biology and evolution. 2014; 32(1):268–74. https://doi.org/10.1093/molbev/msu300 PMID: 25371430

37. Bouckaert R, Vaughan TG, Barido-Sottani J, Duchene S, Fourment M, Gavryushkina A, et al. BEAST 2.5: An Advanced Software Platform for Bayesian Evolutionary Analysis. bioRxiv. 2018.

38. Bouckaert RR, Drummond AJ. bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evolutionary Biology. 2017; 17(1):42. https://doi.org/10.1186/s12862-017-0890-6 PMID: 28166715

39. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic biology. 2018; 67(5):901–4. https://doi.org/10.1093/sysbio/syy032 PMID: 29718447

40. Schliep KP. phangorn: phylogenetic analysis in R. Bioinformatics (Oxford, England). 2011; 27(4):592–3. Epub 2010/12/21. https://doi.org/10.1093/bioinformatics/btq706 PMID: 21169378; PubMed Central PMCID: PMCPmc3035803.

41. Zhang J, Kapli P, Pavlidis P, Stamatakis A. A general species delimitation method with applications to phylogenetic placements. Bioinformatics (Oxford, England). 2013; 29(22):2869–76.

42. Merilä J, Crnokrak P. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology. 2001; 14(6):892–903. https://doi.org/10.1046/j.1420-9101.2001.00348.x

43. Monteiro FA, Donnelly MJ, Beard CB, Costa J. Nested clade and phylogeographic analyses of the Chagas disease vector *Triatoma brasiliensis* in Northeast Brazil. Mol Phylogenet Evol. 2004; 32(1):46–56. Epub 2004/06/10. https://doi.org/10.1016/j.ympev.2003.12.011 PMID: 15186796.

44. Jombart T, Ahmed I. adegenet 1.3–1: new tools for the analysis of genome-wide SNP data. Bioinformatics (Oxford, England). 2011; 27(21):3070–1.

45. Leigh JW, Bryant D. popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution. 2015; 6(9):1110–6.

46. Costa J, Almeida CE, Dujardin JP, Beard CB. Crossing experiments detect genetic incompatibility among populations of *Triatoma brasiliensis* Neiva, 1911 (Heteroptera, Reduviidae, Triatominae). Memórias do Instituto Oswaldo Cruz. 2003; 98(5):637–9. PMID: 12973530

47. Mendonça VJ, Alevi KCC, de Oliveira Medeiros LM, Nascimento JD, de Azeredo-Oliveira MTV, da Rosa JA. Cytogenetic and morphologic approaches of hybrids from experimental crosses between *Tria- toma lenti* Sherlock & Serafim, 1967 and *T. sherlocki* Papa et al., 2002 (Hemiptera: Reduviidae). Infec- tion, Genetics and Evolution. 2014; 26:123–31. https://doi.org/10.1016/j.meegid.2014.05.015 PMID: 24861813

48. Rosa JA, Barata JMS, Barelli N, Santos JLF, Neto B, Miguel F. Sexual distinction between 5th instar nymphs of six species of Triatominae (Hemiptera, Reduviidae). Memórias do Instituto Oswaldo Cruz. 1992; 87(2):257–64.

49. Martínez-Ibarra J, Grant-Guillén Y, Delgadillo-Aceves I, Zumaya-Estrada F, Rocha-Chávez G, Salazar-Schettino P, et al. Biological and genetic aspects of crosses between phylogenetically close species of mexican triatomines (Hemiptera: Reduviidae). Journal of medical entomology. 2011; 48(3):705–7. PMID: 21661335

50. Jiggins CD, Linares M, Naisbit RE, Salazar C, Yang ZH, Mallet J. Sex-linked hybrid sterility in a butterfly. Evolution; international journal of organic evolution. 2001; 55(8):1631–8. Epub 2001/10/03. PMID: 11580022.

51. Abad-Franch F, Pavan MG, Jaramillo-O N, Palomeque FS, Dale C, Chaverra D, et al. *Rhodnius barretti*, a new species of Triatominae (Hemiptera: Reduviidae) from western Amazonia. Memórias do Instituto Oswaldo Cruz. 2013; 108:92–9. https://doi.org/10.1590/0074-0276130434 PMID: 24473808

52. Heitzmann-Fontenelle T. Bionomia comparativa de triatom´ıneos. VI-H´ıbridos de *Triatoma brasiliensis* Neiva, 1911 X *Triatoma lenti*, Sherlocki & Serafim, 1967 (Hemiptera, Reduviidade). Memórias do Instituto Butantan. 1984; 47:175–81.

53. Mendonca VJ, Alevi KCC, Pinotti H, Gurgel-Goncalves R, Pita S, Guerra AL, et al. Revalidation of *Triatoma bahiensis* Sherlock & Serafim, 1967 (Hemiptera: Reduviidae) and phylogeny of the *T. brasiliensis* species complex. Zootaxa. 2016; 4107(2):239–54. https://doi.org/10.11646/zootaxa.4107.2.6 PMID: 27394816

54. Perez R, Hernández M, Quintero O, Scvortzoff E, Canale D, Mendez L, et al. Cytogenetic analysis of experimental hybrids in species of Triatominae (Hemiptera-Reduviidae). Genetica. 2005; 125(2–3):261–70. https://doi.org/10.1007/s10709-005-0369-z PMID: 16247698

55. Ceccarelli S, Balsalobre A, Medone P, Cano ME, Gonçalves RG, Feliciangeli D, et al. DataTri, a database of American triatomine species occurrence. Scientific data. 2018; 5.

56. Roux C, Fraisse C, Romiguier J, Anciaux Y, Galtier N, Bierne N. Shedding light on the grey zone of speciation along a continuum of genomic divergence. PLoS biology. 2016; 14(12):e2000234. https://doi.org/10.1371/journal.pbio.2000234 PMID: 28027292

57. Pita S, Panzera F, Ferrandis I, Galvão C, Gómez-Palacio A, Panzera Y. Chromosomal divergence and evolutionary inferences in Rhodniini based on the chromosomal location of ribosomal genes. Memórias do Instituto Oswaldo Cruz. 2013; 108(3):376–82.

58. Valente VdC, Valente SAdS, Carcavallo RU, Rocha DdS, Galvão C, Jurberg J. Considerações sobre uma nova espécie do gênero *Rhodnius* stal, do Estado do Pará, Brasil (Hemiptera, Reduviidae, Triatominae. Entomol vectores. 2001; 8(1):65–80.

59. Alevi K, Ravazi A, Franco-Bernardes M, Rosa J, Azeredo-Oliveira M. Chromosomal evolution in the pallescens group (Hemiptera, Triatominae). Genetics and Molecular Research. 2015; 14(4):12654–9. https://doi.org/10.4238/2015.October.19.9 PMID: 26505416

60. Bardella VB, Pita S, Vanzela ALL, Galvão C, Panzera F. Heterochromatin base pair composition and diversification in holocentric chromosomes of kissing bugs (Hemiptera, Reduviidae). Memórias do Instituto Oswaldo Cruz. 2016; 111(10):614–24. https://doi.org/10.1590/0074-02760160044 PMID: 27759763

61. Panzera F, Hornos S, Pereira J, Cestau R, Canale D, Diotaiuti L, et al. Genetic variability and geographic differentiation among three species of triatomine bugs (Hemiptera-Reduviidae). The American journal of tropical medicine and hygiene. 1997; 57(6):732–9. PMID: 9430537

62. Panzera F, Ferrandis I, Ramsey J, Ordonez R, Salazar-Schettino P, Cabrera M, et al. Chromosomal variation and genome size support existence of cryptic species of *Triatoma dimidiata* with different epidemiological importance as Chagas disease vectors. Tropical Medicine & International Health. 2006; 11(7):1092–103.

63. Panzera F, Dujardin JP, Nicolini P, Caraccio MN, Rose V, Tellez T, et al. Genomic changes of Chagas disease vector, South America. Emerging Infectious Diseases. 2004; 10(3):438. https://doi.org/10.3201/eid1003.020812 PMID: 15109410

64. Panzera F, Ferreiro MJ, Pita S, Calleros L, Pérez R, Basmadjián Y, et al. Evolutionary and dispersal history of *Triatoma infestans*, main vector of Chagas disease, by chromosomal markers. Infection, Genetics and Evolution. 2014; 27:105–13. https://doi.org/10.1016/j.meegid.2014.07.006 PMID: 25017654

65. Panzera Y, Pita S, Ferreiro M, Ferrandis I, Lages C, Pérez R, et al. High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (Triatominae, Heteroptera). Cytogenetic and Genome Research. 2012; 138(1):56–67. https://doi.org/10.1159/000341888 PMID: 22907389

66. Crossa RP, Hernández M, Caraccio MN, Rose V, Valente SAS, da Costa Valente V, et al. Chromosomal evolution trends of the genus *Panstrongylus* (Hemiptera, Reduviidae), vectors of Chagas disease. Infection, Genetics and Evolution. 2002; 2(1):47–56. PMID: 12798000

67. Gómez-Palacio A, Jaramillo-Ocampo N, Triana-Chávez O, Saldaña A, Calzada J, Pérez R, et al. Chromosome variability in the Chagas disease vector *Rhodnius pallescens* (Hemiptera, Reduviidae, Rhodniini). Memórias do Instituto Oswaldo Cruz. 2008; 103(2):160–4. PMID: 18425268

68. Ueshima N. Cytotaxonomy of the triatominae (Reduviidae: Hemiptera). Chromosoma. 1966; 18(1):97–122.

69. Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. Integrative taxonomy: a multisource approach to exploring biodiversity. Annual review of entomology. 2010; 55:421–38. Epub 2009/09/10. https://doi.org/10.1146/annurev-ento-112408-085432 PMID: 19737081.

4.3 Capítulo III

Estudo proteômico de espermatecas fecundadas de *Rhodnius neglectus* (Hemiptera, Reduviidae)

Resumo

Os triatomíneos são os insetos vetores da doença de Chagas portanto sua importância na dispersão da doença é incontestável, uma vez revelado seu papel como vetor, a subfamília Triatominae ganhou um importante apelo epidemiológico. As fêmeas dos triatomíneos possuem o aparelho reprodutor constituído por um par filamento terminal, um par de ovários com múltiplos ovaríolos, um par de oviduto laterias, oviduto comum, um par de espermatecas e a vagina. Nos triatomíneos a espermateca, é um órgão feminino que desempenha um importante papel na reprodução pois é responsável por armazenar e manter os espermatozoides viáveis para fecundação dos ovócitos. A forma como os espermatozoides são mantidos viáveis durante vários meses não é esclarecida do ponto de vista biológico. Em se tratando de Triatominae acrescem outras dúvidas, pois as suas espermatecas não possuem glândula espermatecal como outras espécies da classe Insecta, ou seja, não tem órgão secretor para manter a viabilidade dos espermatozoides. Por questionar de onde provem as substâncias responsáveis pela manutenção da viabilidade dos espermatozoides na espermateca foi proposto o estudo proteômico das espermatecas das fêmeas fecundas de *R. neglectus* para compreender melhor padrões de expressões proteicas por meio da metodologia *shotgun*. Para tanto executou-se análise por espectrometria de massas com a utilização de um sistema electrospray - Ion trap (ESI/IT), modelo Amazon SL (Bruker Daltonics, Bremen, Germany), acoplado a um sistema Ultra Fast liquid Chromatography (UFLC) (Nexera-Shimadzu Corporation, Tokyo, Japan). Por meio de banco de dados específicos para *R.* neglectus os estudos revelaram 32 proteínas no total, entre as quais proteínas musculares como miosinas e actinas e metabólicas com funções muito bem caracterizadas na literatura como V-ATPases e *HSPs*. Portanto, conclui-se que as espermatecas de *R. neglectus* possuem proteínas que auxiliam a manutenção, nutrição e acidificação dos espermatozoides em suas células epiteliais.

Introdução

A espermateca é um órgão fundamental para a reprodução da classe Insecta inclusive dos triatomíneos, pois armazena e mantém a funcionalidade dos espermatozoides depositados no momento da cópula por vários meses e esse período varia de acordo com a espécie. (PASCINI; MARTINS, 2017; PÉREZ, 1969).

Acredita-se que o primeiro estímulo para a produção dos ovócitos provém das espermatecas após o acasalamento. O par de espermateca, que está ligado ao oviduto comum, armazena e mantém os espermatozoides viáveis até momento da fecundação dos ovócitos. Ao dissecar as espermatecas é possível observar nervos e traqueias conectados a sua forte musculatura com camadas de fibras musculares dispostas horizontalmente e verticalmente (DAVEY, 1965; HUEBNER, 1980; NASCIMENTO et al., 2019b).

A espermateca é um órgão oco circundado por uma parede epitelial que delimita sua luz, que por sua vez é preenchida por uma grande quantidade de espermatozoides. Algumas espécies possuem função secretora em sua parede epitelial fato que auxilia a compreensão de como são mantidos os espermatozoides viáveis por longos períodos de tempo. *Apis melifera* (Linnaeus, 1758) possui glândulas laterais junto às espermatecas que liberam substâncias para nutrir e armazenar os espermatozoides (DEN BOER; BOOMSMA; BAER, 2009). Já *Melipona bicolor* (Lepeletier, 1836) não possui glândulas laterais, mas possui ao redor de sua membrana basal a presença de uma substância amórfica que provavelmente deriva do epitélio pseudoestratificado prismático com células serosas alongadas (CRUZ-LANDIM; YABUKI; IAMONTE, 2003; PASCINI; MARTINS, 2017).

Aspectos morfológicos e histológicos das espermatecas de Triatominae foram estudados e atualmente sabe-se que a espermateca dos gêneros *Rhodnius, Panstrongylus* e *Triatoma* possuem duas porções, a primeira porção conectada ao oviduto comum chamada de porção proximal e a segunda porção mais distante do oviduto, denominada porção distal ou de estoque. As espermatecas de *R. neglectus, R. prolixus, Panstrongylus lignarius* (Walker, 1873), *P. megistus* e *Triatoma tibiamaculata* (Pinto, 1926) são constituídas por epitélio simples colunar e *T. infestans* possui epitélio cuboidal achatado na porção de estoque (NASCIMENTO et al., 2019b).

As espermatecas de triatomíneos estão circundadas por traqueias e corpos adiposos que provavelmente suprem suas necessidades fisiológicas na falta das glândulas espermatecais. A morfologia das espermatecas de *T. infestans* é completamente oval, enquanto que *R. montenegrensis*, *R. nasutus*, *R. neglectus, R. pictipes*, *R. prolixus* possuem espermatecas tubulares,

P. lignarius, *P. megistus*, *Triatoma brasiliensis* (Neiva, 1911), *Triatoma juazeirensis* (Costa & Felix, 2007), *Triatoma sherlocki* (Papa, Jurberg, Carcavallo, Cerqueira & Barata, 2002) e *T. tibiamaculata* possuem as porções anteriores tubulares e as posteriores dilatadas (NASCIMENTO et al., 2017, 2019b).

Algumas questões biológicas podem ser avaliadas por meio do estudo de proteínas. Atualmente sabe-se que determinadas células de triatomíneos são responsáveis por identificar patógenos invasores por meio da síntese de pequenas proteínas relacionadas com o sistema imune do inseto e são sintetizadas no intestino médio e pelo corpo adiposo e posteriormente distribuídas no lúmen do intestino e hemolinfa. Também foi observado que algumas bactérias intestinais são capazes de alterar o desenvolvimento e patogenicidade do protozoário *T. cruzi*, as proteínas que constituem o sistema imune podem influenciar no ciclo evolutivo do parasita e forma como o parasita responde a diferentes bactérias intestinais, essa alteração foi observada em *P. megistus*, *R. prolixus*, *T. infestans* e *T. brasiliensis* (ARAÚJO et al., 2015).

O estudo proteômico do intestino médio de *R. prolixus* apresentou 489 proteínas que foram classificadas em 28 grupos funcionais, como metabolismo de nucleotídeos, carboidratos, citoesqueleto, imunidade e lipídeos. Proteínas importantíssimas no desenvolvimento biológico do vetor e na relação da microbiota intestinal com *T. cruzi*, interação que pode interferir no ciclo epidemiológico e na patogenicidade da doença de Chagas (VIEIRA et al., 2015).

Em *T. infestans* e *Triatoma sordida* (Stal, 1859) as células que constituem os folículos testiculares foram marcadas por fibrilarina em alguns pontos das células germinativas durante o processo de espermatogênese, sendo a fibrilarina uma proteína ácida nuclear que possivelmente está envolvida nos corpos cromáticos que auxiliam o processo de formação dos espermatozoides para perpetuação da espécie (SILISTINO-SOUZA et al., 2012).

A coloração vermelha das glândulas salivares do gênero *Rhodnius* é uma característica proporcionada por meio da presença de uma hemoproteína chamada nitroforina, a presença dessa classe de proteína provavelmente é resultado de processos evolutivos do gênero, a nitroforina e outras proteínas foram destacadas no estudo do transcriptoma da saliva de *R. neglectus* trabalho que resultou em mais de cinco mil sequências codificadas distribuídas em cinco grandes grupos que caracterizam as funções proteicas como proteínas secretoras, manutenção metabólica, transporte de elementos, produto viral e produtos desconhecidos (SANTIAGO et al., 2016).

A análise do perfil proteico de espermatecas de fêmeas virgens e fecundadas da espécie de formiga, *Atta sexdens rubropilosa* (Forel, 1908), mostrou-se diferente esse resultado biológico

é importante para a compreensão do mecanismo de armazenamento e nutrição realiza pelas fêmeas nos espermatozoides contidos nas espermatecas (MALTA et al., 2014).

Dada a importância de estudos proteicos para a compreensão de respostas biológicas determinadas em diferentes momentos, foi proposto o estudo do perfil proteico de espermatecas fecundadas de *R. neglectus* com intuito de auxiliar o entendimento da biologia reprodutiva da subfamília Triatominae.

Materiais e métodos

Extração de proteínas das espermatecas

Os espécimes de *R. neglectus* utilizados no estudo foram oriundos de doação do Dr. Cléber Galvão do Instituto Oswaldo Cruz do Rio de Janeiro para o Insetário de Triatominae de Araraquara mantido na Faculdade de Ciências Farmacêuticas da Universidade Estadual Paulista "Júlio de Mesquita Filho" /UNESP. A colônia de *R. neglectus* foi iniciada em 1985 pelo Dr. Ítalo Sherlock na Fundação Gonçalo Muniz da Bahia.

Para a execução do estudo foram utilizadas 40 fêmeas fecundadas mantidas a 27ºC, as fêmeas foram divididas em quatro recipientes de vidro diferentes, cada recipiente com cinco espécimes de macho para garantir a fecundação. Foram alimentados e armazenadas durante sete dias para garantir a cópula, após sete dias, as fêmeas foram dissecadas com auxílio de tesoura, pinça e bisturi e a fecundação observada com auxílio de lupa. Foram retirados o corpo adiposo e aparelho digestório para completa visualização do aparelho reprodutor. As espermatecas fecundadas foram retiradas e colocadas em microtubos de 1,5 ml e armazenadas a -80ºC.

Posteriormente foi adicionado às espermatecas 1 ml de tampão de Tris-HCL 40mM ph 7.5 gelado, inibidor de protease (0,37 mg/ml- complete mini Sigma-Aldrich) adicionou-se 3,7 mg 1mM para 10 ml de H_2O mili-q. As espermatecas junto com as soluções adicionadas, foram sonicadas com ciclos de 10 segundos para intervalos de 2 minutos no gelo por três repetições.

Em seguida foi realizado centrifugação de 15 minutos a 20.000 g a 4ºC. O sobrenadante retirado e armazenado, ao *pellet* acrescentou 1 ml de tampão de Tris-HCL 40 mM pH 7.5 e realizado a centrifugação nas mesmas condições descritas acima. O sobrenadante desta etapa foi adicionado ao da etapa posterior e acrescentado ácido tricloroacético (TCA) 10% em acetona absoluta gelada, na proporção 1:6 – 1 mL de extrato/ 6 mL da solução TCA 10%- acetona, levado a -20ºC por 12 horas.

Após a adição de soluções, realizou-se centrifugação de 20.000 g por 30 minutos a 4ºC para obtenção do precipitado de proteínas. O *pellet* resultante das etapas anteriores foi lavado quatro vezes com acetona 100% gelada. A amostra foi liofilizada e posteriormente armazenada a

-80ºC que no momento do uso foi ressuspendida em bicarbonato de amônio 50mM, o mesmo utilizado para a digestão tríptica.

Eletroforese por técnica de SDS-PAGE

Os perfis proteicos foram analisados por eletroforese em gel de poliacrilamida com dodecil sulfato de sódio, SDS-PAGE, sob condições redutoras, utilizando sistema de tampão descontínuo (LAEMMLI, 1970; STUDIER, 1973). Os componentes foram primeiramente separados em gel a 14% e em gel de empilhamento a 5% de acrilamida. Para a preparação desse gel foi utilizado solução estoque com 30% acrilamida e 0,8% bis-acrilamida. Para o gel de separação a polimerização foi realizada em solução com 1,5M de Tris- HCl, pH 8,8 e 0,4% de SDS, em presença de 0,1%v/v de N, N, N', N'- tetrametiletilenodiamida (TEMED) e 0,1% de persulfato de amônio. O gel de empilhamento foi polimerizado em presença de 0,5M de TrisHCl pH 6,8 e 0,4% de SDS, além de 0,1% de persulfato de amônio e 0,05% v/v de TEMED. Para o preparo da amostra foram utilizados 30µg de proteína de acordo com a quantificação, diluídos em tampão de amostra (1:4), que é composto de 62,5mM de Tris-HCl [tris (hidroxi-metil) aminometano] pH 6,8, 2% de SDS, 10% de glicerol, 0,5M de ditiotreitol e 0,002% de bromofenol. As proteínas foram dissociadas em banho maria por 3 minutos. A corrida eletroforética foi realizada com tampão composto por Tris 0,075M, glicina 0,57M e SDS 0,1%, pH 8,3, e a voltagem utilizada foi 10mA (50V) até a penetração no gel de separação, e a seguir a 20mA (80V) até o final do gel. A revelação do gel foi feita utilizando Azul de Coomassie G-250, para visualização do perfil de bandas específico.

Digestão tríptica

A digestão das amostras foi realizada partindo-se de 600µg de proteínas de acordo com a quantificação. A partir disso, foram acrescentados 100µL de solução de bicarbonato de amônio 50mM e ureia 7,5M às amostras, mantendo-as à 37°C em banho seco durante 60 minutos. Seguindo-se adição de 10µL de solução de ditiotreiol (DTT) 100mM por mais 60 minutos à 37°C. Continuando o processo adicionou-se 11µL de solução de iodoacetamida (IAA) 400mM, à 25°C, protegido da luz por mais 60 minutos. Finalmente foram adicionados 484µL de solução de bicarbonato de amônio 100mM, 0,6µL de solução de cloreto de cálcio 1M e 1:100 de tripsina (Promega, USA), a reação foi incubada à 37°C de 18 a 24h. Para interromper a reação foram utilizados 5µL de ácido fórmico. As amostras foram secas em *speed-vac* e armazenadas à -20°C até análise.

Espectrometria de Massas LC-MS/MS

Os digestos foram analisados por espectrometria de massas com a utilização de um sistema electrospray - Ion trap (ESI/IT), modelo *Amazon SL* (Bruker Daltonics, Bremen, Germany), acoplado a um sistema *Ultra Fast liquid Chromatography* (UFLC) (Nexera-Shimadzu Corporation, Tokyo, Japan). Foram aplicados 50 µL com auxílio do injetor automático (SIL-20AXR, Shimadzu). As análises cromatográficas foram realizadas sob um gradiente: solução (A) – 0,05% de ácido trifluoroacético (TFA) em água, solução (B) - 0,05% de TFA em acetonitrila (ACN) (2-60% de B (0-90 min), 60-90% de B (90-120min), utilizando-se uma coluna *XBrigdeTM BEH300 C18* (2,1x100mm; 3,5 µm) (Waters). A eluição dos componentes foi monitorada por absorbância a 215 nm, com fluxo de 0,2 mL/min.

Para o controle de aquisição dos dados foram utilizados os softwares *HyStar* e *Trap Control* (BRUKER Daltonics). O espectrômetro de massas foi operado no modo íon positivo, com scan num intervalo de 200 a 2200 m/z. As análises de espectrometria de massas sequenciais, ou seja, espectros de fragmentação em condições de decomposição induzida por colisão (CID) (MS2) foram realizadas utilizando-se os mesmos parâmetros dos experimentos de MS1, exceto a varredura que foi feita num intervalo entre 50 e 2200 m/z. Foi utilizado Hélio como gás de colisão, a uma pressão de 100 kPa. O software *Data Analysis* (Bruker Daltonics) foi utilizado para análise de dados.

Identificação de proteínas

Para a identificação das proteínas, a busca foi executada com o software ProteinScape (versão 3.1, Bruker Daltonics) e o algoritmo Mascot (v2.3, Matrix Science, UK), realizando buscas contra os bancos de dados *Rhodnius neglectus*; Swiss Prot e NCBI.

Parâmetros utilizados:

- Enzima: Tripsina;
- Carbamidometilação (C) como modificação fixa;
- Oxidação (M), como modificação variável;
- Nº de clivagens perdidas pela enzima: 2;
- Massa molecular do tipo: Monoisotópica;
- Massa molecular: sem restrições;
- Erro de tolerância de peptídeos ±0,4Da e erro de tolerância de MSn ± 0,4Da;
- Protonação: +2, +3, +4 para o estado da carga dos peptídeos;

- Tipo de instrumento: ESI-Trap;

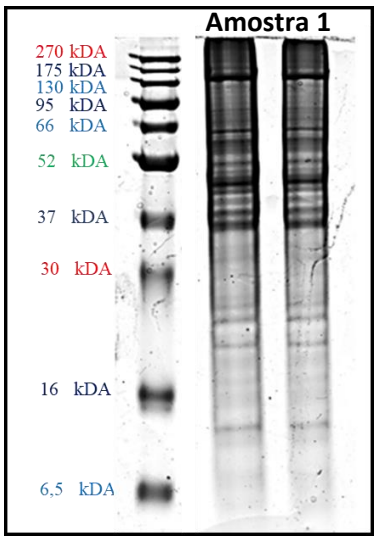
- Protein Scores significativos (p<0,05);

- FDR < 1% (False Discovery Rate) para validar a identificação dos peptídeos.

Resultados

O perfil proteico e a qualidade da extração foram observados por meio do gel SDS-PAGE. No gel de extração é possível visualizar proteínas próximas a 97 kDa, entre 66 kDa e 44kDa, em 30 kDa e próximas a 20 kDa (Figura 1).

Figura 1. Perfil eletroforético de proteínas de espermatecas de *R. neglectus*. Gel SDS-PAGE 12% revelado pelo método Coomasie G-250. Na imagem é possível observar a qualidade da extração da amostra 1 em duplicata com a presença de bandas com massas moleculares que variam de 175 kDa a aproximadamente 15 kDA.



Fonte: Próprio autor.

Após a confirmação da extração das proteínas das espermatecas de *R. neglectus*, a amostra foi preparada para a digestão tríptica e posteriormente aplicada na espectrometria de massa para o *shogtun*. Ao utilizar o banco de dados específico para *R. neglectus* identificou-se 32 proteínas. As proteínas identificadas previamente são descritas na tabela 1.

Tabela 1. Proteínas preditas por espectrometria de massas por meio softwares *HyStar* e *Trap Control* (BRUKER Daltonics) e confrontadas contra o banco de dados de *R. neglectus* (Mascot e ProteinScape) para identificação das sequências de peptídeos. (pI) ponto isoelétrico da proteína; (kDa) massa molecular da proteína. Fonte: Próprio autor.

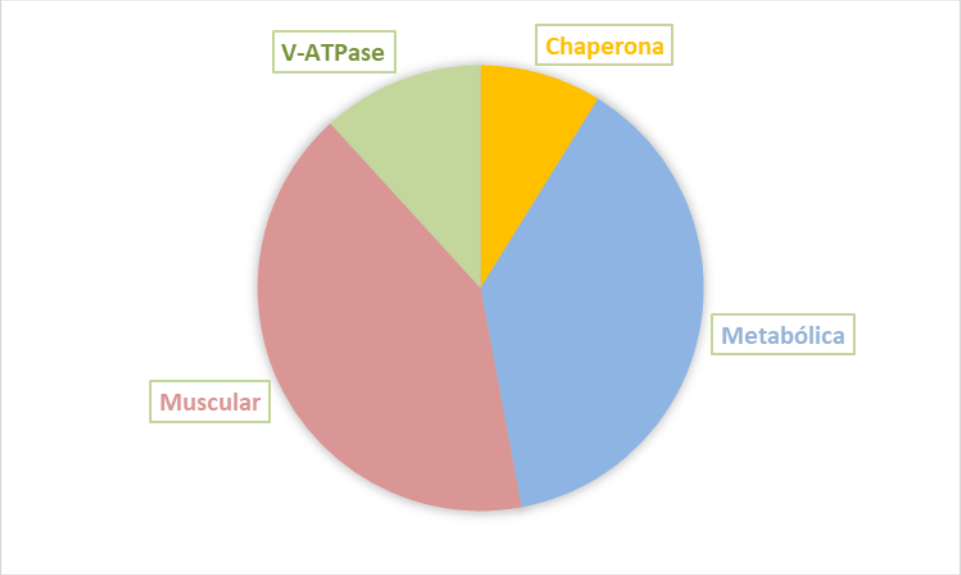
	Código de acesso	Proteínas	Massa (kDa)	Score	Cobertura (%)	pI
1	**JAI53032.1**	Putative myosin class ii heavy chain, partial	159.21	694.3	19,4	5.8
2	**JAI56013.1**	Putative beta-tubulin	50.2	566.2	45.8	4.8
3	**JAI56021.1**	putative actin muscle isoform x2	41.8	503.9	50.8	5.3
4	**JAI54754.1**	putative tubulin beta chain, partial	48.2	367.7	31.3	4.7
5	**JAI55832.1**	putative vacuolar h+-atpase v1 sector subunit b	55.0	352.4	21.8	5.2
6	**JAI54047.1**	putative adaptor protein enigma, partial	57.2	319.2	24.1	7.4
7	**JAI54404.1**	putative aminopeptidase, partial	53.2	290.0	26.4	8.6
8	**JAI55302.1**	putative troponin t skeletal muscle	45.3	276.6	27.2	5.0
9	**JAI53274.1**	putative f0f1-type atp synthase beta subunit	43.1	273.9	21,8	5.1
10	**JAI56007.1**	putative tubulin alpha-1 chain-like protein, partial	50.0	266.2	20.3	5.0
11	**JAI55216.1**	putative vacuolar h+-atpase v1 sector subunit a	67.7	248.0	27.1	5.2
12	**JAI53691.1**	putative actin regulatory gelsolin/villin family	38.7	208.6	32.7	4.9
13	**JAI56019.1**	putative myosin regulatory light chain 2-like protein	22.3	203.8	46.6	4.9
14	**JAI53477.1**	putative small heat shock protein	21.4	192.3	42.5	6.0

15	**JAI54843.1**	putative 4-aminobutyrate aminotransferase, partial	51.9	190.0	14.4	6.5
16	**JAI55337.1**	putative enoyl-coa hydratase	31,5	165.8	29.7	8.8
17	**JAI54140.1**	protein disulfide-isomerase, partial	51.5	165.7	13.8	4.9
18	**JAI53617.1**	putative glycosyl transferase family 8, partial	94.8	164.9	11.2	4.9
19	**JAI52838.1**	putative ca2+-binding actin-bundling protein, partial	60.0	163.1	17.2	5.2
20	**JAI56113.1**	putative small heat shock protein hsp20 family	21.4	150.5	35.1	5.5
21	**JAI55118.1**	putative annexin	35.7	143.9	24.8	5.5
22	**JAI52768.1**	putative beta-spectrin, partial	50.6	128.8	9.9	6.8
23	**JAI54218.1**	putative muscle lim protein mlp84b	48.5	124.3	8.6	8.3
24	**JAI55997.1**	putative superoxide dismutase	15.8	121.2	34.4	5.9
25	**JAI55367.1**	putative cyclophilin type peptidyl-prolyl cis-trans isomerase	17.9	118.2	27.4	8.9
26	**JAI53323.1**	putative f0f1-type atp synthase alpha subunit, partial	46.8	100.8	7.8	9.2
27	**JAI56015.1**	putative beta-spectrin	278.4	89.7	1.7	5.1
28	**JAI55756.1**	putative chaperonin	57.9	72.0	5.4	5.5
29	**JAI56018.1**	putative muscle-specific protein 20	20.3	57.5	12.5	8.4
30	**JAI55853.1**	putative thioredoxin	11.7	48.3	14.3	4.9
31	**JAI55669.1**	putative elongation factor 1-gamma, partial	48.0	36.9	3.1	7.5
32	**JAI54750.1**	putative methylmalonate semialdehyde dehydrogenase	56.6	35.7	4.8	8.2

Uma vez identificadas as sequências de peptídeos das proteínas advindas da extração para *R. neglectus,* os resultados foram confrontados em banco de dados específico, enquanto que *NCBI* e *SwissProt* foram consultados com buscas abertas. Ao todo foram encontradas 32 proteínas

semelhantes para *R. neglectus*, 24 sequências peptídicas semelhantes por meio do *NCBI* e 21 proteínas no *SwissProt*. Realizou-se uma filtragem com *score* maior que 40 e as sequências resultantes estão detalhadas nos apêndices 1, 2 e 3.

Figura 2. Categorização biológica das proteínas encontradas em espermatecas de *R. neglectus*.



Ao categorizar do número total de proteínas preditas encontradas, foram identificadas 14 proteínas musculares, 13 proteínas metabólicas, 4 proteínas da V-ATPases e 3 proteínas de Chaperonas.

Proteínas de diferentes classes proteicas foram encontradas, porém a discussão será focada em proteínas que de alguma maneira podem estar associadas as espermatecas com o intuito de compreender melhor as respostas biológicas desse órgão. É necessário ressaltar que as proteínas encontradas são proteínas preditas, isso significa que suas sequências são semelhantes a outras sequências depositadas em banco de dados e com funções específicas muito bem determinadas.

Discussão

Ao relacionar os resultados obtidos com bancos de dados, principalmente para *R. neglectus* espécie alvo do trabalho algumas proteínas se mostraram em maior abundância como foi o caso das proteínas musculares, resultado esperado devido a espessa camada de músculos da espermateca (HUEBNER, 1980).

A miosina é uma proteína que se movimenta ao longo da cadeia da actina e na presença de ATP é responsável pela contração muscular. Constituem em grande família multigênica que integram sinais bioquímicos e auxiliam a dinâmica citoesquelética, favorecendo os processos de transporte ativo e contração citoplasmática (HEISSLER; SELLERS, 2017).

A actina também identificada no tecido das espermatecas é uma proteína fundamental na organização celular dos eucariotos e sua associação com a miosina gera movimentos celulares e musculares, ou seja, células fagocitárias, ciliadas ou flageladas assim como o tecido muscular realizam seu deslocamento ou contração por meio da interação dessas proteínas. Provavelmente é a proteína mais abundante dos eucariotos, pois possui interações proteína-proteína e sua capacidade de transição entre os estados monoméricos e filamentosos faz dessa proteína uma peça muito importante nas funções celulares, na polaridade assim como na regulação da transcrição (DOMINGUEZ; HOLMES, 2011).

A capacidade de interação proteína-proteína e transição dos estados monoméricos e filamentosos da actina são proporcionados por uma proteína ligante denominada gelsolina. Essa proteína ligante ainda auxilia nos processos de corte, tamponamento, nucleação e agrupamentos de filamentos da actina, são responsáveis pela versatilidade estrutural e ainda estão envolvidas em alguns processos bioquímicos que envolve células normais e células defeituosas da actina (MCGOUGH et al., 2003).

Acredita-se que a fixação dos filamentos de actina para sustentação da membrana plasmática são auxiliados pelas anexinas, proteínas anexas de ligação. Essas proteínas são conhecidas por sua capacidade de ligar os ácidos fosfolipídicos que constituem a membrana citoplasmática e no citosol auxiliam a formação do citoesqueleto celular ao ligar os filamentos de actina. Além disso, alguns estudos com as anexinas indicam a importância dessa proteína para constituição e delimitação do lúmen de órgãos epiteliais (MARTIN-BELMONTE et al., 2007; OZOROWSKI et al., 2012).

O encontro de actina, anexina, geosolina e miosina nas espermatecas de *R. neglectus* fecundadas indicam características musculares desse órgão devido a espessa camada muscular da espermateca. Entretanto, características como transporte ativo de substâncias, delimitação do lúmen e contração muscular estão diretamente relacionados com essas proteínas e por isso não se pode deixar de evidenciar sua relação com a capacidade de secreção e transporte de substâncias pelas células musculares e epiteliais da espermateca.

Outra proteína que desempenha um importante papel na preservação morfológica celular são as espectrinas, essas proteínas também possuem associação com várias organelas celulares e contribuem para a manutenção da estrutura do complexo de Golgi fato que permite a interação no trânsito das proteínas de membrana (DE MATTEIS; MORROW, 2000; HUH et al., 2001). O encontro de polissacarídeos na porção inicial das espermatecas de Triatominae sugerem

uma reserva nutricional para o transporte e preservação dos espermatozoides (NASCIMENTO et al., 2019b), e nesse contexto sugere-se que talvez as espectrinas auxiliem essa função.

As tubulinas como a maioria das proteínas musculares auxiliam a conformação celular e desempenham a função de um esqueleto para as células vivas. Devido a sua estrutura tubular, formada por microtúbulos, possuem capacidade de mudarem a conformação celular e proteica com o auxílio das subunidades α e β que realizam a divisão, motilidade e transporte celular (BALOCH et al., 2019). As β - tubulinas auxiliam a montagem da maioria dos tipos de microtúbulos, mas ao investigar sua relação com a formação dos axonemas que constituem os flagelos dos espermatozoides de *Droshophila* não foi encontrado relação com essa subunidade (HAMMOND; CAI; VERHEY, 2008). Os espermatozoides, armazenados nas espermatecas, perdem o flagelo e permanecem quiescentes por longos períodos (LENSKY; SCHINDLER, 1967). Apesar de estudos anteriores não encontrarem relação da subunidade β – tubulina com a formação dos flagelos dos espermatozoides de *Droshophila*, o encontro das subunidades α e β nas espermatecas fecundadas de Triatominae revelam a necessidade de estudos aprofundados dessas subunidades e sua relação na manutenção e constituição dos espermatozoides nas espermatecas de *R. neglectus*.

Os finos filamentos do musculo estriado das espermatecas são controlados por complexos de tropina, uma proteína ativada na presença de cálcio no tecido (QIU et al., 2003). O musculo é extremamente suscetível ao estresse fisiológico dos organismos e necessita de múltiplos mecanismos para manter sua integridade, neste sentido a proteína Mlp84B participa desse processo de proteção muscular assim como auxilia a ancoragem dos filamentos da actina para a preservação e estruturação do tecido muscular (CLARK; KADRMAS, 2013).

Para a perfeita contração muscular são necessárias outras proteínas metabólicas que liberam energia para as células musculares, como é o caso das V-ATPases, proteínas responsáveis pela bomba de prótons e outras funções biológicas. Essas proteínas em insetos possuem a função de energizar e acidificar as membranas plasmáticas ao fornecer moléculas de Na + /K+-ATPase no transporte ativo secundário dos animais (KLEIN, 1992) e em órgãos como o intestino médio de mariposas (JÄGER et al., 1996).

As V-ATPases possuem duas subunidades principais com domínios citoplasmáticos (F1) e de membrana (F0) que são divididas em várias outras subunidades (HARVEY; NELSON, 1992; RAMALHO-ORTIGÃO et al., 2007). As subdivisões possibilitam a associação e dissociação reversível das subunidades F1 e F0 da V-ATPase, o complexo F0F1 é formado por várias subunidades e permitem a translocação de prótons abaixo de um gradiente eletroquímico para realizar a síntese do ATP (NAKAMOTO; KETCHUM; AL-SHAWI, 1999). A qualidade do

armazenamento dos espermatozoides nas espermatecas fecundadas são completamente dependentes do pH alcalino, nessas condições os espermatozoides são mantidos imóveis enquanto que a mobilidade e ativação dos flagelos são observadas por meio de alterações no pH e presença de substâncias liberadas pela glândula espematecal (LENSKY; SCHINDLER, 1967).

A presença de V-ATPases nas porções proximais e distais das espermatecas de triatomíneos por meio de técnicas de imunolocalização das células epiteliais das espermatecas indicam o controle do pH e acidificação do microambiente da luz espermatecal para a manutenção dos espermatozoides inativos (NASCIMENTO et al., 2019b). O encontro de 4 subunidades da V-ATPase no perfil proteico das espermatecas fecundadas de *R. neglectus* confirmam a relação dessa proteína na forma como as espermatecas mantém os espermatozoides inativos e ativam quando necessário para a fertilização dos ovócitos por longo período de tempo.

Devido a essas proteínas estarem associadas a porção de estoque, local onde provavelmente os espermatozoides são mantidos quiescentes, e a porção proximal, local onde os espermatozoides provavelmente estão ativos para fecundação dos ovócitos (NASCIMENTO et al., 2019b), indicam que as células epiteliais das espermatecas possuem proteínas (V-ATPases) responsáveis pela secreção de substâncias necessárias para o correto armazenamento, nutrição e viabilidade dos espermatozoides. Pode-se correlacionar a presença da subunidade B encontrada neste estudo com a pesquisa realizada por KOTWICA-ROLINSKA et al., 2013 que determinaram a acidificação do lúmen das células epiteliais do ducto deferente por meio da subunidade B fator necessário para a ativação dos espermatozoides quiescentes.

Ao avaliar as alterações climáticas que as fêmeas de Triatominae enfrentam durante sua vida, principalmente mudanças de temperatura que podem refletir na viabilidade dos espermatozoides no lúmen espermatecal, consegue-se compreender o encontro de proteínas de choque térmico no perfil proteico deste estudo. Uma vez que as proteínas conhecidas como *HSPs* (Proteínas de Choque Térmico) possuem função termorreguladora, um mecanismo evolutivo de extrema importância para manter as funções metabólicas intactas após variações climáticas bruscas e garantir o sucesso reprodutivo e a perpetuação da espécie. O encontro dessa proteína nas espermatecas de *R. neglectus* auxiliam a compreensão de como as espermatecas das fêmeas funcionam com variações climáticas bruscas.

As *HSPs* são componentes centrais da rede celular das Chaperonas, uma classe proteica extremamente conhecida e importante que auxilia as proteínas recém-formadas a desempenharem sua função apropriada no organismo, as chaperonas auxiliam os processos de dobragem de proteínas nas células por associação transitória das suas subunidades (MAYER;

BUKAU, 2005), consideradas componentes centrais por desempenharem funções importantes na relação proteína-proteína, dobramentos moleculares, estabilização proteica e prevenção de agregação irreversível (HASANUZZAMAN et al., 2013). As *HSPs* possuem várias famílias e dentre as diversas divisões familiares, as proteínas com baixo peso molecular possuem maior expressão durante o estresse térmico (UJI et al., 2019). Além das funções termoreguladoras, auxiliam no desenvolvimento da oogênese que indica funções nos processos proliferativos dos diferentes estados germinativos (JAGLA et al., 2018; SPIESS et al., 2004).

Alguns estudos evidenciam que as *HSPs* estão associadas aos domínios de ligação e liberação de pequenos peptídeos hidrofóbicos de algumas proteínas e ainda possuem relação com estado de ligação do ATP de baixa afinidade e o estado de ligação do ADP de alta afinidade. Além disso, possuem relação com as funções da V-ATPase que são acompanhadas pelas chaperonas em alguns processos metabólicos (MAYER; BUKAU, 2005).

As *HSPs* encontradas nas espermatecas fecundadas de *R. neglectus* possuem baixo peso molecular e, portanto, podem estar relacionadas ao estresse térmico, uma vez que os espécimes foram mantidos em BOD com temperatura controlada a 27ºC ou a presença dessa proteínas no perfil proteico é devido ao fato de estarem intrinsicamente relacionadas com a espermateca auxiliando no processo reprodutivo dos triatomíneos. Para responder esse questionamento são necessários estudos mais aprofundados da função das *HSPs* e chaperonas nas espermatecas de Triatominae.

Conclusão

Os resultados obtidos fornecem elementos do perfil proteico das espermatecas de *R. neglectus.* Além disso, contribui para a compreensão de como as espermatecas mantém os espermatozoides viáveis por vários meses. As proteínas encontradas e listadas neste estudo foram consideradas importantíssimas para manutenção das funções reprodutivas e biológicas das fêmeas de *R. neglectus* e acredita-se que por conseguinte dos Triatominae. Este estudo permitiu esclarecer a função de proteínas musculares e metabólicas, como a V-ATPase, no epitélio das espermatecas, proteínas que auxiliam a forma como esse órgão nutre e mantém os espermatozoides por determinados períodos de tempo, o que provavelmente contribui para a perpetuação das espécies.

5. CONCLUSÃO FINAL

O estudo dos caracteres morfológicos e citogenéticos de *Rhodnius* sp. resultaram na descrição de *R. taquarussuensis* sp.n. e posteriormente por meio de análises das divergências genéticas e delimitações das espécies pelo estudo da variação de dois genes mitocondriais e quatro nucleares, assim como cruzamentos interespecíficos entre *R. neglectus* e *R. taquarussuensis* foi demonstrado que *R. taquarussuensis* sp. n. trata-se de uma linhagem fenotípica de *R. neglectus*.

Após a publicação de *R. taquarussuensis* sp. n como sinônimo júnior de *R. neglectus*, foi realizado o primeiro estudo do perfil proteico de espermatecas fecundadas de *R. neglectus.* As proteínas encontradas nas possuem funções metabólicas correlacionadas a forma como as espermatecas mantém os espermatozoides viáveis e nutridos até a fecundação dos ovócitos estudo que auxilia a compreensão do processo metabólico necessário para garantir a perpetuação das espécies.

6. REFERÊNCIAS BIBLIOGRÁFICAS

ABAD-FRANCH, F. et al. *Rhodnius barretti*, a new species of triatominae (Hemiptera: Reduviidae) from western Amazonia. **Mem. Inst. Oswaldo Cruz**, v. 108, p. 92–99, 2013.

ANTÔNIO, J. C. **ESTRUTURAÇÃO POPULACIONAL DE Rhodnius neglectus (HEMIPTERA : REDUVIIDAE), NO BIOMA CERRADO E EM ÁREAS DE TRANSIÇÃO CERRADO-**CAATINGA, [s.l.] Instituto Oswaldo Cruz, 2016.

ALBERTO TOSO, M.; FELIPE VIAL, U.; GALANTI, N. Transmisión de la enfermedad de Chagas por vía oral. **Rev. Med. Chile**, v. 139, n. 2, p. 258–266, 2011.

ARAÚJO, C. A. C. et al. Genes encoding defensins of importante Chagas disease vectors used for phylogenetic studies. **Parasitol Res**, v. 114, p. 4503–4511, 2015.

BALOCH, A. H. et al. Microtubule and the Discovery of Tubulin. **EC Neurology**, v. 11, n. 4, p. 233–237, 2019.

BARRETO-SANTANA, D. et al. Biologia comparativa e comportamento alimentar de *Rhodnius neglectus* e *Rhodnius robustus* (Triatominae) sob condições de laboratório Comparative biology and feeding behavior of *Rhodnius neglectus* and *Rhodnius robustus* (Triatominae) under laboratory conditi. **Rev. Soc. Brasileira Med Tropical**, v. 44, n. 4, p. 490–495, 2011.

CARCAVALLO, R. U. et al. Phylogeny of the Triatominae (Hemiptera : Reduviidae) : proposals for taxonomic arrangements. **Entomol Vect**, v. 7, n. 1, p. 1–99, 2000.

CARVALHO, D. B. et al. A novel association between Rhodnius neglectus and the *Livistona australis* palm tree in an urban center foreshadowing the risk of Chagas disease transmission by vectorial invasions in Monte Alto City, São Paulo, Brazil. **Acta Tropica**, v. 130, n. 1, p. 35–38, fev. 2014.

CHAGAS, C. Nova Tripanozomíase humana: Estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp., agente etiológico de nova entidade mórbida do homem. **Mem. Inst. Oswaldo Cruz**, v. 1, p. 159–218, 1909.

CLARK, K. A.; KADRMAS, J. L. *Drosophila melanogaster* muscle LIM protein and alpha-actinin function together to stabilize muscle cytoarchitecture: A potential role for Mlp84B in actin-crosslinking. **Cytoskeleton**, v. 70, n. 6, p. 304–316, jun. 2013.

COURA, J. R.; VIÑAS, P. A. Chagas disease: a new worldwide challenge. **Nature**, v. 465, p. S6-, 23 jun. 2010.

CRUZ-LANDIM, C.; YABUKI, A. T.; IAMONTE, M. Ultrastructure of the spermathecae of *Melipona bicolor bicolor* LEP. (Hymenoptera, Apinae, Meliponini). **Biociências Jornal Uberlândia**, v. 19, n. 1, p. 57–64, 2003.

DA ROSA, J. et al. Study of the external female genitalia of 14 *Rhodnius* species (Hemiptera, Reduviidae, Triatominae) using scanning electron microscopy. **Parasites & Vectors**, v. 7, n. 1, p. 17, 2014.

DA ROSA, J. A. et al. A new species of *Rhodnius* from Brazil (Hemiptera, reduviidae, triatominae). **ZooKeys**, v. 2017, n. 675, p. 1–25, 2017.

DAVEY, K. . Spermatophoro production in *Rhodnius prolixus*. **Quarterly Journal of Microscopical Science**, v. 3–100, p. 221–230, 1959.

DAVEY, K. G. Copulation and egg- production in *Rhodnius prolixus*: the role of the spermathecae.

Journal of Experimental Biology, v. 42, p. 373–378, 1965.

DE MATTEIS, M. A.; MORROW, J. S. Spectrin tethers and mesh in the biosynthetic pathway. **Journal of Cell Science**, v. 113, n. 13, p. 2331–2343, 2000.

DEANE, M. P.; LENZI, H. L.; JANSEN, A. *Trypanosoma cruzi*: Vertebrate and invertebrate cycles in the same mammal host, the opossum *Didelphis marsupialis*. **Mem. Inst. Oswaldo Cruz**, v. 79, n. 4, p. 513–515, 1984.

DE OLIVEIRA, J. et al. Description of a new species of *Nesotriatoma* Usinger, 1944 from Cuba and revalidation of synonymy between *Nesotriatoma bruneri* (Usinger, 1944) and *N. flavida* (Neiva, 1911) (Hemiptera, Reduviidae, Triatominae). **Journal of Vector Ecology**, v. 43, n. 1, p. 148–157, jun. 2018.

DEN BOER, S. P. A.; BOOMSMA, J. J.; BAER, B. *Honey bee* males and queens use glandular secretions to enhance sperm viability before and after storage. **Journal of Insect Physiology**, v. 55, n. 6, p. 538–543, jun. 2009.

DIAS, F. B. S. et al. Ecological aspects of Rhodnius nasutus Stål,. 1859 (Hemiptera: Reduviidae: Triatominae) in palms of the Chapada do Araripe in Ceará, Brazil. **Mem. Inst. Oswaldo Cruz**, v. 103, n. 8, p. 824–830, 2008.

DOMINGUEZ, R.; HOLMES, K. C. Actin Structure and Function. **Annu Rev Biophys**, v. June 9, p. 169–186, 2011.

DORN, P. L. et al. Description of *Triatoma mopan* sp. N. from a cave in belize (hemiptera, reduviidae, triatominae). **ZooKeys**, v. 2018, n. 775, p. 69–95, 2018.

DOS SANTOS SOUZA, E. et al. Description of *Rhodnius marabaensis* sp. N. (Hemiptera, Reduviidade, Triatominae) from Pará State, Brazil. **ZooKeys**, v. 2016, n. 621, p. 45–62, 2016.

GALVÃO, C. et al. A checklist of the current valid species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their geographical distribution, with nomenclatural and taxonomic notes. **Zootaxa**, v. 202, n. 1, p. 1, 30 maio 2003.

GALVÃO, C. **Vetores da doença de Chagas no Brasil**. Curitiba: Sociedade Brasileira de Zoologia, 2014.

GARDIM, S. et al. Multiple mitochondrial genes of some sylvatic Brazilian Triatoma: Non-monophyly of the *T. brasiliensis* subcomplex and the need for a generic revision in the Triatomini. **Infection, Genetics and Evolution**, v. 23, p. 74–79, abr. 2014.

GOMES, C. et al. American trypanosomiasis and Chagas disease: Sexual transmission. **International Journal of Infectious Diseases**, v. 81, p. 81–84, 1 abr. 2019.

GONÇALVES, T. C. M. et al. *Triatoma jatai* sp. nov. in the state of Tocantins, Brazil (Hemiptera: Reduviidae: Triatominae). **Mem. Inst. Oswaldo Cruz**, v. 108, n. (4), p. 429–437, 2013.

GAUNT, M.; MILES, M. The Ecotopes and Evolution of Triatomine Bugs (Triatominae) and their Associated Trypanosomes. **Mem. Inst. Oswaldo Cruz**, v. 95, n. 4, p. 557–565, 2000.

GULLAN, P.; CRANSTON, P. **The Insects: An Outline of Entomology**. 4th ed ed. Oxford: 9600 Garsington Road, 2010.

GURGEL-GONÇALVES, R. et al. Distribuição geográfica, infestação domiciliar e infecção natural de triatomíneos (Hemiptera: Reduviidae) no Estado do Piauí, Brasil, 2008. **Revista Pan-Amazônica de Saúde**, v. 1, n. 4, p. 57–64, dez. 2010.

GURGEL-GONÇALVES, R. **Filogeografia , morfometria e distribuição geográfica potencial**

de populações de *Rhodnius neglectus* (Hemiptera , Reduviidae) no Brasil. [s.l.] Universidade de Brasília, 2008.

GURGEL-GONÇALVES, R.; CUBA, C. A. C. Population structure of *Rhodnius neglectus* Lent and *Psammolestes tertius* Lent & Jurberg (Hemiptera, Reduviidae) in bird nests (Furnariidae) on *Mauritia flexuosa* palm trees in Federal District of Brazil. **Revista Brasileira de Zoologia**, v. 24, n. 1, p. 157–163, mar. 2007.

GURGEL-GONÇALVES, R. et al. Distribuição espacial de populações de triatomíneos (hemiptera: reduviidae) em palmeiras da espécie *Mauritia flexuosa* no Distrito Federal, Brasil. **Rev. Soc. Brasileira de Medicina Tropical**, v. 37, n. 3, p. 241–247, 2004.

HAMMOND, J.; CAI, D.; VERHEY, K. J. Tubulin modifications and their cellular functions. **Curr Opin Cell Biol**, v. 20, n. 1, p. 71–76, 2008.

HARVEY, W. R.; NELSON, N. **V-ATPases**. London: The Company of Biologists Ltd, 1992.

HASANUZZAMAN, M. et al. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. **International Journal of Molecular Sciences**, v. 14, n. 5, p. 9643–9684, 2013.

HEISSLER, S. M.; SELLERS, J. R. Various Themes of Myosin Regulation. **J Mol Biol.**, v. May 8, n. 428, p. 1927–1946, 2017.

HUEBNER, E. Spermathecal ultrastructure of the insect *Rhodnius prolixus* Stal. **Journal of morphology**, v. 166, p. 1–25, 1980.

HUH, G. Y. et al. Calpain proteolysis of αII-spectrin in the normal adult human brain. **Neuroscience Letters**, v. 316, n. 1, p. 41–44, 2001.

HYPSA, V. et al. Phylogeny and biogeography of Triatominae (Hemiptera: Reduviidae): molecular evidence of a New World origin of the Asiatic clade. **Molecular phylogenetics and evolution**, v. 23, n. 3, p. 447–57, jun. 2002.

JÄGER, D. et al. Temporal and spatial distribution of V-ATPase and its mRNA in the midgut of moulting *Manduca sexta*. **Journal of Experimental Biology**, v. 199, p. 1019–27, 1996.

JAGLA, T. et al. Developmental expression and functions of the small heat shock proteins in drosophila. **International Journal of Molecular Sciences**, v. 19, n. 11, p. 1–12, 2 nov. 2018.

JANSEN, A. M. et al. *Trypanosoma cruzi* transmission in the wild and its most important reservoir hosts in Brazil. **Parasites & Vectors**, v. 11, n. 502, p. 1–25, 2019.

JANSEN, A. M.; XAVIER, S. C. DAS C.; ROQUE, A. L. R. Landmarks of the Knowledge and *Trypanosoma cruzi* Biology in the Wild Environment. **Frontiers in Cellular and Infection Microbiology**, v.10:10 , 6 fev. 2020.

JR, A. R.; RASSI, A.; MARIN-NETO, J. A. Chagas heart disease: pathophysiologic mechanisms, prognostic factors and risk stratification Anis. **Mem. Inst. Oswaldo Cruz**, v. 104, n. I, p. 152–158, 2009.

JURBERG, J. et al. Triatoma pintodiasi sp. nov. do subcomplexo *T. rubrovaria* (Hemiptera, Reduviidae, Triatominae). **Revista Pan-Amazônica de Saúde**, v. 4, n. 1, p. 43–56, mar. 2013.

JUSTI, S. et al. Molecular phylogeny of Triatomini (Hemiptera: Reduviidae: Triatominae). **Parasites & Vectors**, v. 7, n. 1, p. 149, 2014.

JUSTI, S. A.; GALVÃO, C. The Evolutionary Origin of Diversity in Chagas Disease Vectors. **Trends in Parasitology**, v. 33, n. 1, p. 42–52, 1 jan. 2017.

KHALIFA, A. Spermatophore production and egg-laying behaviour in *Rhodnius prolixus* Stal. (Hemiptera; Reduviidae). **Parasitology**, v. 40, n. 3–4, p. 283–289, 1950.

KLEIN, U. The insect V-ATPase, A plasma membrane proton oump energizing secondary active transport: Immunological evidence for the occurrence of a V-ATPase in insect ion-transporting epithelia. **The Journal of experimental biology**, v. 172, n. Pt 1, p. 345–354, 1 nov. 1992.

KOTWICA-ROLINSKA, J. et al. Effects of period RNAi on V-ATPase expression and rhythmic pH changes in the vas deferens of *Spodoptera littoralis* (Lepidoptera: Noctuidae). **Insect Biochemistry and Molecular Biology**, v. 43, n. 6, p. 522–532, jun. 2013.

KROPF, S. P.; LACERDA, A. L. **Carlos Chagas**. 22. ed. Rio de Janeiro: Instituto Oswaldo Cruz, 2010.

LACOMBE, D. Estudo anatômico e histológico sobre a subfamília Triatominae (Heteroptera, Reduviidae). Parte VII. Estudo do ducto intestinal do *Triatoma infestans*. **Mem. Inst. Oswaldo Cruz**, v. 55, p. 69–111, 1957.

LAEMMLI, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Nature**, v. 227, p. 680–685, 1970.

LAINSON, R.; SHAW, J. J.; FRALHA, H. Chagas's Disease in the Amazon Basin : I. *Trypanosoma cruzi* infections in silvatic mammals, triatomine bugs and man in the State of Pará, north Brazil. **Transactions of the Royal Medicine and Hygiene**, v. 73, n. 3, p. 193–204, 1979.

LENSKY, Y.; SCHINDLER, H. Motility and reversible inactivation of *Honeybee* spermatozoa in vivo and in vitro. **Les Annales de l'AbeilleLes**, v. 10 (1), p. 5–16, 1967.

LENT, H.; JURBERG, J. O gênero *Rhodnius* Stal, 1859, com um estudo sobre a genitália das espécies (Hemiptera, Reduviidae, Triatominae). **Revista Brasileira de Biologia**, v. 29, p. 487–560, 1969.

LENT, H.; WYGODZINSKY, P. Revision of Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas disease. **Bull Amer Mus Nat Hist**, v. 163, p. 123–529, 1979.

LIMA-CORDÓN, R. A. et al. Description of *Triatoma huehuetenanguensis* sp. N., a potential chagas disease vector (hemiptera, reduviidae, triatominae). **ZooKeys**, v. 2019, n. 820, p. 51–70, 2019.

MALTA, J. et al. Insights into the proteome of the spermatheca of the leaf-cutting and *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). **Florida Entomologist**, v. 97, n. 4, p. 1856–1861, 2014.

MARTIN-BELMONTE, F. et al. PTEN-Mediated Apical Segregation of Phosphoinositides Controls Epithelial Morphogenesis through Cdc42. **Cell**, v. 128, n. 2, p. 383–397, 26 jan. 2007.

MAYER, M. P.; BUKAU, B. Hsp70 chaperones: Cellular functions and molecular mechanism. **Cellular and Molecular Life Sciences**, v. 62, n. 6, p. 670–684, mar. 2005.

MCGOUGH, A. M. et al. The gelsolin family of actin regulatory proteins: Modular structures, versatile functions. **Federation of European Biochemical Societies Letters**, v. 552, p. 75–85, 2003.

MENDONÇA, V. J. et al. Phylogeny of *Triatoma sherlocki* (Hemiptera: Reduviidae:Triatominae) inferred from two mitochondrial genes suggests its location within the *Triatoma brasiliensis* complex. **American Journal of Tropical Medicine and Hygiene**, v. 81, n. 5, p. 858–864, nov. 2009.

MILES, M. A. et al. Chagas's disease in the amazon basin: Ii. the distribution of *Trypanosoma cruzi* zymodemes 1 and 3 in para state, north brazil. **Transactions of the Royal Society of**

Tropical Medicine and Hygiene, v. 75, n. 5, p. 667–674, 1981.

MONGE-MAILLO, B.; LÓPEZ-VÉLEZ, R. Challenges in the management of Chagas disease in Latin-American migrants in Europe. **Clinical Microbiology and Infection**, v. 23, n. 5, p. 290–295, 2017.

MONTEIRO, F. A. et al. Evolution, Systematics, and Biogeography of the Triatominae, Vectors of Chagas Disease. In: **Advances in Parasitology**. [s.l.] Elsevier Ltd, 2018. v. 99p. 265–344.

NAKAMOTO, R. K.; KETCHUM, C. J.; AL-SHAWI, M. K. Rotational coupling in the F0F1 ATP synthase. **Annu. Rev. Biophys. Biomol. Struct**, v. 28, p. 205–234, 1999.

NASCIMENTO, J. D. et al. Morphology of the spermathecae of twelve species of Triatominae (Hemiptera, Reduviidae) vectors of Chagas disease. **Acta Tropica**, v. 176, p. 440–445, 1 dez. 2017.

NASCIMENTO, J. D. et al. Taxonomical over splitting in the *Rhodnius prolixus* (Insecta: Hemiptera: Reduviidae) clade: Are *R. taquarussuensis* (da Rosa et al., 2017) and *R. neglectus* (Lent, 1954) the same species? **PLOS ONE**, v. 14, n. 2, p. e0211285, 7 fev. 2019a.

NASCIMENTO, J. D. et al. Spermathecae: Morphofunctional features and correlation with fat bodies and trachea in six species of vectors of Chagas disease. **Acta Tropica**, v. 197, 1 set. 2019b.

OZOROWSKI, G. et al. Withaferin A binds covalently to the N-terminal domain of annexin A2. **Biol. Chem.**, v. 393, n. 10, p. 1151–1163, 2012.

PASCINI, T. V.; MARTINS, G. F. The insect spermatheca: an overview. **Zoology**, v. 121, p. 56–71, 1 abr. 2017.

PEREIRA-LOURENÇO, A. S.; SANTOS-MALLET, J. R.; FREITAS, S. P. C. Anatomy of the spermatophore in Triatomines (Hemiptera, Reduviidae, Triatominae) and its applications to the study of chagas disease vector biology. **American Journal of Tropical Medicine and Hygiene**, v. 89, n. 4, p. 775–780, out. 2013.

PÉREZ-MOLINA, J. A.; MOLINA, I. Chagas disease. **The Lancet**, v. 391, n. 10115, p. 82–94, 6 jan. 2018.

PÉREZ, R. Estudio sobre la anatomía de *Rhodnius prolixus*. **Revista Venezulana de Sanidad y Asistencia Social**, v. 34, p. 9–98, 1969.

POINAR, G. A primitive triatomine bug, *Paleotriatoma metaxytaxa* gen. et sp. nov. (Hemiptera: Reduviidae: Triatominae), in mid-Cretaceous amber from northern Myanmar. **Cretaceous Research**, v. 93, p. 90–97, 1 jan. 2019.

QIU, F. et al. Troponin C in different insect muscle types: Identification of two isoforms in Lethocerus, Drosophila and Anopheles that are specific to asynchronous flight muscle in the adult insect. **Biochemical Journal**, v. 371, n. 3, p. 811–821, 1 maio 2003.

RAMALHO-ORTIGÃO, J. M. et al. Cloning and characterization of a V-ATPase subunit C from the American visceral leishmaniasis vector *Lutzomyia longipalpis* modulated during development and blood ingestion. **Mem. Inst. Oswaldo Cruz, Rio de Janeiro**, v. 102, n. 4, p. 509–515, 2007.

ROSA, J. DA. et al. Description of *Rhodnius montenegrensis* n. sp. (Hemiptera: Reduviidae: Triatominae) from the state of Rondônia, Brazil. **Zootaxa**, v. 3478, n. 1, p. 62–76, 11 set. 2012.

SANTIAGO, P. B. et al. A Deep Insight into the Sialome of *Rhodnius neglectus*, a Vector of Chagas Disease. **PLOS Neglected Tropical Diseases**, v. 10, n. 4, p. e0004581, 29 abr. 2016.

SANTOS, C. M. DOS et al. Comparative descriptions of eggs from three species of *Rhodnius*

(Hemiptera: Reduviidae: Triatominae). **Mem. Inst. Oswaldo Cruz**, v. 104, n. 7, p. 1012–1018, 2009.

SÁUDE, M. DA. Boletim Epidemiológico. **Secretaria de Vigilância em Saúde Pública**, v. 46, n. 21, p. 46, 2015.

SCHOFIELD, C. J. Biosystematics of the Triatominae. In: **Biosystematics of Haematophagous Insects**. Clarendon ed. [s.l.] Service, M.W., 1988. p. 285–312.

SHIKANAI-YASUDA, M. A.; CARVALHO, N. B. Oral transmission of chagas disease. **Clinical Infectious Diseases**, v. 54, n. 6, p. 845–852, 15 mar. 2012.

SILISTINO-SOUZA, R. et al. Chromatoid body: Remnants of nucleolar proteins during spermatogenesis in triatomine (Heteroptera, Triatominae). **Micron**, v. 43, n. 9, p. 954–960, set. 2012.

SILVA, N. N. et al. Surto epidêmico de doença de Chagas com provável contaminação oral. **Rev. Inst. Med. Trop. Sao Paulo**, v. 10, p. 265–276, 1968.

SOARES BARATA, J. M. ASPECTOS MORFOLÓGICOS DE OVOS DE TRIATOMINAE. **Revista Saúde Pública**, v. 15, p. 490–542, 1981.

SPIESS, C. et al. Mechanism of the eukaryotic chaperonin: protein folding in the chamber of secrets. **Trends Cell Biol**, v. 14, n. 11, p. 598–604, 2004.

STEINDEL, M. et al. Characterization of *Trypanosoma cruzi* isolated from humans, vectors, and animal reservoirs following an outbreak of acute human Chagas disease in Santa Catarina State, Brazil. **Diagnostic Microbiology and Infectious Disease**, v. 60, n. 1, p. 25–32, jan. 2008.

STUDIER, F. . Analysis of bacteriophage T7 early RNAs and proteins on slab gels. **J. Mol. Biol**, v. 79, p. 237–48, 1973.

TARTAROTTI, E.; AZEREDO-OLIVEIRA, M. T. V; CERON, C. R. Phylogenetic approach to the study of triatomines (Triatominae, Heteroptera). **Brazilian Journal of Biology**, v. 66, n. 2 B, p. 703–708, 2006.

UJI, T. et al. Characterization and expression profiles of small heat shock proteins in the marine red alga Pyropia yezoensis. **Cell Stress and Chaperones**, v. 24, n. 1, p. 223–233, 10 jan. 2019.

VARGAS, A. et al. Investigação de surto de doença de Chagas aguda na região extra-amazônica, Rio Grande do Norte, Brasil, 2016. **Cadernos de Saude Publica**, v. 34, n. 1, 2018.

VENTURA-GARCIA, L. et al. Socio-Cultural Aspects of Chagas Disease: A Systematic Review of Qualitative Research. **PLoS Neglected Tropical Diseases**, v. 7, n. 9, 2013.

VIEIRA, L. R. et al. Protein 2DE reference map of the anterior midgut of the blood-sucking bug Rhodnius prolixus. **Proteomics**, v. 00, p. 1–4, 2015.

WHO. **Wordl Health Organization**. Disponível em: <https://www.who.int/chagas/epidemiology/en/>. Acesso em: 22 jul. 2019.

WHO, F. Book Review: Working to Overcome the Global Impact of Neglected Tropical Diseases. **Perspectives in Public Health**, v. 132, n. 4, p. 192–192, 2010.

WIGGLESWORTH, V. The respiration of insects. **Biological reviews**, v. 6, n. 2, p. 181–220, 1931.

WIGGLESWORTH, V. The thoracic gland in *Rhodnius prolixus* (HEMIPTERA) and its role in moulting. **The journal of experimental biology**, v. 29, p. 561–570, 1952.

7. APÊNDICE

Apêndice 1. Identificação das proteínas por meio do software *ProteinScape* (versão 3.1, Bruker Daltonics) e o algoritmo Mascot (v2.3, Matrix Science, UK) para *R. neglectus*.

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Project Info

Name:	Colaboração	**Date:**	Apr 16, 2019

Sample Info & Protocols

Name:	Ju_espermateca
Date:	Nov 5, 2019

Search Result Info

Search Result:	IonTrap_allOrg_2019-11-11 12:01:51		
Location:	/Colaboração/Ju_espermateca/Ju_esp_051119.mgf		
Search Method:	IonTrap_allOrg	**Version:**	1.5
Search Engine(s):	Mascot, 2.3.02	**Ident. Compound(s):**	216/2717
Database(s):	rneglectus, rneglectus_.fasta		
Note:			

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Results

Protein 1: putative myosin class ii heavy chain, partial [Rhodnius neglectus]

Accession:	JAI53032.1	**Score:**	694.3
Database:	rneglectus	**Seq. Coverage [%]:**	19.4
MW [kDa] / pI:	159.2 / 5.8	**No. of Peptides:**	21

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

10	20	30	40	50	60	70	80	90	100
KSPNFQKPKP	PKPGCQAAHF	AIGHYAGVVS	YNITGWLEKN	KDPLNDTVVD	QFKKGSNKLL	IEIFADHPGQ	SGAPDAGGGK	GAKRTKGSAF	LTVSALYKEQ
110	120	130	140	150	160	170	180	190	200
LNNLMTTLKS	TQPHFVRCII	PNELKQPGVI	DSHLVMHQLT	CNGVLEGIRI	CRKGFPNRMV	YPDFKLRYKI	LNPAGVDKEP	DPKKAAAVVL	EATTLDPDQY
210	220	230	240	250	260	270	280	290	300
RLGHTKVFFR	AGVLGQLEEM	RDDRLSKIMG	WLQSYVRGYI	TRKEFKKLQE	QRLSLQVVQR	NLRRYLQLRT	WPWWKMWSKV	KPLLNVANVE	EEMRKLEELV
310	320	330	340	350	360	370	380	390	400
AQTQAALEKE	EKARKEVEAL	NAKLIQEKTD	LLRSLEGEKG	SLSSFQEKVA	KLQAQKTDLE	SQLLDTQERL	QTEEDARNQL	FQQKKKLEQE	SAGLKKDIED
410	420	430	440	450	460	470	480	490	500
LELSMQKTDQ	DKASKEHQIR	NLNDEIAHQD	ELINKLNKEK	KIQSEHNQKT	AEELQAAEDK	INHLTKVKAK	LEQTLDELED	SLEREKKLRG	DVEKAKRKTE
510	520	530	540	550	560	570	580	590	600
GDLKLTQEAV	ADLERNKKEL	EQTIQRKDKE	IASLTAKLED	EQSIVNKTGK	QIKELQSRIE	ELEEEVEAER	QARGKAEKQR	ADLARELEEL	GERLEEAGGA
610	620	630	640	650	660	670	680	690	700
TSAQIELNKK	REAEMSKLRR	DLEEANIQHE	STLANLRKKH	NDAVSEMGDQ	IDQLNKLKTK	VEKEKCQYLC	ELNDVRASID	HLTNEKAATE	KVAKQLQHQI
710	720	730	740	750	760	770	780	790	800
NEVQGKLDEA	NRTLNDFDAA	KKKLSIENSD	LLRQLEEAES	QVSQLSKIKI	SLTTQLEDTK	RLADEEARER	ATLLGKFRNL	EHDLDNLREQ	VEEEAEAKAD
810	820	830	840	850	860	870	880	890	900
IQRQLSKANA	EAQLWRSKYE	SEGIARAEEL	EEAKRKLQAR	LAEAEETIES	LNQKVIALEK	TKQRLATEVE	DLQLEVDRAN	AIANAAEKKA	KAIDKIIGEW
910	920	930	940	950	960	970	980	990	1000
KLKVDDLAAE	LDASQKECRN	YSTELFRLKG	AYEEGQEQLE	AVRRENKNLA	DEVKDLLDQI	GEGGRNIHEI	EKQRKRLEVE	KDELQAALEE	AEAALEQEEN
1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
KVLRSQLELS	QVRQEIDRRI	QEKEEEFENT	RKNHQRALDS	MQASLEAEAK	GKAEALRMKK	KLEADINELE	IALDHANKAN	AEAQKSIKKY	QQQLKDVQTA
1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
LEEEQRARDD	AREQLGIAER	RANALGNELE	ESRTLLEQAD	RGRRQAEQEL	GDAHEQINEL	AAQATSASAA	KRKLEGELQT	LHADLDELLN	EAKNSEEKAK
1210	1220	1230	1240	1250	1260	1270	1280	1290	1300

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

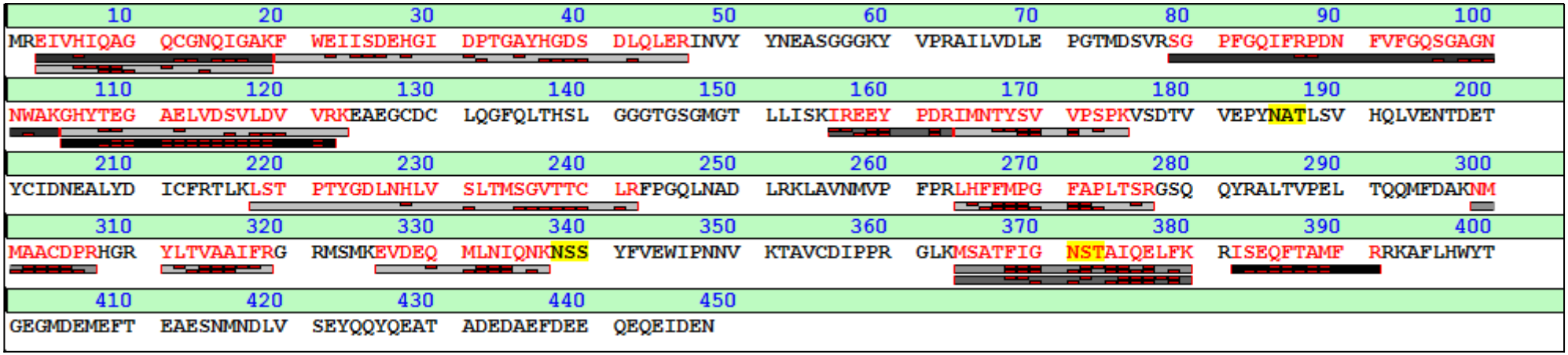
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1157	652.773	2	-114.22	-0.0746	44.40	24.41	1	0.0	0	K.EQLNNLMTTLK.S		99-109	CID
1320	870.854	2	-132.36	-0.1153	48.17	37.78	1	0.0	0	K.VKPLLNVANVEEEMR.K		280-294	CID
1322	686.322	3	-78.20	-0.0537	48.23	27.31	1	0.0	2	R.KLEELVAQTQAALEKEEK.A		295-312	CID
975	774.301	2	-103.33	-0.0800	40.23	29.78	2	0.0	0	K.TDLESQLLDTQER.L		357-369	CID
977	774.284	2	-125.28	-0.0970	40.29	45.48	2	0.0	0	K.TDLESQLLDTQER.L		357-369	CID
161	679.809	2	-139.33	-0.0947	19.93	39.66	1	0.0	3	K.KKLEQESAGLKK.D		385-396	CID
1060	660.752	2	-104.75	-0.0692	42.15	38.83	1	0.0	0	K.DIEDLELSMQK.T		397-407	CID
1662	944.854	2	-131.80	-0.1246	56.70	45.39	1	0.0	1	K.AKLEQTLDELEDSLER.E		469-484	CID
1375	845.303	2	-129.54	-0.1095	49.52	36.12	1	0.0	0	K.LEQTLDELEDSLER.E		471-484	CID
774	737.756	2	-126.07	-0.0930	35.56	50.25	1	0.0	0	R.IEELEEEVEAER.Q		559-570	CID
308	564.17	2	-209.47	-0.1182	24.11	20.91	1	0.0	0	R.ASIDHLTNEK.A		677-686	CID
991	644.319	2	-74.47	-0.0480	40.56	16.2	1	0.0	1	K.KLSIENSDLLR.Q		723-733	CID
1069	580.219	2	-173.19	-0.1005	42.34	22.71	1	0.0	0	K.LSIENSDLLR.Q		724-733	CID
851	787.805	2	-119.36	-0.0940	37.32	25.33	1	0.0	0	R.LAEAEETIESLNQK.V		841-854	CID
1179	815.303	2	-143.66	-0.1171	44.91	45.54	1	0.0	0	R.LATEVEDLQLEVDR.A		865-878	CID
1122	808.311	2	-147.83	-0.1195	43.62	29.07	2	0.0	1	K.LKVDDLAAELDASQK.E		902-916	CID
1119	808.317	2	-140.41	-0.1135	43.56	32.21	2	0.0	1	K.LKVDDLAAELDASQK.E		902-916	CID
1988	971.374	2	-127.07	-0.1234	67.79	56.89	2	0.0	1	K.NLADEVKDLLDQIGEGGR.N		948-965	CID
1986	971.38	2	-120.89	-0.1174	67.73	79.18	2	0.0	1	K.NLADEVKDLLDQIGEGGR.N		948-965	CID
1987	647.931	3	-107.59	-0.0697	67.73	36.89	1	0.0	1	K.NLADEVKDLLDQIGEGGR.N		948-965	CID
852	732.282	2	-100.98	-0.0740	37.33	17.04	1	0.0	0	R.ALDSMQASLEAEAK.G		1037-1050	CID
1473	679.295	3	-95.88	-0.0651	51.89	19.24	1	0.0	1	K.KLEADINELEIALDHANK.A		1061-1078	CID
594	651.745	2	-111.77	-0.0729	31.19	26.23	1	0.0	0	R.ANALGNELEESR.T		1122-1133	CID
1940	793.679	3	-91.44	-0.0726	65.39	19.95	1	0.0	1	R.KLEGELQTLHADLDELLNEAK.N		1173-1193	CID
1296	821.349	2	-108.75	-0.0893	47.67	24.49	1	0.0	0	R.QIEEAEEIAALNLAK.F		1325-1339	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Protein 2: putative beta-tubulin [Rhodnius neglectus]

Accession:	JAI56013.1	**Score:**	566.2
Database:	rneglectus	**Seq. Coverage [%]:**	45.8
MW [kDa] / pI:	50.2 / 4.8	**No. of Peptides:**	14
Modification(s):	Carbamidomethyl		



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
619	911.945	2	-22.00	-0.0201	31.84	24.53	1	0.0	0	R.EIVHIQAGQCGNQIGAK.F	Carbamidomethyl: 10	3-19	CID
617	608.238	3	-122.41	-0.0745	31.78	22.81	1	0.0	0	R.EIVHIQAGQCGNQIGAK.F	Carbamidomethyl: 10	3-19	CID
1476	1034.027	3	-116.63	-0.1206	51.95	63.15	1	0.0	0	K.FWEIISDEHGIDPTGAYHGDSDLQLER.I		20-46	CID
1733	933.327	3	-134.60	-0.1256	58.61	46.66	1	0.0	0	R.SGPFGQIFRPDNFVFGQSGAGNNWAK.G		79-104	CID
1818	979.879	2	-117.90	-0.1155	61.18	98.84	1	0.0	0	K.GHYTEGAELVDSVLDVVR.K		105-122	CID
1740	696.291	3	-104.50	-0.0728	58.81	22.06	1	0.0	1	K.GHYTEGAELVDSVLDVVRK.E		105-123	CID
185	539.136	2	-248.14	-0.1338	20.71	21.64	1	0.0	1	K.IREEYPDR.I		156-163	CID

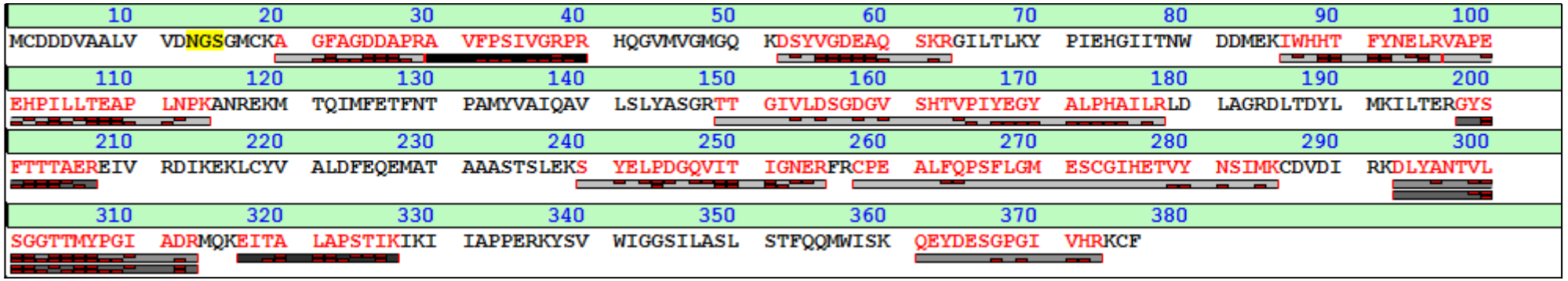
Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
850	668.278	2	-111.45	-0.0745	37.32	29.72	1	0.0	0	R.IMNTYSVVPSPK.V		164-175	CID
1918	912.685	3	-120.17	-0.1097	64.47	23.47	1	0.0	0	K.LSTPTYGDLNHLVSLTMSGVTTCLR.F	Carbamidomethyl: 23	218-242	CID
1775	810.811	2	-136.15	-0.1104	59.86	45.58	1	0.0	0	R.LHFFMPGFAPLTSR.G		264-277	CID
299	533.093	2	-233.22	-0.1244	23.91	30.72	1	0.0	0	K.NMMAACDPR.H	Carbamidomethyl: 6	299-307	CID
1643	527.195	2	-214.69	-0.1132	56.11	30.66	1	0.0	0	R.YLTVAAIFR.G		311-319	CID
740	730.757	2	-135.63	-0.0991	34.72	22.15	1	0.0	0	K.EVDEQMLNIQNK.N		326-337	CID
1810	929.36	2	-123.07	-0.1144	60.96	33.41	2	0.0	0	K.MSATFIGNSTAIQELFK.R		364-380	CID
1804	929.356	2	-127.38	-0.1184	60.71	59.46	2	0.0	0	K.MSATFIGNSTAIQELFK.R		364-380	CID
1312	615.235	2	-110.19	-0.0678	48.04	47.57	1	0.0	0	R.ISEQFTAMFR.R		382-391	CID

Protein 3: putative actin muscle isoform x2 [Rhodnius neglectus]

Accession: JAI56021.1
Database: rneglectus
MW [kDa] / pI: 41.8 / 5.3
Modification(s): Carbamidomethyl

Score: 503.9
Seq. Coverage [%]: 50.8
No. of Peptides: 12



MS/MS Peptide Matches

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

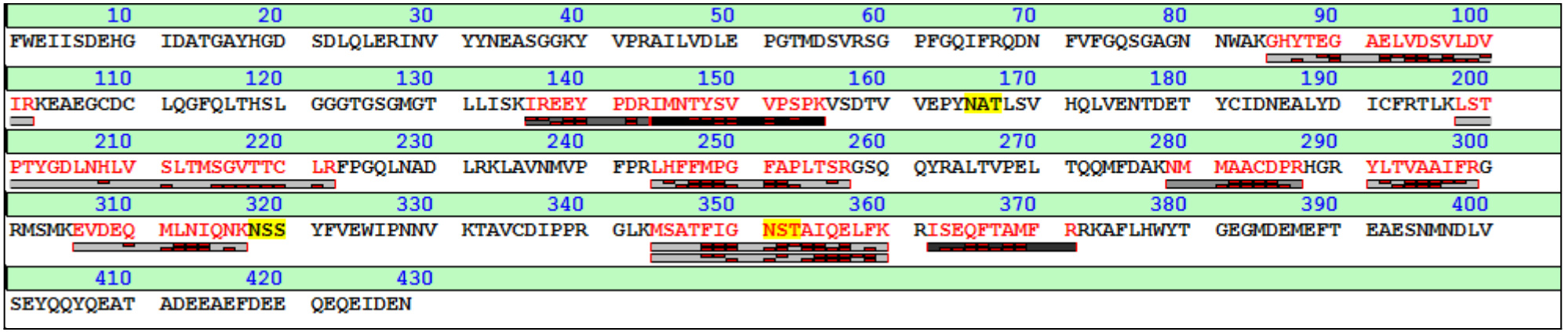
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
270	488.569	2	-324.89	-0.1588	23.07	37.8	1	0.0	0	K.AGFAGDDAPR.A		20-29	CID
1027	599.786	2	-117.34	-0.0704	41.38	17.15	1	0.0	0	R.AVFPSIVGRPR.H		30-40	CID
120	677.691	2	-183.40	-0.1243	18.80	32.39	1	0.0	1	K.DSYVGDEAQSKR.G		52-63	CID
941	758.263	2	-151.93	-0.1152	39.39	38.93	1	0.0	0	K.IWHHTFYNELR.V		86-96	CID
1219	984.435	2	-110.37	-0.1087	45.82	52.05	1	0.0	0	R.VAPEEHPILLTEAPLNPK.A		97-114	CID
1616	1051.102	3	-111.23	-0.1169	55.46	81.49	1	0.0	0	R.TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L		149-178	CID
521	566.653	2	-201.32	-0.1141	29.42	33.74	1	0.0	0	R.GYSFTTTAER.E		198-207	CID
1225	895.825	2	-139.07	-0.1246	45.97	60.88	1	0.0	0	K.SYELPDGQVITIGNER.F		240-255	CID
1849	812.039	4	-105.67	-0.0858	62.03	35.56	1	0.0	0	R.CPEALFQPSFLGMESCGIHETVYNSIMK.C	Carbamidomethyl: 1, 16	258-285	CID
1393	1107.901	2	-124.20	-0.1376	49.98	52.28	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		293-313	CID
1391	1107.888	2	-135.93	-0.1506	49.91	59.66	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		293-313	CID
853	572.225	2	-191.53	-0.1096	37.38	34.42	1	0.0	0	K.EITALAPSTIK.I		317-327	CID
393	496.155	3	-162.32	-0.0805	26.32	19.87	1	0.0	0	K.QEYDESGPGIVHR.K		361-373	CID

Protein 4: putative tubulin beta chain, partial [Rhodnius neglectus]

Accession: JAI54754.1
Database: rneglectus
MW [kDa] / pI: 48.2 / 4.7
Modification(s): Carbamidomethyl

Score: 367.7
Seq. Coverage [%]: 31.3
No. of Peptides: 10

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51



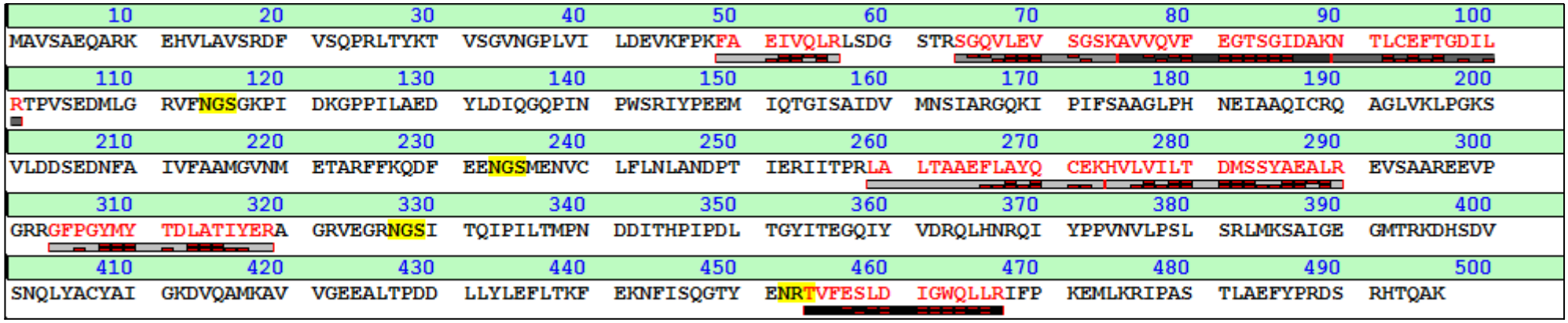
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1927	986.883	2	-120.93	-0.1194	64.87	56.71	1	0.0	0	K.GHYTEGAELVDSVLDVIR.K		85-102	CID
185	539.136	2	-248.14	-0.1338	20.71	21.64	1	0.0	1	K.IREEYPDR.I		136-143	CID
850	668.278	2	-111.45	-0.0745	37.32	29.72	1	0.0	0	R.IMNTYSVVPSPK.V		144-155	CID
1918	912.685	3	-120.17	-0.1097	64.47	23.47	1	0.0	0	K.LSTPTYGDLNHLVSLTMSGVTTCLR.F	Carbamidomethyl: 23	198-222	CID
1775	810.811	2	-136.15	-0.1104	59.86	45.58	1	0.0	0	R.LHFFMPGFAPLTSR.G		244-257	CID
299	533.093	2	-233.22	-0.1244	23.91	30.72	1	0.0	0	K.NMMAACDPR.H	Carbamidomethyl: 6	279-287	CID
1643	527.195	2	-214.69	-0.1132	56.11	30.66	1	0.0	0	R.YLTVAAIFR.G		291-299	CID
740	730.757	2	-135.63	-0.0991	34.72	22.15	1	0.0	0	K.EVDEQMLNIQNK.N		306-317	CID
1810	929.36	2	-123.07	-0.1144	60.96	33.41	2	0.0	0	K.MSATFIGNSTAIQELFK.R		344-360	CID
1804	929.356	2	-127.38	-0.1184	60.71	59.46	2	0.0	0	K.MSATFIGNSTAIQELFK.R		344-360	CID
1312	615.235	2	-110.19	-0.0678	48.04	47.57	1	0.0	0	R.ISEQFTAMFR.R		362-371	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Protein 5: putative vacuolar h+-atpase v1 sector subunit b [Rhodnius neglectus]

Accession:	JAI55832.1	**Score:**	352.5
Database:	rneglectus	**Seq. Coverage [%]:**	21.8
MW [kDa] / pI:	55.0 / 5.2	**No. of Peptides:**	8
Modification(s):	Carbamidomethyl		



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1079	488.139	2	-298.46	-0.1457	42.54	21.94	1	0.0	0	K.FAEIVQLR.L		49-56	CID
344	546.178	2	709.84	0.3874	25.09	18.91	1	0.0	0	R.SGQVLEVSGSK.A		64-74	CID
1047	760.803	2	-129.30	-0.0984	41.82	46.13	1	0.0	0	K.AVVQVFEGTSGIDAK.N		75-89	CID
1431	719.76	2	-129.73	-0.0934	50.89	29.06	1	0.0	0	K.NTLCEFTGDILR.T	Carbamidomethyl: 4	90-101	CID
1514	864.32	2	-135.67	-0.1173	52.92	21.96	1	0.0	0	R.LALTAAEFLAYQCEK.H	Carbamidomethyl: 13	259-273	CID
1832	959.397	2	-116.49	-0.1118	61.63	89.99	1	0.0	0	K.HVLVILTDMSSYAEALR.E		274-290	CID
1716	948.861	2	-89.01	-0.0845	58.09	66.03	1	0.0	0	R.GFPGYMYTDLATIYER.A		304-319	CID
1979	838.877	2	-91.73	-0.0770	67.45	58.43	1	0.0	0	R.TVFESLDIGWQLLR.I		454-467	CID

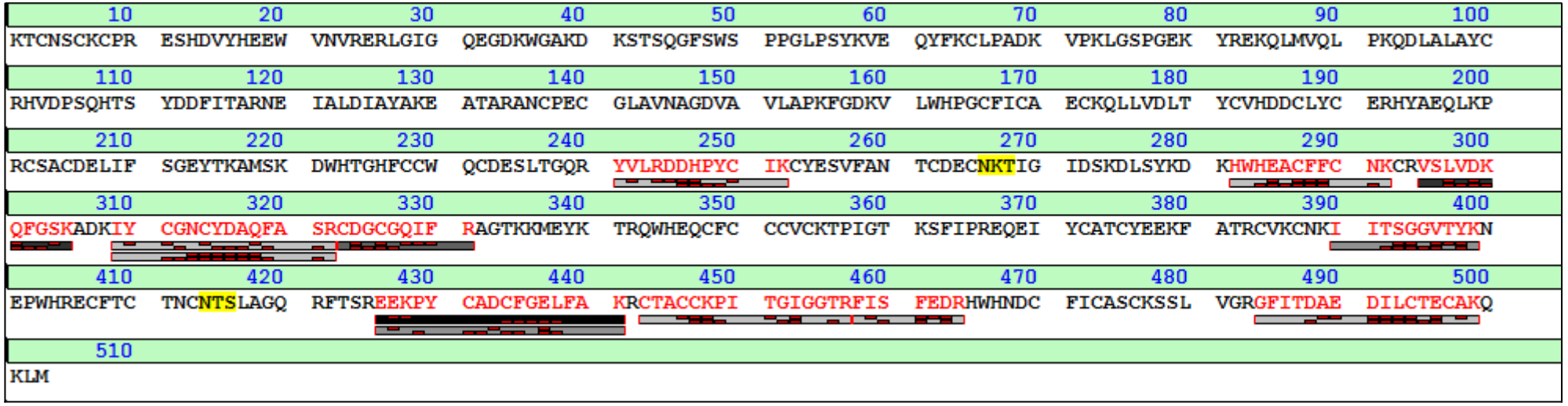
Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type

Protein 6: putative adaptor protein enigma, partial [Rhodnius neglectus]

Accession: JAI54047.1
Database: rneglectus
MW [kDa] / pI: 57.2 / 7.4
Modification(s): Carbamidomethyl

Score: 319.3
Seq. Coverage [%]: 24.1
No. of Peptides: 10



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
680	789.812	2	-99.13	-0.0783	33.38	20.89	1	0.0	1	R.YVLRDDHPYCIK.C	Carbamidomethyl: 10	241-252	CID
865	768.204	2	-149.66	-0.1150	37.63	34.3	1	0.0	0	K.HWHEACFFCNK.C	Carbamidomethyl: 6, 9	282-292	CID
633	604.276	2	-102.09	-0.0617	32.17	30.56	1	0.0	1	R.VSLVDKQFGSK.A		295-305	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

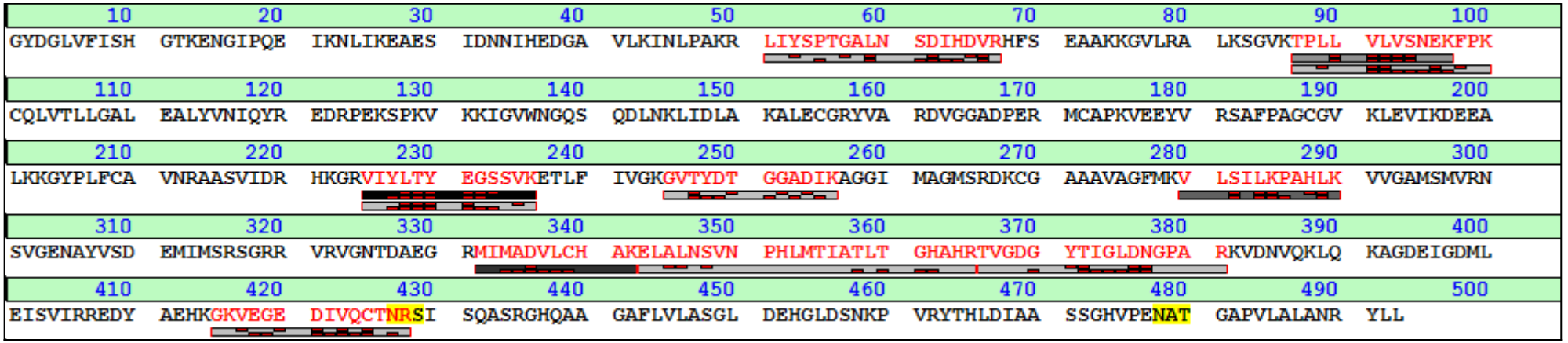
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
647	862.736	2	-145.35	-0.1254	32.48	23.99	2	0.0	0	K.IYCGNCYDAQFASR.C	Carbamidomethyl: 3, 6	309-322	CID
638	862.74	2	-140.71	-0.1214	32.29	49.96	2	0.0	0	K.IYCGNCYDAQFASR.C	Carbamidomethyl: 3, 6	309-322	CID
510	556.622	2	-201.58	-0.1122	29.11	17.27	1	0.0	0	R.CDGCGQIFR.A	Carbamidomethyl: 1, 4	323-331	CID
460	519.677	2	-227.27	-0.1181	27.95	28.61	1	0.0	0	K.IITSGGVTYK.N		390-399	CID
1298	982.323	2	-110.82	-0.1089	47.72	45.61	1	0.0	0	R.EEKPYCADCFGELFAK.R	Carbamidomethyl: 6, 9	426-441	CID
1301	655.226	3	-98.18	-0.0643	47.79	15.99	1	0.0	0	R.EEKPYCADCFGELFAK.R	Carbamidomethyl: 6, 9	426-441	CID
447	826.269	2	-145.55	-0.1203	27.62	39.57	1	0.0	0	R.CTACCKPITGIGGTR.F	Carbamidomethyl: 1, 4, 5	443-457	CID
856	457.038	2	-407.55	-0.1863	37.44	16.36	1	0.0	0	R.FISFEDR.H		458-464	CID
1596	921.795	2	-131.31	-0.1211	54.94	36.12	1	0.0	0	R.GFITDAEDILCTECAK.Q	Carbamidomethyl: 11, 14	484-499	CID

Protein 7: putative aminopeptidase, partial [Rhodnius neglectus]

Accession: JAI54404.1
Database: rneglectus
MW [kDa] / pI: 53.2 / 8.6
Modification(s): Carbamidomethyl

Score: 290.0
Seq. Coverage [%]: 26.4
No. of Peptides: 10

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51



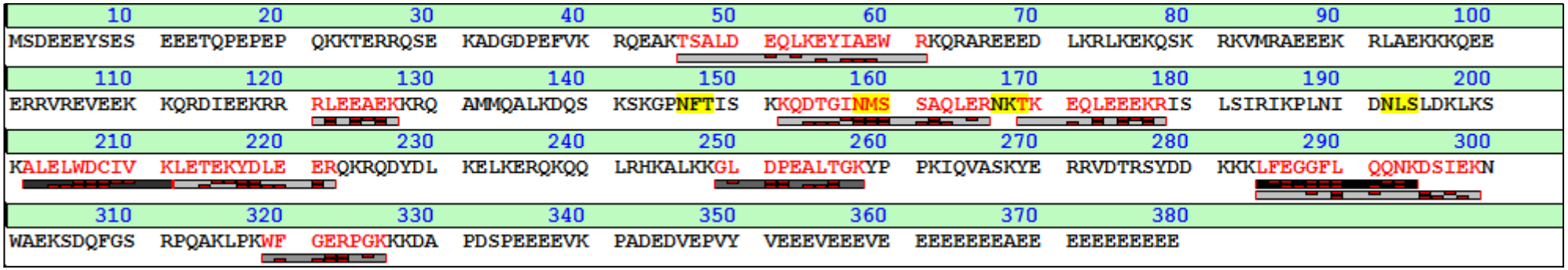
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1118	935.873	2	-121.28	-0.1135	43.51	32.75	1	0.0	0	R.LIYSPTGALNSDIHDVR.H		51-67	CID
1365	606.828	2	-58.57	-0.0355	49.27	25.3	1	0.0	0	K.TPLLVLVSNEK.F		87-97	CID
1540	792.877	2	-119.32	-0.0946	53.57	19.01	1	0.0	1	K.TPLLVLVSNEKFPK.C		87-100	CID
956	679.779	2	-124.65	-0.0847	39.72	29.54	2	0.0	0	R.VIYLTYEGSSVK.E		225-236	CID
957	679.786	2	-114.35	-0.0777	39.77	32.45	2	0.0	0	R.VIYLTYEGSSVK.E		225-236	CID
411	598.72	2	-122.44	-0.0733	26.72	23.22	1	0.0	0	K.GVTYDTGGADIK.A		245-256	CID
1028	609.843	2	-93.89	-0.0573	41.39	19.74	1	0.0	0	K.VLSILKPAHLK.V		280-290	CID
1143	644.747	2	-104.16	-0.0672	44.08	29.37	1	0.0	0	R.MIMADVLCHAK.E	Carbamidomethyl: 8	332-342	CID
1474	832.687	3	-107.09	-0.0892	51.94	22.09	1	0.0	0	K.ELALNSVNPHLMTIATLTGHAHR.T		343-365	CID
844	803.318	2	-98.33	-0.0790	37.20	31.56	1	0.0	0	R.TVGDGYTIGLDNGPAR.K		366-381	CID
395	802.771	2	-146.33	-0.1175	26.33	54.48	1	0.0	1	K.GKVEGEDIVQCTNR.S	Carbamidomethyl: 11	415-428	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Protein 8: putative troponin t skeletal muscle [Rhodnius neglectus]

Accession:	JAI55302.1	**Score:**	276.6
Database:	rneglectus	**Seq. Coverage [%]:**	27.2
MW [kDa] / pI:	45.3 / 5.0	**No. of Peptides:**	10
Modification(s):	Carbamidomethyl		



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1668	651.266	3	-98.54	-0.0642	56.83	24.21	1	0.0	1	K.TSALDEQLKEYIAEWR.K		46-61	CID
27	437.518	2	-495.90	-0.2171	14.59	15.47	1	0.0	1	R.RLEEAEK.K		121-127	CID
606	839.258	2	-186.88	-0.1569	31.52	46.91	1	0.0	1	K.KQDTGINMSSAQLER.N		152-166	CID
62	645.287	2	-79.69	-0.0514	16.57	23.2	1	0.0	2	K.TKEQLEEEKR.I		169-178	CID
1590	623.772	2	-90.81	-0.0566	54.81	30.57	1	0.0	0	K.ALELWDCIVK.L	Carbamidomethyl: 7	202-211	CID
509	712.773	2	-106.36	-0.0758	29.11	37.24	1	0.0	1	K.LETEKYDLEER.Q		212-222	CID
756	500.574	2	-389.63	-0.1951	35.06	18.42	1	0.0	0	K.GLDPEALTGK.Y		249-258	CID
1080	640.772	2	-98.81	-0.0633	42.59	37.94	1	0.0	0	K.LFEGGFLQQNK.D		284-294	CID
1099	926.872	2	-111.78	-0.1036	43.05	27.26	1	0.0	1	K.LFEGGFLQQNKDSIEK.N		284-299	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
566	488.591	2	-332.69	-0.1626	30.60	15.35	1	0.0	0	K.WFGERPGK.K		319-326	CID

Protein 9: putative f0f1-type atp synthase beta subunit [Rhodnius neglectus]

Accession: JAI53274.1
Database: rneglectus
MW [kDa] / pI: 43.1 / 5.1

Score: 273.9
Seq. Coverage [%]: 21.8
No. of Peptides: 6

```
        10        20        30        40        50        60        70        80        90       100
MLTAIGRAAS GALKLGKSAI NPQLLQNEAL RTAGALSATS YATAPQTKGK GAPGKVVAVI GAVVDVQFDD NLPPILNALE VQNRKPRLVL EVAQHLGENT
       110       120       130       140       150       160       170       180       190       200
VRTIAMDGTE GLVRGQDVFD TGFPIRIPVG AETLGRIINV IGEPIDERGP INTDKYSSIH ADAPEFVEMS VEQEILVTGI KVVDLLAPYA KGGKIGLFGG
       210       220       230       240       250       260       270       280       290       300
AGVGKTVLIM ELINNVAKAH GGYSVFAGVG ERTREGNDLY HEMIESGVIS LKDKTSKVAL VYGQMNEPPG ARARVALTGL TVAEYFRDQE GQDVLLFIDN
       310       320       330       340       350       360       370       380       390       400
IFRFTQAGSE VSALLGRIPS AVGYQPTLAT DMGTMQERIT TTKKGSITSV QAIYVPADDL TDPAPATTFA HLDATTVLSR AIAELGIYPA VDPLDSTSRI
       410
MDPNIIGA
```

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1198	839.367	2	-120.03	-0.1008	45.33	66.63	1	0.0	0	R.LVLEVAQHLGENTVR.T		88-102	CID
879	631.763	2	-96.70	-0.0611	37.95	25.4	1	0.0	0	R.TIAMDGTEGLVR.G		103-114	CID
1997	729.365	2	-80.12	-0.0584	68.05	54.62	1	0.0	0	K.TVLIMELINNVAK.A		206-218	CID
2092	641.244	3	-128.88	-0.0827	75.26	22.51	1	0.0	0	R.DQEGQDVLLFIDNIFR.F		288-303	CID
1254	718.303	2	-108.05	-0.0776	46.60	65.95	1	0.0	0	R.FTQAGSEVSALLGR.I		304-317	CID

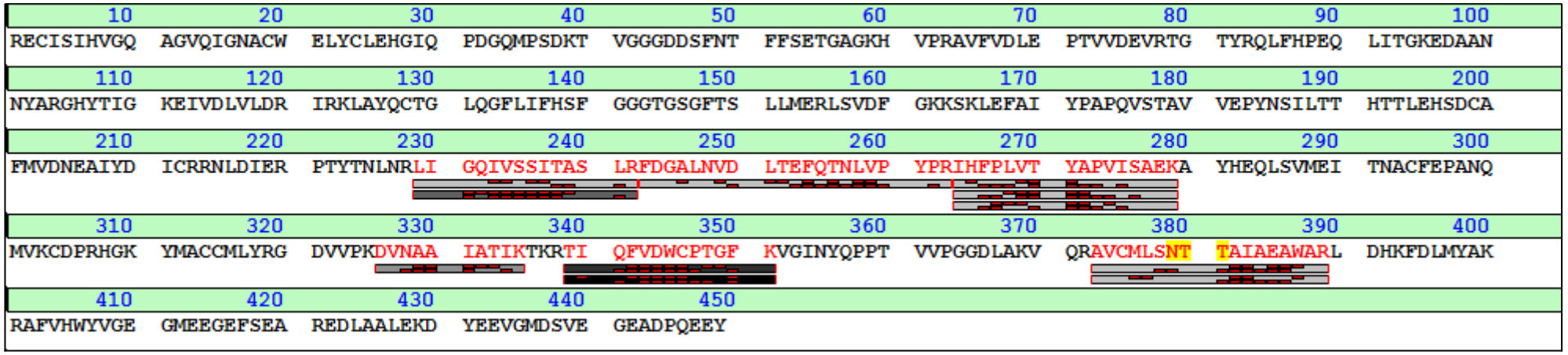
Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1564	994.401	2	-120.05	-0.1194	54.17	38.79	1	0.0	0	R.AIAELGIYPAVDPLDSTSR.I		381-399	CID

Protein 10: putative tubulin alpha-1 chain-like protein, partial [Rhodnius neglectus]

Accession: JAI56007.1
Database: rneglectus
MW [kDa] / pI: 50.0 / 5.0
Modification(s): Carbamidomethyl

Score: 266.2
Seq. Coverage [%]: 20.3
No. of Peptides: 6



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1932	729.359	2	-108.22	-0.0789	65.02	68.11	1	0.0	0	R.LIGQIVSSITASLR.F		229-242	CID
1936	486.575	3	-108.34	-0.0527	65.13	16.16	1	0.0	0	R.LIGQIVSSITASLR.F		229-242	CID
1809	1204.961	2	-121.89	-0.1469	60.91	50.17	1	0.0	0	R.FDGALNVDLTEFQTNLVPYPR.I		243-263	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

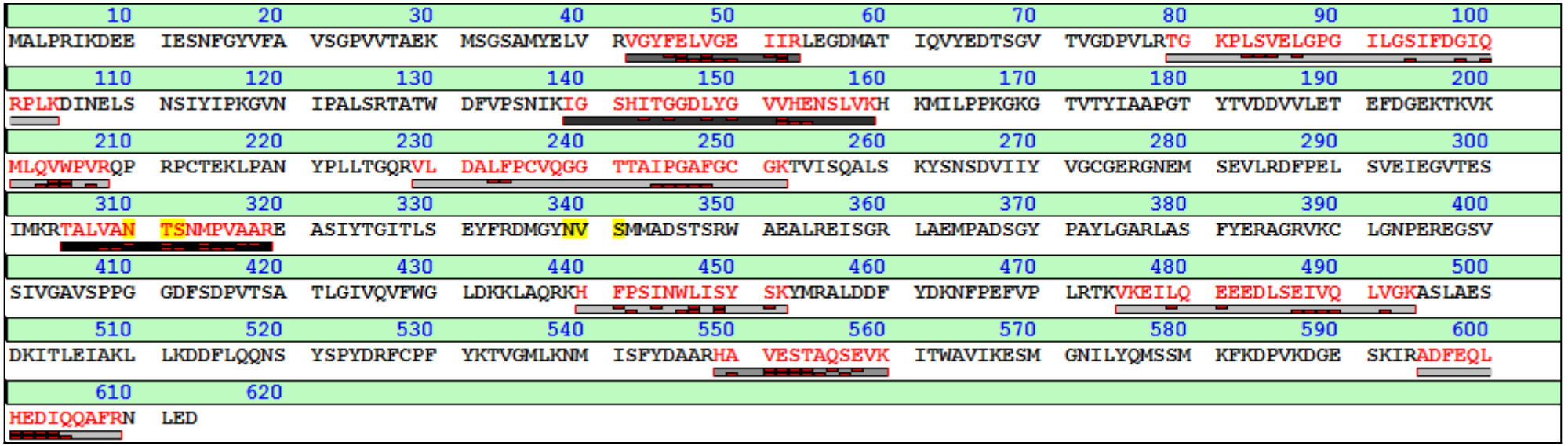
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1634	892.88	2	-135.39	-0.1209	55.91	23.2	3	0.0	0	R.IHFPLVTYAPVISAEK.A		264-279	CID
1611	892.883	2	-132.03	-0.1179	55.33	26.3	3	0.0	0	R.IHFPLVTYAPVISAEK.A		264-279	CID
1631	892.881	2	-134.27	-0.1199	55.85	45.93	3	0.0	0	R.IHFPLVTYAPVISAEK.A		264-279	CID
723	508.182	2	-217.90	-0.1108	34.33	28.53	1	0.0	0	K.DVNAAIATIK.T		326-335	CID
1578	799.795	2	-115.30	-0.0922	54.54	44.59	2	0.0	0	R.TIQFVDWCPTGFK.V	Carbamidomethyl: 8	339-351	CID
1570	799.792	2	-119.05	-0.0952	54.35	45.2	2	0.0	0	R.TIQFVDWCPTGFK.V	Carbamidomethyl: 8	339-351	CID
1598	932.827	2	-138.11	-0.1288	55.00	22.69	2	0.0	0	R.AVCMLSNTTAIAEAWAR.L	Carbamidomethyl: 3	373-389	CID
1604	932.822	2	-143.46	-0.1338	55.14	28.26	2	0.0	0	R.AVCMLSNTTAIAEAWAR.L	Carbamidomethyl: 3	373-389	CID

Protein 11: putative vacuolar h+-atpase v1 sector subunit a [Rhodnius neglectus]

Accession: JAI55216.1
Database: rneglectus
MW [kDa] / pI: 67.7 / 5.2
Modification(s): Carbamidomethyl

Score: 248.0
Seq. Coverage [%]: 27.1
No. of Peptides: 10

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51



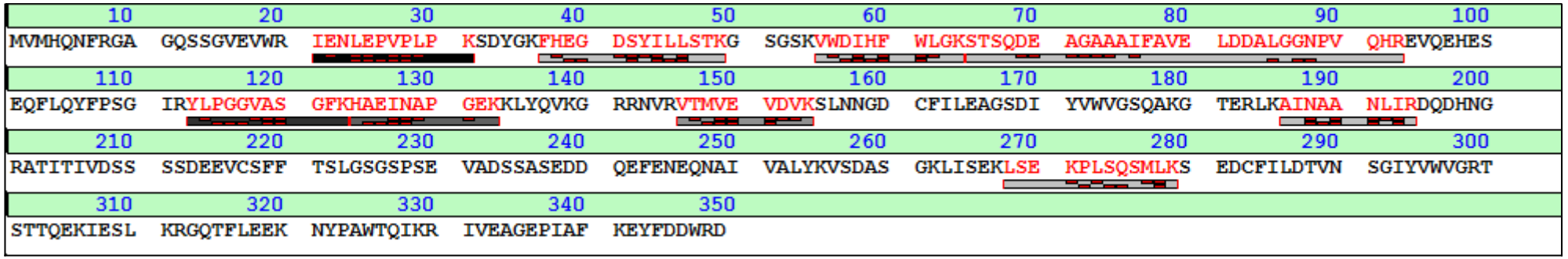
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1895	697.838	2	-71.00	-0.0496	63.66	35.58	1	0.0	0	R.VGYFELVGEIIR.L		42-53	CID
1993	898.085	3	-111.03	-0.0997	67.92	33.44	1	0.0	0	R.TGKPLSVELGPGILGSIFDGIQRPLK.D		79-104	CID
1155	732.303	3	-114.27	-0.0837	44.34	20.55	1	0.0	0	K.IGSHITGGDLYGVVHENSLVK.H		139-159	CID
1354	514.626	2	-316.89	-0.1631	49.02	18.44	1	0.0	0	K.MLQVWPVR.Q		201-208	CID
1697	812.985	3	301.63	0.2451	57.56	20.54	1	0.0	0	R.VLDALFPCVQGGTTAIPGAFGCGK.T	Carbamidomethyl: 8, 22	229-252	CID
754	758.298	2	-135.85	-0.1030	35.04	27.35	1	0.0	0	R.TALVANTSNMPVAAR.E		305-319	CID
1710	796.331	2	-107.99	-0.0860	57.95	15.18	1	0.0	0	K.HFPSINWLISYSK.Y		440-452	CID
1886	766.678	3	-97.57	-0.0748	63.34	24.62	1	0.0	1	K.VKEILQEEEDLSEIVQLVGK.A		475-494	CID
114	643.254	2	-106.90	-0.0688	18.62	35.31	1	0.0	0	R.HAVESTAQSEVK.I		549-560	CID
1426	923.823	2	-126.16	-0.1166	50.76	17.01	1	0.0	0	R.ADFEQLHEDIQQAFR.N		595-609	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Protein 12: putative actin regulatory gelsolin/villin family [Rhodnius neglectus]

Accession:	JAI53691.1	**Score:**	208.6
Database:	rneglectus	**Seq. Coverage [%]:**	32.7
MW [kDa] / pI:	38.7 / 4.9	**No. of Peptides:**	9



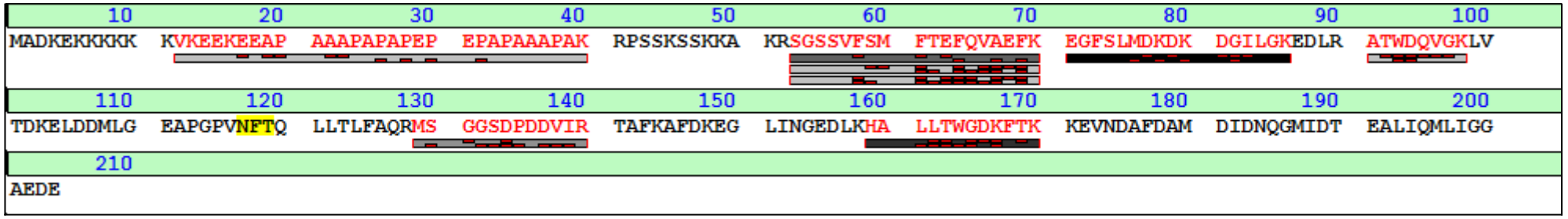
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1101	624.792	2	-114.50	-0.0715	43.11	38.34	1	0.0	0	R.IENLEPVPLPK.S		21-31	CID
1040	755.315	2	-89.80	-0.0678	41.69	26.55	1	0.0	0	K.FHEGDSYILLSTK.G		37-49	CID
1709	650.798	2	-72.97	-0.0475	57.95	15.65	1	0.0	0	K.VWDIHFWLGK.S		55-64	CID
1887	980.358	3	-120.19	-0.1178	63.39	25.71	1	0.0	0	K.STSQDEAGAAAIFAVELDDALGGNPVQHR.E		65-93	CID
881	548.18	2	-210.29	-0.1153	38.00	15.69	1	0.0	0	R.YLPGGVASGFK.H		113-123	CID
170	533.143	2	-237.80	-0.1268	20.25	17.29	1	0.0	0	K.HAEINAPGEK.K		124-133	CID
775	510.102	2	-340.44	-0.1737	35.57	18.03	1	0.0	0	R.VTMVEVDVK.S		146-154	CID
917	478.151	2	-286.04	-0.1368	38.82	32.74	1	0.0	0	K.AINAANLIR.D		186-194	CID
768	680.813	2	-96.74	-0.0659	35.38	18.62	1	0.0	0	K.LSEKPLSQSMLK.S		268-279	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Protein 13: putative myosin regulatory light chain 2-like protein [Rhodnius neglectus]

Accession:	JAI56019.1	**Score:**	203.9
Database:	rneglectus	**Seq. Coverage [%]:**	46.6
MW [kDa] / pI:	22.3 / 4.9	**No. of Peptides:**	6



MS/MS Peptide Matches

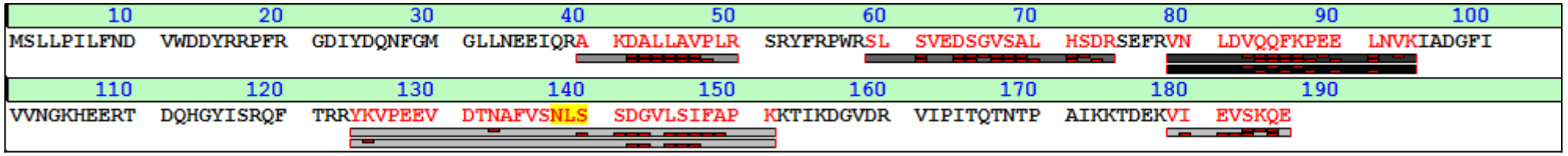
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
487	945.354	3	-144.41	-0.1365	28.54	29.42	1	0.0	2	K.VKEEKEEAPAAAPAPAPEPEPAPAAAPAK.R		12-40	CID
1978	1014.354	2	-118.87	-0.1206	67.38	57.35	2	0.0	0	R.SGSSVFSMFTEFQVAEFK.E		53-70	CID
1980	1014.351	2	-121.83	-0.1236	67.45	64.98	2	0.0	0	R.SGSSVFSMFTEFQVAEFK.E		53-70	CID
1981	676.95	3	440.18	0.2978	67.46	24.13	1	0.0	0	R.SGSSVFSMFTEFQVAEFK.E		53-70	CID
1094	876.838	2	-122.55	-0.1075	42.98	15.48	1	0.0	2	K.EGFSLMDKDKDGILGK.E		71-86	CID
423	452.618	2	-246.93	-0.1118	27.03	17.81	1	0.0	0	R.ATWDQVGK.L		91-98	CID
455	624.707	2	-116.66	-0.0729	27.82	30.43	1	0.0	0	R.MSGGSDPDDVIR.T		129-140	CID
1082	708.809	2	-107.70	-0.0763	42.66	45.73	1	0.0	1	K.HALLTWGDKFTK.K		159-170	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Protein 14: putative small heat shock protein [Rhodnius neglectus]

Accession: JAI53477.1
Database: rneglectus
MW [kDa] / pI: 21.4 / 6.0

Score: 192.4
Seq. Coverage [%]: 42.5
No. of Peptides: 5



MS/MS Peptide Matches

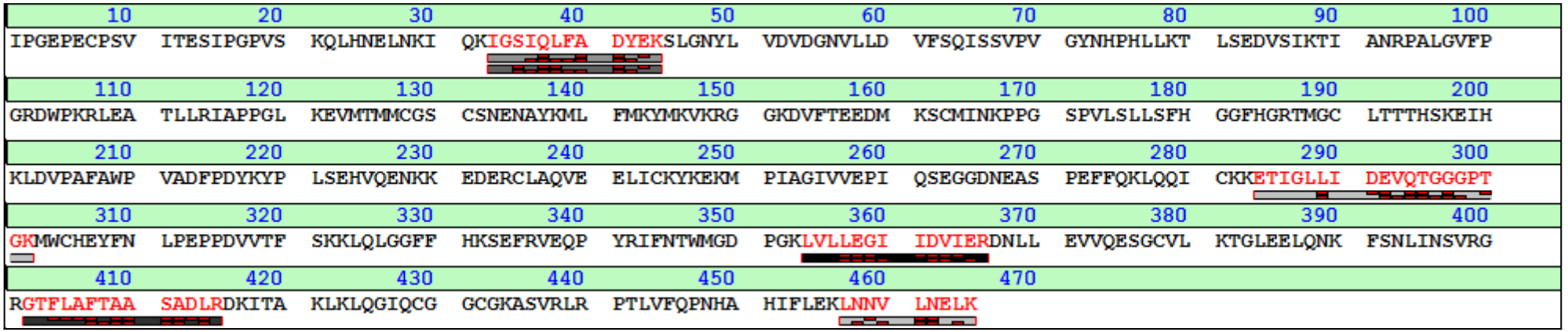
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1140	583.788	2	-134.32	-0.0784	44.02	43.66	1	0.0	1	R.AKDALLAVPLR.S		40-50	CID
718	829.801	2	-122.48	-0.1016	34.21	56.96	1	0.0	0	R.SLSVEDSGVSALHSDR.S		59-74	CID
1204	950.408	2	-109.80	-0.1044	45.50	17.71	2	0.0	0	R.VNLDVQQFKPEELNVK.I		79-94	CID
1194	950.4	2	-118.22	-0.1124	45.25	38.47	2	0.0	0	R.VNLDVQQFKPEELNVK.I		79-94	CID
1958	1009.381	3	-134.47	-0.1358	66.32	15.64	2	0.0	1	R.YKVPEEVDTNAFVSNLSSDGVLSIFAPK.K		124-151	CID
1957	1009.382	3	-133.48	-0.1348	66.26	24.86	2	0.0	1	R.YKVPEEVDTNAFVSNLSSDGVLSIFAPK.K		124-151	CID
211	466.067	2	-410.46	-0.1914	21.43	28.4	1	0.0	1	K.VIEVSKQE.-		179-186	CID

Protein 15: putative 4-aminobutyrate aminotransferase, partial [Rhodnius neglectus]

Accession: JAI54843.1
Database: rneglectus
MW [kDa] / pI: 51.9 / 6.5

Score: 190.0
Seq. Coverage [%]: 14.4
No. of Peptides: 5

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51



MS/MS Peptide Matches

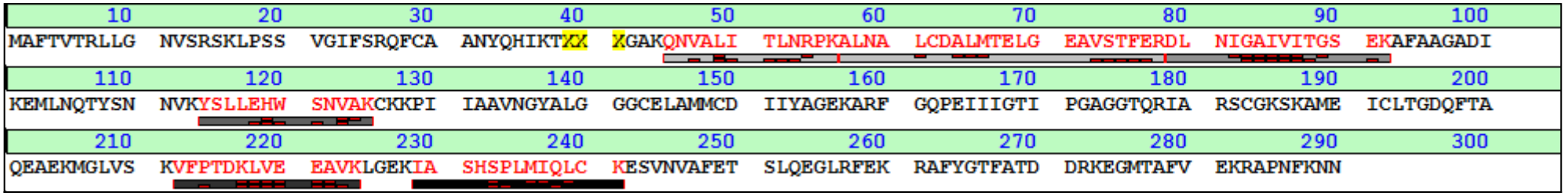
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1379	692.276	2	-123.30	-0.0854	49.59	38.89	2	0.0	0	K.IGSIQLFADYEK.S		33-44	CID
1383	692.33	2	-45.31	-0.0314	49.72	38.94	2	0.0	0	K.IGSIQLFADYEK.S		33-44	CID
1457	942.863	2	-144.53	-0.1363	51.49	36.43	1	0.0	0	K.ETIGLLIDEVQTGGGPTGK.M		284-302	CID
2086	741.373	2	-104.55	-0.0775	74.92	37.34	1	0.0	0	K.LVLLEGIIDVIER.D		354-366	CID
1554	720.802	2	-105.03	-0.0757	53.96	57.11	1	0.0	0	R.GTFLAFTAASADLR.D		402-415	CID
1053	528.664	2	-268.59	-0.1420	41.95	20.21	1	0.0	0	K.LNNVLNELK.-		457-465	CID

Protein 16: putative enoyl-coa hydratase [Rhodnius neglectus]

Accession: JAI55337.1
Database: rneglectus
MW [kDa] / pI: 31.5 / 8.8
Modification(s): Carbamidomethyl

Score: 165.8
Seq. Coverage [%]: 29.7
No. of Peptides: 6

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
906	683.832	2	-116.82	-0.0799	38.57	23.03	1	0.0	0	K.QNVALITLNRPK.A		45-56	CID
2189	804.327	3	-79.43	-0.0639	81.88	28.23	1	0.0	0	K.ALNALCDALMTELGEAVSTFER.D	Carbamidomethyl: 6	57-78	CID
1415	715.338	2	-84.54	-0.0605	50.44	47.46	1	0.0	0	R.DLNIGAIVITGSEK.A		79-92	CID
1222	723.81	2	-86.25	-0.0624	45.94	19.46	1	0.0	0	K.YSLLEHWSNVAK.C		114-125	CID
1171	737.852	2	-80.26	-0.0592	44.72	30.9	1	0.0	1	K.VFPTDKLVEEAVK.L		212-224	CID
1126	749.328	2	-95.34	-0.0714	43.70	16.68	1	0.0	0	K.IASHSPLMIQLCK.E	Carbamidomethyl: 12	229-241	CID

Protein 17: protein disulfide-isomerase, partial [Rhodnius neglectus]

Accession:	JAI54140.1	**Score:**	165.7
Database:	rneglectus	**Seq. Coverage [%]:**	13.8
MW [kDa] / pI:	51.5 / 4.9	**No. of Peptides:**	5

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

10	20	30	40	50	60	70	80	90	100
DHILVEFYAP	WCGHCKALAP	QYAKAAEKLS	ELNSQIKLAK	VDATTEGELA	EKFNVRGYPT	LKFFRKGQQV	EYTGGRQAED	IVSWLLKKTG	PPAKSLSSVD
110	120	130	140	150	160	170	180	190	200
EAKAFIDEHP	VVVIGYFKDP	ECEGAKRFLD	VASTVDDHPF	GIVSDNALFS	ELSVEEDKVI	LYKKFDDGKS	EFSGSLEDPN	ELTKFVASES	LPLIVEFNHE
210	220	230	240	250	260	270	280	290	300
TAQKIFGGDI	KSHLLLFLSK	KLGHFDEHLE	PIKPVAKEHK	GELLFVVVNA	DETDHQRILE	FFGIAETEVP	TMRLIRLEED	MSKFKPDTDD	LGPDSIKAFV
310	320	330	340	350	360	370	380	390	400
KAFLEGTLKE	HLPSQTLPED	WDKHPVKVLV	STNFDSVVFD	TEKDVLVEFY	APWCGHCKQL	APIYDKLGEA	FSEKKDVVIA	KIDATANELE	HTKISSFPTI
410	420	430	440	450	460				
KLYKKGDNQG	INYEGERTLE	GLTKFLESGG	EYKGGEPASE	KTEEEEEEED	DHPKKDEL				

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
298	631.705	2	-164.87	-0.1042	23.86	31.79	1	0.0	0	K.VDATTEGELAEK.F		41-52	CID
1450	529.22	2	-196.21	-0.1039	51.35	22.0	1	0.0	0	K.SHLLLFLSK.K		212-220	CID
1872	926.877	2	-110.37	-0.1023	62.93	44.48	1	0.0	0	R.ILEFFGIAETEVPTMR.L		258-273	CID
763	774.279	2	-134.34	-0.1040	35.25	35.67	1	0.0	0	K.FKPDTDDLGPDSIK.A		284-297	CID
407	671.264	2	-107.07	-0.0719	26.65	31.78	1	0.0	0	K.IDATANELEHTK.I		382-393	CID

Protein 18: putative glycosyl transferase family 8, partial [Rhodnius neglectus]

Accession: JAI53617.1
Database: rneglectus
MW [kDa] / pI: 94.8 / 4.9

Score: 164.9
Seq. Coverage [%]: 11.2
No. of Peptides: 6

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

10	20	30	40	50	60	70	80	90	100
NTAWVTLATN	DTYCLGALVL	ANSLKRVNTV	HQLAVLITPG	VSQSMRQQLA	KVFNVVKEVD	VLDSGDEANL	ALIARPELGV	TFTKLHCWNL	TQFSKCVFLD
110	120	130	140	150	160	170	180	190	200
ADVLVVQNCD	ELFEREELSA	APDVGWPDCF	NSGVFVFLPS	KDTFKALIDC	ALGRGSFDGG	DQGLLNTFFN	DWPTKDIKKH	LPFIYNMVST	ASYSYLPAFK
210	220	230	240	250	260	270	280	290	300
LFGSQVKIVH	FIGSNKPWLQ	SGSAATSSLS	GFLETWWNIF	NSHVAQALST	DMISNCTFDD	VRWHNHSSSM	HSPTPVHQLF	HDPVRGVHLG	QEAPQQEERH
310	320	330	340	350	360	370	380	390	400
QFIDPWEEIT	VDQMQSTTRE	HCAQSISEGR	YSNYSPQRRE	DCVVGNSEVS	LDTIQRFGEI	RLTGEDQSSI	GHCTNGQCRN	EPVSCTVVSL	PSTCLQNISE
410	420	430	440	450	460	470	480	490	500
LETGLQSISV	QESGLQNMSE	QETVNVYCQT	QSQEAQINIP	NLNNFPKDSP	FQFHHQAAQN	IPSTSTYSSM	CPLSSIQPAS	LPIDMPSNCP	QSCSLQQQQS
510	520	530	540	550	560	570	580	590	600
TSRTGLSQEV	GLAGALAGGE	RSAIEDVWRR	QNWEQGVIDY	MGRDSFENIW	KKISESVGNK	ASVPGTAPSA	PLTEAGEKSV	TPPAVPAESK	EVPSQQIAKE
610	620	630	640	650	660	670	680	690	700
PTGSAVAPPV	KAEQTAEGAP	VCAEPPTVDK	TTSPTEKKEE	APKTEIEPAK	ECITSDTAES	AKVTSPTSVE	SAKETAQDLL	KTDAPATMAA	PGLPSEITPP
710	720	730	740	750	760	770	780	790	800
LVGEPPRTDL	PTQLLPAVPE	LPSEAKTEIH	ADTKLLQEEK	LKAAPSETKE	PELKSSADTA	GKEVTEAAVA	KQEVPKTEAS	LPTPAEGAKP	ETTQPGIEVP
810	820	830	840	850	860	870	880		
KDTTSVPSKA	DIIDAKADEA	KMSKPEATSS	AAPKTEDVKT	LPTEVKPTQE	APKDGSEATE	KPSKDDSKQK	KNTNRS		

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1493	717.328	3	-95.52	-0.0685	52.40	29.43	1	0.0	0	R.VNTVHQLAVLITPGVSQSMR.Q		27-46	CID
1727	958.043	3	-131.99	-0.1265	58.35	15.98	2	0.0	0	K.EVDVLDSGDEANLALIARPELGVTFTK.L		58-84	CID
1721	958.045	3	-129.90	-0.1245	58.22	27.54	2	0.0	0	K.EVDVLDSGDEANLALIARPELGVTFTK.L		58-84	CID
60	416.995	2	-543.60	-0.2268	16.37	29.8	1	0.0	0	K.ISESVGNK.A		553-560	CID
436	591.689	2	-224.52	-0.1329	27.36	16.71	1	0.0	0	K.SVTPPAVPAESK.E		579-590	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
410	817.788	2	-155.55	-0.1272	26.71	45.41	1	0.0	1	K.SSADTAGKEVTEAAVAK.Q		755-771	CID
483	769.785	2	-181.66	-0.1399	28.41	16.02	1	0.0	0	K.TLPTEVKPTQEAPK.D		840-853	CID

Protein 19: putative ca2+-binding actin-bundling protein, partial [Rhodnius neglectus]

Accession: JAI52838.1
Database: rneglectus
MW [kDa] / pI: 60.0 / 5.2

Score: 163.1
Seq. Coverage [%]: 17.2
No. of Peptides: 6

```
10        20        30        40        50        60        70        80        90        100
EKSFEDWLLS EMMRLERLEH LAQKFKHKAD INEEWTRGKE EMLQSGDFRQ CRLNELKALK KKHEAFESDL AAHQDRVEQI AAIAGELNAL RYHDCDTVNS
110       120       130       140       150       160       170       180       190       200
RCKRICDQWD RLGSLTQQRR SNLDEAEKIL EKIDVLHLEF AKRAAPFNNW LDGTREDLVD MFIVHTMEEI QGLLEAHSQF KATLGEADKE YTSIVALVKE
210       220       230       240       250       260       270       280       290       300
VEATVHKYHI PGGLENPYTT LTANDLTVKW NDVRQLVPQR DSTLQTELRK QQNNEMLRRQ FAEKANQVGP WIERQMDAVT AIGMGLQGSL EDQLHRLKEY
310       320       330       340       350       360       370       380       390       400
EQGVFAYKPH IEELEKIHQA VQEGMIFENR YTQYTMETLR VGWEQLLTSI NRNINEVENQ ILTRDSKGIT QEQLNEFRAS FNHFDKNRTG RLAPDEFKSC
410       420       430       440       450       460       470       480       490       500
LVSLGYSIGK DRQGEIDFQR ILAVVDPNNT GYVHFDAFLD FMTRESTDTD TAEQVIDSFR ILAADKPYIL PDELRRELPP DQAEYCIKRM PAYKGPNSVP
510       520
GALDYMSFST ALYGESDL
```

MS/MS Peptide Matches

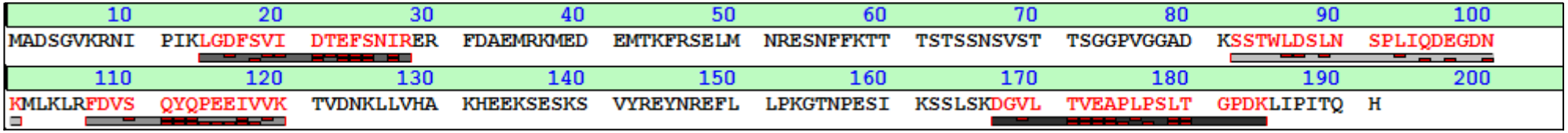
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1985	772.276	2	-95.32	-0.0736	67.72	30.66	1	0.0	0	K.SFEDWLLSEMMR.L		3-14	CID
1497	954.415	2	-109.85	-0.1049	52.47	40.93	1	0.0	1	K.ATLGEADKEYTSIVALVK.E		182-199	CID

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1129	817.332	3	-113.67	-0.0929	43.76	15.46	1	0.0	1	R.LKEYEQGVFAYKPHIEELEK.I		297-316	CID
1712	708.308	2	-109.70	-0.0777	58.01	37.2	1	0.0	0	R.VGWEQLLTSINR.N		341-352	CID
827	667.777	2	-92.22	-0.0616	36.81	23.21	1	0.0	0	K.GITQEQLNEFR.A		368-378	CID
1314	907.309	2	-109.06	-0.0990	48.05	15.63	1	0.0	0	R.ESTDTDTAEQVIDSFR.I		445-460	CID

Protein 20: putative small heat shock protein hsp20 family [Rhodnius neglectus]

Accession: JAI56113.1
Database: rneglectus
MW [kDa] / pI: 21.4 / 5.5

Score: 150.5
Seq. Coverage [%]: 35.1
No. of Peptides: 4



MS/MS Peptide Matches

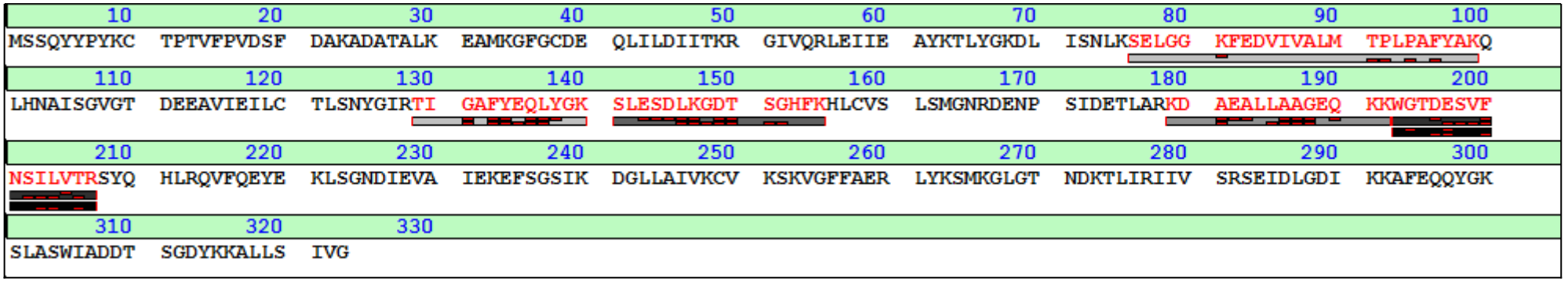
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1617	856.835	2	-108.68	-0.0931	55.47	39.91	1	0.0	0	K.LGDFSVIDTEFSNIR.E		14-28	CID
1496	740.271	3	-111.57	-0.0826	52.47	23.28	1	0.0	0	K.SSTWLDSLNSPLIQDEGDNK.M		82-101	CID
1159	840.827	2	-119.63	-0.1006	44.46	54.03	1	0.0	0	R.FDVSQYQPEEIVVK.T		107-120	CID
1448	905.363	2	416.97	0.3774	51.30	33.31	1	0.0	0	K.DGVLTVEAPLPSLTGPDK.L		167-184	CID

Protein 21: putative annexin [Rhodnius neglectus]

Accession: JAI55118.1
Database: rneglectus
MW [kDa] / pI: 35.7 / 5.5

Score: 143.9
Seq. Coverage [%]: 24.8
No. of Peptides: 5

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
2116	866.382	3	291.84	0.2528	77.35	26.43	1	0.0	1	K.SELGGKFEDVIVALMTPLPAFYAK.Q		76-99	CID
1313	695.254	2	-146.81	-0.1021	48.05	26.46	1	0.0	0	R.TIGAFYEQLYGK.S		129-140	CID
803	810.798	2	-121.88	-0.0988	36.21	22.77	1	0.0	1	K.SLESDLKGDTSGHFK.H		141-155	CID
544	736.336	2	-99.65	-0.0734	30.02	39.18	1	0.0	2	R.KDAEALLAAGEQKK.W		179-192	CID
1671	862.354	2	-95.23	-0.0821	56.90	22.39	2	0.0	0	K.WGTDESVFNSILVTR.S		193-207	CID
1675	862.345	2	-105.66	-0.0911	57.02	29.06	2	0.0	0	K.WGTDESVFNSILVTR.S		193-207	CID

Protein 22: putative beta-spectrin, partial [Rhodnius neglectus]

Accession: JAI52768.1
Database: rneglectus
MW [kDa] / pI: 50.6 / 6.8
Modification(s): Carbamidomethyl

Score: 128.8
Seq. Coverage [%]: 9.9
No. of Peptides: 4

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

```
        10         20         30         40         50         60         70         80         90        100
MMNNGIDQYG DGYMEPEEEW EREGLLDPAW EKQQKKTFTA WCNSHLRKAG TSIENIEEDF RNGLKLMLLL EVISGETLPK PDRGKMRFHK IANVNKALDF
       110        120        130        140        150        160        170        180        190        200
IASKGVKLVS IGAEEIVDGN LKMTLGMIWT IILRFAIQDI SVEEMTAKEG LLLWCQRKTA PYKNVNVQNF HLSFKDGLAF CALIHRHRPD LIDYNKLSKD
       210        220        230        240        250        260        270        280        290        300
NPLQNLNTAF DVAEKYLDIP RMLDPEDMTN TAMPDERVIM TYVXXXXXAF SGAQKAETAA NRICKVLKVN QENERLMEEY ERLASDLLEW IRRTMPWLES
       310        320        330        340        350        360        370        380        390        400
RVTDNSLAGV QKKLEEYRTY RRKLKPPRVE QKAKLETNFN TLQTKLRLSN RPAYMPTEGK MVSDIANAWK GLENAEKSFE DWLLSEMMRL ERLEHLAQKF
       410        420        430        440
KHKADIHEEW TRGKEEMLQS GDFRQCRLNE LKALK
```

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1410	587.743	2	-106.34	-0.0625	50.31	19.19	1	0.0	0	K.EGLLLWCQR.K	Carbamidomethyl: 7	149-157	CID
1199	723.777	2	-139.60	-0.1011	45.37	26.93	1	0.0	0	K.NVNVQNFHLSFK.D		164-175	CID
1785	608.279	2	-100.66	-0.0612	60.06	52.04	1	0.0	0	R.LASDLLEWIR.R		283-292	CID
1985	772.276	2	-95.32	-0.0736	67.72	30.66	1	0.0	0	K.SFEDWLLSEMMR.L		378-389	CID

Protein 23: putative muscle lim protein mlp84b [Rhodnius neglectus]

Accession: JAI54218.1
Database: rneglectus
MW [kDa] / pI: 48.5 / 8.3
Modification(s): Carbamidomethyl

Score: 124.3
Seq. Coverage [%]: 8.6
No. of Peptides: 3

10	20	30	40	50	60	70	80	90	100
MCGKFLDSTN	CAEHEGELYC	KVCHGRKYGP	KGYGFGGGAG	CLSMDKGEHL	GNAEASRASA	ETPVRAIAKA	PEGEGCPRCG	GYVYAAEQML	ARGRGWHREC
110	120	130	140	150	160	170	180	190	200
FKCAECSKRL	DSVNCCEGPD	KDIYCKVCYG	KRFGPKGYGY	GQGGGALQSD	PSVNGEVPAP	RTTTIDTACI	KAAPGQGCPR	CGGVVFAAEQ	VLAKGREWHR
210	220	230	240	250	260	270	280	290	300
KCFKCRDCNK	TLDSIIACDG	PDKDVYCKTC	YGKKWGPHGY	GFACGSGFLQ	TDGLTEEEIS	SARPFYNPDT	TSIRAPPGEG	CPRCGGMVFA	AEQQLAKGTM
310	320	330	340	350	360	370	380	390	400
WHKICFNCAQ	CHRPLDSMLA	CDGPDKEIYC	KACYGKNFGP	KGFGYGHSPT	LVSTNGESTV	AYCDARPITG	VKATDGKGCL	RCGFEVYAAE	QMISKHRVWH
410	420	430	440	450	460				
KRCFNCGDCH	RSLDSTNLND	GPDGDIYCRG	CYGRHFGPRG	VGFGLGAGAL	TMA				

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
727	1036.795	2	-137.95	-0.1430	34.41	42.01	1	0.0	0	K.FLDSTNCAEHEGELYCK.V	Carbamidomethyl: 7, 16	5-21	CID
67	415.52	2	-485.82	-0.2020	16.84	17.24	1	0.0	0	R.ASAETPVR.A		58-65	CID
1470	724.805	2	-106.15	-0.0769	51.81	65.02	1	0.0	0	R.CGGVVFAAEQVLAK.G	Carbamidomethyl: 1	181-194	CID

Protein 24: putative superoxide dismutase [Rhodnius neglectus]

Accession: JAI55997.1
Database: rneglectus
MW [kDa] / pI: 15.8 / 5.9
Modification(s): Carbamidomethyl

Score: 121.2
Seq. Coverage [%]: 34.4
No. of Peptides: 4

10	20	30	40	50	60	70	80	90	100
MPIKAVCVLK	GETVKGTVYF	EQESPNAEVK	LSGEVAGLQK	GLHGFHVHEF	GDNTNGCTSA	GAHFNPDNKE	HGAPTDETRH	VGDLGNVCAG	EDGVAKVGIC
110	120	130	140	150	160				
DKAISLCGPL	SIIGRTLVVH	ADPDDLGKGG	HELSKTTGNA	GARLACGVIG	ITKA				

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

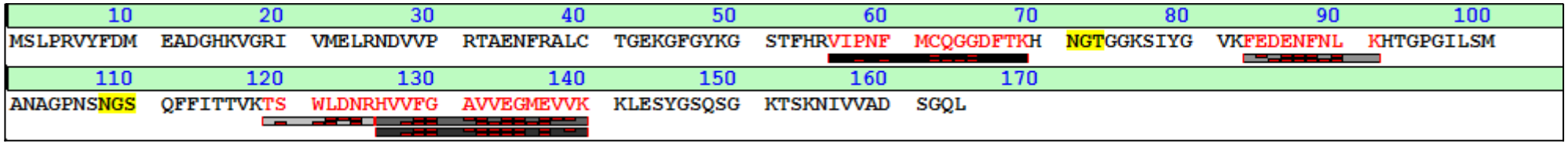
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
453	501.102	2	-364.93	-0.1829	27.76	22.16	1	0.0	0	K.LSGEVAGLQK.G		31-40	CID
562	849.278	2	-142.71	-0.1212	30.48	45.64	1	0.0	0	R.HVGDLGNVCAGEDGVAK.V	Carbamidomethyl: 9	80-96	CID
1701	678.82	2	-98.73	-0.0670	57.69	33.91	1	0.0	0	K.AISLCGPLSIIGR.T	Carbamidomethyl: 5	103-115	CID
724	690.313	2	-70.83	-0.0489	34.34	19.48	1	0.0	0	R.TLVVHADPDDLGK.G		116-128	CID

Protein 25: putative cyclophilin type peptidyl-prolyl cis-trans isomerase [Rhodnius neglectus]

Accession: JAI55367.1
Database: rneglectus
MW [kDa] / pI: 17.9 / 8.9
Modification(s): Carbamidomethyl

Score: 118.2
Seq. Coverage [%]: 27.4
No. of Peptides: 4



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1227	807.278	2	-121.59	-0.0982	46.01	18.43	1	0.0	0	R.VIPNFMCQGGDFTK.H	Carbamidomethyl: 7	56-69	CID
800	578.177	2	-159.92	-0.0925	36.15	31.18	1	0.0	0	K.FEDENFNLK.H		83-91	CID
598	446.087	2	-297.14	-0.1326	31.32	15.97	1	0.0	0	K.TSWLDNR.H		119-125	CID
1451	800.367	2	-80.96	-0.0648	51.36	51.48	2	0.0	0	R.HVVFGAVVEGMEVVK.K		126-140	CID
1453	800.355	2	-95.95	-0.0768	51.42	52.58	2	0.0	0	R.HVVFGAVVEGMEVVK.K		126-140	CID

Protein 26: putative f0f1-type atp synthase alpha subunit, partial [Rhodnius neglectus]

Accession:	JAI53323.1	**Score:**	100.8
Database:	rneglectus	**Seq. Coverage [%]:**	7.8
MW [kDa] / pI:	46.8 / 9.2	**No. of Peptides:**	3

```
        10        20        30        40        50        60        70        80        90       100
MALISARVIT SVARQFTSSV PQVGNLSWPA SQNTFRAIHV SCSHRAAEIS SILEERILGT PPKTDLEETG RVLSIGDGIA RVYGLKNIQA DEMVEFSSGL
       110       120       130       140       150       160       170       180       190       200
KGMALNLEPD NVGVVVFGND KLISEGDVVK RTGAIVDVPV GEDLLGRVVD ALGNPIDGKG PLASKKRMRV GVKAPGIIPR ISVREPMQTG IKAVDSLVPI
       210       220       230       240       250       260       270       280       290       300
GRGQRELIIG DRQTGKTALA IDTIINQKRF NDADEEKKKL YCIYVAIGQK RSTVAQIVKR LTDTGSMKYT IIVSATASDA APLQYLAPYS GCAMGEHLRD
       310       320       330       340       350       360       370       380       390       400
NGKHALIIYD DLSKQAVAYR QMSLLLRRPP GREAYPGDVF YLHSRLLERA AKMNDANGGG SLTALPVIET QAGDVSAYIP TNVISITDGQ IFLETELFYK
       410       420       430       440
GIRPAINVGL SVSRVGSAAQ TKAMKQVAGS MKLEL
```

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1078	609.264	2	-95.59	-0.0582	42.54	31.94	1	0.0	0	R.AAEISSILEER.I		46-56	CID
780	599.261	2	-110.67	-0.0663	35.69	27.51	1	0.0	0	R.VVDALGNPIDGK.G		148-159	CID
1055	644.284	2	-103.67	-0.0668	42.01	41.39	1	0.0	0	K.HALIIYDDLSK.Q		304-314	CID

Protein 27: putative beta-spectrin [Rhodnius neglectus]

Accession:	JAI56015.1	**Score:**	89.7
Database:	rneglectus	**Seq. Coverage [%]:**	1.7
MW [kDa] / pI:	278.4 / 5.1	**No. of Peptides:**	3

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

10	20	30	40	50	60	70	80	90	100
MDQITPKEVR	ILETADDIQE	RREQVLGRYG	DFKSDARAKR	QKLEDSRRFQ	YFKRDADELE	SWINEKLQAA	SDESYKDPTN	LQAKIQKHQA	FEAEVAAHSN
110	120	130	140	150	160	170	180	190	200
AIVMLDNTGK	EMINQGHYAS	DVIERRLEEL	HKLWELLLSR	LAEKGMKLQQ	ALVLVQFLRQ	CDEVMFWIKD	KEMFVTTDEF	GQDLEHVEVL	QRKFDEFQKD
210	220	230	240	250	260	270	280	290	300
MASQEYRVTE	VNELADKLVS	DGHPEREMIF	KRKEELNEAW	TRLRQQALMR	QEKLFGAHEI	QRLNRDADET	VAWIAEKDVV	LSSDDYGRDL	ASVQTLQRKH
310	320	330	340	350	360	370	380	390	400
EGVERDLAAL	EDKVSTLGKE	ADRLCTIHSD	HAAQIQAKRA	EIVSYWERLT	AKAKERRQKL	DESYYLHRFL	ADFRDLISWI	NDMKAIISAD	ELAKDVAGAE
410	420	430	440	450	460	470	480	490	500
ALLERHQEHK	GEIDAREDSF	RSTAEAGEVL	LERNHYAAAE	VREKLHVLES	EKSALLSLWD	DRRLLNEQCM	DLQLFYRDTE	QADTWMAKQE	AFLANDDLGD
510	520	530	540	550	560	570	580	590	600
SLDSVEALIK	KHEDFEKSLA	AQEEKIKALD	EFATKLIECQ	HYAADDVAQR	RSLLLERRSA	LLDKSSERRA	ILDDSFTLQQ	FERDCDETKG	WINEKLKFAT
610	620	630	640	650	660	670	680	690	700
DDSYLDPTNL	NGKVQKQQNF	EQELNANSSR	MEEITSTGQE	LIEADHYASD	RIRSRMEDIV	QLWEVLVAAT	EKKGSKLQEA	SQQQQFNRTV	EDIELWFTEI
710	720	730	740	750	760	770	780	790	800
EGQLHSEDYG	KDLTSVQNLQ	KKHALLEADV	GSHQDRIEGI	RVAAAQFVER	GHFDAENIKA	KQEAVTERYA	ALQKPMSIRK	QKLLDSLRVQ	QLFRDIEDEE
810	820	830	840	850	860	870	880	890	900
AWIREKEPVA	ASTNRGRDLI	GVQNLMKKHQ	AVLAEINNHE	SRMSAVTQAG	DQMIDEGHFS	SEEIKKRTAD	LNAHWLQLKE	KALQRKQDLE	DSLQAHQYFA
910	920	930	940	950	960	970	980	990	1000
DANEAESWMK	EKEPMVVNED	FGKDEDSAEA	LLKKHEALVS	DLEAFGNTIV	ALSQQAELCK	QQETPVIDVT	GKECVMALYD	YTEKSPREVS	MKKGDVLTLL
1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
NSNNKDWWKV	EVNDRQGFVP	AAYVKKMEAG	LTASQQNLAD	SSSIAARQSQ	IEQQYKRLLD	LAKERQNKLN	ETVKAYVLVR	EAAELATWIK	DKENHAQVQD
1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
IGEDLEQVEV	MQKKFDDFQS	DLKANEVRLA	EMNEIAMQLM	TLGQTEAAVK	IQTQLQDLNE	KWTSLQQLTE	ERATQLGSAH	EVQRFHRDVD	ETKDWIQEKE
1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
EALNNDDLGK	DLRSVQALQR	KHEGLERDLA	ALGDKIKQLD	ETANRLMQTH	PETAEQTYAK	QRDINDEWTQ	LTAKANSRKE	RLLDSYDLQR	FLSDYRDLMS

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1881	714.33	2	-154.37	-0.1103	63.21	39.74	1	0.0	0	K.LQQALVLVQFLR.Q		148-159	CID
1213	705.314	2	-94.17	-0.0664	45.69	26.59	1	0.0	0	R.TADLNAHWLQLK.E		868-879	CID
1723	702.316	3	-107.56	-0.0755	58.28	23.37	1	0.0	1	K.KIEELGAILEEHLILDNR.Y		2205-2222	CID

Protein 28: putative chaperonin [Rhodnius neglectus]

Accession: JAI55756.1
Database: rneglectus
MW [kDa] / pI: 57.9 / 5.5
Modification(s): Carbamidomethyl

Score: 72.0
Seq. Coverage [%]: 5.4
No. of Peptides: 2

10	20	30	40	50	60	70	80	90	100
MMSLNPIRIL	KPEAEEEKGE	MARLSNFVGA	IAVGDLVKST	LGPKGMDKIL	ASYGPRGRGK	VEVTNDGATI	LKAIGVDNPA	AKILVNMAKV	QDDEVGDGTT
110	120	130	140	150	160	170	180	190	200
SVTVLAAELL	REAERMIDRK	IHPQTIISGY	RKAASVALEA	LEAAANDNSS	NDEKFQKDLH	NLAMTALSSK	ILNLHKEKFS	KLVVDAVLRL	KGSGNLSAIQ
210	220	230	240	250	260	270	280	290	300
VIKKTGGCLS	DSFLDDGFLL	DKKVGQFQPK	RIENAKILIA	NTPMDTDKIK	VFGSRVRVES	VAMIAELELA	EKEKMKDKVN	KILSHNCNVF	VNRQLIYNYP
310	320	330	340	350	360	370	380	390	400
EQLFADAKIM	AIEHADFDGI	ERLALVTGGE	IVSTFDSPET	TKLGECDLIE	EVMVGEDKLL	RFSGVKLGEA	CSIVIRGATQ	QIVDEAERSL	HDALCVLVTT
410	420	430	440	450	460	470	480	490	500
VRDKRTIFGG	GSAEMIMALA	VSDAANKVVG	KESVAMEAYS	RALLQIPMTI	SDNGGFDSSY	LITSLRAAHI	EGKVSAGLDM	TNGEIGDMGE	SGVLESFAVK
510	520	530	540						
RQMILSASEA	TEVILRVDDI	IKAAPRKRTE	DRGYC						

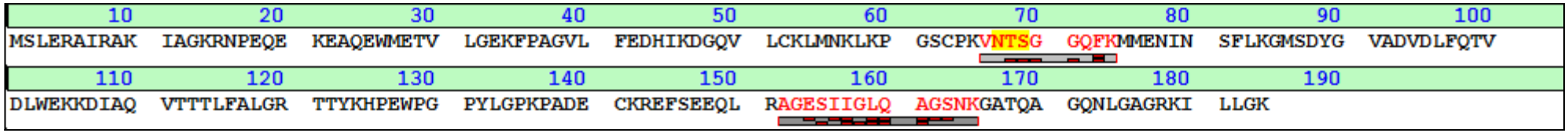
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1687	751.851	2	-108.38	-0.0815	57.30	51.26	1	0.0	0	R.LSNFVGAIAVGDLVK.S		24-38	CID
1600	792.314	2	-149.33	-0.1183	55.02	20.76	1	0.0	0	R.SLHDALCVLVTTVR.D	Carbamidomethyl: 7	389-402	CID

Protein 29: putative muscle-specific protein 20 [Rhodnius neglectus]

Accession: JAI56018.1
Database: rneglectus
MW [kDa] / pI: 20.3 / 8.4

Score: 57.6
Seq. Coverage [%]: 12.5
No. of Peptides: 2



MS/MS Peptide Matches

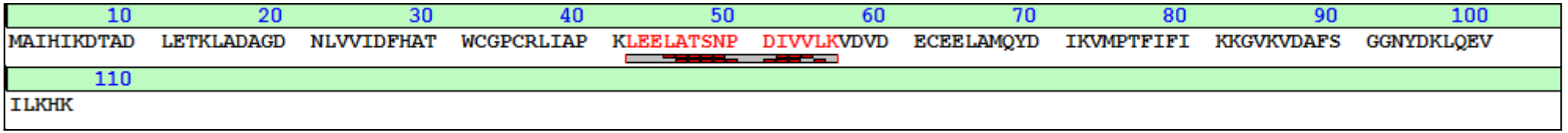
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
128	469.094	2	-312.26	-0.1465	19.00	17.13	1	0.0	0	K.VNTSGGQFK.M		66-74	CID
744	672.779	2	-119.67	-0.0805	34.79	40.42	1	0.0	0	R.AGESIIGLQAGSNK.G		152-165	CID

Protein 30: putative thioredoxin [Rhodnius neglectus]

Accession: JAI55853.1
Database: rneglectus
MW [kDa] / pI: 11.7 / 4.9

Score: 48.3
Seq. Coverage [%]: 14.3
No. of Peptides: 1

Protein Report
IonTrap_allOrg_2019-11-11 12:01:51



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1261	820.865	2	-114.38	-0.0939	46.84	48.34	1	0.0	0	K.LEELATSNPDIVVLK.V		42-56	CID

Apêndice 2. Identificação das proteínas por meio do software *ProteinScape* (versão 3.1, Bruker Daltonics) e o algoritmo Mascot (v2.3, Matrix Science, UK) para *SwissProt*.

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Project Info

Name:	Colaboração	**Date:**	Apr 16, 2019

Sample Info & Protocols

Name:	Ju_espermateca
Date:	Nov 5, 2019

Search Result Info

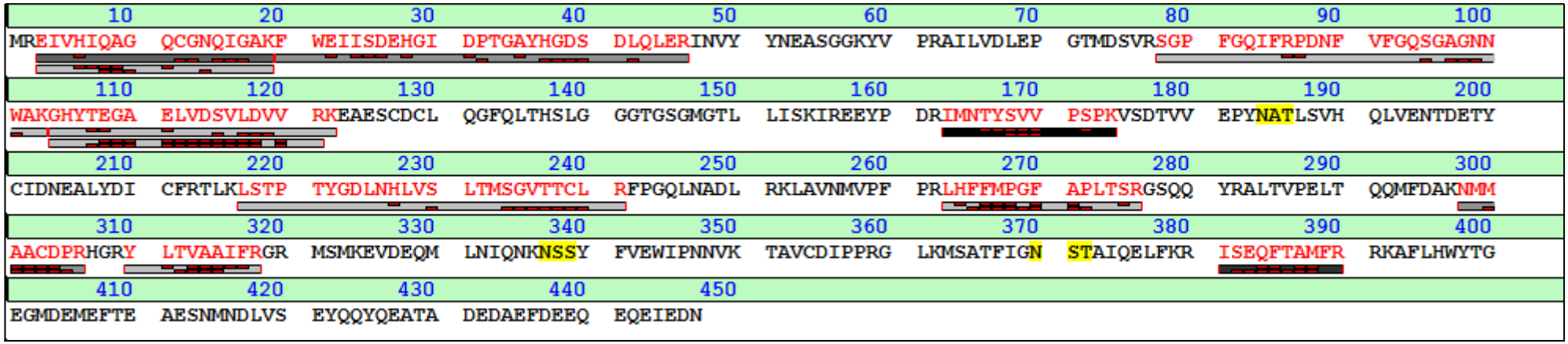
Search Result:	IonTrap_allOrg_2019-11-11 12:08:16		
Location:	/Colaboração/Ju_espermateca/Ju_esp_051119.mgf		
Search Method:	IonTrap_allOrg	**Version:**	
Search Engine(s):	Mascot, 2.3.02	**Ident. Compound(s):**	119/2717
Database(s):	SwissProt, SwissProt_57.15.fasta		
Note:			

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Results

Protein 1: Tubulin beta-1 chain OS=Manduca sexta PE=2 SV=1

Accession:	TBB1_MANSE	**Score:**	566.2
Database:	SwissProt	**Seq. Coverage [%]:**	37.6
MW [kDa] / pI:	50.2 / 4.8	**No. of Peptides:**	11
Modification(s):	Carbamidomethyl		



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
619	911.945	2	-22.00	-0.0201	31.84	24.53	1	0.0	0	R.EIVHIQAGQCGNQIGAK.F	Carbamidomethyl: 10	3-19	CID
617	608.238	3	-122.41	-0.0745	31.78	22.81	1	0.0	0	R.EIVHIQAGQCGNQIGAK.F	Carbamidomethyl: 10	3-19	CID
1476	1034.027	3	-116.63	-0.1206	51.95	63.15	1	0.0	0	K.FWEIISDEHGIDPTGAYHGDSDLQLER.I		20-46	CID
1733	933.327	3	-134.60	-0.1256	58.61	46.66	1	0.0	0	R.SGPFGQIFRPDNFVFGQSGAGNNWAK.G		78-103	CID
1818	979.879	2	-117.90	-0.1155	61.18	98.84	1	0.0	0	K.GHYTEGAELVDSVLDVVR.K		104-121	CID
1740	696.291	3	-104.50	-0.0728	58.81	22.06	1	0.0	1	K.GHYTEGAELVDSVLDVVRK.E		104-122	CID

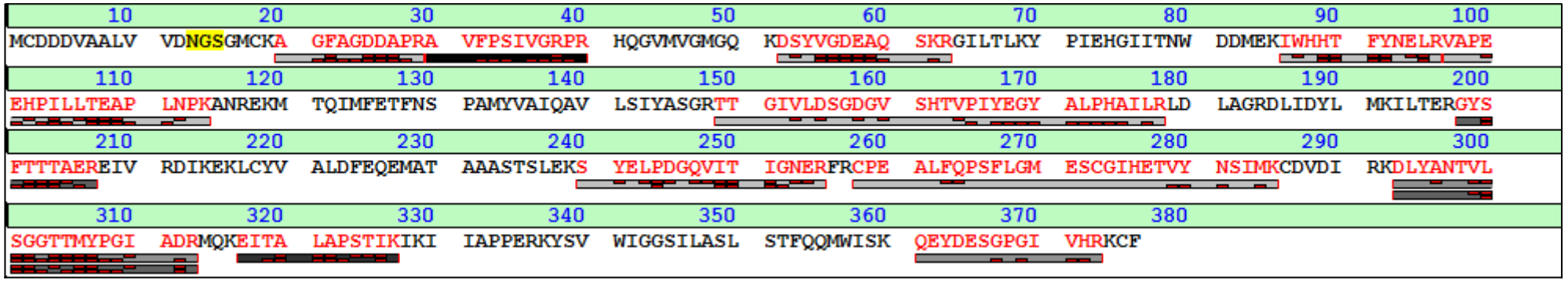
Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
850	668.278	2	-111.45	-0.0745	37.32	29.72	1	0.0	0	R.IMNTYSVVPSPK.V		163-174	CID
1918	912.685	3	-120.17	-0.1097	64.47	23.47	1	0.0	0	K.LSTPTYGDLNHLVSLTMSGVTTCLR.F	Carbamidomethyl: 23	217-241	CID
1775	810.811	2	-136.15	-0.1104	59.86	45.58	1	0.0	0	R.LHFFMPGFAPLTSR.G		263-276	CID
299	533.093	2	-233.22	-0.1244	23.91	30.72	1	0.0	0	K.NMMAACDPR.H	Carbamidomethyl: 6	298-306	CID
1643	527.195	2	-214.69	-0.1132	56.11	30.66	1	0.0	0	R.YLTVAAIFR.G		310-318	CID
1312	615.235	2	-110.19	-0.0678	48.04	47.57	1	0.0	0	R.ISEQFTAMFR.R		381-390	CID

Protein 2: Actin, clone 211 OS=Artemia sp. PE=2 SV=1

Accession: ACT2_ARTSX
Database: SwissProt
MW [kDa] / pI: 41.8 / 5.3
Modification(s): Carbamidomethyl

Score: 503.9
Seq. Coverage [%]: 50.8
No. of Peptides: 12



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
270	488.569	2	-324.89	-0.1588	23.07	37.8	1	0.0	0	K.AGFAGDDAPR.A		20-29	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1027	599.786	2	-117.34	-0.0704	41.38	17.15	1	0.0	0	R.AVFPSIVGRPR.H		30-40	CID
120	677.691	2	-183.40	-0.1243	18.80	32.39	1	0.0	1	K.DSYVGDEAQSKR.G		52-63	CID
941	758.263	2	-151.93	-0.1152	39.39	38.93	1	0.0	0	K.IWHHTFYNELR.V		86-96	CID
1219	984.435	2	-110.37	-0.1087	45.82	52.05	1	0.0	0	R.VAPEEHPILLTEAPLNPK.A		97-114	CID
1616	1051.102	3	-111.23	-0.1169	55.46	81.49	1	0.0	0	R.TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L		149-178	CID
521	566.653	2	-201.32	-0.1141	29.42	33.74	1	0.0	0	R.GYSFTTTAER.E		198-207	CID
1225	895.825	2	-139.07	-0.1246	45.97	60.88	1	0.0	0	K.SYELPDGQVITIGNER.F		240-255	CID
1849	812.039	4	-105.67	-0.0858	62.03	35.56	1	0.0	0	R.CPEALFQPSFLGMESCGIHETVYNSIMK.C	Carbamidomethyl: 1, 16	258-285	CID
1393	1107.901	2	-124.20	-0.1376	49.98	52.28	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		293-313	CID
1391	1107.888	2	-135.93	-0.1506	49.91	59.66	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		293-313	CID
853	572.225	2	-191.53	-0.1096	37.38	34.42	1	0.0	0	K.EITALAPSTIK.I		317-327	CID
393	496.155	3	-162.32	-0.0805	26.32	19.87	1	0.0	0	K.QEYDESGPGIVHR.K		361-373	CID

Protein 3: Myosin heavy chain, muscle OS=Drosophila melanogaster GN=Mhc PE=1 SV=4

Accession: MYSA_DROME
Database: SwissProt
MW [kDa] / pI: 224.3 / 5.9

Score: 368.2
Seq. Coverage [%]: 6.7
No. of Peptides: 10

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

10	20	30	40	50	60	70	80	90	100
MPKPVANQED	EDPTPYLFVS	LEQRRIDQSK	PYDSKKSCWI	PDEKEGYLLG	EIKATKGDIV	SVGLQGGEVR	DIKSEKVEKV	NPPKFEKIED	MADMTVLNTP
110	120	130	140	150	160	170	180	190	200
CVLHNLRQRY	YAKLIYTYSG	LFCVAINPYK	RYPVYTNRCA	KMYRGKRRNE	VPPHIFAISD	GAYVDMLTNH	VNQSMLITGE	SGAGKTENTK	KVIAYFATVG
210	220	230	240	250	260	270	280	290	300
ASKKTDEAAK	SKGSLEDQVV	QTNPVLEAFG	NAKTVRNDNS	SRFGKFIRIH	FGPTGKLAGA	DIETYLLEKA	RVISQQSLER	SYHIFYQIMS	GSVPGVKDIC
310	320	330	340	350	360	370	380	390	400
LLTDNIYDYH	IVSQGKVTVA	SIDDAEEFSL	TDQAFDILGF	TKQEKEDVYR	ITAAVMHMGG	MKFKQRGREE	QAEQDGEEEG	GRVSKLFGCD	TAELYKNLLK
410	420	430	440	450	460	470	480	490	500
PRIKVGNEFV	TQGRNVQQVT	NSIGALCKGV	FDRLFKWLVK	KCNETLDTQQ	KRQHFIGVLD	IAGFEIFEYN	GFEQLCINFT	NEKLQQFFNH	IMFVMEQEEY
510	520	530	540	550	560	570	580	590	600
KKEGINWDFI	DFGMDLLACI	DLIEKPMGIL	SILEEESMFP	KATDQTFSEK	LTNTHLGKSA	PFQKPKPPKP	GQQAAHFAIA	HYAGCVSYNI	TGWLEKNKDP
610	620	630	640	650	660	670	680	690	700
LNDTVVDQFK	KSQNKLLIEI	FADHAGQSGG	GEQAKGGRGK	KGGGFATVSS	AYKEQLNSLM	TTLRSTQPHF	VRCIIPNEMK	QPGVVDAHLV	MHQLTCNGVL
710	720	730	740	750	760	770	780	790	800
EGIRICRKGF	PNRMMYPDFK	MRYQILNPRG	IKDLDCPKKA	SKVLIESTEL	NEDLYRLGHT	KVFFRAGVLG	QMEEFRDERL	GKIMSWMQAW	ARGYLSRKGF
810	820	830	840	850	860	870	880	890	900
KKLQEQRVAL	KVVQRNLRKY	LQLRTWPWYK	LWQKVKPLLN	VSRIEDEIAR	LEEKAKKAEE	LHAAEVKVRK	ELEALNAKLL	AEKTALLDSL	SGEKGALQDY
910	920	930	940	950	960	970	980	990	1000
QERNAKLTAQ	KNDLENQLRD	IQERLTQEED	ARNQLFQQKK	KADQEISGLK	KDIEDLELNV	QKAEQDKATK	DHQIRNLNDE	IAHQDELINK	LNKEKKMQGE
1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
TNQKTGEELQ	AAEDKINHLN	KVKAKLEQTL	DELEDSLERE	KKVRGDVEKS	KRKVEGDLKL	TQEAVADLER	NKKELEQTIQ	RKDKELSSIT	AKLEDEQVVV
1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
LKHQRQIKEL	QARIEELEEE	VEAERQARAK	AEKQRADLAR	ELEELGERLE	EAGGATSAQI	ELNKKREAEL	SKLRRDLEEA	NIQHESTLAN	LRKKHNDAVA
1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
EMAEQVDQLN	KLKAKAEHDR	QTCHNELNQT	RTACDQLGRD	KAAQEKIAKQ	LQHTLNEVQS	KLDETNRTLN	DFDASKKKLS	IENSDLLRQL	EEAESQVSQL

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

MS/MS Peptide Matches

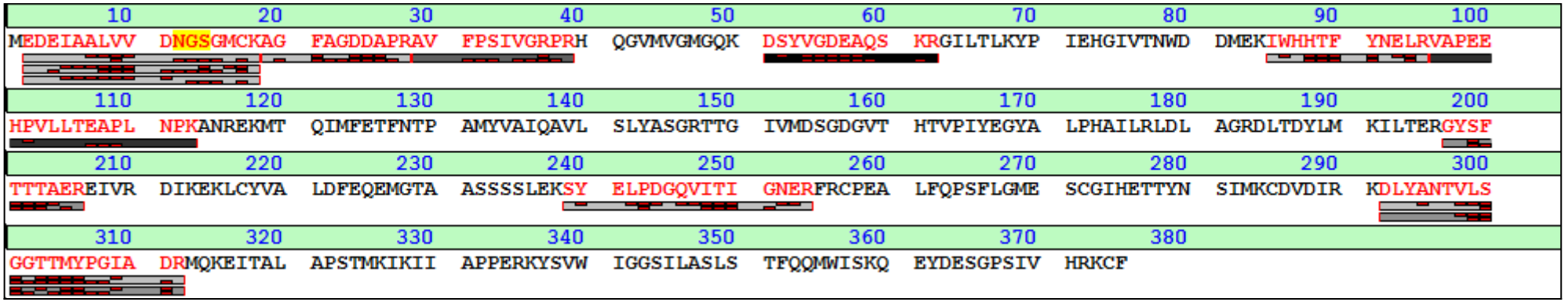
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1662	944.854	2	-131.80	-0.1246	56.70	45.39	1	0.0	1	K.AKLEQTLDELEDSLER.E		1024-1039	CID
1375	845.303	2	-129.54	-0.1095	49.52	36.12	1	0.0	0	K.LEQTLDELEDSLER.E		1026-1039	CID
774	737.756	2	-126.07	-0.0930	35.56	50.25	1	0.0	0	R.IEELEEEVEAER.Q		1114-1125	CID
1069	580.219	2	-173.19	-0.1005	42.34	22.71	1	0.0	0	K.LSIENSDLLR.Q		1279-1288	CID
851	787.805	2	-119.36	-0.0940	37.32	25.33	1	0.0	0	R.LAEAEETIESLNQK.C		1396-1409	CID
1122	808.311	2	-147.83	-0.1195	43.62	29.07	2	0.0	1	K.LKVDDLAAELDASQK.E		1457-1471	CID
1119	808.317	2	-140.41	-0.1135	43.56	32.21	2	0.0	1	K.LKVDDLAAELDASQK.E		1457-1471	CID
1988	971.374	2	-127.07	-0.1234	67.79	56.89	2	0.0	1	K.NLADEVKDLLDQIGEGGR.N		1503-1520	CID
1986	971.38	2	-120.89	-0.1174	67.73	79.18	2	0.0	1	K.NLADEVKDLLDQIGEGGR.N		1503-1520	CID
1987	647.931	3	-107.59	-0.0697	67.73	36.89	1	0.0	1	K.NLADEVKDLLDQIGEGGR.N		1503-1520	CID
852	732.282	2	-100.98	-0.0740	37.33	17.04	1	0.0	0	R.ALDSMQASLEAEAK.G		1592-1605	CID
1473	679.295	3	-95.88	-0.0651	51.89	19.24	1	0.0	1	K.KLEADINELEIALDHANK.A		1616-1633	CID
1296	821.349	2	-108.75	-0.0893	47.67	24.49	1	0.0	0	R.QIEEAEEIAALNLAK.F		1880-1894	CID

Protein 4: Actin, cytoplasmic 1 OS=Oreochromis mossambicus GN=actb PE=2 SV=1

Accession: ACTB_OREMO
Database: SwissProt
MW [kDa] / pI: 41.7 / 5.3
Modification(s): Carbamidomethyl

Score: 361.4
Seq. Coverage [%]: 33.6
No. of Peptides: 9

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16



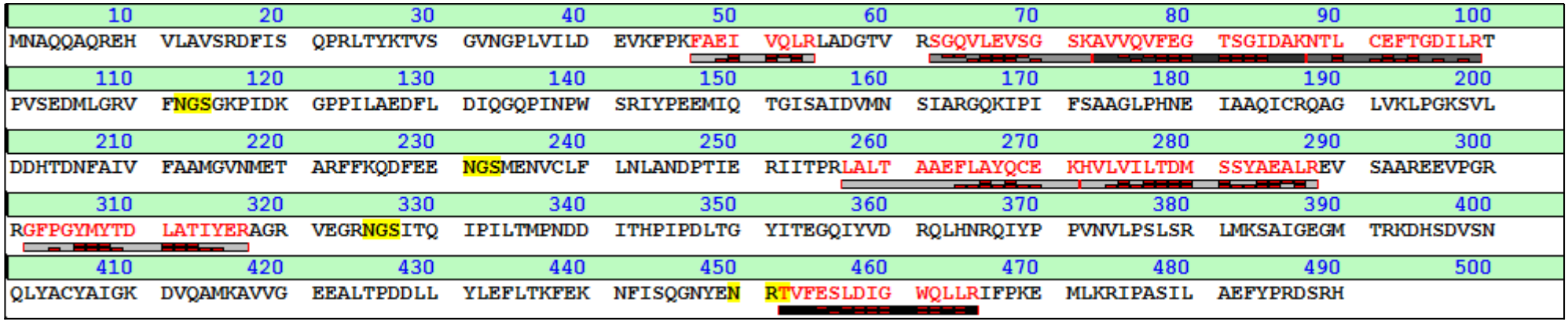
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1203	904.281	2	-146.70	-0.1327	45.45	25.27	3	0.0	0	M.EDEIAALVVDNGSGMCK.A	Carbamidomethyl: 16	2-18	CID
1185	904.75	2	371.87	0.3363	45.05	33.21	3	0.0	0	M.EDEIAALVVDNGSGMCK.A	Carbamidomethyl: 16	2-18	CID
1246	904.293	2	-133.43	-0.1207	46.46	64.45	3	0.0	0	M.EDEIAALVVDNGSGMCK.A	Carbamidomethyl: 16	2-18	CID
270	488.569	2	-324.89	-0.1588	23.07	37.8	1	0.0	0	K.AGFAGDDAPR.A		19-28	CID
1027	599.786	2	-117.34	-0.0704	41.38	17.15	1	0.0	0	R.AVFPSIVGRPR.H		29-39	CID
120	677.691	2	-183.40	-0.1243	18.80	32.39	1	0.0	1	K.DSYVGDEAQSKR.G		51-62	CID
941	758.263	2	-151.93	-0.1152	39.39	38.93	1	0.0	0	K.IWHHTFYNELR.V		85-95	CID
1138	651.963	3	-97.11	-0.0633	43.96	16.42	1	0.0	0	R.VAPEEHPVLLTEAPLNPK.A		96-113	CID
521	566.653	2	-201.32	-0.1141	29.42	33.74	1	0.0	0	R.GYSFTTTAER.E		197-206	CID
1225	895.825	2	-139.07	-0.1246	45.97	60.88	1	0.0	0	K.SYELPDGQVITIGNER.F		239-254	CID
1393	1107.901	2	-124.20	-0.1376	49.98	52.28	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		292-312	CID
1391	1107.888	2	-135.93	-0.1506	49.91	59.66	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		292-312	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Protein 5: V-type proton ATPase subunit B OS=Drosophila melanogaster GN=Vha55 PE=1 SV=1

Accession:	VATB_DROME	**Score:**	352.5
Database:	SwissProt	**Seq. Coverage [%]:**	22.0
MW [kDa] / pI:	54.5 / 5.3	**No. of Peptides:**	8
Modification(s):	Carbamidomethyl		



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1079	488.139	2	-298.46	-0.1457	42.54	21.94	1	0.0	0	K.FAEIVQLR.L		47-54	CID
344	546.178	2	709.84	0.3874	25.09	18.91	1	0.0	0	R.SGQVLEVSGSK.A		62-72	CID
1047	760.803	2	-129.30	-0.0984	41.82	46.13	1	0.0	0	K.AVVQVFEGTSGIDAK.N		73-87	CID
1431	719.76	2	-129.73	-0.0934	50.89	29.06	1	0.0	0	K.NTLCEFTGDILR.T	Carbamidomethyl: 4	88-99	CID
1514	864.32	2	-135.67	-0.1173	52.92	21.96	1	0.0	0	R.LALTAAEFLAYQCEK.H	Carbamidomethyl: 13	257-271	CID
1832	959.397	2	-116.49	-0.1118	61.63	89.99	1	0.0	0	K.HVLVILTDMSSYAEALR.E		272-288	CID
1716	948.861	2	-89.01	-0.0845	58.09	66.03	1	0.0	0	R.GFPGYMYTDLATIYER.A		302-317	CID
1979	838.877	2	-91.73	-0.0770	67.45	58.43	1	0.0	0	R.TVFESLDIGWQLLR.I		452-465	CID

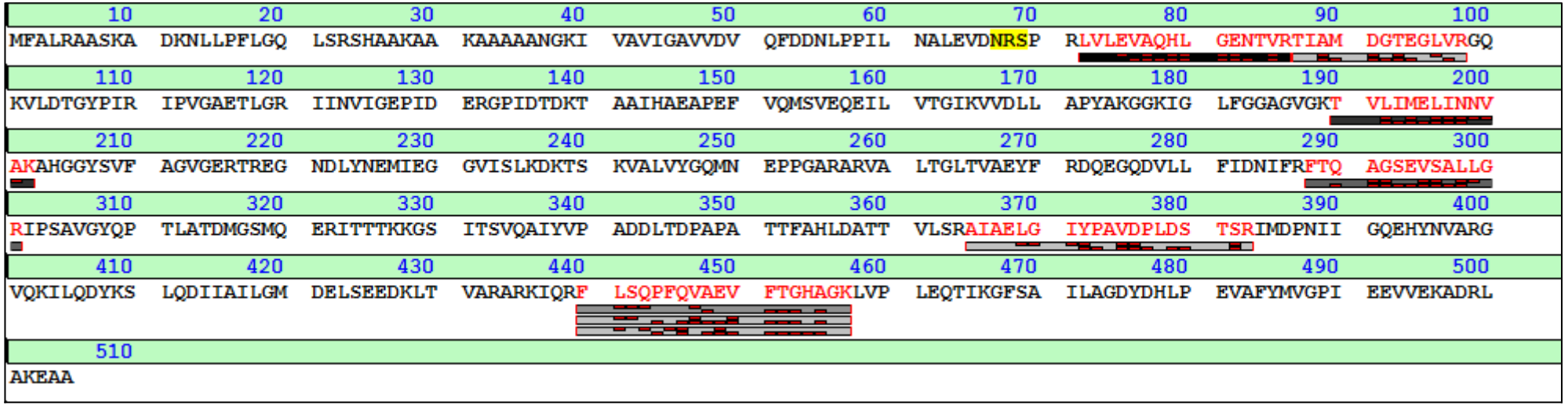
Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type

Protein 6: ATP synthase subunit beta, mitochondrial OS=Drosophila melanogaster GN=ATPsyn-beta PE=1 SV=3

Accession: ATPB_DROME
Database: SwissProt
MW [kDa] / pI: 54.1 / 5.1

Score: 350.6
Seq. Coverage [%]: 18.0
No. of Peptides: 6



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1198	839.367	2	-120.03	-0.1008	45.33	66.63	1	0.0	0	R.LVLEVAQHLGENTVR.T		72-86	CID
879	631.763	2	-96.70	-0.0611	37.95	25.4	1	0.0	0	R.TIAMDGTEGLVR.G		87-98	CID
1997	729.365	2	-80.12	-0.0584	68.05	54.62	1	0.0	0	K.TVLIMELINNVAK.A		190-202	CID

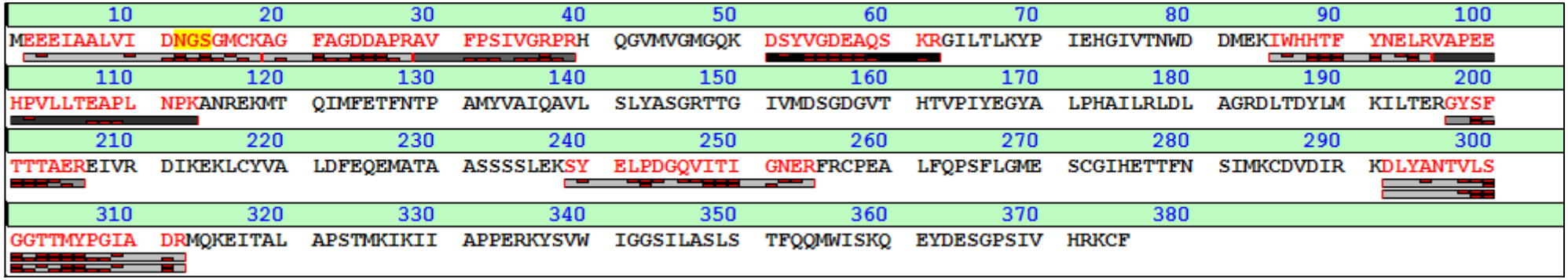
Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1254	718.303	2	-108.05	-0.0776	46.60	65.95	1	0.0	0	R.FTQAGSEVSALLGR.I		288-301	CID
1564	994.401	2	-120.05	-0.1194	54.17	38.79	1	0.0	0	R.AIAELGIYPAVDPLDSTSR.I		365-383	CID
1686	981.913	2	-95.98	-0.0942	57.29	63.17	2	0.0	0	R.FLSQPFQVAEVFTGHAGK.L		440-457	CID
1684	981.918	2	-90.89	-0.0892	57.24	76.66	2	0.0	0	R.FLSQPFQVAEVFTGHAGK.L		440-457	CID
1680	654.938	3	-105.74	-0.0693	57.16	17.65	1	0.0	0	R.FLSQPFQVAEVFTGHAGK.L		440-457	CID

Protein 7: Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1

Accession: ACTG_HUMAN
Database: SwissProt
MW [kDa] / pI: 41.8 / 5.3
Modification(s): Carbamidomethyl

Score: 337.6
Seq. Coverage [%]: 33.6
No. of Peptides: 9



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1338	918.325	2	-113.59	-0.1043	48.63	40.59	1	0.0	0	M.EEEIAALVIDNGSGMCK.A	Carbamidomethyl: 16	2-18	CID
270	488.569	2	-324.89	-0.1588	23.07	37.8	1	0.0	0	K.AGFAGDDAPR.A		19-28	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

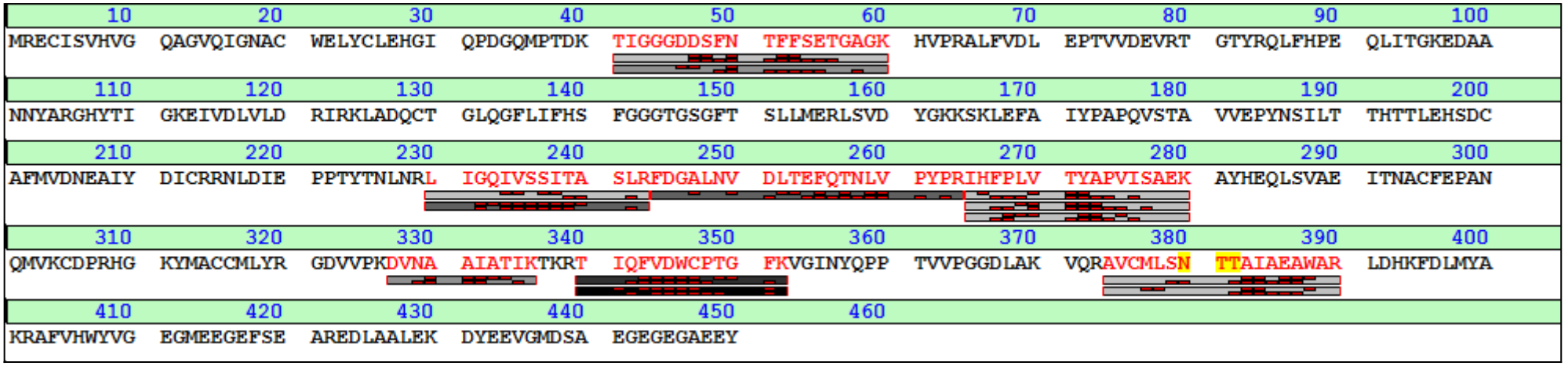
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1027	599.786	2	-117.34	-0.0704	41.38	17.15	1	0.0	0	R.AVFPSIVGRPR.H		29-39	CID
120	677.691	2	-183.40	-0.1243	18.80	32.39	1	0.0	1	K.DSYVGDEAQSKR.G		51-62	CID
941	758.263	2	-151.93	-0.1152	39.39	38.93	1	0.0	0	K.IWHHTFYNELR.V		85-95	CID
1138	651.963	3	-97.11	-0.0633	43.96	16.42	1	0.0	0	R.VAPEEHPVLLTEAPLNPK.A		96-113	CID
521	566.653	2	-201.32	-0.1141	29.42	33.74	1	0.0	0	R.GYSFTTTAER.E		197-206	CID
1225	895.825	2	-139.07	-0.1246	45.97	60.88	1	0.0	0	K.SYELPDGQVITIGNER.F		239-254	CID
1393	1107.901	2	-124.20	-0.1376	49.98	52.28	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		292-312	CID
1391	1107.888	2	-135.93	-0.1506	49.91	59.66	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		292-312	CID

Protein 8: Tubulin alpha chain OS=Bombyx mori PE=2 SV=1

Accession: TBA_BOMMO
Database: SwissProt
MW [kDa] / pI: 49.9 / 5.0
Modification(s): Carbamidomethyl

Score: 319.2
Seq. Coverage [%]: 24.7
No. of Peptides: 7

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16



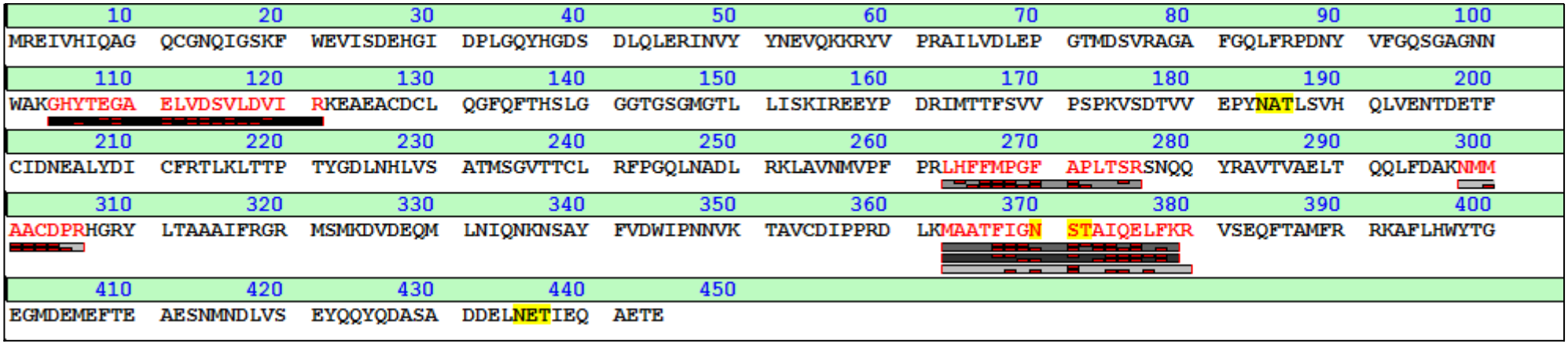
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1366	1004.327	2	-122.61	-0.1232	49.28	47.39	2	0.0	0	K.TIGGGDDSFNTFFSETGAGK.H		41-60	CID
1371	1004.313	2	-136.55	-0.1372	49.41	52.98	2	0.0	0	K.TIGGGDDSFNTFFSETGAGK.H		41-60	CID
1932	729.359	2	-108.22	-0.0789	65.02	68.11	1	0.0	0	R.LIGQIVSSITASLR.F		230-243	CID
1936	486.575	3	-108.34	-0.0527	65.13	16.16	1	0.0	0	R.LIGQIVSSITASLR.F		230-243	CID
1809	1204.961	2	-121.89	-0.1469	60.91	50.17	1	0.0	0	R.FDGALNVDLTEFQTNLVPYPR.I		244-264	CID
1634	892.88	2	-135.39	-0.1209	55.91	23.2	3	0.0	0	R.IHFPLVTYAPVISAEK.A		265-280	CID
1611	892.883	2	-132.03	-0.1179	55.33	26.3	3	0.0	0	R.IHFPLVTYAPVISAEK.A		265-280	CID
1631	892.881	2	-134.27	-0.1199	55.85	45.93	3	0.0	0	R.IHFPLVTYAPVISAEK.A		265-280	CID
723	508.182	2	-217.90	-0.1108	34.33	28.53	1	0.0	0	K.DVNAAIATIK.T		327-336	CID
1578	799.795	2	-115.30	-0.0922	54.54	44.59	2	0.0	0	R.TIQFVDWCPTGFK.V	Carbamidomethyl: 8	340-352	CID
1570	799.792	2	-119.05	-0.0952	54.35	45.2	2	0.0	0	R.TIQFVDWCPTGFK.V	Carbamidomethyl: 8	340-352	CID
1598	932.827	2	-138.11	-0.1288	55.00	22.69	2	0.0	0	R.AVCMLSNTTAIAEAWAR.L	Carbamidomethyl: 3	374-390	CID
1604	932.822	2	-143.46	-0.1338	55.14	28.26	2	0.0	0	R.AVCMLSNTTAIAEAWAR.L	Carbamidomethyl: 3	374-390	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Protein 9: Tubulin beta chain OS=Onchocerca gibsoni GN=TBB PE=3 SV=1

Accession:	TBB_ONCGI	**Score:**	232.0
Database:	SwissProt	**Seq. Coverage [%]:**	13.3
MW [kDa] / pI:	49.9 / 4.9	**No. of Peptides:**	5
Modification(s):	Carbamidomethyl, Oxidation		



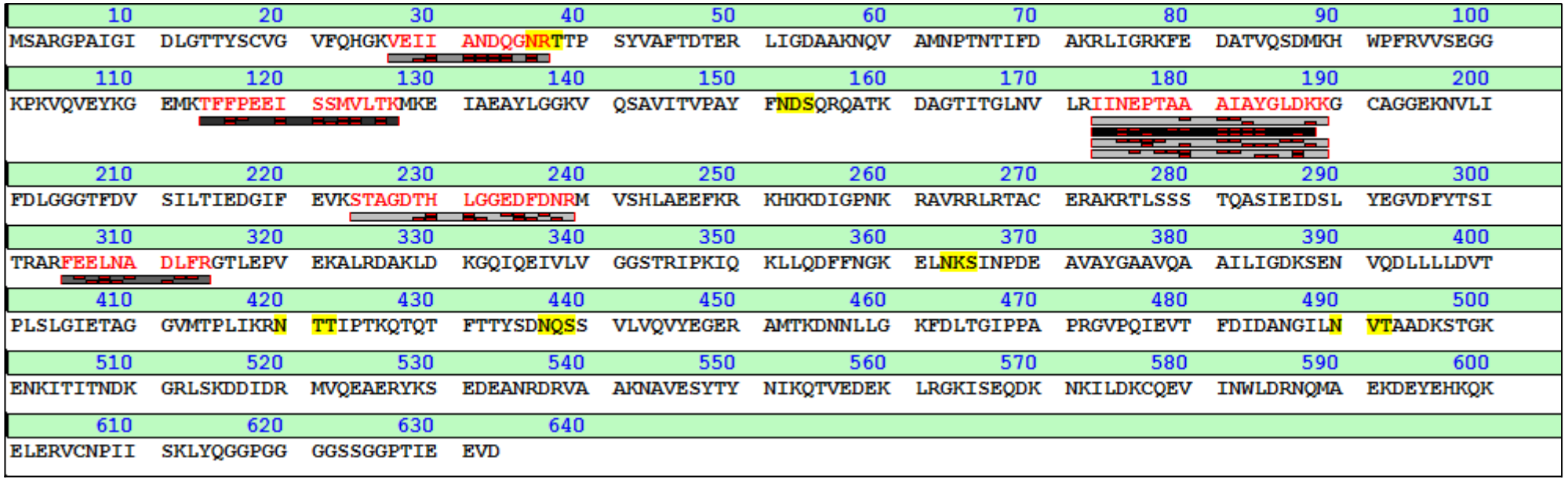
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1927	986.883	2	-120.93	-0.1194	64.87	56.71	1	0.0	0	K.GHYTEGAELVDSVLDVIR.K		104-121	CID
1775	810.811	2	-136.15	-0.1104	59.86	45.58	1	0.0	0	R.LHFFMPGFAPLTSR.S		263-276	CID
299	533.093	2	-233.22	-0.1244	23.91	30.72	1	0.0	0	K.NMMAACDPR.H	Carbamidomethyl: 6	298-306	CID
1810	929.36	2	-123.07	-0.1144	60.96	34.89	2	0.0	0	K.MAATFIGNSTAIQELFK.R	Oxidation: 1	363-379	CID
1804	929.356	2	-127.38	-0.1184	60.71	60.73	2	0.0	0	K.MAATFIGNSTAIQELFK.R	Oxidation: 1	363-379	CID
1711	1007.412	2	-112.11	-0.1129	57.96	16.63	1	0.0	1	K.MAATFIGNSTAIQELFKR.V	Oxidation: 1	363-380	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Protein 10: Heat shock-related 70 kDa protein 2 OS=Rattus norvegicus GN=Hspa2 PE=2 SV=2

Accession:	HSP72_RAT	**Score:**	223.9
Database:	SwissProt	**Seq. Coverage [%]:**	10.7
MW [kDa] / pI:	69.6 / 5.5	**No. of Peptides:**	6



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
384	614.738	2	-129.56	-0.0797	26.12	53.07	1	0.0	0	K.VEIIANDQGNR.T		27-37	CID
1779	814.812	2	-127.19	-0.1036	59.93	38.46	1	0.0	0	K.TFFPEEISSMVLTK.M		114-127	CID
1308	830.351	2	-120.71	-0.1002	47.92	35.21	1	0.0	0	R.IINEPTAAAIAYGLDK.K		173-188	CID

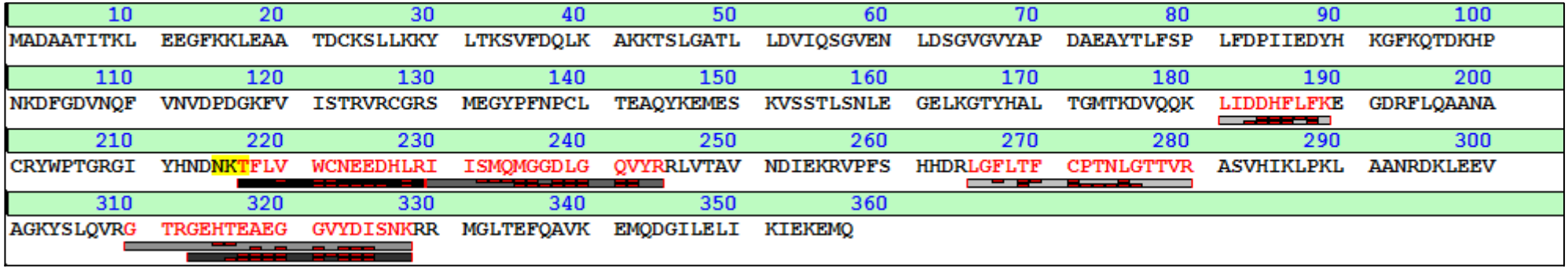
Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1196	894.392	2	-119.31	-0.1067	45.31	17.84	2	0.0	1	R.IINEPTAAAIAYGLDKK.G		173-189	CID
1186	894.385	2	-127.14	-0.1137	45.06	40.21	2	0.0	1	R.IINEPTAAAIAYGLDKK.G		173-189	CID
1190	596.603	3	-109.35	-0.0652	45.12	16.86	1	0.0	1	R.IINEPTAAAIAYGLDKK.G		173-189	CID
451	846.243	2	-145.83	-0.1234	27.69	35.47	1	0.0	0	K.STAGDTHLGGEDFDNR.M		224-239	CID
1359	627.293	2	-29.77	-0.0187	49.14	21.46	1	0.0	0	R.FEELNADLFR.G		305-314	CID

Protein 11: Arginine kinase OS=Carcinus maenas PE=2 SV=1

Accession: KARG_CARMA
Database: SwissProt
MW [kDa] / pI: 40.2 / 6.4
Modification(s): Carbamidomethyl

Score: 223.1
Seq. Coverage [%]: 20.2
No. of Peptides: 6



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1290	574.23	2	-140.95	-0.0809	47.54	40.91	1	0.0	0	K.LIDDHFLFK.E		181-189	CID
1327	859.799	2	-119.08	-0.1024	48.36	24.84	1	0.0	0	K.TFLVWCNEEDHLR.I	Carbamidomethyl: 6	217-229	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1264	834.312	2	-124.43	-0.1038	46.91	47.23	1	0.0	0	R.IISMQMGGDLGQVYR.R		230-244	CID
1748	898.872	2	-111.04	-0.0998	59.07	31.11	1	0.0	0	R.LGFLTFCPTNLGTTVR.A	Carbamidomethyl: 7	265-280	CID
418	673.881	3	-152.42	-0.1027	26.91	21.34	1	0.0	1	R.GTRGEHTEAEGGVYDISNK.R		310-328	CID
437	853.273	2	-133.39	-0.1138	27.37	57.72	1	0.0	0	R.GEHTEAEGGVYDISNK.R		313-328	CID

Protein 12: Arginine kinase OS=Homarus gammarus PE=1 SV=4

Accession: KARG_HOMGA
Database: SwissProt
MW [kDa] / pI: 40.0 / 6.0
Modification(s): Carbamidomethyl

Score: 180.6
Seq. Coverage [%]: 17.7
No. of Peptides: 5

```
        10        20        30        40        50        60        70        80        90       100
MADAATIAKL EEGFKKLEAA TDCKSLLKKY LSKDIFDSLK AKKTSLGATL LDVIQSGVEN LDSGVGIYAP DAEAYSLFAP LFDPIIEDYH KGFKQTDKHP
       110       120       130       140       150       160       170       180       190       200
AKDFGDVSKF INVDPEGTFV ISTRVRCGRS MEGYPFNPCL TEAQYKEMEE KVSSTLSGLE GELKGSYFPL TGMTKEVQQK LIDDHFLFKE GDRFLQAANA
       210       220       230       240       250       260       270       280       290       300
CRYWPAGRGI YHNDNKTFLV WCNEEDHLRI ISMQMGGDLG QVYRRLVSAV NDIEKRVPFS HHDRLGFLTF CPTNLGTTVR ASVHIKLPKL AANREKLEEV
       310       320       330       340       350       360
AAKFSLQVRG TRGEHTEAEG GIYDISNKRR MGLTEFQAVK EMQDGILELI KIEKEM
```

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1290	574.23	2	-140.95	-0.0809	47.54	40.91	1	0.0	0	K.LIDDHFLFK.E		181-189	CID
1327	859.799	2	-119.08	-0.1024	48.36	24.84	1	0.0	0	K.TFLVWCNEEDHLR.I	Carbamidomethyl: 6	217-229	CID
1264	834.312	2	-124.43	-0.1038	46.91	47.23	1	0.0	0	R.IISMQMGGDLGQVYR.R		230-244	CID
616	544.198	2	-193.50	-0.1053	31.74	36.5	1	0.0	0	R.LVSAVNDIEK.R		246-255	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1748	898.872	2	-111.04	-0.0998	59.07	31.11	1	0.0	0	R.LGFLTFCPTNLGTTVR.A	Carbamidomethyl: 7	265-280	CID

Protein 13: Heat shock cognate 71 kDa protein OS=Danio rerio GN=hspa8 PE=2 SV=1

Accession: HSP7C_DANRE
Database: SwissProt
MW [kDa] / pI: 70.9 / 5.2

Score: 151.7
Seq. Coverage [%]: 7.9
No. of Peptides: 4

```
10        20        30        40        50        60        70        80        90        100
MSKGPAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL IGDAAKNQVA MNPTNTVLDA NRLNGRQFDD GVVQSDMKHW PFNVINDNSR
110       120       130       140       150       160       170       180       190       200
PKVQVEYKGE SKSFYPEEIS SMVLTKMKEI AEAYLGKTVS NAVITVPAYS NDSQRQATKD AGTISGLNVL VIINEPTAAA IAYGLDKKVG AERNVLIFDL
210       220       230       240       250       260       270       280       290       300
GGGSFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVNH FITEFKRKHK KDISDNKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA
310       320       330       340       350       360       370       380       390       400
RFEELNADLF RGTLDPVEKA LRDAKMDKAQ IHDIVLVGGS TRIPKIQKLL QDYFNGKELN KSINPDEAVA YGAAVQAAIL SGDKSENVQD LLLLDVTPLS
410       420       430       440       450       460       470       480       490       500
LGIETAGGVM TVLIKRNTTI PTKQTQTFTT YSDNQPGVLI QVYEGERAMT KDNNLLGKFE LTGIPPAPRG VPQIEVTFDI DANGIMNVSA VDKSTGKENK
510       520       530       540       550       560       570       580       590       600
ITITNDKGRL SKEDIERMVQ EAEKYKAEDD VQRDKVSAKN GLESYAFNMK STVEDEKLKG KISDEDKQKI LDKCNEVIGW LDKNQTAERE EFEHQQKELE
610       620       630       640       650
KVCNPIITKL YQSAGGMPGG MPEGMPGGFP GAGAAPGGGS SGPTIEEVD
```

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
384	614.738	2	-129.56	-0.0797	26.12	53.07	1	0.0	0	K.VEIIANDQGNR.T		26-36	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
451	846.243	2	-145.83	-0.1234	27.69	35.47	1	0.0	0	K.STAGDTHLGGEDFDNR.M		221-236	CID
1359	627.293	2	-29.77	-0.0187	49.14	21.46	1	0.0	0	R.FEELNADLFR.G		302-311	CID
902	733.341	2	-93.69	-0.0687	38.50	41.74	1	0.0	0	K.AQIHDIVLVGGSTR.I		329-342	CID

Protein 14: Alpha-actinin, sarcomeric OS=Anopheles gambiae GN=Actn PE=3 SV=2

Accession: ACTN_ANOGA
Database: SwissProt
MW [kDa] / pI: 106.5 / 5.6
Modification(s): Carbamidomethyl

Score: 151.0
Seq. Coverage [%]: 6.4
No. of Peptides: 5

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

10	20	30	40	50	60	70	80	90	100
MMENGGYVGQ	YGGEENYMEQ	EEEWEREGLL	DPAWEKQQKK	TFTAWCNSHL	RKAGTSIENI	EDDFRNGLKL	MLLLEVISGE	TLPKPDRGKM	RFHKIANVNK
110	120	130	140	150	160	170	180	190	200
ALDFIASKGV	KLVSIGAEEI	VDGNLKMTLG	MIWTIILRFA	IQDISVEEMT	AKEGLLLWCQ	RKTAPYKNVN	VQNFHLSFKD	GLAFCALIHR	HRPDLIDYSK
210	220	230	240	250	260	270	280	290	300
LSKDNPLENL	NTAFDVAEKY	LDIPRMLDPD	DLINTPKPDE	RAIMTYVSCY	YHAFQGAQQP	GSTPFVIHLT	KTGLSYRFFV	RLFAAETAAN	RICKVLKVNQ
310	320	330	340	350	360	370	380	390	400
ENERLMEEYE	RLASDLLEWI	RRTMPWLNSR	QSDSTLAGVQ	KKLEEYRTYR	RKHKPPRVEQ	KAKLETNFNT	LQTKLRLSNR	PAYMPTEGKM	VSDITNSWKG
410	420	430	440	450	460	470	480	490	500
LEHAEKAFEE	WLLAETMRLE	RLEHLAQKFK	HKADTHEDWT	KGKEEMLQSQ	DFRNCKLNEL	KALKKKHEAF	ESDLAAHQDR	VEQIAAIAQE	LNTLEYHDCA
510	520	530	540	550	560	570	580	590	600
SVNARCQRIC	DQWDRLGALT	QRRRQGLDEA	ERILEKIDLL	HLEFAKRAAP	FNNWLDGARE	DLVDMFIVHT	MEEIQGLIQA	HDQFKATLGE	ADKEFNVIIG
610	620	630	640	650	660	670	680	690	700
LVRDAEAIVK	QEQVPGGLVN	PYTTLSADLI	SRKWSEVRAL	VPQRDQTLAN	ELRKQQNNEM	LRRQFAEKAN	AVGPWIERQM	DAVTAIGMGI	SGSLEEQLHR
710	720	730	740	750	760	770	780	790	800
LKEYEQAVYA	YKPSIEELEK	IHQAVQESMI	FENRYTHYTM	ETLRVGWEQL	LTSINRNINE	VENQILTRDS	KGITQEQLTE	FRSSFNHFDK	NRTGRLAPEE
810	820	830	840	850	860	870	880	890	900
FKSCLVSLGY	SIGKDKQGDM	DFQRILAVVD	PNASGYVQFD	AFLDFMTRES	TDTDTAEQVI	DSFRILASDR	PYILPDELRR	ELPPDQAEYC	IQRMPPYKGP
910	920	930							
NAIPGALDYM	SFSTALYGES	DL							

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1410	587.743	2	-106.34	-0.0625	50.31	19.19	1	0.0	0	K.EGLLLWCQR.K	Carbamidomethyl: 7	153-161	CID
1199	723.777	2	-139.60	-0.1011	45.37	26.93	1	0.0	0	K.NVNVQNFHLSFK.D		168-179	CID
1785	608.279	2	-100.66	-0.0612	60.06	52.04	1	0.0	0	R.LASDLLEWIR.R		312-321	CID
1712	708.308	2	-109.70	-0.0777	58.01	37.2	1	0.0	0	R.VGWEQLLTSINR.N		745-756	CID

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1314	907.309	2	-109.06	-0.0990	48.05	15.63	1	0.0	0	R.ESTDTDTAEQVIDSFR.I		849-864	CID

Protein 15: Tubulin beta-1 chain OS=Brugia pahangi PE=3 SV=1

Accession: TBB1_BRUPA
Database: SwissProt
MW [kDa] / pI: 50.2 / 4.7
Modification(s): Carbamidomethyl

Score: 141.3
Seq. Coverage [%]: 8.0
No. of Peptides: 3

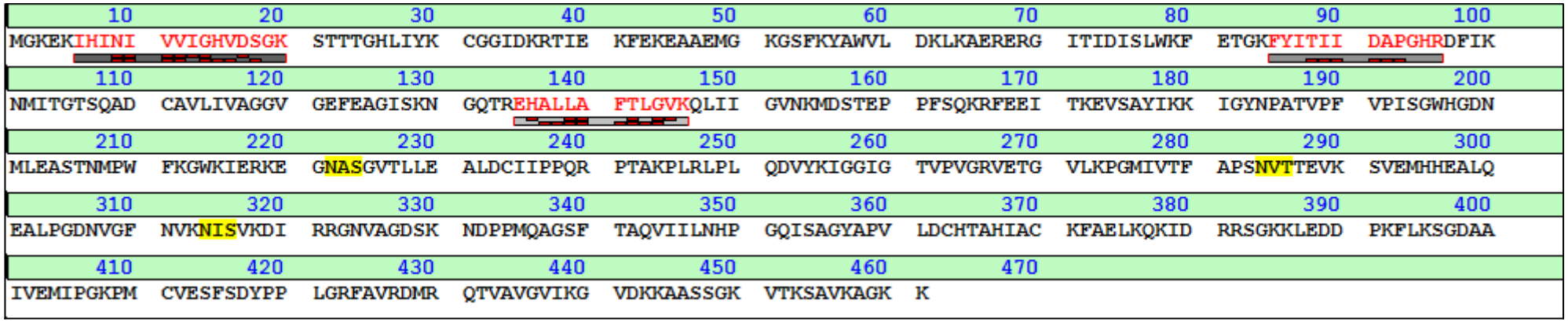
```
        10         20         30         40         50         60         70         80         90        100
MREIVHVQAG QCGNQIGAKF WEVISDEHGV QPDGTYKGDS DLQIERINVY YNEANGGKYV PRAVLVDLEP GTMDSIRGGE FGQLFRPDNF VFGQSGAGNN
       110        120        130        140        150        160        170        180        190        200
WAKGHYTEGA ELVDNVLDVI RKEAEGCDCL QGFQLTHSLG GGTGSGMGTL LISKIREEYP DRIMSSFSVV PSPKVSDVVL EPYNATLSVH QLVENTDETF
       210        220        230        240        250        260        270        280        290        300
CIDNEALYDI CFRTLKLANP TYGDLNHLVS VTMSGVTTCL RFPGQLNADL RKLAVNMVPF PRLHFFMPGF APLSARDAAA YRALNVAELT QQMFDAKNMM
       310        320        330        340        350        360        370        380        390        400
AACDPRHGRY LTVAAMFRGR MSMREVDEQM MQVQNKNSSY FVEWIPNNVK TAVCDIPPRG LKMSATFIGN TTAIQELFKR ISEQFTAMFR RKAFLHWYTG
       410        420        430        440        450
EGMDEMEFTE AESNMNDLVS EYQQYQDATA DEEGDLQEGE SEYIEQEE
```

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
299	533.093	2	-233.22	-0.1244	23.91	30.72	1	0.0	0	K.NMMAACDPR.H	Carbamidomethyl: 6	298-306	CID
1846	936.365	2	-125.17	-0.1172	61.97	41.33	1	0.0	0	K.MSATFIGNTTAIQELFK.R		363-379	CID
1312	615.235	2	-110.19	-0.0678	48.04	47.57	1	0.0	0	R.ISEQFTAMFR.R		381-390	CID

Protein 16: Elongation factor 1-alpha, oocyte form OS=Xenopus laevis GN=eef1ao PE=1 SV=1

Accession:	EF1A2_XENLA	**Score:**	141.0
Database:	SwissProt	**Seq. Coverage [%]:**	8.5
MW [kDa] / pI:	50.1 / 9.2	**No. of Peptides:**	3

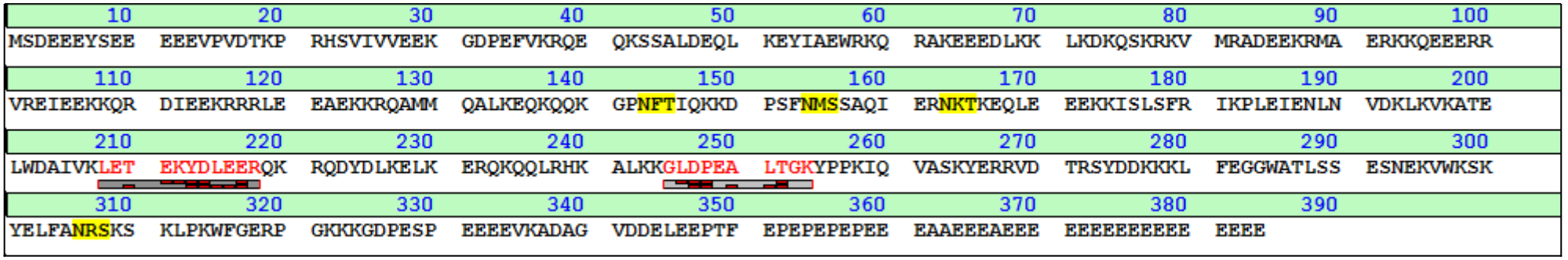


MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1208	800.878	2	-105.02	-0.0841	45.57	59.07	1	0.0	0	K.IHINIVVIGHVDSGK.S		6-20	CID
961	701.729	2	-211.60	-0.1485	39.85	28.03	1	0.0	0	K.FYITIIDAPGHR.D		85-96	CID
1518	649.834	2	-66.15	-0.0430	52.99	53.92	1	0.0	0	R.EHALLAFTLGVK.Q		135-146	CID

Protein 17: Troponin T OS=Periplaneta americana GN=TNT PE=2 SV=1

Accession:	TNNT_PERAM	**Score:**	86.5
Database:	SwissProt	**Seq. Coverage [%]:**	5.5
MW [kDa] / pI:	45.9 / 5.0	**No. of Peptides:**	2



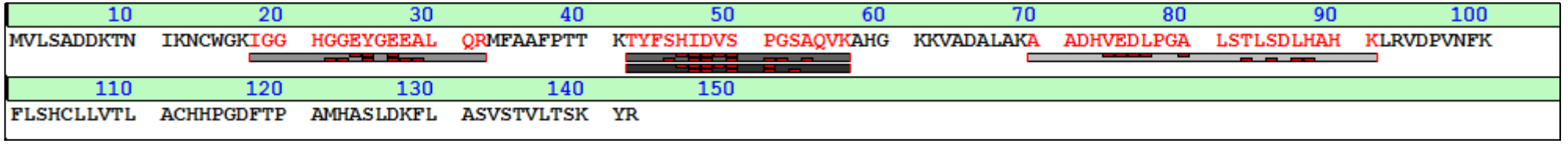
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
509	712.773	2	-106.36	-0.0758	29.11	37.24	1	0.0	1	K.LETEKYDLEER.Q		208-218	CID
756	500.574	2	-389.63	-0.1951	35.06	18.42	1	0.0	0	K.GLDPEALTGK.Y		245-254	CID

Protein 18: Hemoglobin subunit alpha-1/2 OS=Rattus norvegicus GN=Hba1 PE=1 SV=3

Accession: HBA_RAT
Database: SwissProt
MW [kDa] / pI: 15.3 / 7.8

Score: 83.4
Seq. Coverage [%]: 37.3
No. of Peptides: 3



MS/MS Peptide Matches

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
463	786.794	2	-101.28	-0.0797	28.01	16.88	1	0.0	0	K.IGGHGGEYGEEALQR.M		18-32	CID
905	868.342	2	-108.39	-0.0941	38.56	40.04	2	0.0	0	K.TYFSHIDVSPGSAQVK.A		42-57	CID
904	868.334	2	-117.60	-0.1021	38.51	45.7	2	0.0	0	K.TYFSHIDVSPGSAQVK.A		42-57	CID
1279	766.303	3	-112.04	-0.0859	47.24	20.82	1	0.0	0	K.AADHVEDLPGALSTLSDLHAHK.L		70-91	CID

Protein 19: ATP synthase subunit alpha OS=Rickettsia bellii (strain OSU 85-389) GN=atpA PE=3 SV=1

Accession: ATPA_RICB8
Database: SwissProt
MW [kDa] / pI: 56.4 / 5.6

Score: 68.9
Seq. Coverage [%]: 4.5
No. of Peptides: 2

10	20	30	40	50	60	70	80	90	100
MKLKPIEVAD	ILQKEIANIN	CLSELEEVGQ	VINVGDGIAK	IYGLANVQSG	EVVEFESGVK	GLVLNLENDS	VDAVIMGDDN	QVQQGDKVKR	TKEVLEVLVG
110	**120**	**130**	**140**	**150**	**160**	**170**	**180**	**190**	**200**
TALLGRVVDA	LGNPIDGKGD	IKSKEYRHIE	MKAPGIIDRA	SVSEPVQTGI	KVIDLLIPIG	RGQRELIIGD	RQTGKTAIAI	DTIINQKKAH	SLNDEKDKIY
210	**220**	**230**	**240**	**250**	**260**	**270**	**280**	**290**	**300**
CIYVAIGQKR	SSVAQIVKKL	EDAGAMDYTI	IVSATASEAA	ALQFVAPYAA	CSMGEYFRDN	GKHALIIYDD	LSKHAVAYRQ	ISLLLRRPPG	REAYPGDVFY
310	**320**	**330**	**340**	**350**	**360**	**370**	**380**	**390**	**400**
LHSRLLECAA	KMSEEKGGGS	LTALPIIETQ	AGDVSAYIPT	NVISITDGQI	FLESELFYKG	IRPAVNVGIS	VSRVGSAAQI	KAMKQVAGSI	KLELAQFREL
410	**420**	**430**	**440**	**450**	**460**	**470**	**480**	**490**	**500**
ESFLQFGSDL	DSATKAQIEH	GKRLVEILKQ	AQYHPFSVEE	QIISIYAGTK	KYLINIPVER	IKEFEEKMLS	EIKQNQKDIL	ESIKSEKRIT	EENEQKLKTF
510	**520**								
LENFVKDFVK	ID								

MS/MS Peptide Matches

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
780	599.261	2	-110.67	-0.0663	35.69	27.51	1	0.0	0	R.VVDALGNPIDGK.G		107-118	CID
1055	644.284	2	-103.67	-0.0668	42.01	41.39	1	0.0	0	K.HALIIYDDLSK.H		263-273	CID

Protein 20: Spectrin alpha chain OS=Drosophila melanogaster GN=alpha-Spec PE=1 SV=2

Accession: SPTCA_DROME
Database: SwissProt
MW [kDa] / pI: 278.1 / 5.1

Score: 63.1
Seq. Coverage [%]: 0.5
No. of Peptides: 1

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

10	20	30	40	50	60	70	80	90	100
MENFTPKEVK	ILETVEDIQE	RREQVLSRYN	DFKIETRQKR	EKLEDSRRFQ	YFKRDADELE	SWIHEKLQAA	SEESYRDPTN	LQAKIQKHQA	FEAEVSAHSN
110	120	130	140	150	160	170	180	190	200
AIVSLDNTGQ	EMINQQHFAS	ESIQVRLDEL	HKLWELLLSR	LAEKGLKLQQ	ALVLVQFLRQ	CEEVMFWIKD	KETFVTADEF	GQDLEHVEVL	QRKFDEFQKD
210	220	230	240	250	260	270	280	290	300
MASQEYRVTE	VNQLADKLVQ	DGHPERDTIT	KRKEELNEAW	QRLKQLAIVR	QEKLFGAHEI	QRFNRDADET	VAWIAEKDVV	LSSDDYGRDL	ASVQALQRKH
310	320	330	340	350	360	370	380	390	400
EGVERDLAAL	EDKVSTLGAE	AQRLCSIHAD	HSDQIRDKQA	EIANYWQSLT	TKARERKQKL	DESYYLHRFL	ADFRDLVSWI	NGMKAIISAD	ELAKDVAGAE
410	420	430	440	450	460	470	480	490	500
ALLERHQEHK	GEIDAREDSF	KLTTESGQKL	LEREHYAAAE	IQEKLAALEN	DKSSLLSLWE	DRRILYEQCM	DLQLFYRDTE	QADTWMAKQE	AFLANEDLGD
510	520	530	540	550	560	570	580	590	600
SLDSVEALIK	KHEDFEKSLA	AQEEKIKALD	IFATKLIDGQ	HYAADDVAQR	RQMLLARRAA	LQEKSSKRRQ	LLEDSNRYQQ	FERDCDETKG	WISEKLKFAT
610	620	630	640	650	660	670	680	690	700
DDSYLDPTNL	NGKMQKHQNF	EHELNANKSR	IEDITNVGTE	LIEKQHYAAD	QINTRMQEIV	VLWETLVQAS	DKKGTKLNEA	CQQQQFNRTI	EDIELWLSEI
710	720	730	740	750	760	770	780	790	800
EGQLLSEDHG	KDLTSVQNLQ	KKHALLEADV	MAHQDRIESI	KVAANKFIES	GHFDADNIRN	KEGNLSARYA	ALAAPMGERK	QHLLDSLQVQ	QLFRDLEDEA
810	820	830	840	850	860	870	880	890	900
AWIREKEPIA	ASTNRGRDLI	GVQNLIKKHQ	AVLAEINNHE	ARLLNVISSG	ENMLKDQPFA	SDDIRQRLEA	LQEQWNTLKE	KSSQRKQDLD	DSLQAHQYFA
910	920	930	940	950	960	970	980	990	1000
DANEAESWMR	EKEPIATGSD	YGKDEDSSEA	LLKKHEALVS	DLEAFGNTIQ	ALQEQAKNCR	QQETPVVDIT	GKECVVALYD	YTEKSPREVS	MKKGDVLTLL
1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
NSNNKDWWKV	EVNDRQGFVP	AAYIKKIDAG	LSASQQNLVD	NHSIAKRQNQ	INSQYDNLLA	LARERQNKLN	ETVKAYVLVR	EAADLAQWIR	DKENHAQIAD
1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
VVGEDLEEVE	VLQKKFDDFN	DDLKANEVRL	ANMNEIAVQL	TSLGQTEAAL	KIQTQMQDLN	EKWNNLQTLT	AEKASQLGSA	HEVQRFHRDI	DETKDWIAEK
1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
ANALNNDDLG	KDLRSVQTLQ	RKHEGVERDL	AALRDKIRQL	DETANRLMQS	HPDTAEQTYA	KQKEINEMWD	QIITKSTARK	EKLLDSYDLQ	RFLSDYRDLL

Protein Report
IonTrap_allOrg_2019-11-11 12:08:16

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1881	714.33	2	-154.37	-0.1103	63.21	39.74	1	0.0	0	K.LQQALVLVQFLR.Q		148-159	CID

Protein 21: Fructose-bisphosphate aldolase OS=Drosophila melanogaster GN=Ald PE=1 SV=5

Accession: ALF_DROME
Database: SwissProt
MW [kDa] / pI: 39.0 / 7.0

Score: 47.7
Seq. Coverage [%]: 3.0
No. of Peptides: 1

```
        10         20         30         40         50         60         70         80         90        100
MTTYFNYPSK ELQDELREIA QKIVAPGKGI LAADESGPTM GKRLQDIGVE NTEDNRRAYR QLLFSTDPKL AENISGVILF HETLYQKADD GTPFAEILKK

       110        120        130        140        150        160        170        180        190        200
KGIILGIKVD KGVVPLFGSE DEVTTQGLDD LAARCAQYKK DGCDFAKWRC VLKIGKNTPS YQSILENANV LARYASICQS QRIVPIVEPE VLPDGDHDLD

       210        220        230        240        250        260        270        280        290        300
RAQKVTETVL AAVYKALSDH HVYLEGTLLK PNMVTAGQSA KKNTPEEIAL ATVQALRRTV PAAVTGVTFL SGGQSEEEAT VNLSAINNVP LIRPWALTFS

       310        320        330        340        350        360        370
YGRALQASVL RAWAGKKENI AAGQNELLKR AKANGDAAQG KYVAGSAGAG SGSLFVANHA Y
```

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1068	597.267	2	-126.30	-0.0754	42.33	47.74	1	0.0	0	K.VTETVLAAVYK.A		205-215	CID

Apêndice 3. Identificação das proteínas por meio do software *ProteinScape* (versão 3.1, Bruker Daltonics) e o algoritmo Mascot (v2.3, Matrix Science, UK) para *NCBI*.

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

Project Info

Name:	Colaboração	**Date:**	Apr 16, 2019

Sample Info & Protocols

Name:	Ju_espermateca
Date:	Nov 5, 2019

Search Result Info

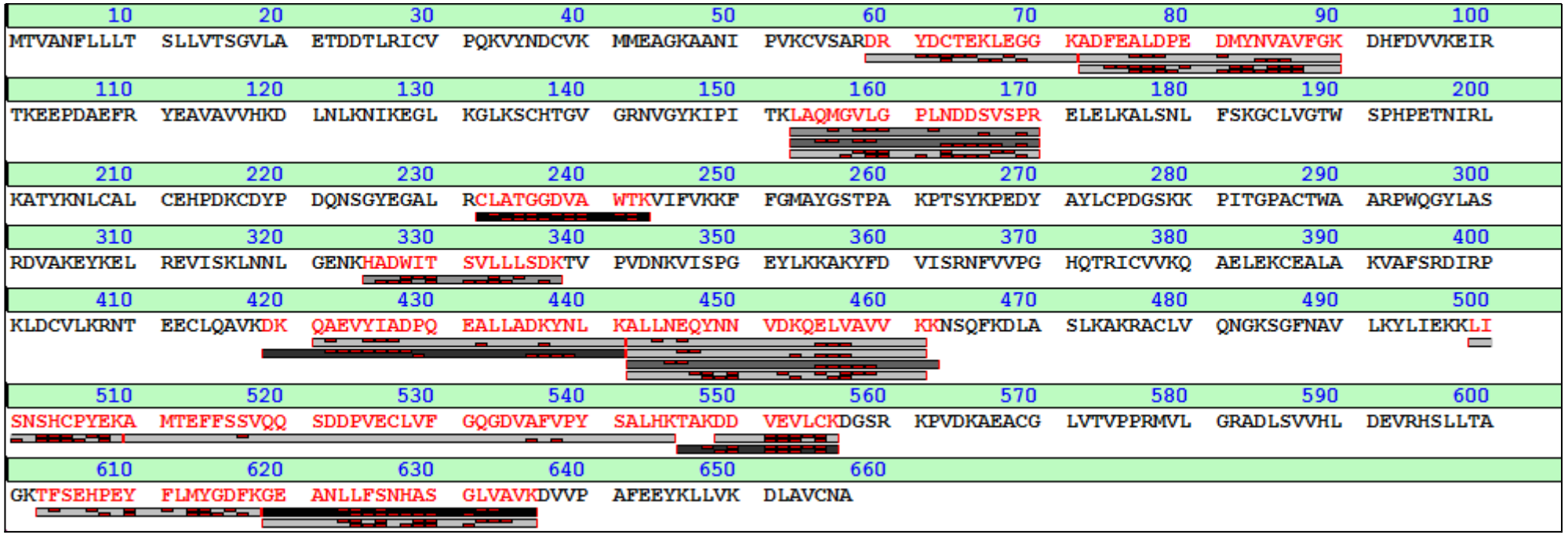
Search Result:	IonTrap_allOrg_2019-11-06 16:28:01		
Location:	/Colaboração/Ju_espermateca/Ju_esp_051119.mgf		
Search Method:	IonTrap_allOrg	**Version:**	1.4
Search Engine(s):	Mascot, 2.3.02	**Ident. Compound(s):**	111/2717
Database(s):	NCBInr, ncbinr_.fasta		
Note:			

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

Results

Protein 1: transferrin [Rhodnius prolixus]

Accession:	gi|156891049	**Score:**	752.3
Database:	NCBInr	**Seq. Coverage [%]:**	32.3
MW [kDa] / pI:	72.8 / 7.4	**No. of Peptides:**	15
Modification(s):	Carbamidomethyl		



MS/MS Peptide Matches

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
241	785.713	2	-189.52	-0.1489	22.27	35.91	1	0.0	2	R.DRYDCTEKLEGGK.A	Carbamidomethyl: 5	59-71	CID
1728	1065.861	2	-119.20	-0.1271	58.42	67.11	1	0.0	0	K.ADFEALDPEDMYNVAVFGK.D		72-90	CID
1731	710.913	3	-114.58	-0.0815	58.48	33.7	1	0.0	0	K.ADFEALDPEDMYNVAVFGK.D		72-90	CID
1294	934.861	2	-127.68	-0.1194	47.61	44.99	1	0.0	0	K.LAQMGVLGPLNDDSVSPR.E		153-170	CID
1305	623.571	3	-136.31	-0.0850	47.86	29.25	2	0.0	0	K.LAQMGVLGPLNDDSVSPR.E		153-170	CID
1297	623.573	3	-133.10	-0.0830	47.68	34.93	2	0.0	0	K.LAQMGVLGPLNDDSVSPR.E		153-170	CID
743	639.746	2	-101.57	-0.0650	34.78	47.32	1	0.0	0	R.CLATGGDVAWTK.V	Carbamidomethyl: 1	232-243	CID
1803	799.34	2	-116.15	-0.0929	60.70	35.31	1	0.0	0	K.HADWITSVLLLSDK.T		325-338	CID
1293	879.033	3	-105.00	-0.0923	47.57	54.79	1	0.0	2	K.DKQAEVYIADPQEALLADKYNLK.A		419-441	CID
1364	797.985	3	-124.89	-0.0997	49.27	27.63	1	0.0	1	K.QAEVYIADPQEALLADKYNLK.A		421-441	CID
1210	1143.999	2	-104.24	-0.1193	45.62	24.71	1	0.0	1	K.ALLNEQYNNVDKQELVAVVK.K		442-461	CID
1207	762.998	3	-109.12	-0.0833	45.56	51.81	2	0.0	1	K.ALLNEQYNNVDKQELVAVVK.K		442-461	CID
1205	762.986	3	-124.84	-0.0953	45.50	59.18	2	0.0	1	K.ALLNEQYNNVDKQELVAVVK.K		442-461	CID
1091	805.696	3	-103.73	-0.0836	42.91	28.15	1	0.0	2	K.ALLNEQYNNVDKQELVAVVKK.N		442-462	CID
280	674.233	2	-131.57	-0.0887	23.32	27.64	2	0.0	0	K.LISNSHCPYEK.A	Carbamidomethyl: 7	499-509	CID
1972	1002.103	4	-119.04	-0.1193	66.93	28.28	1	0.0	0	K.AMTEFFSSVQQSDDPVECLVFGQGDVAFVPYSALHK.T	Carbamidomethyl: 18	510-545	CID
469	639.237	2	-135.97	-0.0869	28.14	45.23	1	0.0	1	K.TAKDDVEVLCK.D	Carbamidomethyl: 10	546-556	CID
458	489.071	2	-333.28	-0.1631	27.89	33.09	1	0.0	0	K.DDVEVLCK.D	Carbamidomethyl: 7	549-556	CID
1581	1005.843	2	-107.11	-0.1078	54.61	22.39	1	0.0	0	K.TFSEHPEYFLMYGDFK.G		603-618	CID
1307	913.899	2	-101.31	-0.0926	47.91	43.08	2	0.0	0	K.GEANLLFSNHASGLVAVK.D		619-636	CID
1302	913.873	2	-129.76	-0.1186	47.79	52.09	2	0.0	0	K.GEANLLFSNHASGLVAVK.D		619-636	CID

Protein 2: PREDICTED: myosin heavy chain, muscle isoform X15 [Halyomorpha halys]

Accession: gi|939672556
Database: NCBInr
MW [kDa] / pI: 224.7 / 5.8
Score: 560.9
Seq. Coverage [%]: 7.1
No. of Peptides: 11

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

10	20	30	40	50	60	70	80	90	100
MASKGPAGMQ	KGADDPDPTP	YLFVSLEQKR	IDQTKPYDAK	KACWVPDEAE	GFVLGEIQGT	KGDIVSVKLP	SMEEKNFKKE	QVFQVNPPKF	EKVEDMADLT
110	120	130	140	150	160	170	180	190	200
YLNDASVLYN	LKQRYYAKLI	YTYSGLFCVA	INPYKRFPVY	TMRCAKLYRG	KRRNEVPPHI	FAISDGAYVN	MLTNKENQSM	LITGESGAGK	TENTKKVIAY
210	220	230	240	250	260	270	280	290	300
FATVGASTKK	EEASEKKGTL	EDQVVQTNPV	LEAFGNAKTV	RNDNSSRFGK	FIRIHFGPSG	KLAGADIETY	LLEKARVISQ	QTLERSYHIF	YQIMSGAVKG
310	320	330	340	350	360	370	380	390	400
VKDMCLLSNN	IQDYYFIAQG	KTTIPGVDDG	EEMQLTDEAF	NVLGFTQQEK	DDIYKITAAV	MHMGCMKFKQ	RGREEQAEAD	GLEEGIRVGK	LLGIEGEELY
410	420	430	440	450	460	470	480	490	500
KNLLKPRIKV	GNEFVTQGRN	VSQVTYSVGA	MSKGMFDRLF	KFLVKKCNET	LDTKQKRQHF	IGVLDIAGFE	IFDYNGFEQL	CINFTNEKLQ	QFFNHHMFVL
510	520	530	540	550	560	570	580	590	600
EQEEYKKEGI	NWAFIDFGMD	LLACIELIEK	PMGILSILEE	ESMFPKATDK	TFEDKLNTNH	LGKSPNFQKP	KPPKPGCQAA	HFAIGHYAGV	VSYNITGWLE
610	620	630	640	650	660	670	680	690	700
KNKDPLNDTV	VDQYKKGSNK	LLCEIFADHP	GQSAPAGADA	GGKGGRGKKG	GGFATVSSSY	KEQLNNLMTT	LRSTQPHFVR	CIIPNELKQP	GVIDSHLVMH
710	720	730	740	750	760	770	780	790	800
QLTCNGVLEG	IRICRKGFPN	RMAYPDFKLR	YKILNPAGVE	KLTDEKAMAG	VILESTGLDP	DMYRLGHTKV	FFRAGVLGQM	EELRDERLSR	IISWMQSYIR
810	820	830	840	850	860	870	880	890	900
GYLCRKEFKK	LQEQRLALQI	VQRNLRRYLA	LRTWNWWKMW	SKVKPLLNVA	NVEEEMRKLE	ELVAQTQAAL	EKEEKARKEV	EALNAKLLQE	KTELLRNLEG
910	920	930	940	950	960	970	980	990	1000
EKGTLGSLQE	RSAKLQAQKA	DLESQLMDTQ	ERLQQEEDAR	NQLFQQKKKL	EQENGSLKKD	IEDLELSVTK	SDQDKASKEH	QIRNLNDEIA	HQDELINKLN
1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
KEKKIQSEHN	QKTAEELQAA	EDKINHLTKV	KAKLEQTLDE	LEDSLEREKK	LRGDVEKAKR	KTEGDLKLTQ	EAVADLERNK	KELEQTIQRK	DKEMASLTAK
1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
LEDEQGIVNK	TGKQIKELQA	RIEELEEEVE	AERQARGKAE	KQRADLAREL	EELGERLEEA	GGATSAQIEL	NKKREAEMSK	LRRDLEEANI	QHESTLANLR
1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
KKHNDAVSEM	GDQIDQLNKL	KTKVEKEKCQ	YLCELNDLRA	SIDHLTNEKA	ATEKIAKQLQ	HQLNEVQGKL	DESNRSLNDF	DAAKKKLSIE	NSDLLRQLEE

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

MS/MS Peptide Matches

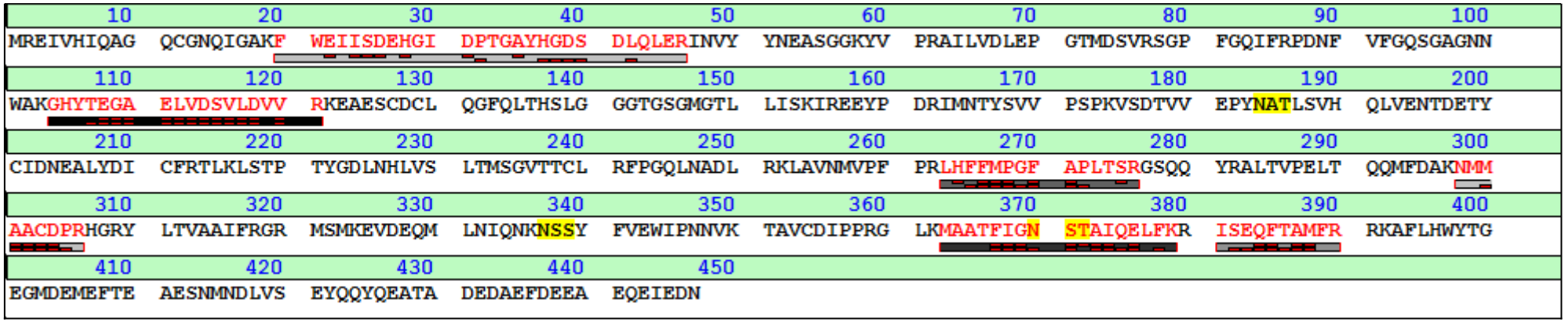
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1093	664.284	2	-124.11	-0.0825	42.93	24.15	1	0.0	0	K.VIAYFATVGASTK.K		197-209	CID
1320	870.854	2	-132.36	-0.1153	48.17	37.78	1	0.0	0	K.VKPLLNVANVEEEMR.K		843-857	CID
1662	944.854	2	-131.80	-0.1246	56.70	45.39	1	0.0	1	K.AKLEQTLDELEDSLER.E		1032-1047	CID
1375	845.303	2	-129.54	-0.1095	49.52	36.12	1	0.0	0	K.LEQTLDELEDSLER.E		1034-1047	CID
774	737.756	2	-126.07	-0.0930	35.56	50.25	1	0.0	0	R.IEELEEEVEAER.Q		1122-1133	CID
308	564.17	2	-209.47	-0.1182	24.11	20.91	1	0.0	0	R.ASIDHLTNEK.A		1240-1249	CID
851	787.805	2	-119.36	-0.0940	37.32	25.33	1	0.0	0	R.LAEAEETIESLNQK.V		1404-1417	CID
1179	815.303	2	-143.66	-0.1171	44.91	45.54	1	0.0	0	R.LATEVEDLQLEVDR.A		1428-1441	CID
1119	808.317	2	-140.41	-0.1135	43.56	32.21	2	0.0	1	K.LKVDDLAAELDASQK.E		1465-1479	CID
1988	971.374	2	-127.07	-0.1234	67.79	56.89	2	0.0	1	K.NLADEVKDLLDQIGEGGR.N		1511-1528	CID
1986	971.38	2	-120.89	-0.1174	67.73	79.18	2	0.0	1	K.NLADEVKDLLDQIGEGGR.N		1511-1528	CID
594	651.745	2	-111.77	-0.0729	31.19	26.23	1	0.0	0	R.ANALGNELEESR.T		1685-1696	CID

Protein 3: PREDICTED: tubulin beta-1 chain [Amyelois transitella]

Accession: gi|913328978
Database: NCBInr
MW [kDa] / pI: 50.1 / 4.8
Modification(s): Carbamidomethyl, Oxidation

Score: 540.3
Seq. Coverage [%]: 21.3
No. of Peptides: 6

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

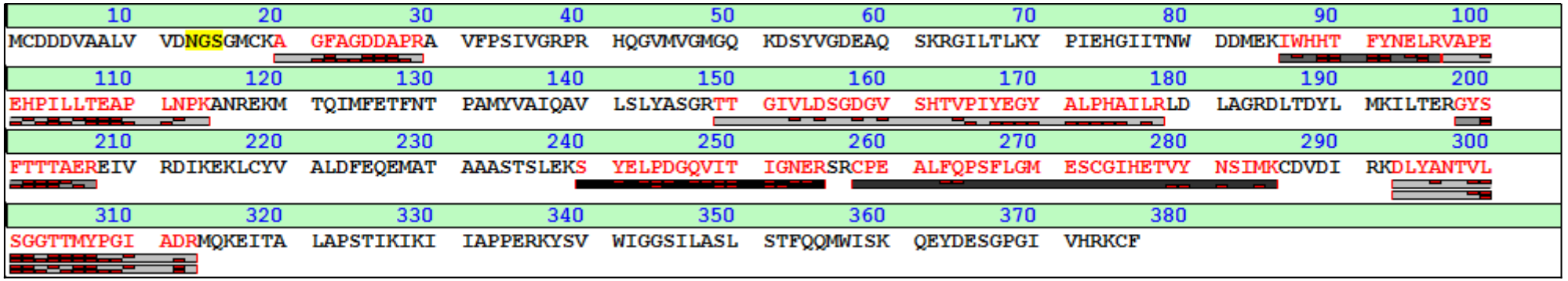
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1476	1034.027	3	-116.63	-0.1206	51.95	63.15	1	0.0	0	K.FWEIISDEHGIDPTGAYHGDSDLQLER.I		20-46	CID
1818	979.879	2	-117.90	-0.1155	61.18	98.84	1	0.0	0	K.GHYTEGAELVDSVLDVVR.K		104-121	CID
1775	810.811	2	-136.15	-0.1104	59.86	45.58	1	0.0	0	R.LHFFMPGFAPLTSR.G		263-276	CID
299	533.093	2	-233.22	-0.1244	23.91	30.72	1	0.0	0	K.NMMAACDPR.H	Carbamidomethyl: 6	298-306	CID
1804	929.356	2	-127.38	-0.1184	60.71	60.73	2	0.0	0	K.MAATFIGNSTAIQELFK.R	Oxidation: 1	363-379	CID
1312	615.235	2	-110.19	-0.0678	48.04	47.57	1	0.0	0	R.ISEQFTAMFR.R		381-390	CID

Protein 4: actin 1 [Laodelphax striatella]

Accession: gi|480474671
Database: NCBInr
MW [kDa] / pI: 41.7 / 5.3
Modification(s): Carbamidomethyl

Score: 503.9
Seq. Coverage [%]: 38.3
No. of Peptides: 8

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

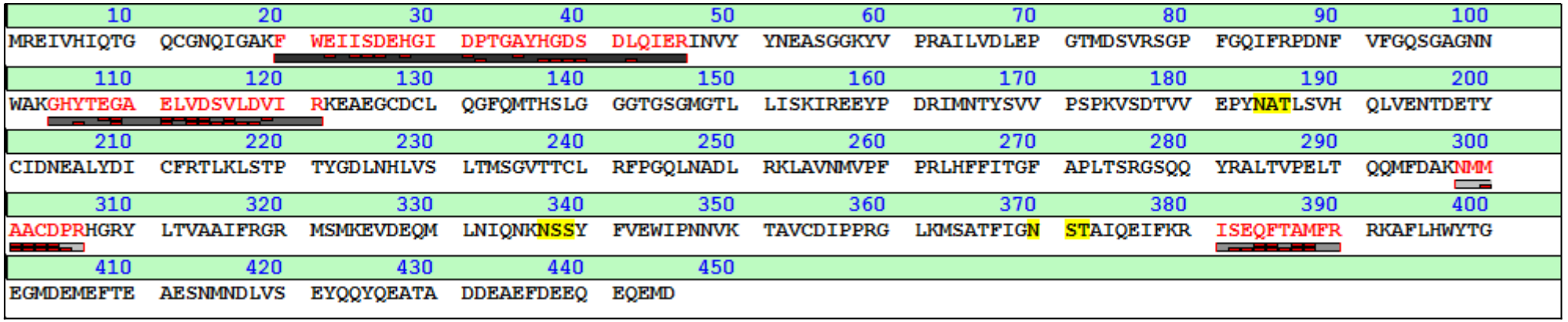
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
270	488.569	2	-324.89	-0.1588	23.07	37.8	1	0.0	0	K.AGFAGDDAPR.A		20-29	CID
941	758.263	2	-151.93	-0.1152	39.39	38.93	1	0.0	0	K.IWHHTFYNELR.V		86-96	CID
1219	984.435	2	-110.37	-0.1087	45.82	52.05	1	0.0	0	R.VAPEEHPILLTEAPLNPK.A		97-114	CID
1616	1051.102	3	-111.23	-0.1169	55.46	81.49	1	0.0	0	R.TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L		149-178	CID
521	566.653	2	-201.32	-0.1141	29.42	33.74	1	0.0	0	R.GYSFTTTAER.E		198-207	CID
1225	895.825	2	-139.07	-0.1246	45.97	60.88	1	0.0	0	K.SYELPDGQVITIGNER.S		240-255	CID
1849	812.039	4	-105.67	-0.0858	62.03	35.56	1	0.0	0	R.CPEALFQPSFLGMESCGIHETVYNSIMK.C	Carbamidomethyl: 1, 16	258-285	CID
1393	1107.901	2	-124.20	-0.1376	49.98	52.28	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		293-313	CID
1391	1107.888	2	-135.93	-0.1506	49.91	59.66	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		293-313	CID

Protein 5: PREDICTED: tubulin beta-1 chain-like [Cimex lectularius]

Accession: gi|939249199
Database: NCBInr
MW [kDa] / pI: 50.0 / 4.8
Modification(s): Carbamidomethyl

Score: 388.1
Seq. Coverage [%]: 14.4
No. of Peptides: 4

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1476	1034.027	3	-116.63	-0.1206	51.95	63.15	1	0.0	0	K.FWEIISDEHGIDPTGAYHGDSDLQIER.I		20-46	CID
1927	986.883	2	-120.93	-0.1194	64.87	56.71	1	0.0	0	K.GHYTEGAELVDSVLDVIR.K		104-121	CID
299	533.093	2	-233.22	-0.1244	23.91	30.72	1	0.0	0	K.NMMAACDPR.H	Carbamidomethyl: 6	298-306	CID
1312	615.235	2	-110.19	-0.0678	48.04	47.57	1	0.0	0	R.ISEQFTAMFR.R		381-390	CID

Protein 6: PREDICTED: tubulin beta-4B chain [Papilio machaon]

Accession: gi|943951663
Database: NCBInr
MW [kDa] / pI: 49.5 / 4.8
Modification(s): Carbamidomethyl

Score: 357.3
Seq. Coverage [%]: 11.5
No. of Peptides: 4

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

10	20	30	40	50	60	70	80	90	100
MREIVHIQAG	QCGNQIGAKF	WEVISDEHGI	DPTGAYQGDS	DLQLERINVY	YNEATGGKYV	PRAVLVDLEP	GTMDSVRSGP	FGQIFRPDNF	VFGQSGAGNN
110	120	130	140	150	160	170	180	190	200
WAKGHYTEGA	ELVDSVLDVV	RKEAESCDCL	QGFQLTHSLG	GGTGSGMGTL	LISKIREEYP	DRIMNTFSVV	PSPKVSDTVV	EPYNATLSVH	QLVENTDETF
210	220	230	240	250	260	270	280	290	300
CIDNEALYDI	CFRTLKLTTP	TYGDLNHLVS	ATMSGVTTCL	RFPGQLNADL	RKLAVNMVPF	PRLHFFMPGF	APLTSRGSQQ	YRALSVPELT	QQMFDSKNMM
310	320	330	340	350	360	370	380	390	400
AACDPRRGRY	LTVAAVFRGR	MSMKEVDEQM	LNIQNKNSSY	FVEWIPNNVK	TAVCDIPPRG	LKMSATFIGN	TTAIQELFKR	ISEQFTAMFR	RKAFLHWYTG
410	420	430	440	450					
EGMDEMEFTE	AESNMNDLVS	EYQQYQDATA	DDEGEFDEEV	EE					

MS/MS Peptide Matches

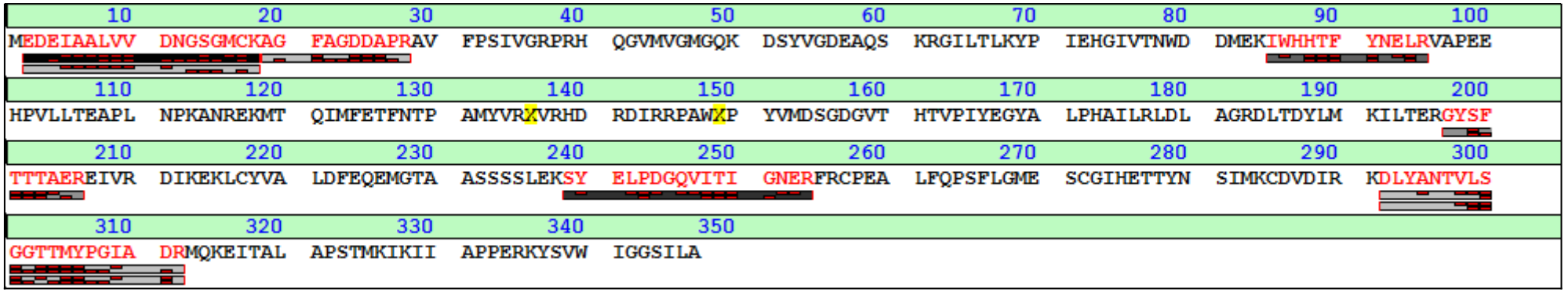
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1818	979.879	2	-117.90	-0.1155	61.18	98.84	1	0.0	0	K.GHYTEGAELVDSVLDVVR.K		104-121	CID
1775	810.811	2	-136.15	-0.1104	59.86	45.58	1	0.0	0	R.LHFFMPGFAPLTSR.G		263-276	CID
299	533.093	2	-233.22	-0.1244	23.91	30.72	1	0.0	0	K.NMMAACDPR.R	Carbamidomethyl: 6	298-306	CID
1312	615.235	2	-110.19	-0.0678	48.04	47.57	1	0.0	0	R.ISEQFTAMFR.R		381-390	CID

Protein 7: PREDICTED: LOW QUALITY PROTEIN: actin, cytoplasmic 1 [Larimichthys crocea]

Accession: gi|734634023
Database: NCBInr
MW [kDa] / pI: 38.9 / 5.5
Modification(s): Carbamidomethyl

Score: 345.0
Seq. Coverage [%]: 24.5
No. of Peptides: 6

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

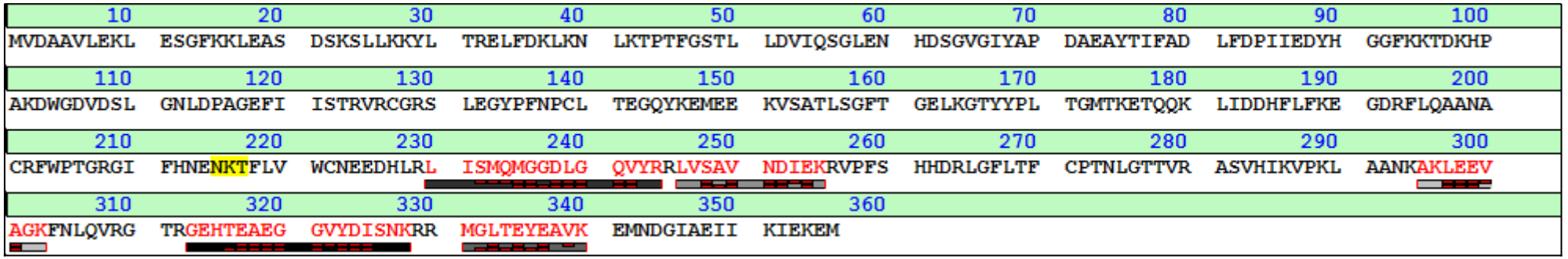
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1185	904.75	2	371.87	0.3363	45.05	33.21	3	0.0	0	M.EDEIAALVVDNGSGMCK.A	Carbamidomethyl: 16	2-18	CID
1246	904.293	2	-133.43	-0.1207	46.46	64.45	3	0.0	0	M.EDEIAALVVDNGSGMCK.A	Carbamidomethyl: 16	2-18	CID
270	488.569	2	-324.89	-0.1588	23.07	37.8	1	0.0	0	K.AGFAGDDAPR.A		19-28	CID
941	758.263	2	-151.93	-0.1152	39.39	38.93	1	0.0	0	K.IWHHTFYNELR.V		85-95	CID
521	566.653	2	-201.32	-0.1141	29.42	33.74	1	0.0	0	R.GYSFTTTAER.E		197-206	CID
1225	895.825	2	-139.07	-0.1246	45.97	60.88	1	0.0	0	K.SYELPDGQVITIGNER.F		239-254	CID
1393	1107.901	2	-124.20	-0.1376	49.98	52.28	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		292-312	CID
1391	1107.888	2	-135.93	-0.1506	49.91	59.66	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		292-312	CID

Protein 8: arginine kinase [Triatoma infestans]

Accession: gi|197310855
Database: NCBInr
MW [kDa] / pI: 40.0 / 5.9
Modification(s): Carbamidomethyl

Score: 332.6
Seq. Coverage [%]: 14.3
No. of Peptides: 4

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

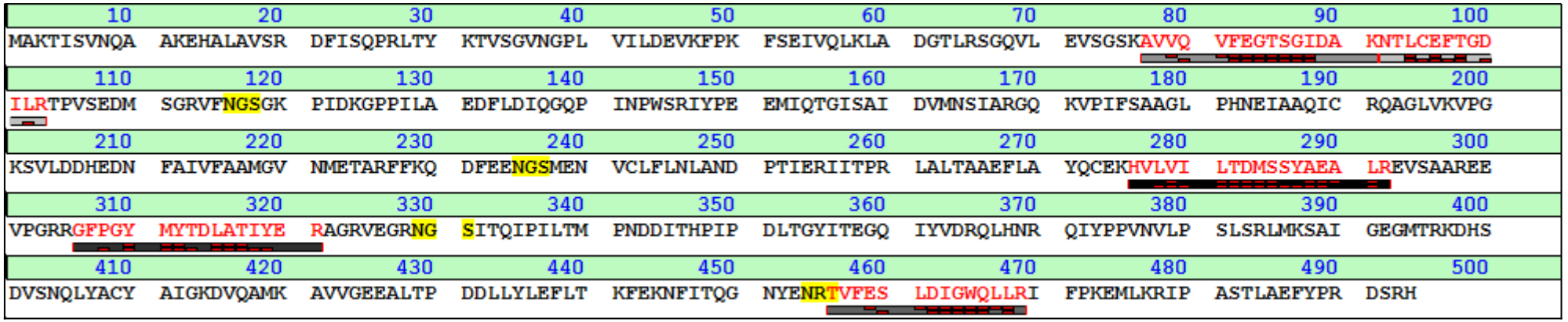
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1264	834.312	2	-124.43	-0.1038	46.91	47.23	1	0.0	0	R.LISMQMGGDLGQVYR.R		230-244	CID
616	544.198	2	-193.50	-0.1053	31.74	36.5	1	0.0	0	R.LVSAVNDIEK.R		246-255	CID
437	853.273	2	-133.39	-0.1138	27.37	57.72	1	0.0	0	R.GEHTEAEGGVYDISNK.R		313-328	CID
797	570.701	2	-145.25	-0.0829	36.08	29.02	1	0.0	0	R.MGLTEYEAVK.E		331-340	CID

Protein 9: V-type proton ATPase subunit B [Papilio xuthus]

Accession: gi|914615693
Database: NCBInr
MW [kDa] / pI: 54.8 / 5.3
Modification(s): Carbamidomethyl

Score: 330.5
Seq. Coverage [%]: 15.0
No. of Peptides: 5

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

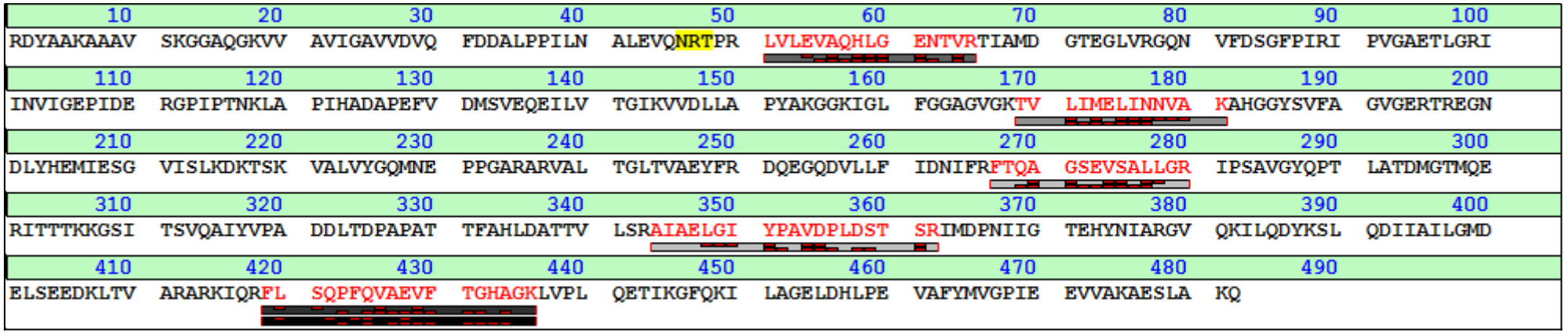
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1047	760.803	2	-129.30	-0.0984	41.82	46.13	1	0.0	0	K.AVVQVFEGTSGIDAK.N		77-91	CID
1431	719.76	2	-129.73	-0.0934	50.89	29.06	1	0.0	0	K.NTLCEFTGDILR.T	Carbamidomethyl: 4	92-103	CID
1832	959.397	2	-116.49	-0.1118	61.63	89.99	1	0.0	0	K.HVLVILTDMSSYAEALR.E		276-292	CID
1716	948.861	2	-89.01	-0.0845	58.09	66.03	1	0.0	0	R.GFPGYMYTDLATIYER.A		306-321	CID
1979	838.877	2	-91.73	-0.0770	67.45	58.43	1	0.0	0	R.TVFESLDIGWQLLR.I		456-469	CID

Protein 10: ATP synthase subunit beta, mitochondrial, partial [Trachymyrmex zeteki]

Accession: gi|1012972782
Database: NCBInr
MW [kDa] / pI: 51.7 / 5.1

Score: 328.1
Seq. Coverage [%]: 16.4
No. of Peptides: 5

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

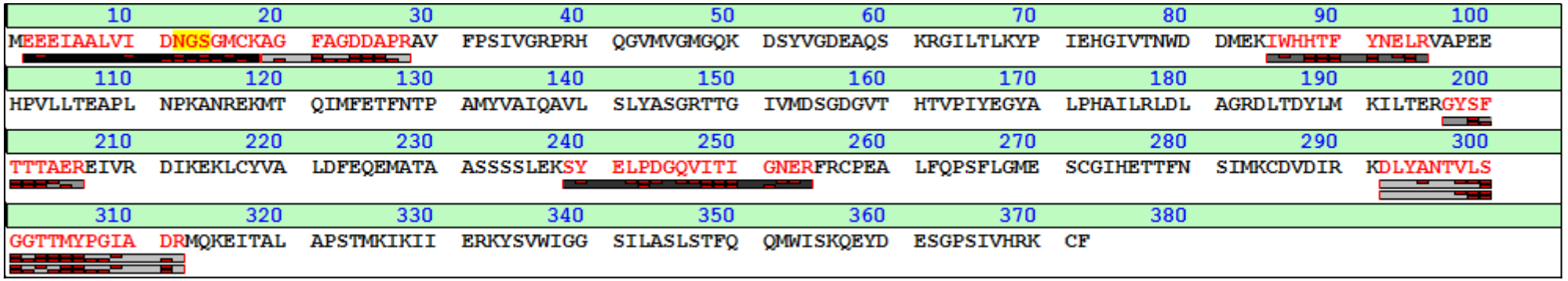
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1198	839.367	2	-120.03	-0.1008	45.33	66.63	1	0.0	0	R.LVLEVAQHLGENTVR.T		51-65	CID
1997	729.365	2	-80.12	-0.0584	68.05	54.62	1	0.0	0	K.TVLIMELINNVAK.A		169-181	CID
1254	718.303	2	-108.05	-0.0776	46.60	65.95	1	0.0	0	R.FTQAGSEVSALLGR.I		267-280	CID
1564	994.401	2	-120.05	-0.1194	54.17	38.79	1	0.0	0	R.AIAELGIYPAVDPLDSTSR.I		344-362	CID
1686	981.913	2	-95.98	-0.0942	57.29	63.17	2	0.0	0	R.FLSQPFQVAEVFTGHAGK.L		419-436	CID
1684	981.918	2	-90.89	-0.0892	57.24	76.66	2	0.0	0	R.FLSQPFQVAEVFTGHAGK.L		419-436	CID

Protein 11: actin, cytoplasmic 1 [Cricetulus griseus]

Accession: gi|537136955
Database: NCBInr
MW [kDa] / pI: 41.5 / 5.3
Modification(s): Carbamidomethyl

Score: 321.1
Seq. Coverage [%]: 22.8
No. of Peptides: 6

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

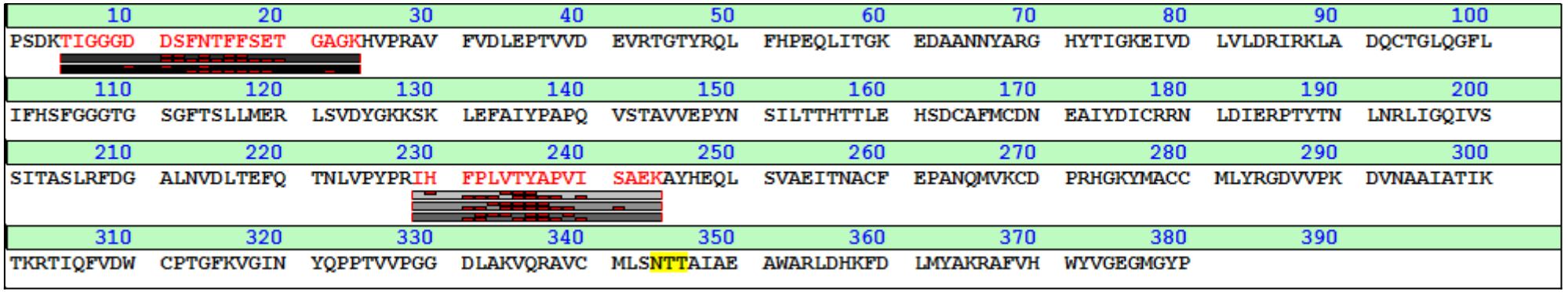
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1338	918.325	2	-113.59	-0.1043	48.63	40.59	1	0.0	0	M.EEEIAALVIDNGSGMCK.A	Carbamidomethyl: 16	2-18	CID
270	488.569	2	-324.89	-0.1588	23.07	37.8	1	0.0	0	K.AGFAGDDAPR.A		19-28	CID
941	758.263	2	-151.93	-0.1152	39.39	38.93	1	0.0	0	K.IWHHTFYNELR.V		85-95	CID
521	566.653	2	-201.32	-0.1141	29.42	33.74	1	0.0	0	R.GYSFTTTAER.E		197-206	CID
1225	895.825	2	-139.07	-0.1246	45.97	60.88	1	0.0	0	K.SYELPDGQVITIGNER.F		239-254	CID
1393	1107.901	2	-124.20	-0.1376	49.98	52.28	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		292-312	CID
1391	1107.888	2	-135.93	-0.1506	49.91	59.66	2	0.0	0	K.DLYANTVLSGGTTMYPGIADR.M		292-312	CID

Protein 12: alpha-tubulin, partial [Locusta migratoria]

Accession: gi|401757809
Database: NCBInr
MW [kDa] / pI: 42.2 / 6.1
Modification(s): Carbamidomethyl

Score: 319.2
Seq. Coverage [%]: 9.5
No. of Peptides: 2

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1366	1004.327	2	-122.61	-0.1232	49.28	47.39	2	0.0	0	K.TIGGGDDSFNTFFSETGAGK.H		5-24	CID
1371	1004.313	2	-136.55	-0.1372	49.41	52.98	2	0.0	0	K.TIGGGDDSFNTFFSETGAGK.H		5-24	CID
1634	892.88	2	-135.39	-0.1209	55.91	23.2	3	0.0	0	R.IHFPLVTYAPVISAEK.A		229-244	CID
1611	892.883	2	-132.03	-0.1179	55.33	26.3	3	0.0	0	R.IHFPLVTYAPVISAEK.A		229-244	CID
1631	892.881	2	-134.27	-0.1199	55.85	45.93	3	0.0	0	R.IHFPLVTYAPVISAEK.A		229-244	CID

Protein 13: heat shock cognate protein [Riptortus pedestris]

Accession: gi|501290073
Database: NCBInr
MW [kDa] / pI: 71.4 / 5.4
Modification(s): Carbamidomethyl

Score: 267.6
Seq. Coverage [%]: 8.9
No. of Peptides: 4

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

10	20	30	40	50	60	70	80	90	100
MAKAPAVGID	LGTTYSCVGV	FQHGKVEIIA	NDQGNRTTPS	YVAFTDTERL	IGDAAKNQVA	MNPNNTIFDA	KRLIGRRFDD	VTVQSDMKHW	PFEVISDGGK
110	120	130	140	150	160	170	180	190	200
PKIQVQYKGE	NKTFFPEEIS	SMVLTKMKET	AEAYLGKTVT	NAVVTVPAYF	NDSQRQATKD	AGTISGLNVL	RIINEPTAAA	IAYGLDKKGS	GERNVLIFDL
210	220	230	240	250	260	270	280	290	300
GGGTFDVSIL	TIEDGIFEVK	STAGDTHLGG	EDFDNRMVNH	FVQEFKRKYK	KDLTQNKRAL	RRLRTACERA	KRTLSSSTQA	SIEIDSLYEG	TDFYTSITRA
310	320	330	340	350	360	370	380	390	400
RFEELNADLF	RSTMEPVEKS	LRDAKMDKAQ	IHDIVLVGGS	TRIPKVQKLL	QDFFNGKELN	KSINPDEAVA	YGAAVQAAIL	HGDKSEEVQD	LLLLDVTPLS
410	420	430	440	450	460	470	480	490	500
LGIETAGGVM	TALIKRNTTI	PTKQTQTFTT	YSDNQPGVLI	QVYEGERAMT	KDNNLLGKFE	LTGIPPAPRG	VPQIEVTFDI	DANGILNVTA	IEKSTGKENK
510	520	530	540	550	560	570	580	590	600
ITITNDKGRL	SKEEIERMVN	DAEKYKAEDD	KQKAVIQAKN	TLESYCFNMK	STVEDEKLKD	KIPEADKNTI	LEKCNEVIRW	LDANQLAEKE	EFEHKQKELE
610	620	630	640	650	660				
QLCNPIITKL	YQSGGMPGGM	PGGMPGGMGG	FPGGAPNAGG	AAGPTIEEVD					

MS/MS Peptide Matches

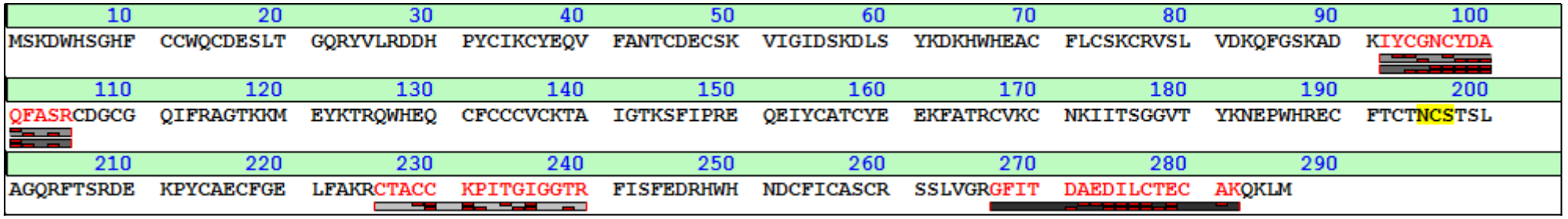
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
384	614.738	2	-129.56	-0.0797	26.12	53.07	1	0.0	0	K.VEIIANDQGNR.T		26-36	CID
1186	894.385	2	-127.14	-0.1137	45.06	40.21	2	0.0	1	R.IINEPTAAAIAYGLDKK.G		172-188	CID
451	846.243	2	-145.83	-0.1234	27.69	35.47	1	0.0	0	K.STAGDTHLGGEDFDNR.M		221-236	CID
902	733.341	2	-93.69	-0.0687	38.50	41.74	1	0.0	0	K.AQIHDIVLVGGSTR.I		329-342	CID

Protein 14: PREDICTED: four and a half LIM domains protein 2-like isoform X5 [Halyomorpha halys]

Accession: gi|939664394
Database: NCBInr
MW [kDa] / pI: 32.8 / 8.1
Modification(s): Carbamidomethyl

Score: 223.0
Seq. Coverage [%]: 15.7
No. of Peptides: 3

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
647	862.736	2	-145.35	-0.1254	32.48	23.99	2	0.0	0	K.IYCGNCYDAQFASR.C	Carbamidomethyl: 3, 6	92-105	CID
638	862.74	2	-140.71	-0.1214	32.29	49.96	2	0.0	0	K.IYCGNCYDAQFASR.C	Carbamidomethyl: 3, 6	92-105	CID
447	826.269	2	-145.55	-0.1203	27.62	39.57	1	0.0	0	R.CTACCKPITGIGGTR.F	Carbamidomethyl: 1, 4, 5	226-240	CID
1596	921.795	2	-131.31	-0.1211	54.94	36.12	1	0.0	0	R.GFITDAEDILCTECAK.Q	Carbamidomethyl: 11, 14	267-282	CID

Protein 15: myosin heavy chain isoform 2 [Daphnia pulex]

Accession: gi|321476144
Database: NCBInr
MW [kDa] / pI: 223.2 / 5.9

Score: 195.9
Seq. Coverage [%]: 2.1
No. of Peptides: 4

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

10	20	30	40	50	60	70	80	90	100
MPPKKDMGPD	PDPAQYLFVS	LEMKRADQTK	PYDGKKATWV	PCEKDSYQLG	EITGTKGDLV	VVKVADGNEK	MVKKDQCFPV	NPPKFEKVED	MADLTYLNDA
110	120	130	140	150	160	170	180	190	200
AVLHNLRQRY	YHKLIYTYSG	LFCVAINPYK	RFPIYTQRVI	KMYIGKRRNE	VPPHIFCISD	GAYMDMLTNH	ENQSMLITGE	SGAGKTENTK	KVIAYMASVG
210	220	230	240	250	260	270	280	290	300
ASTKKPKEGE	VKKGNLEDQI	VQTNPVLEAF	GNAKTTRNDN	SSRFGKFIRI	HFGNSGKLAG	ADIETYLLEK	ARVISQQALE	RSYHIFYQIM	SGKLPTLKAM
310	320	330	340	350	360	370	380	390	400
CSLSDNIYDY	PFVSQGKVTV	PSIDDSEEMQ	MADEAFEILG	MGEQRPEIWK	ITAAVMHFGT	MKFKQRGREE	QADPDGTQEG	ENVAKMMGVD	GPQLYMNFLK
410	420	430	440	450	460	470	480	490	500
PRIKVGNEFV	TQGRNVNQVV	YSIGAMAKAI	FDRLFKWLVK	RVNETLETGQ	KRVTFIGVLD	IAGFEIFDYN	GFEQLCINFT	NEKLQQFFNH	HMFVLEQEEY
510	520	530	540	550	560	570	580	590	600
KREGIEWTFI	DFGMDLQNTI	DLLEKPMGVL	SILEEESMFP	KATDQTFAEK	LNNNHLGKSA	SFVKPKPAKA	GCKEAHFAIA	HYAGTVPYNI	TGWLEKNKDP
610	620	630	640	650	660	670	680	690	700
LNDTVVDQFK	KGSSKLVQEI	FADHPGQSGG	KEEAKGGKRG	KGGGFSTVSS	AYREQLNGLM	KTLNATSPHF	IRCIIPNETK	SPGVIDSHLV	MHQLTCNGVL
710	720	730	740	750	760	770	780	790	800
EGIRICRKGF	PNRMVYPDFK	HRYMILAPNE	MKAEPDERKA	AKICLEKIAL	DPEWYRIGHT	KVFFKAGVLG	QLEEMRDDKL	AKIITWMQSF	IRGYHTRKQY
810	820	830	840	850	860	870	880	890	900
KQLQDQRVAL	CVVQRNLRSY	LQMRTWAWYR	LWQKVKPLLN	VTRVEDEIKA	LEDKAAAAQA	NFEKEEKLRK	ELETNLAKLT	KEKEDLLNRL	QAESGTVADF
910	920	930	940	950	960	970	980	990	1000
HDKQNKLMSQ	KADLESQLSD	TQERLQQEED	ARNQLFQNKK	KLEQEASGLK	KDIEDLELAL	QKTETDKATK	DHQIRNLNDE	IAHQDELINK	LNKEKKHMQE
1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
VNQKTAEDLQ	ASEDKVNHLN	KVKAKLEQTL	DELEDSLERE	KKLRADIEKN	KRKTEGDLKL	TQEAVADLER	NKKELEQTIQ	RKDKEIASLN	AKLEDEQSLV
1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
GKLQKQIKEL	QSRIEELEEE	VEAERQARAK	AEKQRADLAR	ELEELGERLE	EAGGATAAQI	ELNKKREAEL	SKLRRDLEES	NIQHESVLSN	LRKKHNDAVS
1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
EMSEQIDQLN	KMKAKAEKDR	SQFAGENNDL	RAAMDHVSSD	KAAAEKMTKM	LQQQLNEIQS	KLDEANRSLN	DFDVQKKKLT	IENSDYLRQL	EDAESQVSQL

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
161	679.809	2	-139.33	-0.0947	19.93	39.66	1	0.0	3	K.KKLEQEASGLKK.D		940-951	CID
1662	944.854	2	-131.80	-0.1246	56.70	45.39	1	0.0	1	K.AKLEQTLDELEDSLER.E		1024-1039	CID
1375	845.303	2	-129.54	-0.1095	49.52	36.12	1	0.0	0	K.LEQTLDELEDSLER.E		1026-1039	CID
774	737.756	2	-126.07	-0.0930	35.56	50.25	1	0.0	0	R.IEELEEEVEAER.Q		1114-1125	CID

Protein 16: PREDICTED: alpha-actinin, sarcomeric isoform X2 [Acyrthosiphon pisum]

Accession: gi|328703083
Database: NCBInr
MW [kDa] / pI: 103.7 / 5.6
Score: 185.7
Seq. Coverage [%]: 3.8
No. of Peptides: 3

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

10	20	30	40	50	60	70	80	90	100
MITMNSHSND	IDYSNGYMEP	EEEWEREGLL	DPAWEKQQKK	TFTAWCNSHL	RKAGTAIENI	EEDFRNGLKL	MLLLEVISGE	TLPKPDRGKM	RFHKIANVNK
110	120	130	140	150	160	170	180	190	200
ALDFIASKGV	KLVSIGAEEI	VDGNLKMTLG	MIWTIILRFA	IQDISVEEMT	AKEGLLLWCQ	RKTAPYKNVN	VQNFHLSFKD	GLAFCALIHR	HRPDLIDYHK
210	220	230	240	250	260	270	280	290	300
LSKDNPLQNL	NTAFDVAEKY	LDIPRMLDPE	DMTNTAMPDE	RAIMTYVSSY	YHCFSGAQKA	ETAANRICKV	LKVNQENERL	MEEYERLASD	LLEWIRRTLP
310	320	330	340	350	360	370	380	390	400
WLQSRQADNS	LSGVQKRLEE	YRTYRRKHKP	PRVEQKAKLE	TNFNTLQTKL	RLSNRPAYMP	TEGKMVSDIA	NAWKGLEQSE	KSFEDWLLSE	MMRLERLEHL
410	420	430	440	450	460	470	480	490	500
AQKFKAKADT	HEDWTRGKEE	MLQSSDFRQC	KLNDLKALKK	KHEAFESDLA	AHQDRVEQIA	AIAHELNSLE	YHDSTSVNIR	CQRICDQWDR	LGSLTQKRRT
510	520	530	540	550	560	570	580	590	600
DLDDAEKILE	KIDILHLEFA	KRAAPFNNWL	DGTREDLVDM	FIVHTVEEIQ	GLIDAHGQFK	ATLSDADKEY	NSIIGLVKDV	ESTVQKYQIP	GGLQNPYTTL
610	620	630	640	650	660	670	680	690	700
TSSDLSKKWS	EVKHLVPQRD	TTLQAELRKQ	QNNEMLRRQF	AEKSNQVGPW	IERQMDAVTA	IGMGLQGSLE	DQLHQLKQYE	QNVFAYKPHI	EELEKIHQAV
710	720	730	740	750	760	770	780	790	800
QEGMIFENRY	TQYTMETLRV	GWEQLLTSIN	RNVNEVENQI	LTRDSKGITQ	EQLNEFRASF	NHFDKNRTGR	LAPEEFKSCL	VSIGYSIGKD	RQGEIDFQRI
810	820	830	840	850	860	870	880	890	900
LAVVDPNSTG	YVHFDAFLDF	MTRESTDTDT	AEQVIDSFRI	LAGDKPYILS	DELRRELPPD	QAEYCIQRMA	PYKGVNAVPG	ALDYMSFSTA	LYGESDL

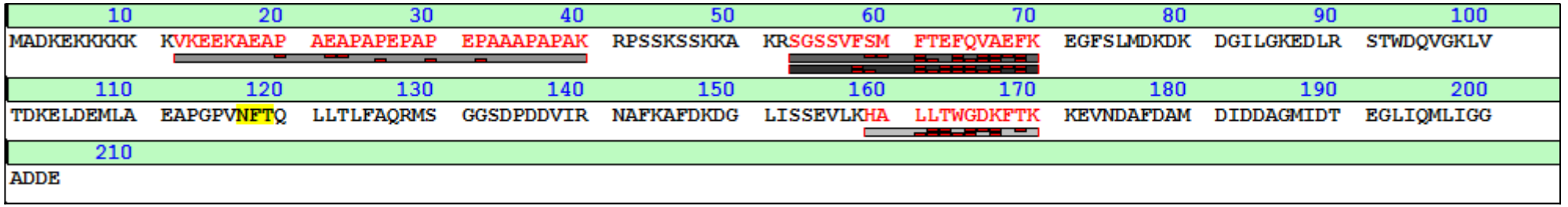
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1199	723.777	2	-139.60	-0.1011	45.37	26.93	1	0.0	0	K.NVNVQNFHLSFK.D		168-179	CID
1785	608.279	2	-100.66	-0.0612	60.06	52.04	1	0.0	0	R.LASDLLEWIR.R		287-296	CID
1712	708.308	2	-109.70	-0.0777	58.01	37.2	1	0.0	0	R.VGWEQLLTSINR.N		720-731	CID

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

Protein 17: PREDICTED: myosin regulatory light chain 2 [Halyomorpha halys]

Accession: gi|939657543
Database: NCBInr
MW [kDa] / pI: 22.2 / 4.9
Score: 164.9
Seq. Coverage [%]: 28.9
No. of Peptides: 3



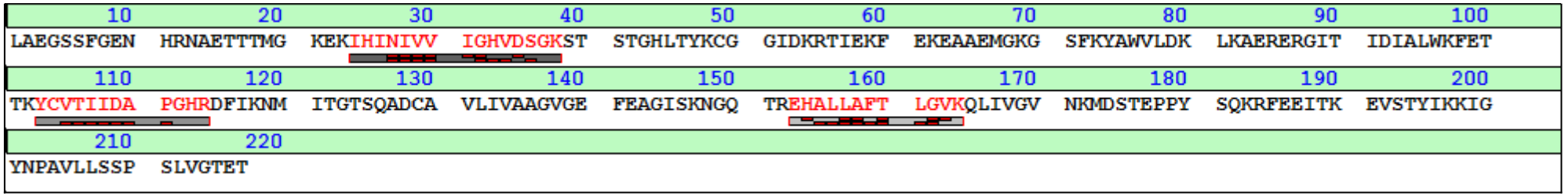
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
487	945.354	3	-144.41	-0.1365	28.54	23.74	1	0.0	2	K.VKEEKAEAPAEAPAPEPAPEPAAAPAPAK.R		12-40	CID
1978	1014.354	2	-118.87	-0.1206	67.38	57.35	2	0.0	0	R.SGSSVFSMFTEFQVAEFK.E		53-70	CID
1980	1014.351	2	-121.83	-0.1236	67.45	64.98	2	0.0	0	R.SGSSVFSMFTEFQVAEFK.E		53-70	CID
1082	708.809	2	-107.70	-0.0763	42.66	45.73	1	0.0	1	K.HALLTWGDKFTK.K		159-170	CID

Protein 18: elongation factor 1 alpha, partial [Paralichthys olivaceus]

Accession: gi|81157921
Database: NCBInr
MW [kDa] / pI: 23.7 / 7.9
Modification(s): Carbamidomethyl
Score: 150.0
Seq. Coverage [%]: 18.0
No. of Peptides: 3

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1208	800.878	2	-105.02	-0.0841	45.57	59.07	1	0.0	0	K.IHINIVVIGHVDSGK.S		24-38	CID
961	701.729	2	539.23	0.3782	39.85	36.97	1	0.0	0	K.YCVTIIDAPGHR.D	Carbamidomethyl: 2	103-114	CID
1518	649.834	2	-66.15	-0.0430	52.99	53.92	1	0.0	0	R.EHALLAFTLGVK.Q		153-164	CID

Protein 19: PREDICTED: V-type proton ATPase catalytic subunit A [Halyomorpha halys]

Accession: gi|939644958
Database: NCBInr
MW [kDa] / pI: 67.8 / 5.2
Modification(s): Carbamidomethyl

Score: 141.5
Seq. Coverage [%]: 4.2
No. of Peptides: 1

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

10	20	30	40	50	60	70	80	90	100
MALPRIKDED	QESKFGYVFG	VSGPVVTAEK	MSGSAMYELV	RVGYFELVGE	IIRLEGDMAT	IQVYEETSGV	TVGDPVLRTG	KPLSVELGPG	ILGSIFDGIQ
110	120	130	140	150	160	170	180	190	200
RPLKDINEIS	NSIYIPKGVN	IPALSRSAAW	EFQPTNIKVG	SHITGGDLYG	VVHENTLVKH	KMILPPRAKG	TVTYLAAPGN	YTVDDVVLET	EFDGEKTKFT
210	220	230	240	250	260	270	280	290	300
MLQVWPVRQP	RPVTEKLPAN	YPLLTGQRVL	DALFPCVQGG	TTAIPGAFGC	GKTVISQALS	KYSNSDVIIY	VGCGERGNEM	SEVLRDFPEL	SVEIDGVTES
310	320	330	340	350	360	370	380	390	400
IMKRTALVAN	TSNMPVAARE	ASIYTGITLS	EYFRDMGYNV	SMMADSTSRW	AEALREISGR	LAEMPADSGY	PAYLGARLAS	FYERAGRVKC	LGNPEREGSV
410	420	430	440	450	460	470	480	490	500
SIVGAVSPPG	GDFSDPVTSA	TLGIVQVFWG	LDKKLAQRKH	FPSINWLISY	SKYMRALDDF	YDKNFPEFVP	LRTKVKEILQ	EEEDLSEIVQ	LVGKASLAES
510	520	530	540	550	560	570	580	590	600
DKITLEIAKL	LKDDFLQQNS	YSPYDRFCPF	YKTVGMLKNM	ITFYDLARHA	VESTAQSEKK	ITFAVIKESM	GNILYQMSSM	KFKDPVKDGE	SKIRADFEQL
610	620								
QEDIQQAFRN	LED								

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1993	898.085	3	-111.03	-0.0997	67.92	33.44	1	0.0	0	R.TGKPLSVELGPGILGSIFDGIQRPLK.D		79-104	CID

Protein 20: Muscle LIM protein Mlp84B [Melipona quadrifasciata]

Accession: gi|925676200
Database: NCBInr
MW [kDa] / pI: 91.1 / 8.8
Modification(s): Carbamidomethyl

Score: 131.9
Seq. Coverage [%]: 4.4
No. of Peptides: 3

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

10	20	30	40	50	60	70	80	90	100
MALERQVRAK	IAAKRNPEQE	KEAQEWIESI	LGKKFPPGEA	FEDVIKDGQV	LCHLMNKISP	GSISKINTSG	GQFKMMENIN	AFQKALKDYG	VADVDVFQTV
110	120	130	140	150	160	170	180	190	200
DLWEKKDIAQ	VVTTLFALGR	TTYKHPEWKG	PYLGPKPADE	CKREFTEEQL	RAGETVIGLQ	AGSNKGATQS	GQSIGATQWW	LKFKVVVIRG	GTLEAVKCNI
210	220	230	240	250	260	270	280	290	300
LMMRIQINFD	ADGYNKSVDK	DRNYFMFENP	KLHQNPAPPE	EPDISNHLRL	RREERIKFRQ	TFAVHQSFSR	EEASDRESQR	ETGIDKMPFK	PVEHPKCPKC
310	320	330	340	350	360	370	380	390	400
GKSVYAAEER	VAGGLKWHKM	CFKCGLCGKL	LDSTNCTEHE	GELFCKVCHG	RKFGPKGYGF	GGGAGTLSMD	QGEHLKSSDP	MIQQHDPPFG	NEIGRSKGSL
410	420	430	440	450	460	470	480	490	500
LRTRGPRACV	CVTYMSEKSD	LYKRHVFRDV	ARGSNAILEP	RAIAKAPEGE	GCPRCGGYVY	AAEQMLARGR	YATERDSDRR	VMAMVRVVAP	YKATATPMAC
510	520	530	540	550	560	570	580	590	600
YPKKFGPRGI	GHAGVMWIGL	QCDIEDEGDA	APRTTVIDTA	VIKAPPGKGC	PRCGGVVFAA	EQVLAKGREW	HRKCYKCRDC	SKTLDSIIAC	DGPDKDVYCK
610	620	630	640	650	660	670	680	690	700
TCYGKKWGPH	GYGFACGSGF	LQTDGLTEEE	ISASRPFYNP	DTTAIKAPAG	QGCPRCGGMV	FAAEQQLAKG	TMWHKKCFNC	AECHRPLDSM	LACDGPDKEI
710	720	730	740	750	760	770	780	790	800
HCRSCYSKLF	GPKGFGFGHT	PTLVSTNGDH	APSYIDAKPQ	VGQKRTDGNG	CARCGYPVYA	AEQMISKNRL	WHKRCFSCAE	CHRSLDSTNL	NDGPDGDIYC
810	820	830							
RGCYNRNFGP	KGVGFGMGAG	TLTMA							

MS/MS Peptide Matches

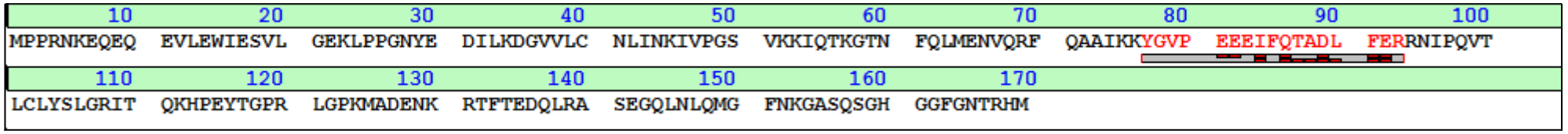
Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
744	672.779	2	-119.67	-0.0805	34.79	40.42	1	0.0	0	R.AGETVIGLQAGSNK.G		152-165	CID
148	462.588	2	-295.44	-0.1367	19.46	26.43	1	0.0	0	K.SVYAAEER.V		303-310	CID
1470	724.805	2	-106.15	-0.0769	51.81	65.02	1	0.0	0	R.CGGVVFAAEQVLAK.G	Carbamidomethyl: 1	553-566	CID

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

Protein 21: PREDICTED: myophilin [Trichogramma pretiosum]

Accession: gi|936694571
Database: NCBInr
MW [kDa] / pI: 19.1 / 7.8
Modification(s): Carbamidomethyl

Score: 127.5
Seq. Coverage [%]: 10.1
No. of Peptides: 1



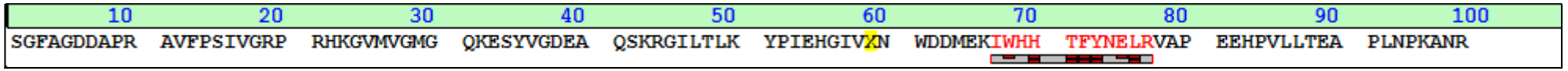
MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1824	1021.86	2	-126.15	-0.1289	61.31	59.98	1	0.0	0	K.YGVPEEEIFQTADLFER.R		77-93	CID

Protein 22: actin, partial [Brevinucula verrillii]

Accession: gi|343952816
Database: NCBInr
MW [kDa] / pI: 11.0 / 6.5
Modification(s): Oxidation

Score: 116.6
Seq. Coverage [%]: 11.2
No. of Peptides: 1



MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
941	758.263	2	-151.93	-0.1152	39.39	38.93	1	0.0	0	K.IWHHTFYNELR.V		67-77	CID

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

Protein 23: paramyosin, long form [Riptortus pedestris]

Accession:	gi|501294930	**Score:**	107.9
Database:	NCBInr	**Seq. Coverage [%]:**	1.6
MW [kDa] / pI:	102.8 / 5.5	**No. of Peptides:**	1

10	20	30	40	50	60	70	80	90	100
MPLASSTRQT	KTYTYRSTGG	GGTTDVNIEY	SADLSALSRL	EDKIRLLSED	LESERELRQR	IEREKADLSV	QVLSLQERLE	EAEGGAESQF	EINKKRDTEL
110	120	130	140	150	160	170	180	190	200
LKLRKLLEDV	HLESEETAHL	LRKKHQEVVV	DFQEQLDLVS	KAKSKAEKEK	AKFQQEVYEL	LSQVESANKE	RIISIKHAEK	LEVTIHELNI	RIEELNRTIV
210	220	230	240	250	260	270	280	290	300
DITSHKTRLS	QENIELTKEV	QDLKVNIENV	TYLRSQVASQ	LEDARRRLEE	DERRRATLES	SLHQVEVELE	STRVQLEEEA	EARLDLERQL	VKANGDALTW
310	320	330	340	350	360	370	380	390	400
KSKYDSEAAA	RAEEVDEIRK	KYTIRLQEQE	EHIETLIVKI	NNLEKQKSRL	QSEVEVLIID	LEKANNSARE	FSKRVEQLER	IHVELKSRLD	ETTIALDQTQ
410	420	430	440	450	460	470	480	490	500
RDLRTRTQEI	QRLNHELEKT	REQKDSLARE	NKKLADDLHD	AKNTMAEMNR	RLHELEIELR	RLENEREELS	AAYREAEAGR	KAEEQRVMRL	SAEFNQFRHE
510	520	530	540	550	560	570	580	590	600
AEKRIQEKDE	EIEIIRKQTS	IEIEQLNARV	AEAETKLKTE	VIRIKKKLQV	HITELELSLD	VANKNNIQLQ	QTIKKQSLQL	TELQAHYDET	TRQLAVTVDQ
610	620	630	640	650	660	670	680	690	700
LAIASRKVQS	LTAEIEEIRG	NYEQALRAKR	SAEQMYEDAQ	SRINELTTIN	VNISAQRAKI	EQELASCAAD	YEEVTKELKI	ADERYQKVQI	ELKHTVEHLH
710	720	730	740	750	760	770	780	790	800
EEQERIIKIE	TIKKSLEIEV	KNLTVRLEEV	EANAIVGGRR	IISKLEARIK	DMELELDEEK	RRHAETVKIL	RKKERQIKEI	IIQSEEDHKN	LTMLQEALDK
810	820	830	840	850	860	870	880	890	
TNQKVSIYKR	QLNEQEGMSQ	QSVTRVRRFQ	RELEAAEDRA	DTAESNLSLI	RAKHRTFVTT	STVPGSQVYL	VKETHTSETY		

MS/MS Peptide Matches

Protein Report
IonTrap_allOrg_2019-11-06 16:28:01

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1021	854.845	2	-133.85	-0.1144	41.25	30.38	1	0.0	0	R.LQEQEEHIETLIVK.I		326-339	CID

Protein 24: hypothetical protein DAPPUDRAFT_309281 [Daphnia pulex]

Accession: gi|321459762
Database: NCBInr
MW [kDa] / pI: 59.3 / 9.3

Score: 100.8
Seq. Coverage [%]: 4.0
No. of Peptides: 2

```
        10         20         30         40         50         60         70         80         90        100
MLSARLVSSV ARQLPKNVPK VARRSLPAIQ TAVRQLHVTP TAKAAEISSI LEERILGAAP KENLEETGRV LSIGDGIARV YGLKNIQAEE MVEFSSGLKG
       110        120        130        140        150        160        170        180        190        200
MALNLEPDNV GVVVFGNDKL IKEGDIVKRT GAIVDVPIGA ELLGRVVDAL GNPIDGKGSL AGAKRARVGV KAPGIIPRIS VREPMQTGIK AVDSLVPIGR
       210        220        230        240        250        260        270        280        290        300
GQRELIIGDR QTGKTAIAID AIINQKRFND GADEKKKLYC IYVAIGQKRS TVAQIVKRLT DSDAMKYTIV VAATASDAAP LQFLAPYSGC AMGEYFRDNG
       310        320        330        340        350        360        370        380        390        400
KHALIIYDDL SKQAVAYRQM SLLLRRPPGR EAYPGDVFYL HSRLLERAAK MNEVHGGGSL TALPVIETQA GDVSAYIPTN VISITDGQIF LETELFYKGI
       410        420        430        440        450        460        470        480        490        500
RPAINVGLSV SRVGSAAQTR AMKQVAGSMK LELAQYREVA AFAQFGSDLD ASTQQLLSRG VRLTELLKQG QYVPMAIEEQ VPVIYCGVRG YLDKLDPSKI
       510        520        530        540        550
TAFEKEFLQH IKTTHADVLT AIAKDGKITD ETDAKLKKIV TEFLATFQA
```

MS/MS Peptide Matches

Cmpd.	m/z meas.	z	Δ m/z [ppm]	Δ m/z [Da]	Rt [min]	Score	No. of Cmpds.	Site [%]	P	Sequence	Modification	Range	Type
1078	609.264	2	-95.59	-0.0582	42.54	31.94	1	0.0	0	K.AAEISSILEER.I		44-54	CID
1055	644.284	2	-103.67	-0.0668	42.01	41.39	1	0.0	0	K.HALIIYDDLSK.Q		302-312	CID

8. ANEXOS

Anexo 1. COMITÊ DE ÉTICA

CEUA
Comissão de Ética no Uso de Animais
Faculdade de Ciências Farmacêuticas - UNESP

C E R T I F I C A D O

Certificamos que a proposta intitulada "**Manutenção do Insetário de Triatominae**", registrada com o Protocolo CEUA/FCF/CAr: 18/2019, sob a responsabilidade do pesquisador João Aristeu da Rosa, cuja equipe é composta pelos seguintes pesquisadores: Juliana Damieli Nascimento, Jader de Oliveira, Heloísa Pinotti, André Luiz Rodrigues Menezes, Tiago Belintani e Vinícius Fernandes de Paiva que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado "ad-referendum" pelo Coordenador da COMISSÃO DE ÉTICA NO USO DE ANIMAIS da Faculdade de Ciências Farmacêuticas do Campus de Araraquara da UNESP em 21 de janeiro de 2020.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	Fevereiro de 2022
Espécie/linhagem/raça	Ave – Pato (doméstico) /Camundongo heterogênico Swiss
Nº de animais	50 patos /140 camundongos
Peso/Idade	Pato 4,5 kg / Camundongos 20-30 g
Sexo	Machos e Fêmeas
Origem	Camundongos: Biotério Central do Câmpus de Botucatu Patos: criadores da região de Araraquara
Registro CIAEP	02.00082.2019

Araraquara, 21 de janeiro de 2020.

Prof Dr Marcelo Tadeu Marin
Coordenador da CEUA

Anexo 2. DECLARAÇÃO DE DIREITOS AUTORAIS

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **ESTUDO DOS ASPECTOS MORFOLÓGICOS, FILOGENÉTICOS E PROTEÔMICO DE RHODNIUS NEGLECTUS (HEMIPTERA, REDUVIIDAE) - STUDY OF ASPECTS MORPHOLOGICAL, PHYLOGENETIC AND PROTEOMIC OF RHODNIUS NEGLECTUS (HEMIPTERA, REDUVIIDAE)**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 09/04/2020

Assinatura : _____
Nome do(a) autor(a): **Juliana Damieli Nascimento**
RG n.° 46.375.555-8

Assinatura : _____
Nome do(a) orientador(a): **Prof. Dr. João Aristeu da Rosa**
RG n.° 4.473.812