Published in final edited form as:

Ticks Tick Borne Dis. 2015 April ; 6(3): 424–434. doi:10.1016/j.ttbdis.2015.03.012.

Identification of 24 h *Ixodes scapularis* immunogenic tick saliva proteins

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Abstract

Ixodes scapularis is arguably the most medically important tick species in the United States. This tick transmits 5 of the 14 human tick-borne disease (TBD) agents in the USA: *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *B. miyamotoi*, *Babesia microti*, and Powassan virus disease. Except for the Powassan virus disease, *I. scapularis*-vectored TBD agents require more than 24 h post attachment to be transmitted. This study describes identification of 24 h immunogenic *I. scapularis* tick saliva proteins, which could provide opportunities to develop strategies to stop tick feeding before transmission of the majority of pathogens. A 24 h fed female *I. scapularis* phage display cDNA expression library was biopanned using rabbit antibodies to 24 h fed *I. scapularis* female tick saliva proteins, subjected to next generation sequencing, *de novo* assembly, and bioinformatic analyses. A total of 182 contigs were assembled, of which ~19% (35/182) are novel and did not show identity to any known proteins in GenBank. The remaining ~81% (147/182) of contigs were provisionally identified based on matches in GenBank including ~18% (27/147) that matched protein sequences previously annotated as hypothetical and putative tick saliva proteins. Others include proteases and protease inhibitors (~3%, 5/147), transporters and/or ligand binding proteins (~6%, 9/147), immunogenic tick saliva housekeeping enzyme-like (17%, 25/147), ribosomal protein-like (~31%, 46/147), and those classified as miscellaneous (~24%, 35/147). Notable among the miscellaneous class include antimicrobial peptides (microplusin and ricinusin), myosin-like proteins that have been previously found in tick saliva, and heat shock tick saliva protein. Data in this study provides the foundation for in-depth analysis of *I. scapularis* feeding during the first 24 h, before the majority of TBD agents can be transmitted.

Keywords

Ixodes scapularis; Biopanning; 24 h tick saliva immuno-transcriptome; Tick vaccine targets

Introduction

Ticks are obligate blood-feeding ectoparasites that vector viruses, bacteria, and protozoa. Inflicting many negative consequences on their hosts, ticks are considered to be one of the most medically and veterinary important arthropods and rank second only to mosquitoes in terms of disease transmission (Sonenshine, 1993). There are at least 53 tick species frequently found to parasitize animals and humans (Dantas-Torres et al., 2012). It is estimated that ticks cause between 13.9 and 18.7 billion US dollars in damages worldwide annually (de Castro, 1997). With the habitat ranges of ticks quickly expanding due to the movement of humans and migration of animals as well as an increase in suitable environments due to climate changing, it is plausible to suggest that total cost of damages will only increase (Madder et al., 2012).

In the past, ticks and tick-borne diseases were solely associated with veterinary medicine, however from the discovery of the causative agent of Lyme disease in the 1980s, the importance of ticks to human health was further emphasized (Benach et al., 1983; Burgdorfer et al., 1982, 1983). The Centers for Disease Control and Prevention lists 14 human disease agents transmitted by ticks in the United States. Of those 14, the deer tick, *Ixodes scapularis* transmits causative agents for four diseases: human granulocytic anaplasmosis, human babesiosis, Lyme disease and Powassan virus disease. Specifically, *I. scapularis* transmits lineage II of the Powassan virus disease, the deer tick virus (Dupuis II et al., 2013). Lyme borreliosis, the most prevalent vector-borne disease in the northern hemisphere, was originally estimated to affect 30,000 however now it is thought to affect 300,000 people per year in the United States (CDC, 2013). Recently it was discovered that *Borrelia miyamotoi* is transmitted by *I. scapularis* and linked to human illness as seen in Russia and the U.S. (Gugliotta et al., 2013; Krause et al., 2013; Platonov et al., 2011; Scoles et al., 2001). Another concerning factor to human public health, *I. scapularis* has demonstrated the ability to co-transmit pathogens and co-infect hosts with *Borrelia burgdorferi* and *Anaplasma phagocytophilum* (Levin and Fish, 2000).

Large-scale tick control by acaricide treatment represents the main way ticks are eliminated from hosts. However, this method is only a short-term solution as tick resistance to these chemicals is quickly emerging. The development of new acaricides is time consuming and expensive. Acaricides also contaminate the environment and animal feed as well as posing as health risks to humans (Graf et al., 2004). The concept of immunizations against ticks appears to be a practical solution and has been demonstrated with anti-tick vaccines against *Rhipicephalus microplus* (de la Fuente et al., 2007; Olds et al., 2013; Willadsen, 2004). However, this vaccine remains the only commercially available vaccine against ticks. With new molecular biology technologies and bioinformatic analyses advances, the process of identifying suitable universal tick antigenic targets has become more promising.

Tick vaccine development is centered on two main strategies including targeting “exposed” or “concealed” antigens. “Exposed” antigens are secreted in tick saliva and are exposed to the host at the feeding site whereas concealed antigens are hidden from the host immune system therefore failing to trigger an immune response. We propose that immunizing hosts with exposed antigens would eliminate the need for booster vaccinations because the host’s

immune system would be naturally primed by repetitive tick feeding. “Concealed” antigens would require repeated immunizations to maintain elevated antibody titers, making their use impractical. Except for viruses such as the Powassan virus disease that are transmitted within minutes of tick attaching onto host skin, the majority of TBD need more than 24 h after attachment to be successfully transmitted to host (Ebel and Kramer, 2004; McQuiston et al., 2000). For instance, *B. burgdorferi*, the causative agent of Lyme disease, invades the tick’s salivary glands to enter the host 48 h post-attachment (De Silva & Fikrig, 1995). Likewise, studies have also shown that *A. phagocytophilum* transmission occurs between 24 and 48 h after tick attachment, while *Babesia microti* migrate to the salivary glands after 2–3 days of tick attachment and multiply to 10,000 sporozoites (des Vignes et al., 2001; Hodzic et al., 1998; Katavolos et al., 1998; Kjemtrup and Conrad, 2000). The purpose of this study was to identify *I. scapularis* immunogenic tick saliva proteins that are secreted into the host during the first 24 h after tick attachment onto host skin. Immunization against 24 h *I. scapularis* tick saliva proteins could impede the tick feeding process before the majority of pathogens would be transmitted to the host.

Materials and methods

Ticks

For this study, unfed *I. scapularis* ticks were purchased from the tick laboratory at Oklahoma State University. In our lab, ticks were maintained in tick chambers at room temperature, >85% relative humidity and fed on New Zealand white rabbits according to the animal use protocol approved by Texas A&M University IACUC. Female *I. scapularis* ticks were mated prior to feeding. Female ticks were presumed mated when found paired up with male ticks. Ticks were restricted to feed on the tops of rabbits’ ears using an orthopedic stockinet tick containment device adhered onto rabbit skin with Kamar Adhesive (Kamar Products Inc., Zionsville, IN). Female ticks were manually detached with forceps at the 24 h time point. Ticks were then washed in diethylpyrocarbonate (DEPC) treated water to remove pieces of rabbit skin and hair. The tick mouthparts were also inspected for any remaining rabbit skin tissue. Following cleaning, ticks were air dried on paper towels, pooled in groups of 8–10, chopped up using scissors and subsequently disrupted using a sonic dismembrator model 100 (Fischer Scientific, Itasca, IL) in 1 mL of TRIzol (Life Technologies, Carlsbad, CA, USA). Finally, ticks were stored at –80 °C until total RNA extraction. Whole tick RNA was used in this experiment to ensure that all salivary proteins were identified, including those translated elsewhere and subsequently transported to the salivary gland for tick feeding.

Preparing of 24 h *Ixodes scapularis* cDNA expression phage display library

Tick messenger RNA extraction, cDNA synthesis, phage display libraries preparation, and phage selection through biopanning protocol were performed as previously described (Radulovi et al., 2014). Total RNA was extracted by using TRIzol reagent according to manufacturer’s protocol and mRNA was isolated from total RNA with Straight A’sTM mRNA isolation system (Novagen, Madison, WI, USA). cDNA synthesis was performed using OrientExpressTM Oligo(dT) cDNA Synthesis Kit (Novagen, Madison, WI, USA) starting from a total of 4 µg of mRNA. The quality of cDNA was checked by amplification

of actin gene sequence as previously described, followed by preparation of 24 h fed female *I. scapularis* parent phage display expression cDNA library using T7 Select System (Novagen, Madison, WI, USA) (Radulovi et al., 2014). In order to verify the quality of the phage display library, the individual lengths of the cloned cDNA sequences were checked by PCR amplifications using T7 “up” and “down” primers provided in T7 Select System kit. *Escherichia coli* BLT5403 strain grown in M9TB liquid and LB solid media with carbenicillin (final concentration 50 µg/mL) was used as host cells for T7 library phages. Plate lysate amplification protocol was used for amplification of parent library. Selection of phages containing cloned cDNA sequence encoding antigenic tick proteins secreted through saliva during first 24 h post attachment was performed with the biopanning protocol using antibodies to 24 h fed *I. scapularis* females (Radulovi et al., 2014).

Production of rabbit antibodies to tick saliva proteins was previously described (Mulenga et al., 2013). Briefly, pre-mated female ticks were placed on rabbit ears to feed for 24 h. A total of 15 specimens were fed per ear. After 24 h ticks were manually removed and rabbits were re-infested with unfed pre-mated females. A 24 h feeding cycle was repeated 4 times within one week, followed by a one-week break. The entire cycle was repeated four times before rabbit exsanguination and serum collection. The antibody response to tick saliva protein was verified by western blot analysis using proteins extracted from unfed ticks as well as from ticks at different stages of engorgement. As a negative control we used serum obtained from rabbits before tick feeding. The biopanned library was established after four rounds of biopanning. As a control for nonspecific binding of phages to produced antibodies, a control biopanned library was established using rabbit serum obtained prior to tick feeding as a source of antibodies.

Next generation sequencing, de novo assembly, and sequence analysis

24 h fed female and control libraries were subjected to next generation sequencing as previously described (Radulovi et al., 2014). Illumina HiSeq2000 system with the following parameters was used: paired-end sequencing, read length of 100 bp, and 800,000 reads per sample. Sequence reads trimmed at the default 0.05 limit value were *de novo* assembled using CLC Genomics Workbench software version 6.0.2 (CLC Bio-Qiagen, Cambridge, MA, USA). All other parameters were set to the default settings. Contigs that were found in the control biopanned library were eliminated from further analysis due to non-specificity. Annotation of assembled contigs was performed by using BlastX homology search during June 2014. There is a possibility that findings may change as new sequences are deposited in GenBank. Lastly, signal peptide prediction was performed on the SignalP 4.1 server (<http://www.cbs.dtu.dk/services/SignalP/>). For contigs that encoded partial protein sequences, exact matches in GenBank were used.

Results and discussion

In our study, a total of 3.7 million reads were obtained from the next generation sequencing of *I. scapularis* 24 h fed female parent phage display library, yielding 9134 contigs after *de novo* assembly (unpublished). Immuno-screening of the parent library with antibodies to 24 h *I. scapularis* tick saliva proteins yielded a cDNA library that was enriched for 24 h *I. scapularis* tick saliva immunogenic protein-encoding cDNAs. Illumina sequencing of this

library yielded 720,000 reads and successfully assembled 182 contigs. Specificity of this immuno-screening method was confirmed with sequencing results of the control biopanned library showing only the presence of phage-originated sequences (results not shown). Of the 182 contigs, 35 did not show amino acid identities to annotated proteins in GenBank. The remaining 147 contigs were provisionally annotated on the basis of their identity to protein sequences in GenBank and include those showing identity to hypothetical tick proteins, proteases, protease inhibitors, binding proteins and transporters, enzymes, ribosomal proteins, and proteins of miscellaneous functions as summarized in Fig. 1. Of the 147 proteins, signal peptides were confirmed for 17 (marked with an asterisk sign, Tables 1–5). This analysis is partial given that the majority of sequences in this study and their matches in GenBank are partial protein sequences. We anticipate that once full sequences are obtained the majority of proteins in this study will have signal peptides. These preliminary results aim to provide a foundation for further investigation of tick saliva proteins and could be further supported by the recombinant expression of select proteins and their functional characterization. However, this is outside the aims of this particular study. For clarity, the remaining part of this results and discussion section is divided based on provisionally identified protein groups.

I. scapularis tick saliva proteins of unknown function

Table 1 lists 27 contig sequences that show similarity to tick protein sequences of unknown function in the GenBank database. It is notable that of the 27 contigs, 26 show similarity exclusively to *I. scapularis* tick proteins. This could be explained by the fact that the *I. scapularis* genome has been sequenced. The lone sequence, GBSR01000175, shows identity to *Haemaphysalis qinghaiensis* (ABQ96857). It is interesting to note that this protein was also identified from an immune-screen (direct submission). Although tick saliva proteins in Table 1 are of unknown function, they could represent key immunogenic tick saliva proteins that control important physiological functions during the early phase of tick attachment. It is also important to note that these proteins do not have any identity to non-tick proteins therefore removing the possibility that immune responses against these proteins could cross-react with the host. In most transcriptome studies, the majority of sequences are of unknown function. This begins the process of functional analysis for the 27 proteins in Table 1. Data here shows that these proteins are immunogenic and are injected into the host during the first 24 h of *I. scapularis* tick feeding.

I. scapularis tick saliva immunogenic proteases and protease inhibitors

Central to numerous biological processes, proteases in ticks are specifically linked to embryo development, blood meal feeding, and blood meal digestion in the midgut (Fagotto, 1995; Franta et al., 2010; Mendiola et al., 1996; Pohl et al., 2008). Silencing of select tick proteases has confirmed their importance to tick function and negatively affected tick feeding and reproduction (Karim et al., 2005; Miyoshi et al., 2004). Similar results were demonstrated by feeding ticks on animals immunized with recombinant proteases (Leal et al., 2006). Table 2A lists GBSR01000043 and GBSR01000097 provisionally identified as legumain and cathepsin B-like cysteine proteases respectively. This finding indicates that cysteine-like proteases are present in *I. scapularis* tick saliva at the beginning of tick feeding.

Commonly found in the Phylum Arthropoda, legumain-like proteases or asparaginyl endopeptidases, are vacuolar processing enzymes that specifically hydrolyze asparaginyl bonds. Legumain-like proteases have been found among secreted enzymes in the mite secretome (Schicht et al., 2013; Xu et al., 2014). In regards to ticks, legumain-like proteases have been found in *Rhipicephalus microplus*, *Amblyomma maculatum*, *Haemaphysalis longicornis*, and *I. ricinus* (Abdul Alim et al., 2007; Karim et al., 2011; Kongsuwan et al., 2010; Sojka et al., 2007). The primary cleavage of hemoglobin in *I. ricinus* midgut occurs with the assistance of legumain (Horn et al., 2009). Legumain-like proteases appear to be common among other parasites such as *Schistosoma mansoni* and *Trichomonas vaginalis*, which is understandable due to their shared goal of modulating their host's immune system while acquiring nutrients (Dalton et al., 1997; Rendón-Gandarilla et al., 2013).

GBSR01000097 showed similarity to cathepsin B in several hard ticks species and one soft tick species. This protease is hypothesized to be active at the tick-host interface. Ubiquitously expressed in mammals, cathepsin B is involved in protein turnover and degradation, antigen presentation and processing, proenzyme activation, hormone maturation, and extracellular matrix remodeling (Brömme and Wilson, 2011; Honey and Rudensky, 2003; Lecaille et al., 2002; Roberts, 2005). Ticks are pool feeders that successfully feed by disrupting host tissue and ingesting blood that accumulates at the feeding site, triggering host tissue repair response (Lavoipierre, 1965). Cathepsin B cysteine proteases could interrupt tissue repair response by eliminating cellular mediators of inflammation and degradation of the extracellular matrix. In addition to host blood meal digestion, cysteine proteases have been shown to play a role in disease transmission in ticks (Tsuji et al., 2008). Aside from ticks, other parasites such as *Spirometra manosi* and *Euclinostomum heterostomum* have cysteine proteases as a major element of their excretory-secretory immuno-proteome (Dan Dan et al., 2013; Shareef and Abidi, 2014). Other parasites like *Trypanosoma b. brucei*, appear to need cathepsin B for survival due to its role in breaking down endocytosed transferrin for iron acquisition (Cotton et al., 2014). RNAi studies of cathepsin B led to clearance of these parasites from the blood stream in mice (Abdulla et al., 2008). It is notable that findings in our study are consistent with findings in *A. americanum* where both legumain and cathepsin B-like proteases were found among 24–48 h immunogenic tick saliva proteins (Radulovic et al., 2014). Similarly, cysteine proteases were found in *R. microplus* proteome (Tirloni et al., 2014).

I. scapularis protease inhibitors

Secretion of protease inhibitors in tick saliva has been documented in several tick genera and species (Chalaise et al., 2011; Imamura et al., 2006; Prevot et al., 2006). In parasite–host interactions, it is hypothesized that parasites including ticks, use protease inhibitors to overcome host defenses and supports the presence of secreted protease inhibitors in *I. scapularis* tick saliva (Armstrong, 2006). Table 2B lists GBSR01000015 and GBSR01000069 as respectively similar to *Dermacentor variabilis* and *D. andersoni* Kunitz type serine protease inhibitors, as well as GBSR01000156, which provisionally identified as a tick carboxypeptidase inhibitor (TCI). Kunitz-domain serine protease inhibitor transcripts are one of the most abundant protein families in tick salivary gland transcriptomes (Schwarz et al., 2014). In hard ticks, Kunitz-BPTI (bovine pancreatic trypsin inhibitor) proteins

function as regulators of host blood, angiogenesis, and wound healing (Islam et al., 2009; Paesen et al., 2009). TCI was speculated to be secreted during feeding and demonstrated a fibrinolysis function in *R. bursa* (Arolas et al., 2005). Consistent with data here, TCI was also found among 24–48 h *A. americanum* immunogenic tick saliva proteins (Radulović et al., 2014). Beyond this, the role(s) of TCI in tick feeding is unknown.

I. *scapularis* tick saliva transporters and/or ligand binding proteins

Table 3 lists nine contigs that were provisionally identified as *I. scapularis* tick saliva transporters and/or binding proteins that are thought to be involved in molecular interactions at the tick–host interface. Most notable are ferritin and hemelipoglycoprotein, which are important to heme metabolism.

Ferritin—GBSR01000061 was provisionally identified as ferritin, which plays a vital role in iron homeostasis in every organism. Iron is a necessary but potentially toxic element due to its ability to catalyze the propagation of reactive oxygen species and generate highly reactive molecules that can lead to damage of cellular macromolecules, cell death and tissue injury (Papanikolaou and Pantopoulos, 2005; Wang and Pantopoulos, 2011). During feeding, ticks can ingest 200–600 times their unfed body weight, exposing them to massive quantities of iron, requiring ticks to have a strategy to maintain iron homeostasis (Rajput et al., 2006). It is important to note that the ferritin being discussed here is injected into the host during the first 24 h of *I. scapularis* feeding, during the period when blood meal uptake is minimal (Sonenshine, 1993). From this perspective, it is plausible that the ferritin reported here may not function to eliminate iron from the tick. Ferritins have also been implicated in immune response and oxidative stress (Ong et al., 2006; Orino et al., 2001). In addition to the role of storage in mammals, insect ferritins have shown to assist in iron transport (Pham and Winzerling, 2010). Ferritin has been previously identified in several tick species including *Ornithodoros moubata*, *Ixodes ricinus*, *H. longicornis* and *R. microplus* (Galay et al., 2014; Hajdusek et al., 2010; Kopáček et al., 2003). Silencing of ferritin has demonstrated negative effects on tick feeding and tick reproduction (Galay et al., 2013, 2014). Immunization trials against ferritin where tick infestations were reduced indicate that tick ferritins are essential for successful feeding (Hajdusek et al., 2010).

Two subtypes of ferritin have been described in ticks, ferritin 1 and ferritin 2. Intracellular ferritin 1, a common heavy chain type ferritin, is indicated to serve as the main iron storage ferritin whereas extracellular ferritin 2 is primarily expressed in the gut and acts as an iron-transporter (Hajdusek et al., 2009; Kopáček et al., 2003). In accordance with our results, data from *A. americanum* saliva also suggests the presence of a third ferritin (Radulović et al., 2014). GBSR01000061 shows 100% amino acid identity to ferritin 1 but also shares 51% identity with the ferritin 3 (NP 572854) homologue in *Drosophila melanogaster*. GBSR01000061 also contains high conservation between hard and soft tick species which is supported by previous sequence analyses which display conservation of ferritin in eight species of hard ticks (Xu et al., 2004).

Hemelipoglycoprotein—Hemelipoproteins (HeLp) bind heme as well as serving as a transport protein for cholesterol and various types of fatty acids (Maya-Monteiro et al.,

2004). HeLp has been identified in other tick species' saliva, indicating that it could have a necessary role in tick feeding and survival (Díaz-Martín et al., 2013; Madden et al., 2003; Maya-Monteiro et al., 2000). Tirloni et al. (2014) speculated that HeLp could decrease inflammation by removing heme molecules that are released during hemolysis and accumulate at the feeding site (Graça-Souza et al., 2002; Tirloni et al., 2014). The pro-inflammatory and oxidative properties of heme could make its presence unfavorable to successful tick feeding. Experimental results have also shown that HeLp can reduce oxidative damages from heme toxicity in addition to transporting heme to tick tissues (Bentinger et al., 2007; Graça-Souza et al., 2006).

I. scapularis tick saliva immunogenic enzymes

Table 4 lists 25 immunogenic enzymes that were identified in *I. scapularis* tick saliva. GBSR01000011 shows identity to peptidyl-prolyl cis-trans isomerase which is also known as cyclophilin A (CypA), a ubiquitously expressed intracellular protein that promotes protein folding (Fischer and Schmid, 1990). CypA was first recognized as a cell receptor for the potent immunosuppressive drug cyclosporin A (CsA) which upon binding, reduces T cell activation (Liu et al., 1992). CypA has also demonstrated to be a powerful regulator of Ca^{2+} in activated platelets (Elvers et al., 2012). Furthermore, CypA may play a role in the tick immune response to pathogens (Maeda et al., 2013).

Oxidative stress is induced in response to injury from tick feeding therefore ticks need biological machinery to combat the resulting reactive oxidative species (ROS) (Rojkind et al., 2002). In Table 4, 5 (GBSR01000042, GBSR01000095, GBSR01000133, GBSR01000159, and GBSR01000181) of 25 enzymes are related to coping with oxidative stress, indicating that ticks indeed do have a mechanism to combat ROS. Superoxide dismutases are in the family of antioxidant enzymes that remove ROS from the cellular environment to prevent oxidative stress (Marklund, 1985). Evidence indicates that exogenous superoxide dismutases could act as a method to treat neutrophil-mediated inflammation with an increased focus on the therapeutic application of superoxide dismutase for inflammatory diseases (Yasui and Baba, 2006). GBSR01000042 has similarity to superoxide dismutase that sequesters the metals copper and zinc. This is interesting because copper accumulates at sites of inflammation and accelerates the synthesis and activity of superoxide dismutase Cu-Zn (Beveridge et al., 1985; Harris, 1992; Milanino et al., 1993). Zinc also has shown to increase wound healing, therefore if the tick has superoxide dismutases in its saliva, potentially the binding of zinc could counteract wound healing (Lansdown et al., 2007). This enzyme in tick saliva could represent a method to overcome the host's response to oxidative stress and injury induced by the wound the tick creates while feeding.

GBSR01000133 shows identity to glutathione S-transferase (GST), proteins that have been characterized in many organisms and show to play important roles in oxidative stress response, xeno-biotic and endogenous compounds metabolism (Sheehan et al., 2001). GSTs have been previously analyzed in ticks and shown to play a role in oxidant defenses in eggs (Freitas et al., 2007). GBSR01000133 appears to have similar identity in several tick species, implying that GSTs serve a crucial function for tick survival. GBSR01000159 was

identified with similar anti-oxidant functions to GST, whereas GBSR01000181 showed unique antioxidant defense towards oxidation of proteins and lipids (Bentinger et al., 2007; Beyer, 1992; Imai and Nakagawa, 2003).

Interestingly, while GBSR01000014 identifies with *I. scapularis*, GBSR01000135 shows provisional identity to acetylcholinesterase in *R. sanguineus*. Acetylcholinesterases have also been shown to be secreted in other blood feeding arthropods such as *Cimex lectularius* and *Triatoma matogrossensis* saliva (Ribeiro et al., 2012). Acetylcholinesterase in *R. microplus* was hypothesized to affect wound healing and immune response by decreasing acetylcholine in tissue (Temeyer et al., 2013). Finally, in addition to anti-oxidant enzymes, two contigs, GBSR01000047 and GBSR01000103, identified as E3 ubiquitin ligase which was previously characterized in *I. scapularis* and shows ability to restrict bacterial colonization in the tick (Severo et al., 2013).

Ribosomal and other proteins of miscellaneous function

Table 5A lists ribosomal proteins that make up ~31% of the contigs identified in *I. scapularis* tick saliva. High numbers of housekeeping proteins such as ribosomes have previously been reported in the tick saliva proteomes of *I. ricinus*, *R. microplus*, *D. andersoni*, and *A. americanum* (Chmelar et al., 2008; Mudenda et al., 2014; Radulovic et al., 2014; Tirloni et al., 2014). The high prevalence of ribosomal proteins is intriguing and could indicate that they serve as more than simple housekeeping functions in ticks. Additional functions of ribosomal proteins have become evident, including anti-inflammatory activity (Abreu-Velez et al., 2013; Lv et al., 2013; Poddar et al., 2013). GBSR01000004 and GBSR01000006, respectively have 77% and 71% identity to two separate ribosomal proteins in *Ailuropoda melanoleuca* which have each been characterized and shown anti-cancer function by inhibiting cell growth and proliferation activity (Li et al., 2014; Su et al., 2012). A vaccine based on combinations of ribosomal proteins have shown success in against *Leishmania* parasite lesions, supporting the idea of a ribosomal vaccine against ticks (Ramirez et al., 2014). We would like to caution the limitation of data in this study presented here. Further empirical data will be needed to verify if putative functions of ribosomal proteins are indeed involved in tick feeding. Another interesting study would be secondary structural analysis to investigate if folding patterns of putative ribosomal proteins are similar to mammals, which could be a strong indication for functional conservation.

Notable miscellaneous function proteins from Table 5B include GBSR01000038 and GBSR01000105 which showed similarity to defensin proteins which are antimicrobial agents of innate immunity (Ganz, 2003). Defensins have been previously identified in hard ticks including *I. scapularis* (Fogaça et al., 2004; Hynes et al., 2005; Johns et al., 2001; Saito et al., 2009). Finding defensins in *I. scapularis* saliva correlates with the analysis of *A. americanum* immune-proteome (Radulovic et al., 2014). Another aspect of successful feeding focuses on preventing microbial colonization at the feeding site. Defensin has been attributed to clearing *Borrelia* from *D. marginatus* ticks, demonstrating an evident borrelial activity (Chrudimská et al., 2014). In addition to defensin, heat shock proteins could also provide protection against bacterial colonization as well as inflammation (Pockley, 2003). GBSR01000070 and GBSR01000104 identify with heat shock proteins,

with GBSR01000070 showing conservation in several hard and soft tick species. Finally, it is important to note that GBSR0100002 and GBSR01000150 show similarity to myosin alkali chain proteins which have previously been identified in salivary glands as immunogenic (Francischetti et al., 2005). This is proof of concept that proteins identified from this study can be identified as immunogenic proteins.

Conclusion

This study expands on previous studies that identified 24 h *I. scapularis* immunogenic tick saliva proteins (Narasimhan et al., 2007a) and provides us with opportunities to identify valuable candidates for the development of anti-tick vaccines that may affect early tick feeding events including pathogen transmission. Indeed Narasimhan et al. (2007a) demonstrated that immunity against *I. scapularis* saliva proteins affected *B. burgdorferi* transmission. The *I. scapularis* genome sequence project and several transcriptomes have provided sequence resources that allowed for identification of a large number of proteins in this study (Hill and Wikel, 2005; Ribeiro et al., 2006; Valenzuela et al., 2002). Given that we screened whole tick libraries, it is possible that some of the proteins identified in our study have dual roles in the midgut as well as the salivary glands but even so, this could make them increasingly more valuable to the tick during feeding.

Other studies have identified *I. scapularis* nymph tick saliva proteins (Das et al., 2001; Narasimhan et al., 2007a; Schuijt et al., 2011). Presumably these studies identified fewer numbers of tick saliva proteins because of differences in experimental approaches. Our study and those of others used tick immune serum in animals that were repeatedly infested. However, the key difference is that in our study we raised antibodies specifically to the 24 h feeding time point, while other previous studies used antibodies to replete fed tick saliva proteins. In this study, our goal was to select antigens injected into the host at the start of feeding and not throughout feeding. We believe that this could explain why we observed minimal overlap in types of identified proteins. It is also possible that the apparent differences in types of proteins identified could be explained by the fact that adult ticks and nymphs may secrete different types of proteins.

Although there is minimal overlap between our study and other *I. scapularis* tick saliva protein studies, there are similarities that could point out proteins used by both adults and nymphs to feed. For instance, glutathione S-transferase identified in Das et al. (2001) as well as in our study, was shown to be important in *B. burgdorferi* transmission (Narasimhan et al., 2007b). It is also interesting to note that in our study, we identified protease inhibitors similar to the profiles of immuno-dominant proteins that bound antibodies to nymph tick saliva proteins (Das et al., 2001). This study contributes to the identification of immunogenic adult *I. scapularis* tick saliva proteins and provides the foundation for in depth analyses on the role(s) of 24 h saliva proteins in tick feeding. Nymphs play a major role in the transmission of tick-borne disease pathogens to humans therefore proteins in this study that were also found in nymphs could represent some of the targets that warrant further investigation.

Acknowledgments

This work was supported by the National Institute of Allergy and Infectious Diseases/National Institutes of Health (NIAID/NIH) grants (AI081093 and AI093858) to AM.

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Fig. 1.

Classifications based on putative function of provisionally identified 24 h *Ixodes scapularis* immunogenic tick saliva proteins: a total of 182 *de novo* assembled contigs were searched against tick sequence entries in GenBank for provisional identification. N-TSP = novel *I. scapularis* tick saliva proteins which did not match protein sequences in GenBank; UF-TSP = *I. scapularis* tick saliva proteins that matched previously annotated hypothetical or putative tick saliva proteins; PPI = proteases and protease inhibitors; TL = transporters and ligand binding proteins; IHE = immunogenic house keeping-like enzymes; RP = ribosomal proteins; MF = proteins of miscellaneous function.

Table 1*I. scapularis* tick saliva proteins of unknown function.

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000001 ^a	Secreted salivary gland peptide, putative <i>Ixodes scapularis</i>	XP_002410662	6e-55
GBSR01000006 ^a	Hypothetical protein IScW_ISCW000821 <i>Ixodes scapularis</i>	XP_002400584	1e-24
GBSR01000026	Hypothetical protein IScW_ISCW018974 <i>Ixodes scapularis</i>	XP_002434901	1e-23
GBSR01000031	Hypothetical protein IScW_ISCW011437 <i>Ixodes scapularis</i>	XP_002412053	3e-09
GBSR01000036	Conserved hypothetical protein <i>Ixodes scapularis</i>	XP_002414210	1e-62
GBSR01000050	Conserved hypothetical protein <i>Ixodes scapularis</i>	XP_002416605	3e-04
GBSR01000052	Hypothetical protein IScW_ISCW017886 <i>Ixodes scapularis</i>	XP_002406616	8e-33
GBSR01000064	Hypothetical protein IScW_ISCW010425 <i>Ixodes scapularis</i>	XP_002399967	9e-08
GBSR01000078	Hypothetical protein IScW_ISCW000670 <i>Ixodes scapularis</i>	XP_002408865	1e-37
GBSR01000087 ^a	Putative salivary secreted protein <i>Ixodes scapularis</i>	AAV66696	8e-17
GBSR01000088	Conserved hypothetical protein <i>Ixodes scapularis</i>	XP_002405938	9e-105
GBSR01000092	Fed tick salivary protein 5 <i>Ixodes scapularis</i>	AAV63539	6e-37
GBSR01000096	Hypothetical protein IScW_ISCW018708 <i>Ixodes scapularis</i>	XP_002434355	3e-05
GBSR01000098 ^a	Secreted salivary gland peptide, putative <i>Ixodes scapularis</i>	XP_002436188	1e-07
GBSR01000101	Conserved hypothetical protein <i>Ixodes scapularis</i>	XP_002409473	4e-51
GBSR01000102 ^a	Hypothetical protein IScW_ISCW021555 <i>Ixodes scapularis</i>	XP_002411977	2e-10
GBSR01000116	Conserved hypothetical protein <i>Ixodes scapularis</i>	XP_002405372	5e-22
GBSR01000117	Hypothetical protein IScW_ISCW005479 <i>Ixodes scapularis</i>	XP_002435815	2e-27
GBSR01000119 ^a	Putative secreted salivary protein <i>Ixodes scapularis</i>	AAV66634	6e-63
GBSR01000129	Hypothetical protein IScW_ISCW008326 <i>Ixodes scapularis</i>	XP_002400389	1e-08
GBSR01000132	Hypothetical protein IScW_ISCW019938 <i>Ixodes scapularis</i>	XP_002400845	0.21
GBSR01000138	Hypothetical protein IScW_ISCW021147 <i>Ixodes scapularis</i>	XP_002405418	2e-69
GBSR01000146	Hypothetical protein IScW_ISCW021355 <i>Ixodes scapularis</i>	XP_002411707	2e-04
GBSR01000148	Hypothetical protein IScW_ISCW001796 <i>Ixodes scapularis</i>	XP_002400162	1e-47
GBSR01000167	Conserved hypothetical protein <i>Ixodes scapularis</i>	XP_002407622	5e-78
GBSR01000174	Secreted salivary gland peptide, putative <i>Ixodes scapularis</i>	XP_002402427	1e-89
GBSR01000175	Unknown <i>Haemaphysalis qinghaiensis</i>	ABQ96857	1e-11

^aSignal peptide confirmed.

Table 2A*I. scapularis* tick saliva immunogenic proteases.

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000043 ^a	Legumain-like protease precursor <i>Ixodes ricinus</i>	AAS94231	6e-35
GBSR01000097	Cathepsin B endopeptidase, putative <i>Ixodes scapularis</i>	XP_002404474	6e-112
	Cathepsin B-like cysteine protease form 1 <i>Ixodes ricinus</i>	ABO26563	7e-108
	Putative cathepsin B-like cysteine protease form 1 <i>Dermacentor variabilis</i>	ACF35525	4e-84
	Longipain <i>Haemaphysalis longicornis</i>	BAF43801	2e-61
	Midgut cysteine proteinase 1 <i>Rhipicephalus appendiculatus</i>	AAO60044	6e-65
	Cathepsin B-like protein <i>Argas monolakensis</i>	ABI52787	7e-30

^aSignal peptide confirmed.

Table 2B*I. scapularis* tick saliva immunogenic protease inhibitors.

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000015 ^a	Dathoxin-4 <i>Dermacentor andersoni</i>	ABZ89564	7e-14
	Putative Kunitz-BPTI protein <i>Dermacentor variabilis</i>	ACF35511	7e-14
GBSR01000069 ^a	Serine proteinase inhibitor, putative <i>Ixodes scapularis</i>	XP_002434145	2e-52
GBSR01000156	Carboxypeptidase inhibitor precursor, putative <i>Ixodes scapularis</i>	XP_002408813	6e-52

^aSignal peptide confirmed.

Table 3*I. scapularis* tick saliva transporters and/or ligand binding proteins.

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000044	Cationic amino acid transporter, putative <i>Ixodes scapularis</i>	XP_002404803	0.12
GBSR01000049	GTP-binding protein CRFG/NOG1, putative <i>Ixodes scapularis</i>	XP_002407133	4e-107
GBSR01000060 ^a	Peritrophic membrane chitin binding protein, putative <i>Ixodes scapularis</i>	XP_002405106	2e-40
GBSR01000061	Ferritin, putative <i>Ixodes scapularis</i>	XP_002414546	8e-28
	Ferritin <i>Ixodes ricinus</i>	AAC19131	2e-26
	Ferritin heavy chain-like protein <i>Dermacentor andersoni</i>	AAR21568	3e-24
	Ferritin <i>Rhipicephalus sanguineus</i>	AAQ54715	3e-24
	Ferritin <i>Amblyomma americanum</i>	AAQ54708	3e-24
	Ferritin <i>Ornithodoros moubata</i>	AAC19132	9e-24
	Ferritin <i>Hyalomma asiaticum asiaticum</i>	AAS66655	1e-23
	Ferritin heavy-chain <i>Argas monolakensis</i>	ABI52633	6e-23
GBSR01000071 ^a	GGY domain-containing protein, putative <i>Ixodes scapularis</i>	XP_002411974	3e-09
GBSR01000081	Oligopeptide transporter, putative <i>Ixodes scapularis</i>	XP_002414468	0.70
GBSR01000142 ^a	Hemelipoglycoprotein precursor, putative <i>Ixodes scapularis</i>	XP_002415017	1e-26
GBSR01000173	NIK- and IKKB-binding protein, putative <i>Ixodes scapularis</i>	XP_002409303	0.21
GBSR01000180	Apoptosis-promoting RNA-binding protein TIA-1/TIAR, putative <i>Ixodes scapularis</i>	XP_002414860	3e-41

^aSignal peptide confirmed.

Table 4

I. scapularis tick saliva immunogenic enzymes.

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000009	DNA-directed RNA polymerase 2A, putative <i>Ixodes scapularis</i>	XP_002402558	3e-31
GBSR01000011 ^a	Peptidyl-prolyl cis-trans isomerase, putative <i>Ixodes scapularis</i>	XP_002411993	2e-102
	Cyclophilin B <i>Haemaphysalis longicornis</i>	BAG41814	3e-90
GBSR01000014	Acetylcholinesterase, putative <i>Ixodes scapularis</i>	XP_002413109	1.3
GBSR01000020	ATP-dependent RNA helicase, putative <i>Ixodes scapularis</i>	XP_002435440	6e-37
GBSR01000025	FYVE finger-containing phosphoinositide kinase, fyv1, putative <i>Ixodes scapularis</i>	XP_002400440	0.18
GBSR01000027	F1F0 ATP-synthase subunit Cf6, putative <i>Ixodes scapularis</i>	XP_002399676	1e-40
GBSR01000042 ^a	Superoxide dismutase Cu-Zn, putative <i>Ixodes scapularis</i>	XP_002414082	1e-86
GBSR01000047	E3 ubiquitin ligase, putative <i>Ixodes scapularis</i>	XP_002401000	2e-49
GBSR01000085	Nucleoside diphosphate kinase, putative <i>Ixodes scapularis</i>	XP_002416191	1e-56
	Nucleoside diphosphate kinase <i>Ornithodoros parkeri</i>	ABR23367	1e-40
GBSR01000095	Protein disulfide-isomerase <i>Ixodes scapularis</i>	AAV66973	9e-99
	Protein disulfide isomerase <i>Haemaphysalis longicornis</i>	ABS50238	3e-82
	Protein disulfide isomerase <i>Amblyomma variegatum</i>	ABD16189	1e-76
	Protein disulfide isomerase 3 <i>Haemaphysalis qinghaiensis</i>	ACA84006	2e-31
GBSR01000100	N-acetyltransferase, putative <i>Ixodes scapularis</i>	XP_002412276	5e-69
	N-acetyltransferase <i>Ornithodoros coriaceus</i>	ACB70303	9e-58
GBSR01000103	E3 ubiquitin ligase, putative <i>Ixodes scapularis</i>	XP_002400201	1e-18
GBSR01000114	Glutamyl-tRNA synthetase, cytoplasmic, putative <i>Ixodes scapularis</i>	XP_002434363	7e-20
GBSR01000133	Glutathione S-transferase, putative <i>Ixodes scapularis</i>	XP_002401749	2e-58
	Glutathione S-transferase <i>Rhipicephalus sanguineus</i>	AGK29895	5e-53
	Glutathione S-transferase <i>Rhipicephalus microplus</i>	ADQ01063	8e-52
	Glutathione S-transferase mu class <i>Rhipicephalus annulatus</i>	ABR24785	9e-52
	Putative glutathione S-transferase <i>Dermacentor variabilis</i>	ACF35504	2e-50
	Glutathione S-transferase <i>Haemaphysalis longicornis</i>	AAQ74441	2e-48
GBSR01000134	Acetylcholinesterase <i>Rhipicephalus sanguineus</i>	AAP49301	0.66
GBSR01000135	Cyclin-dependent kinase 11 <i>Rhipicephalus microplus</i>	AHF48780	3e-40
GBSR01000144	Homocysteine S-methyltransferase, putative <i>Ixodes scapularis</i>	XP_002403007	0.44
GBSR01000145	ATP synthase C subunit <i>Ixodes scapularis</i>	AAV66884	7e-56
	ATP synthase c-subunit <i>Ixodes pacificus</i>	AAT92216	4e-55
GBSR01000149	RNAse H, putative <i>Ixodes scapularis</i>	XP_002402054	1e-25
GBSR01000153	Cytidine deaminase, putative <i>Ixodes scapularis</i>	XP_00241033	5e-47
GBSR01000157	F1F0 ATP-synthase subunit Cf6, putative <i>Ixodes scapularis</i>	XP_002399676	6e-38
GBSR01000158	26S proteasome non-ATPase regulatory subunit, putative <i>Ixodes scapularis</i>	XP_002410756	6e-55
GBSR01000159	Phospholipid-hydroperoxide glutathione peroxidase, putative <i>Ixodes scapularis</i>	XP_002401221	3e-70
	Phospholipid-hydroperoxide glutathione peroxidase <i>Rhipicephalus microplus</i>	ABA62391	2e-59
	Phospholipid-hydroperoxide glutathione peroxidase <i>Dermacentor variabilis</i>	ABA62390	2e-58
GBSR01000171	Ornithine decarboxylase, putative <i>Ixodes scapularis</i>	XP_002413144	1e-70
	TPA: ornithine decarboxylase <i>Amblyomma variegatum</i>	DAA34383	4e-52

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000181	NADH:CoQ oxidoreductase subunit B18, putative <i>Ixodes scapularis</i>	XP_002405984	1e-59

^aSignal peptide confirmed.

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Table 5A

Ribosomal proteins.

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000003	Ribosomal protein L11, putative <i>Ixodes scapularis</i>	XP_002409414	8e-137
	60S ribosomal protein L11 <i>Ornithodoros parkeri</i>	ABR23487	3e-127
GBSR01000004	Ribosomal protein L31, putative <i>Ixodes scapularis</i>	XP_002403582	8e-78
	Ribosomal protein L31 <i>Dermacentor variabilis</i>	ACF35537	2e-74
	Ribosomal protein L31 <i>Argas monolakensis</i>	ABI52770	8e-73
GBSR01000012	Ribosomal protein S24, putative <i>Ixodes scapularis</i>	XP_002434386	2e-04
	Ribosomal protein S24 <i>Dermacentor variabilis</i>	AAAY66904	4e-04
GBSR01000013	60S ribosomal protein L32, putative <i>Ixodes scapularis</i>	XP_002399507	3e-58
	60S ribosomal protein L2/L8 <i>Ornithodoros coriaceus</i>	ACB70396	1e-85
	TPA: putative ribosomal protein L8e <i>Amblyomma variegatum</i>	DAA34056	4e-45
GBSR01000021	RecName: Full = 40S ribosomal protein SA <i>Ornithodoros parkeri</i>	A6NA00	1e-73
	TPA: 40S ribosomal protein SA <i>Amblyomma variegatum</i>	DAA34051	2e-67
GBSR01000024	60S ribosomal protein L27, putative <i>Ixodes scapularis</i>	XP_002434022	2e-82
	60S ribosomal protein L27 <i>Argas monolakensis</i>	ABI52762	1e-70
GBSR01000029	Ribosomal protein L6, putative <i>Ixodes scapularis</i>	XP_002400555	2e-113
	Ribosomal protein L6 <i>Ixodes pacificus</i>	AAT92170	7e-113
	TPA: 60S ribosomal protein L6 <i>Amblyomma variegatum</i>	DAA34118	1e-47
GBSR01000032	Ribosomal protein L35, putative <i>Ixodes scapularis</i>	XP_002410105	2e-80
	60S ribosomal protein L35-like protein <i>Dermacentor variabilis</i>	ACF35541	7e-74
GBSR01000033	40S ribosomal protein S13 <i>Ixodes scapularis</i>	AAAY66954	5e-73
	40S ribosomal protein S13 <i>Argas monolakensis</i>	ABI52720	2e-70
GBSR01000034	Regulator of ribosome synthesis, putative <i>Ixodes scapularis</i>	XP_002406973	2e-114
GBSR01000037	40S ribosomal protein S7-like <i>Ixodes scapularis</i>	AAAY66963	1e-86
	TPA: truncated 40S ribosomal protein S7 <i>Amblyomma variegatum</i>	DAA34567	7e-66
GBSR01000053	40S ribosomal protein S28 <i>Ornithodoros parkeri</i>	ABR23349	2e-26
GBSR01000055	Ribosomal protein S17 <i>Ixodes scapularis</i>	AAAY66942	5e-54
	Ribosomal protein S17 <i>Argas monolakensis</i>	ABI52710	2e-49
	Ribosomal protein S17 <i>Dermacentor variabilis</i>	ACF35534	3e-48
GBSR01000057	Ribosomal protein L37A <i>Ixodes scapularis</i>	AAAY66836	4e-23
	Ribosomal protein L37A <i>Ornithodoros parkeri</i>	ABR23427	4e-21
GBSR01000058	60S ribosomal protein L10, putative <i>Ixodes scapularis</i>	XP_002399224	2e-49
	60S ribosomal protein L10 <i>Ornithodoros parkeri</i>	ABR23479	3e-36
	60S ribosomal protein L10 <i>Ornithodoros moubata</i>	AGJ90349	8e-12
GBSR01000059	Ribosomal protein L21, putative <i>Ixodes scapularis</i>	XP_002403588	9e-74
	Ribosomal protein L21 <i>Ornithodoros moubata</i>	AAS55946	7e-63
	Ribosomal protein L21 <i>Ornithodoros parkeri</i>	ABR23438	1e-61
	Ribosomal protein L21 <i>Ornithodoros coriaceus</i>	ACB70301	1e-60
GBSR01000063	Ribosomal protein L4, putative <i>Ixodes scapularis</i>	XP_002402322	1e-40
GBSR01000065	RecName: Full = 40S ribosomal protein S21 <i>Ixodes scapularis</i>	Q4PM64	2e-54

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000067	40S ribosomal protein S21 <i>Argas monolakensis</i>	ABI52671	5e-49
	Ribosomal protein S21 <i>Ornithodoros coriaceus</i>	ACB70312	6e-44
	Ribosomal protein L22, putative <i>Ixodes scapularis</i>	XP_002412444	3e-52
	60S ribosomal protein L22 <i>Ornithodoros parkeri</i>	ABR23370	5e-48
GBSR01000068	60S ribosomal protein L22 <i>Ornithodoros coriaceus</i>	ACB70329	9e-41
	Ribosomal protein L18a <i>Ixodes scapularis</i>	AAV66898	3e-42
	60S ribosomal protein L18a <i>Ornithodoros parkeri</i>	ABR23382	3e-36
	60S ribosomal protein L9, putative <i>Ixodes scapularis</i>	XP_002407167	1e-25
GBSR01000073	60S ribosomal protein L9 <i>Ornithodoros parkeri</i>	ABR23490	2e-23
	RecName: Full = 60S ribosomal protein L17 <i>Ixodes scapularis</i>	Q4PM54	7e-36
	RecName: Full = 60S ribosomal protein L17 <i>Argas monolakensis</i>	Q09JW2	7e-35
	Ribosomal protein, putative <i>Ixodes scapularis</i>	XP_002404931	6e-136
GBSR01000082	60S ribosomal protein L7 <i>Argas monolakensis</i>	ABI52733	1e-114
	60S ribosomal protein L14, putative <i>Ixodes scapularis</i>	XP_002403086	2e-46
	RecName: Full = 40S ribosomal protein S3a <i>Ixodes scapularis</i>	Q4PM31	3e-104
	40S ribosomal protein S3a <i>Argas monolakensis</i>	ABI52667	3e-94
GBSR01000091	TPA: 40S ribosomal protein S3a <i>Amblyomma variegatum</i>	DAA34106	1e-83
	40S ribosomal protein S9, putative <i>Ixodes scapularis</i>	XP_002416200	4e-57
	Ribosomal processing protein, putative <i>Ixodes scapularis</i>	XP_002408200	1e-17
	TPA: putative ribosomal protein L8e <i>Amblyomma variegatum</i>	DAA34056	3e-42
GBSR01000093	60S ribosomal protein L2/L8 <i>Ornithodoros coriaceus</i>	ACB70396	2e-39
	Conserved hypothetical protein <i>Ixodes scapularis</i>	XP_002402767	4e-24
	40S ribosomal protein S3 <i>Ornithodoros parkeri</i>	ABR23477	1e-19
	40S ribosomal protein S3 <i>Ornithodoros parkeri</i>	ABR23477	6e-97
GBSR01000107	Ribosomal protein S15Aa <i>Ixodes scapularis</i>	AAV66923	1e-41
	Ribosomal protein S26, putative <i>Ixodes scapularis</i>	XP_002401133	2e-56
	40S ribosomal protein S26 <i>Argas monolakensis</i>	ABI52751	2e-53
	Ribosomal protein L27, putative <i>Ixodes scapularis</i>	XP_002399339	4e-30
GBSR01000112	ribosomal protein L26 <i>Ixodes scapularis</i>	AAV66956	4e-39
	60S ribosomal protein L126 <i>Ornithodoros parkeri</i>	ABR23381	2e-37
	40S ribosomal protein S2/30S <i>Ornithodoros parkeri</i>	ABR23354	5e-161
	40S ribosomal protein, putative <i>Ixodes scapularis</i>	XP_002400781	1e-149
GBSR01000122	TPA: 40S ribosomal protein S2 <i>Amblyomma variegatum</i>	DAA34096	1e-117
	60S ribosomal protein L10a <i>Ixodes scapularis</i>	AAV66960	2e-27
	60S ribosomal protein L10A <i>Ornithodoros parkeri</i>	ABR23359	9e-24
	Ribosomal protein S18 <i>Ixodes ricinus</i>	AAQ21388	1e-64
GBSR01000140	Ribosomal protein S18 <i>Ornithodoros parkeri</i>	AAV66885	1e-64
	Ribosomal protein L21, putative <i>Ixodes scapularis</i>	XP_002403588	9e-93
	Ribosomal protein L21 <i>Ornithodoros parkeri</i>	ABR23438	2e-85
	Ribosomal protein L21 <i>Ornithodoros moubata</i>	AAS55946	7e-85
GBSR01000154	Ribosomal protein L21 <i>Ornithodoros coriaceus</i>	ACB70301	2e-85
	40S ribosomal protein S6, putative <i>Ixodes scapularis</i>	XP_002434746	5e-32

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000163	RecName: Full = 40S ribosomal protein S21 <i>Ixodes scapularis</i>	Q4PM64	6e-46
	40S ribosomal protein S21 <i>Argas monolakensis</i>	ABI52671	1e-43
	Ribosomal protein S21 <i>Ornithodoros coriaceus</i>	ACB70312	4e-41
GBSR01000168	Ribosomal protein L30 <i>Ixodes pacificus</i>	AAT92174	2e-62
	Ribosomal protein L30 <i>Ornithodoros parkeri</i>	ABR23430	2e-62
	Ribosomal protein L30 <i>Ornithodoros coriaceus</i>	ACB70334	5e-62
GBSR01000169	40S ribosomal protein S12, putative <i>Ixodes scapularis</i>	XP_002410600	8e-71
	40S ribosomal protein S12 <i>Ornithodoros parkeri</i>	ABR23482	4e-80
	40S ribosomal protein S12 <i>Dermacentor variabilis</i>	AAP04352	3e-78
GBSR01000170	RecName: Full = 40S ribosomal protein S23 <i>Dermacentor variabilis</i>	Q86FP7	9e-81
	40S ribosomal protein S23 <i>Argas monolakensis</i>	ABI52808	4e-80
	40S ribosomal protein S23, putative <i>Ixodes scapularis</i>	XP_002412862	3e-78
GBSR01000177	60S ribosomal protein L23 <i>Ixodes scapularis</i>	AAY66949	7e-56
GBSR01000182	Ribosomal protein L35, putative <i>Ixodes scapularis</i>	XP_002410105	2e-41
	60S ribosomal protein L35-like protein <i>Dermacentor variabilis</i>	ACF35541	5e-41

* Signal peptide confirmed.

Table 5B

Proteins of miscellaneous function.

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000002	Myosin alkali light chain protein <i>Haemaphysalis longicornis</i>	ADN34300	2e-41
	Myosin alkali light chain protein <i>Haemaphysalis qinghaiensis</i>	AAV41826	3e-45
	Myosin light chain 1, putative <i>Ixodes scapularis</i>	XP_002414092	2e-28
GBSR01000007	Peptidoglycan recognition protein, putative <i>Ixodes scapularis</i>	XP_002413091	1e-07
GBSR01000008	Alpha-1 collagen type III, putative <i>Ixodes scapularis</i>	XP_002400135	6e-22
GBSR01000010	Tropomyosin invertebrate, putative <i>Ixodes scapularis</i>	O97162	1e-40
	RecName: Full = Tropomyosin <i>Rhipicephalus microplus</i>	Q8IT89	1e-11
	RecName: Full = Tropomyosin <i>Haemaphysalis longicornis</i>	Q8IT89	1e-11
	Unknown <i>Haemaphysalis qinghaiensis</i>	ABQ96858	1e-11
	Cyclophilin B <i>Haemaphysalis longicornis</i>	BAG41814	3e-90
GBSR01000022	Glycoprotein gC1qBP, putative <i>Ixodes scapularis</i>	XP_002400595	7e-93
	TPA: glycoprotein gC1qBP <i>Amblyomma variegatum</i>	DAA34112	2e-39
GBSR01000035	Transcription factor containing NAC and TS-N domains, putative <i>Ixodes scapularis</i>	XP_002413138	3e-63
	TPA: transcription factor <i>Amblyomma variegatum</i>	DAA34590	1e-54
GBSR01000038 ^a	Ricinusin, putative <i>Ixodes scapularis</i>	XP_002400090	3e-59
	Microplusin preprotein-like <i>Ixodes scapularis</i>	AAV66716	3e-58
	Ricinusin <i>Ixodes ricinus</i>	ABB79785	5e-50
GBSR01000040	Nono protein, putative <i>Ixodes scapularis</i>	XP_002401784	5e-29
GBSR01000045	Membrane protein, putative <i>Ixodes scapularis</i>	XP_002411759	5e-28
GBSR01000046	Neurofilament heavy polypeptide, putative <i>Ixodes scapularis</i>	XP_002413564	3e-40
GBSR01000056	Sorting nexin, putative <i>Ixodes scapularis</i>	XP_002402470	4e-25
GBSR01000066	tgf beta receptor associated protein-1, putative <i>Ixodes scapularis</i>	XP_002433616	5e-78
GBSR01000070	Heat shock protein <i>Haemaphysalis longicornis</i>	AFI64328	5e-22
	Heat shock protein <i>Haemaphysalis doenitzi</i>	AFI64330	6e-22
	Hsp90 protein, putative <i>Ixodes scapularis</i>	XP_002414808	8e-21
	Heat shock protein 90 <i>Ornithodoros moubata</i>	AGJ90347	5e-20
	TPA: heat shock protein 90 <i>Amblyomma variegatum</i>	XP_002403553	1e-07
GBSR01000076	Elongation factor 1 gamma, putative <i>Ixodes scapularis</i>	XP_002410199	3e-86
GBSR01000077	EF-Hand domain-containing protein, putative <i>Ixodes scapularis</i>	XP_002406497	8e-28
GBSR01000086	Alpha tubulin, putative <i>Ixodes scapularis</i>	XP_002434310	8e-100
GBSR01000090	Density-regulated protein, putative <i>Ixodes scapularis</i>	XP_002402211	8e-81
GBSR01000104	Hsp90 protein, putative <i>Ixodes scapularis</i>	XP_002414808	2e-130
GBSR01000105 ^a	Microplusin preprotein-like <i>Ixodes scapularis</i>	AAV66495	3e-47
GBSR01000124	Vacuolar sorting protein VPS28, putative <i>Ixodes scapularis</i>	XP_002402690	6e-44
GBSR01000125	Death-associated protein <i>Ixodes scapularis</i>	XP_002409376	6e-38
	Death-associated protein <i>Argas monolakensis</i>	ABI52760	2e-27
GBSR01000127	Translation initiation factor 3, subunit B, putative <i>Ixodes scapularis</i>	XP_002414993	6e-81
GBSR01000131	Alternative splicing factor ASF/SF2, putative <i>Ixodes scapularis</i>	XP_002415663	2e-31
	TPA: alternative splicing factor ASF/SF2 <i>Amblyomma variegatum</i>	DAA34416	5e-31

Accession #	Provisional identification in GenBank	Accession #	E-values
GBSR01000136	Actin, putative <i>Ixodes scapularis</i>	XP_002408110	5e-53
	Actin <i>Haemaphysalis longicornis</i>	AAP81255	1e-52
	Actin <i>Ornithodoros moubata</i>	AAS55945	1e-52
	Actin <i>Rhipicephalus microplus</i>	AAP79880	2e-52
	Actin <i>Rhipicephalus appendiculatus</i>	AAP81256	2e-52
	Actin <i>Hyalomma asiaticum</i>	ADJ56346	2e-52
	Actin <i>Ixodes persulcatus</i>	BAF98180	8e-51
	Actin <i>Ixodes ricinus</i>	AAX11193	3e-50
GBSR01000139	TPA: translation initiation factor 2 beta subunit <i>Amblyomma variegatum</i>	DAA34350	1e-09
GBSR01000143	Mapmodulin, putative <i>Ixodes scapularis</i>	XP_002407054	8e-05
GBSR01000147	Transmembrane protein, putative <i>Ixodes scapularis</i>	XP_002406433	4e-30
GBSR01000150	Myosin regulatory light chain <i>Ixodes pacificus</i>	AAT92161	3e-54
GBSR01000151	Splicing factor 3A, putative <i>Ixodes scapularis</i>	XP_002404980	4e-16
GBSR01000161 ^a	Cuticular protein, putative <i>Ixodes scapularis</i>	XP_002434576	2e-17
GBSR01000162	Synaptobrevin 1–2, putative <i>Ixodes scapularis</i>	XP_002404323	2e-69
	Synaptobrevin <i>Rhipicephalus microplus</i>	AHH29557	3e-55
GBSR01000164	Growth hormone-inducible soluble protein, putative <i>Ixodes scapularis</i>	XP_002400004	4e-19
GBSR01000165	Staufen, putative <i>Ixodes scapularis</i>	XP_002433902	2e-85
GBSR01000172	Chaperonin complex component, TCP-1 delta subunit, putative <i>Ixodes scapularis</i>	XP_002399659	5e-05
GBSR01000179	gar2, putative <i>Ixodes scapularis</i>	XP_002402869	1e-04

^aSignal peptide confirmed.