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The sialotranscriptome of the gopher-tortoise tick, *Amblyomma tuberculatum*

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Abstract

The gopher tortoise tick, *Amblyomma tuberculatum*, is known to parasitize keystone ectotherm reptile species. The biological success of ticks requires precise mechanisms to evade host hemostatic and immune responses. Acquisition of a full blood meal requires attachment, establishment of the blood pool, and engorgement of the tick. Tick saliva contains molecules which counter the host responses to allow uninterrupted feeding on the host. RNASeq of the salivary glands of *Amblyomma tuberculatum* ticks were sequenced resulting in 138,030 pyrosequencing reads which were assembled into 29,991 contigs. A total of 1875 coding sequences were deduced from the transcriptome assembly, including 602 putative secretory and 982 putative housekeeping proteins. The annotated data sets are available as a hyperlinked spreadsheet. The sialotranscriptome assembled for this tick species made available a valuable resource for mining novel pharmacological activities and comparative analysis.

Keywords

Tick; salivary glands; transcriptome; sialome; Amblyomma tuberculatum; hematophagy

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Introduction

The gopher tortoise (*Gopherus polyphemus*), a keystone species native to the Southeastern United States, is listed as threatened under the US Endangered Species Act in the Western part of its range (Service, 1987) and the Eastern populations have been classified as a candidate for federal listing as a threatened species (Dodd Jr, 1995; Service, 2015). The gopher tortoise is a keystone species because its burrows are utilized by 350 vertebrate and invertebrate commensals (Catano and Stout, 2015; Johnson et al., 2017).

Amblyomma tuberculatum Marx, 1894 is commonly known as gopher tortoise tick for parasitizing the gopher tortoise. The gopher tortoise ticks feed for several days or weeks on the gopher tortoises and obtain a vast blood meal up to 100 times their unfed weight. Tick hematophagy (blood-feeding) is usually divided into slow feeding and fast feeding. Tick attachment involves the penetration of the skin by the chelicerae and the secretion of salivary cement, which helps affix the tick at the feeding site. Tick saliva also assists in feeding, its complex mixture of compounds disarming the host's attempts to establish hemostasis and tissue repair. Tick saliva composition, as revealed by sialotranscriptome (from the Greek, sialo means saliva), indicates the presence of over 5,000 putative secreted peptides, containing representatives of dozens of protein families (Chmelar et al., 2016; Gulia-Nuss et al., 2016; Karim and Ribeiro, 2015; Karim et al., 2011). Sialomes of ixodid ticks are an invaluable resource for the tick biologists and will help in comparative analysis to better understand the complex biology of tick hematophagy and evolution. Very limited work has been done to investigate tick feeding on gopher tortoises (Budachetri et al., 2016; Cooney and Hays, 1972; Ennen and Qualls, 2011). In this study, we report an annotated catalogue of salivary gland expressed transcripts from adult female *A. tuberculatum* resulting from over 138,000 pyrosequencing sequences, representing, to our knowledge, the first analysis of a tick sialome from a parasite exclusively feeding on an ectotherm. This study is also relevant by describing the sialome of a tick feeding on its natural host, because tick sialomes may change according to the particular host that the tick is feeding (Narasimhan et al., 2019). This study complements the sialotranscriptome of other *Amblyomma* species (Aljamali et al., 2009; Batista et al., 2008; Esteves et al., 2017; Garcia et al., 2014; Karim and Ribeiro, 2015; Karim et al., 2011; Maruyama et al., 2010; Moreira et al., 2017; Ribeiro et al., 2011).

Materials and Methods

Ethics Statement

All animal experiments were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol for the collection of ticks from Gopher tortoises was approved by the Institutional Animal Care and Use Committee of the University of Southern Mississippi (protocol # 10042001).

Ticks

All ticks were collected directly from gopher tortoises that were trapped using a collapsible Tomahawk^{R} Model 18 Live Trap (81.28 X 25.4 X 30.48) covered with burlap and pine

needles to provide shade. The site of collection was from Wiggins Airport (Wiggins, Mississippi) [30°50’32” N, −89°09’37”W] and Crossroads site within the DeSoto National Forest, MS [30°57’24”N, −89°06’32”W]. Ticks were manually pulled from the tortoise using fine forceps. The partially blood fed ticks were brought into the lab and cleaned as described earlier (Budachetri et al., 2016). Ticks were identified using standard morphological keys. Tick salivary glands were dissected from 10–12 engorged female ticks. The dissecting solution was ice cold 100 mM 3-(N-Morpholino)-propanesulfonic acid (MOPS) buffer containing 20 mM ethylene glycol bis-(β-aminoethyl ether)-N, N, N’, N’ -tetraacetic acid (EGTA), pH 6.8. After removal, glands were washed gently in the same ice-cold buffer. The dissected SGs were stored immediately after dissection in RNAlater (Ambion Inc., Austin, TX, USA) prior to isolating mRNA.

RNA and Library preparation

These were done essentially as reported before (Karim et al., 2011). Briefly, RNA Poly A+ mRNA was isolated using the Illustra™ QuickPrep micro mRNA purification kit (GE Healthcare, Piscataway, NJ, USA. mRNA quality was determined by lab-on-chip analysis using the 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). The mRNA quantity was determined by Nanodrop.

Library preparations for GS FLX titanium (Roche/454 Life Sciences, Branford, CT, USA) sequencing were developed in the Center for Genomics and Bioinformatics, Indiana University. The cDNA library was normalized using Trimmer cDNA normalization kit (Evrogen, Moscow, Russia) according to manufacturer’s instructions. Normalized DNA was fragmented by sonication, and ends were enzymatically blunted and ligated to customized 454 adaptors. Emulsion PCR reactions were performed according to the manufacturer (Roche 454 Life Sciences). Sequencing adapters (A and B) were automatically removed from the reads using signal processing software (Roche 454 Life Sciences).

Bioinformatics Analysis

Assembly of all reads were done using the assemblers Abyss and Soapdenovo-Trans with every other kmer (-k program switch) parameter from 17 to 85 (Birol et al., 2009; Liu et al., 2011; Luo et al., 2012; Miller et al., 2010; Simpson et al., 2009). Resulting contigs were re-assembled by a pipeline of blastn and cap3 assembler (Huang and Madan, 1999) as described earlier (Karim et al., 2011). Coding sequences were extracted based on blastx (Altschul et al., 1997) results deriving from several database matches, including a subset of the non-redundant protein database of the National Center for Biotechnology Information (NCBI) containing tick and other invertebrate sequences, as well as the Swissprot and Gene Ontology (GO) databases. The longest open reading frame was also extracted if it had a signal peptide indicative of secretion as evaluated by version 3.0 of the SignalP program (Nielsen et al., 1999).

Data sharing

All coding sequences are available for browsing in additional file S1 which also contains hyperlinks to several databases, as explained previously (Karim et al., 2011; Ribeiro et al., 2004). This project was registered at the National Center for Biotechnology Information

(NCBI) under Bioproject ID PRJNA595760 and Biosample ID SAMN13569030. Deducted coding sequences and their translations were deposited to DDBJ/EMBL/GenBank under the accession GIDH00000000. The version described in this paper is the first version, GIDH01000000.

Results and Discussion

A total of 138,030 pyrosequencing reads were assembled into 29,991 contigs. A subset of this data containing 1,875 coding sequences larger than 150 nucleotides and mapping at least 2 reads was assembled from 64,852 reads. (Spreadsheet S1 and Table 1). This subset maps 47 % of all raw reads. Five broad categories of expressed genes are observed in spreadsheet S1 and summarized in Tables 2. The putatively secreted (S) category contained ~ 59% of the reads and ~32% CDS; the housekeeping (H) category had ~39% reads with ~52% CDS; ~2% of reads were not classifiable, constituting 265 contigs which were assigned to the unknown (UK) class; Transposable elements (TE class) accounted for 25 CDS and 93 reads, and 2 rickettsial reads derived from microbes.

The following is a guide to browsing spreadsheet S1.

Housekeeping (H) Genes

A total of 982 CDS ascribed to H-class genes expressed in the SGs of *Am. tuberculatum* were further characterized into 22 subgroups according to function (Table 3 and spreadsheet S1). CDS of the H class represents 52.37% of all CDS and 39.32% of total reads (Table 3). As shown in Table 3, detoxification and oxidant metabolism / detoxification enzymes contain altogether 624 reads which include sulfotransferases and selenoproteins. Sulfotransferases have been shown to play a role in dopamine detoxification (Pichu et al., 2011; Yalcin et al., 2010), a neurotransmitter associated with tick salivary secretion (Kaufman, 1978). Enzymes which neutralize oxidative and endoplasmic reticulum (ER) stress such as superoxide dismutase (cytoplasmic), superoxide dismutase (mitochondrial), glutathione peroxidase, thioredoxin peroxidase, salivary selenoprotein precursor, selenoprotein K and selenoprotein T are also present in the sialotranscriptome of *A. tuberculatum*. Carboxypeptidases and leukotriene hydrolases were also detected under amino acid metabolism related genes. Energy and lipid metabolism genes altogether contain 2,450 reads including pyrophosphatases which promotes the rapid hydrolysis of inorganic pyrophosphate and activates fatty acids for beta oxidation. Several immunity-related proteins such as TNF receptor-associated factor-like protein, Toll like receptors, interferon alpha-inducible proteins etc. were annotated. Other additional classes of housekeeping sequences related to protein synthesis machinery, energy metabolism, protein modification, signal transduction, protein export and transcription machinery were also found abundantly expressed in salivary glands.

Secreted (S) class of expressed genes

A total of 602 CDS attributed to secreted (S)-class genes expressed in the SGs of *Am. tuberculatum* were further characterized into several subgroups based on their function (Table 3 and Spreadsheet S1). It covers 32.1% of CDS and 58.5% of total reads. Among the

secreted (S) class of expressed genes are metalloproteases, M13 peptidases, lipocalins, Kunitz domain containing proteins, TIL domain containing proteins, evasins and 8.9 kDa protein family, altogether covering for ~86% of the reads attributed to the S class.

Metalloproteases—Metalloproteases of the reprolysin superfamily have been previously described in tick sialomes. In *I. scapularis*, a recombinant enzyme of this family was shown to have fibrinolytic properties and to inhibit angiogenesis (Francischetti et al., 2003, 2005). The tick metalloproteases are distinct reprolysins that can be readily identified by the domain cd04272 from the CDD, annotated as "ZnMc_salivary_gland_MPs, Zinc-dependent metalloprotease, salivary gland_MPs. Metalloproteases secreted by the salivary glands of arthropods". The *A. tuberculatum* sialotranscriptome reveals 16 CDS coding for members of this family, accruing 1.32% of the reads associated with the S class (Table 3). Phylogenetic analysis of near full-length *A. tuberculatum* sequences of this class with their best matches from the Acari database reveals 5 distinct clades with strong bootstrap support (named I-V on Supplementary figure 1), and one clade with less bootstrap support named A (Supplementary. figure 1). Clades I-IV and A are exclusively metastriate, while clade V has both prostriate and metastriate sequences, distinctly arranged in subclades. Five *A. tuberculatum* sequences are found in clades I, V and A. AtSigP-642 belongs to clade I, a clade characterized by metastriate sequences containing a carboxyterminal extension rich in Ser and Thr as visible within the red box in supplemental alignment S1. The Netoglyc server (Hansen et al., 1998) indicates 15 sites for N-galactosylation of the carboxyterminus of AtSigP-642, representing these metalloproteases to have a mucin tail, as previously pointed out before (Francischetti et al., 2009). AtSigP-168 and AtSigP-210 are closely related sequences (~10% amino acid difference) found in clade A and may represent alleles or more probably a recent gene duplication. AtSig-2297 is related to a *R. pulchellus* metalloprotease, with strong bootstrap support also in clade A. Finally, AtSigP-1811 is found in the metastriate subclade of clade V and may represent descent of the most primitive type of metalloprotease that gave rise to the prostriate expansion.

M13 metalloproteases/peptidases of the neprilysin family are usually membrane bound ecto enzymes associated with the hydrolysis of peptide hormones (Ouimet, 2004). Previous disclosure of tick sialomes (Francischetti et al., 2011; Karim et al., 2011) revealed highly expressed members of this family suggesting their role in their hosts, and indeed they lack indication of transmembrane domains or glyco inositol-phosphate anchors. The sialotranscriptome of *A. tuberculatu*m reveals 12 CDS coding for M13 zinc peptidases, accreting 855 reads, or 2.27 % (Table 3) of all reads associated with secreted products, more than the classical salivary metalloproteases that have been characterized in *I. scapularis* and *I. ricinus* as fibrinolytic and anti-angiogenic enzymes (Francischetti et al., 2003; Kotsyfakis et al., 2015; Ribeiro et al., 2006; Schwarz et al., 2013); these are represented in the *A. tuberculatum* sialotranscriptome by 16 CDS assembled from 498 reads. The phylogenetic reconstruction of the M13 peptidases from this sialotranscriptome data (Supplementary figure 2) showed Clades I and II to contain distinct subclades of vertebrates and invertebrates, indicating the ancient origin of these two subfamilies. Clade III contains insect and worm sub clades, and clades IV and V are purely from ticks. Two of the *A. tuberculatum* sequences form a subclade of clade V, where two copies of M13 sequences are

also found for *A. maculatum* and *A. triste*, signifying a gene duplication with possible gene conversion maintaining the paralogs within species-specific clades. This contrasts with clade IV sequences, where two or more gene products are represented for *R. pulchellus*, *A. cajennense* (sensu lato) and *A. triste*, but the subclades are multi-specific, indicating their equal evolutionary rate of change after the species diverged from the common ancestor.

Lipocalin Family—Proteins of the lipocalin family have been found in tick sialomes as product of dozens of genes in each species (Kim et al., 2016; Mans et al., 2008; Paesen et al., 1999; Paesen et al., 2000; Sangamnatdej et al., 2002; Tirloni et al., 2014). A few have been characterized functionally which collectively pointed their kratagonistic role (from the Greek kratos = seize) in sequestering host agonists such as serotonin, histamine, leukotrienes and thromboxanes (Andersen and Ribeiro, 2017; Ribeiro and Arca, 2009). The sialotranscriptome of *A. tuberculatum* identified 76 CDS coding for members of this family, representing 45% of the reads attributed to the secreted class (Table 3). Alignment of 26 *A. tuberculatum* sequences comprised of more than 150 amino acids with 549 best matches of the Acari database (Supplemental alignment S2) reveals 8 blocks of sequence similarities that are separated by insertions (marked with red lines on the top of the alignment). These insertions are usually flanked by glycine or proline residues (shown within the red boxes in the alignment). The first represents the signal peptide, distinguishable by the turquoise color of the leucine and other hydrophobic amino acids. Because lipocalins contain eight-stranded antiparallel-barrel arranged beta strands (Schlehuber and Skerra, 2005), the 7 blocks of conservation after the signal peptide represent these helices, perhaps the larger block (number 5) containing 2 helices, and the insertions represent loops between the helices. The phylogenetic reconstruction is complex and shallow, producing twelve large clades (marked A-L in the supplemental figure), most having under 50% bootstrap support (Supplementary. figure. 3). This may derive from the fast evolution of this group of lipocalin-coding genes that makes phylogenetic reconstruction difficult. However, sixteen clades with strong bootstrap support are shown, named with Roman numerals. Most clades are metastriate specific and group closely related species, thus within a shorter evolutionary time. The few clades with prostriate sequences are marked “Prot” on the phylogenetic tree. The alignment and phylogeny indicate the complexity of the tick lipocalin superfamily, but at the same time points to robust clades of sequences that could indicate a common function, helping to choose sequences for functional characterization of these proteins.

Kunitz domain containing proteins—These protease inhibitors are the second largest family among secreted salivary proteins. In ticks, Kunitz inhibitors possess one to seven Kunitz domains and most of them have anti-coagulant property which inhibit various proteases of the coagulation cascade (Assumpcao et al., 2016; Chmelar et al., 2012; Corral-Rodríguez et al., 2009; Francischetti et al., 2004; Francischetti et al., 2002; Mans et al., 2002; Nazareth et al., 2006; Paesen et al., 2007; Valdes et al., 2013). Apart from protease inhibition, Kunitz domains also inhibit ion channel (Castaneda and Harvey, 2009; Harvey, 1997; Kwong et al., 1995; Lucchesi and Moczydlowski, 1991). A study on *Rhipicephalus appendiculatus* tick has suggested a vasodilator function of these proteins by activating maxiK channels in an *in vitro* system (Paesen et al., 2009). Other members of this family

also bind to integrin and demonstrate anti-platelet activity (Chmelar et al., 2019; Mans et al., 2002).

These properties make Kunitz-Domain Protease Inhibitors significantly important target molecules in tick research. A Kunitz-domain protein Ir-CPI (*Ixodes ricinus* contact phase inhibitor), a very efficient inhibitor of the coagulation cascade (Decrem et al., 2009) is being used for pre-clinical testing. Some of the Kunitz inhibitors such as Ixolaris and Amblyomin-X have also demonstrated anti-cancer therapeutic potential (Barboza et al., 2015; Carneiro-Lobo et al., 2009; Carneiro-Lobo et al., 2012; Chudzinski-Tavassi et al., 2016; de Oliveira Ada et al., 2012; de Souza et al., 2016). Highest potential of these inhibitors lies in their anti-hemostatic properties which could be exploited in drug development for several human diseases. In this study, these proteins accrued 8.98% of the total reads i.e. 3388, and numbered 42 CDS (Table 3).

Despite the diversity of the Kunitz domain containing peptides in ticks, the phylogenetic reconstruction of *A. tuberculatum* protein sequences (Supplementary figure 4) with their best matching sequences from the NCBI Acari database indicates 9 robust clades of sequences (100% bootstrap support) containing at least one *A. tuberculatum* sequence and one or more sequences from other tick species, all from metastriate ticks except clade IX were sequences from *I. ricinus* and *I. scapularis* are also found, and may represent uniquely conserved sequences, probably of a housekeeping function. Clade VII is interesting as it has three clear sub-clades, (A) containing *A. maculatum*, *A. triste*, *A. americanum* and *A. tuberculatum* sequences, (B) containing only *A. maculatum* and *A. triste* sequences, and (C) containing *R. pulchellus*, *A. americanum*, *A. cajennense* (sensu lato), *A. parvum* and *H. marginatum* sequences. The only species appearing in both sub clades A and C is *A. americanum*, the sole sequence of subclade A being the most basal in the clade VII, whereas the sequences from *A. maculatum and A. triste* are found in the sister clades indicating evolutionarily closer relationship than those of *A. americanum* and *A. tuberculatum.* (Supplementary figure 4)

8.9 kDa protein family—This is a protein family of secreted peptides of molecular weight near 10 kDa but can also have proteins of double size containing two domains. This protein family is exclusive to hard ticks. An earlier work (Francischetti et al., 2009) has described the identification of sixty members of this protein family from prostriate and metastriate ticks. The *A. tuberculatum* sialotranscriptome reveals 19 CDS coding for members of this family, accruing 12.63% of the reads i.e. 4,763 reads associated with the secreted class. In a previous study, phylogenetic analysis of these proteins has shown robust clades with metastriate and prostriate sequences indicative of multiple round of gene duplication and faster evolution of these proteins (Karim et al. 2011).

Evasins—Evasins are considered as potential target molecules in tick research because they inhibit inflammatory responses (Charo and Ransohoff, 2006) associated with some chemokine classes. Evasins are a family of chemokine-binding proteins which were first identified in *Rhipicephalus sanguineus* (Deruaz et al., 2008; Frauenschuh et al., 2007) and more recently biochemically characterized in other tick species (Hayward et al., 2017). Evasin homologs are present in *Amblyomma* and *Ixodes* species, but homology in *Ixodes* is

weaker (Frauenschuh et al., 2007). Evasin-1 and evasin-4 bind specifically to CC chemokines, and evasin-3 binds to CXC chemokines (Deruaz et al., 2008; Frauenschuh et al., 2007). Evasin-1 has therapeutic potential due to its ability to inhibit T-cell, neutrophil, macrophage migration and the production of inflammatory cytokines (Chemlar et al. 2019). The *A. tuberculatum* sialotranscriptome reveals 20 CDS coding for members of this family, accruing 15.39% of the reads i.e. 5,804 reads associated with the secreted class. In a previous study, phylogenetic analysis indicated the diversity of the Evasin family, corroborated in this study by the finding of at least 18 different genes coding for evasins within *A. maculatum* (Karim et al., 2011).

Conclusion

In this study, the sialotranscriptome of adult female *A. tuberculatum* collected directly from gopher tortoises, we report an annotated catalogue of expressed transcripts from over ~138,000 pyrosequencing reads assembled into 29,991 contigs. Putatively secreted (S) category and housekeeping (H) category both together cover for 98% of the mapped reads, and 84% of the coding sequences (CDS). 41% of the reads from the putative housekeeping class were unknown conserved. Many orphan proteins have been found in this study which are secretory in nature, indicative of *Amblyomma*-specific proteins. Information related to the mechanism of tick feeding on ectotherm gopher tortoises is limited. Comparisons of the sequences of the different proteins families with those from ticks feeding on mammalian hosts may shed light on the evolution of blood feeding by ticks. This study will help in understanding the complex biology of tick hematophagy. On the other hand, knowledge on the physiology of hemostasis, inflammation and immunity in tortoises is virtually unknown when compared to the same subjects in mammals. The repertoire of salivary proteins from *A. tuberculatum* may result in the development of tools to investigate tortoise hemostasis and inflammation, as happened by the use of a bird-feeding vampire bat salivary protein which helped to determine the plasminogen activation pathway in birds (Cartwright and Hawkey, 1969).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Aljamali MN, Hern L, Kupfer D, Downard S, So S, Roe BA, Sauer JR, Essenberg RC, 2009 Transcriptome analysis of the salivary glands of the female tick Amblyomma americanum (Acari: Ixodidae). Insect molecular biology 18, 129–154. [PubMed: 19320755]

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ, 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402. [PubMed: 9254694]

Andersen JF, Ribeiro JM, 2017 Salivary Kratagonists: Scavengers of Host Physiological Effectors During Blood Feeding, Arthropod Vector: Controller of Disease Transmission. Elsevier, London, pp. 51–63.

Assumpcao TC, Ma D, Mizurini DM, Kini RM, Ribeiro JM, Kotsyfakis M, Monteiro RQ, Francischetti IM, 2016 In Vitro Mode of Action and Anti-thrombotic Activity of Boophilin, a Multifunctional Kunitz Protease Inhibitor from the Midgut of a Tick Vector of Babesiosis, *Rhipicephalus microplus*. PLoS neglected tropical diseases 10, e0004298. [PubMed: 26745503]

Barboza T, Gomes T, Mizurini DM, Monteiro RQ, Konig S, Francischetti IM, Signoretti PV, Ramos IP, Gutfilen B, Souza SA, 2015 (99m)Tc-ixolaris targets glioblastoma-associated tissue factor: in vitro and pre-clinical applications. Thromb Res. 136, 432–439. doi: 410.1016/j.thromres.2015.1005.1032. Epub 2015 Jun 1016. [PubMed: 26070446]

Batista IF, Chudzinski-Tavassi AM, Faria F, Simons SM, Barros-Batestti DM, Labruna MB, Leao LI, Ho PL, Junqueira-de-Azevedo IL, 2008 Expressed sequence tags (ESTs) from the salivary glands of the tick Amblyomma cajennense (Acari: Ixodidae). Toxicon 51, 823–834. [PubMed: 18243270]

Birol I, Jackman SD, Nielsen CB, Qian JQ, Varhol R, Stazyk G, Morin RD, Zhao Y, Hirst M, Schein JE, Horsman DE, Connors JM, Gascoyne RD, Marra MA, Jones SJ, 2009 *De novo* transcriptome assembly with ABySS. Bioinformatics (Oxford, England) 25, 2872–2877.

Budachetri K, Gaillard D, Williams J, Mukherjee N, Karim S, 2016 A snapshot of the microbiome of *Amblyomma tuberculatum* ticks infesting the gopher tortoise, an endangered species. Ticks and tick-borne diseases 7, 1225–1229. [PubMed: 27460902]

Carneiro-Lobo TC, Konig S, Machado DE, Nasciutti LE, Forni MF, Francischetti IM, Sogayar MC, Monteiro RQ, 2009 Ixolaris, a tissue factor inhibitor, blocks primary tumor growth and angiogenesis in a glioblastoma model. J Thromb Haemost 7, 1855–1864. [PubMed: 19624457]

Carneiro-Lobo TC, Schaffner F, Disse J, Ostergaard H, Francischetti IM, Monteiro RQ, Ruf W, 2012 The tick-derived inhibitor Ixolaris prevents tissue factor signaling on tumor cells. J Thromb Haemost 10, 1849–1858. [PubMed: 22823596]

Cartwright T, Hawkey C, 1969 Activation of Blood Fibrinolytic Mechanism in Birds by Saliva of Vampire Bat (Diaemus youngi). J Physiol-London 201, P45-+.

Castaneda O, Harvey AL, 2009 Discovery and characterization of cnidarian peptide toxins that affect neuronal potassium ion channels. Toxicon 54, 1119–1124. [PubMed: 19269305]

Catano CP, Stout IJ, 2015 Functional relationships reveal keystone effects of the gopher tortoise on vertebrate diversity in a longleaf pine savanna. Biodivers Conserv 24, 1957–1974.

Charo IF, Ransohoff RM, 2006 The many roles of chemokines and chemokine receptors in inflammation. New England Journal of Medicine 354, 610–621.

Chmelar J, Calvo E, Pedra JH, Francischetti IM, Kotsyfakis M, 2012 Tick salivary secretion as a source of antihemostatics. Journal of proteomics 75, 3842–3854. [PubMed: 22564820]

Chmelar J, Kotal J, Karim S, Kopacek P, Francischetti IM, Pedra JH, Kotsyfakis M, 2016. Sialomes and Mialomes: A Systems-Biology View of Tick Tissues and Tick-Host Interactions. Trends Parasitol. 32, 242–254. doi: 210.1016/j.pt.2015.1010.1002. Epub 2015 Oct 1028. [PubMed: 26520005]

Chmelar J, Kotal J, Kovarikova A, Kotsyfakis M, 2019 The Use of Tick Salivary Proteins as Novel Therapeutics. Frontiers in Physiology 10. [PubMed: 30740057]

Chudzinski-Tavassi AM, Morais KLP, Pacheco MTF, Pasqualoto KFM, de Souza JG, 2016 Tick salivary gland as potential natural source for the discovery of promising antitumor drug candidates. Biomed Pharmacother 77, 14–19. [PubMed: 26796259]

Cooney JC, Hays KL, 1972 Bionomics of Gopher Tortoise Tick, *Amblyomma tuberculatum* Marx. Journal of medical entomology 9, 239–&. [PubMed: 5057497]

Corral-Rodríguez MÁ, Macedo-Ribeiro S, Pereira PJB, Fuentes-Prior P, 2009 Tick-derived Kunitz-type inhibitors as antihemostatic factors. Insect biochemistry and molecular biology 39, 579–595. [PubMed: 19631744]

de Oliveira Ada S, Lima LG, Mariano-Oliveira A, Machado DE, Nasciutti LE, Andersen JF, Petersen LC, Francischetti IM, Monteiro RQ, 2012 Inhibition of tissue factor by ixolaris reduces primary tumor growth and experimental metastasis in a murine model of melanoma. Thromb Res 130, e163–170. [PubMed: 22683021]

de Souza JG, Morais KL, Anglés-Cano E, Boufleur P, de Mello ES, Maria DA, Origassa CST, de Campos Zampolli H, Câmara NOS, Berra CM, 2016 Promising pharmacological profile of a Kunitz-type inhibitor in murine renal cell carcinoma model. Oncotarget 7, 62255. [PubMed: 27566592]

Decrem Y, Rath G, Blasioli V, Cauchie P, Robert S, Beaufays J, Frère J-M, Feron O, Dogné J-M, Dessy C, 2009 Ir-CPI, a coagulation contact phase inhibitor from the tick *Ixodes ricinus*, inhibits thrombus formation without impairing hemostasis. Journal of Experimental Medicine 206, 2381–2395.

Deruaz M, Frauenschuh A, Alessandri AL, Dias JM, Coelho FM, Russo RC, Ferreira BR, Graham GJ, Shaw JP, Wells TN, Teixeira MM, Power CA, Proudfoot AE, 2008 Ticks produce highly selective chemokine binding proteins with antiinflammatory activity. The Journal of experimental medicine 205, 2019–2031. [PubMed: 18678732]

Dodd CK Jr, 1995 Reptiles and amphibians in the endangered longleaf pine ecosystem.

Ennen JR, Qualls CP, 2011 Distribution and Habitat Utilization of the Gopher Tortoise Tick (Amblyomma tuberculatum) in Southern Mississippi. Journal of Parasitology 97, 202–206.

Esteves E, Maruyama SR, Kawahara R, Fujita A, Martins LA, Righi AA, Costa FB, Palmisano G, Labruna MB, Sa-Nunes A, Ribeiro JMC, Fogaca AC, 2017 Analysis of the Salivary Gland Transcriptome of Unfed and Partially Fed Amblyomma sculptum Ticks and Descriptive Proteome of the Saliva. Frontiers in cellular and infection microbiology 7. [PubMed: 28164039]

Francischetti IM, Mather TN, Ribeiro JM, 2003 Cloning of a salivary gland metalloprotease and characterization of gelatinase and fibrin(ogen)lytic activities in the saliva of the Lyme disease tick vector Ixodes scapularis. Biochemical and biophysical research communications 305, 869–875. [PubMed: 12767911]

Francischetti IM, Mather TN, Ribeiro JM, 2004 Penthalaris, a novel recombinant five-Kunitz tissue factor pathway inhibitor (TFPI) from the salivary gland of the tick vector of Lyme disease, *Ixodes scapularis*. Thrombosis and haemostasis 91, 886–898. [PubMed: 15116248]

Francischetti IM, Mather TN, Ribeiro JM, 2005 Tick saliva is a potent inhibitor of endothelial cell proliferation and angiogenesis. Thrombosis and haemostasis 94, 167–174. [PubMed: 16113800]

Francischetti IM, Sa-Nunes A, Mans BJ, Santos IM, Ribeiro JM, 2009 The role of saliva in tick feeding. Frontiers in bioscience 14, 2051–2088.

Francischetti IM, Valenzuela JG, Andersen JF, Mather TN, Ribeiro JM, 2002 Ixolaris, a novel recombinant tissue factor pathway inhibitor (TFPI) from the salivary gland of the tick, Ixodes scapularis: identification of factor X and factor Xa as scaffolds for the inhibition of factor VIIa/tissue factor complex. Blood 99, 3602–3612. [PubMed: 11986214]

Francischetti IMB, Anderson JM, Manoukis N, Pham VM, Ribeiro JMC, 2011 An insight into the sialotranscriptome and proteome of the coarse bontlegged tick, *Hyalomma marginatum rufipes*. Journal of proteomics 74, 2892–2908. [PubMed: 21851864]

Frauenschuh A, Power CA, Deruaz M, Ferreira BR, Silva JS, Teixeira MM, Dias JM, Martin T, Wells TN, Proudfoot AE, 2007 Molecular cloning and characterization of a highly selective chemokine-binding protein from the tick *Rhipicephalus sanguineus*. The Journal of biological chemistry 282, 27250–27258. [PubMed: 17640866]

Garcia GR, Gardinassi LG, Ribeiro JM, Anatriello E, Ferreira BR, Moreira HN, Mafra C, Martins MM, Szabo MP, de Miranda-Santos IK, Maruyama SR, 2014 The sialotranscriptome of *Amblyomma triste, Amblyomma parvum* and *Amblyomma cajennense* ticks, uncovered by 454-based RNA-seq. Parasit Vectors 7, 430. [PubMed: 25201527]

Gulia-Nuss M, Nuss AB, Meyer JM, Sonenshine DE, Roe RM, Waterhouse RM, Sattelle DB, de la Fuente J, Ribeiro JM, Megy K, Thimmapuram J, Miller JR, Walenz BP, Koren S, Hostetler JB, Thiagarajan M, Joardar VS, Hannick LI, Bidwell S, Hammond MP, Young S, Zeng Q, Abrudan JL, Almeida FC, Ayllon N, Bhide K, Bissinger BW, Bonzon-Kulichenko E, Buckingham SD, Caffrey DR, Caimano MJ, Croset V, Driscoll T, Gilbert D, Gillespie JJ, Giraldo-Calderon GI, Grabowski JM, Jiang D, Khalil SM, Kim D, Kocan KM, Koci J, Kuhn RJ, Kurtti TJ, Lees K, Lang EG, Kennedy RC, Kwon H, Perera R, Qi Y, Radolf JD, Sakamoto JM, Sanchez-Gracia A, Severo MS, Silverman N, Simo L, Tojo M, Tornador C, Van Zee JP, Vazquez J, Vieira FG, Villar M, Wespiser AR, Yang Y, Zhu J, Arensburger P, Pietrantonio PV, Barker SC, Shao R, Zdobnov EM, Hauser F, Grimmelikhuijzen CJ, Park Y, Rozas J, Benton R, Pedra JH, Nelson DR, Unger MF, Tubio JM, Tu Z, Robertson HM, Shumway M, Sutton G, Wortman JR, Lawson D, Wikel SK, Nene VM, Fraser CM, Collins FH, Birren B, Nelson KE, Caler E, Hill CA, 2016 Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease. Nature communications 7, 10507.

Hansen JE, Lund O, Tolstrup N, Gooley AA, Williams KL, Brunak S, 1998 NetOglyc: prediction of mucin type O-glycosylation sites based on sequence context and surface accessibility. Glycoconj J 15, 115–130. [PubMed: 9557871]

Harvey AL, 1997 Recent studies on dendrotoxins and potassium ion channels. General pharmacology 28, 7–12. [PubMed: 9112070]

Hayward J, Sanchez J, Perry A, Huang C, Valle MR, Canals M, Payne RJ, Stone MJ, 2017 Ticks from diverse genera encode chemokine-inhibitory evasin proteins. Journal of Biological Chemistry 292, 15670–15680.

Huang X, Madan A, 1999 CAP3: A DNA sequence assembly program. Genome Res. 9, 868–877. [PubMed: 10508846]

Johnson SA, Ober HK, Adams DC, 2017 Are keystone species effective umbrellas for habitat conservation? A spatially explicit approach. J Nat Conserv 37, 47–55.

Karim S, Ribeiro JM, 2015 An Insight into the sialome of the Lone Star tick, *Amblyomma americanum*, with a glimpse on its time dependent gene expression. PLoS ONE 10, e0131292. [PubMed: 26131772]

Karim S, Singh P, Ribeiro JM, 2011 A deep insight into the sialotranscriptome of the gulf coast tick, *Amblyomma maculatum*. PLoS ONE 6, e28525. [PubMed: 22216098]

Kaufman WR, 1978 Actions of some transmitters and their antagonists on salivary secretion in a tick. Am. J. Physiol. 235, R76–81. [PubMed: 677342]

Kim TK, Tirloni L, Pinto AFM, Moresco J, Yates JR, Vaz ID, Mulenga A, 2016 Ixodes scapularis Tick Saliva Proteins Sequentially Secreted Every 24 h during Blood Feeding. PLoS neglected tropical diseases 10.

Kotsyfakis M, Schwarz A, Erhart J, Ribeiro JM, 2015 Tissue- and time-dependent transcription in *Ixodes ricinus* salivary glands and midguts when blood feeding on the vertebrate host. Scientific reports 5, 9103. [PubMed: 25765539]

Kwong PD, McDonald NQ, Sigler PB, Hendrickson WA, 1995 Structure of beta 2-bungarotoxin: potassium channel binding by Kunitz modules and targeted phospholipase action. Structure 3, 1109–1119. [PubMed: 8590005]

Liu Y, Schmidt B, Maskell DL, 2011 Parallelized short read assembly of large genomes using de Bruijn graphs. BMC bioinformatics 12, 354. [PubMed: 21867511]

Lucchesi KJ, Moczydlowski E, 1991 On the interaction of bovine pancreatic trypsin inhibitor with maxi Ca(2+)-activated K+ channels. A model system for analysis of peptide-induced subconductance states. The Journal of general physiology 97, 1295–1319. [PubMed: 1714938]

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu SM, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam TW, Wang J, 2012 SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. GigaScience 1, 18. [PubMed: 23587118]

Mans BJ, Louw AI, Neitz AW, 2002 Savignygrin, a platelet aggregation inhibitor from the soft tick *Ornithodoros savignyi*, presents the RGD integrin recognition motif on the Kunitz-BPTI fold. The Journal of biological chemistry 277, 21371–21378. [PubMed: 11932256]

Mans BJ, Ribeiro JM, Andersen JF, 2008 Structure, function, and evolution of biogenic aminebinding proteins in soft ticks. The Journal of biological chemistry 283, 18721–18733. [PubMed: 18445596]

Maruyama SR, Anatriello E, Anderson JM, Ribeiro JM, Brandao LG, Valenzuela JG, Ferreira BR, Garcia GR, Szabo MPJ, Patel S, Bishop R, de Miranda-Santos IKF, 2010 The expression of genes coding for distinct types of glycine-rich proteins varies according to the biology of three metastriate ticks, Rhipicephalus (Boophilus) microplus, Rhipicephalus sanguineus and Amblyomma cajennense. BMC genomics 11. [PubMed: 20053297]

Miller JR, Koren S, Sutton G, 2010 Assembly algorithms for next-generation sequencing data. Genomics 95, 315–327. [PubMed: 20211242]

Moreira HNS, Barcelos RM, Vidigal PMP, Klein RC, Montandon CE, Maciel TEF, Carrizo JFA, de Lima PHC, Soares AC, Martins MM, Mafra C, 2017 A deep insight into the whole transcriptome of midguts, ovaries and salivary glands of the *Amblyomma sculptum* tick. Parasitology international 66, 64–73. [PubMed: 27789388]

Narasimhan S, Booth CJ, DePonte K, Wu M-J, Liang X, Mohanty S, Kantor F, Fikrig E, 2019 Host-specific expression of *Ixodes scapularis* salivary genes. Ticks and tick-borne diseases 10, 386–397. [PubMed: 30545615]

Nazareth RA, Tomaz LS, Ortiz-Costa S, Atella GC, Ribeiro JM, Francischetti IM, Monteiro RQ, 2006 Antithrombotic properties of Ixolaris, a potent inhibitor of the extrinsic pathway of the coagulation cascade. Thrombosis and haemostasis 96, 7–13. [PubMed: 16807644]

Nielsen H, Brunak S, von Heijne G, 1999 Machine learning approaches for the prediction of signal peptides and other protein sorting signals. Protein engineering 12, 3–9. [PubMed: 10065704]

Ouimet T, 2004 Neprilysin 2: a novel messenger peptide-inactivating metalloprotease. Protein and peptide letters 11, 479–489. [PubMed: 15544569]

Paesen GC, Adams PL, Harlos K, Nuttall PA, Stuart DI, 1999 Tick histamine-binding proteins: isolation, cloning, and three- dimensional structure. Molecular cell 3, 661–671. [PubMed: 10360182]

Paesen GC, Adams PL, Nuttall PA, Stuart DL, 2000 Tick histamine-binding proteins: lipocalins with a second binding cavity. Biochim. Biophys. Acta 1482, 92–101. [PubMed: 11058751]

Paesen GC, Siebold C, Dallas ML, Peers C, Harlos K, Nuttall PA, Nunn MA, Stuart DI, Esnouf RM, 2009 An ion-channel modulator from the saliva of the brown ear tick has a highly modified Kunitz/BPTI structure. Journal of molecular biology 389, 734–747. [PubMed: 19394347]

Paesen GC, Siebold C, Harlos K, Peacey MF, Nuttall PA, Stuart DI, 2007 A tick protein with a modified Kunitz fold inhibits human tryptase. Journal of molecular biology 368, 1172–1186. [PubMed: 17391695]

Pichu S, Yalcin EB, Ribeiro JM, King RS, Mather TN, 2011 Molecular characterization of novel sulfotransferases from the tick, *Ixodes scapularis*. BMC Biochem 12, 32. [PubMed: 21708020]

Ribeiro JM, Alarcon-Chaidez F, Francischetti IM, Mans BJ, Mather TN, Valenzuela JG, Wikel SK, 2006 An annotated catalog of salivary gland transcripts from *Ixodes scapularis* ticks. Insect Biochem. Mol. Biol. 36, 111–129. [PubMed: 16431279]

Ribeiro JM, Anderson JM, Manoukis NC, Meng Z, Francischetti IM, 2011 A further insight into the sialome of the tropical bont tick, Amblyomma variegatum. BMC genomics 12, 136. [PubMed: 21362191]

Ribeiro JM, Topalis P, Louis C, 2004 AnoXcel: an *Anopheles gambiae* protein database. Insect molecular biology 13, 449–457. [PubMed: 15373803]

Ribeiro JMC, Arca B, 2009 From sialomes to the sialoverse: An insight into the salivary potion of blood feeding insects. Adv Insect Physiol 37, 59–118.

Sangamnatdej S, Paesen GC, Slovak M, Nuttall PA, 2002 A high affinity serotonin- and histamine-binding lipocalin from tick saliva. Insect Mol. Biol. 11, 79–86. [PubMed: 11841505]

Schlehuber S, Skerra A, 2005 Lipocalins in drug discovery: from natural ligand-binding proteins to “anticalins”. Drug Discov Today 10, 23–33. [PubMed: 15676296]

Schwarz A, von Reumont BM, Erhart J, Chagas AC, Ribeiro JM, Kotsyfakis M, 2013 De novo *Ixodes ricinus* salivary gland transcriptome analysis using two next-generation sequencing methodologies. Faseb J 27, 4745–4756. [PubMed: 23964076]

Service, U.S.F.a.W., 1987 Endangered and threatened wildlife and plants; determination of threatened status for the gopher tortoise (Gopherus polyphemus). Federal Register 52, 25376–25380.

Service, U.S.F.a.W., 2015 Endangered and threatened wildlife and plants; review of native species that are candidates for listing as Endangered or Threatened; Annual notice of findings on resubmitted petitions; Annual description of progress on listing actions. Federal Register 80, 80584–80614.

Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I, 2009 ABySS: a parallel assembler for short read sequence data. Genome research 19, 1117–1123. [PubMed: 19251739]

Tirloni L, Reck J, Terra RM, Martins JR, Mulenga A, Sherman NE, Fox JW, Yates JR 3rd, Termignoni C, Pinto AF, da Silva Vaz I Jr., 2014 Proteomic analysis of cattle tick Rhipicephalus (Boophilus) microplus saliva: A comparison between partially and fully engorged females. PLoS ONE 9, e94831. [PubMed: 24762651]

Valdes JJ, Schwarz A, Cabeza de Vaca I, Calvo E, Pedra JH, Guallar V, Kotsyfakis M, 2013 Tryptogalinin is a tick Kunitz serine protease inhibitor with a unique intrinsic disorder. PLoS ONE 8, e62562. [PubMed: 23658744]

Yalcin EB, Stangl H, Pichu S, Mather TN, King RS, 2010 Monoamine neurotransmitters as substrates for novel tick sulfotransferases, homology modeling, molecular docking, and enzyme kinetics. ACS Chem Biol 6, 176–184. [PubMed: 21043483]

Table 1:

Length statistics for raw reads, assembled contigs and extracted coding sequences (CDS)

	Raw reads	Contigs	Extracted CDS (1)
Total number of sequences	138,030	29,991	1,875
Average length	323.73	335.23	443.45
Median size	459	471	501
L50	417	400	393
< 100	31,527	8,977	0
101–200	11,090	2,634	168
201–300	8,112	1,282	410
301–400	13,957	2,121	381
401–500	60,992	10,189	332
501–600	12,305	2,063	209
601–700	28	947	174
701–1000	14	1,254	146
1001–2000	5	510	52
2001–3000	0	13	3
3001–5000	0	1	0
Total	138,030	29,991	1,875

(1)Only CDS larger than 150 nt with 2 or more reads were retained

The CDS derived from assembly of 64,852 reads, or 46.98% of all raw reads

Table 2:

Five broad categories of expressed genes from the sialotranscriptome of *Amblyomma tuberculatum*

Class	Number of CDS	Number of reads	%CDS	% Reads
Secreted	602	37,724	32.107	58.489
Housekeeping	982	25,362	52.373	39.322
Transposons	25	93	1.333	0.144
Rickettsia	1	2	0.053	0.003
Unknown	265	1,317	14.133	2.042
Total	1,875	64,498	100	100

Table 3:

General classification of extracted coding sequences (CDS) from the sialotranscriptome of *Amblyomma tuberculatum*

Class	Number of CDS[1]	Total reads[2]
Putative secreted protein		
Enzymes		
5′-nucleotidase	2	41
Dipeptidyl peptidase (Kininase?)	2	5
Endonuclease	1	27
Sphingomyelinase and lipases	2	12
Metalloproteases	16	498
M13 peptidases - may be for peptide processing	12	855
Protease inhibitor domains		
Serpins	1	23
Kunitz domain containing	42	3388
Kazal domain	2	18
TIL domain	18	1005
Cystatins	5	77
Thyropins	2	23
Basic tail	9	507
Lipocalins	76	17105
Antigen 5	2	8
Immunity related		
Antimicrobial peptides		
Defensins	4	23
Microplusin	6	200
5.3 kDa peptide	1	4
Pathogen recognition domains		
ML domain	3	43
Peptidoglycan recognition	1	15
Ixoderins	2	8
Mucins	20	194
Tick specific proteins		
Glycine rich superfamily	21	594
8.9 kDa protein family	19	4763
Evasins	20	5804
12 kDa family	2	4
Other secreted (many may be fragments of larger proteins with false signal P)	311	2480
Housekeeping proteins		
Cytoskeletal proteins	31	446
Detoxification	16	501
Oxidant metabolism/Detoxification	11	123

Class	Number of CDS[1]	Total reads[2]
Extracellular matrix	11	59
Immunity	8	60
Amino acid metabolism	15	188
Carbohydrate metabolism	20	175
Energy metabolism	75	2212
Intermediary metabolism	1	3
Lipid metabolism	29	238
Nucleotide metabolism	11	101
Nuclear regulation	30	446
Protein export	70	745
Protein modification	64	1058
Proteasome machinery	50	620
Protein synthesis machinery	145	5696
Signal transduction	89	911
Storage	4	225
Transcription factor	16	153
Transcription machinery	75	651
Transporters and channels	46	342
Unknown conserved	116	8206
Unknown conserved membrane protein	49	2203
Unknown product	265	1317
Transposable element	25	93
Rickettsial	1	2
Total	1875	64498

1) Total number of coding sequences found for the indicated class.

2) Total number of illumina reads mapping to the total number of CDS of the indicated class.